

Examination of *Helicobacter pylori* DNA in tap waters and bottled drinking waters

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Abstract

Aim: *Helicobacter pylori* is bacterium which colonizes the human gastric mucosa and known to affect half of the world's population. *H. pylori* is defined as the aetiological agent of peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. It has been suggested that the transmission of bacterium occurs via fecal-oral, oral-oral, and gastro-oral routes. In the present study, we aimed to investigate the presence of *H. pylori* DNA in drinking and tap water samples.

Materials and Methods: Sixty-six different trademarked drinking and 36 different tap water samples were collected from March 2019 - February 2020 all around Turkey. All water samples were filtrated using 0.22 µM membrane filters and filters were incubated in Brain Heart Infusion Broth for a half-hour at room temperature. Then, bacterial DNA was extracted. To detect *H. pylori* DNA, *ureC* (*glmM*) gene was investigated by Polymerase Chain Reaction (PCR). The PCR products were visualized in 1.5 % agarose gel electrophoresis.

Results: In this study, *H. pylori* DNA was not detected in any of the water samples tested.

Conclusion: In the present study, it was suggested that drinking and tap waters do not have a role in the transmission of *H. pylori* in this geographical area. Investigation of 500 mL of bottled waters could be one of the most obvious limitations in this study. Therefore it may be concluded that different water sources such as 19L flagon bottled drinking waters, water samples from various tanks, well waters, groundwater, and surface waters from rivers, lakes, or seas may be useful for clarifying the role of water for transmission route of this pathogen.

Keywords: *H.pylori* DNA; drinking water; tap water; water transmission

INTRODUCTION

Helicobacter pylori colonizes human gastric mucosa which causes gastritis, peptic ulcer disease, and mucosa-associated lymphoid tissue lymphoma (1-3). World Health Organisation defined the bacterium as a class I carcinogen in 1994 (4). It is established that infection rates are 70-90% of the population in developing countries and below 20% in some developed countries (1,5). *H. pylori* strains have different virulence factors that are responsible for various clinical manifestations. One of the important virulence factors is the adaptation ability to unfavorable conditions of the stomach, particularly by urease enzyme (5-9).

Although natural reservoir and host of the bacterium are completely unknown; human is the only defined reservoir of bacterium. Transmission routes of bacterium have been defined by fecal-oral, oral-oral, and gastro-oral (3,8,9). Previous epidemiologic studies suggested that water is an important source for transmission of *H. pylori* (3,8,10-16). Regardless of water transmission, some important properties of bacteria come into prominence to preserve

infectious properties such as bacterium synthesizes DNA and RNA at minimum levels and transforms into coccoid forms rather than spiral forms (8,9,17). On the other hand, some previous studies suggested that *H. pylori* may show resistance to some disinfectants. In relation to this, Baker et al (2002) and Lin et al (2016) shown that *H. pylori* is more resistant to chlorination and ozonation than *E. coli*. It is well known that the ability of biofilm formation also provides resistance to disinfection applications (18,19).

Another advantage of bacterium is related to survival and growth in free-living amoebae. It was reported that *Acanthamoeba castellanii* provides a niche for *H. pylori* and protects against environmental conditions (20,21). In some countries, evidence supports the existence of a strong relationship between the infection rates and exposing tap waters to wastewaters; this situation were known to correlate with socioeconomic status and poor hygienic conditions in developing countries (1,3,8). There are many studies reporting *H. pylori* detection in drinking, well, and tap waters in different regions of the world (1,10,13,15). In the present study, we aimed to examine

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DISCUSSION

It is well known that *H. pylori* colonizes nearly half of the world population's gastric mucosa. In regard to transmission routes of bacteria, investigations focusing on water transmission are few. Evidence related with water transmission of *H. pylori* based upon detection of bacterial DNA (24,25) and coccoid forms in water (15,26), the survival of bacteria in artificially contaminated water (27), and growth ability of bacteria in water (13,16).

Some authors suggest that although the virulence of bacterial coccoid forms in water is low, these forms still have urease activity and can adhere to human epithelial cells (3,11). These findings give us the reason to examine the presence of *H. pylori* DNA in drinking and tap water samples in our country. Isolation of bacteria and/or detection of bacterial DNA in drinking, surface, well, and tap water samples were shown in many studies all around the world (10-16,28,29).

In relation to transmission routes and the acquisition of bacterium, some approaches were also proposed. The association between *H. pylori* and *Acanthamoeba* was reported by Winiecka-Krusnell et al. (2002). They found that *Acanthamoeba castellanii* provides a niche for *H. pylori* for nearly eight weeks. Thus, it was suggested that this symbiotic lifestyle protects the bacteria against environmental conditions and even disinfection (20).

Another proposed approach is related to the ability of biofilm formation. This property is known as an advantage to survive and attach on surfaces of pipe systems (30-32). Azavedo et al. (2006) reported that bacteria adhered to various plumbing materials such as copper and stainless steel (33).

Moreover, *H. pylori* strains were found to be more resistant to chlorination and ozonation than *E. coli* (18,19). It is well known that *E. coli* is a coliform bacterium which is defined as a biological indicator for detecting fecal contamination of water samples. Therefore, some authors suggest that the examination of traditional microorganisms in water may not be enough to protect people from exposure to other unexpected microorganisms such as *H. pylori* (3,18,19,34).

Other researchers also believe that there is a relationship between *H. pylori* transmission and consumption of untreated well or spring waters (35,36). High infection rates in developing countries may be related to the consumption of contaminated water in poor sanitary conditions. In a study by Bahrami et al. (2013) it was shown that dental units' water samples can become a public health concern by contamination with *H. pylori* (13). In accordance with Bahrami's study, several authors reported that there are many direct or indirect risk factors for *H. pylori* infections associated with aquatic environments such as swimming in rivers, fresh waters, streams, and marine waters, using contaminated groundwaters as water supplies, and the ingestion of contaminated undercooked vegetables (3,36,37). Therefore, we may think that the social and economic statuses of the countries are the

major determinant for the prevalence of water-originated transmission of this bacterium.

Several studies on the isolation of *H. pylori* strains from different parts of the world showed that the detection rates of this bacterium could be variable depends on the geographical regions and origins of waters examined. *H. pylori* were detected in 20% of water samples in Switzerland (well waters and tap waters), while this rate was found as 26% in England (waters from distributions systems) and 50% in Peru (drinking waters) (10,11,38). The detection rates of *H. pylori* reported as 4-40% from drinking waters and tap waters in the Middle East countries (2,12,13,24). In our study, there was no *H. pylori* DNA in any of 102 water samples; which is consistent with the results of some other studies (15,39,40). Thus, we suggest that these results can also prove the effectiveness of disinfection of city tap waters and drinking waters for our country in our study's frame.

CONCLUSION

Although we examined *H. pylori* in water samples by using PCR which is a very sensitive and specific molecular technique, we could not detect *H. pylori* DNA in any of the samples. The source of water samples we examined were disposable bottled drinking waters and chlorinated tap waters which was the most important limitation of our study. Therefore, it may be concluded that examination of surface, well, river, lake, or seawaters and 19L flagon bottled drinking waters and samples from various tanks, may be useful for clarifying the role of water for transmission route of this pathogen.

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Competing Interests: The authors declare that they have no competing interest.

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