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Frequency and seasonality of viable *Helicobacter pylori* in drinking water in Dhamar Governorate, Yemen

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Abstract
Helicobacter pylori is an important and common bacterial pathogen in humans. The accumulated evidence of *H. pylori*'s existence in water from different environmental sources suggests a water-borne transmission route. This study aimed at investigating the occurrence of *H. pylori* in different water sources used by human populations in Dhamar Governorate, Yemen. 250 samples were randomly collected from the municipal water supply network, wells, and springs. The samples were processed, plated onto modified campy-blood agar, and incubated under microaerobic conditions for 4-10 days. Bacterial identification was based on morphological properties and biochemical tests. Bacteriological analysis showed that 9.6% and 13.2% of tap and surface water samples were contaminated with *H. pylori*, respectively. Despite a higher frequency in samples from rural areas, these were not significantly (p=0.068) more contaminated than the samples from urban areas. Regarding the seasonal variations of *H. pylori* detection, 85.71% of positive samples were detected in the late winter and spring seasons (February to May). To conclude, *H. pylori* transmission through water is likely to occur in Dhamar Governorate. Further prospective studies are highly recommended to provide further evidence and a clearer picture of *H. pylori* transmission.

Introduction
Helicobacter pylori has been recognized as a major pathogen in humans for nearly four decades. Although treating infected individuals and improving living standards in communities have reduced transmission of infection, *H. pylori* remains the most common human bacterial pathogen, infecting about half of the world's population (Hooi *et al*., 2017). It is also associated with gastrointestinal diseases that range from gastritis to neoplastic diseases such as mucosa-associated lymphoid tissue lymphomas and gastric cancer (Paradowska *et al*., 2010; Abadi and Kusters, 2016).
A great deal has been learned about the epidemiology, infection, biology, pathogenesis, diagnosis, and treatment of *H. pylori*; however, major gaps in our knowledge remain to be filled. The precise mode of infection transmission remains unclear, despite the fact that many epidemiological studies have identified different potential risk factors (Kusters *et al*., 2006; Chmiela and Kupcinskas, 2019). Although *H. pylori* infection occurs worldwide, its prevalence varies widely within and between countries. For instance, 70–90% of people in developing countries have *H. pylori*, while only 25–50% of those in developed countries are infected (Almashhadany and Mayass 2018; Almashhadany *et al*., 2022). Fortunately, *H. pylori* prevalence is decreasing as a result of improved sanitary conditions and treatment procedures (Azevedo *et al*., 2007, Niknam *et al*., 2014).
Different bacterial pathogens, such as fast-growing atypical mycobacteria, *Burkholderia pseudomallei*, and *H. pylori*, might be transmitted directly by drinking water, especially in developing regions (Ashbolt, 2004). The main reservoir of *H. pylori* is humans, particularly the human stomach; however, environmental or animal reservoirs have also been investigated as sources of *H. pylori* infection (Al-Mashhadany and Mayass, 2017). Food and water sources have been suggested as reservoirs outside the human gastrointestinal tract, and *H. pylori* or its DNA has been detected in these sources (Khalifa *et al*., 2010; Momtaz *et al*., 2014). It is worth mentioning that *H. pylori* can survive in water for prolonged periods (Enroth and Engstrand 1995; Al-Mashhadany, 2018; Al-Mashhadany, 2020).
The fact that *H. pylori* may be transmitted through water and contaminated food is posing a major problem in public health (Al-Mashhadany *et al*., 2018; Vesga *et al*., 2018, Al-Mashhadany, 2018). Epidemiological studies in Peru and other developing countries strongly support the transmission of *H. pylori via* water, as evidenced by its detection through polymerase chain reaction (PCR), suggesting that water can be an important vehicle for *H. pylori* infection (Hulten *et al*., 1996, Boehnke *et al*., 2018). The source of H. *pylori* in drinking water has been attributed to fecal contamination (Talaei *et al*., 2015).
Various findings that support the hypothesis that *H. pylori* is a water-borne pathogen have been reported. It has been mentioned that individuals who consume raw vegetables are more likely to acquire *H. pylori* (Chen *et al*., 2005; Yahaghi *et al*., 2014). The association of the infection with the consumption of raw vegetables is additional indirect evidence of the presence of *H. pylori* in the water

used for the irrigation of these vegetables (Abdel-Latif and Abouzied, 2016). Studies from developing countries with low socioeconomic status and poor management of drinking water suggest that environmental factors play important roles in *H. pylori* epidemiology (Aziz *et al*., 2015). Indeed, an association between the prevalence of *H. pylori* in Peruvian children and the source of drinking water was shown more than three decades ago (Klein *et al*., 1991).
H. pylori infection in Yemen is primarily acquired in early childhood (Al-Mashhadany and Mayass, 2017). Its transmission routes are debated; therefore, this work aimed to study the occurrence of *H. pylori* in drinking water in Dhamar Governorate and to determine its occurrence in drinking water for months during the period of study.

Materials and Methods

Sampling sites and study design

Dhamar Governorate is located in the middle west of Yemen at 2400-2500 m above sea level and has a small main city. Tap water samples were collected from the governmental municipal water network in the city, while surface and ground water samples were taken from small natural springs/streams and wells in the villages around the city. These natural sources are used as drinking water for villagers. The samples (1 L each) were collected randomly between February and October 2021 to assess the frequency of detecting culturable *H. pylori* in different seasons.

Samples collection and processing

A total of 250 drinking water samples (136 tap water and 114 surface water) were collected from different places in Dhamar Governorate, Yemen. The samples were collected in sterile bottles and transported to the laboratory inside an icebox containing ice on the internal walls (Al-Mashhadany and Mayass, 2017). Samples were filtered through a 42 mm diameter 0.45 μm pore-sized nylon membrane. The membrane was transferred onto a sterile 140-mm Petri dish containing 25 mL of phosphate-buffered saline and the organisms were resuspended using a cell scraper to rub the surface of the membrane and elute the depositions off the surface. A volume of 10 mL was transferred into a sterile container and stored at 4°C until culture within half an hour (Whale *et al*., 2003).

Detection of Helicobacter pylori

The stored volume (10 mL) was centrifuged at 4000 rpm for 30 minutes, and the upper 5 mL of the supernatant was discarded (Whale *et al*., 2003). Then 20 μL of the sample was spread onto modified campy-blood agar with Dent's supplement and incubated under microaerobic conditions (O_2 5%, CO_2 5%, H_2 2%, N_2 88%) for 4-10 days (Al-Mashhadany and Mayass, 2017; Almashhadany *et al*., 2022). The identification of *H. pylori* isolates was started by colony morphology on the agar plates and confirmed by biochemical tests according to a published protocol (Whale *et al*., 2003). Suspected small, round, and translucent colonies of *H. pylori* were purified by subculture and then identified by the mentioned biochemical tests. The biochemical tests included catalase test, oxidase test, urease test, indole test, growth in 1% glycine, growth in 3.5% sodium chloride, triple sugar iron agar reactions, H_2S production, cephalothin/nalidixic acid sensitivity/resistance, and hippurate hydrolysis test (Table 1).

Statistical analysis

Data were analyzed using SPSS software version 25 (IBM SPSS Inc, Chicago, IL, USA). Confidence intervals of prevalence were calculated using the "exact" Clopper-Pearson method at an α level of 0.05. The chi-square test was applied to test the difference between groups.

Results

Out of 250 samples of different types of drinking water, 28 (11.2%) gave a positive result for the isolation of *H. pylori*. This result includes 13/136 (9.6%) positive samples from tap water and 15/114 (13.2%) positive samples from surface water (Table 2). Statistical analysis using the chi-square test

showed no significant differences between sources in terms of the occurrence of *H. pylori* among tap water and surface water samples ($p=0.370$, $\chi^2=0.80$). Additionally, it is estimated that a maximum of 15.78% of drinking water may be contaminated with *H. pylori*.

Occurrence of Helicobacter pylori in water according to collection sites

In rural drinking water, *H. pylori* was found at a higher frequency in comparison to drinking water from urban areas. However, that difference is not statistically significant ($p=0.068$, $\chi^2=3.33$). Table 3 shows the details of *H. pylori* prevalence in drinking water according to collection sites.

Isolation of Helicobacter pylori from water according to the type of water sources

Fourteen of 136 tap water samples (10.3%), six out of 80 ground water samples from wells (7.5%), and eight of 34 samples from spring water (23.5%) were found to be contaminated with H. pylori (Figure 1).

Monthly variations in the rate of Helicobacter pylori isolation

The rate of positive samples detected during the study period varied widely, with 85.71% of positive samples being detected in the late winter and spring seasons (February to May). The highest rate of isolation from drinking water was in April (33.3%), with an observed decrease in the isolation rate in earlier and later months and no isolation could be obtained from late summer to late autumn (August to October). There is a significant difference between seasons in terms of the isolation of *H. pylori* from water ($p<0.01$). (Figure 2).

Discussion

Humans are the principal source of *H. pylori* transmission, and control measures that can be applied to protect drinking water supplies include preventing contamination through human waste and providing adequate disinfection since *H. pylori* is sensitive to oxidizing disinfectants (Castillo-Rojas *et al*., 2004). As the transmission of *H. pylori* through water has become increasingly suspected, the World Health Organization (WHO) now refers to this microorganism as a water contaminant (WHO 2022). *H. pylori* acquisition is being linked to the quality of the drinking water, particularly the consumption of untreated well water (Bellack *et al*., 2006; Doyle, 2012, Plonka *et al*., 2014).

The detection of *H. pylori* in water varies between studies that used different detection tests. The current findings are consistent with the study results previously obtained in Dhamar Governorate (Almashhadany and Mayass, 2017), where the total occurrence of *H. pylori* in water samples was 11.61%. However, we found fewer positive samples than reported by Hegarty *et al*. (1999), who found that actively respiring *H. pylori* were present in 60% of the surface water samples (n=62) and 65% of the shallow groundwaters samples. The identity of *H. pylori* isolates was confirmed by 16S rRNA PCR (Hegarty *et al*., 1999). On the other hand, our findings are higher than those found in similar studies from Iraq (n=471) (Al-Sulami *et al*., 2012), Pakistan (n=50) (Khan *et al*., 2012), and Iran (n=400) *(Ranjbar et al*., 2016), where the detection rate ranged between 1.77% and 3.63% only. Such variations are expected to be linked to differences in geographical locations, human activities near water sources, and detection methodologies. Several studies have been performed to assess the viability of *H. pylori* in water since it has been suggested that the coccoid form of *H. pylori* is responsible for transmission in the environment (Kayali *et al*., 2018). Moreover, *H. pylori* could survive disinfection practices normally used in drinking water treatment in a viable but non-culturable form (Moreno *et al*., 2007), which would allow the bacterium to go undetected by culture methods.

Regarding water types, our results are lower than those reported by Ahmed *et al*. (2007) in South India, who found that the prevalence of *H. pylori* infection among people who drank water from wells was 92% compared with 74.8% of those who drank tap water ($p<0.001$). *H. pylori* infection prevalence was found to be higher in people with a low clean water index (CWI) (88.2%) than in those with a higher CWI (33.3%) ($p<0.001$).

The seasonality of *H. pylori* is not well understood. Our seasonality results are not in agreement with the study by Ranjbar *et al* (2016) performed in Iran, who found that the samples collected in the summer season had the highest prevalence of *H. pylori* (4.54%). A significant statistical difference was seen in the prevalence of *H. pylori* between the warm and cold seasons of the year ($p<0.05$). In another study carried out in Venezuela, the findings showed that *H. pylori* infection frequency was significantly higher during the rainy season (96%) than during the dry season *(Domínguez-Bello et al*., 2002).

Conclusions

Water is an important vehicle for the transmission of numerous pathogens, including *H. pylori*. In Dhamar Governorate, transmission of *H. pylori* through water is very likely to occur, as viable cells of this pathogen have been recovered through traditional bacteriological analysis. Surface water is more frequently contaminated than municipal water supplies. Regarding the seasonality of *H. pylori*, the highest detection rates occur during winter and spring, while no positive samples were detected during autumn. Further prospective studies are highly recommended to provide additional evidence and a clearer picture of *H. pylori* transmission.

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Table 1. Biochemical tests confirming the identity of *Helicobacter pylori.*

Biochemical Tests	Results	Biochemical Tests	Results
Catalase	Positive	H_2S production in (TSI)	Negative
Oxidase	Positive	TSI with lead acetate paper	Positive
Urease	Positive	Nalidixic acid	Resistance
Indole	Negative	Cephalothin	Sensitive
Growth in 1% Glycine	Negative	Hippurate Hydrolysis	Negative
Growth in 3.5% NaCl	Negative		

TSI, triple sugar iron agar.

Table 2. Isolation of *Helicobacter pylori* from drinking water types in Dhamar Governorate.

Water type	No. examined	Positive samples		95% confidence interval
		n	%	
Tap water	136	13	9.6	5.19 – 15.79
Surface and ground water	114	15	13.2	7.56 – 20.77
Total	**250**	**28**	**11.2**	**7.57 – 15.78**

Table 3. Isolation of *Helicobacter pylori* in drinking water according to collection sites.

Collection site	No. examined	Positive samples		95% confidence intervals
		n	%	
Urban	130	10	7.7	3.75 – 13.69
Rural	120	18	15.0	9.14 – 22.67
Total	**250**	**28**	**11.2**	**7.57 – 15.78**

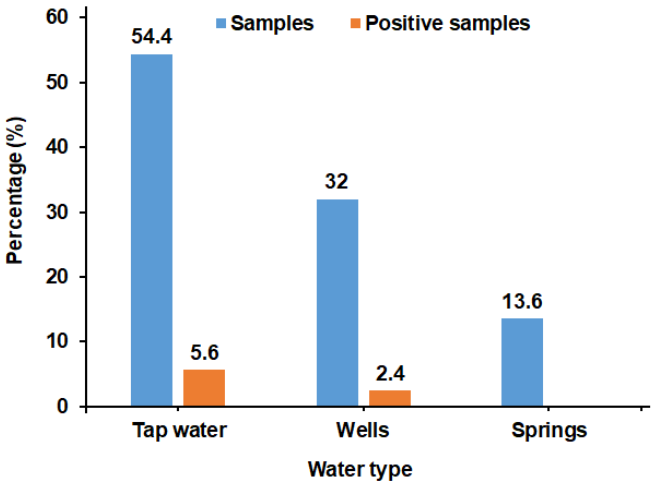

Figure 1. Collected samples and *Helicobacter pylori*-positive samples. Percentages of collected samples from different sources along with the culture-positive samples.

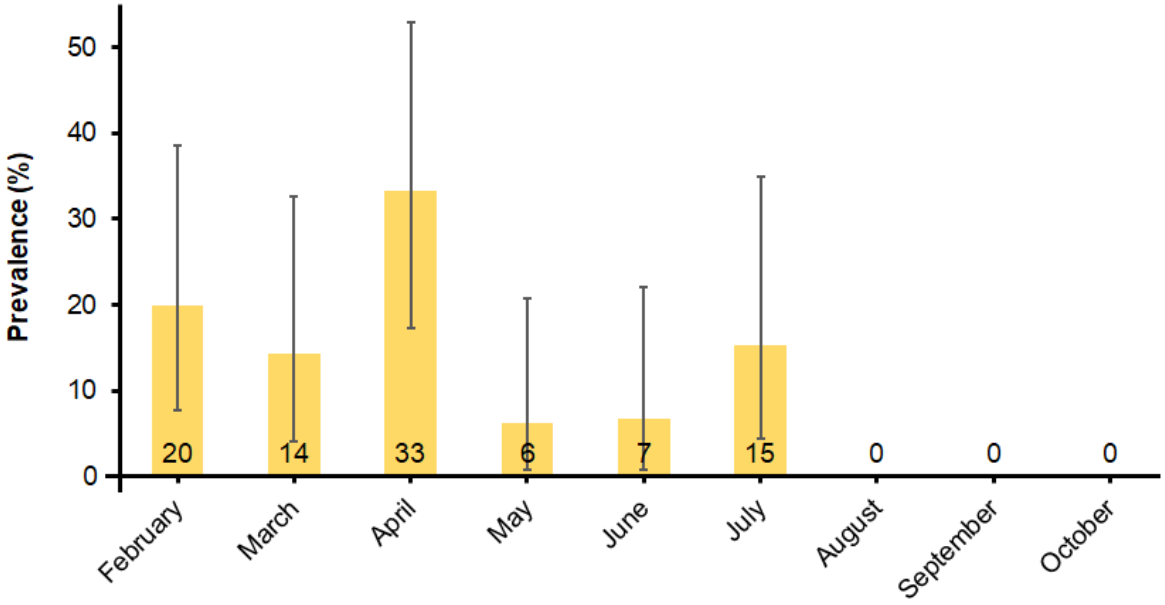

Figure 2. Relationship between months and prevalence of *Helicobacter pylori* in water during the period of the study. Error bars represent 95% confidence intervals.