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Original Article

Detection of *Helicobacter pylori* DNA in Some Egyptian Water Systems and Its Incidence of Transmission to Individuals

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Abstract

Background: The current study aimed to detect the presence of *Helicobacter pylori* in some water systems in Egypt as well as in blood samples of patients suffering from stomach ulcers.

Methods: Fifty-one water samples collected from some Egyptian governorates; Giza, Alexandria, Monofia, Beheira, Minya, Sohag, was subjected to physical and chemical analysis. Urease gene ureC, a highly specific sequence in *H. pylori* DNA was tracked in tested water samples and then in blood samples of patients' resident in areas with infected water supply using PCR technique. Data were all fed to the computer using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test.

Results: Two samples, belonging to Abu El Matamir-Beheira, and Sidi Bishr-Alexandria, showed positive results for presence of *H. pylori*. These two samples gave a positive result for the second time, on applying PCR technique for the cultures isolated under microaeroplilic conditions. Blood samples (173) were withdrawn from individuals living in the two areas with infected water supply. As diagnosed by ELISA, positive tests for *H. pylori* were recorded in 33.3% and 33.8% of patients suffering from ulcers in Abu El Matamir-Beheira, and Sidi Bishr-Alexandria, respectively.

Conclusion: In developing countries such as Egypt, there is a big possibility for *H. pylori* to be transmitted via drinking water, hence, causing an epidemic infection, particularly in dense populated areas.

Keywords: *Helicobacter pylori*, Egypt, Water systems, Urease gene ureC, ELISA

Introduction

Helicobacter pylori causes gastritis and peptic ulcers in millions of people around the world (1). It has also been involved in stomach cancer to such a level that WHO has classified it as a Class I Carcinogen (2, 3). Up to 85% of people infected with *H. pylori*, never experience symptoms or problems (4), and acute infection can show an acute gastritis with abdominal pain or nausea (5).

According to Aziz et al.(6), "Over the preceding years and to date, the definitive mode of human infection by *Helicobacter pylori* has remained largely unknown". According to the investigation of many studies, contaminated water was found to be the main causative of transmission and infection (6,7). "The infection rate is especially higher in developing countries (80-90%), where contaminated water, combined with social hardships and poor sanitary conditions, plays a key role " (6). This pathogen was tracked in different water systems either by cultivable, serological or molecular techniques (1, 7). However, molecular techniques proved to be the most accurate, specific and fast

(7, 8). In other words, one could detect DNA from a single infectious agent, targeting highly specific genes in the *H. pylori* DNA like the urease gene (7, 9).

In Egypt, the River Nile is considered the main source of drinking water. It supplies 56.8 billion m^3 of freshwater every year, which represents 97% of all renewable water resources in Egypt. Contamination of drinking water in Egypt was and still a sound problem of a very frightening hygiene risk, threatening the health of the Egyptian community (10, 11). A social problem like this should be strongly and properly faced. It was thus aimed in this study to assess the prevalence of *H. pylori* in different water sources in Egypt by detection of DNA. It was also aimed to measure the incidence of disease transmission to individuals.

Experimental Section

Studied areas

The study was conducted in six governorates in Egypt (Fig. 1). All characterized by dense population and high incidence of gastric disease and cancer (12- 14). The geographic variation of studied areas is believed to be largely socioeconomic. The source of drinking water for all of them is the River Nile or its branches, except for Abu El Matamir-Beheira and Al Sadat-Monofia. A brief illustration for each studied area is as follows:

Beheira Governorate is located in the northern part of Egypt in the Nile Delta, and is one of largest areas with relatively poor citizens. Thus two cities were selected; Abu El Matamir and Kafr El-Dawwar. The Mahmoudia canal (river) goes through Kafr el-Dawwar north to Alexandria. It branches west from the Nile and is the main source of drinking water and irrigation there.

Alexandria Governorate is located in the northern part of Egypt, directly on the Mediterranean Sea, making it one of the most important harbours in Egypt. Sidi Bishr representing the largest district in the city of Alexandria was chosen for the current study. El Ebrahimia is another Alexandrian district, but a more developed than Sidi Bishr.

Monofia Governorate is located in the northern part of the country in the Nile Delta, to the north of the capital Cairo. Al Sadat City is located 94 kilometres north-west of Cairo, and is as one of the largest industrial cities in Egypt.

Giza Governorate situated on the west bank of the Nile River opposite to the capital Cairo. It includes a stretch of the left bank of the Nile Valley around Giza, and acquires a large stretch of desert. El Mohandessin is a district in Giza City, the capital of Giza Governorate, and considered as one of the most prestigious and expensive areas in both Giza and Cairo.

Minya Governorate located in Upper Egypt. It has great wealth of important archaeological sites and considered as an important agricultural and industrial region. Minya City the capital is located approximately 245 km south of Cairo on the western bank of the Nile River, which flows north through the city.

Sohag Governorate is one of the governorates of Upper Egypt. It is located in the southern part of the country, and covers a stretch of the Nile Valley. Sohag City is the capital and lies on the western bank of the Nile on a fertile agricultural plain.

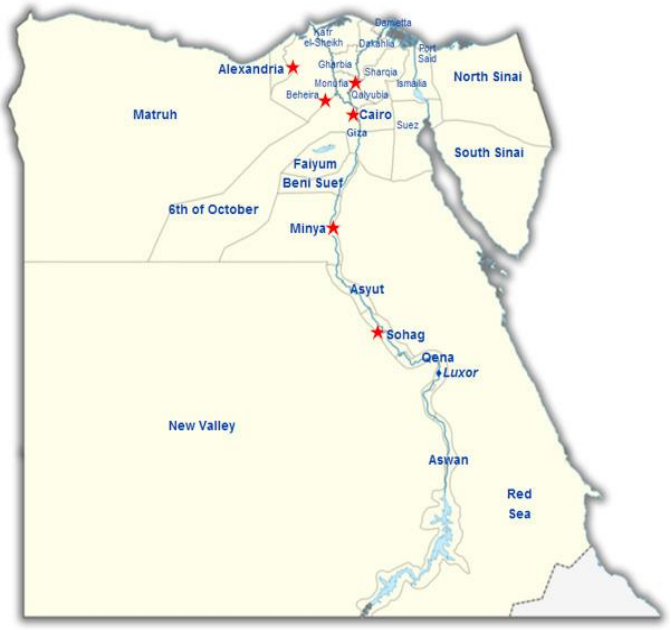

Fig. 1: A map illustrating the Egyptian districts under study

Water samples

Fifty-one water samples were collected from studied areas. The number of samples was sufficient to cover studied areas, depending on the nature of the area and type of its water source (Table 1). The number of samples taken was related to the density of population. For example, seven samples were taken from high-populated areas, and lower number from less populated ones.

Table 1: Source and of type of water samples under investigation

Source	Type	Number of samples
Abu El Matamir-Beheira.	Ground water	7
Kafr El Dawar-Beheira.	Al Mahmoudia River	7
Kafr El Dawar-Beheira.	Tap Water	6
El Ebrahimia-Alexandria.	Tap Water	7
Sidi Bishr-Alexandria.	Tap Water	7
Al Sadat-Monofia.	Ground water	6
El Mohandessin-Giza.	Tap Water	7
Minya	Tap Water	2
Sohag	Tap Water	2

Sample analysis and preparation

Water samples were collected in sterile 150 mL polypropylene tubes, transported to the laboratory in an ice chest and stored at 4°C for further analysis. They were analyzed using electrolyte analyzed (Easy Lyte analyzer Medica Coropration-USA). Alkalinity and hardness were titrated using sulfuric acid at normality 0.02N, according to Buret titration method 8221 (HACH company, USA).

For sample preparation, dechlorination of water was performed using 0.1% (wv^{-1}) sodium thiosulfate (Sigma). Prefiltration was done, using AP 25µm prefilters (Millipore Corp) with 0.8µm pore size and 25 µm diameter. This process improves the filtration and causes big reduction in the number of bacteria other than *H. pylori* (15). Water samples were then filtered through Millipore fluoropore FHLP filters (Millipore Corp), with pore size 0.5 µm and 25µm diameter, using a swinnex filter holder to collect the bacterial cells under vacuum. Each filter was soaked first in ethanol until it became transparent (≤ 1min) and then transferred with the laminated surface to collect the bacterial cells. After filtration, the filter was folded and transferred with a sterile forceps into a 0.6 mL Gene Amp reaction tube (Perkin-Elmer Cetus) containing 100 µL sterile distilled water, with the cell coated surface facing inside. The tubes were stored at -20°C until further processing.

Isolation and cultivation of H.pylori

For isolation of bacteria, each membrane of the filtered samples was then immersed into 2mL of tryptic soy broth (TSB) for 1 h. Each 2mL TSB was placed on the surface of Dent's selective medium (16) with the cell coated surface facing the media, incubated at 37°C for 72 h under microaerophilic conditions in the presence of 5–15% oxygen and 5% carbon dioxide. Developed colonies were tested for *H.pylori* by PCR.

DNA extraction

For DNA extraction, the Freeze-thaw-lysis (17) approach was adopted. This technique is used in the absence of lysozyme. It was used for both the bacterial cells collected by filtration of water through the fluoropore FHLP filters directly and the isolated colonies on Dent's medium. Without removing the filters, bacterial cells in the tubes were lysed and the DNA was released by repeated cycles (about 5 to 10 cycles) of freezing in an ethanol- dry ice bath and thawing at 50°C in a water bath. The tubes were then cooled in an ice bath for 30 min.

Polymerase Chain Reaction

In the present study, the PCR (18) was performed with primers for urease gene ureC5' – AAG CTT TTA GGG GTG TTA GGG GTT T– 3' and 5' – AAG CTT ACT TTC TAA CAC TAA CGC – 3', indicative of *H. pylori* infection (19). Amplification was performed in a final volume of 50 µL of PCR mixture containing 0.8 µm of each primer, 10 mM of each deoxy nucleotide triphosphate (dATP, dGTP, dTTP and dCTP), 10 mM tris HCl, 50 mM KCl, 0.1% triton X– 100, 1.5 mM $MgCl_2$, one unit of DNA polymerase (Fermentas) and 10 µL of template DNA. DNA amplification was carried out as follows: denaturation at 94°C for 5 min in the first cycle, followed by annealing for 30 sec at 60°C, extension for 2 min at 72°C, and denaturation for 30 sec at 94°C for a total of 40 PCR cycles. The extension for the last cycle was increased to 5 min to ensure complete extension of the amplified fragment. The PCR products were resolved by 2% agarose gel electrophoresis (20).

Detection of H. pylori in blood samples

A total of 173 blood samples were collected from patients with stomach ulcers living in areas with infected water supply as proven in the present work as follows: sixty-eight kindly provided by Al-Quds lab (Sidi Bishr-Alexandria) and one hundred and five samples from Dr. Abdel-Raouf Mahmoud's Lab (Abu El Matamir-Beheira).
H.pylori in blood samples collected from patients was detected according to the protocol of *H.pylori* IgG-ELISA Kit of Bioactivia-Germany using an enzyme immunoassay as directed by the manufacturer (Premier Platinum HpSA; Meridian Diagnostics Inc., Cincinnati, Ohio).

Statistical analysis of the data

Data were fed to the computer using IBM *SPSS software package version 20.0*. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test.

Chi-Square test

It tests the association between qualitative nominal variables, it is performed mainly on frequencies. It determines whether the observed frequencies differ significantly from expected frequencies.

Results

Physical and chemical characteristics of water sources

Investigation of physical and chemical properties of water samples revealed the data shown in Table 2, where all types of water had a normal to slightly alkaline pH ranging from 7.2 to 8.4. Nile River water was characterized by highest pH and turbidity (pH 7.9-8.4, turbidity 2.5-3.4 NTU). Nile River samples were found to be the warmest of a temperature 27°C, whereas the tap water samples of other different districts had less temperature degrees ranging between 20 and 22°C. The ground water samples, were the coldest (17°C). Regarding the total alkalinity and total hardness, the two ground water samples with the Nile River were the highest with the following order: Ground water from Al Sadat > ground water Abu El Matamir > Kafr El Dawar. Again, the two ground water samples and that of Nile River were the highest in sodium, calcium, and magnesium ions concentration, where the Nile River sample was the highest in sodium (38-41mgL^{-1}), and the ground water from Al Sadat was the highest in calcium (62-72mgL^{-1}). On the other hand, the ground water samples from Abu El Matamir was the highest in magnesium (50mgL^{-1}) followed by ground water from Al Sadat (27-29mgL^{-1}). On the other hand, chlorine ions were in their higher level in all tap water samples in addition to samples from Nile River (40-58mgL^{-1}). Chlorine in ground water samples was found in its lower level.

Detection of H. pylori DNA in water samples

In this study, direct detection of ureC gene in water samples, using PCR technique, provided positive data in only two samples (tap water of Sidi Bishr-Alexandria and ground water of Abu El Matamir-Beheira), representing only 3.92% of the total samples.

Table 2: Physical and chemical characteristics of tested water samples in different districts in Egypt

Parameter	Unit	Ground water		Nile		Tap water				
		Abu El Matamir-Beheira	Al Sadat-Monofia	Kafr El Dawar-Beheira	Kafr El Dawar-Beheira	El Ebrahimia-Alexandria	Sidi Bishr Alexandria	El Mo-handessin-Cairo	Minya	Sohag
pH		7.2-7.5	7.3-7.8	7.9-8.4	7.4-7.7	7.5	7.6	7.25	7.19	7.22
Turbidity	NTU	0.78	0.45-0.7	2.5-3.4	0.55-0.98	0.77-0.87	0.9	0.57	1.08	1.05
Temperature	ºC	17.5	17	27	21	22	22	20.4	22	21.5
Total Alka-linity	mgL^{-1}	130-150	219-240	89-120	5-12	9-12	7-9	9-12	12	12
Total Hard-ness	mgL^{-1}	110-138	121-149	35-55	10-16	5-8	4-6	6-10	15-17	10
Sodium	mgL^{-1}	22-25	28-32	38-41	12-14	11	10-15	15	17	16
Calcium	mgL^{-1}	30-38	62-72	47-50	23	35	31.5	27	37	34
Magnesium	mgL^{-1}	27-29	50	9-11	3-5	6	5.5	7	8	7
Chlorine	mgL^{-1}	14-18	23-29	44-89	31-57	40-48	41-48	55.1	57.7	55.2

However, applying another booster PCR to magnify the product of the first PCR run, led to the detection of *H.pylori* in the same water samples. Same results were obtained when tracking the DNA of bacteria grown on Dent's medium applying PCR technique.

Detection of H. pylori in blood samples.

The prevalence of *H.pylori* in patients resident in the same districts where the pathogen was detected in water samples was evaluated. Blood samples were collected from 173 patients suffering from ulcers (68 from Sidi Bishr district, and 105 from Abu El Matamir city), and diagnosed by ELISA.

With respect to age, in Abu El Matamir, Beheira, *H.pylori* was demonstrated in 50% of the children and 31.6% of adults, at P= 0.001. While in Sidi Bishr district, the percent of infection was zero among children and 34.8% in adults, at P=0.091. Generally, no significant difference was recognized among the total incidence of infection in the two investigated areas, which was about 33.3% in Abu El Matamir while that calculated in Sidi Bishr area is 33.8% at P = 0.842.

Discussion

Physical and chemical characteristics of water sources

Physical and chemical properties of water samples were investigated in order to determine the quality and characteristics of the tested water samples and correlating them with the incidence of presence of *H.pylori* detected later by PCR technique. It was shown that, all types of tested water had a normal to slightly alkaline pH, and within a normal range of temperatures, according to the Egyptian weather. Nile River water was characterized by highest pH and turbidity due to the industrial disposal. A high level of total alkalinity and total hardness was also recorded in Nile River, in addition to the two ground water samples. The high level of alkalinity and hardness in Nile River is in accordance with that recorded by Ali et al. (21). Again, the two ground water samples and that of Nile River were the highest in sodium, calcium, and magnesium ions concentration. These recorded levels of magnesium are in accordance with those of total hardness and very logic for ground waters. Chlorine ions were found to be higher in all tap water samples in addition to samples from Nile River, rather than in the other water samples. This is in accordance with the fact that Egyptian government uses chlorine as the main disinfectant in drinking water systems. Conclusively, all the recorded levels for physical and chemical water properties were found to be in the normal and expected ranges.

Detection of H. pylori DNA in water samples

The PCR assay applied in this work specifically targets a region of the ureC gene, which has been shown to be unique and essential for the growth

of *H. pylori*. Results can be obtained rapidly, unlike culture, which takes days to weeks (1, 3).

In the current study, the low incidence of direct detection of *H. pylori*, represented in the two samples of tap water of Sidi Bishr-Alexandria and ground water of Abu El Matamir-Beheira, could be attributed to water characteristics including hardness, alkalinity, pH and temperature, which might have led to DNA denaturation. Ground water of Abu El Matamir-Beheira contains low levels of chloride ions (chlorine) which might be the reason for the detection of *H.pylori* in this sample. On the other hand, *H.pylori* was demonstrated in tap water of Sidi Bishr-Alexandria, which contained high level of chlorine, which is commonly used as disinfectant in Egypt to ensure the safe distribution of water to people. Although studies conducted by Baker et al. (22) have shown that *H. pylori* is inactivated by chlorine, their conclusions were based on the lack of recovery using standard culture plating methods. They also, concluded that *H. pylori* have the ability to resist and tolerate disinfectants in the distribution systems and because of that it may be transmitted via water borne route. Giao et al. (23) confirmed that *H. pylori* can survive chlorination and stay undetectable using the culture methods.

Detection of *H.pylori* DNA in tap water of Sidi Bishr-Alexandria is not surprising in such dense populated area, with low sanitation practice and observed poverty. Abu El Matamir-Beheira relies on ground water as the major source of drinking water. The presence of *H.pylori* in ground water may be explained by Azevedo et al. (24) statement, "the notion about the inability of the bacterium to survive alone in running water, but to develop a symbiotic relationship and form complex structures on contact surfaces, makes it rational to assume that groundwater is a reservoir for *H. pylori* due to its stagnant nature".

Similar observations were reported in similar developing areas in the world. Khan et al. (25) reported the presence of DNA *H. pylori* in 4% of samples collected from municipal drinking water of Karachi, Pakistan. In another study, Brahami et al. (6) reported positive for the presence of *H. pylori* data in 4% of tap water samples examined in Isfahan province, Iran. In addition, the presence of *H.pylori* DNA in ground water in Iraq City was reported by Horiuchi et al. (26). Moreover, the detection of *Helicobacter* DNA, in the water distribution system in England by PCR technique was found by Watson et al. (15) who also stated that viable cells were not isolated and not cultured. Linke et al. (27), showed that in Germany, drinking water biofilms can act as a reservoir for *H. pylori* which increases the concerns about the role of biofilms as vectors for pathogens.

Detection of H. pylori in blood samples

On investigating the presence of *H. pylori* in blood samples of stomach ulcer patients resident in the same districts where the pathogen was detected in water samples, no significant difference was recognized among the total incidence of infection among the two areas, in spite of that Alexandria is a well-developed city if compared with Abu El Matamir city, having a more advanced water distributing system in addition to health care program. Besides, Alexandrians are highly educated, having a relatively high incomes compared to their candidates in Abu El Matamir. However, both cities, Alexandria and Abu El Matamir, were found to have the same rate of infection by *H.pylori* among their individuals.

Conclusion

This study shows some evident on the transmission of *H. pylori* by drinking water in Egypt. However, *H. pylori* prevalence is more pronounced between different communities, often located within the same country or region, and separated based on sanitation, overall hygiene, and standards of living. The high prevalence of *H. pylori,* particularly among children, is alarming and requires more attention from the government to control its transmission.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission,

redundancy, etc.) have been completely observed by the authors.

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