Isolation and identification of *Helicobacter pylori* from drinking water in Basra governorate, Iraq

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استفراد وتشخيص جرثومة الـمَلْويَّة البوّابية في مياه الشرب في محافظة البصرة بالعراق

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الخلاصة: إن طرز انتقال العدوى بالملويَّة البوابية مازالت غير واضحة. وقد جُمعت 198 عينة لمياه الشرب من 22 منطقة في محافظة البصرة خلال الفترة من تشرين الأول/ أكتوبر 2006 حتى تموز/ يوليو 2007. وقِيسَ تركيز الكلور المتبقي وكذلك عدد جراثيم القولونيات الإجمالية والبرازية. وقد حصل الباحثون على 469 مزرعة جرثومية، بعد استزراع العينات على مُسْتَنْبَت كولومبيا الأغار بالدم المحوَّر، وأمكن التعرُّف فيها على 173 مستفردةً جرثومية، أربع عشرة منها كانت لأنواع الـمَلْويَّة، منها عشرة فقط للمَلْويّة البوّابية (2.0٪ من إجمالي المستفرَدات). وتم اختبار هذه المستفرَدات من حيث حساسيتها للمضادات الحيوية وقدرتها على تحمل الكلور بتركيز 0.5 مغ/ ل. وهذه – حسب معلومات الباحثين – هي أول مرة يتم الإبلاغ فيها عن وجود جرثومة الملوية البوابية في مياه الشرب المعالَجة.

ABSTRACT The mode of the transmission of *Helicobacter pylori* infection remains poorly understood. A total of 198 samples of drinking water from 22 districts of Basra governorate were collected during the period October 2006 to July 2007. The concentration of residual chlorine was measured and the numbers of total and faecal coliforms were counted. On modified Columbia urea agar, 469 bacterial cultures were obtained, of which 173 isolates were identified. Only 14 isolates were *Helicobacter* spp., of which 10 were *H. pylori* (2.0% of the total isolates). These isolates were tested for antibiotic susceptibility as well as ability to tolerate chlorine at 0.5 mg/L. To our knowledge, this is the first report of the occurrence of *H. pylori* in treated municipal drinking water.

Isolation et identification d'*Helicobacter pylori* dans l'eau potable du gouvernorat de Bassora (Iraq)

RÉSUMÉ Le mode de transmission d'une infection à *Helicobacter pylori* reste mal connu. Cent quatre-vingt-dix-huit échantillons d'eau potable provenant de 22 districts du gouvernorat de Bassora ont été recueillis entre octobre 2006 et juillet 2007. La concentration de chlore résiduel a été mesurée et le nombre de coliformes totaux et fécaux ont été comptés. Sur les 469 cultures bactériennes obtenues à partir de la gélose Columbia modifiée à l'urée, 173 isolats ont été identifiés. Seuls 14 d'entre eux correspondaient à *Helicobacter* spp., dont 10 à *H. pylori* (2 % du total des isolats). La sensibilité aux antibiotiques de ces isolats a été testée, de même que leur tolérance au chlore à une concentration de 0,5 mg/l. À notre connaissance, il s'agit du premier signalement d'*H. pylori* dans de l'eau potable municipale traitée.

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Introduction

Helicobacter pylori, originally classified as *Campylobacter pylori*, is a Gram-negative, microaerophilic, spiral-shaped, motile bacterium associated with gastritis, peptic ulcer, duodenal ulcer and chronic gastritis. It is also implicated in the development of gastric cancer [1–3]. The mode of transmission of *H. pylori* remains poorly understood. It has been suggested that the housefly has the potential to transmit the bacterium, especially in areas of the world with poor sanitation [4]. Other likely transmission routes are faecal–oral, iatrogenic or oral–oral [5]. While drinking water contaminated with faeces has been proposed as a source of infection, *H. pylori* has not been isolated from water except in some instances in which it was detected using polymerase chain reaction (PCR) on samples from Colombia, Lima and Peru [6,7].

Three epidemiological studies in South America have linked transmission to food and water. In Chile, more than 60% of 1815 Chileans younger than 35 years old and of lower socioeconomic groups were found to be *H. pylori* seropositive [8]. A study of 407 children aged 2 months to 12 years in Peru also concluded that water was the vehicle of infection, because children who used the municipal water supply had a higher prevalence of *H. pylori* infection than children who used private wells [9]. Furthermore, an increased risk of infection was observed in children who swam in rivers and streams in the southern Colombian Andes [10]. While all these studies confirmed the possibility of *H. pylori* transmission via water, efforts to isolate the bacterium from water have been unsuccessful [11,12].

This study in Basra, Iraq, aimed to isolate *H. pylori* from treated drinking water and investigate the relationship of *H. pylori* to total and faecal coliforms as well as its susceptibility to several antibiotics and to chlorine.

Methods

Enumeration and identification of *H. pylori*

A total of 198 samples of drinking water from 22 districts in Basra governorate were collected during the period October 2006 to July 2007.

The concentration of residual chlorine for each sample was measured using a chlorine meter (Lovibond 2000). Aliquots of 250 mL from each sample were filtered by the membrane filtration technique using 47 mm cellulose acetate filters with a nominal pore size of 0.22 µm (Sartorius). The filter papers were cultured on modified Columbia urea agar medium [13] consisting of Columbia agar supplemented with 1% haemin, 5% urea solution, 4 µg of vancomycin and 0.12 mg of phenol red and incubated at 37 °C for 5–7 days under microaerophilic conditions (5% CO_2, 10% H_2, 85% N_2) for the isolation of *H. pylori*.

H. pylori was identified using biochemical tests which included: the catalase, oxidase and urease tests, tests for hydrogen sulphide (H_2S) production, nitrate reduction, growth with 3.5% NaCl, growth with 1% glycine, growth at varying temperatures (25 °C and 42 °C), growth on peptone-starch-dextrose agar and sensitivity to cephalothin and nalidixic acid.

H. pylori isolates were tested for their antibiotic susceptibility according to the method of Piddock [14] using 7 antibiotics disks including tetracycline, ampicillin, amoxicillin, erythromycin, kanamycin, gentamicin and rifampicin (Bioanalyze).

The isolates of *H. pylori* were exposed to 0.5 mg/L concentrations of chlorine for 4 different time periods (10s, 20s, 40s and 60s) [15].

Enumeration of coliform bacteria

The concentrations of coliform bacteria were determined by filtering 2 × 10 mL volumes of each sample using 47 mm cellulose acetate filters with a nominal pore size of 0.45 µm (Sartorius). The filters were cultured on m-FC agar and m-Endo agar for detection of faecal and total coliforms respectively.

Results

A total of 198 water samples were collected from 22 different districts during the period of the study which extended over the winter and summer seasons.

Measurement of residual chlorine concentration in the water samples showed that 41.3% of samples were free of chlorine. Figure 1 shows the residual chlorine concentrations in the districts in the winter and summer seasons. In some of the districts the concentrations varied greatly between the seasons and were usually higher in summer than winter.

Only 14.1% of the samples conformed to World Health Organization criteria for water quality of zero fecal and total coliforms [16] (Table 1). Only 80/198 isolates were positive for the 3 biochemical tests for *H. pylori* positivity (urease, catalase and oxidase). On completing the other biochemical tests only 14 isolates were characterized as *Helicobacter* spp.: 4 *H. mustelae* and 10 *H. pylori* (Table 2). Therefore isolates of *H. pylori* comprised 2.0% of the total isolates and 5.0% of the total samples. The presence of these isolates was higher in certain districts than others, especially in Old Basra, Al-Jamiayat, Al-Zubair and Al-Ma'aqal.

Antibiotic susceptibility tests showed that 80% of *H. pylori* isolates were susceptible to tetracycline, 50% to ampicillin and amoxicillin, 40% to kanamycin, gentamicin and rifampicin and 30% to erythromycin. Inactivation of *H. pylori* isolates by chlorination showed that *H. pylori* were not sensitive to chlorine, since the final numbers of bacterial colonies were high after each period of chlorine exposure, i.e. less than 1-log reduction (Table 3).

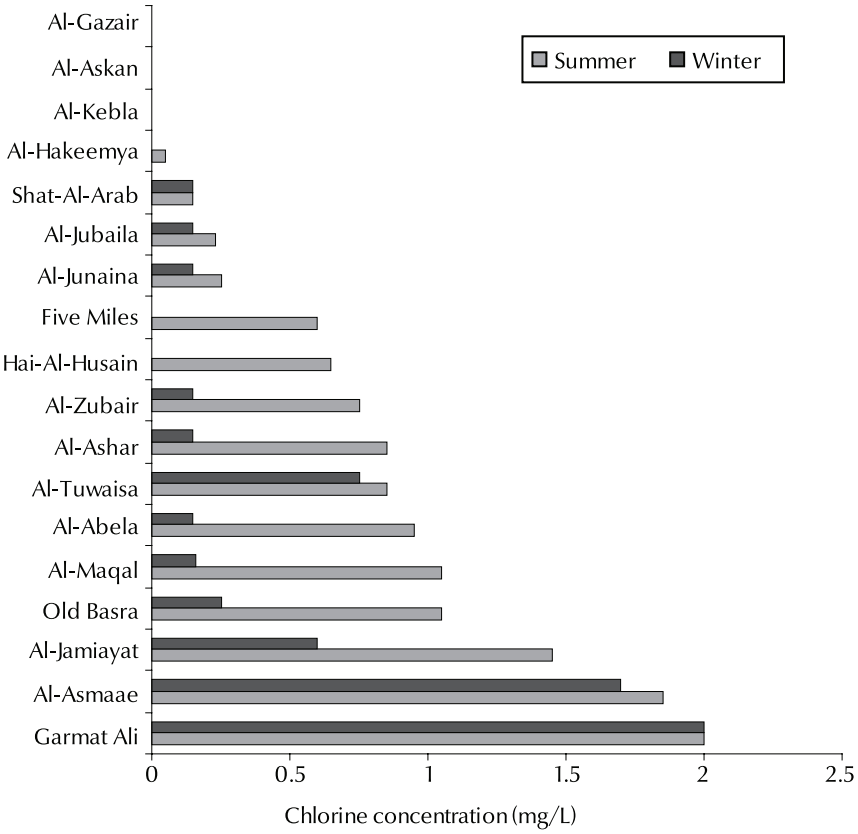

Figure 1 **Residual chlorine concentrations of water samples from Basra during winter and summer seasons (*n* = 198)**

Table 1 **Mean concentrations of total and faecal coliforms in water samples isolated from different districts of Basra (*n* = 198)**

District	Total coliforms (CFU/100 mL)	Faecal coliforms (CFU/100 mL)
Abu-Al-Khaseeb	1000	1300
Al-Ashar	415	393
Al-Tuwaisa	322	302
Al-Zubair	208	254
Al-Eskan	197	207
Al-Jazair	380	200
Old Basra	236	177
Al-Hussain Q	106	125
Al-Hakeemya	60	109
Al-Qibla	1500	100
Five Miles	5	92
Shatt-Al-Alarab	113	76
Al-Guzaiza	187	57
Al-Maaqal	81	48
Al-Abela	7	30
Al-Jamiayat	16	24
Al-Jubaila	525	20
Garmat-Ali	45	14
Al-Asmaaee	14	10
Al-Qurna	0	2
Al-Junaina	10	0

CFU = colony-forming units.

Discussion

Culture is considered the gold standard for detection of bacteria, but the method is not sensitive, and is specific only if additional testing is performed on the isolates. However, the application of molecular biology identification methods has potential drawbacks [16]. The method of choice involves PCR amplification of specific *H. pylori* genes such as *ureA* and *ureC*. Although this technique appears to be sensitive, it lacks specificity [17]. Therefore other approaches have been reported involving the use of *H. pylori* 16s *rRNA* [18,19] or different sets of primers [16].

The present study was intended as a preliminary probing for the presence of *H. pylori* in drinking water. A more comprehensive research project on both drinking water and sewage is underway, for which a combination of biochemical and PCR using *ureA* primer gene and *rRNA* gene primer is applied.

The mode of transmission of *H. pylori* remains an area of discussion. Increased risk of infection has been associated with contaminated drinking water [9] and the consumption of uncooked vegetables irrigated with untreated sewage [8]. Currently the role of water in dissemination of this pathogen remains problematic since *H. pylori* is a fastidious organism and has been difficult to isolate from environmental sources such as water [15].

Studies of the presence of *H. pylori* in the aquatic environment have relied on molecular methods using PCR, immunomagnetic separation and autoradiography [20,21]. These studies suggest that the organism may survive in water for an extended period of time and that *H. pylori* infection is spread by contaminated water [15]. The present study succeeded in isolating *H. pylori* from chlorine-treated drinking water by using culture methods. We can link this to the low concentration of chlorine in the water samples, permitting it to grow and survive in large numbers,

Table 2 **Biochemical tests of 14 isolates of *Helicobacter* spp.**

Isolate no.	Catalase	Oxidase	Urease	Nitrate reduction	H_2S	Growth with 3.5% NaCl	Growth on PSD agar	Growth on 1% glycine	Growth at 42 °C	Growth at 25 °C	Cephalothin resistant	Nalidixic acid resistant
H. pylori												
1	+	+	+	–	–	–	+	–	+	–	+	+
2	+	+	+	+	–	–	+	–	+	–	+	+
3	+	+	+	–	–	–	+	–	–	–	+	–
4	+	+	+	–	–	–	+	–	–	–	+	–
5	+	+	+	–	–	–	+	–	–	–	+	+
6	+	+	+	–	–	–	+	–	–	–	–	+
7	+	+	+	–	–	–	+	–	+	–	–	+
8	+	+	+	–	–	–	+	–	–	–	+	+
9	+	+	+	+	–	–	+	–	–	–	+	+
10	+	+	+	–	–	–	+	–	–	–	+	+
H. mustelae												
1	+	+	+	+	–	–	–	+	+	–	+	–
2	+	+	+	+	–	–	–	+	+	–	+	–
3	+	+	+	+	–	–	–	+	+	–	+	–
4	+	+	+	+	–	–	–	+	+	–	–	–

PSD = peptone-starch-dextrose.

and to the water distribution system, since this bacterium has the ability to form biofilms in waterpipes [22,23]. Furthermore, the waterpipes suffer from breaks and corrosion at many sites, which cause drinking water contamination by sewage infiltration and rain leakage into the system [24,25].

The higher isolation rate of this bacterium in some districts than others may be due to the increased breaks and corrosion sites in the water supply systems which raise the rate of contamination. In some of these samples *H. pylori* were detected in the absence of coliforms, suggesting the shortcomings of these indicators for pathogenic bacteria.

Another explanation for the high isolation rate of *H. pylori* is the use of modified Columbia urea agar medium, which may enhance the growth of this bacterium.

Isolates of *H. pylori* were 80% tetracycline sensitive. This resistance may be a result of mutation in the *16rRNA* gene, which is the target for this antibiotic [26]. For kanamycin, gentamicin, erythromycin and rifampicin the resistance could be a result of gene mutation such as mutation of the *rpoB* gene which is the target for rifampicin. Generally antibiotic resistance is considered the main problem associated with *H. pylori* treatment since antibiotic-resistant strains have become prevalent throughout the world and are the main cause of failure in *H. pylori* treatment.

Increased resistance of *H. pylori* to chlorine may be attributed to the growth of bacterial cells in the form of biofilms that make these cells acquire greater resistance to disinfectants than free cells [27]. However, some studies found that *H. pylori* isolates were resistant to chlorine and ozone but sensitive to monochloramine disinfection [28].

To our knowledge, this is the first report of the isolation of *H. pylori* in municipal treated drinking water and this could be of epidemiological significance. Further research is needed to establish which factors affect the ability of *H. pylori* to survive in distribution systems and be isolated from drinking water, such as the bacterial strains, density of bacteria in the distribution systems, type of waterpipe materials, efficiency of disinfection process and the techniques and materials used for culture.

Table 3 Chlorine inactivation of *Helicobacter pylori* isolates in 0.5 mg/L chlorine at different exposure times

Isolate no.	Exposure time				
	Initial (log_{10} CFU/mL)	10 s (log_{10} CFU/mL)	20 s (log_{10} CFU/mL)	40 s (log_{10} CFU/mL)	60 s (log_{10} CFU/mL)
1	3.07	2.93	2.51	2.13	1.85
2	4.15	1.01	3.13	1.09	0.95
3	5.16	4.95	4.81	4.57	3.91
4	2.11	2.09	1.33	1.15	1.85
5	5.61	2.15	3.88	3.60	3.47
6	4.81	4.67	2.56	2.31	0.02
7	3.75	3.51	3.55	3.19	3.65
8	UC	UC	2.16	1.25	0.91
9	2.15	1.35	1.17	0.57	1.01
10	4.95	4.88	3.11	2.15	1.01

CFU = colony-forming units; UC = uncountable.

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Progress on sanitation and drinking-water: 2010 update

The above-mentioned report describes the status and trends with respect to the use of safe drinking-water and basic sanitation and progress made towards the Millennium Development Goals' drinking-water and sanitation target.

As the world approaches 2015, it becomes increasingly important to identify who are left behind and to focus on the challenges of addressing their needs. This report therefore shows disparities: the gap between progress in providing access to drinking-water versus sanitation; the divide between urban and rural populations in terms of the services provided; differences in the way different regions are performing, bearing in mind that they started from different baselines; and disparities between different economic strata in society.

The information presented in this report includes data from household surveys and censuses completed during the period 2007–2008. It also contains datasets from earlier surveys and censuses that have become available to the Joint Monitoring Programme (JMP) since the publication of the previous JMP report in 2008.

Further information about this and other WHO publication is available at: http://www.who.int/publications/en/