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ORIGINAL ARTICLE

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Correlation among fecal indicator bacteria and physicochemical parameters with the presence of *Helicobacter pylori* DNA in raw and drinking water from Bogotá, Colombia

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Abstract

Background: The quality of raw and drinking water is a matter of considerable concern due to the possibility of fecal contamination. To assess the quality and public health risk of different types of water, the fecal indicator bacteria (FIB) are used. However, some pathogens, such as *Helicobacter pylori*, may be present in water when FIB cannot be found. *H pylori* is recognized as the causative agent of chronic gastritis, peptic and duodenal ulcers, and gastric cancer. The aim of this study was to evaluate the relationships among physicochemical parameters, FIB concentrations, and the presence of *H pylori* DNA in raw and drinking water from Bogotá, Colombia.

Materials and Methods: A total of 310 water samples were collected 1 day per week from July 2015 to August 2016, and physicochemical parameters (pH, turbidity, conductivity, and residual free chlorine) were measured. Presence of *H pylori* DNA was determined and quantified by quantitative polymerase chain reaction (qPCR). Fecal indicator bacteria (total coliforms, *Escherichia coli*, and spores of sulfite-reducing Clostridia) were enumerated by using standard culture techniques.

Results: Thirty of 155 (31%) raw water samples and forty-eight of 155 (38.7%) drinking water samples were positive for the presence of *H pylori*. No statistically significant relationships were found between physicochemical parameters or FIB with the presence or absence of *H pylori* in any sample ($P < 0.05$).

Conclusions: This study provides evidence of the presence of *H pylori* DNA in raw and drinking water in Bogotá, and shows that the detection and enumeration of FIB and physicochemical parameters in water do not correlate with the risk of contamination with *H pylori*.

KEYWORDS
drinking water, fecal indicator bacteria, *Helicobacter pylori*, physicochemical parameters, qPCR, raw water

1 | INTRODUCTION

Microbiological contamination of water can occur along a water supply system, from the water source(s) to the treatment plant. Water utilities monitor water quality at sources and treatment plants[1] and take necessary action if required. However, outbreaks of waterborne disease can appear as a result of the entrance of pathogens into distribution systems.[2]

Thus, the drinking water may contain at least small amounts of some contaminants. For these reasons, in 2016 the US Environmental Protection (EPA) published the final Contaminant Candidate List 4 (CCL 4), which includes 97 chemicals and 12 microbial contaminants (Adenovirus, Caliciviruses, Enterovirus, Hepatitis A virus, *Campylobacter jejuni*, *Escherichia coli* (0157), *Helicobacter pylori*, *Legionella pnuemophila*, *Micobacterium avium*, *Salmonella enterica*, *Shiguella sonnei*, and *Naegleria fowleri*). These contaminants are significant pathogens for public health due to its association with multiple diseases.[3]

Among these contaminants, *H pylori* is a microorganism that infects humans, with a global prevalence of 50%.[4-6] In developing countries, about 70-90% of adults show serological evidence of current or past infection with *H pylori*.[7] The rate of infection in Colombia is estimated to reach 70%-80% of the general adult population.[8] Infection rates are considerably lower in the developed world; for example, the rate of infection in the United States is estimated at 10-25% of the general adult population.[9] *H pylori* is recognized as the causative agent of chronic gastritis, peptic, and duodenal ulcers, and the etiologic agent in gastric cancers[4,6,10] and, in 1994, the World Health Organization International Agency for Research on Cancer designated *H pylori* as a Class I carcinogen and issued a gastric cancer warning.[11]

Although *H pylori* has been proposed as a new drinking water contaminant, few studies have standardized procedures for its detection in water. This is because *H pylori* is a dimorphic Gram-negative bacillus, which shows a spiral form in its cultivable state, but can adopt a coccoid morphology under conditions of stress,[12] entering a viable but nonculturable (VBNC) state, in which the bacterium is unable to grown on agar plates by using conventional culture methods.[13] VBNC cells retain membrane integrity, and contain undamaged genetic information. These forms have been suggested to be the way in which *H pylori* survives in environment.[14]

Although the form of transmission of the *H pylori* has not been precisely defined, and the oral-oral or oral–fecal routes are proposed, the role of the presence of viable coccoid but nonculturable forms in the environment is not yet understood.[9,15] While it has not been demonstrated that *H pylori* can be transmitted through drinking water, there is increasing interest in knowing how the presence of this contaminant can influence water quality and whether or not it may have an impact on public health. Especially, because *H pylori* is a frequent colonizer of the human stomach.

The presence of *H pylori* DNA in drinking water, surface water, groundwater, and wastewater has been previously reported.[15-17] Moreover, there has been some controversy about the efficiency of chlorination and ozonation disinfection methods to inactivate some pathogens resistant to chlorine, such as *H pylori*.[12,18]

Recently, different independent studies have isolated and cultured *H pylori* from wastewater and drinking water.[5,16,17,19,20] In addition, *H pylori* has been reliably detected in diverse water sources, such as rivers, lakes, drinking water, municipal, and residual waters, among others, by using molecular biology techniques such as PCR, quantitative real-time polymerase chain reaction (qPCR), and fluorescent in situ hybridization.[17,21-25]

Detecting a waterborne pathogen is difficult, and thus, standard methods are directed to detect nonpathogenic organisms commonly found in human feces. Traditional assessments of microbiological water quality have focused on the presence of fecal indicator bacteria (FIB), whose concentration or density is related to the health risk posed by this water. Fecal indicator bacteria are nonpathogenic organisms, abundant in human and animal feces where pathogenic organisms can be found.[26] Fecal indicator bacteria presence is usually associated with agricultural operations, cattle management, or human habitation. Determination of FIB is, at the moment, the most used method to predict the presence of pathogens in water resources and drinking water[9] but these indicator organisms are only weakly associated with the presence of some pathogens.[27,28] Moreover, measurements of fecal indicator organisms in water can be highly variable.[29] Traditionally, culture techniques have been commonly used to enumerate *E coli* and total coliforms in raw and drinking water.[30,31] However, some authors have pointed out that some pathogens, such as *H pylori*, can be able to survive when these indicators are inactivated.[5,32,33] Thus, the enumeration of coliform bacteria as indicators of drinking water quality may not correlate accurately with the risk this water possesses to consumers.[27,32,34] There are few studies that look for indicator microorganisms different from the traditional ones to monitor water quality.

Consequently, pathogens as *H pylori* are not directly monitored in water. However, a predictive value of water quality indicators with regard to the presence of pathogens has not been established or quantified and, the correlation has not been estimated between them. In addition, given of the difficulty of determining *H pylori* in raw and drinking water, it was suggested that the variations presented by the physicochemical parameters (pH, turbidity, conductivity, and residual free chlorine) could give some indicative of the presence of it pathogen, taking into account that these parameters are the ones that are routinely measured in the entrance and exit of the water treatment plants. Therefore, in this study, we analyzed the relationships between water quality and presence of *H pylori* DNA in raw water and drinking water from Bogotá city, Colombia, due to the absence of information related to this issue. A specific aim of this study was to determine whether a numerical correlation could be found between counts of FIB (total coliforms, *E coli*, and spores of sulfite-reducing Clostridia) and physicochemical parameters (pH, turbidity, conductivity, and residual free chlorine) in raw and drinking water and *H pylori* DNA detection. We evaluated the presence of FIB by using conventional microbiological analyses and the *H pylori* DNA was detected through the qPCR.

2 | METHODS

2.1 | Water sampling

Sample collection and characterization of water physical, chemical, and biological quality of raw and drinking water were carried out using standard methodologies.[35]

Between July 2015 and August 2016, a total of 310 samples coming from both, raw water (n = 155) and drinking water (n = 155), were taken with a weekly frequency. From each sample point, different amounts of water were taken into sterile bottles, as described above.

The raw water samples were taken from 3 three different points of the catchment of superficial waters, it which possess a different concentration of fecal contamination, and which it is subjected to a disinfection treatment with chlorine, and after is distributing as drinking water to Bogotá city, Colombia. Drinking water samples were collected from three different points of the same city.

For *H pylori* detection in drinking water samples, the "Moore swab" method was used.[35,36] Briefly, a swab was kept in contact with drinking water flow for 72 hours, removed and placed into a sterile bottle. For analysis of *H pylori* in raw water samples, 300 mL of water was collected into 500 mL sterile bottles.

Samples for determining total coliforms, *E coli*, and spores of sulfite-reducing Clostridia (SSRC) were taken following the Standard Methods 9006 A-3 protocol.[35] Briefly, for the analysis of total coliforms and *E coli* from raw water, 200 mL was sampled into 300 mL sterile bottles. From drinking water, 700 mL was collected into 1 L sterile bottles. For the analysis of spores of sulfite-reducing Clostridia, 500 mL was sampled from both, raw and drinking water, and held in anaerobic conditions until processing. All raw and drinking water samples were held at 4°C and processed within a few hours.

2.2 | Culture conditions and bacterial strain

The reference strains *E coli* ATCC 25992 and *Salmonella enterica subsp* enterica serovar Entiritidis 13076 ATCC were cultured in Nutrient Agar (Merk, Germany) and incubated under aerobic conditions at 36 ± 2°C for 24 hours. *Clostridium perfringens* 262 CMPUJ strain was cultured in Nutrient Agar (Merk, Germany) and incubated under anaerobic conditions (AnaeroGen™ 2.5 L, Thermo Scientific, USA) at 37 ± 2°C for 24 hours. Reference strains were used as positive controls for quantification of FIB.

2.3 | Detection of *H pylori* using qPCR analysis and DNA sequencing

Each raw water sample was centrifuged at 3000×g for 20 minutes, and the pellet was resuspended in 2 mL of phosphate-buffered saline (PBS 1×: 130 mmol/L sodium chloride, 10 mmol/L sodium phosphate, pH 7.2).

For the drinking water samples, the swab was transferred to 200 mL of Brucella Broth (Becton Dickinson BBL™, USA) supplemented (BBS) with 0.4% Isovitalex (Becton Dickinson BBL™, USA) and 0.2% Dent (Oxoid, USA) for sample elution and incubated at 37°C under microaerophilic conditions (5% O_2, 11% CO_2, 85% N_2) for 24 hours. After this pre-enrichment step, each sample was centrifuged at 3000×g for 20 minutes and the pellet was resuspended in PBS 1×.

Both, raw and drinking PBS suspension samples were concentrated by immunomagnetic separation (IMS) according to Enroth and Engstrand.[37] The bead-bacterium aggregates were finally resuspended in PBS 1× and subsequently analyzed by qPCR. Quantitative real-time polymerase chain reaction positive results were confirmed by sequencing. DNA was purified from a 1 mL aliquot of each IMS concentrated sample using the DNeasy Blood & Tissue kit (Qiagen, USA), according to the manufacturer's instructions.

Specific *H pylori* qPCR was carried out using a set of primers to amplify a 372 bp fragment of the *vac*A gene (VacF: 5´-GGC ACA CTG GAT TTG TGG CA- 3´ y vacR: 5´-CGC TCG CTT GAT TGG ACA GA- 3´).[38] For qPCR analysis, the final reaction volume of 20 µL contained 2 µL of Light-Cycler® FastStart DNA SYBR Green I (Roche Applied Science, Spain), 1.6 µL of $MgCl_2$ (50 mM), 0.5 µL of each primer (20 mM), and 2 µL of DNA template. A positive control with *H pylori* DNA strain NCTC 11637 and a control of external contamination, consisting of qPCR mix without DNA were included in each qPCR analysis, and as negative control used *E coli* DNA strain ATCC 25992.

The qPCR was run under the following conditions: initial DNA denaturation step at 95°C for 10 minutes followed by 40 cycles of: 95°C for 10 seconds, 62°C for 5 seconds, and 72°C for 16 seconds; and finally, one cycle at 72°C for 15 seconds and one at 40°C for 30 seconds.[16] All raw and drinking water samples and controls were run in duplicate. A standard curve was constructed with 10 to 10^6 genome copies of *H pylori* NCTC 11637 DNA16. Given that each *H pylori* genome has one copy of *vac*A gene, we assumed that one genome copy of *H pylori* was equivalent to one genomic unit (GU)[39].

The qPCR products were analyzed in 2% (w/v) agarose gel electrophoresis prepared with 1× TAE Buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0 ± 0.2), and stained with 0.02% SYBR® Safe-DNA Gel Stain (Invitrogen, USA), at run 80 V for 1 hour. The gel was visualized through the Gel Doc™ XR+ Imaging System Molecular Imager (BIO-RAD, USA). 100 bp Plus DNA ladder (Invitrogen™ by Life Technologies™, USA) was used as a molecular weight marker.

The homology between the amplified sequences and the corresponding *H pylori vac*A gene fragment was performed by sequencing, using the Sanger method (Macrogen, Korea). Sequences were compared to the sequences published in GenBank according to Altschul et al[40] by using BLAST software alignment tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4 | Detection and enumeration of fecal indicator bacteria

The FIB monitored in both raw and drinking water samples were *E coli*, total coliforms, and spores of sulfite-reducing Clostridia. For the enumeration of *E coli* and total coliforms, membrane filtration ISO 9308-1:2014 method[41] was used. Briefly, the raw water samples were serial 10-fold diluted (10^{-1}-10^{-4}), and subsequently, all the dilutions were filtered. One hundred mL of each drinking

water sample was directly filtered. Then, the filters were put on Chromocult® Coliform agar (Merck, Germany), and incubated at 36 ± 2°C for 21 ± 3 hours. The enumeration of the spores of sulfite-reducing Clostridia was performed also by membrane filtration ISO 6461-2:1986 method.[42] Both, raw and drinking water samples were heated a 75°C for 15 minutes. Afterward, the raw water samples were serial 10-fold diluted (10^{-1}-10^{-4}) and all the dilutions were filtered. Aliquots of 100 mL of the drinking water samples were directly filtered. The filters were placed on SPS (Sulfite polymyxin sulfadiazine) agar (Merck, Germany) and incubated under microaerophilic conditions in anaerobic jars (Oxoid, UK) with a gas-generating envelope (AnaeroGen™ 2.5 L, Thermo Scientific, USA) at 37 ± 2°C for 44 ± 4 hours.

2.5 | Physicochemical parameters analysis

Water quality was evaluated from the physicochemical parameters obtained from the diary routine control of both raw and drinking water samples, by using the Standard Methods protocols.[35] pH and conductivity were measured by electrometric method (SM 4500-H-B and SM 2510-B, respectively). Turbidity was measured by nephelometric method (SM 2130-B), and in the drinking water, the free residual chlorine was measured by the Ferrous Titrimetric method–DPD (SM 4500-CL-F).

2.6 | Statistical analysis of the data

All statistical analyses were conducted in IBM SPSS Statistics software package version 24.0.[43] The data were analyzed with Bivariate statistical test.[44] Qualitative data were described by using number and percent. Differences in data values were considered significant at $P < 0.05$. Descriptive statistics were used to examine the distribution of *H pylori* contamination, fecal indicator bacteria, and physicochemical parameters.

In order to determine whether there is a correlation between the concentration of FIB (total coliforms, *E coli*, and spores of sulfite-reducing *Clostridium*) or physicochemical parameters (pH, conductivity, turbidity, and free residual chlorine) and the presence of *H pylori*, the Spearman correlation coefficient (ρ) and the Tau-b Kendall correlation coefficient were used. For all the analysis, *H pylori* was used as independent variable and the fecal indicator bacteria and physicochemical parameters were used as dependent variables.

3 | RESULTS

3.1 | *Helicobacter pylori* in raw and drinking water

During the sampling period (July 2015 to August 2016), *H pylori* DNA was detected by qPCR in the raw and drinking water samples, with 31% (30/155) and 38.7% (48/155) of positive samples, respectively (Figure 1A, B). From July to December (2015) *H pylori* was detected in all samples from both, raw and drinking water. Among the raw water samples collected in 2016, *H pylori* DNA was detected in all samples, except in February ones. In drinking water samples from July and August of 2016, *H pylori* DNA was not detected. In the rest of samples, it was detected in at least in one water sample.

In the raw water, *H pylori* DNA was quantified in 13 (8.4%) of the positive samples, with concentrations ranging between 1.28×10^1 and 4.69×10^2 genomic units (GU) per mL (Figure 2A, B). In the drinking water samples, *H pylori* DNA could only be quantified in 20 (12.9%) of the positive samples, with concentration values ranging from 5.77 to 2.12×10^3 GU per sample (Table 1 and Table 2). In all the remaining positive samples, C_t values were above the reliability threshold (>35 cycles). Sequencing of all amplicons showed that the sequence of all of them was 98%-100% similar to a fragment of *H pylori vac*A gene sequence (GenBank accession numbers AF049653.1-CP003904.1 AJ438914.1-U95971.1).

3.2 | Fecal indicator bacteria in raw and drinking water

Throughout the sampling period, FIB were detected in all the raw water samples. Total coliforms were present in a range between 2.4×10^1 and 5.7×10^4 CFU/100 mL; *E coli* between 2.0 and

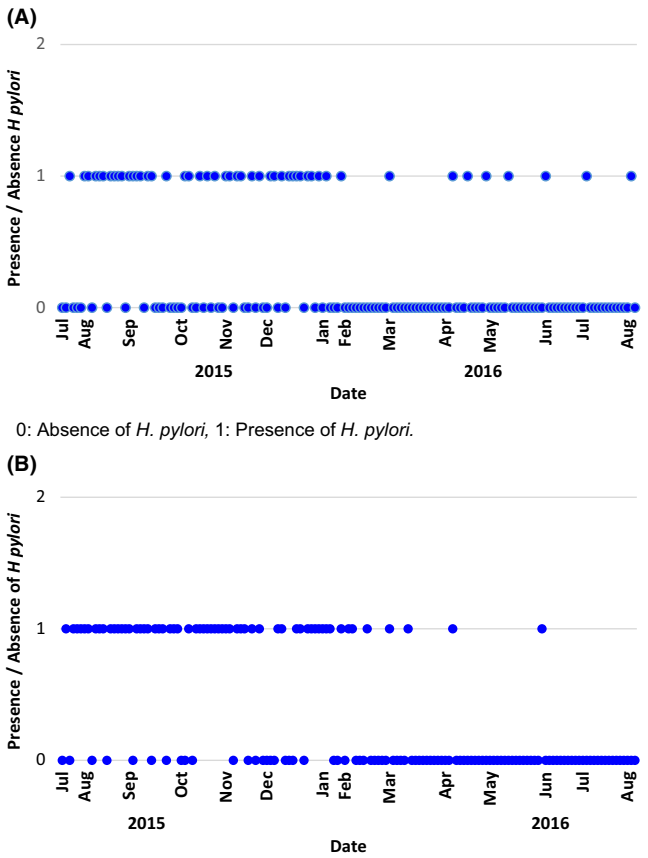

FIGURE 1 Presence/absence of *Helicobacter pylori* DNA detected by qPCR in water samples. (A) Presence/absence of *H pylori* DNA in raw water from July 2015 to August 2016. (B) Presence/absence of *H pylori* DNA in drinking water from July 2015 to August 2016. 0, Absence of *H pylori*; 1, Presence of *H pylori*

3.0×10^3 CFU/100 mL and spores of sulfite-reducing Clostridia from 1.0 to 5.5×10^3 CFU/100 mL. A high concentration of total coliforms was found in comparison with *E coli* and spores of sulfite-reducing Clostridia (Table 1). In the drinking water samples, no FIB were detected throughout the sampling period (Table 2).

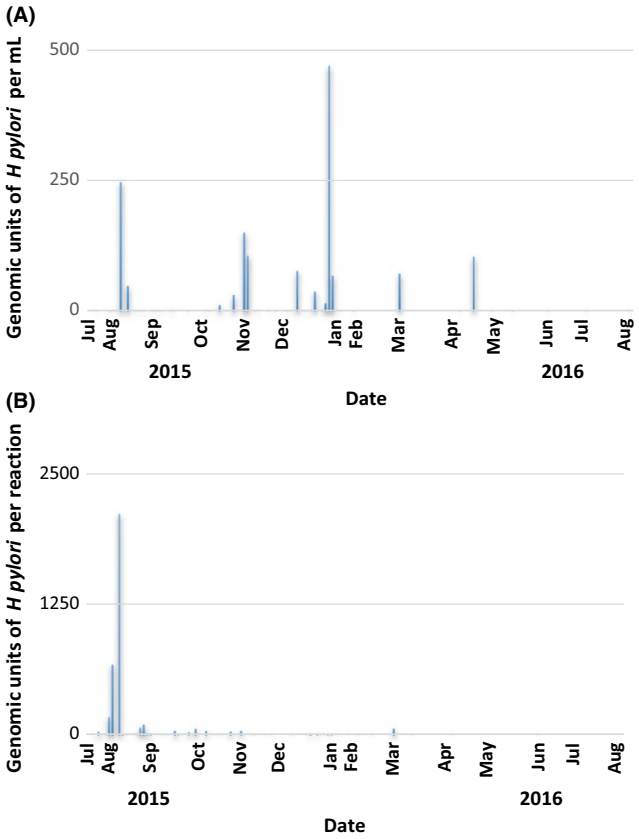

FIGURE 2 Genomic units of *Helicobacter pylori* DNA detected by qPCR in water samples. (A) Genomic units of *H pylori* DNA per mL in raw water from July 2015 to August 2016. (B) Genomic units of *H pylori* DNA per reaction in drinking water from July 2015 to August 2016

3.3 | Physicochemical parameters in raw and drinking water

The raw water quality parameters were those permitted by Colombian regulations for raw water samples to be used as a source of water for drinking water. The drinking water analyzed parameters were those required by Colombian regulations regarding water suitable for human consumption (Table 1; Table 2, respectively). According to the World Health Organization (WHO), the residual free chlorine in drinking water should be between 0.2 and 1.0 mg/L.[45] However, Colombian regulations allow chlorine concentrations between 0.3 and 2.0 mg/L[46] (3).

3.4 | Associations among fecal indicator bacterial, physicochemical parameters, and *H pylori* in raw and drinking water

Statistical analysis of the results for raw water did not show a significant association among fecal indicator bacteria concentration, pH, and the presence/absence of *H pylori* DNA. We found a negative association, but not statistically significant, among turbidity, conductivity, and the presence of *H pylori* (Table 3). In the case of the drinking water, we did not find statistically significant association among fecal indicator bacteria concentration, conductivity, turbidity, pH, and residual free chlorine with the presence/absence of *H pylori* DNA (Table 4).

4 | DISCUSSION

Currently, US Environmental Protection Agency (EPA) includes *H pylori* in its Contaminant Candidate List (CCL), which comprises chemical and microorganisms contaminants that are known to be present in drinking water systems and are suspected to pose public health risk.[3,47] However, data available on the presence of *H pylori* and the type of the water treatment in drinking water are limited. Thus, a quantitative microbial risk assessment (QMRA) has not yet been

TABLE 1 Characteristics of raw water samples from July 2015 to August 2016

	N	Range	Median	Mean
H pylori genome units/mL	155	1.28×10^1-4.69×10^2	7.5×10^1	7.99×10^1
Turbidity (NUT)	155	1.5-47.1	7.1	7.6
Conductivity (µS/cm)	155	8.1-110	41	41.6
pH	155	6.1-7.26	6.8	6.69
Total coliforms (CFU/100 mL)	155	2.4×10^1-5.7×10^4	4.48×10^2	7.55×10^2
E coli (CFU/100 mL)	155	2.0-3.0×10^3	5.6×10^1	6.20×10^1
Spores of sulfite-reducing Clostridia (CFU/100 mL)	155	1.0-5.5×10^3	5.1×10^1	3.91×10^1

µS/cm, micro Siemens/centimeter; CFU/mL, colony forming unit/milliliter; N, samples number; NUT, nephelometric unit turbidity.

TABLE 2 Characteristics of drinking water samples from July 2015 to August 2016

	N	Range	Median	Mean
H pylori genome units/reaction	155	5.77-2.12 × 10^3	3.73 × 10^1	4.05 × 10^1
Turbidity (NUT)	155	0.1-1.3	0.24	0.26
Conductivity (µS/cm)	155	34-150	68.3	73.1
pH	155	6.39-7.95	6.77	6.83
Free chlorine residual (FAC) (mg/L)	155	0.79-2.0	1.66	1.59
Total coliforms (CFU/100 mL)	155	<1*	<1*	<1*
E coli (CFU/100 mL)	155	<1*	<1*	<1*
Spores of sulfite-reducing Clostridia (CFU/100 mL)	155	<1*	<1*	<1*

µS/cm, micro Siemens/centimeter; CFU/mL, colony forming unit/milliliter; mg/L, milligram/liter; N, samples number; NUT, nephelometric unit turbidity.
*Detection limit of method analysis.

carried out for *H pylori* in the water[48] and *H pylori* remains as a candidate while a standardized method for its detection and identification in environmental samples is implemented.

Multiple studies have confirmed the occurrence of *H pylori* in environmental and drinking waters around the world.[5,16,17,49] Lu et al[21] cultured *H pylori* from a sample from a canal along the US/Mexico border that was heavily contaminated with untreated raw sewage. Vesga et al[17] also successfully cultured *H pylori* from influent and effluent water samples from drinking water treatment plants from Bogotá (Colombia). Together, these studies suggest that conventional chlorine disinfection treatments for water potability do not assurance the elimination of all pathogens, as is the case with *H pylori*.

A few studies have shown poor correlation between the presence of these organisms with that of fecal indicators bacteria such as total coliforms or *E coli*.[50-53] Thus, the presence of *H pylori* in water may not be accurately assessed by the use of traditional fecal indicator bacterial detection methods.

In this study, we used the Moore swab method, and a pre-enrichment step, to increase the effectiveness of detection of *H pylori* in drinking water. This method has proven to be useful for isolating different pathogen and enteric bacteria from low contaminated waters, surface water, drinking water, and soil.[17,54,55]

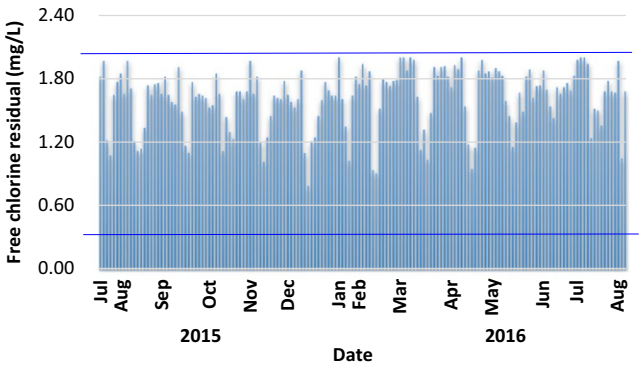

FIGURE 3 Residual free chlorine in drinking water from July 2015 to August 2016. The upper line in the free chlorine residual graph represents the upper limit of the Colombian regulations recommendation for FAC (2.0 mg/L), and the lower line represents the minimum recommended FAC residual (0.3 mg/L)

During the sampling period (July 2015 to August 2016), 31% (30/155) of the raw water samples and 38.7% (48/155) of the drinking water samples were positive for the presence of *H pylori* DNA by qPCR. Although we were able to quantify *H pylori* DNA in 12.9% (20/155) of the drinking water samples, with mean levels between 5.77 and 2.12 × 10^3 GU/reaction, these results do not represent the real level of contamination of the waters, as the sampling method included a pre-enrichment step. In the raw water samples, the concentration of *H pylori* DNA could only be quantified in 8.4% (13/155) of the samples, with concentrations between 1.28 × 10^1 and 4.69 × 10^2 GU/mL.

Although the presence of DNA in a sample is not indicative of the presence of viable cells, we considered that detection of *H pylori* DNA by qPCR followed by specific sequencing of the amplicons ensured us to detect the presence of *H pylori* in the samples, which was the aim of this work. It is widely known that the cultivation of this bacterium from environment is very difficult. Moreover, under adverse environmental circumstances *H pylori* cells enter in a Viable But Not Culturable stage in which culture is not possible.[16] Molecular methods for estimating the presence of exclusively viable cells in water, such as detection of mRNA, PMA-PCR, or DVV-FISH have been previously used in very few occasions and need further investigations.[56]

In the last decades, many studies have focused on the relationship between water contamination indicators and pathogens. *Clostridium sp* has been proposed as an indicator of the presence of *Giardia* cysts and *Cryptosporidium* oocysts in environmental samples, due to the formation of spores that would have a similar resistance structures as these parasites.[57-59] It has been previously suggested that *H pylori* survives in the environment in a viable but nonculturable form.[60,61] Considering that this forms could be more resistant than culturable cells in environmental samples and that their behavior may vary in

TABLE 3 Statistical data showing the relationship of fecal indicator bacteria, turbidity, pH, and conductivity, with the presence/absence of *Helicobacter pylori* in raw water

	Tau-b Kendall correlation coefficient			Spearman correlation coefficient		
Parameter	Tau-b Kendall correlation coefficient	Sig. (bilateral)	N	Spearman correlation coefficient	Sig. (bilateral)	N
H pylori						
Turbidity	−0.115	0.083	155	−0.140	0.083	155
Conductivity	−0.085	0.203	155	−0.103	0.204	155
pH	0.004	0.952	155	0.005	0.952	155
Total coliform	−0.088	0.182	155	−0.107	0.183	155
E coli	−0.096	0.154	155	−0.115	0.154	155
Spores of sulfite-reducing Clostridia	−0.047	−0.047	155	−0.057	0.479	155

N, samples number.

relation to traditional indicators of fecal contamination (total coliforms and *E coli*), in this study the presence of the spores of sulfite-reducing Clostridia was evaluated as a possible indicator of the presence and or absence of *H pylori* in raw and drinking water. In raw water samples, the concentration of the spores of sulfite-reducing *Clostridia* was found to range between 1.0 and 5.5×10^3 CFU/100 mL, and in drinking water samples they were not detected. Statistical analysis showed that there is no direct relationship between the detection of spores of sulfite-reducing Clostridia and the presence/absence of *H pylori* in both, raw or drinking water. This suggests that the presence of *H pylori* in water samples cannot be evaluated by detecting the spores of sulfite-reducing *Clostridia* and those variations that may occur in the concentration cannot be taken as indicative to suppose the presence or absence of *H pylori* in raw or drinking water.

Colombian regulations (Decree 1594, 1984)[62] stipulate minimum physical, chemical, and microbiological requirements of water to consider it as drinking water. In our study, we found that the values of the average concentration of total coliforms in the raw water samples evaluated are within the maximum values allowed (maximum value 2×10^4 CFU/100 mL).

Quantification of fecal indicator bacteria in all the drinking water samples complied the values established in the resolution 2115,[46] which states that the maximum value accepted for total coliforms and *E coli* must be 0 or <1 CFU/100 mL, using the membrane filtration technique. Statistical analysis showed that there was no direct relationship between assessed indicators of fecal contamination and the presence of *H pylori* in the raw and drinking water samples. This suggests that the presence of *H pylori* in water cannot be evaluated by detecting the fecal indicator bacteria and variations that may occur in the concentration of total coliforms or *E coli* cannot be taken as indicative for assuming the presence or absence of *H pylori* in the raw or drinking water.

There are limited data on the association of the fecal indicators bacteria and the presence of *H pylori* in water. Braganca et al[63] and Baker & Hegarty,[50] in their work with biofilms, showed that *H pylori* was more resistant to chlorination than *E coli*. Hegarty et al[64] were

TABLE 4 Statistical data showing the relationship of fecal indicator bacteria, turbidity, pH, conductivity, and free available chlorine residual, with the presence/absence of *Helicobacter pylori* in drinking water

	Tau-b Kendall correlation coefficient			Spearman correlation coefficient		
Parameter	Tau-b Kendall correlation coefficient	Sig. (bilateral)	N	Spearman correlation coefficient	Sig. (bilateral)	N
H pylori						
Turbidity	−0.005	0.937	155	−0.006	0.937	155
Conductivity	0.015	0.820	155	0.018	0.821	155
pH	0.048	0.466	155	0.059	0.468	155
Free Chlorine residual (FAC)	−0.082	0.215	155	−0.100	0.216	155
Total coliform	0.0	0.0	155	0.0	0.0	155
E coli	0.0	0.0	155	0.0	0.0	155
Spores of sulfite-reducing Clostridia	0.0	0.0	155	0.0	0.0	155

N, samples number.

the first to evaluate the relationship of the presence of *H pylori* and *E coli* in waters. They did not find any correlation between the presence of *H pylori* DNA in drinking water and the presence of *E coli*, and suggested that this indicator may fail when it is used as the only test of water potability.

In this study, we did not either found statistically significant relationships between the physicochemical parameters (turbidity, conductivity, pH, and residual free chlorine) and the presence/absence of *H pylori* in the raw and drinking water samples. This suggests that the presence/absence of *H pylori* in water does not depend on the turbidity, conductivity, pH, and residual free chlorine and variations that may occur in the values of this physicochemical parameters cannot be used as indicative for assuming the presence or absence of *H pylori* in the raw or drinking water.

In this study, different detection and quantification methods were used for the microorganisms evaluated (culture for detection and quantification of FIB and qPCR for *H pylori* DNA). Therefore, it is proposed to carry out new studies, using molecular methods for the detection of FIB. Furthermore, the "Moore swab" method was used for the concentration of *H pylori* and standardized qPCR for the detection and quantification of DNA from drinking water samples. These methods can be used in future research to determine the levels in which *H pylori* is found and the possible effects that its presence in drinking water may have on public health.

5 | CONCLUSIONS

To our knowledge, this is the first long-time sampling study of raw water and drinking water contamination with *H pylori* in Bogotá, Colombia. During 1-year sampling period, we detected *H pylori* DNA in 31% of the raw water samples and 38.7% of the drinking water samples, using qPCR, which suggests that there is continued contamination of the raw water with *H pylori*.

While *H pylori* was often found in samples containing FIB, the presence and abundance of FIB was not predictive of *H pylori* DNA present or absence. Furthermore, we found no significant relationship between physicochemical parameters and *H pylori* presence in both raw and drinking water.

Our results highlight the importance of performing periodic controls for the detection of specific pathogens in water, since the only control of fecal contamination by traditional indicators such as total coliforms, *E coli*, or spores of sulfite-reducing *Clostridia*, cannot be indicative of the presence of pathogens of great relevance for public health, as *H pylori*.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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