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Research article

Occurrence of *Helicobacter pylori* in drinking water sources and antimicrobial resistance profile in the central region of Peru

María Custodio [a,*], Raúl Montalvo-Otivo [a], Jhonatan Crispín-Ayala [a], Jeampier Bendezu-Meza [a], Pilar Herrera-Quintana [a], Heidi De la Cruz [b], Javier Huarcaya [b]

[a] Facultad de Medicina Humana, Universidad Nacional del Centro del Perú, Av. Mariscal Castilla N° 3989-4089, Huancayo, Peru
[b] Laboratorio de Investigación de Aguas, Universidad Nacional del Centro del Perú, Av. Mariscal Castilla N° 3989-4089, Huancayo, Peru

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ABSTRACT

Introduction: Contamination of drinking water by *Helicobacter pylori* can cause serious diseases, including cancer. The determinants of the infection rate are socioeconomic status, low standard of living and overcrowding. In addition, exposure to environmental sources contaminated with feces, such as water and vegetables, is another risk factor for infection. We analyzed the occurrence of *H. pylori* in drinking water sources and the antimicrobial resistance profile in central Peru.

Methods: Water samples were collected from taps in four provinces of the Junín region. Previously, biofilm sampling was performed from the internal surface of the taps. The samples were cultured on modified brain heart infusion blood agar at 37 °C under microaerophilic conditions for seven days. Antibiotic sensitivity of *H. pylori* was determined by the Kirby Bauer diffusion method.

Results: The results revealed that pH (9.25) and turbidity (5.15 NTU) exceeded the Peruvian environmental quality standards for drinking water. The amount of free chlorine residual in the *H. pylori* positive water samples ranged from 0.02 to 0.12 mg/L. *H. pylori* was present in 2/192 tap water samples (1.04 %) and in 3/192 tap biofilm samples (1.56 %). It was observed that 100 % of *H. pylori* isolates from water samples from the Chilca district showed resistance to nalidixic acid and 66.67 % to both amoxicillin and chloramphenicol. Resistance to nalidixic acid of *H. pylori* isolates obtained from biofilm samples from taps in the El Tambo district ranged from 66.67 % to 100 %.

Conclusion: The study findings reveal that water samples and tap biofilms in the Chilca, El Tambo and Huamancaca chico districts in the Junín region harbor *H. pylori*. They also reveal variability in the pattern of resistance to more than one antibiotic tested from one district to another.

1. Introduction

Universal access to safe drinking water is a basic human right that remains one of the elusive Sustainable Development Goals (SDGs). The decision to incorporate a specific goal on water (SDG-6) among the 17 SDGs is a clear recognition that water is not only

* Corresponding author.
E-mail address: mcustodio@uncp.edu.pe (M. Custodio).

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part of many other SDGs but, in many respects, their precondition [1]. The supply of good quality drinking water is hampered by a number of challenges, including microbial contamination [2]. The World Health Organization [3] reports that 3.3 % of global deaths are related to water quality. Drinking water contamination can cause more than 50 serious diseases, including digestive diseases, infectious diseases, cancer, etc. Among the etiological agents of gastrointestinal diseases with high disease burden is *Helicobacter pylori*, a class I carcinogen (responsible for non-cardiac gastric cancer and gastric mucosa associated lymphoma) [4,5].

Helicobacter pylori is a Gram-negative, microaerophilic, flagellated bacterium that asymptomatically colonizes the stomachs of 50 % of the world's population [6,7]. The prevalence varies from one country to another, in some developing countries the prevalence of *H. pylori* infection is higher than 80 % and in developed countries it is lower than 20 % [8]. Worldwide, the prevalence of *H. pylori* is 70.1 % in Africa, 69.4 % in South America, 66.6 % in Western Asia, 34.3 % in Western Europe and 37.1 % in North America [9]. The determinants of the infection rate are the socioeconomic level and living conditions in early childhood, primarily [10]. Another important factor is the mode of transmission, with horizontal transmission being the most predominant in developing countries as opposed to transmission through family members in developed countries [11]. Infection with this bacterium in humans has been associated with the development of gastritis, gastric or duodenal ulcers, adenocarcinoma, lymphoma or primary gastric cancer and gastrointestinal diseases [12], depending on the presence or absence of genetic virulence factors, including cytotoxin-associated gene A (CagA) and vacuolizing cytotoxin (VacA) [13].

The U.S. Environmental Protection Agency has included *H. pylori* on the Candidate List of Microbial Contaminants [13]. The route of transmission of *H. pylori* remains uncertain. However, evidence supports that transmission of this bacterium is by both the fecal-oral and oral-oral routes [14]. Several studies have shown that low living standards and overcrowding are the main risk factors for contracting *H. pylori* infection, constituting a multifactorial risk. In addition, exposure to environmental sources contaminated with feces, such as water and vegetables, is another risk factor for *H. pylori* infection [15]. It has been repeatedly reported that this bacterium can survive for an extended period of time in drinking water distribution systems due to its ability to form biofilms (structures that provide a stable environment under stressful conditions) [16,17]. Consumption of water from untreated sources, such as river, spring and well water is believed to be associated with the dynamics of *H. pylori* transmission and infection. As well as, ingestion of contaminated raw or undercooked vegetables and ability of *H. pylori* to survive in unfavorable temperature conditions (4–15 °C) over a wide pH range [18,19].

The presence of *H. pylori* in drinking water represents a potential public health risk [8]. In Peru, many communities lack access to reliable sources of drinking water or sanitation services. Therefore, the population using water from these sources would be at higher risk of becoming infected with *H. pylori*. Several studies have reported the presence of *H. pylori* DNA in treated and untreated drinking water and wastewater samples using molecular techniques in the city of Lima [20]. Quantitative polymerase chain reaction (qPCR) has been used to detect and measure *H. pylori* from gastric biopsies, drinking water, and water biofilm samples [16,21]. For example, in a study of drinking tap water to measure *H. pylori* by qPCR they found that 20.3 % of water samples contained *H. pylori* [22]. Another

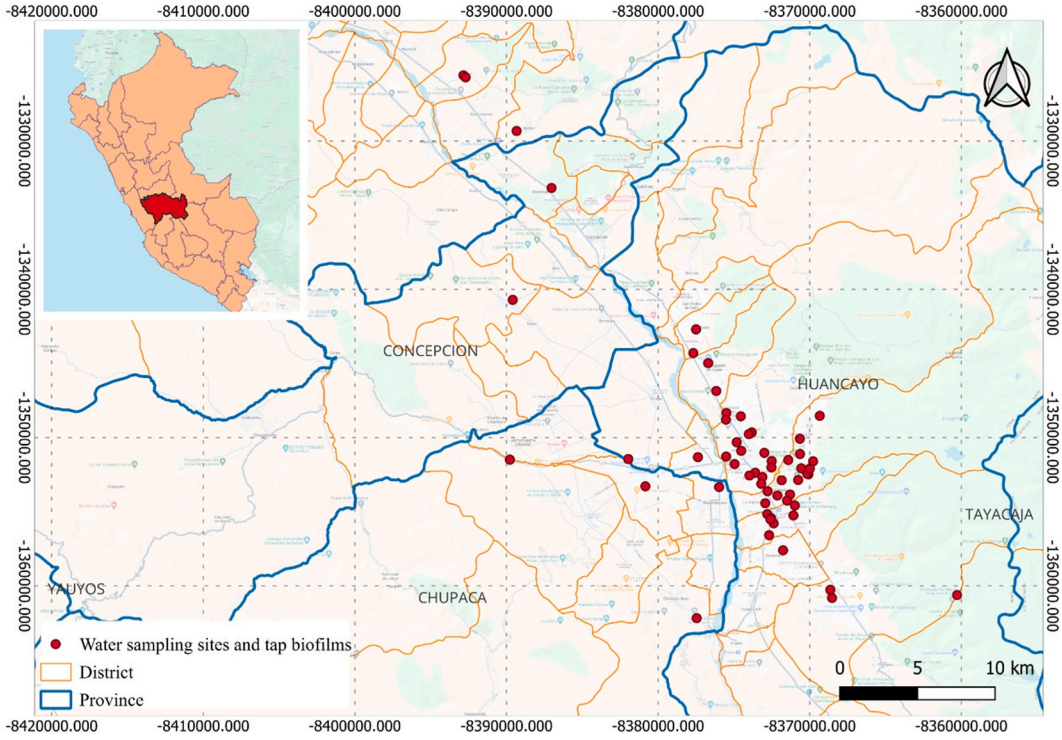

Fig. 1. Location map of the study area and water and tap biofilm sampling sites.

study reports that the highest amount of *H. pylori* found in drinking water was 1.6E6 genome copy/L. However, to our knowledge, there is no study in other Peruvian cities on this bacterium in drinking water. Therefore, the objective of this study was to analyze the occurrence of *H. pylori* in drinking water sources and the antimicrobial resistance profile in the central region of Peru.

2. Materials and methods

2.1. Study area and population description

The study area is located in the central region of the Peruvian Andes, in the Junín region, which covers an area of 44,329 km^2, representing 3.4 % of the national territory, at latitude 11°40′13.51″ south and longitude 74°56′13.85″ west with an altitude ranging from 360 to 5000 masl (Fig. 1). It covers two natural regions, the highlands with 20,821 km^2 (47 %) and the jungle with 23,508 km^2 (53 %), with a population of 1,246,038 inhabitants (4.2 % of the national population) [23]. Population growth in recent decades has been concentrated in the provinces of Huancayo (603,163 inhabitants), Jauja (88,002 inhabitants), Concepción (58,982 inhabitants) and Chupaca (58,012 inhabitants) [24]. The concentration of population in these provinces is generated to develop productive activities and services. In general, there are drinking water service providers throughout the Junín region. Drinking water in this region comes mainly from surface water bodies and groundwater [25].

2.2. Collection and preparation of water samples

A total of 192 tap water samples were collected in four provinces of the Junin region in central Peru between August and October 2023. Previously, the homeowners gave their informed consent for the collection of water samples from their respective taps. Prior to the collection of the water samples, biofilms were sampled from the internal surface of the taps using sterile swabs and deposited in their respective test tubes containing 3 ml of brain heart infusion broth and selective supplement (BHI-Dent) [26]. Then, at each tap water was allowed to run for 1 min before collecting the sample. For the detection of *H. pylori* each sample consisted of 100 ml of tap water collected in 250 ml sterile glass bottles containing 0.5 g of sodium thiosulfate for dechlorination. While for the determination of physicochemical parameters of water, 750 ml water was collected from each tap. All samples were collected under aseptic conditions and transferred to the laboratory on ice within 2 h of collection. Water physicochemical parameters such as pH, temperature, electrical conductivity (EC) and total dissolved solids (TDS) were measured with the WTW Multi3630 IDS multiparameter equipment and turbidity with the HI98703-01 turbidity meter.

2.3. Analysis of drinking water quality parameters

Measurement of pH, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) was performed by electrometric methods using the 3630 IDS WTW portable multiparameter equipment. The pH was measured using a pH probe (Sentix 940) according to SM 4500-H, EC/TDS using an EC probe (Tetracon 925) according to SM 2510B and DO with a DO probe (FDO 925) according to ASTM D888-12 TEST Method C. Turbidity (NTU) measurement was performed using the HANNAH HI98703-01 turbidity meter according to SM 2130 B nephelometric method. Temperature (°C) was measured using YOWEXA YET 71OI thermometer equipment.

2.4. Isolation and biochemical analysis of Helicobacter pylori

Water samples were centrifuged at 3000 rpm for 5 min, the supernatant was decanted and the sediment was resuspended in 2 ml of BHI-Dent for 1 h. Samples were cultured on modified brain heart infusion blood agar plates containing selective supplement (trimethoprim 30 mg/L and vancomycin 10 mg/L) at 37 °C under microaerophilic conditions for seven days. Biofilm swab samples contained in 2 mL of BHI-Dent were incubated under similar conditions as the water samples. After incubation time had elapsed, the plates were examined to determine the characteristic appearance of the *H. pylori* colonies (0.5 - 1 mm in diameter, circular, convex, translucent and weakly hemolytic). Biochemical analysis was performed by urease tests, oxidase and catalase tests. The presence of *H. pylori* was confirmed by Gram staining (gram-negative organisms with predominance of coccoid forms), biochemical tests (urease, oxidase and catalase tests) [27], and antibiotic sensitivity. The color change on urea agar to a deep purple color was considered as an indicator of *H. pylori* growth in the medium (positive urease test). Positive reaction to the oxidase test was evidenced by the turning of colonies to a blue/purple color. Catalase activity in the isolated strains was assessed by the drop technique. Briefly, 3 % hydrogen peroxide (H_2O_2) was added to a pure colony on a slide. The formation of oxygen bubbles was considered a positive result. For comparison, an *H. pylori* strain from a clinical sample was used.

2.5. Multi-antibiotic resistance profile of Helicobacter pylori

Antibiotic sensitivity of *H. pylori* isolates was determined by the Kirby Bauer diffusion method. Inocula of *H. pylori* colonies were prepared in Tryptic Soy Broth and incubated at 37 °C under microaerophilic conditions until a turbidity equal to 0.5 McFarland (equivalent to 2×10^8) was obtained by UV/VIS spectrophotometer reading at 625 nm (absorbance was in the range of 0.08–0.1). Sterile swabs were then dipped into the inocula and spread on Mueller-Hinton agar plates (BD DifcoTM, USA) and sheep blood (8 % v/v), and the antibiotic discs (Nalidixic acid (30 μg) - NA30, Amoxicillin (25 μg) - AMX25, Azithromycin (15 μg) - AZM15, Ciprofloxacin

(5 μg) - CIP5, Chloramphenicol (30 μg) - C30, Gentamicin (10 μg) - GM10, Tetracycline (30 μg) - TE30) were plated. Plates were incubated at 37 °C under microaerophilic conditions for 72 h (Fig. 2). The diameters of the inhibition zones were measured and the results were interpreted as susceptible (S), intermediate (I) or resistant (R), according to the standards of the National Committee for Clinical Laboratory Standards [28,29].

3. Results

3.1. Analysis of the physicochemical characteristics of tap water

Table 1 presents a statistical summary of the mean values of the physicochemical parameters of tap water quality obtained in four provinces of the Junín region (Huancayo, Chupaca, Concepción and Jauja provinces). In the province of Chupaca, the highest mean values of temperature (18.70 °C) and pH (8.05) were recorded in the Ahuac and lower Chongos districts, respectively. Meanwhile, the highest mean values of electrical conductivity (1163.67 μS/cm), turbidity (1.00 NTU), TDS (745 mg/L) and DO (7.03 mg/L) were recorded in Huamancaca Chico and Chupaca. In Concepción, the highest mean values of physicochemical water quality parameters were recorded in the Matahuasi district, except for the mean values of temperature and pH, which were recorded in the Aco. In Huancayo, the highest mean values for temperature (19.05 °C) were recorded in the Chilca district, pH (9.25) in Huancayo, EC (1447.33 μS/cm), TDS (927.33 mg/L), DO (7.63 mg/L) and turbidity (21.23 NTU) in the El Tambo district. In Jauja, the highest mean values of temperature (18.87 °C), EC (566.33 μS/cm), TDS (363 mg/L) were recorded in the Apata district. While the pH (7.93), DO (6.47 mg/L) and turbidity (5.15 NTU) values in the El Mantaro district were highest in the Apata district. In contrast, the pH (9.25) and turbidity (5.15 NTU) recorded in Huancayo and Jauja provinces exceeded the Peruvian environmental quality standards for drinking water. In the *H. pylori* positive water samples, the amount of free chlorine residual ranged from 0.02 to 0.12 mg/L.

3.2. Detection of Helicobacter pylori in tap water and biofilms

H. pylori was present in 2/192 tap water samples (1.04 %) and in 3/192 tap biofilm samples (1.56 %) from households in the provinces included in the study. Twenty percent of the total samples (tap water and tap biofilm) that were positive for *H. pylori* came from Chilca district, Huancayo province (code 16Hu-Ch-a) and the other 20 % of the samples came from Huamancaca chico district, Chupaca province (code 6Ch-Hu-a). Sixty percent of the total samples that were positive for *H. pylori* corresponded to biofilm samples from taps that came from the districts of El Tambo (codes 23Hu-Et-h and 24Hu-Et-h) and Chilca (code 9Hu-Ch-h) (Table 2).

3.3. Multi-antibiotic resistance profile of Helicobacter pylori

Antibiotic resistance of *H. pylori* was tested by the Kirby Bauer diffusion method as described in the materials and methods section. Table 3 shows the percentages of *H. pylori* isolates corresponding to sensitive (no in vitro resistance), intermediate and resistant phenotypes to each antibiotic. 100 % of the *H. pylori* isolates obtained from water samples from the Chilca district showed resistance to nalidixic acid and 66.67 % to both amoxicillin and chloramphenicol. Of the *H. pylori* isolates, 33.33 % showed intermediate

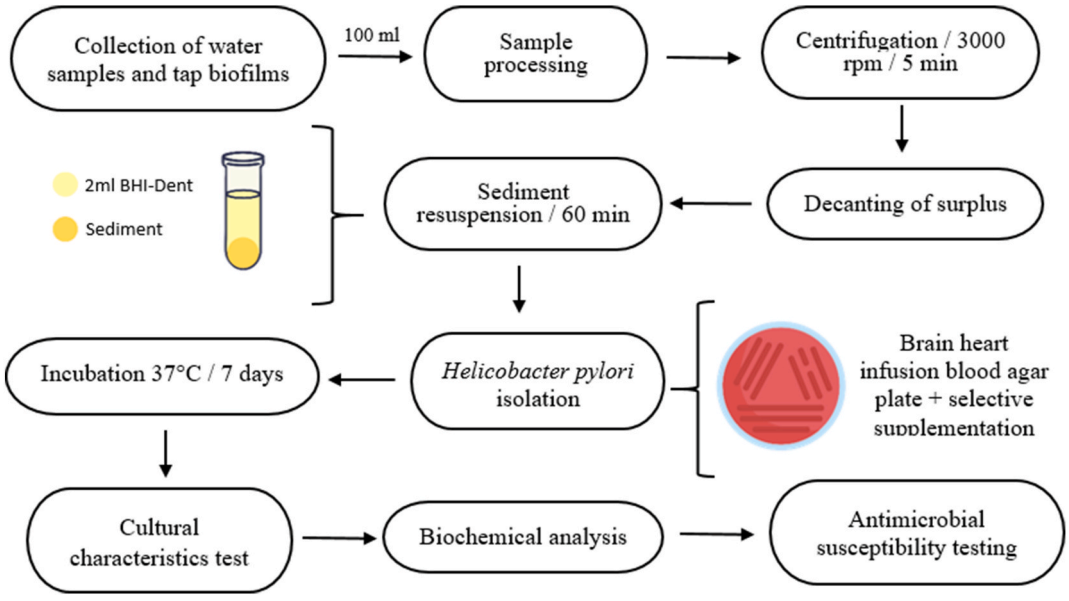

Fig. 2. Methodological design to isolate *Helicobacter pylori* from tap water and biofilms.

Table 1
Mean and standard deviation (SD) of physicochemical parameters of tap water in provinces of the Junín region.

Province	Code	Temperature (°C)	pH	EC (μS/cm)	TDS (mg/L)	DO (mg/L)	Turbidity (NTU)
Chupaca	1Ch-Ah	17.93 (0.06)	8.02 (0.01)	950.67 (0.58)	608.33 (0.58)	6.72 (0.04)	0.47 (0.04)
	2Ch-Cb	13.43 (0.35)	7.81 (0.01)	731.33 (0.58)	468.33 (0.58)	6.2 (0.14)	0.72 (0.02)
	3Ch-Ch	18.70 (0.00)	7.86 (0.01)	914.67 (0.58)	585.67 (0.58)	6.42 (0.02)	0.96 (0.02)
	4Ch-Ch	17.70 (0.10)	7.80 (0.00)	1104.67 (0.58)	707.00 (0.00)	5.93 (0.03)	0.53 (0.06)
	5Ch-Ch	17.40 (0.10)	8.05 (0.00)	756.00 (1.00)	484.67 (0.58)	7.03 (0.06)	1.00 (0.19)
	6Ch-Hu	12.73 (0.40)	7.90 (0.01)	1163.67 (0.58)	745.00 (1.00)	6.40 (0.03)	0.67 (0.06)
Concepción	7Co-Ac	16.27 (0.06)	8.07 (0.01)	425.00 (0.00)	272.00 (0.00)	6.33 (0.29)	0.33 (0.02)
	8Co-Ma	15.67 (0.25)	7.92 (0.02)	871.00 (0.00)	557.33 (0.58)	6.61 (0.05)	0.35 (0.03)
Huancayo	9Hu-Ch	16.53 (0.06)	8.18 (0.30)	568.00 (1.00)	364.33 (0.58)	6.23 (0.07)	0.61 (0.02)
	10Hu-Ch	18.53 (0.06)	7.77 (0.00)	587 0.00 (0.00)	376.00 (0.00)	6.86 (0.05)	0.34 (0.08)
	11Hu-Ch	16.50 (0.20)	7.83 (0.04)	1039.00 (0.00)	665.00 (0.00)	6.54 (0.02)	0.30 (0.03)
	12Hu-Ch	19.07 (0.21)	7.74 (0.02)	835.33 (0.58)	535.00 (0.00)	6.62 (0.01)	0.35 (0.06)
	13Hu-Ch	16.63 (0.06)	7.85 (0.00)	940.67 (0.58)	602.00 (0.00)	6.56 (0.07)	0.35 (0.04)
	14Hu-Ch	17.60 (0.10)	7.86 (0.02)	912.33 (0.58)	584.00 (0.00)	7.03 (0.02)	3.18 (0.16)
	15Hu-Ch	18.73 (0.21)	7.67 (0.01)	1031.00 (0.00)	660.00 (0.00)	6.31 (0.01)	0.60 (0.03)
	16Hu-Ch	18.27 (0.15)	7.91 (0.00)	904.67 (0.58)	578.67 (0.58)	6.96 (0.05)	0.65 (0.07)
	17Hu-Ch	18.33 (0.06)	8.00 (0.01)	774.67 (0.58)	495.67 (0.58)	7.12 (0.06)	0.25 (0.01)
	18Hu-Et	18.83 (0.15)	7.84 (0.02)	1447.33 (0.58)	927.33 (1.53)	6.27 (0.03)	0.68 (0.03)
	19Hu-Et	17.53 (0.06)	7.86 (0.01)	760 (0.00)	486.00 (0.00)	6.54 (0.02)	0.51 (0.04)
	20Hu-Et	18.70 (0.10)	6.94 (1.15)	790.33 (0.58)	506.33 (0.58)	6.61 (0.01)	0.40 (0.05)
	21Hu-Et	16.30 (0.10)	7.80 (0.10)	1022 (1.00)	655.00 (0.00)	6.46 (0.01)	0.58 (0.09)
	22Hu-Et	16.53 (0.12)	7.91 (0.01)	786.33 (0.58)	504.00 (1.00	6.48 (0.07)	0.35 (0.02)
	23Hu-Et	18.13 (0.06)	7.93 (0.00)	731.67 (0.58)	468.33 (0.58)	7.01 (0.11)	1.03 (0.15)
	24Hu-Et	16.00 (0.17)	7.87 (0.01)	798.00 (1.00)	511.00 (0.00)	6.07 (0.09)	1.31 (0.07)
	25Hu-Et	16.93 (0.06)	7.87 (0.00)	630.00 (0.00)	403.00 (0.00)	6.48 (0.01)	1.24 (0.04)
	26Hu-Et	17.90 (0.00)	7.63 (0.01)	792.00 (0.00)	507.00 (0.00)	5.74 (0.03)	0.79 (0.03)
	27Hu-Et	12.90 (0.72)	7.87 (0.01)	765.33 (0.58)	489.67 (0.58)	6.11 (0.03)	21.23 (1.89)
	28Hu-Et	18.20 (0.10)	7.83 (0.00)	982.67 (0.58)	629.00 (0.00)	6.71 (0.08)	0.27 (0.13)
	29Hu-Et	18.27 (0.06)	7.90 (0.00)	912.67 (0.58)	584.00 (0.00)	6.75 (0.13)	0.44 (0.03)
	30Hu-Et	15.30 (0.30)	8.01 (0.03)	790.33 (0.58)	505.33 (0.58)	6.24 (0.04)	0.84 (0.04)
	31Hu-Et	14.90 (0.20)	7.82 (0.01)	978.67 (0.58)	626.33 (0.58)	6.52 (0.02)	0.21 (0.02)
	32Hu-Et	18.23 (0.21)	8.06 (0.00)	899.67 (0.58)	575.67 (0.58)	7.13 (0.11)	0.51 (0.24)
	33Hu-Et	17.53 (0.06)	7.95 (0.00)	987.33 (0.58)	631.67 (0.58)	6.51 (0.24)	0.37 (0.07)
	34Hu-Et	18.30 (0.00)	8.28 (0.00)	772.00 (0.15)	494.00 (0.15)	7.63 (7.80)	0.94 (1.09)
	35Hu-Et	18.90 (0.00)	7.85 (0.01)	737.00 (0.00)	471.67 (0.58)	6.12 (0.04)	1.28 (0.10)
	37Hu-Et	15.20 (0.20)	7.87 (0.12)	801.33 (3.51)	513.67 (1.53)	6.63 (0.04)	0.33 (0.02)
	37Hu-Hh	15.07 (0.25)	8.08 (0.02)	83,600 (1.73)	535.67 (1.15)	6.52 (0.19)	0.36 (0.03)
	38Hu-Hn	16.67 (0.15)	8.13 (0.01)	950.33 (1.15)	608.67 (0.58)	6.68 (0.03)	0.26 (0.03)
	39Hu-Hn	18.30 (0.10)	7.60 (0.35)	965.33 (0.58)	617.00 (0.00)	6.76 (0.02)	0.63 (0.03)
	40Hu-Hn	14.47 (0.25)	7.96 (0.01)	923.67 (1.15)	591.33 (0.58)	6.25 (0.01)	1.56 (0.05)
	41Hu-Hu	17.27 (0.15)	9.25 (0.02)	610.33 (1.15)	391.33 (0.58)	6.28 (0.06)	0.42 (0.03)
	42Hu-Hu	15.13 (0.15)	7.63 (0.03)	752.67 (1.15)	482.00 (0.00)	6.42 (0.03)	0.67 (0.05v
	43Hu-Hu	18.33 (0.06)	7.82 (0.00)	968.00 (1.00)	619.33 (0.58)	6.68 (0.12)	0.47 (0.09)
	44Hu-Hu	18.73 (0.06)	8.12 (0.03)	578.00 (0.00)	370.00 (0.00)	6.38 (0.01)	2.93 (0.03)
	45Hu-Hu	18.63 (0.06)	7.74 (0.02)	599.67 (0.58)	383.67 (0.58)	6.92 (0.02)	0.72 (0.03)
	46Hu-Hu	17.37 (0.06)	8.02 (0.04)	718.67 (0.58)	460.00 (0.00)	6.46 (0.02)	0.99 (0.05)
	47Hu-Hu	17.53 (0.06)	8.06 (0.00)	699.00 (0.00)	447.00 (0.00)	6.42 (0.03)	1.77 (0.04)
	48Hu-Hu	16.87 (0.06)	8.00 (0.00)	773.33 (0.58)	495.00 (0.00)	6.44 (0.18)	1.02 (0.02)
	49Hu-Hu	15.90 (0.30)	8.03 (0.01)	817.33 (0.58)	523.00 (0.00)	6.57 (0.11)	1.65 (0.31)
	50Hu-Hu	18.10 (0.10)	7.87 (0.00)	736.67 (0.58)	471.00 (0.00)	7.16 (0.09	0.77 (0.18)
	51Hu-Hu	18.03 (0.06)	7.90 (0.00)	798.00 (0.00)	511.00 (0.00)	6.83 (0.09))	0.61 (0.13)
	52Hu-Hu	18.23 (0.06)	7.96 (0.00)	807.67 (0.58)	517.00 (0.00)	6.74 (0.12)	0.62 (0.11)
	53Hu-Hu	18.10 (0.10)	8.11 (0.00)	671.33 (1.15)	429.67 (0.58)	6.87 (0.05)	0.33 (0.08)
	54Hu-Hu	18.60 (0.00)	7.70 (0.01)	1207.00 (0.00)	772.33 (0.58)	6.14 (0.01)	4.36 (0.03)
	55Hu-Mi	17.10 (0.20)	7.87 (0.02)	619.67 (0.58)	397.67 (0.58)	6.42 (0.12)	0.78 (0.04)
	56Hu-Pi	17.20 (0.10)	8.12 (0.03)	948.33 (3.06)	607.33 (0.58)	6.10 (0.04)	0.68 (0.07)
	57Hu-Sc	18.37 (0.15)	7.86 (0.00)	957.00 (2.65)	612.00 (1.00)	6.76 (0.05)	0.20 (0.01)
	58Hu-Sc	17.93 (0.35)	7.98 (0.00)	643.67 (0.58)	402.00 (0.00)	6.65 (0.11)	0.41 (0.10)
	59Hu-Sc	18.40 (0.10)	7.80 (0.00)	699.00 (0.00)	447.67 (0.58)	6.51 (0.08)	0.84 (0.06)
	60Hu-Sa	16.30 (0.10)	8.15 (0.01)	749.00 (0.00)	479.00 (0.00)	6.54 (0.02)	0.9 (0.02)
	61Hu-Sa	17.83 (0.25)	8.01 (0.00)	594.00 (0.00)	380.00 (0.00)	6.62 (0.05)	0.65 (0.07)
Jauja	62Ja-Ap	18.87 (0.15)	7.67 (0.02)	566.33 (0.58)	363.00 (0.00)	6.40 (0.03)	0.77 (0.07)
	63Ja-Em	16.33 (0.12)	7.87 (0.15)	378.33 (0.58)	243.00 (0.00)	6.43 (0.12)	5.15 (0.05)
	64Ja-Em	17.20 (0.10)	7.93 (0.02)	564.00 (0.00)	361.00 (0.00)	6.47 (0.03)	3.85 (0.02)
Peruvian drinking water [30]		Δ3	6.5–8.5	1500	1000	≥6	5

Table 2
Detection of *Helicobacter pylori* in tap water and tap biofilms in provinces of the Junín region.

Province	Code	Tap water (a)			Tap biofilms (h)		
		Urease test	Catalase test	Oxidase test	Urease test	Catalase test	Oxidase test
Chupaca	1Ch-Ah	–	–	–	+	+	–
	2Ch-Cb	–	–	–	–	–	–
	3Ch-Ch	–	–	–	–	–	–
	4Ch-Ch	–	–	–	+	+	–
	5Ch-Ch	–	–	–	–	–	–
	6Ch-Hu	+	+	+	–	–	–
Concepción	7Co-Ac	–	–	–	–	–	–
	8Co-Ma	–	–	–	–	–	–
Huancayo	9Hu-Ch	+	+	–	+	+	+
	10Hu-Ch	–	–	–	–	–	–
	11Hu-Ch	–	–	–	–	–	–
	12Hu-Ch	–	–	–	–	–	–
	13Hu-Ch	–	–	–	–	–	–
	14Hu-Ch	–	–	–	–	–	–
	15Hu-Ch	–	–	–	–	–	–
	16Hu-Ch	+	+	+	–	–	–
	17Hu-Ch	–	–	–	–	–	–
	18Hu-Et	–	–	–	–	–	–
	19Hu-Et	–	–	–	–	–	–
	20Hu-Et	–	–	–	–	–	–
	21Hu-Et	–	–	–	–	–	–
	22Hu-Et	–	–	–	–	–	–
	23Hu-Et	–	–	–	+	+	+
	24Hu-Et	–	–	–	+	+	+
	25Hu-Et	–	–	–	–	–	–
	26Hu-Et	–	–	–	–	–	–
	27Hu-Et	–	–	–	–	–	–
	28Hu-Et	–	–	–	–	–	–
	29Hu-Et	–	–	–	–	–	–
	30Hu-Et	–	–	–	–	–	–
	31Hu-Et	–	–	–	–	–	–
	32Hu-Et	–	–	–	–	–	–
	33Hu-Et	–	–	–	–	–	–
	34Hu-Et	–	–	–	–	–	–
	35Hu-Et	–	+	+	–	–	–
	37Hu-Et	–	–	–	–	–	–
	37Hu-Hh	–	–	–	–	–	–
	38Hu-Hn	+	+	–	–	–	–
	39Hu-Hn	–	–	–	–	–	–
	40Hu-Hn	–	–	–	–	–	–
	41Hu-Hu	–	–	–	–	–	–
	42Hu-Hu	–	–	–	–	–	–
	43Hu-Hu	–	–	–	–	–	–
	44Hu-Hu	–	–	–	–	–	–
	45Hu-Hu	–	–	–	–	–	–
	46Hu-Hu	–	–	–	–	–	–
	47Hu-Hu	+	+	–	–	–	–
	48Hu-Hu	–	–	–	–	–	–
	49Hu-Hu	–	–	–	–	–	–
	50Hu-Hu	–	–	–	–	–	–
	51Hu-Hu	–	–	–	+	+	–
	52Hu-Hu	–	–	–	–	–	–
	53Hu-Hu	–	–	–	–	–	–
	54Hu-Hu	–	–	–	–	–	–
	55Hu-Mi	–	–	–	–	–	–
	56Hu-Pi	–	–	–	–	–	–
	57Hu-Sc	–	–	–	–	–	–
	58Hu-Sc	–	–	–	–	+	+
	59Hu-Sc	–	–	–	–	–	–
	60Hu-Sa	–	–	–	–	–	–
	61Hu-Sa	–	–	–	–	–	–
Jauja	62Ja-Ap	+	+	–	–	–	–
	63Ja-Em	–	–	–	–	–	–
	64Ja-Em	–	–	–	–	–	–

+: positive test, -: negative test.

susceptibility to amoxicillin, azithromycin and chloramphenicol, and 66.67 % to tetracycline. Likewise, 100 % of the isolates of this bacterium were sensitive to ciprofloxacin and gentamicin, 66.67 % to azithromycin, and 33.33 % to tetracycline. Seventy-five percent and 50 % of the *H. pylori* isolates obtained from water samples from the Huamancaca chico district showed resistance to nalidixic acid and amoxicillin, respectively. Twenty-five percent of the isolates showed intermediate susceptibility to nalidixic acid, amoxicillin, azithromycin, ciprofloxacin, chloramphenicol, and gentamicin. Seventy-five percent of *H. pylori* isolates were susceptible to azithromycin, ciprofloxacin, chloramphenicol and gentamicin, 50 % to tetracycline and 25 % to amoxicillin. Resistance of *H. pylori* isolates from biofilms from taps in the El Tambo district to tetracycline ranged from 33.33 % to 66.67 % and to nalidixic acid ranged from 66.67 % to 100 %.

4. Discussion

Declining water quality due to various stressors has become an issue of global concern. In poor sanitary environments, bacterial pathogens and indicators of fecal contamination can enter the water supply system, leading to outbreaks of waterborne diseases [31, 32]. The physicochemical water quality parameters evaluated in this study did not exceed Peru's environmental quality standards for drinking water, except for pH and turbidity. Routine water quality monitoring is performed based on physicochemical parameters and the detection of fecal indicator bacteria (*Escherichia coli* and thermotolerant coliforms) that include these standards. Although the transmission of *H. pylori* through water is increasingly suspected and the World Health Organization now refers to this microorganism as a water contaminant [33] it is still not included as an indicator bacterium for contamination.

H. pylori is a bacterium that infects the stomach and can cause problems such as gastritis, ulcers and gastric cancer. This bacterium can withstand challenging conditions such as microoxygenation (between 2 - 5 % oxygen) and pH levels ranging from 4.5 to 9.0. It can even survive up to two weeks at a temperature of 4 °C [34]. While *H. pylori* is easily adapted to the stomach environment, in the context of water it can enter a viable but non-culturable state, making it difficult to detect [35]. It is important to clarify that in this study the ideal physicochemical conditions of *H. pylori* have been found in the water sources where the bacteria were identified. The concentrations of free residual chlorine detected in this study were lower than the values established in the Peruvian standard (≥0.5 mg/L); revealing a deficient sanitation in the drinking water distribution system. However, *H. pylori* present variable resistance to chlorine, which affects its control in water treatment systems. Research indicates that while free chlorine can inactivate *H. pylori*, this pathogen demonstrates greater resistance compared to other bacteria such as *Escherichia coli* (Baker et al., 2002). Our results also agree with those reported by Liu et al. [34] who indicate that *H. pylori* can adhere to different materials and coexist with other bacteria on pipes, faucets, and water surfaces suggesting possible survival in water distribution systems.

Other studies also show that chlorine is effective in eliminating *H. pylori*, mainly when it reaches appropriate levels (0.5–2 mg/L), indicating that standard disinfection practices can control its presence in drinking water [36]. Although efficiency depends on other factors such as pH, presence of organic matter, and contact time. The longer the contact time, the more effective the removal. Chlorine is most effective at low pH (acidic), where the active form of chlorine, hypochlorous acid (HOCl), which has greater disinfecting power, predominates. If the pH is too high, chlorine is transformed into hypochlorite ion (OCl^-), which is less effective in eliminating this bacterium [37]. The presence of organic matter in the water decreases the bactericidal activity of chlorine because much of the available chlorine reacts with this substance, decreasing the amount of free chlorine available to attack the bacteria. At suboptimal

Table 3
Multiple antibiotic susceptibility profile of *Helicobacter pylori* isolates.

Province District	Sample code	ASP	Antibiotic						
			NA30 Nº (%)	AMX25 Nº (%)	AZM15 Nº (%)	CIP5 Nº (%)	C30 Nº (%)	GM10 Nº (%)	TE30 Nº (%)
Huancayo	16Hu-Ch-a[a]	S	0	0	2 (66.67)	3 (100)	0	3 (100)	1 (33.33)
Chilca		I	0	1 (33.33)	1 (33.33)	0	1 (33.33)	0	2 (66.67)
		R	3 (100)	2 (66.67)	0	0	2 (66.67)	0	0
Chupaca	6Ch-Hu-a[a]	S	0	1 (25)	3 (75)	3 (75)	3 (75)	3 (75)	2 (50)
Huamancaca chico		I	1 (25)	1 (25)	1 (25)	1 (25)	1 (25)	1 (25)	0
		R	3(75)	2 (50)	0	0	0	0	2 (50)
Huancayo	9Hu-Ch-h[b]	S	0	0	1 (33.33)	2 (66.67)	0	1 (33.33)	0
Chilca		I	1 (33.33)	1 (33.33)	2 (66.67)	1 (33.33)	2 (66.67)	1 (33.33)	3 (100)
		R	2 (66.67)	2 (66.67)	0	0	1 (33.33)	1 (33.33)	0
Huancayo		S	0	2 (66.67)	2 (66.67)	2 (66.67)	1 (33.33)	3 (100)	1 (33.33)
El Tambo	23Hu-Et-h[b]	I	0	0	1 (33.33)	1 (33.33)	1 (33.33)	0	0
		R	3 (100)	1 (33.33)	0	0	1 (33.33)	0	2 (66.67)
	24Hu-Et-h[b]	S	0	2 (66.67)	2 (66.67)	3 (100)	2 (66.67)	2 (66.67)	1 (33.33)
		I	1 (33.33)	0	1 (33.33)	0	0	1 (33.33)	1 (33.33)
		R	2 (66.67)	1 (33.33)	0	0	1 (33.33)	0	1 (33.33)

NA30: Ácido Nalidíxico (30 μg), AMX25: Amoxicilina (25 μg), AZM15: Azitromicina (15 μg), CIP5: Ciprofloxacina (5 μg), C30: Cloranfenicol (30 μg), GM10: Gentamicina (10 μg) y TE30: Tetraciclina (30 μg).
ASP: Antimicrobial susceptibility profile.
[a] *H. pylori* isolates obtained from water samples.
[b] *H. pylori* isolates obtained from tap biofilm samples.

chlorine concentrations, *H. pylori* has the ability to enter a state of non-culturable viability, in which the bacteria cannot replicate or be detected by conventional methods, but can still maintain the ability to cause infections if conditions become favorable again [38].

Our results also reveal the presence of *H. pylori* in drinking water samples (1.04 %) and tap biofilms (1.56 %) from two of the four provinces included in the study. Although it is true that these results show low frequency values compared to those found in other studies at the global level, the results of the study show that *H. pylori* is still present in the water samples from two of the four provinces included in the study [39,40]. The presence of *H. pylori* in drinking water and tap biofilms would indicate that disinfection and distribution systems are not efficient. Which translates, as lack of efficient methods for water purification, the use of drinking water captured by piping from the water body, the possibility of the presence of bacterial colonies as biofilms in the pipes for water transfer and the possibility of leakage of wastewater to drinking water sources. Our results are also supported by Ref. [41] who point out that domestic contamination of drinking water is possible when hygienic conditions are deficient. On the other hand, our findings are similar than those found in Iran by Ref. [42], where the detection rate ranged from 1.77 % to 3.63 %.

H. pylori infection is one of the most common chronic infections worldwide (44.3 %), with differences associated with the socioeconomic level of countries [43]. The prevalence of *H. pylori* varies widely within and between countries, reaching the highest levels (80 %) in developing countries [44,45]. These differences in *H. pylori* prevalence probably reflect the level of urbanization, sanitation, access to drinking water and various socioeconomic levels. However, studies conducted in the three Peruvian geographic regions reveal no significant differences between *H. pylori* prevalence and low socioeconomic level. In our study, 40 % of water samples that were positive for *H. pylori* were observed in peri-urban households in the Chilca and Huamancaca chico districts, where the levels of free residual chlorine in tap water were below the optimal range established by the WHO (0.2 - 1.0 mg/L) [46]. These results are similar to those of Hasanvand et al. [32], who found *H. pylori* in drinking water samples with low free chlorine residual levels (0.04 mg/L) and even at levels (0.58 mg/L) that were within the WHO recommended range. The presence of *H. pylori* in tap biofilms in the El Tambo and Chilca districts reflects the fact that old drinking water distribution systems are a source of contamination of the water supplied. Studies supporting our findings report that 1.8 billion people have used a contaminated drinking water source [1].

The resistance of *H. pylori* to nalidixic acid (100 %), amoxicillin (66.67 %) and chloramphenicol (66.67 %), independent of sample type (water or tap biofilms) reveals the presence of virulent and resistant strains. Furthermore, the prevalence of resistance against human-derived antimicrobial agents in *H. pylori* from drinking water samples could confirm the routes of contamination of water of human origin. Our findings also reveal variability of the resistance pattern from one district to another and resistance to more than one of the antibiotics tested. Similar antimicrobial resistance findings have been reported in previous studies in clinical isolates of *H. pylori* in Lima, Peru [21]. However, the resistance revealed by this Peruvian study is closely dependent on its use. Other important data from our study were the susceptibility shown by *H. pylori* isolates to gentamicin (100 %), azithromycin (66.67 %) and ciprofloxacin (66.67 %).

Co-infection of *H. pylori* and *E. coli* in water is an under-researched topic, but some studies have looked at the simultaneous presence of both bacteria in water, particularly in contaminated or inadequately treated water. *E. coli* is a classic fecal indicator in water systems, as it is present in the intestines of humans and animals, and its presence in water generally indicates fecal contamination. *H. pylori* is a gastric bacterium, present in untreated or insufficiently chlorinated water. The main route of transmission of *H. pylori* involves human-to-human contact, but contaminated water could act as a means of transmission [47]. Some studies have suggested that both *H. pylori* and *E. coli* can coexist in water contaminated with human or animal feces, particularly in regions with poor sanitary infrastructures, because both bacteria can be present in the human digestive tract. In some countries where access to treated drinking water is limited, both pathogens have been detected in surface water and groundwater. Studies have shown that *H. pylori* infection can not only affect gastric microecology, but also change the intestinal flora [48]. The relationship between *H. pylori* and *E. coli* is complex and there is evidence to suggest that *H. pylori* infection can influence the gut microbiota and potentially affect the presence and behavior of *E. coli* [49,50].

5. Conclusion

In conclusion, water samples and tap biofilms in the Chilca, El Tambo and Huamancaca chico districts in the Junín region harbor *H. pylori*. Viable cells of this pathogen have been recovered through enrichment isolation, selection and identification methods. The resistance of *H. pylori* isolates to nalidixic acid obtained from tap biofilm samples ranged from 66.67 % to 100 %. Finally, the findings of this study provide a first approach to the problem of *H. pylori* contamination of tap water in this region of the country. Despite the limitations, our results highlight the role of tap water with low levels of free residual chlorine in the transmission of *H. pylori*, which is why improvements in the sanitary conditions of the distribution and purification systems of this elementary liquid should be encouraged. Further prospective studies on the dynamics of this tap water contaminant are strongly recommended.

CRediT authorship contribution statement

María Custodio: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Raúl Montalvo-Otivo:** Investigation, Conceptualization. **Jhonatan Crispín-Ayala:** Methodology. **Jeampier Bendezu-Meza:** Methodology. **Pilar Herrera-Quintana:** Project administration, Methodology, Formal analysis. **Heidi De la Cruz:** Project administration, Methodology, Formal analysis. **Javier Huarcaya:** Project administration, Methodology, Formal analysis.

Data share statement

Data will be made available on request.

Ethics and consent statement

The study was reviewed and approved by the Research Ethics Committee of the National University of Central Peru (CEI-UNCP) with approval number: [CEI-UNCP-N°054–2023], dated June 8, 2023. Before proceeding to sample collection, all participants were informed of the purpose of the study and their consent was obtained.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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