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# Water quality and phenotypic antimicrobial resistance in isolated of *E. coli* from water for human consumption in Bagua, under One Health approach

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ABSTRACT

The One Health approach acknowledges the interconnection between human health, animal health, and environmental health, recognizing that these domains are closely intertwined, as many diseases affecting humans are also common in animals. Water acts as a vehicle for the transmission of such diseases, highlighting the significance of monitoring the quality of water intended for human consumption. In 2022, a research study was conducted to evaluate the water quality for human consumption in Bagua, Amazonas Region. The physicochemical analysis indicated that most parameters were within normal range, except for residual chlorine, which was predominantly absent. Microbiological analysis revealed the presence of total coliforms and *E. coli*. Phenotypic characterization of *E. coli* isolates exhibited resistance to the several antibiotics, including nalidixic acid, gentamicin, amoxicillin plus clavulanic acid, norfloxacin, and ciprofloxacin. These findings indicate a compromised production of water for human consumption, as per the water quality regulations in Peru. The presence of fecal contamination poses a significant microbiological risk to consumers. These results underscore the breakdown of the human-environment-animal interface within the One Health approach, thereby endangering public health.

## 1. Introduction

The One Health approach acknowledges the interconnectedness of human health, animal health, and environmental health, as 70 % of diseases that affect humans are shared with animals, and water serves as a vehicle for disease transmission[1]. Water, being an essential element for human existence, is a non-renewable resource that is increasingly scarce. Its quality and management for human consumption present one of the biggest challenges today. Disturbingly, approximately two million people worldwide, mostly children under the age of five, die each year due to waterborne diseases resulting from the consumption of contaminated water[2,3]. This

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highlights the significant disruption of the interface between human health and environmental health. To address this issue, it is crucial to emphasize pollution control and approach the problem from the perspective of One Health, examining the interactions among people, domestic animals, wildlife, plants, and the environment[4,5].

Water-associated diseases are a leading cause of morbidity and mortality, particularly in developing countries[6–8]. Therefore, ensuring an adequate supply of drinking water for human consumption is critical in reducing disease transmission[9], including zoonotic waterborne diseases [10]. Individuals who rely on water for human consumption but lack access to a safe and sufficient supply are at risk of consuming contaminated food and water, as well as encountering contaminated water during bathing or other activities[11].

In Peru, anemia rates have been alarmingly high in various regions for many years. For instance, in the Puno Region, 7 out of every 10 children are affected by anemia, while in the Amazonas Region, the figure stands at 4 out of every 10 children[12–15]. These statistics, accepted by both the central government and local authorities, highlight the adverse impact of anemia on the development of children living in extreme poverty. Additionally, acute diarrheal diseases contribute to infant mortality in these regions.

One of the contributing factors to these circumstances is the quality of water for human consumption, as it shows values above the standards[16].

The responsible entities for providing drinking water fail to do so, necessitating the implementation of additional re-chlorination steps throughout the distribution system. These steps are crucial to maintain residual chlorine levels that ensure the microbiological quality of the water[17].

Inadequate access to safe drinking water is also a leading cause of diarrhea, which contributes to malnutrition[18,19].

It is important to consider that the processes for purifying drinking water for human consumption involve several stages of evaluation, including physical, chemical, microbiological, and heavy metal parameters, as established by sanitary regulations in Peru [20,21]. Among these parameters, certain ones are prioritized and referred to as mandatory controls, such as pH, temperature, conductivity, residual chlorine, turbidity, and microbiological analysis.

An evaluation conducted in the city of Puno revealed that the residual chlorine in the water distribution system exceeds 0.5 mg/L. However, it is important to consider that historical variations in diarrheal diseases are associated with temperature, and the sustained presence of residual chlorine ensures good bacteriological status of water[22]. Nevertheless, the presence of traces of certain inorganic chemical compounds raises concerns regarding the acceptability of water for human consumption, particularly in high Andean rural communities of Peru[23]. Fecal contamination of drinking water is common in peri-urban communities in Peru[17,24–28]. Thus,

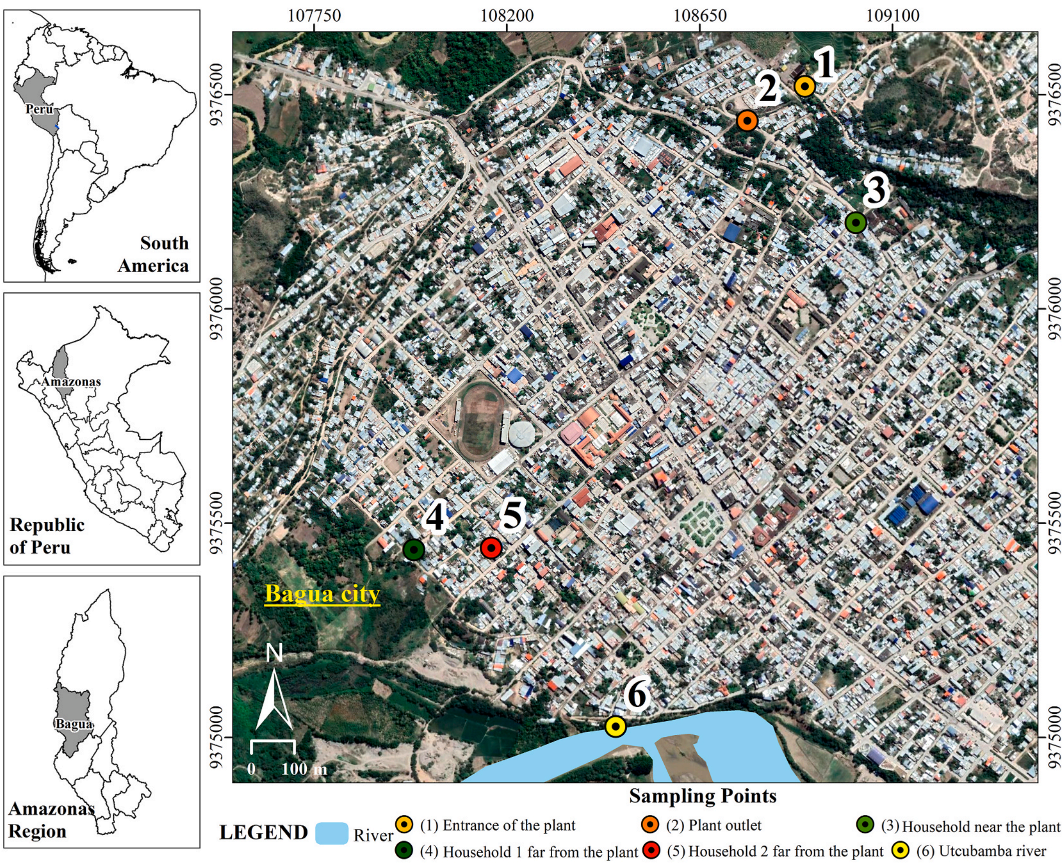


**Fig. 1.** Geographical location of the research work.

ensuring sufficient, safe, and continuous access to drinking water is crucial for human health, well-being, and development[29]. Therefore, the inclusion of additional re-chlorination steps throughout the water distribution system for human consumption is necessary[17]. Pathogens will always pose a significant challenge to human health, especially in developing regions[30], making this an opportunity to implement actions aligned with the One Health approach [1].

There is a lack of data on antimicrobial-resistant *E. coli* and other microorganisms present in environmental water, hindering our understanding of their development and spread using the One Health approach. It is crucial to comprehend the prevalence, distribution, and characteristics of bacteria present in surface waters. Additionally, the research on antimicrobial-resistant bacteria in natural watersheds is limited, and its impact on human health and food safety remains poorly understood. Addressing this critical data gap is necessary to comprehend antimicrobial resistance and mitigate its spread[31].

In the analysis of water samples, it was found that *E. coli* isolates exhibit a higher frequency of resistance to tetracycline, ampicillin, streptomycin, and nalidixic acid[32]. Similarly, a prevalence of 16.3 % of antibiotic resistance factors in *E. coli* isolates from drinking water samples has been reported. These samples can act as reservoirs for antimicrobial resistance genes, which can be transferred between human and animal populations through waste, leading to environmental contamination[33], thereby breaking the One Health approach.

Furthermore, significant resistance characteristics were observed in *E. coli* isolates from water samples. The highest levels of resistance were found against ampicillin, followed by amoxicillin, tetracyclines, chloramphenicol, and piperacillin. These isolates demonstrated resistance to multiple drugs[34]. Another study reported that *E. coli* isolates from water samples showed resistance to tetracycline, ampicillin, piperacillin, trimethoprim/sulfamethoxazole, and chloramphenicol. These findings indicate that drinking water sources could serve as reservoirs of antibiotic resistance, potentially posing a risk to public health[35].

Considering the above, it is crucial to identify the parameters that should be monitored as a general rule to determine the quality of water consumed by the population of the city of Bagua. This research work aims to provide insights into the water quality for human consumption and the presence of antimicrobial resistance factors, while highlighting its relationship with the One Health framework.

## 2. Materials and methods

### 2.1. Delimitation of the study

The study was conducted in Bagua, the capital of the province of the same name in the Amazonas Region of Peru (Fig. 1). The drinking water service in Bagua is provided by the "Empresa municipal de agua potable y alcantarillado de Bagua" S.A. (EMAPAB). The Bagua drinking water system (DWS) operates with a conventional treatment plant.

### 2.2. Sampling procedure

Sampling was carried out in November 2022 with three replicates at each sampled point to analyze both the physical-chemical and microbiological parameters of the water after DWS treatment. The sampling points were selected non-probabilistically and randomly, identifying households near and far from the treatment plant, collected from the following locations, duly georeferenced (see Table 1): the treatment plant, various points in the distribution system (including a household near and two household far from the treatment plant), the Bagua drinking water system, and a sample from the Utcubamba River, which runs through the city of Bagua. The criteria established by the Ministry of Health[20,21] were followed during the sampling process.

### 2.3. Assessed parameters

The measured variables adhered to the guidelines set by Peruvian regulations[20]. These variables encompassed both physical-chemical aspects (such as conductivity, residual chlorine, temperature, turbidity and pH) and microbiological factors (including total coliforms and *E. coli*). Allowable limits for water for human consumption are detailed in Table 2. The physical-chemical parameters were evaluated at the collection point, whereas microbiological and heavy metal analyses were performed in the laboratory within a maximum time frame of 6 h from the moment of sample collection.

**Table 1**
Sampling locations in the city of Bagua.

| Nº | Sample point | Georeferenced (UTM) | | Altitude |
|---|---|---|---|---|
| | | **East** | **North** | |
| **1** | Entrance of the plant | 773674 | 9377123 | 487 |
| **2** | Plant outlet | 773539 | 9377044 | 470 |
| **3** | Household near the plant | 773790 | 9376804 | 462 |
| **4** | Household 1 far from the plant | 772752 | 9376052 | 425 |
| **5** | Household 2 far from the plant | 772932 | 9376054 | 423 |
| **6** | Utcubamba River | 773218 | 9375635 | 401 |

**Table 2**
Permissible legal limits for water intended for human consumption as per Peruvian regulation (DS N° 031-2010-SA)[20].

| Parameter | Analysis Method | Legal limit |
|---|---|---|
| | | (DS N° 031-2010-SA, MINSA 2011) |
| Physical-chemical | | |
| turbidity | | 5 NTU |
| residual chlorine | | 250 mg $Cl^-$/L |
| temperature | | n.a. |
| conductivity (25 °C) | | 1500 µhmo/cm |
| pH | | 6.5–8.5 |
| Microbiological | | |
| total coliforms | | 0 CFU/100 ml at 35 °C |
| fecal coliforms and *E. coli* | | 0 CFU/100 ml at 44.5 °C |
| Heavy metals | | |
| Mn | | 0.4 mg/L |
| Fe | | 0.3 mg/L |
| Zn | | 3.0 mg/L |
| Cu | | 2.0 mg/L |
| As | | 0.010 mg/L |

n.a., not applicable; NTU, nephelometric turbidity units; CFU, colony forming units.

#### 2.3.1. *Equipment and methods*

The following equipment and methods were used for parameter determination:

- pH, conductivity, and temperature were measured using the HI9828 Multiparameter (HANNA) equipment.
- Turbidity was measured using the TN-100 portable Turbidimeter (EUTECH).
- Residual chlorine was measured using the POCKET II CLORIMETER (HACH, USA) with DPD (diethyl-*p*-phenyldiamine) tablets as a reagent.
- Heavy metals were tested using Semiquantitative Test Strips. The specific reagents and protocols recommended by the manufacturer were used for each metal: Zn (Quantofix® Zink, 91310), Mn (Visocolor® HE, 920055), Cu (Quantofix® Copper, 91304), Fe (Quantofix® Total iron, 91344), and As (Quantofix® Arsen sensitive, 91345).

The microbiological examination was conducted at the molecular biology laboratory of Universidad Nacional Intercultural Fabiola Salazar Leguía de Bagua (UNIFSLB). For sample collection, sterilized containers were used, with each sample consisting of 100 ml of treated water. To detect and quantify total coliforms and *E. coli*, the *m*-ColiBlue 24 broth (M00PMCB24, HACH Company, Loveland, USA) was employed, with an incubation period of 24 h at 35 °C and 44.5 °C, respectively. The m-FC broth without rosolic acid (MHA00FCR2, Millipore, MA, USA) was used to detect and quantify fecal coliforms, with an incubation period of 24 h at 44.5 °C. In cases where *E. coli* strains were detected, they were isolated using two culture media: MacConkey (MC) and eosin methylene blue (EMB). The subsequent step involved determining the antimicrobial phenotypic resistance of the isolates.

### 2.4. *Determination of antibiotic resistance phenotypes*

The antimicrobial phenotypic resistance of all isolated *E. coli* strains from the sampled locations was determined. The disk diffusion method, following the guidelines recommended by the Clinical Laboratory Standards Institute[36], was utilized to determine the antibiotic resistance phenotypes. Six antibiotics were tested: nalidixic acid (NA, 30 µg), gentamicin (GEN, 10 µg), chloramphenicol (C, 30 µg), amoxicillin + clavulanic acid (AMC, 30 µg), norfloxacin (NX, 10 µg), and ciprofloxacin (CIP, 5 µg). The interpretation criteria based on the diameter of the inhibition zones were as follows (in mm): NA30: R (resistant) $\leq$ 13, S (susceptible) $\geq$ 19; GEN10: R $\leq$ 12, S $\geq$ 15; C30: R $\leq$ 12, S $\geq$ 18; AMC30: R $\leq$ 13, S $\geq$ 18; NX10: R $\leq$ 12, S $\geq$ 17; CIP5: R $\leq$ 15, S $\geq$ 21. In each assay, the reference strain *Pseudomonas aeruginosa* was used for quality control.

### 2.5. *Statistical analysis*

A statistical analysis was conducted to examine the variations between the sampling points and the investigated parameters for descriptive purposes. Compliance with the assumptions of normality (Shapiro-Wilk) was checked, followed by Pearson's correlation analysis. The data were analyzed using Minitab 17 software with a 95 % confidence interval[37].

## 3. Results

### 3.1. *Physicochemical parameters*

The evaluated physicochemical parameters, including pH, temperature, turbidity, and conductivity, were found to be within the permissible limits set by the water quality regulations for human consumption in Peru [20]. However, notable statistical disparities were observed between each sampling point for all parameters, except for temperature, it is evident that there is no linear association between the variables according to Table 4. It is noteworthy that the levels of residual chlorine did not meet the requirements set by Peruvian regulations, which stipulate values ranging from 0 to 0.6 mg/L-1, except for a solitary point identified at the outlet of the water treatment plant in one of the three samples. The statistical analysis revealed significant differences among the sampling points. The minimum allowable value for residual chlorine is 0.5 mg/L-1 according to the Peruvian regulations[20], as shown in Table 3. Therefore, the water does not meet the requirements for drinking water and suitability for human consumption.

### 3.2. Heavy metals

The presence of only one heavy metal, iron (Fe), was detected in all sampled points, including the river, with a concentration of 2 mg/L. However, the sampling point does not appear to be a decisive factor in its presence. Significant differences among each sampling point were observed in the statistical analysis. Pearson correlation analysis revealed a strong correlation between turbidity, pH, and total dissolved solids, as shown in Table 4.

### 3.3. Microbiological analysis

During the microbiological analysis, it was noted that the majority of the sampled points, including the river, demonstrated the presence of total coliforms, which was anticipated. Likewise, *E. coli* was detected in all sampled points except for one point situated at the water treatment plant's outlet. The concentrations of total coliforms varied from 0 to greater than 200 CFU/100 ml, while the concentrations of *E. coli* ranged from 0 to 146 CFU/100 ml. Significant statistical disparities in bacterial growth were observed between each sampled point ($p < 0.05$).

A robust correlation was identified between pH and the presence of both total coliforms and *E. coli.* Furthermore, a highly significant correlation was observed between the concentrations of total coliforms and *E. coli,* confirming the expected relationship (Table 4).

### 3.4. Antibiotic resistance phenotypes

A total of 7 *E. coli* strains were isolated from the samples, representing different sampling points. Among these strains, all of them exhibited resistance to nalidixic acid, gentamicin, chloramphenicol, amoxicillin plus clavulanic acid, and ciprofloxacin. However, there were variations in the phenotypic behavior for norfloxacin among the strains isolated from different sampling points and cultured in different media. Interestingly, one strain isolated from a house far from the treatment plant and cultured in EMB medium showed susceptibility to norfloxacin, whereas another strain from the same location and culture medium exhibited resistance to norfloxacin (Table 5).

## 4. Discussion

The levels of heavy metals detected in the water samples were generally within the recommended values, except for iron (Fe), which exceeded the permissible limits set by Peruvian regulations. The presence of elevated levels of Fe in the water samples may pose a potential risk to public health. This could be attributed to the age and material of the pipes, as well as the lack of maintenance, which has been observed in some reported locations[38].

All the physicochemical parameters measured in the water samples were determined to be within the recommended ranges, except for residual chlorine. The absence of residual chlorine was observed in all sampled points in Bagua, except for one specific point at the outlet of the water treatment plant, where its presence was reported as per the recommendations of the health authority.

The absence or inadequate concentration of residual chlorine indicates a potential risk of microbial contamination in the drinking

**Table 3**
Statistical analysis among the sampled points.

| Sampling point | chlorine* (mg/L) | pH* | Turbidity UNT | TDS* (mg/L) | Conductivity* umhos/cm | T °C | Fe* (mg/L) | Total coliforms UFC/100 ml | *E. Coli* UFC/100 ml |
|---|---|---|---|---|---|---|---|---|---|
| Entrance of the plant | 0 ± 0 | 7.9 ± 0.6 | 3.3 ± 5.7 | 43.4 ± 25.4 | 64.9 ± 42 | 26 ± 0 | 2 ± 0 | 123 ± 92 | 5 ± 9 |
| Plant outlet | 0.2 ± 0.3 | 7.9 ± 0.7 | 0 ± 0 | 30.3 ± 29.6 | 309.3 ± 193.9 | 26.5 ± 0.5 | 2 ± 0 | 0 ± 0 | 0 ± 0 |
| Household near the plant | 0 ± 0.1 | 7.9 ± 1.4 | 0 ± 0 | 46 ± 38.4 | 207.7 ± 190.5 | 26.2 ± 0.3 | 2 ± 0 | 67 ± 115 | 21 ± 35 |
| Household 1 far from the plant | 0 ± 0 | 8 ± 1.3 | 0.3 ± 0.6 | 49.5 ± 42.3 | 128.1 ± 36.9 | 26.3 ± 0.3 | 2 ± 0 | 57 ± 45 | 17 ± 29 |
| Household 2 far from the plant | 0 ± 0 | 7.7 ± 1 | 1.9 ± 2.1 | 42.9 ± 35.6 | 184 ± 157.9 | 26 ± 0 | 2 ± 0 | 9 ± 8 | 1.3 ± 2 |
| Utcubamba River | 0 ± 0 | 8.9 ± 1.8 | 111.9 ± 104.6 | 154.8 ± 131.9 | 346 ± 18 | 26.5 ± 0.5 | 2 ± 0 | 169 ± 93 | 52 ± 82 |

TDS: Total dissolved solids. * Statistically significant differences.

**Table 4**
Pearson's correlation between the evaluated parameters.

| | chlorine | pH | Turbidity | TDS | CE | T° | T. coliforms |
|---|---|---|---|---|---|---|---|
| pH | −0.227 | | | | | | |
| Turbidity | −0.252 | 0.979 | | | | | |
| TDS | −0.360 | 0.980 | 0.990 | | | | |
| Conductivity | 0.482 | 0.606 | 0.625 | 0.556 | | | |
| T° | 0.514 | 0.606 | 0.514 | 0.470 | 0.776 | | |
| T. coliforms | −0.389 | 0.943 | 0.896 | 0.937 | 0.499 | 0.495 | |
| *E. Coli* | −0.389 | 0.943 | 0.896 | 0.937 | 0.499 | 0.495 | 1.000 |

**Table 5**
Phenotypic characterization of resistance of strains of *E. coli*.

| Source | CM | Antibiotic resistance phenotypes | | | | | |
|---|---|---|---|---|---|---|---|
| | | NA | GEN | C | AMC | NK | CIP |
| EP | MC | R | R | R | R | R | R |
| | | R | R | R | R | R | R |
| HCP | | R | R | R | R | I | R |
| H1FFP | | R | R | R | R | R | R |
| | | R | R | R | R | R | R |
| H2FFP | | R | R | R | R | I | R |
| RU | | R | R | R | R | I | R |
| EP | EMB | R | R | R | R | R | R |
| | | R | R | R | R | R | R |
| HCP | | R | R | R | R | I | R |
| H1FFP | | R | R | R | R | R | R |
| | | R | R | R | R | R | R |
| H2FFP | | R | R | R | R | S | R |
| RU | | R | R | R | R | R | R |

R, resistant. I, intermediate. S, susceptible.
RU, Utcubamba river. EP, entrance of the plant. HCP, household near the plant. H1FFP, Household 1 far from the plant. H2FFP, Household 2 far from the plant.
CM, culture medium. MC, MacConkey. EMB, eosin methylene blue
NA, nalidixic acid. GEN, gentamicin. C, Chloramphenicol. AMC, amoxicillin + clavulanic acid. NK, norfloxacin. CIP, ciprofloxacin.

water, as confirmed by the microbiological analysis, which revealed the presence of total coliforms and *E. coli* in most cases[20,28]. This indicates that the water intended for human consumption in Bagua is contaminated with fecal matter, posing a high microbiological risk, consistent with Peruvian health regulations and similar to findings reported in studies conducted in Ethiopia[39], as well as other studies by Morales et al. [40]; Gil et al. [25]; Miranda et al. [27]; Bigoni et al. [24]; Shamimuzzaman et al. [41]; Samo et al. [42]; Heitzinger et al. [26]; Duressa et al. [43] and Titilawo et al. [44]. These studies also emphasized the need for adequate water treatment for human consumption, particularly to address the contamination resulting from the infiltration of animal waste.

The microbiological conditions of the drinking water in Bagua not only contribute to the challenge of reducing high rates of childhood anemia at the regional and national levels, which has significant implications for child and maternal health[45], but also pose risks of waterborne epidemics [46] and outbreaks of foodborne diseases [47]. These conditions highlight the importance of considering the interconnectedness of humans, animals, and the environment, as emphasized in the One Health approach[1]. Therefore, it is crucial for the entity responsible for providing the drinking water service to ensure the presence of residual chlorine at levels recommended for final consumers [20,48,49], as reported in previous studies [22]. Chlorine has proven to be an effective disinfectant against bacteria, whether they are resistant or susceptible to antibiotics[50–55].

Previous studies have reported that microbial contamination is common in intermittent water service systems[56,57]. In the case of Bagua, where households receive water service for a shorter duration (4 h/day compared to 8 h/day in most big urban cities)[58], it is likely that the water quality is compromised [17]. This is due to many households storing water in concrete/Eternit tanks to cope with insufficient supply, which are inadequately maintained and result in the proliferation of rodent feces. These inadequate storage practices alter the chemistry and microbiology of the water[59]. Urgent actions are necessary for the relevant authorities to provide drinking water with the required microbiological quality, ensuring continuous and uninterrupted service for 24 h a day, as well as the implementation of water management systems[60].

The occurrence of multiresistant *E. coli* strains, particularly to lactamases, in water samples is a common observation[61], which aligns with our findings. Similar patterns of resistance have been reported for chloramphenicol in Georgia, USA[62], and for amoxicillin, gentamicin, and ciprofloxacin in Peru [63,64]. These resistance factors can facilitate the transfer of antimicrobial resistance genes to other bacteria[65].

Immediate actions are required to provide safe drinking water and prevent waterborne diseases. This requires the implementation of robust surveillance systems and awareness campaigns to mitigate the contamination of water intended for human consumption[44]. It is important to note that a significant proportion of communicable diseases are associated with an unsafe and inadequate water supply[41,43]. Lack of access to high-quality water and poor hygiene practices contribute to approximately half of all cases of malnutrition and anemia[66–69]. Therefore, addressing these issues is crucial not only for human health but also for the overall well-being of the population. Also, the lack of reagents, due to the COVID-19 pandemic, prevented the genotypic characterization of the isolates, which will be carried out this year.

It is essential to adopt a One Health approach, which considers the interconnectedness of animals, humans, and the environment, to prevent disease[1]. This approach should also be applied to the provision of drinking water, aiming to eliminate factors contributing to antimicrobial resistance. By doing so, we can ensure the delivery of safe and clean water to the population, promoting public health and well-being. Studies with longer monitoring periods and strategies for implementation should be proposed to help reduce the burden of antibiotic resistance, as well as its impact on human and planetary health[70].

In Bagua and the native communities there is a scarcity of studies that can guide the current governing bodies. However, there is evidence of a high prevalence of digestive diseases (>30 %), particularly in children over 10 years of age[71]. This study aims to provide a framework for corrective actions to be taken by local and national governments as well as NGOs. In this regard, this research stands as a pioneering in Amazonas with the goal of ameliorating the health issues faced by the population.

## 5. Conclusions

The findings of this study indicate that the majority of sampled points within the drinking water system did not meet the minimum recommended concentration of residual chlorine set by the national authority (0.5 mg/L). This lack of residual chlorine raises concerns regarding the microbiological safety of the water for human consumption, as evidenced by the presence of fecal contamination in the form of total coliforms and *E. coli*. Such contamination disrupts the interconnectedness of the man-animal-environment interface, which is a key aspect of the One Health approach, and poses a significant risk to public health. Since the presence of fecal contamination in the water serves as both an environmental disruptor and a potential source of disease transmission, it presents two main concerns: First, it disrupts the environmental balance; Second, there is a risk of disease transmission to both animals and humans. The latter can occur either through animal vectors or direct contact, ultimately affecting environmental, human, and animal health. This perspective aligns with the One Health approach.

Furthermore, it was observed that most of the isolated *E. coli* strains exhibited phenotypic resistance to multiple antibiotics, including nalidixic acid, gentamicin, amoxicillin plus clavulanic acid, norfloxacin, and ciprofloxacin. This highlights the presence of antimicrobial resistance in the studied water samples, which has implications for the effectiveness of treatment and control strategies.

In summary, the insufficient levels of residual chlorine and the presence of antibiotic-resistant *E. coli* strains in the drinking water system raise significant concerns regarding the safety and quality of the water supply. It is crucial that prompt actions are taken by the appropriate authorities to address these issues and guarantee the delivery of safe and uncontaminated drinking water, prioritizing the protection of public health.

### Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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### CRediT authorship contribution statement

**Pompeyo Ferro:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft. **Eli Morales:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Euclides Ticona:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Polan Ferró-Gonzales:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Anderson Oblitas:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Ana Lucia Ferró-Gonzáles:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft.

## 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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