UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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Monitoring the seasonal dynamics of the microbial load on the San Pedro River

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RESUMEN

La contaminación de los recursos hídricos afecta a todo el mundo representando un problema en la salud mundial, esta contaminación pude ser causada por numerosos agentes microbianos *como Escherichia coli* y sus patotipos (EHEC, EAEC, EIEC, EPEC)*, Giardia* spp*., Cryptosporidium* spp*., Helicobacter pylori, Campylobacter jejuni, C. upsaliensis, C. coli, Mycobacterium tuberculosis,*y *M. leprae*. El objetivo de este estudio fue monitorear la dinámica estacional (invierno, transición y verano) en la carga microbiana en tres puntos de muestreo (un punto de control antes de Quito y dos puntos contaminados en Quito) a lo largo del rio San Pedro, por medio de análisis microbiológicos y moleculares. El cultivo de coliformes y *E. coli* se realizó mediante el método de la gota en medio cultivo Chromocult agar, mientras que la identificación y validación de los microorganismos se realizó mediante Reacción en cadena de Polimerasa (PCR) y secuenciamiento Sanger. Los resultados obtenidos demostraron que la concentración de coliformes y *E. coli* en los tres puntos de muestreo y épocas de colecta excedieron los límites establecidos por la legislación Americana, Europea y Brasileña. Adicionalmente, se identificaron microorganismos tales como *Giardia* spp*., Criptosporidium* spp*., Mycobacterium tuberculosis, Mycobacterium leprae y* patotipos de *E. coli* (EHEC, EAEC y EPEC); siendo los más persistentes *Giardia* spp*., Criptosporidium* spp*., Mycobacterium tuberculosis* y EHEC. Este estudio confirma la prevalencia de diferentes microorganismos a lo largo del rio San Pedro que pueden ser perjudiciales para la salud humana. Se recomienda realizar estudios futuros donde se identifiquen las fuentes puntuales de contaminación del rio San Pedro.

Palabras clave: recursos hídricos**,** coliformes totales, Rio San Pedro, *Giardia* spp*., Criptosporidium* spp*., Mycobacterium tuberculosis, Mycobacterium leprae,* y patotipos de *E. coli.*

ABSTRACT

The contamination of natural water resources is affecting worldwide, representing a global health problem. This contamination can be caused by numerous microbial agents such as *Escherichia coli* and their pathotypes (EHEC, EAEC, EIEC, EPEC)*, Giardia* spp.*, Cryptosporidium* spp.*, Helicobacter pylori, Campylobacter jejuni, C. upsaliensis, C. coli, Mycobacterium tuberculosis,* and *M. leprae*. The main goal of the present study was to monitor the seasonal dynamics (rainy, transitional, and dry) in the microbial load at three sampling points (one control point before Quito and two contaminated points within Quito) along the San Pedro River, through classical microbiological and molecular analyses. The cultivation of total coliforms and *E. coli* was carried out by the drop counting method in chromogenic culture media while the identification and validation of the remaining microorganisms was carried out by Reaction Polymerase chain (PCR) and Sanger sequencing, respectively. The results demonstrated high levels of total coliforms and *E. coli* at the three sampling points during the three collection seasons exceeding the limits established by the American, European, and Brazilian legislations. In addition, numerous pathogens, such as *Giardia* spp., *Cryptosporidium* spp.*, M. tuberculosis, M. leprae,* and three pathotypes of *E. coli* (EHEC, EAEC, and EPEC), were identified. The most persistent microorganisms were *Giardia* spp.*, Cryptosporidium* spp.*, Mycobacterium tuberculosis,* and EHEC. This study confirms the prevalence of different microorganisms along the San Pedro River that can be harmful to human health. It is recommended to carry out future studies to determine the punctual source of contamination in the San Pedro River.

Keywords: water resources, total coliforms, San Pedro River, *Giardia* spp.*, Cryptosporidium* spp.*, Mycobacterium tuberculosis, Mycobacterium leprae, E. coli* pathotypes.

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1. INTRODUCTION

1.1. Global context

Nowadays, water pollution is becoming one of the biggest problems worldwide, causing approximately 14000 people's deaths by day around the world and affecting developed countries and developing countries. The main factors that directly affect water quality are vegetation, soil type, climate change, the incidence of precipitation, flow conditions, and human activities. However, human activities, such as agriculture, industrial activities, and municipalities, are the main activities that cause massive pollution of water (F. Chaudhry & M. Malik, 2017).

Water is a critical source for any living organism, human activities, and even more for food production. It is known that around 40% of the food supply requires water irrigation and most of the industrial process depends on water. Nonetheless, water quality is decreasing because of urbanization, industrial activity, and population growth (Halder & Islam, 2015). The continuous discharge of untreated water contributes to chemical compound accumulation and microbial proliferation such as pathogen, commensal, or opportunistic microorganisms (Dobrowsky, De Kwaadsteniet, et al., 2014).

1.2. Ecuadorian Context

Contaminated natural freshwater resources are the third greatest source of transmission of infectious diseases (Muniesa et al., 2006). In Ecuador, only 83% of the population has access to potable water but may not always be drinkable quality water. In rural regions, the situation is even worse, where only 53.9% of the population has potable water (National Institute of Statistics and Censuses, 2017; Vinueza et al., 2021). Part of the population uses river water for various domestic activities, including laundry, personal hygiene, and, on occasion, food preparation (El Comercio, 2019).

Natural water sources such as rivers are the most affected by anthropogenic activities such as population growth, agriculture, industrial activity, and sewage. These main activities altered the water quality increasing the concentration of microorganisms impacting human health as a result of the lack of wastewater treatment plants in Ecuador.

1.3. Pichichincha Province

San Pedro River one of the Ecuadorian rivers located in Pichincha province is also affected by water pollution. Pichincha province is one of the twenty-four provinces and it is located in the capital of Ecuador (Quito). It was estimated that in 2020 Pichincha harboured approximately 3.228.233 habitants (INEC, 2013). It is well-known that most industrial and domestic waste produced in Pichincha province ends in four main rivers, more exactly Machangara, Monjas, Guallabamba, and San Pedro Rivers (Gomez et al ., 2014). In addition, only 3.38% of wastewater was treated in the metropolitan district of Quito until 2020 (EPMAS, 2020)

The Metropolitan Public Company for Drinking Water and Sanitation has implemented a program for the decontamination of the rivers of the Metropolitan District of Quito (EPMAS, 2020), contributing to the improvement of the life quality of the population. The intervention area is in the upper basin of the Guayllabamba River, where the Metropolitan District of Quito constructed 32 urban parishes and 33 rural parishes. However, there are still no wastewater sanitation plans for the San Pedro River.

1.4. San Pedro River

San Pedro River is born in the foothills of the Ilinizas, crossing the areas of Machachi, Sangolqui, and Cumbaya, and finally converging into the Machangara River to create the Guayllabamba River (El Comercio, 2021). The river crosses rural and urban areas, and, as the river moves ahead of the cities, water pollution increases dramatically. San Pedro River is considered one of the four main rivers of Quito being one of the most

contaminated in the metropolitan district of Quito due to domestic and industrial wastewater. Nowadays, only 3% of wastewater is treated, which is a problem as the San Pedro River exceeds the limits of contaminants such as fecal matter, bacteria, metals, fats, oils, and chemicals (Machado, 2023).

1.5. Objectives

1.5.1. General objective

Evaluate the influence of seasonal dynamics (rainy, transitional, and dry seasons) in the microbial load in three sampling points at San Pedro River.

1.5.2. Specific objectives

- Quantify the microbial load of *Escherichia coli* and total coliforms in the three sampling points at San Pedro River during three seasons (rainy, transitional, and dry).
- Identified the presence of relevant pathogens in the three sampling points at San Pedro River during three seasons (rainy, transitional, and dry) by polymerase chain reaction (PCR) and Sanger sequencing of the following microorganisms: *Giardia* spp.*, Cryptosporidium* spp., *Helicobacter pylori, Campylobacter jejuni, C.upsaliensis, C. coli*, enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), *Mycobacterium tuberculosis,* and *M. leprae.*
- Establish the persistent or punctual contaminations of all evaluated microorganisms on the different sampling points during the seasons.

1.6. Justification

In the last two years, studies have shown that the water quality of the Pichincha rivers is decreasing due to population growth and industrialization (Borja-Serrano et al., 2020; Vinueza et al., 2021) These studies were limited by the number of sampling points

as well as the different seasons of the year. For these reasons, it was important to perform a longitudinal study of the San Pedro River in three different seasons (rainy, transitional, and dry) to confirm the prevalence of pathogens that could represent a risk to human health. According to Ecuadorian legislation, the evaluation of water quality in rivers must be performed by specific analysis such as chemicals and microbiological parameters.

Microbiological parameters involve the identification of pathogens that can be harmful to human health, as well as *E. coli* and total coliform count. This study seeks to raise awareness in the competent authorities as well as in the scientific community. In addition, the present study aims to demonstrate that poor wastewater management influences the prevalence of pathogenic microorganisms along the San Pedro River at different times of the year. It is important to mention that the San Pedro River is a water source used for numerous economic activities such as agriculture and cattle raising.

2. METHODS

This study was conducted within a research group and shares methods with the project titled “Monitoring Microbial Load in the Seasonal Dynamics of the San Pedro River” performed by Alison Cabrera.

2.1.Sample site and collection

Water samples were collected from three sampling points in the San Pedro River located in the province of Pichincha, Ecuador (see Figure 1). Samples from points 1 and 2 were taken from urban sites with high proximity to population and several contamination effluents while point 0 located outside of Quito was used as control. Samples were collected between November 2022 and July 2023 on two different dates during each of the three seasons, more exactly, rainy, transitional, and dry seasons (Appendix 1). Samples were collected by duplicated in glass jars of 800 ml capacity each, previously sterilized by autoclaving at 121ºC. Samples were collected by immersing the bottles in superficial water to a depth of 0.30 m and opening the lid once they were completely submerged. To preserve the samples, they were transported in a cooler with ice packs at 4ºC to the Microbiology Institute at the Universidad San Francisco de Quito (IM-USFQ).

2.2.Sample preparation for microbiological analysis

All samples destinated for microbial analysis were filtered using a vacuum pump under aseptic conditions (Chemical Duty Pump, Millipore, Merck, Burlington, MA, USA) through a 0.45 µm nitrocellulose membrane (Millipore, Merck, Burlington, MA, USA). The subsequent procedures were adapted from previous studies (Borja-Serrano et al., 2020; Vinueza et al., 2021a). Once at least 100 mL of water was filtered, the membrane was removed from the equipment with sterile tweezers and placed in a Falcon tube with 20 ml of sterile distilled water. For resuspending the particles and microorganisms, the Falcon tube was vortexed for 10-15 minutes at maximum speed. The membrane was then removed, and the Falcon tubes were

centrifuged at 7000 rpm for 15 minutes. The supernatant was discarded, and the pellet was suspended in 2 mL of sterile distilled water. Each sample was divided into 3 aliquots of 500 µL.

2.3.Cultivation of *Escherichia coli* and coliforms

For the total count of *Escherichia coli* and coliforms, serial dilutions from one of the aliquots were cultivated in Chromocult Agar culture medium (Merck; Biolab, Wadeville, Gauteng, South Africa) by the 3-drop culture technique according to previously optimized protocols (Borja-Serrano et al., 2020; Herigstad et al., 2001; Naghili et al., 2013; Vinueza et al., 2021a). Briefly, a volume of 10 µL of each sample and their serial 10-fold dilutions (from 10^{-1} until 10^{-4}) was deposited horizontally on the upper edge of the media petri dish in triplicate (so-called 3-drop culture). The petri dish was then turned upside down, allowing each sample to drop down without touching the bottom edge or joining each other. Finally, the petri dishes were incubated at 37°C for 24-48 h.

2.4.DNA extraction procedure

DNA extraction from the samples was performed following the manual of the commercial PowerSoil DNA Pro Kit (Qiagen, Venlo, Netherlands) following the manufacturer's instructions.

2.5.Molecular identification of pathogens

DNA samples were applied in polymerase chain reaction (PCR) assays using previously optimized primers for the identification of *E. coli* pathotypes, *Giardia* and *Cryptosporidium* spp., *Helicobacter pylori*, *Campylobacter* species (more exactly, *C. jejuni, C. coli,* and *C. upsaliensis*), *Mycobacterium leprae*, and *Mycobacterium tuberculosis*. Briefly, for each pathogen, a PCR Master mix was realized consisting of a final volume of 15.00 µL with 3.00 µL of 5X Green GoTaq Flexi buffer (1X final concentration; Promega, Madison, USA), 0.90-

1.80 μL of $MgCl_2$ (1.50-3.00 mM final concentration; Promega, Madison, USA), 0.30-0.60 μL of dNTP Mix (0.20-0.40 mM final concentration; Promega, Madison, USA), 0.30-0.75 μL of each PCR primer (0.20-0.50 μM final concentration; 0.10-0.15 μL of GoTaq Flexi DNA polymerase (0.50 U final concentration; Promega, Madison, USA), 1.00 -2.00 μL of template DNA, and the remaining volume of DNA-free water. In the case of nested PCR assays, the second PCR Master mix contained the same volumes as the first PCR Master mix except for 0.08 μL of GoTaq Flexi DNA polymerase. The PCR analysis was performed in a thermocycler (Bio-Rad Laboratories Inc.) using the primers and PCR conditions shown in Table 1. All samples were randomly performed in triplicate with different negative and positive controls that were provided by the Microbiology Institute at Universidad San Francisco de Quito (Borja-Serrano et al., 2020; Vinueza et al., 2021).

The PCR products were visualized using electrophoresis with 1.5% agarose gel and staining with SYBR Safe, except for *Mycobacterium tuberculosis* using electrophoresis with 2.0% agarose gel.

3. RESULTS

3.1. *Escherichia coli* and total coliforms counts San Pedro River

The count of *E. coli* and total coliforms were analyzed on the San Pedro River through three different collection points during three seasons (see Figure 2 and Table 2). As shown in Table 2, the average amount of *Escherichia coli* and total coliforms in all collection points and seasons exceed the limits allowed by the European Union guidelines (European Union Law, 2006), United States of America standard values of the Recreational Water Quality Criteria (EPA, 2012) and Brazilian guidelines for bathing waters under Resolution CONAMA nº 274 of 29 November 2000 (Ambiente, 2001).

As shown in Figure 2, the levels of *E. coli* and total coliforms demonstrated the same trend of results, where SP1 and SP2 points showed superior microbial levels when compared to the control SP0 point. However, the microbial loads of the control SP0 point were superior than initially expected during all seasons. During the rainy season, the sampling point with the major amount of *E. coli* was SP2 with 1.52×10^7 CFU per 100mL while the highest level of total coliforms was observed in the SP1 point with 1.72×10^9 CFU per 100mL.

Regarding the transitional season, the SP1 point evidenced the highest levels of *E. coli* and total coliforms with 9.02×10^7 and 2.32×10^8 CFU per 100mL, respectively. On the other hand, the SP2 point had the highest microbial load of *E. coli* with 4.53×10^7 CFU per 100 mL and total coliforms with 2.40×10^8 CFU per 100 mL in the dry season. It is important to note that the highest microbial load was obtained by SP1 point in the rainy season showing an increment of log CFU of 2 when compared to *E.coli* and a superior amount of 1 log CFU at least with the remaining microbial loads from other seasons and points (see Figure 2).

3.2. Molecular identification of pathogens on superficial waters of San Pedro River

All samples from the San Pedro River were further characterized by the molecular identification of numerous pathogens on superficial waters through polymerase chain reaction (PCR) assays (see Table 3). Both *Cryptosporidium* and *Giardia* parasites were found in all three seasons, where the first parasite was identified at SP1 and SP2 points of the rainy season but only at SP1 and SP2 points of the dry and transitional seasons, respectively; while *Giardia* spp. was detected at SP1 and SP2 points in both rainy and transition seasons but only identified at SP1 point in dry season. Moreover, three-quarters of *E. coli* pathotypes were punctually detected in San Pedro River over time, more exactly, EAEC at SP2 point in the rainy season, EHEC at SP2 point in both rainy and dry seasons, and EPEC at SP1 point in the transitional season.

Mycobacterium tuberculosis was the most prevalent pathogen in the San Pedro River, being identified at all collection points in the rainy season and SP1 point in both transitional and dry seasons. Finally, *Mycobacterium leprae* was detected at the SP2 point in both transitional and dry seasons. Nevertheless, it is important to note that *Helicobacter pylori*, *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter upsaliensis*, and *E. coli* pathotype EIEC were not identified by PCR assays in any collection point of the present study during the three seasons.

4. DISCUSSION

4.1. Fecal coliform bacteria in San Pedro River

In the present study, *Escherichia coli* and total coliform levels were above the permitted limits in the three collection points (SP0, SP1, and SP2) during three seasons (rainy, transitional, and dry). More exactly, the total microbial counts exceed the limits established by the European Union guidelines (European Union Law, 2006), United States Protection Agency (Recreational Water Quality Criteria, 2012) as well as Ecuadorian legislation (TULSMA, 2015). Our results are in agreement with previous studies performed in countries from Latin America, such as Chile (Fierro et al., 2021), Mexico (Pérez Castresana et al., 2018), and Brazil (Giowanella et al., 2015), as shown in (Table 4). However, the highest microbial load was observed in SP1 during the rainy season with a total coliforms average of 1.72×10^9 CFU per 100 mL, while the average lowest amount of coliforms was 1.67×10^5 CFU per 100 mL in SP0 in the same season.

The highest levels of total coliforms exceeded the results reported in Mexico (1.86×10^8 CFU per 100 mL) (Pérez Castresana et al., 2018), Chile (3.94×10^8 CFU per 100 mL)(Fierro et al., 2021). On the other hand, our study reveals that the highest *E.coli* count was determined in SP1 during the transitional season (9.00×10^7 CFU per 100 mL), and the lowest *E.coli* count was also observed in SP0 during the rainy season (1.00×10^5 CFU per 100 mL). Previous studies showed similar results as the case of Białka River in Poland (8.00×10^8 CFU per 100 mL) in comparison with our highest *E. coli* value in SP1, while Egypt (1.72×10^5 CFU per 100 mL) reported analogous results when compared with our lowest amount of *E.coli* in the San Pedro River. According to Pérez Castresana et al. the main reason for high concentrations of fecal bacteria is usually due to anthropogenic pollution (mainly from sewer discharges) and farming (Pérez Castresana et al., 2018), justifying the highest amount of fecal bacteria found in SP1 point at the San Pedro River as it crosses a huge urban zone and agricultural activity is

nearby. Finally, the highest concentration of fecal bacteria (*E. coli* and total coliforms) in SP1 during the rainy season could be attributed to the precipitation increasing surface runoffs that carry microbial contaminants to the water load in the river, as previously described (Ling et al., 2017).

4.2. Prevalences of microbial primary pathogens

There is a wide range of primary pathogens that can be found in wastewater, which are not usually detected in the standard surveillance of natural freshwater resources or the lack of wastewater treatment. The most common pathogens found in river loads are *E. coli* pathotypes, as well as *Mycobacterium*, *Cryptosporidium*, and *Giardia* genera. In the present work, some of these pathogens were identified in the three seasons, more exactly, *Giardia* and *Cryptosporidium* spp. except for the control point SP0. The main cause of *Giardia* and *Cryptosporidium* spp. transmission is fecal contamination derived from infected hosts (humans and/or animals) releasing a huge number of transmissive oocysts on wastewater discharges (Castro-Hermida et al., 2009). However, the continuous prevalence of *Giardia* spp. in SP1 suggested endemic infection among the human population and animals (cattle or even pets). The viability of *Giardia* spp. oocytes increase with rainfall due to the oocyte morphology allowing its survival during harsh weather conditions (Pinto-Duarte et al., 2022). Likewise, *Cryptosporidium* oocytes can survive at low temperatures (4°C) for at least a year (Castro-Hermida et al., 2009). According to a study realized in Ecuador (San Fernando, Azuay province), it was determined a prevalence of 93.3% of *Cryptosporidium* spp. and 76.7% of *Giardia* spp. in calves and, additionally, the local water collection systems evidenced *Cryptosporidium* and *Giardia* concentrations of 5 and 10 oocytes per 100ml (Palacios, 2017), respectively.

Furthermore, the present study identified *Mycobacterium* species in water samples during the three seasons, specifically *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

M. tuberculosis was identified in all collection points, including the control point. This pathogen could be transmitted in a direct way (air transmission) or an indirect way (fecal-oral transmission) with wastewater discharge being the most common transmission. In fact, it is reported that approximately 20% of the patients with *M. tuberculosis* could present extra-pulmonary manifestations shedding in fecal and urine that finally ends in river loads (Mtetwa et al., 2022). Additionally, *M. tuberculosis* is a big problem in livestock activity as it is reported in India that 84.3% of tested cattle are positive for *M. tuberculosis* postulating cows as the main source of transmission (Sweetline Anne. et al., 2017). So, it is plausible that Ecuadorian livestock represent a source of transmission, and it could explain the positive samples obtained in the SP0 control point being near an area with livestock activity, meanwhile, positive samples in the SP2 point could be associated with the wastewater discharge from a near hospital suggesting a high probability of having wastewater contamination with fecal shedding from infected patients. *M. leprae* was also detected in SP2 point during the transitional and rainy season, so it is also probable that contaminated fluids of patients ended up in the San Pedro River through wastewater discharges.

M. leprae is a bacteria responsible for leprosy and its transmission occurs while being in contact with a patient infected or fomites (such as fluids) (Holanda et al., 2017). However, some cases had been reported where patients did not report being in contact with an infected human suggesting transmission via environmental sources due to the ability of *M. leprae to* survive under favorable environmental conditions and being already found in water and soil (Holanda et al., 2017). A previous study analyzed 30 samples of water from five cities of Ceará (Brazil) where 23 (76.7%) of the samples were positive for *M. leprae*. The authors postulated that fluid secretions from patients with multibacillary (MB) leprosy were the source of transmission via wastewater discharges and *M. leprae* were able to survive for 45 days until eight months inside amoebas (Arraes et al., 2017). Finally, three-fourths of *E. coli* pathotypes

were identified in the San Pedro River, more exactly, enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC). The EHEC was the most prevalent pathotype in our group set being detected in SP2 during rainy and dry seasons, followed by EPEC and EAEC pathotypes only identified in SP1 during transitional and in SP2 during rainy season, respectively. Our results agreed with the literature being EHEC the most prevalent pathotype found among environmental samples (Stanford et al., 2016) However, EHEC was only detected in the SP2 point which is the most contaminated collection point of the present study and, as previously referred, nearby a hospital, where several patients are frequently treated of gastrointestinal (GI)-related infections. The EPEC is also a frequent *E. coli* pathotype found in natural freshwater resources. In Mexico, Edith Chávez-Bravo et al. (2020) identified an EPEC prevalence of 85% in Atoyac River located in the City of Puebla. Moreover, the present study identified EPEC presence SP1 point, which surroundings are principal urban zones as well as farming, suggesting a different contamination source from EHEC. Finally, EAEC was also detected at SP2 point only during the rainy season, suggesting punctual and/or fluctuating contamination of the San Pedro River. However, a previous study conducted by Vinueza et al. (2021) also reported the presence of EAEC in the Machangara River, which is an *E. coli* pathotype frequently found in developing countries such as Ecuador. Our results demonstrated a serious public health concern about the persistence and punctual microbial contaminations.

5. CONCLUSIONS

In summary, the microbial analysis revealed the influence of the season in the total count of *E. coli* and coliforms in the San Pedro River, as well as the sampling point, where the highest contamination of total coliforms levels was obtained in the rainy season at the sampling point SP1. Regarding the transitional season, the SP1 point evidenced the highest levels of *E. coli*. Additionally, it is important to mention that the three sampling points exceeded the limits allowed by the European Union, the United States, the Brazilian, and the Ecuadorian legislations. The molecular analysis showed the presence of pathogens that could cause serious problems in the Public Health system.

The most prevalent pathogens found in all seasons were *Giardia* spp. and *Cryptosporidium* spp. Meanwhile, three *E. coli* pathotypes were identified, more exactly EAEC at SP2 point in the rainy season, EHEC at SP2 point in both rainy and dry seasons, and finally EPEC at SP1 point in the transitional season. Moreover, *Mycobacterium tuberculosis* was the most prevalent pathogen found in all seasons and sampling points, but *Mycobacterium leprae* was only detected at the SP2 point in transitional and dry seasons. The prevalence of these opportunistic and primary pathogens could be associated with several factors such as sewage discharges, agricultural activity, temperature, anthropogenic activity, and lack of water treatment plants among others. Further studies should identify the main contamination sources of the sampling points in order to take action in the implementation of water treatment plants on critical contamination sources.

TABLES

Table 1. Primers and PCR cycling parameters for the detection of various potential bacterial pathogens.

Organism	Primer name	Primer sequence (5′–3′)	PCR cycling parameters	Gene (size [bp])	References
Single PCR assays					
Universal	Forward: fDD2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG	3 min at 94°C; 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1.5 min	*16S* rRNA (1600)	(Dobrowsky, De Kwaadstenie t, et al., 2014; Dobrowsky, van Deventer, et al., 2014)
	Reverse: rPP2	CCAAGCTTCTAGACGGITACCTTGTTACGACTT			
Helicobacter pylori	Forward:	GCGGGATAGTCAGTCAGGTG	2 min at 94°C; 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min	*16S* rRNA (706)	(Valenzuela & Machado, 2016)
	Reverse:	AAGATTGGCTCCACTTCGCA			
Campylobacter spp.	Forward: IpxA*C. coli*	AGA CAA ATA AGA GAG AAT CAG	2 min at 94°C; 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 45 s	lpxA gene (391)	(Klena et al., 2004)
	Forward: IpxAC. *jejuni*	ACA ACT TGG TGA CGA TGT TGT A		lpxA gene (331)	
	Forward: pxAC. *upsaliensis*	AAG TCG TAT ATT TTC YTA CGC TTG TGT G		lpxA gene (206)	
	Reverse: lpxARKK2m	CAA TCA TGD GCD ATA TGA SAA TAH GCC AT			
EAEC	Forward: AggRKs1	GTATACACAAAAGAAGGAAGC	2 min at 95°C; 35 cycles of 95°C for 1 min, 54°C for 1 min, 72°C for 1 min	*aggR* (254)	(Ramírez Castillo et al., 2013)
	Reverse: AggRkas2	ACAGAATCGTCAGCATCAGC			
EHEC	Forward: VTcomU	GAGCGAAATAATTTATATGTG		*stx* (518)	
	Reverse: Vtcomd	TGATGATGGCAATTCAGTAT			
EPEC	Forward: SK1	CCCGAATTCGGCACAAGCATAAGC		*eae* (881)	
	Reverse: SK2	CCCGGATCCGTCTCGCCAGTATTCG			
EIEC	Forward: IpaIII	GTTCCTTGACCGCCTTTCCGATACCGTC		*ipaH* (619)	
	Reverse: IpaIV	GCCGGTCAGCCACCCTCTGAGAGTAC			
	Forward: S13	CTCCACCTGGACCGGCGAT		*pra* (531)	

Mycobacterium leprae	Reverse: S62	GACTAGCCTGCCAAGTCG	5 min at 95°C; 30 cycles of 94°C for 2 min, 58°C for 1 min, 72°C for 2min		(Arunagiri et al., 2017)
Nested PCR assays					
Mycobacterium tuberculosis	Forward: Mpb1	TCCGCTGCCAGTCGTCTTCC	5 min at 95°C; 30 cycles of 95°C for 30 s, 54°C for 30 s, 72°C for 30 s	*MPB64* (240)	(Madhavan et al., 2000)
	Reverse: Mpb2	GTCCTCGCGAGTCTAGGCCA			
	Forward: Mpb3	ATTGTGCAAGGTGAACTGAG	5 min at 95°C; 35cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s	*MPB64* (200)	
	Reverse: Mpb4	AGCATCGAGTCGATCGCGGA			
Criptosporidium spp.	Forward: Cry 15	GTAGATAATGGAAGAGATTGTG	10 min at 95°C; 45 cycles of 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 50 seconds	*COWP* (550)	(Salza, 2014; Yu et al., 2009)
	Reverse: Cry 9	GGACTGAAATACAGGCATTATCTT			
	Forward: Cowpnest F	TGTGTTCAATCAGACACAGC	10 min at 95°C; 32 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 50 s.	*COWP* (311)	
	Reverse: Cowpnest R	TCTGTATATCCTGGTGGG			
Giardia spp.	Forward: AL3543	AAATTATGCCTGCTCGTCG	5 min at 94°C; 35 cycles of 94°C for 45s, 50°C for 45 s, 72°C for 1 min	*TPI* (605)	(Salza, 2014)
	Reverse: AL3546	CAAACCTTTTCCGCAAACC			
	Forward: AL3544	CCCTTCATCGGTGGTAACTT	5 min at 94°C; 35 cycles of 94°C for 45s, 53°C for 30 s, 72°C for 1 min	*TPI* (530)	
	Reverse: AL3545	GTGGCCACCACTCCCGTGCC			

Table 2. The average amount of *Escherichia coli* and total coliforms in the San Pedro River across the three seasons and water classification applied to bathing-water standards by USA, European, and Brazilian guidelines.

Season	Sample code	GPS Coordinates	*Escherichia coli* (CFU per 100 mL ± SD)	Total coliforms (CFU per 100 mL ± SD)	USA guidelines (*E. coli*: ≤100-126 CFU per 100 mL[a]; No values are given for total coliforms)	European guidelines (*E. coli*: ≤500 CFU per 100 mL[b]; No values are given for total coliforms)	Brazilian guidelines (*E. coli*: ≤800 CFU per 100 mL; Faecal (thermotolerant) coliforms: ≤1000 CFU per 100 mL[c]; No values are given for total coliforms:)
Rainy	SP0	0°35'43.4"S 78°37'26.1"W	1.00E+05 (0.00E+00)	1.67E+05 (9.40E+04)	Not acceptable	Not acceptable	Not acceptable
	SP1	0°19'48"S 78°27'35"W	1.20E+07 (1.70E+07)	1.72E+09 (2.37E+09)	Not acceptable	Not acceptable	Not acceptable
	SP2	0°12'29"S 78°25'12"W	1.52E+07 (1.15E+07)	6.85E+07 (7.28E+07)	Not acceptable	Not acceptable	Not acceptable
Transitional	SP0	0°35'43.4"S 78°37'26.1"W	2.22E+06 (2.99E+06)	4.05E+06 (5.58E+06)	Not acceptable	Not acceptable	Not acceptable
	SP1	0°19'48"S 78°27'35"W	9.00E+07 (8.04E+07)	2.32E+08 (7.28E+07)	Not acceptable	Not acceptable	Not acceptable
	SP2	0°12'29"S 78°25'12"W	4.85E+07 (5.40E+07)	1.20E+08 (2.05E+07)	Not acceptable	Not acceptable	Not acceptable
Dry	SP0	0°35'43.4"S 78°37'26.1"W	1.78E+05 (9.19E+04)	2.33E+05 (1.18E+05)	Not acceptable	Not acceptable	Not acceptable
	SP1	0°19'48"S 78°27'35"W	1.23E+07 (4.22E+06)	3.15E+07 (3.96E+06)	Not acceptable	Not acceptable	Not acceptable
	SP2	0°12'29"S 78°25'12"W	4.53E+07 (5.37E+07)	2.40E+08 (2.96E+08)	Not acceptable	Not acceptable	Not acceptable

Legend: SD – Standard deviation values; [a] Recreational Water Quality Criteria U.S. EPA, 1976. [b] Council of the European Union (2006). "Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC"). [c] Brazilian guidelines for bathing waters established by Resolution CONAMA n° 274 of 29 November 2000.

Table 3. Seasonal detection of pathogenic microorganisms by conventional PCR in the three sampling points on the San Pedro River.

		Season								
Microorganism	***gene***	**Rainy**			**Transitional**			**Dry**		
		SP0	*SP1*	*SP2*	*SP0*	*SP1*	*SP2*	*SP0*	*SP1*	*SP2*
Universal	*16srRNA*	X	X	X	X	X	X	X	X	X
Cryptsporidum spp.	*COWP*	-	X	X	-	-	X	-	X	-
Giardia spp.	*TPI*	-	X	X	-	X	X	-	X	-
EAEC	*aggR*	-	-	X	-	-	-	-	-	-
EHEC	*stx*	-	-	X	-	-	-	-	-	X
EPEC	*ege*	-	-	-	-	X	-	-	-	-
EIEC	*ipaH*	-	-	-	-	-	-	-	-	-
Helicobacter pylori	*16srRNA*	-	-	-	-	-	-	-	-	-
Campylobacter jejuni	IpxAC	-	-	-	-	-	-	-	-	-
Campylobacter coli	IpxAC	-	-	-	-	-	-	-	-	-
Campylobacter upsaliensis	IpxAC	-	-	-	-	-	-	-	-	-
M. tuberculosis	Mpb64	X	X	X	-	X	-	-	X	-
M. leprae	pra	-	-	X	-	-	X	-	-	-

Legend: sampling collection points- SP0- El Chaupi; SP1- San Pedro de Taboada; SP2- Cumbaya. The presence of microorganisms is represented with (X) and the absence with (-).

Table 4. Comparison of the *E. coli* and coliforms counting and the presence of *E. coli* pathotypes and parasites in other urban areas worldwide.

N°	Country	Study Group (n)	Parasites		Counting		*E. coli* pathotypes				References
			Giardia spp.	*Cryptosporidium* spp.	*E. coli* (CFU/mL)	Total coliforms (CFU/mL)	EHEC	EPEC	EIEC	EAEC	
1	Ecuador	2	X	X	0 - 5.83×10^8	5.00×10^3 - 1.13×10^9	X	X	X	X	This study
2	Colombia*	2	-	X	4.30×10^1 - 9.30×10^1	3.00 - 1.10×10^3	NA	NA	NA	NA	(Alarcón et al., 2005; Cely-Ramírez et al., 2021)
3	Brazil*	2	X	X	2.00 - 2.75	2.70×10^1 - 2.74×10^2	NA	X	NA	X	(Freitas et al., 2015; Manoel et al., 2020), (Giowanella et al., 2015)
4	Chile*	1	NA	NA	1.61×10^1 - 3.02×10^3	6.54×10^1 - 3.94×10^5	NA	NA	NA	NA	(Fierro et al., 2021)
5	Mexico*	1	NA	NA	1.86×10^5 - 2.60×10^5	1.86×10^5 - 3.20×10^5	NA	X	NA	X	(Pérez Castresana et al., 2018), (Ramírez Castillo, Avelar González, Garneau, Márquez Díaz, et al., 2013)
6	USA	1	X	X	1.86×10^3 - 3.20×10^3	1.86×10^3 - 2.60×10^3	NA	NA	NA	NA	(Dreelin et al., 2014), (Staley et al., 2014)
7	Canada	1	X	X	1.00 - 1.25×10^4	1.00 - 3.25×10^4	NA	N	NA	NA	(Payment et al., 2000)

8	Poland*	1	X	X	NR - 8.00×10^4	1.20×10^1 - 9.50×10^4	NA	NA	NA	NA	(Bojarczuk et al., 2018)
9	Croatia*	1	NA	NA	1.00 - 2.57×10^6	1.50 - 1.73×10^7	NA	NA	NA	NA	(Filipović Marijić et al., 2018)
10	Italy*	7	X	X	3.00×10^{-2} – 4.10×10^2	0 – 1.30×10^2	X	NA	NA	X	(Briancesco and Bonadonna, 2005), (Ferronato et al., 2013),
11	India*	1	NA	NA	2.00×10^2 - 5.80×10^4	5.00×10^2 - 1.20×10^5	NA	NA	NA	NA	(Mariya et al., 2019)
12	Indonesia	1	NA	NA	1.80 - 7.90×10^5	1.80 - 3.50×10^5	NA	NA	NA	NA	(Puspitasari and Hadi, 2022)
13	Malaysia	3	NA	X	NR - 6.10×10^3	NR - 7.20×10^3	NA	NA	NA	X	(Bilung et al., 2017; Wong et al., 2022), (Bong et al., 2022)
14	Nigeria*	3	X	X	4.50×10^1 - 3.45×10^2	2.90×10^2 - 2.40×10^3	NA	NA	NA	X	(Squire and Ryan, 2017; Titilawo et al., 2020), (Kabiru et al., 2015)
15	Ghana*	1	NA	NA	2.34×10^6 - 9.29×10^6	1.57×10^{10} - 9.10×10^{11}	NA	NA	NA	NA	(Apau et al., 2022)

16	Egypt*	2	X	X	1.72×10^2 - 5.87×10^2	2.70×10^2 - 9.81×10^2	NA	NA	NA	NA	(Squire and Ryan, 2017; Abdelhafiz et al., 2021)
17	Korea*	2	X	X	1 - 9.80×10^2	1 - 5.04×10^3	NA	NA	NA	NA	(Seo et al., 2019)

Legend: *These studies/articles include the physicochemical parameters of water. ** All these studies used NPM/100mL for counting. According to Ecuadorean legislation, the limit for *E. coli* and total coliforms for recreational water use is 200 MPN/100mL and 1000 MPN/100mL respectively. Annex I, Book VI of the TULSMA reformed on the Acuerdo Ministerial 97 on July 30, 2015 (see Table 6) (TULSMA, 2015).

FIGURES

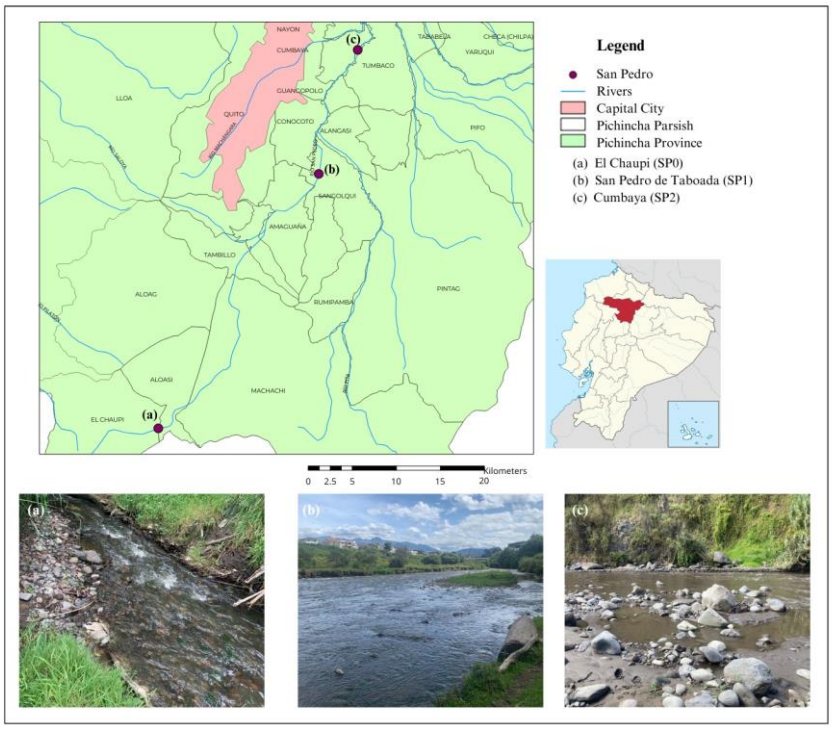

Figure 1: General map of the San Pedro River with sample collection point for chemical and microbiological analysis. (d) El Chaupi, control point (e) Sangolquí and (f) Cumbayá. The map was made with ArcGIS Desktop software (version 10.8 available online.

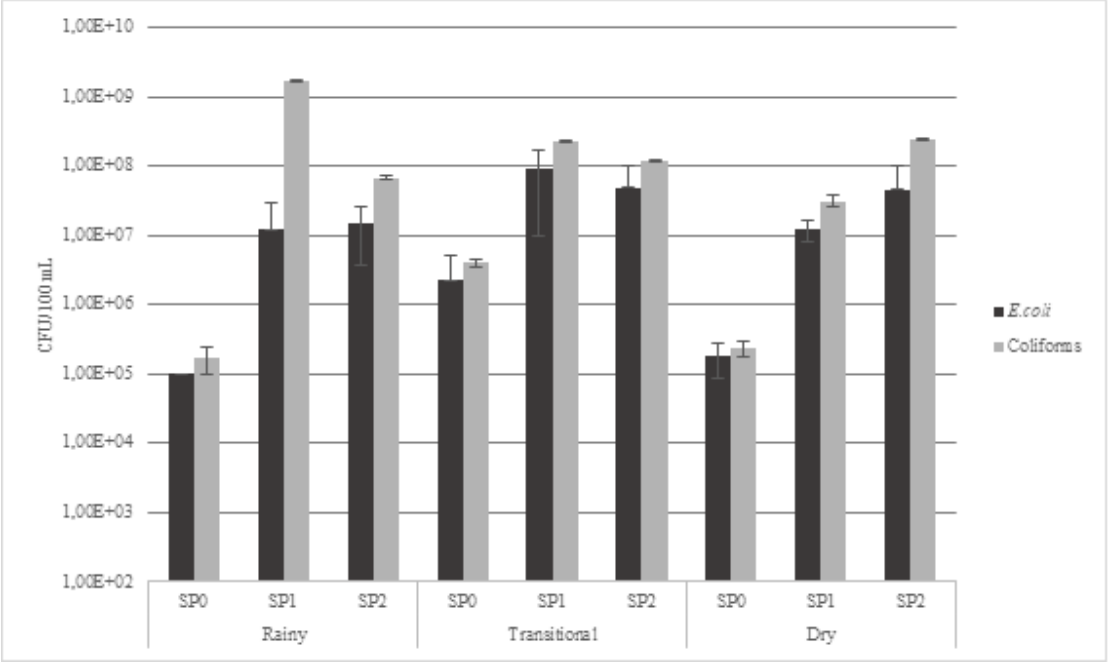

Figure 2. Average count of *E.coli* and total coliforms on San Pedro River in different sampling points during rainy, transitional, and dry seasons. Sampling collection points were the following: SP0 - El Chaupi point; SP1 - San Pedro de Taboada point; and, SP2 - Cumbaya point.

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APPENDIX

Appendix 1. Selection of the San Pedro River and their collection samples for microbial analysis.

Sample Code	River	GPS Coordinates	City (Province)	Region	Season	Collection sampling	Mean annual flow ($m^3 s^{-1}$) [a]	Monthly average temperature (°C)[b]	Name of INAMHI Stations	GPS Coordinates of INAMHI Stations	Height of INAMHI Stations (m)
SP0	San Pedro	0°35'43.4"S 78°37'26.1"W	El Chaupi (Pichincha)	Andean	Rainy	12/11/2022 26/11/2022	3.1006	16,01	H0159 San Pedro en Machachi	0°27"43" S / 78°32'42"W	2680
					Transitional	12/03/2023 18/03/2023					
					Dry	17/06/2023 01/07/2023					
SP1	San Pedro	0°19'48"S 78°27'35"W	San Pedro de Taboada (Pichincha)	Andean	Rainy	12/11/2022 26/11/2022	N/A	16.01	H0159 San Pedro en Machachi	0°27"43" S / 78°32'42"W	2680
					Transitional	12/03/2023 18/03/2023					
					Dry	17/06/2023 01/07/2023					
SP2	San Pedro	0°12'29"S 78°25'12"W	Cumbayá (Pichincha)	Andean	Rainy	14/11/2022 28/11/2022	N/A	16.01	H0159 San Pedro en Machachi	0°27"43" S / 78°32'42"W	2680
					Transitional	10/03/2023 17/03/2023					
					Dry	16/06/2023 30/06/2023					

Legend: [a] Data from the National Institute of Meteorology and Hydrology (INAMHI, https://www.inamhi.gob.ec/biblioteca/). ‘‘Determining the microbial and chemical contamination in Ecuador’s main rivers’’ Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8531378/; [b] J.Pilalumbo (2020). “Estudio de la calidad de agua del río San Pedro, ubicado dentro del distrito metropolitano de Quito en el período 2013-2019” Retrieved from: http://repositorio.utc.edu.ec/bitstream/270000/7084/1/pc-001049.