Research Paper

Virulence associated factors in bacteria from water bodies in Belém, Pará, Brazil: bacteriological composition and threat to public health

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ABSTRACT

A lack of sewage treatment contaminates water bodies threatening human health by spreading waterborne gastroenteritis. This is a particular problem for developing countries, where the risks associated with surface water contamination remain largely unknown. To understand the risk associated with sewage contamination of water bodies, we evaluated the microbiological indicators of water quality and isolated bacterial strains from water bodies from the city of Belém, Pará, Brazil. The strains were identified by biochemical and serological tests and polymerase chain reactions (PCRs). The thermotolerant coliforms and *Escherichia coli* presented values above 1,000 (NMP/100 mL) biweekly from August 2012 to November 2015, without a significant statistical difference between sampling periods (Kruskal–Wallis $p > 0.05$). The water of the Tucunduba river presented contamination levels similar to those in a sewage pumping station (Dunn test $p > 0.05$). From 240 bacterial isolates, we identified 163 *Vibrio cholerae*, 8 *Vibrio mimicus*, 24 *E. coli*, and 5 *Salmonella* spp. The isolates of *V. cholerae* demonstrated *N*-acetylglucosamine (NAG) profile (Non-O1 and Non-O139) and 18 expressed the *stn*/*sto* gene. No *E. coli* was shown to be potentially pathogenic. The results revealed that water bodies in Belém were constantly contaminated by sewage and fecal microorganisms, including the potential circulation of pathogens in viable and cultivable form.

Key words | microbiological indicators, pathogenic bacteria, surface water, water pollution

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HIGHLIGHTS

- Constant pollution of urban water bodies in Belém, North of Brazil.
- Water body with bacteriological contamination index similar to sewage effluents.
- Circulation of potential pathogens in their viable and cultivable form.
- *Vibrio cholerae* NAG, with expression of important virulence genes in waterborne gastroenteritis.

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INTRODUCTION

The 21st century has been marked by the challenges of monitoring aquatic ecosystems, considering the intense polluting activities that have severely compromised biodiversity and directly or indirectly impacted human health (Salem & Amin 2012).

Despite the advances made in recent years, insufficient or non-existent basic sanitation in large metropolises still causes an important environmental impact, especially concerning the intense pollution of water bodies receiving domestic and industrial effluents.

In Brazil, a survey performed by the National Sanitation Information System – SNIS, published in 2015, revealed that 34 million Brazilians live without treated water in their homes, 102 million are not assisted with sewage collection, and only 42.67% of all sewage generated in the country is treated before being discharged into water bodies. This context contributes to explain the fecal-oral transmission diseases responsible for 87% of hospitalizations related to inadequate sanitation conditions from 2000 to 2013 (IBGE 2015).

More than 5 million people die each year from water-related diseases affected by microbial infections, where 90% are children under five, mainly in developing countries. According to WHO (2004), 88% of these diseases are directly or indirectly related to water supply issues that are often inadequate, associated with poor or non-existent sanitation conditions (Cabral 2010).

In Brazil, the microbiological indicators of freshwater quality are regulated by the National Council of the Environment (CONAMA 357/2005) that establishes that water microbiological standards for thermotolerant coliforms should not exceed 1,000 MPN/100 mL. Despite the existing regulations, most of the freshwater bodies in Brazil lack continuous monitoring. The lack of monitoring combined with insufficient or non-existent basic sanitation (particularly sewage treatment) represents a threat to public health with the risk of increasing the dissemination of pathogenic bacteria (e.g., *Vibrio cholera*).

Belém is located on river peninsulas and a very anthropized region, with disorganized occupations and serious sanitation problems. Historically, Belém experienced huge changes in the city's natural environment, especially on water bodies, creating a favorable environment for disseminating potentially pathogenic bacteria of importance to public health (PMB 2014). Therefore, this study aimed to evaluate the bacteriological quality of surface water, together with an evaluation of their pathogenic potential in water bodies located in the urban area of Belém-Pará. For that, we considered the four main drainage basins in Belém municipality: Tucunduba, Guajará, Una, and Guamá.

METHODOLOGY

Study area, sampling period, and sample collection

Belém is the capital of the state of Pará, which is located between the geographical coordinates latitude 01°23′06″S and longitude 48°29′05″W. According to IBGE, in 2016, the estimated population was 1,446,042 inhabitants, with a territorial area of 1,059,458 km^2. The municipality is placed in the Amazon region, with hot and humid weather, high rainfall with an average annual precipitation of 2,889 mm. Belém has 13 hydrographic basins in its territory. The city's base is under fluvial peninsulas, with the Guamá River to the south and the Guajará Bay to the west. However, nowadays, only 15% of the original vegetation coverage remains preserved. Besides, Belém transformed rivers into channels, which receive rainwater and untreated sewage (Matos *et al.* 2011; PMB 2014).

The sampling occurred biweekly from August 2012 to November 2015 in the following sampling points (Figure 1): Ver-o-Peso Market (01°27′8″S; 48°30′13″W), in the commerce district, corresponding to water samples from Guajará Bay; Açaí Port (01°28′35.6″S; 48°29′40.2″W), in the Jurunas region; corresponding to water samples from the Guamá river, in the Federal University of Pará – UFPA basic campus bridge (01°28′26.1″S; 48°27′18.1″W), in the Guamá region, samples from the Tucunduba stream; and one of untreated sewage water located in the UNA Sewage

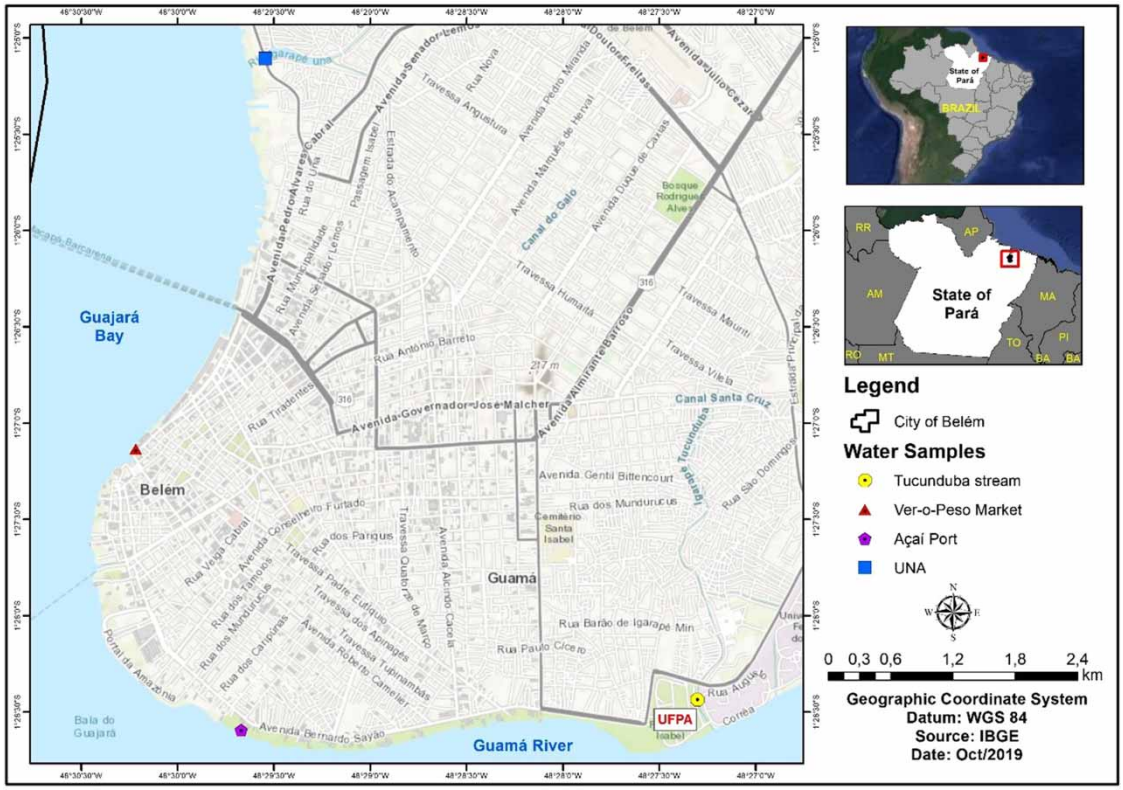

Figure 1 | Geographical location of the sampling points for analysis of the microbiological indicators in Belém-PA, Brazil.

Lifting Station (01°25′06.3″S; 48°29′32.6″W), in the Telégrafo region. All samples followed the procedures described in the 23rd edition of Standard Methods for the Examination of Water and Wastewater, methodology SM-1060 (APHA/AWWA/WEF 2012).

For the collection of water samples, polyethylene bottles or NASCO® sterile bags (250 mL) were used, packed in an isothermal box with recyclable ice ($T < 6$ °C), and transported to the Environmental Microbiology Laboratory in Ananindeua-PA (SAMAM/IEC/SVS) for microbiological analysis.

Quantification of microbiological indicators and bacterial isolation

The Most Probable Number (MPN) of Total Coliforms and *Escherichia coli* were determined using the chromogenesis substrate method (ONPG-MUG, COLILERT®). The Thermotolerant Coliforms were quantified through a modification in the technique consisting of incubating the QUANTI-TRAY cards in a water bath with a constant temperature of 44.5 °C.

The isolation of bacteria from Enterobacteriaceae and Vibrionaceae families in water samples followed the recommendations described in the Standard Methods for Examination of Water and Wastewater (APHA/AWWA/WEF 2012) and the technical standards L5.507, L5.218, and L5.232 of Environmental Company of the state of São Paulo – CETESB. Briefly, a volume of 5 liters of water from each selected point was collected in sterile containers and transported in Styrofoam boxes with ice to the laboratory within a maximum of 2 h after collection. The samples were then concentrated in a cellulose ester membrane with 0.45 m porosity divided into equal parts and inoculated in enrichment media (APT, pH70 Rappaport/425 °C–18 h, APA, pH 85).

The microbes that grew in the enrichment media were sown in three different selective-indicator media: (i) Thiosulfate-Citrate-Bile-Saccharose Agar (TCBS), (ii) *Salmonella* Shigella Agar (SS), and (iii) MacConkey Agar (MC) for

bacterial isolation, followed by biochemical and serological identification. The isolates obtained at that phase were initially identified as *Vibrio cholerae*, *E. coli*, *Salmonella* spp., or *Vibrio mimicus*. For confirming their classification, all the bacteria were re-isolated, identified, and confirmed by the biochemical and serological methods, following the same initial isolation protocol with selective media. All the bacterial isolates were stored in the Biorepository of the Environmental Microbiology Laboratory of the Evandro Chagas Institute.

Virulence factors of *V. cholerae* and *E. coli* isolates

The bacterial isolates of *V. cholerae* and *E. coli* had their DNA exposed through the lysis obtained after thermal shock according to the recommendations of Baloda *et al.* (1995) for use in polymerase chain reaction (PCR) in a Vereti™ 96-Well ThermalCycler (Applied Biosystems – US).

Multiplex PCR (*E. coli*) and conventional PCR (*V. cholerae*) were used to investigate the factors associated with virulence, as recommended by Aranda *et al.* (2004) and Sá *et al.* (2012). For multiplex PCR, seven pairs of primers were used for *E. coli* (Table 1). For conventional PCR, four pairs of primers were used for *V. cholerae*, available at the Environmental Microbiology Laboratory of the Evandro Chagas Institute (Table 1).

The reference strains used as positive controls were *E. coli*: EPEC E2348/69 (*eae* and *bfp* gene), EAEC O42 (*aggR* gene), ETEC H10407 (*elt* and *est* gene), EIEC EDL1284 (*ipaH* gene), and EHEC EDL931 (*stx* gene) and *E. coli* K12 DH5α as the negative control. The presence of bands was identified in electrophoresis gel.

Climate data

Previous studies showed that precipitation influences one of the drainage basin's water quality investigated (Alencar *et al.* 2019). Therefore, to evaluate climatic variables' role in the microbiological indicators and the virulence factors, we included the following variables in our analysis: precipitation, temperature, and relative humidity. The climatological variables were obtained from the Meteorological Database for Teaching and Research – BDMEP of the National Institute of Meteorology – INMET. The data were selected for the period under study from the meteorological station Belém-PA code 82191.

Table 1 | Oligonucleotides used in multiplex PCR (*Escherichia coli*) and conventional PCR (*Vibrio cholerae*) and their respective amplification products

Target gene	Oligonucleotide sequence (5′-3′)	Reference
eae	CTGAACGGCGATTACGCGAA CGAGACGATACGATCCAG	Aranda *et al.* (2004)
bfpA	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	Aranda *et al.* (2004)
aggR	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	Toma *et al.* (2003)
elt	GGCGACAGATTATACCGTGC CGGTCTCTATATTCCCTGTT	Aranda *et al.* (2004)
est	ATTTTTMTTTCTGATTTRTCTT CACCCGGTACARGCAGGATT	Aranda *et al.* (2004)
ipaH	GTTCCTTGACCGCCTTTCC GATACCGTCGCCGGTCAGCC ACCCTCTGAGAGTAC	Aranda *et al.* (2004)
*stx*1/*stx*2	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	Toma *et al.* (2003)
tcp H-B	GGTGACTTTGTGTGGTTAAATG CCATAATCCGACACCTTG	Sá *et al.* (2012)
ctxAB	GCAGTCAGGTGGTCTTATTGC TCCAGATATGCAATCCTCAG	Sá *et al.* (2012)
stn/sto	GAGAAACCTATTCATTGCA GCAAGCTGGATTGCAAC	Vicente *et al.* (1997)
Zot	TAAACCTTGAACGCATAG CGCCCATAGACCACGATA	Sá *et al.* (2012)

Statistical analysis

The microbiological analysis results and the descriptive analysis were performed using Microsoft Office Excel/2010 software. For determining the normality and homogeneity of the data, we used the Shapiro–Wilks test and Bartlet's test.

To check for differences between sampling sites, the Kruskal–Wallis test was performed. The test assumes that the variables do not follow a normal distribution and check the differences between ranks of values. Then, the Dunn test was selected to perform multiple comparisons between different sites.

Spearman's correlation was used to search for possible correlations between microbiological indicators (total coliforms, thermotolerant coliforms, and *E. coli*) and climatic

variables. All analyses were performed using the BioEstat 5.0 program (Ayres *et al.* 2008), adopting $p < 0.05$ as the significance level.

RESULTS AND DISCUSSION

The quantitative microbiological analysis revealed high levels of water contamination. Total coliforms (MPN/100 mL) ranged from 1.32×10^4 (Min) in the Açaí Port to 2.42×10^8 (Max) in the UNA (the sewage pumping station). Concentrations of thermotolerant coliforms ranged from 4.10×10^3 (Min) at the Açaí Port to 9.80×10^7 (Max) at UNA. For *E. coli*, the variation was between 3.10×10^3 (Min) at the Açaí Port and 6.87×10^7 (Max) at UNA.

The concentrations of thermotolerant coliforms and *E. coli* exceeded 1,000 per 100 mL in 100% of the sampling at the four points of the study, as demonstrated by the temporal dispersion (Figure 2). Moreover, the concentrations of microbiological indicators evidenced high contamination levels that remained constant throughout the months of study. *E. coli* has high fecal specificity and cannot reproduce outside its primary habitat, surviving about 1 day in water, 1.5 days in sediment, and 3 days in soil (Mugnai *et al.* 2014). Therefore, the constant detection of thermotolerant coliforms and *E. coli* in the studied environments suggests

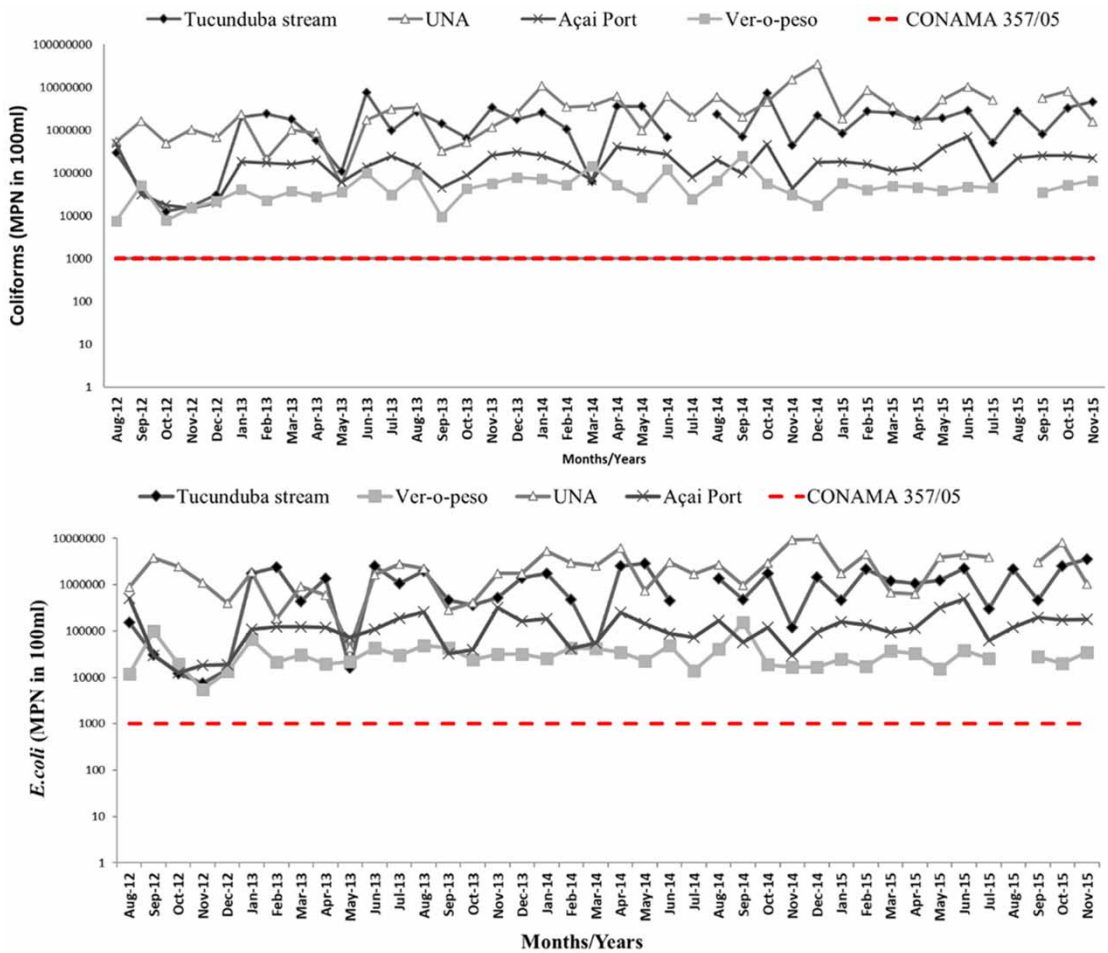

Figure 2 | Monthly quantification of Most Probable Number in 100 mL (MPN/100 mL) of *E. coli* and thermotolerant coliforms in the study points between August 2012 and November 2015 in the four sampling locations in Belém-PA, Brazil. Red dashed line indicates the CONAMA 357/05 standard for water quality (1,000 MPN/100 mL). Please refer to the online version of this paper to see this figure in color: https://doi.org/10.2166/washdev.2021.239.

a continuous source of contamination in the water bodies of Belém.

Beyond the extreme fecal contamination rates at all study sites, the statistical analysis highlighted the difference in bacterial concentration levels between sites. The concentration levels found in Tucunduba river – MO08 were similar to those found in UNA – MO13 (Kruskal–Wallis $p > 0.05$), a point selected to represent raw sewage. Therefore, there is an alarming anthropogenic impact on the water quality of the Tucunduba river.

Matos *et al.* (2009) and Matos *et al.* (2011) pointed to the historical and growing disorderly urbanization around the Tucunduba basin as one factor that accelerated the water bodies' pollution in Belém. A considerable amount of domestic and industrial waste is constantly dumped in the stream, corroborating this study's findings.

When analyzing the climatic variables (precipitation, temperature, and relative humidity), the months of December, January, February, March, April, and May presented higher rainfall rates, which is considered the rainy season. In contrast, in June, July, August, September, October, and November, the local precipitation decreases, followed by increased temperature. The medium relative humidity was shown with small variations, reaching the maximum in February/2014 (89%) and the minimum in November/2015 (73%).

The statistical correlation between these climatic variables at the points and their respective levels of microbiological indicators, for the most part, did not demonstrate significant correlations for Tucunduba stream, Ver-o-Peso, and Açaí Port ($p > 0.05$; Table 2). This result suggests that the input of water contaminant is constant throughout the years, reducing the influence of climate variables.

The survey resulted in a total of 240 strains from Enterobacteriaceae and Vibrionaceae families. Initially, a total of 166 were identified as *V. cholerae*, 54 of *E. coli*, 11 of *Salmonella* spp., and 9 of *V. mimicus*. The re-isolation with biochemical and serological methods confirmed that 200 strains, among which 163 (81.5%) *V. cholerae*, all did not agglutinate against the specific antiserum O1/O139, therefore classified as Non-O1/Non-139 8 (4%) *V. mimicus*, 24 (12%) *E. coli*, and 5 (2.5%) *Salmonella* spp. (Figure 3). The species with the highest number of confirmed strains was *V. cholerae* Non-O1/Non-O139 (81.5%), which is directly associated with the fact that this microorganism is natural to the aquatic environment (Baker-Austin *et al.* 2017).

Our study did not identify any strains of serogroups O1 and O139 – the cholera epidemic strains and etiological agents. Sá *et al.* (2007), in an extensive study of cholera in Belém between the years 1999 and 2006, also did not obtain any isolates of *V. cholerae* O1/O139.

Table 2 | Spearman correlation analysis between the microbiological indicators and the climatic variables

Sampling site	Climatic variable	TC	TTC	EC
Tucunduba MO08	Rainfall (mm)	$p = 0.39, r = -0.13$	$p = 0.30, r = 0.16$	$p = 0.54, r = 0.09$
	Temperature (°C)	$p = 0.79, r = 0.04$	$p = 0.72, r = 0.05$	$p = 0.88, r = 0.02$
	Relative air humidity	$p = 0.17, r = 0.36$	$p = 0.69, r = 0.06$	$p = 0.59, r = 0.08$
	Insolation	$p = 0.91, r = -0.01$	$p = 0.95, r = -0.00$	$p = 0.91, r = -0.01$
Ver-o-Peso MO12	Rainfall (mm)	$p = 0.96, r = 0.00$	$p = 0.34, r = 0.15$	$p = 0.20, r = 0.20$
	Temperature (°C)	$p = 0.86, r = -0.03$	$p = 0.30, r = -0.16$	$p = 0.20, r = -0.20$
	Relative air humidity	$p = 0.89, r = -0.02$	$p = 0.44, r = 0.12$	$p = 0.32, r = 0.16$
	Insolation	$p = 0.43, r = 0.12$	$p = 0.80, r = 0.04$	$p = 0.41, r = -0.13$
UNA MO13	Rainfall (mm)	$p = 0.67, r = -0.06$	$p = 0.95, r = 0.00$	$p = 0.35, r = -0.15$
	Temperature (°C)	$p = 0.51, r = 0.10$	$p = 0.79, r = -0.04$	$p = 0.76, r = 0.04$
	Relative air humidity	$p = 0.26, r = 0.18$	$p = 0.83, r = -0.03$	$p = 0.32, r = -0.16$
	Insolation	$p = 0.78, r = -0.04$	$p = 0.16, r = 0.22$	$p = 0.05, r = -0.30$
Açaí Port MO14	Rainfall (mm)	$p = 0.09, r = -0.26$	$p = 0.69, r = 0.06$	$p = 0.51, r = 0.10$
	Temperature (°C)	$p = 0.54, r = 0.09$	$p = 0.71, r = -0.05$	$p = 0.68, r = -0.06$
	Relative air humidity	$p = 0.83, r = 0.03$	$p = 0.55, r = 0.09$	$p = 0.64, r = 0.07$
	Insolation	$p = 0.40, r = 0.13$	$p = 0.54, r = 0.09$	$p = 0.54, r = 0.09$

TC, Total coliforms; TTC, Thermotolerant coliforms; EC, *Escherichia coli*.

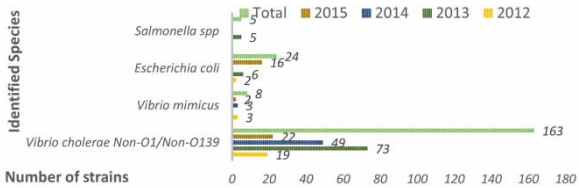

Figure 3 | Total distribution of the reactivation and identification of strains according to species per year of study in Belém-PA, Brazil.

Sá *et al.* (2012), during environmental monitoring in the municipality of Belém, obtained a strain of *V. cholerae* O1 from surface water samples in the Tucunduba stream. However, their molecular study did not detect the main virulence genes determining this strain's confirmation as, in fact, *V. cholerae* O1 toxigenic.

Following the previous study of Sá *et al.* (2012), we also sampled the same point in our research. Therefore, we confirmed the efficiency of the methods used, especially pointing to potentially pathogenic strains circulating in this aquatic environment. The study corroborates that serogroup classification does not define this species' pathogenicity; however, it highlights its circulation in waters characteristic of the Amazon region.

The information available on the incidence of cholera in Brazil (the Ministry of Health, available through the public platform of the Information System of Aggravates Notification – Sinan Net) revealed that, in the last 10 years, only two cases of cholera occurred in Brazil, one in 2011 notified by the municipality of São Paulo and the other notified by the municipality of Canoas, Rio Grande do Sul. All cases were imported and later evolved to cure. Given that the recorded cases were imported, we highlight the risk of spreading the disease, given the high contamination levels.

V. cholerae Non-O1/Non-O139 represented the largest number of identified and confirmed strains. These strains' pathogenicity is not yet widely known, but they are known to cause human infections mediated by toxins other than the choleric toxin (Siboni *et al.* 2016).

It is estimated that 1–3.4% of acute diarrheal diseases in developing countries, as much as in developed countries, are caused by these species, associated in most cases with consumption of seafood and contaminated water (Deshayes *et al.* 2015). Seafood is one of the major products of Ver-o-Peso Market, thus posing a threat of food contamination in that location.

V. mimicus was isolated from the sampling points in different years (2012, 2014, and 2015). This bacterium's importance as a pathogen was recognized after cases of severe diarrhea associated with the *V. mimicus* in Costa Rica (Campos *et al.* 1996). *V. mimicus* freshwater's presence may pose a threat to aquatic animals and humans coming in contact with the water contaminated with this bacterium. Under this situation, the presence of *V. mimicus* together with *V. cholerae* may be high, and the transmission of the organism through such types of water is very likely.

The results of this study raise important questions about the circulation of these strains in the aquatic environments studied, especially in the context of public health, since information on clinical infections by these species at the local and regional levels may be underestimated due to under-diagnosis and lack of knowledge of these infections as an emerging disease, as recent studies have shown (Deshayes *et al.* 2015; Zmeter *et al.* 2018). All environmental isolates of *V. cholerae* Non-O1/Non-O139 were negative for *ctx*, *tcp*, and *zot* genes.

On the other hand, 18 (11%) isolates were positive for the *stn/sto* gene, producing an amplification of 216 pb (Figure 4) as expected. Although it is known that the pathogenic mechanism of *V. cholerae* Non-O1/Non-O139 is multifactorial, with several virulence factors involved in different infections, Zago *et al.* (2017) highlight thermostable enterotoxin as responsible for most cases of gastroenteritis, thus playing a key role in human infections.

According to Morris *et al.* (1990), the expression of thermostable enterotoxin by *V. cholerae* Non-O1/Non-O139 varies from one geographical area to another. Based on

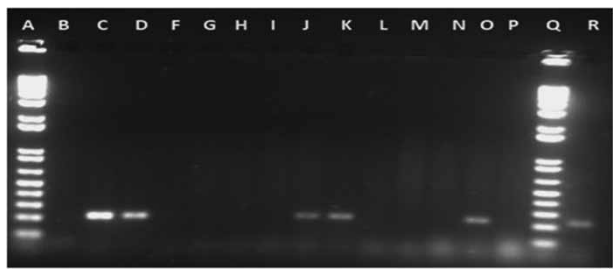

Figure 4 | Agarose gel electrophoresis 2% of the PCR product of *V. cholerae* isolated from the four sampling locations in Belém-PA, Brazil. Line B – negative control to *stn/sto* gene and line R – positive control to *stn/sto* gene. Lines C, D, J, K, and O showed as positive samples for that gene. Lines A and Q – molecular weight markers 1 kb.

these results, we confirmed the circulation of pathogenic strains in aquatic environments.

It should also be noted that the discrepancy in the number of isolates between the points, especially in the UNA, which presented low isolation, about 7.5%, is explained by the fact that these are raw sewage samples. Sato *et al.* (1995) highlight bacterial competitiveness as an interfering factor in the survival of microorganisms in highly polluted environments. The low percentages of isolation in sewage effluents were also reported by Sá *et al.* (2007).

Among the bacterial species identified, the percentages of *E. coli* and *Salmonella* spp., 12% ($n = 24$) and 2.5% ($n = 5$), respectively, were relatively low, considering the study period from similar surveys. Cho *et al.* (2018) investigated the prevalence of *E. coli* in a watershed in northeast Georgia and obtained 496 isolates.

However, it is important to emphasize that some bacterial species can enter a viable, non-cultivable state, such as *E. coli* and *Salmonella* spp. They are capable of presenting an adaptive cellular stage, conferring greater survival in totally unfavorable environments. This process makes the formation of colonies impossible and the cultivation with traditional approaches. Therefore, the difficulty in isolating these species through the methodology used can be explained by the characteristic conditions mentioned above. Nevertheless, the circulation of *Salmonella* spp. is confirmed in the studied environments, suggesting contamination of fecal origin since this bacterium lives in the intestinal tract of animals and humans (Michael & Schwarz 2016).

The circulation of *Salmonella* spp. in the environment, especially in water, configures a source of contamination, increasing the risks of infection because all *Salmonella*s, to some degree, are pathogenic bacteria (Tortora *et al.* 2012).

Chen *et al.* (2013) emphasized that controlling these infections is extremely difficult due to the high tolerance of the bacteria to environmental stress, the wide distribution, multiple drug resistance, and especially its high adaptability. Therefore, the best approach is to treat the sewage to avoid the spread of those microbes.

None of the 24 confirmed strains of *E. coli* was positive for the virulence factors surveyed (*eae*, *bfpA*, *aggR*, *elt*, *est*, *ipaH*, *stx1/stx2*), confirming the commensality of these strains since they are part of the intestinal microbiota and is the predominant aerobic organism of the gastrointestinal tract (Smati *et al.* 2015).

In summary, our research showed the extreme rates of contamination of surface waters in the urban area of Belém, due to the indiscriminate release of intense pollutant loads in these water bodies, evidencing the insufficient/non-existent sanitation conditions in the municipality, especially regarding sanitary management. This context represents significant risks to public health and highlights the need for guidelines to improve the quantitative and qualitative aspects of water, and emphasizes the need to integrate society in this process as an ally to environmental management.

This study also confirmed the relevance of environmental health monitoring research as an important tool in identifying risks to public health from constant environmental degradation.

CONCLUSIONS

The concentrations of microbiological indicators were, throughout the study period, above the maximum limits established for fresh waters of class 2, showing the existence of a continuous source of pollution. Total coliforms (MPN/100 mL) ranged from 1.32×10^4 to 2.42×10^8 and the concentrations of thermotolerant coliforms ranged from 4.10×10^3 to 9.80×10^7. In summary, the concentrations of thermotolerant coliforms exceeded 1,000 per 100 mL.

There is intense contamination of fecal origin in all the water bodies studied, associated with the discharge of sewage *in natura*. For *E. coli*, the variation ranged from 3.10×10^3 to 6.87×10^7.

The Tucunduba stream presented bacteriological contamination levels similar to those found in raw sewage samples. The climatic variables do not interfere significantly in the contamination rates, confirming sewage's continuous input through the year. There is the circulation of *V. cholerae* Non-O1/Non-O139 pathogens in the surface waters studied detected by virulence factors.

In summary, the extreme levels of contamination with coliforms in surface waters from the urban area of Belém allow the circulation of *V. cholerae* Non-O1/Non-O139 pathogens and pose a threat to public health. Our results

evidenced the poor sanitation conditions in the municipality, especially regarding sanitary management.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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