JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Prevalence, diversity, and antimicrobial resistance of *Salmonella* spp. isolated from river sediments in Northwest Mexico

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Abstract

Introduction: *Salmonella* is a major foodborne pathogen widely distributed in the environment. Surface water, soil, and sediments may confer a protective effect on *Salmonella* against non-host conditions.
Methodology: This study focused on determining the prevalence of *Salmonella* spp. in river sediments from Sinaloa central region by the Most Probable Number (MPN) technique and determining the antimicrobial resistance profile by Kirby-Bauer assay.
Results: Results showed the prevalence of *Salmonella* from 37.5 to 62.5% of the samples, oscillating from 0.322 to 20 MPN/4g, with August being the month with the highest levels. *In silico* geno-serotyping reveals the presence of *Salmonella* serotypes Typhi, Javiana, Ohio, Montevideo, Oranienburg, Pomona, Agona, Livingstone, Weltevreden, Anatum, and Minnesota. The most prevalent serotypes in river sediments were Pomona, Montevideo, and Oranienburg. Almost all isolates showed resistance to erythromycin, rifampin, and penicillin.
Conclusions: This study reveals the prevalence and distribution of *Salmonella enterica* in river sediments, which may represent a potential niche for establishment and survival in the environment and become a potential contamination source.

Key words: *Salmonella*; river sediments; antimicrobial resistance.

J Infect Dev Ctries 2024; 18(12):1875-1882. doi:10.3855/jidc.19938

(Received 29 January 2024 – Accepted 09 April 2024)

Introduction

Salmonella is a major foodborne pathogen, causing around 95.1 million cases worldwide and 1.35 million *Salmonella* infections per year in the United States of America, commonly associated with contaminated food, beverages, and drinking water [1,2]. *Salmonella* is a widespread microorganism in diverse environments, given its ubiquitous nature, it is possible to detect *Salmonella* from environmental settings, such as rivers, lakes, seawater, soils, and sediments. This reveals the necessity of studying the mechanisms and strategies for the survival of *Salmonella* and epidemiological surveillance [3,4]. Nonetheless, *Salmonella* diversity varies from each region, either in prevalence or serotype distribution [5-7]. Liu *et al.* (2015) mentioned that contaminated river sediments with enteric microorganisms such as *Salmonella* may potentially hazard native benthic microbiota and human health [8].

Salmonella has the ability for long-term survival outside the host, overcoming the exposition against the natural conditions of the river water [9]. In this sense, Fish and Pettibone (1995) demonstrated *Salmonella* could survive for 56 days in water and sediment microcosms [10]. Moore *et al.* (2003) evaluated the survival of *Salmonella enterica* in freshwater and sediments, suggesting that *Salmonella* can survive from 54 to 119 days [11]. Further studies suggested that temperature may affect long-term survival in environmental niches, which deal with differences in the persistence of enteric bacteria, such as *Escherichia coli* and *Salmonella* [12]. Siddiqhee *et al.* (2018) detected *E. coli* and *Salmonella* in sediments, which survived for 21 days, regardless of temperature and conditions of continuous and extended desiccation, periodic inundation, and constant inundation [13].

Another critical issue is the antimicrobial resistance of *Salmonella*, which has been suggested as a global concern in public health. Based on the World Health

Organization (WHO), *Salmonella* should be a top priority in surveillance studies of antimicrobial resistance in foodborne bacteria [14]. Studies performed by Patel *et al.* (2020) evaluated the presence of *Salmonella* in river water and their sediments from shrimp-farms source water in south India, reporting 28.7% of positive samples from river water and 25.5% from sediments in the farm environment [15]. In the same study, strains showed resistance against sulfonamide, tetracycline, chloramphenicol, and furazolidone. Previous studies by our research team have been focused on the isolation and antimicrobial resistance of *Salmonella* from river water. López-Cuevas *et al.* (2009) reported 12 strains that showed resistance against tetracycline and only one strain resistant to streptomycin [16]. On the other hand, Jiménez-Edeza *et al.* (2011) reported diverse antimicrobial patterns, including resistance to ampicillin, neomycin, chloramphenicol, or streptomycin [17]. Further studies reported the isolation and antimicrobial resistance in 23 *Salmonella* serotypes, including Anatum, Oranienburg, and Saintpaul, which showed diverse resistance patterns against different antibiotics such as ampicillin, neomycin, chloramphenicol and ceftazidime [18]. This study aimed to isolate and enumerate *Salmonella* from river sediments along the river paths and evaluate their resistance profile against first-choice-use antibiotics.

Methodology

Sample collection

Sediment samples were collected monthly from June 2018 to March 2019 from 11 sites along the Humaya and Tamazula rivers and their convergence to form the Culiacán River, finally in an estuarine environment. Rivers sampled from in this study are very important because their path begins from two main dams, López-Mateos and Sanalona, which represent the main source of water supply for domestic human consumption, cattle industry, and agriculture, finishing in an estuarine environment that is very important for fisheries activities. These sites have been continuously designed as river water quality monitoring sampling stations. Samples of sediments (100 g) were collected approximately 30 cm from the edge using a sterile spoon and placed in sterile bags. Samples were transported in an icebox at refrigeration temperature (4 ºC) to the laboratory of food and environmental microbiology of the Laboratorio Nacional para la Investigación en Inocuidad Alimentaria (LANIIA) from the Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD), Culiacán Station.

Isolation of Salmonella

Salmonella was isolated from sediments using the methodology established by the United States Environmental Protection Agency (U.S. EPA 2006) [19]. Briefly, 30 g of each sample was added with 270 mL of phosphate-buffered solution and mixed for 2 min. Another aliquot of 30 g was used to adjust the results of MPN/4g in dry base.

From each sample, aliquots of 20, 10, and 1 mL were inoculated in 10 mL 3X, 5 mL 3X, and 10 mL 1X Tryptic Soy Broth (TSB), respectively, and incubated at 37 ºC for 24 hours. Thereafter, from each tube, six aliquots of 30 µL were collected and placed in Modified Semisolid Rappaport-Vassiliadis (MSRV) plates (Difco, USA), and incubated at 42 ºC for 24 hours. From the previous step, presumptive *Salmonella* colonies were isolated by streaking on xylose lysine deoxycholate agar (XLD, Difco, USA) and incubated at 37 ºC during 24 hours. Biochemical confirmation was performed using LIA (Lysine Iron Agar, Difco, USA), TSI agar (Triple Sugar Iron, Difco, USA), and Urea broth as indicated (Difco, USA).

Confirmation by PCR

For final confirmation, colonies of *Salmonella* identified as positive by biochemical tests were confirmed by PCR. Briefly, the PCR mix was composed of 5.0 µL Master Mix, 0.2 µL of each forward (ACACCTCCTCTTCTCACCAGCGTATC) and reverse (CGGCTTTGATTTCCGCCACCAGA) primer for *pfk* gene designed for this study (Sigma-Aldrich), 1.6 µL water and 3.0 µL of DNA. PCR protocol was performed in a thermal cycler (Master cycler, Eppendorf, Germany) under the following amplification conditions: 1 cycle of 94 °C for 15 seconds, 35 cycles of 94 °C for 3 seconds, 50 °C for 10 seconds, and 74 °C for 35 seconds and finally an extension cycle of 74 °C for 2 minutes and 45 °C for 2 seconds. The amplified fragment size was 178 bp, and PCR products were separated by electrophoresis in 1% agarose gels containing gel red, and amplified DNA bands were visualized using a UV Transilluminator (UVP, U.S.A).

Sequencing and in silico serotyping of Salmonella spp. isolates

Salmonella isolates recovered from river sediment were grown for 24 hours on TSB at 37 °C. Genomic DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and the DNA was assessed and quantified by a Nanodrop 2000c instrument

(Thermo Fisher Scientific, Delaware, USA). Genomic DNA from each isolate was quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and the concentration was adjusted to 0.2 ng/µL, and 1 ng was used to prepare genomic DNA libraries using the Nextera XT Library Preparation Kit (Illumina, San Diego, CA, USA). Genomes of all isolates were sequenced, 21 genomes using a MiSeq (Illumina USA) platform to obtain 300 bp paired-end reads, at the Earlham Institute (Norwich, UK) (Table 1). The read quality was analyzed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc), and cleanup was performed with fastp v0.20.1 [20]. The reads were assembled into contigs using SPAdes v3.13.0 [21]. Also, all strains were evaluated with *Salmonella In Silico* Typing Resource (SISTR) v1.0.2 [22] was employed to perform serovar predictions of the assembled genome sequences for each *Salmonella* isolate. The default parameters were specified for using a combination of both serogroup-specific probes and core genome multilocus sequence typing (cgMLST) analysis for the serovar prediction (Table 1).

Antimicrobial susceptibility test

The Kirby-Bauer disk diffusion method on Mueller-Hinton agar was performed to evaluate the antimicrobial susceptibility of *Salmonella* following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [25]. Antibiotics evaluated were contained in disks at different concentrations per each antibiotic as indicated by the manufacturer (Becton-Dickinson, USA): Tetracycline (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Sulphamethoxazole-Trimethoprim (25 µg), Azithromycin (15 µg), Gentamicin (10 µg), Amikacin (30 µg), Penicillin (10 IU), Ciprofloxacin (5 µg), Trimethoprim (5 µg) and Rifampicin (5 µg).

Results

Salmonella isolation from river sediments

From a total of 77 collected samples, 37 samples (48%) were positive for *Salmonella* distributed along the river path, which suggests a high prevalence of *Salmonella* in river sediments. The distribution of positive samples ranged from 37.5% to 62.5% in 10 of 11 sampling sites (Figure 1). Sampling sites F and C had the highest percentage of positive samples (62.5%), followed by sites I, G, and B (50.0%), and five sites

Figure 1. *Salmonella* distribution in river sediments along the study area.

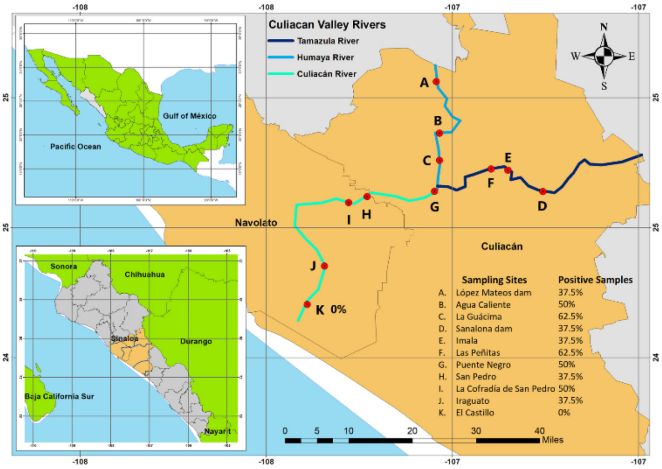


Table 1. Genomic sequences of *Salmonella*.

Strain	Serovar cgMLST	GenBank Accession	Genome size (Mb)	Contigs	Isolation source	Reference
JCS-02	Javiana	JAOBPV000000000	4.5	44	México: Sinaloa 2019	This study
JCS-03	Minnesota	JAOBQF000000000	5.0	123	México: Sinaloa 2019	This study
JCS-04	Pomona	JAOBPZ000000000	5.3	339	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-05	Oranienburg	JALPLR000000000	5.0	245	México: Sinaloa 2019	González-Torres *et al.* [2023]
JCS-06	Montevideo	JAOBQD000000000	4.5	44	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-07	Pomona	JAOBPY000000000	5.4	48	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-08	Pomona	JAOBPX000000000	4.9	71	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-14	Minnesota	JAOBQE000000000	5.6	219	México: Sinaloa 2019	This study
JCS-19	Typhi	NA	4.8	1	México: Sinaloa 2019	This study
JCS-22	Livingstone	JAOBPU000000000	4.7	6	México: Sinaloa 2019	This study
JCS-23	Agona	JAOBPT000000000	5.0	311	México: Sinaloa 2019	This study
JCS-24	Weltevreden	JAOBPS000000000	5.8	106	México: Sinaloa 2019	This study
JCS-25	Pomona	JAOBPW000000000	5.6	773	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-26	Ohio	JAOBPR000000000	4.8	115	México: Sinaloa 2019	This study
JCS-27	Montevideo	JAOBQC000000000	5.4	1392	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-28	Montevideo	JAOBQB000000000	5.6	167	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]
JCS-29	Anatum	JAOBPQ000000000	4.7	69	México: Sinaloa 2019	This study
JCS-30	Oranienburg	JALPLS000000000	4.6	80	México: Sinaloa 2019	González-Torres *et al.* [2023]
JCS-31	Oranienburg	JALPLT000000000	4.7	54	México: Sinaloa 2019	González-Torres *et al.* [2023]
JCS-32	Oranienburg	JALPLU000000000	4.7	53	México: Sinaloa 2019	González-Torres *et al.* [2023]
JCS-34	Montevideo	JAOBQA000000000	5.8	221	México: Sinaloa 2019	Garrido-Palazuelos *et al.* [2024]

Table 2. Concentrations of *Salmonella* in river sediments.

Sampling site	Latitude/Altitude, N/W	Months						
		Jun	Jul	Aug	Nov	Dec	Jan	Feb
A	25º 02' 46.9", 107º 23' 50.5"	0.381	-	0.81	0.736	-	-	0.423
B	24º 55' 44.2", 107º 23' 14.9"	1.231	7.239	4.153	3.538	-	-	0.322
C	25º 52' 10.2", 107º 24' 37.1"	1.374	5.856	2.205	0.496	-	-	-
D	25º 41' 54.8", 108º 38' 41.3"	2.278	20	2.888	-	-	-	-
E	24º 51' 11.7", 107º 13' 17.2"	0.345	-	2.632	3.073	-	-	0.322
F	24º 51' 41.8", 107º 15' 21.1"	3.575	1.035	5.4	-	-	0.423	0.698
G	24º 48' 24.3", 107º 24' 34.4"	2.606	-	10.608	-	-	5.68	-
H	24º 47' 06.2", 107º 33' 31.7"	-	-	1.135	1.205	-	-	-
I	24º 46'47.3", 107º 35' 39.8"	0.365	5.818	4.578	0.776	-	-	-
J	24º 37' 39.2", 107º 39' 39.2"	0.375	0.74	-	0.429	-	-	-
K	24º 32'39.7", 107º 42' 22.8"	-	-	-	-	-	-	-

with 37.5% (Figure 1). Contrary to the results in most sampling sites, sampling site K, located on the coast of Navolato, Sinaloa, was the unique site where *Salmonella* was undetected.

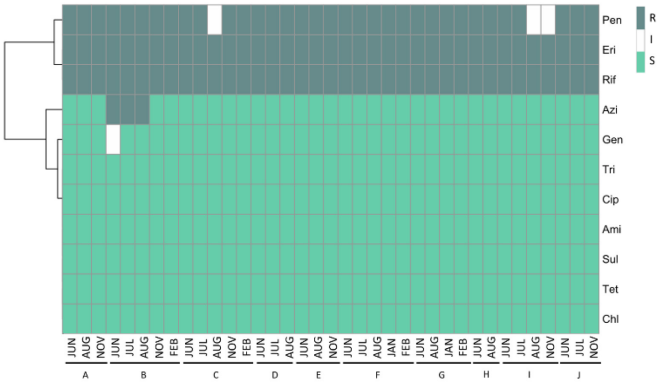
Concentrations of *Salmonella* showed variable levels in river sediments from June 2018 to February 2019 in 10 sampling sites. July and August were the months with the highest concentrations of *Salmonella* in river sediments from site D (20.000 MPN/4g in July), site F (5.400 MPN/4g in August), site B (7.239 MPN/4g in July), site G (10.608 MPN/4g in August), site C (5.856 MPN/4g in July) and site I (5.818 MPN/4g in July) (Table 2).

On the other hand, January and February showed the lowest number of positive samples and *Salmonella* concentrations, with two positive samples in January (2/11) and four positive samples in February (4/11), respectively. Furthermore, *Salmonella* was not detected in all sampling sites in December (0/11). Additionally, concentrations of *Salmonella* during this period (December-February) ranged from 0.322 to 5.680 MPN/4g across sites A, B, E, F, and G, respectively (Table 2). This decrease was probably due to low temperatures and the scarce movement of runoff due to the absence of rain in this season.

Salmonella serotypes distribution in river sediments

Results from genomic analysis based on *in silico* geno-serotyping showed a diversity of typhoidal and non-typhoidal *Salmonella* serotypes along the study area, detecting 11 serotypes, namely Typhi, Javiana, Ohio, Montevideo, Oranienburg, Pomona, Agona, Livingstone, Weltevreden, Anatum, and Minnesota. The most prevalent serotypes in river sediments were Pomona, Montevideo, and Oranienburg, with 5, 4, and 4 strains for each serotype, respectively, showing similar distribution along the path of the Humaya River. Additionally, site B, belonging to the Humaya River, represented the most diverse with Anatum, Minnesota, and Montevideo (Table 3). Interestingly, *Salmonella* Oranienburg was distributed from site A to site I, being

Figure 2. Antimicrobial resistance in *Salmonella* isolated from river sediments.



Pen: Penicillin; Eri: Erythromycin; Rif: Rifamycin; Azi: Azithromycin; Gen: Gentamycin; Tri: Trimethoprim; Cip: Ciprofloxacin; Ami: Amikacin; Sul: Sulfamethoxazole-Trimethoprim; Tet: Tetracycline; Chl: Chloramphenicol. R: Resistant; I: Intermedium; S: Sensitive. A, B, C, D, E, F, G, H, I, J: Sampling sites.

Table 3. *Salmonella* serotype distribution in the study area.

Sampling site	Latitude/Altitude N/W	Serotype	Month of isolation
A	25º 02' 46.9", 107º 23' 50.5"	Oranienburg, Montevideo	June, November
B	24º 55' 44.2", 107º 23' 14.9"	Anatum, Minnesota, Montevideo	November, July, June
C	25º 52' 10.2", 107º 24' 37.1"	Pomona, Livingstone, Oranienburg	June, August, November
D	25º 41' 54.8", 108º 38' 41.3"	Javiana	June
E	24º 51' 11.7", 107º 13' 17.2"	Minnesota, Montevideo	June, November
F	24º 51' 41.8", 107º 15' 21.1"	Pomona, Typhi	June, August
G	24º 48' 24.3", 107º 24' 34.4"	Pomona, Agona, Montevideo	June, August, January
H	24º 47' 06.2", 107º 33' 31.7"	Weltevreden, Oranienburg	August, November
I	24º 46'47.3", 107º 35' 39.8"	Pomona, Oranienburg	August, November
J	24º 37' 39.2", 107º 39' 39.2"	Ohio	November
K	24º 32'39.7", 107º 42' 22.8"	ND	

similar to *Salmonella* Pomona, such distribution pattern from site B (Humaya River) to site I, including sampling site F (Tamazula River) (Table 3).

Antimicrobial resistance of Salmonella isolated from sediments

Salmonella's antimicrobial resistance isolated from sediments showed a constraint but homogeneous resistance. All strains from all sampling sites and months were resistant to Erythromycin and Rifamycin. On the other hand, only one strain, isolated from site B showed intermedium resistance to Gentamicin (Figure 2). In contrast to the limited resistance profile, 92% of strains (34/37) showed susceptibility to 8 of 11 antibiotics under evaluation, such as tetracycline, chloramphenicol, and ciprofloxacin (Figure 2). Resistance to macrolides such as erythromycin has been poorly reported for non-typhoidal *Salmonella*.

Discussion

Salmonella isolation from river sediments

The presence and concentrations of *Salmonella* in river sediments in this study may be favored by the combination of high environmental temperatures and the beginning of the rain season, which results in eutrophication processes that contribute to generating host conditions for the establishment and abundance of *Salmonella* in river sediments [26,27]. In this regard, the trend in the physicochemical parameters in the studied area was as follows: temperature from 25.21 to 28.6 ºC, pH from 7.31 to 7.95, soluble dissolved solids from 96.14 to 1880 ppm, salinity from 70 to 1080 ppm, and conductivity around 210.70 to 2824.57 µS/cm; which may favor the survival and establishment of microbial populations, including *Salmonella*. On the contrary, the coastal sampling site K was unique in that it was not possible to detect the presence of *Salmonella* in this study; in this regard, our results differ from studies that suggest that *Salmonella* is prevalent in marine environments and can survive for 21 days in estuarine sediments [28,13].

Antimicrobial resistance of Salmonella isolated from sediments

Results for antimicrobial resistance obtained in this study may suggest a limited but highly homogeneous spectrum of resistance among isolates regardless of the sampling site. This resistance may be attributed to gene acquisition during the establishment of *Salmonella* in river sediments [29].

Previous studies reported the isolation of *Salmonella* Typhimurium, Give, Anatum, Agona, Infantis, Oranienburg, and Minnesota from river sediments, which were susceptible to ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole, tetracycline, streptomycin, and gentamycin [16]. Almeida *et al.* (2018) identified the presence of the *mphA* gene in *Salmonella* strains resistant to macrolides [30]. We suggest that *Salmonella* strains in our study may contain genes for antimicrobial resistance [23,24], which could be acquired by horizontal transference mediated by mobile elements or by the same selective pressure exerted by the presence of antibiotics in the water of the analyzed rivers. In this regard, Burgueño-Román *et al.* (2019) detected the presence of antimicrobial gene families in seven *Salmonella* serotypes isolated from the same study area, which may favor the resistance against aminoglycosides, β-lactams, and sulfonamides [31].

Cucak *et al.* (2018) isolated and evaluated 15 serotypes of *Salmonella* from soils and river sediments in Serbia, including Brandenburg, Enteritidis, and Wien. Eighty-five percent of strains were sensitive to 20 antibiotics, including penicillin, cephalosporins, fluoroquinolones, aminoglycosides, and amphenicols [32]. These authors suggest that the environmental origin of *Salmonella* strains determines the ability to persist in non-host environments, such as river sediments [32]. Micallef *et al.* (2012) isolated and evaluated strains of *Salmonella* from water, sediments, and soil in tomato farms, detecting diverse serotypes such as Newport, Javiana, Lille, Typhimurium, and Tennesee, reporting the resistance against several antibiotics such as cefotaxime, tetracycline, amoxicillin/clavulanic acid and ampicillin [33].

Benevides *et al.* (2020) identified antimicrobial resistance profiles for 22 *Salmonella* serotypes, such as Oranienburg, Agona, and Livingstone, isolated from egg-producer farm samples in Brazil [34]. They reported that all strains showed resistance against at least one antimicrobial, including 77 strains resistant against streptomycin, 66 against sulphonamide, and 32 against ciprofloxacin. *Salmonella* Oranienburg was the most susceptible to antimicrobials, showing resistance solely to streptomycin. Previous studies by Carvalho *et al.* (2013) reported antimicrobial resistance in *Salmonella* Braenderup, Panama, and Infantis, isolated from shrimp farms in Brazil, with results ranging from 23% strains resistant against at least one antimicrobial, 20% against at least two antimicrobials, being ampicillin, oxytetracycline, and tetracycline the least effective antimicrobials [35].

In this sense, Aljindan *et al.* (2020) reported an increasing pattern in the antimicrobial resistance levels

among *Salmonella* clinical strains isolated from 2011 to 2018 against antibiotics such as ampicillin, amoxicillin, amoxicillin/clavulanic acid, and ciprofloxacin [36]. That resistance significantly increased from 26.4% for strains isolated in 2011 compared to 37.8% for the strains isolated in 2018; nonetheless, this behavior may be variable between the serotype and isolation origin. Bassani *et al.* (2021) reported that antimicrobial resistance of *Salmonella* Heidelberg is not affected by the time of isolation, based on the antimicrobial resistance profile against gentamycin, tetracycline, nalidixic acid, and ciprofloxacin among strains isolated from poultry farms in Brazil, during 2006 and 2016 [37].

Díaz-Torres *et al.* (2020) reported the isolation and characterization of *Salmonella* Agona, Typhimurium, and Weltevreden from surface lake water in Jalisco, México, showing 16 antimicrobial resistance profiles against eight antibiotics, where 94.8% of strains were resistant to at least one antibiotic, including gentamycin, tetracycline, sulfamethoxazole, and streptomycin [38].

The presence of antimicrobials in water represents a health risk because it can lead to the emergence of resistant microorganisms. For example, more than 70% of unaltered molecules of tetracycline are excreted into the environment due to its low volatility and high hydrophilicity. According to Daghrir and Drogui (2013), this phenomenon can be a determining factor for the exposure of microorganisms to non-lethal antibiotic concentrations, favoring the development of resistant strains [39]. Based on the results, our study suggests that *Salmonella* serotypes are well-adapted and established in this kind of ecological niche. However, it is necessary to perform further analysis focused on identifying and quantifying antibiotics in this environment to better associate the phenotypic traits of *Salmonella* strains with the chemical quality of water and sediments.

Conclusions

This study reveals the distribution and diversity of *Salmonella* serotypes in river sediments, representing an important matrix for the survival and establishment in subtropical environments. Additionally, the reduced spectrum of resistance against antimicrobials in all *Salmonella* may be favorable when the microorganism re-enters contact with a human host, given their susceptibility against the most commonly used antibiotics.

Acknowledgements

The authors thank Bs. Célida Isabel Martínez Rodríguez, who provided technical support in this study.

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Conflict of interests: No conflict of interests is declared.