

Assessment of antibiotic resistant coliforms from bioaerosol samples collected above a sewage-polluted river in La Paz, Bolivia

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ABSTRACT

Antimicrobial resistance is a global health risk, and the presence of resistant bacteria in the environment may be an indicative of fecal pollution. The objective of this study has been to assess the antibiotic resistance of airborne coliforms near a highly impacted urban river that may contain high levels of fecal waste. The pilot study has been located within an Andean river basin, the Choqueyapu River basin, which flows through La Paz city in Bolivia. Bioaerosol samples have been collected using liquid impingement and plated on mTEC agar. Coliforms have been detected within 80% of the air samples. The resistance profiles of coliforms present in 20 air samples have been determined by using a modified Kirby-Bauer disk diffusion test against amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, meropenem, sulfamethoxazole-trimethoprim, and tetracycline, antibiotics commonly used to treat gram-negative infection. Broad patterns of antibiotic resistance have been observed throughout the study, with coliforms from at least one sample exhibiting resistance to each of the tested antibiotics. Resistance to sulfamethoxazole and amoxicillin-clavulanic acid has been the most commonly observed, with coliforms in 73% and 60% of samples which helps to demonstrate resistance to these antibiotics, respectively. This study provides insight into the prevalence of airborne, antibiotic resistant coliforms near concentrated fecal waste streams and this highlights an underappreciated hazard and the potential exposure risk in areas where fecal waste may become aerosolized at any given time.

1. Introduction

Antimicrobial resistance to antibiotics is an emerging public-health risk. It occurs when a microorganism evolves to resist the effects of an antimicrobial agent and it has the capability to multiply within its presence (Gaze and Depledge, 2017). The Review on Antimicrobial Resistance (O'Neill Commission, 2014) projects indicates that antimicrobial resistant infections may become the leading cause for death at a worldwide level by 2050. This problem has worsened with the widespread use of antibacterial agents to prevent infections not only among humans, but also among animals and even among food-producing plants (Bhardwaj et al., 2015). In fact, bacteria have already possessed several genetic mechanisms to resist the effects of antimicrobial agents even before the appearance of antibiotics. As an antecedent to this matter, the environment is a substantial pool of antibiotic resistance genes which have evolved over millions of years (Friedman et al., 2016).

The spread of resistance is associated with the heavy consumption and misuse of antimicrobial agents among humans, animals and within

various agricultural practices, along with poor sanitation whose effect is very much related to the overcrowding of cities (Bartoloni et al., 2004). The problem in developing countries is more complex due to the existence of more factors that ought to be considered: higher numbers of immunocompromised patients, the unavailability to second- and third-line drugs, poor hygiene, unreliable quarantine measures and policy implementation issues (Chaudhary, 2016).

Waste streams from pharmaceutical manufacturing companies and healthcare facilities; they are believed to be significant sources of antibiotic residues and antibiotic resistant bacteria, respectively. Wastewater discharge from urban areas and runoff from agricultural feeding operations introduce subinhibitory concentrations of antibiotics into the environment (Gandolfi et al., 2011), which are sufficient to select resistant genes (Gullberg et al., 2011). The concern about this issue has led to the consideration of resistant genes, which are regarded as environmental pollutants themselves (Kümmerer, 2009).

Bacteria in natural environments act as reservoirs of resistant genes, and therefore, it is the focus of an increasing research. It is well known that microorganisms which are present in natural and anthropogenic

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environments, can be released and it can remain airborne for long periods of time (Kasprzyk, 2008). Airborne biological particles (bioaerosols) refer to the fraction of airborne particulate matter. As Gandolfi et al. (2013) stated, these groups of biological particles may play a critical role in the negative effects of aerosols on biological systems. Studies aimed at detecting the potential harmful effects from these airborne microorganisms, which have been reported in various publications (D'Arcy et al., 2012; Eames et al., 2009; Eduard et al., 2012; Tang, 2009). However, the state of knowledge from outdoor environments still needs to be developed.

The World Health Organization (2014) has identified seven bacteria of international concern regarding antimicrobial resistance, including *Escherichia coli*, a representative species from the Enterobacteriaceae family of the gram-negative bacteria. The proportion of *E. coli* resistant bacteria to common antibacterial agents exceeded 50% in many WHO regions (Chaudhary, 2016). As Griffin et al. (2001) described, *E. coli* are able to survive outside the human body for a limited amount of time, making these bacteria an ideal indicator to assess environmental conditions within fecal contamination. Moreover, *E. coli* is a useful indicator of the spread of the acquired antibiotic resistance genes in environmental bacterial communities. Several studies have used these bacteria as a sensitive indicator in the surveillance of antimicrobial

resistance (Bartoloni et al., 2004, 1998; Calva et al., 1996).

Some studies have been conducted in Bolivia to assess this emerging threat. In its Global Report on Surveillance of Antimicrobial Resistance, the World Health Organization reviewed the status of surveillance and information available for Bolivia, including a report of 47% of 8259 *E. coli* isolates from the urinary tract infections demonstrating resistance to fluoroquinolones. Bartoloni et al. (2004) and Pallecchi et al. (2007) studied antibiotic resistant bacteria from communities with minimal antibiotic exposure, detecting unexpected high levels of antibiotic resistance in *E. coli* isolates. Moreover, Poma et al. (2016) and Guzman-Otazo et al. (2019), evaluated the occurrence of enteropathogenic bacteria and its resistance in river water, soil and vegetables samples within the La Paz River basin. They demonstrated that the La Paz River basin was heavily polluted with a high density of thermotolerant coliforms and that it was a reservoir of multiple antibiotic resistant enteropathogens. However, little is known about the presence of bioaerosols in the environment surrounding the river. This study has been designed to fill this gap in our understanding to exposure to antimicrobial resistant bacteria in the La Paz River basin.

The aim of this study was to assess the resistance profiles of coliforms present in bioaerosol samples, which have been collected adjacent to the Choqueyapu River, an urban river that receives heavy

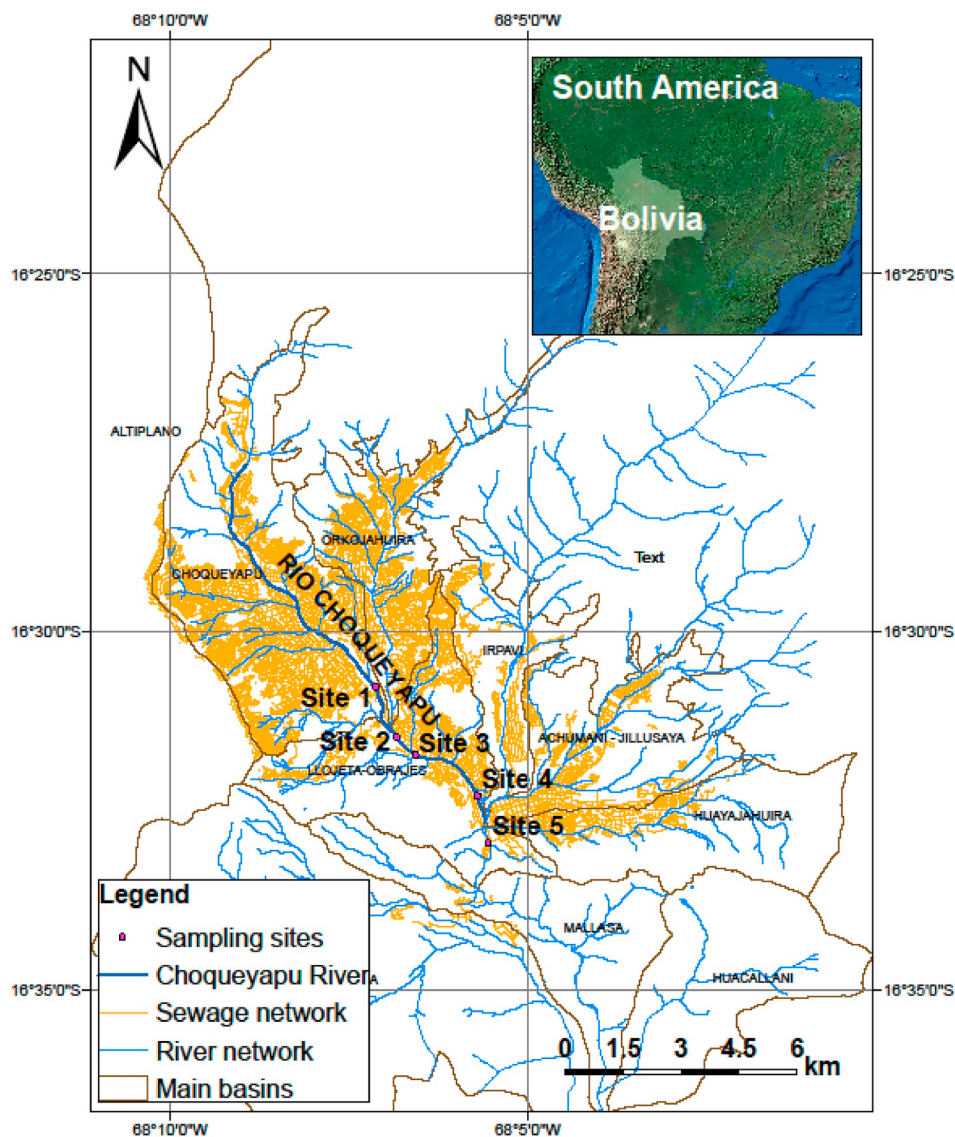


Fig. 1. Sampling sites along Choqueyapu River (Río Choqueyapu).

Table 1

Diameters used to categorize Enterobacteriaceae as susceptible, intermediate, or resistant. Data obtained from Performance Standards for Antimicrobial Susceptibility Testing from the Clinical and Laboratory Standards Institute.

Antibiotic	Antibiotic class	Disc Content	Interpretation categories and diameter of the zone of inhibition, [mm]		
			Susceptible	Intermediate	Resistant
Amoxicillin-clavulanic acid	β -lactam combination agents	20/10 μ g	≥ 18	14–17	≤ 13
Ciprofloxacin	Quinolones and Fluoroquinolones	5 μ g	≥ 21	16–20	≤ 15
Gentamicin	Aminoglycosides	10 μ g	≥ 15	13–14	≤ 12
Meropenem	Carbapenems	10 μ g	≥ 23	20–22	≤ 19
Sulfamethoxazole-trimethoprim	Folate pathway antagonist	1.25/23.75 μ g	≥ 16	11–15	≤ 10
Tetracyclin	Tetracyclines	30 μ g	≥ 15	12–14	≤ 11

discharges and sludge from domestic, commercial, and industrial wastewater. The assessment included a quantitative analysis of antibiotic resistant coliforms and multidrug resistant coliforms (defined as resistant to at least two different classes of antibiotics), which are present in air samples.

2. Materials and methods

2.1. Study area and sampling points

The Choqueyapu River is part of the La Paz River basin network (La Paz, Bolivia), in the headwaters of the Amazon River basin. It begins in the Pampalarama lagoon and flows towards the southern part of the city, where it merges with a number of tributaries. The Choqueyapu River flows through La Paz city, receiving untreated wastewater which is discharged from domestic, commercial, and industrial sources (Guzman-Otazo et al., 2019; Poma et al., 2016).

Bioaerosol samples were collected from five different points along the bank of the Choqueyapu River, as shown in Fig. 1. The first point (Site 1) was located at the output of a long culvert which crosses an important part of the city (16°30'46.8" S, 68°7'6.9" W). From the second point onwards, rivers flow along open channels. The second sampling site (Site 2) was located after the confluence of the two main tributaries of the Choqueyapu River (16°31'28.5" S, 68°6'49.8" W); the third point (Site 3) was located close to a hospital (16°31'43.8" S, 68°6'33.8" W); the fourth point (Site 4) was located at a footbridge which crosses the Choqueyapu River (16°32'18.1" S, 68°5'42.0" W); the fifth point (Site 5) was located next to the Amor de Dios bridge at the outlet of the basin (16°32'57.6" S, 68°5'32.7" W). Five samples have been collected at each site. The analysis shown in this paper presents the data collected in five sampling events between November and December of 2018. The samples were collected during days when there was not any pluvial precipitation registered for at least one day before sampling (De La Rosa et al., 2002).

2.2. Bioaerosol collecting and bacteriological method

Bioaerosol samples have been collected by using the AGI-30 liquid impingement device (Ace Glass, Vineland, New Jersey), which collects airborne particles into a liquid (Gandolfi et al., 2013; Ko et al., 2009; May and Harper, 1957). The collecting medium in the AGI-30 impinger was a Tween mixture that contained 1% peptone, 0.01% Tween 80, and 0.005% antifungal prepared in distilled water (Chen and Li, 2010; Ko et al., 2009; Li et al., 2010; Thorne et al., 1992; Chang and Chou, 2011). Each AGI-30 impinger contained 20 ml of the Tween mixture. The flow rate of the impinger was maintained at 12 l/min and the sampling time was 30 min, for a total sampling volume of 0.36 m³ of air per sample.

The AGI-30 impingers had been transported to the laboratory on ice at 4 °C (Chinivasagam and Blackall, 2005). The transportation time varied between 3 h and 30 min depending on the sampling schedule. The AGI-30 impingers were stored at 1 °C in a refrigerator in the laboratory for periods that were below an hour before processing. The

isolation of coliforms was performed by using the membrane filtration technique described in the Compendium of Standard Methods for the Analysis of Water and Wastewater (American Public Health Association, 1999) using filtration membranes with a porosity of 0.45 μ m. The membranes were placed over the surface of a plate with M-Tec Agar (selective gram-negative, lactose-fermenting bacteria and *E. coli* strains) using an aseptic technique to ensure that the samples were not contaminated. The filtration was performed by taking the 20 ml of Tween mixture at once. Dilutions had not been performed, because it was deemed unnecessary due to the concentration of CFU found. The inoculated plates were inverted and incubated at 37 °C for a term of 20 h. Colonies were counted after 20 h, while quantifying the presence of coliforms considering the selectivity under the presence of the M-Tec agar. For the data analysis, the airborne microbial concentration of coliforms had been estimated by dividing the number of CFUs detected in the impinger liquid by the product of the sampling airflow rate and duration (Ko et al., 2009).

2.3. Antimicrobial susceptibility testing

The Kirby-Bauer Disk Diffusion Susceptibility Test is a standardized method, which is provided to determine the resistance of pathogenic bacteria against various antimicrobial agents. The Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Hudzicki, 2009) published by the American Society of Microbiology was used for this study. Two Mueller-Hinton plates have been used to perform the test for each positive sample. A positive sample was any plate with detectable coliform CFUs. A sterile loop has been used to transfer one to five coliform colonies to a sterile saline tube until the turbidity of the solution matched a 0.5 McFarland standard. We modified the original protocol to examine the resistance among multiple coliform colonies on a per-sample basis. Coliforms are a group of bacteria – including *Klebsiella pneumoniae*, other *Klebsiella*, as well as *E. coli* – and our aim was to assess resistance profiles of this group in a given air sample. We took this non-standard approach because people are not exposed to single bacteria but to combinations of them, which are present in the environment, including

Table 2
Concentration of coliform isolates per site.

	Sample	Site				
		Site 1	Site 2	Site 3	Site 4	Site 5
Concentration of coliforms (CFU/m ³)	1	94,44	25,00	19,44	2,78	0,00
	2	47,22	5,56	36,11	5,56	5,56
	3	130,56	108,33	0,00	0,00	0,00
	4	13,89	11,11	2,78	2,78	11,11
	5	75,00	11,11	2,78	2,78	0,00
Mean concentration of coliforms (CFU/m ³)		72,22	32,22	12,22	2,78	3,33
Maximum concentration of coliforms (CFU/m ³)		130,56	108,33	36,11	5,56	11,11
Minimum concentration of coliforms (CFU/m ³)		13,89	5,56	0,00	0,00	0,00

Table 3
Antibiotic susceptibility profile for coliform isolates per site.

Number of positive samples	Site 1			Site 2			Site 3			Site 4			Site 5		
	Resistant (%)	Intermediate (%)	Susceptible (%)	Resistant (%)	Intermediate (%)	Susceptible (%)	Resistant (%)	Intermediate (%)	Susceptible (%)	Resistant (%)	Intermediate (%)	Susceptible (%)	Resistant (%)	Intermediate (%)	Susceptible (%)
Antibiotic															
Amoxicillin-clavulanic acid	80	0	20	80	0	20	75	0	25	25	0	75	0	0	100
Ciprofloxacin	20	0	80	20	0	80	0	0	100	25	0	75	0	0	100
Gentamicin	0	0	100	20	0	80	0	0	100	25	0	75	0	0	100
Meropenem	20	10	70	0	20	80	0	0	100	0	0	100	0	0	100
Sulfamethoxazole-trimethoprim	80	0	20	80	0	20	50	0	50	75	0	25	75	25	0
Tetracyclin	40	0	60	40	10	50	0	12,5	87,5	25	25	50	25	0	75

Table 4

Analysis of variance of percentage of antibiotic resistant coliform per site.

Source of variability	Sum of squares	Degrees of freedom	Average of squares	F	Critical value for F
Antibiotic	14006.67	5	2801.33	7.39	2.71
Site	3625.00	4	906.25	2.39	2.87
Error	7585.00	20	379.25		
Total	25216.67	29			

potentially through bioaerosols. The solution has been spread over the surface of the Mueller-Hinton plate and it was left to dry off before the placing of the antibiotic discs. The antibiotic discs were placed on the plates by using sterile forceps, while pushing them to ensure the complete contact between the media and the disc (Hudzicki, 2009). The plates were inverted and incubated at 37 °C for 18 h. The plates were checked to measure the diameter of the inhibition zone for each antibiotic and then compared to the Performance Standards for Antimicrobial Susceptibility Testing from the [Clinical and Laboratory Standards Institute \(2018\)](#). The bacteria were determined as susceptible, intermediately resistant, or resistant according to the values shown on [Table 1](#).

Six types of antibiotics were tested using the Kirby-Bauer method. The selected antibiotics (20/10 µg amoxicillin-clavulanic acid, 5 µg ciprofloxacin, 10 µg gentamicin, 10 µg meropenem, 1.25/23.75 µg sulfamethoxazole-trimethoprim, 30 µg tetracyclin), are commonly used in medical practice. Negative controls were prepared by using blank discs and ensuring that the inhibition zone non-existent around the control discs.

3. Results

The results of the enumeration of coliforms differ from 130,56 CFU/m³ to 0 CFU/m³ as shown in [Table 2](#). The analysis of the data collected from the isolation of coliform colonies from bioaerosol samples indicates that 80% of the 25 samples were positive for coliforms.

After the identification of colonies, these 20 positive samples were tested in duplicate to test the antibiotic resistance by using the Kirby-Bauer Disk Diffusion Susceptibility Test in order to assess the resistance profile. The analysis of data for each sample site is shown on [Table 3](#) and [Table 4](#). The information focused on the effectiveness of the antibiotics is shown on [Table 5](#). The multidrug resistance of the strains is evaluated on [Table 6](#).

The comparison of resistant bacteria, which isolates percentages from one site to another, was not statistically significant, as determined by the analysis of variance for one applied variable. An antibiotic is a significant factor according to the same analysis. [Table 3](#) shows the results of the analysis at a confidence level of 95%.

Broad patterns of antibiotic resistance were observed, with at least one sample exhibiting resistance to each of the tested antibiotics. Sulfamethoxazole-trimethoprim and amoxicillin-clavulanic acid have the highest resistant profiles, with 73% y 60% of resistant samples respectively, according to the information of [Table 5](#). The studied samples showed multidrug resistance in 62,5% of the analyzed cases since they are resistant in a range of two to four of the tested substances.

4. Discussion

This comparison is based on previous studies and reports on antibiotic resistance of coliforms along water bodies, including some in the Choqueyapu River. However, as of our knowledge, there are few studies focused on airborne particles, and even fewer in which coliforms are the reference strain to be used as an air quality environmental indicator. The percentage of resistance to a specific antibiotic of outdoor particles will differ not only from levels of environmental pollution, but it will also depend on the temporality of the sampling, closely related to

Table 5
Summary of coliform antibiotic resistance profile.

Antibiotic	Resistant		Intermediate		Susceptible	
	Coliform samples	Percentage (%)	Coliform samples	Percentage (%)	Coliform samples	Percentage (%)
Amoxicillin-clavulanic acid	24	60	0	0	16	40
Ciprofloxacin	6	15	0	0	34	85
Gentamicin	4	10	0	0	36	90
Meropenem	2	5	3	8	35	88
Sulfamethoxazole-trimethoprim	29	73	1	3	10	25
Tetracyclin	11	28	4	10	25	63

Table 6
Coliform samples which are multi-drug resistant.

Resistant profile		Coliform samples n = 40	Percentage (%)
Does not exhibit		5	12,5
One antibiotic		10	25
Sub-total		15	37,5
Multi-drug resistance	Two antibiotics	15	37,5
	Three antibiotics	4	10
	Four antibiotics	6	15
Sub-Total		25	62,5

environmental and weather conditions. Nevertheless, the results obtained in the reviewed studies suggest that the environment is a source of resistance to take into count to understand this global problem.

We cannot say specifically how many isolates are resistant, but by combining isolates and subjecting them to resistance testing, we are selecting the most resistant strains in this group of coliforms. Furthermore, by simultaneously testing multiple isolates from this group, we select for the most resistant profile in a given sample. As a result of this, airborne coliform samples are resistant to antibiotics that are commonly used in medical practices by showing high levels of antibiotic resistance and by being multi-resistant to a range of two to four antibiotics in most of the cases.

Within the reviewed academic works, the study conducted by Gandolfi et al. (2011) in the urban area of Milan is the closest to the one analyzed in our study when contrasting the tested antibiotics. Gandolfi et al. (2011) collected samples during winter and summer along an urban area predominantly affected by urban traffic, and disk diffusion was performed using ten antibiotics, which has in common the analysis of resistance to ciprofloxacin, gentamicin, and tetracycline. The level of antibiotic resistance from Enterobacteriaceae to these antibiotics is not significant with percentages of resistant isolates below 10%, in contrast with our results which define percentages of resistance in coliforms above 10% for these tested antibiotics. This shows the difference between the presence of an open sewer system on an urban location and its effects on the quality of the environment regarding public health.

Studies conducted in Mexico showed different resistance levels from water samples (Rivera Tapia et al., 2006) and a broad variety of environmental matrices as well (Junco Díaz et al., 2006). In the first case, around 15% of the isolated strains of Enterobacteriaceae were resistant to sulfamethoxazole-trimethoprim, in contrast to the 73% of resistant strains found in airborne particles near to the Choqueyapu River. Moreover, *Escherichia* strains showed multi-resistance to two or more antibiotics in 50% of the disk diffusion tests performed by Rivera Tapia et al. (2006), in contrast to the 63% of multidrug resistant isolates of the study conducted in Mexico. The susceptibility of the strains tested by Junco Díaz et al. (2006) were found to be significant for the common antibiotics used in the Kirby Bauer Test. In the same study, there are considerable levels of resistance that had been found against sulfamethoxazole-trimethoprim (8% of the *Vibrio cholerae* isolates and 9% of the *Aeromonas* sp. isolates) and for Tetracycline (with 14% of the

Aeromonas sp. isolates and 20% of the *Salmonella* sp isolates resistant). In contrast, we found a higher resistance to sulfamethoxazole-trimethoprim and tetracycline from coliform isolates tested for airborne particles near to the Choqueyapu River (73% y 28%, respectively). It is worth considering that the main difference between these studies was the medium from which the bacteria were collected. In the first case water samples were used for the study, in the second a broad variety of environmental matrices (a low percentage of them were taken from air samples); in our case, the medium was from airborne samples. The fact that resistance is higher in this study is highly concerning considering that bacteria has better conditions to persist in a water media than in the atmosphere. This might suggest that the quality of the basin is not adequate for human's interaction and it is in fact damaging to their health.

5. Conclusions

According to these results, airborne coliform samples are resistant to antibiotics that are commonly used in medical practice, such as sulfamethoxazole-trimethoprim and amoxicillin-clavulanic acid. The results did not only showed that coliform samples had high levels of antibiotic resistance, but also that the samples were multi-resistant to a range of two to four antibiotics in 62,5% of the analyzed samples. This phenomenon could possibly be explained by the polluting discharges with low concentration of antibiotic agents. These low concentrations may be enough to wipe out weak or non-resistant coliforms, but it would allow for stronger resistant coliforms to persist and they would contribute to the dissemination of this antibiotic resistance. This study provides an overview of the prevalence of resistant bacteria on the bank of the Choqueyapu River. As a result, the performed assessment provides additional information to understand the risks attached to the predominance of pollution and its effect on the environment. Given the complex interactions between human activities and the potential development of antimicrobial resistance investigated in this paper, further research is needed to evaluate the risks that may be detected downstream among the sampling sites, in agriculture, drinking water and the expected efficiency of the wastewater treatment plant to be implemented downstream of the Choqueyapu River.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2020.113494>.

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