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# Coliforms and antibiotic-resistant bacteria in water from rivers and wells at Curitibanos, Santa Catarina

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**Abstract**

The quality of water used for human consumption related to the absence of coliforms, which, which may cause diseases and exhibit antimicrobial resistance, a frequent issue in places with poor or non-existent environmental sanitation. The present study evaluated the concentrations of total and thermotolerant coliforms in rivers and wells in Curitibanos (SC), as well as the phenotypic resistance to four antibiotics (Ampicillin, Ampicillin+Sulbactam, Ciprofloxacin and Tetracycline), during four seasons of the year. Analysis of variance was performed and means were separated by the Scott-Knott test, at 5% error probability. In rivers, the highest values of fecal coliforms were recorded in summer and winter, and resistance to ampicillin and ampicillin+sulbactam was higher in autumn, while resistance to tetracycline was more prevalent in summer. In the wells, the highest averages of fecal coliforms occurred in summer, autumn and winter, and the highest levels of resistance were observed against ampicillin, in autumn. Overall, rivers had more compromised water quality, compared to wells, emphasizing the need to preserve aquatic resources in order to decrease evolution of resistance to antibiotics and diseases and deaths cause by superbacteria that may be ingested with contaminated water.

*Keywords: Escherichia coli*, multiresistance, water resources, antimicrobial resistance

# Coliformes e bactérias resistentes a antibióticos em águas de rios e poços em Curitibanos, Santa Catarina

**Resumo**

A qualidade da água usada para consumo humano está ligada à ausência de coliformes, que podem causar doenças e exibir resistência a antimicrobianos, problema frequente em regiões com saneamento ambiental precário ou inexistente. O presente estudo avaliou a concentração de coliformes totais e termotolerantes em rios e poços em Curitibanos (SC), bem como a resistência fenotípica a quatro antibióticos (Ampicilina, Ampicilina+Sulbactam, Ciprofloxacina e Tetraciclina), durante quatro estações do ano. A análise de variância foi realizada e as médias foram separadas pelo teste de Scott-Knott, a 5% de probabilidade de erro. Nos rios, os maiores valores de coliformes fecais foram registrados no verão e inverno, e a resistência à ampicilina e à ampicilina+sulbactam foi maior no outono, enquanto a resistência à tetraciclina foi mais prevalente no verão. Nos poços, as maiores médias de coliformes fecais ocorreram no verão, outono e inverno, e os maiores níveis de resistência foram observados frente à ampicilina, no outono. De maneira geral, os rios apresentaram maior comprometimento de qualidade da água, em comparação aos poços, reforçando a necessidade da preservação de recursos hídricos para diminuir a evolução da resistência a antibióticos e doenças e óbitos causados por superbactérias ingeridas com água contaminada.

*Palavras-chave: Escherichia coli*, multirresistência, recursos hídricos, resistência antimicrobiana

## Introduction

Water has become one of the most endangered environmental resources throughout the world due to high levels of physical, chemical and microbiological pollution that compromise its use (Sahin, Sivri, Akpinar, Cincin, & Sonmez, 2021; Ma, Shen, Tang, Li, & Dai, 2022). According to the United Nations (2020), most of global population does not have access to potable water, and has no alternative other than using water from ponds, rivers and wells. However, consumption of non-potable water may cause health problems due to ingestion of microorganisms that cause diarrhea, leptospirosis, hepatitis A and many verminosis (Chique *et al*., 2021; Ma *et al*., 2022). Data from the World Health Organization estimate that 3.4 million people die every year due to diseases acquired from contaminated water (World Health Organization, 2019).

In order to indicate fecal contamination in the water, it is necessary to verify the presence of thermotolerant coliforms, with *Escherichia coli* as the main representative, a strictly enteric bacterium (Madigan, Bender, Buckley, Sattley, & Stahl, 2021). This aspect is directly related to the release of untreated industrial, hospital and domestic effluents into water bodies, which represents an important route in the transmission of fecal contaminants through the environment (Meirelles-Pereira *et al*., 2002).

Most recently, another aspect of water microbiology has been investigated, which is microbial resistance to antibiotics (Ana, Madriaga, & Espino, 2021). Historically, it was believed that antibiotic-resistant bacteria were mainly acquired in hospitals and clinics, but further research indicated their presence in food, soil and water (Skandalis *et al*., 2021). In addition, the conventional treatment adopted by most water treatment plants is inefficient in removing emerging contaminants, which include antibiotics and substances capable of harming the health of humans and animals (Cartaxo *et al*., 2020).

Furthermore, it is known that infections caused by resistant bacteria are more serious when compared to sensitive bacteria, have a greater interval of resolution of the infection and are less responsive to treatments (World Health Organization, 2017). Hence, ingestion of untreated water with resistant *E. coli* strains can be related to increased chances of developing incurable infections, leading to serious health conditions and, ultimately, death (Dafale, Srivastava, & Purohit, 2020).

Occurrence of antibiotic-resistant *E. coli* has been extensively studied in urban drinking water (O'Flaherty, Borrego, Balcazar, & Cummins, 2018), but is poorly explored at rural areas, where people have limited or no access to treated water. A few reports on this topic were performed on either, wells or rivers, and point to concerning levels of antibiotic-resistance in *E. coli* in distinct regions of Brazil (França, & Melloni, 2014; Helena *et al*., 2019; Colet, Pieper, Kaufmann, Schwambach, & Pletsch, 2021). Information on seasonal *E. coli* contamination can be useful to predict the quality of water bodies at certain times of the year. Our objective was to evaluate the contamination of water from wells and rivers by *E. coli* in different seasons of the year and to identify the resistance of these microorganisms to four types of antibiotics (ampicillin, ampicillin+sulbactam, ciprofloxacin and tetracycline) in Curitibanos – SC.

## Materials and Methods

This study was carried on in the city of Curitibanos, Santa Catarina - SC, Brazil (27°16'58"S, 50°35'02"W), located at 987 meters above sea level. Five locations along Marombas and Pessegueirinho rivers and five wells located along both rivers were chosen as sampling sites (Table 1). Water from Marombas River is used for public consumption in the city, after treatment performed by a State Company. It is considered one of the most important medium-large size rivers in central part of the state and flows through 11 cities. Pessegueirinho River is entirely placed within Curitibanos, and receives most of pollutant discharges from urban areas. As most of both rivers flow through farms and rural areas where people do not have access to treated water, most population capture water to irrigate crops, farming, wash clothes, and also to drink without previously treating through physical, chemical or biological processes.

**Table 1**. Geographic coordinates of water sampling points in Curitibanos, Santa Catarina, Brazil.

| Site | Location | Latitude (S) | Longitude (W) |
|---|---|---|---|
| 1 | Pessegueirinho River source | 27°18'56.0" | 50°32'28.1" |
| 2 | Well near Pessegueirinho source | 27°18'50.1" | 50°33'11.7" |
| 3 | Pessegueirinho River middle course, after deposition of sewage and wastewater | 27°16'18.9" | 50°34'30.2" |
| 4 | Well near Pessegueirinho middle course | 27°16'26.6" | 50°34'35.8" |
| 5 | Pessegueirinho River delta, where it meets Rio Marombas | 27°12'06.6" | 50°34'18.2" |
| 6 | Well near Pessegueirinho delta | 27°12'49.4" | 50°33'54.8" |
| 7 | Marombas River middle course, near to Water Treatment Plant | 27°12'04.2" | 50°34'07.1" |
| 8 | Well near Water Treatment Plant | 27°12'28.2" | 50°32'31.9" |
| 9 | Marombas River after junction with Pessegueirinho River | 27°11'44.3" | 50°35'04.6" |
| 10 | Well in a property near point 9 | 27°12'16.7" | 50°35'12.2" |

Samples were collected throughout 12 months, contemplating four seasons: Spring 2020, Summer 2020/2021, Autumn 2021 and Winter 2021. In each sampling site, three 100mL portions of water were taken (each one was considered a repetition), with pre-sterilized flasks containing 10% sodium thiosulfate; hence 30 samples were collected at each season of the year.

The number of coliforms was estimated by the Multiple Tubes Technique (American Public Health Association, 2012; Ministério do Desenvolvimento Urbano e Meio Ambiente, 2013) and followed all federal agency requirements for water quality assessment in Brazil. Total coliforms were estimated in water from wells, whereas fecal coliforms were estimated in both, rivers and wells (Ministério do Desenvolvimento Urbano e Meio Ambiente, 2005; Conselho Nacional do Meio Ambiente, 2005). Water samples from rivers were first introduced into nine tubes with Lauryl Tryptose Broth, each containing a Durham tube. First, three 10 mL portions of water were disposed in three tubes containing 10mL double-strength medium. Then, three 1mL aliquots of water were introduced into three tubes with 10mL single-strength medium. Finally, three portions of 0.1mL were disposed in three tubes, each at 35.0 °C, 48 hours. Based on presence of microbial growth and gas production, positive tubes were identified and used to inoculate tubes with EC broth. Incubation was performed at 44.5 °C for 24 hours. Total number of positive EC tubes from in each dilution series was used to calculate the MPN - Most Probable Number – of fecal coliforms (Blodgett, 1998). Mean values of thermotolerant coliforms in river waters were calculated from five sampling sites (1,3,5,7 and 9).

Water samples from wells were first analyzed with five tubes, each with 10mL single-strength Lauryl Tryptose Broth. Incubation conditions, selection of positive tubes and procedures for fecal coliforms were the same applied to water samples from rivers. Inoculation of Brilliant Green 2% Bile Broth, followed by incubation at 35 °C for 48 hours, allowed detection of total coliforms, and quantification was expressed as Most Probable Number – MPN, of total coliforms (Conselho Nacional do Meio Ambiente, 2013). Mean values of total and thermotolerant coliforms in waters from wells were calculated from five sampling sites (2,4,6,8 and 10).

Fecal coliforms (*E. coli*) from each site were isolated with MacConkey Agar at 35°C, 24 hours. In this process, one positive tube from each repetition was used to inoculate one Petri Dish by streaking. From each Petri Dish, four individual *E. coli* colonies were selected to be tested for antimicrobial resistant test, based on methods of Bortoloti, Melloni, Marques, Carvalho and Andrade (2018). Each colony was inoculated in a tube with Muller Hinton Broth, and kept at 35 °C overnight (CLSI, 2018).

Antibiotic susceptibility test was performed through the disk diffusion method. Antibiotics tested were ampicillin, tetracycline, ciprofloxacin, ampicillin+sulbactam. Those are representatives of drugs from class A, B, C and D to treat infections caused by members of *Enterobacteriaceae* in standard health protocols of Brazil (Laborclin, 2019). Tubes with Muller Hinton Broth were used to inoculate plates with Muller Hinton Agar using a pre-sterilized swab. After inoculation, disks with each antibiotic were introduced and plates were kept at 35 °C for 16 hours. Control plates were also established, and those were inoculated with *Escherichia coli* ATCC® 25922 for quality control purposes (CLSI, 2018). Diameter of inhibition zone around each disk was measured and values were compared to standard ranges in order to define each bacteria colony as resistant or not (Laborclin, 2019). Percentage of isolates that were resistant to each antibiotic was then determined.

For statistical analysis, water samples from rivers and wells were analyzed as separate datasets. Sampling site and season were considered as sources of variation. Analysis of Variance was performed at 5% probability level. When differences among means were identified, the Scott-Knott test was applied to separate and rank means (Pr<Fc=0.05). Statistics was carried on with Sisvar software (Ferreira, 2011).

## Results and discussion

Fecal coliforms were detected in all river samples, and means were affected by both, sampling site and season (Pr>Fc=0.0391). Summer and winter were the seasons at which highest number of fecal coliforms was detected, at both Pessegueirinho and Marombas rivers (Table 2; Figure 1).

**Table 2.** Mean values of thermotolerant coliforms (*E. coli*) (MPN $100mL^{-1}$) in river waters from five sampling sites and four seasons between 2020 and 2021. Mean values of total and thermotolerant coliforms in waters from wells at five sampling sites and four seasons between 2020 and 2021. Curitibanos, Santa Catarina, Brazil.

| Site | Spring 2020 | Summer 2020 | Autumn 2021 | Winter 2021 |
|---|---|---|---|---|
| | Thermotolerant coliforms - Rivers | | | |
| 1 | 10.87$^{Ab*}$ | 560.00$^{Ba}$ | 43.67$^{Ab}$ | 24.47$^{Bb}$ |
| 3 | 441.67$^{Ab}$ | 786.67$^{Ba}$ | 233.33$^{Ab}$ | >1100.00$^{Aa}$ |
| 5 | 39.00$^{Ab}$ | >1100.00$^{Aa}$ | 401.33$^{Ab}$ | >1100.00$^{Aa}$ |
| 7 | 15.47$^{Ac}$ | 590.00$^{Bb}$ | 174.33$^{Ac}$ | >1100.00$^{Aa}$ |
| 9 | 45.33$^{Ab}$ | >1100.00$^{Aa}$ | 616.67$^{Aa}$ | 1100.00$^{Aa}$ |
| | Total coliforms - Wells | | | |
| 2 | 0.00$^{Ca}$ | 0.00$^{Ba}$ | 0.00$^{Ba}$ | 0.00$^{Ba}$ |
| 4 | >16.00$^{Aa}$ | >16.00$^{Aa}$ | >16.00$^{Aa}$ | >16.00$^{Aa}$ |
| 6 | 0.00$^{Cb}$ | 2.43$^{Bb}$ | >16.00$^{Aa}$ | >16.00$^{Aa}$ |
| 8 | 5.50$^{Bb}$ | 1.47$^{Bb}$ | 13.73$^{Aa}$ | 3.17$^{Bb}$ |
| 10 | 6.13$^{Bb}$ | >16.00$^{Aa}$ | 13.73$^{Aa}$ | >16.00$^{Aa}$ |
| | Thermotolerant coliforms - Wells | | | |
| 2 | 0.00$^{Ba}$ | 0.00$^{Ba}$ | 0.00$^{Ca}$ | 0.00$^{Ca}$ |
| 4 | >16.00$^{Aa}$ | >16.00$^{Aa}$ | 13.73$^{Aa}$ | >16.00$^{Aa}$ |
| 6 | 0.00$^{Bc}$ | 2.43$^{Bb}$ | 5.50$^{Ba}$ | 3.17$^{Bb}$ |
| 8 | 1.47$^{Ba}$ | 1.47$^{Ba}$ | 0.00$^{Ca}$ | 2.433$^{Ba}$ |
| 10 | 0.73$^{Bb}$ | >16.00$^{Aa}$ | 4.13$^{Bb}$ | >16.00$^{Aa}$ |

Means followed by the same letters and numbers are not different according to the Scott-Knott test. Uppercase letters indicate comparisons inside a column, lowercase letters refer to comparisons inside a line. Legend of sites: 1) Pessegueirinho River source; 2) Well near Pessegueirinho source; 3) Pessegueirinho River middle course, after deposition of sewage and wastewater; 4) Well near Pessegueirinho middle course; 5) Pessegueirinho River delta, where it meets Rio Marombas; 6) Well near Pessegueirinho delta; 7) Marombas River middle course, near to Water Treatment Plant; 8) Well near Water Treatment Plant; 9) Marombas River after junction with Pessegueirinho River; 10) Well in a property near Marombas River after junction with Pessegueirinho River.

Regarding water from wells, total coliforms were identified at all places and seasons, except site 2. Number of both types of coliforms was affected by both, location of the well and season of the year (Pr>Fc=0,0001 and Pr>Fc=0,0001, correspondingly) and data are shown in Table 2. Well number 4, which is located close to Pessegueirinho middle course, was the most contaminated water source. Overall, lowest frequency of microbiological pollution was observed at Spring.

In rivers, resistance of fecal coliforms against ampicillin and ampicillin+sulbactam was different among seasons (Pr>Fc=0.0003 and Pr>Fc=0.0006), but not among sampling sites. Highest percentages of bacterial resistance to both drugs were recorded at autumn (Figure 2).

Values of resistance to ciprofloxacin were very low (overall mean of 2.08%) and were not different among sampling sites or seasons (Pr<Fc=0.4340). On the other

hand, resistance to tetracycline was different among both, sampling locations and time of the year (Pr>Fc=0.0391). The highest means were 50 and 75% at the middle and lower course of Pessegueirinho River during summer (Table 3; Figure 1).

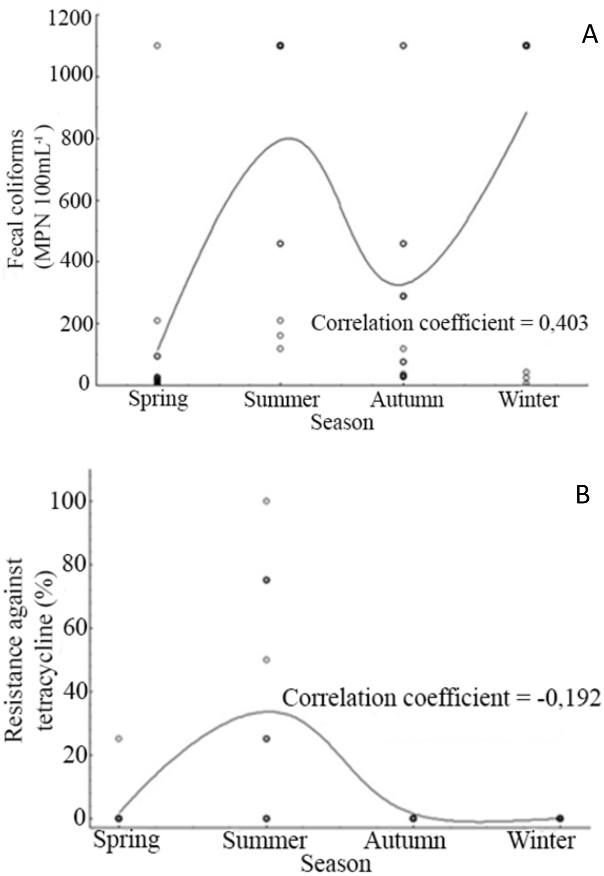


**Figure 1.** Correlation graph between fecal coliforms (A) and resistance (%) to tetracycline (B) in river waters from five sampling sites and four seasons between 2020 and 2021.

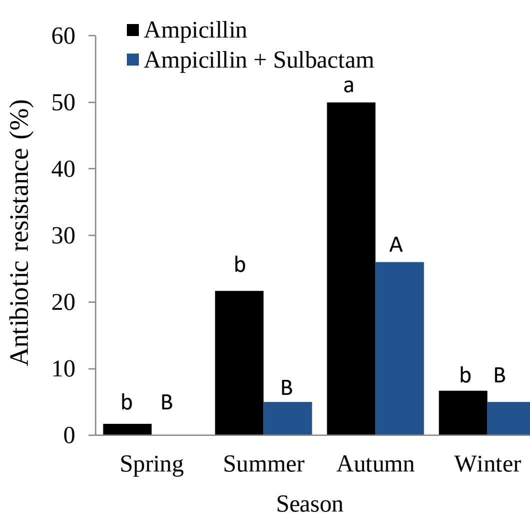


**Figure 2.** Percentage of fecal coliforms isolates that were resistant to ampicillin and ampicillin+sulbactam at spring, summer, autumn and winter. Means followed by the same letters and numbers are not different according to the Scott-Knott test. Lowercase letters indicate comparisons regarding ampicillin, uppercase letters refer to comparisons regarding ampicillin+ sulbactam.

Concerning bacteria isolated from wells, the percentages of isolates resistant to ampicillin was different among wells and seasons (Pr>Fc=0,0081). Highest mean values were observed at well number 6, at summer and autumn (Table 3). Resistance to ampicillin + sulbactam was less than 3% and was not affected by location or season (Pr>Fc=0,0547). The same was observed regarding ciprofloxacin (Pr>Fc=0,4665) and tetracycline (Pr>Fc=0,6977).

Our study has explored, for the first time, quality of water regarding coliforms and resistance against antibiotics in different water sources in the municipality of Curitibanos – SC, along the time frame of one year. Results from this research highlight very concerning aspects about microbiological pollution in rural areas, what may impose serious long-term effects on human and animal health, as well as environmental contamination.

The majority of sampling sites exhibited higher frequency of total and fecal coliforms at summer and winter. This might be associated to increased rainfall that is commonly observed at this region, between June and September. Higher precipitation may be associated to runoff of coliforms from soil to river and percolate to wells, and contribute to higher concentrations of bacteria in water, as observed by Helena *et al.* (2019), in which a positive correlation was observed between the presence of *E. coli* in the water and the existence of slopes on land adjacent to wells used to supply water intended for human consumption.

**Table 3.** Mean values of resistance (%) to tetracycline and ampicillin in river waters from five sampling sites and four seasons between 2020 and 2021. Curitibanos, Santa Catarina, Brazil.

| Site | Spring 2020 | Summer 2020 | Autumn 2021 | Winter 2021 |
|---|---|---|---|---|
| | | Tetracycline | | |
| 1 | 0.00 Aa* | 16.67 Ba | 0.00 Aa | 0.00 Aa |
| 3 | 8.33 Ab | 75.00 Aa | 0.00 Ab | 0.00 Ab |
| 5 | 0.00 Ab | 50.00 Aa | 0.00 Ab | 0.00 Ab |
| 7 | 0.00 Aa | 25.00 Ba | 0.00 Aa | 0.00 Aa |
| 9 | 0.00 Aa | 8.33 Ba | 0.00 Aa | 0.00 Aa |
| | | Ampicillin | | |
| 2 | 0.00 Aa* | 0.00 Ba | 0.00 Ba | 0.00 Aa |
| 4 | 8.33 Aa | 0.00 Ba | 33.33 Aa | 0.00 Aa |
| 6 | 0.00 Ab | 66.67 Aa | 100.00 Aa | 33.33 Ab |
| 8 | 0.00 Ab | 8.333 Bb | 0.00 Bb | 50.00 Aa |
| 10 | 0.00 Ab | 33.33 Ab | 0.00 Bb | 16.67 Aa |

Means followed by the same letters and numbers are not different according to the Scott-Knott test. Uppercase letters indicate comparisons inside a column, lowercase letters refer to comparisons inside a line. Legend of sites: 1) Pessegueirinho River source; 2) Well near Pessegueirinho source; 3) Pessegueirinho River middle course, after deposition of sewage and wastewater; 4) Well near Pessegueirinho middle course; 5) Pessegueirinho River delta, where it meets Rio Marombas; 6) Well near Pessegueirinho delta; 7) Marombas River middle course, near to Water Treatment Plant; 8) Well near Water Treatment Plant; 9) Marombas River after junction with Pessegueirinho River; 10) Well in a property near Marombas River after junction with Pessegueirinho River.

In most places around those rivers and wells, landscape is very hilly. Farms with cattle, swine and other animals are very common, and animals are allowed to freely move and defecate in all areas, as well as those close to water source. As animal feces are contaminated with both types of coliforms and are deposited on soil surface, rainfall will transport those microorganisms towards lower parts of the landscape, reaching rivers and wells as final destination. For instance, Invik *et al*. (2019) have reported that occurrence of *E. coli* in wells is highly related to rainfall and was associated to presence of animals in neighboring areas. Valenzuela, Almonacid and Barrientos (2009) observed that presence of both total and fecal coliforms was increased by precipitation in rural areas with cattle. At times, levels of bacteria may be so high that will lead to contamination of groundwater. When studying water from an aquifer in Argentina, Urseler *et al*. (2019) evaluated 62 samples, and 8% were contaminated with *E. coli*. The authors emphasized a strong influence of land use, related to high animal populations and wastewater treatment infiltration. In Brazil, Moretto *et al*. (2022) also related presence of *E. coli* to presence of animals that graze in areas surrounding two rivers. Positive correlations among precipitation and occurrence of *E. coli* in river waters were observed by Abreu and Cunha (2017) and Medeiros, Silva and Lins (2018), as well. Overall, values of coliforms in our study were higher than those reported by the referred authors in Brazil.

Overall, rivers showed more frequent occurrence of antibiotic-resistant bacteria than wells. This suggests that communities that drink water from wells are using a source that is safer than rivers, which is a positive aspect. However, it reveals a very serious environmental problem in two of the most significant rivers that flow through Curitibanos. Furthermore, summer and autumn were seasons at which bacteria resistant to ampicillin were more present at all sampling sites. At those same seasons, rivers were more contaminated with bacteria resistant to ampicillin+sulbactam. This emphasizes that water from those places must be carefully considered at summer and autumn, mainly when collected for drinking purposes. Regarding resistance to the other two drugs, both types of water sources are safe for consumption at any time of the year.

Occurrence of antibiotic-resistance microorganisms in rivers is a subject that has received increased attention in science community for the past years, given its consequences on all spectrum of one-health concept. In fact, previous studies in Brazil and other countries have revealed thoughtful numbers regarding this subject. In India, Diwan *et al.* (2018) registered highest percentage of *E. coli* resistance to ampicillin in autumn. In Spain, Pérez *et al*. (2022) performed evaluations in the Revion River throughout one year, and reported higher frequency of resistance against ampicillin at winter. About ciprofloxacin, levels were low and constant throughout all seasons (a pattern that is similar to our current results).

In Brazil, França and Melloni (2014) found high levels of resistance against ampicillin (37%), whereas ciprofloxacin was one of the drugs agains which coliforms were more sensitive. Böger *et al*. (2021) detected resistance of *E. coli* to several antibiotics in Brazil, but the most worrying aspect was the presence of beta-lactamase producing bacteria, what makes them resistant to drugs that are combined even with beta-lactamase inhibitors. The same characteristic was detected in rivers at Curitibanos, once resistance to ampicillin+sulbactam was frequently observed. This is an alarming fact concerning development and spread of multi-resistant strains, because even broad-spectrum drugs are no longer effective to kill those bacteria.

Hence, use and consumption of water from those sources, mainly from rivers, require attention and sanitation practices to decrease levels of coliforms. In ingested by humans or animals, resistant bacteria may cause infections that will no longer be treated by certain antibiotics, or even spread resistance genes to other microbes that are native components of human microbiota (Huddleston, 2014).

Also, a very impacting research on this topic showed that 2/3 of rivers worldwide are contaminated with antibiotics, and levels were up to 300 times higher than those considered safe (Wilkinson, & Boxall, 2019). Authors discuss that those rivers, from 72 countries, will in fact trigger development of superbacteria, a global concern, because people will be at risk of life-threatening diseases.

Our study revealed high-risk sites and seasons that are not safe for water consumption regarding concentration of coliforms and occurrence of antibiotic-resistant isolates. However, further research is needed to investigate and determinate: 1) causes and sources of microbiological pollution; 2) correlations with land use and antibiotic use by population; 3) outcomes and consequences on animal and human health. Altogether, these efforts will contribute to decrease widespread contamination of river systems and promote better quality of life of people who do not have access to treated water and adequate sanitation practices.

## Conclusion

Water from most wells and rivers in Curitibanos is not safe for use without previous treatment, and imposes serious risks to people and animals due to occurrence of total and fecal coliforms, as well as antibiotic-resistance. Use of water resources should be avoided at specific seasons due to highest frequency of resistance against ampicillin in bacteria. Observation of resistance to a broad spectrum drug (ampicillin+sulbactam) is concerning for its potential to multidrug resistance, that is strongly related to non-treatable infections and may impose serious problems to public health systems.

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