


Water quality monitoring in southern Brazil and the assessment of risk factors related to contamination by coliforms and *Escherichia coli*

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ABSTRACT

The potability of water, including underground sources, is constantly affected by human activities. To assess water quality and water security in rural and urban areas of southern Brazil, a quantitative, retrospective analysis of water samples collected monthly by the Brazilian health authorities (19,687 samples from 2013 to 2021) was performed. In rural areas, 5,979 water samples (77.54%) were found to be contaminated by coliform bacteria and 3,431 (44.50%) by *Escherichia coli*. In addition, 1,616 (20.95%) of the contaminated samples were significantly correlated with rainfall amount. In urban areas, 1,268 (10.95%) of the samples contained coliform bacteria and 293 (2.53%) of these samples contained *E. coli*, with the factor of rainfall associated with 1,081 samples (9.33%) with bacterial contamination. In terms of physicochemical parameters, turbidity exceeded the national standard (5 uT) in 448 (2.32%) samples and fluoride fell below the required level (0.8 mg/L) in 106 samples (0.54%). The presence of free residual chlorine (0.2–2.0 mg/L) was verified in 846 samples (14.38%) in rural areas and in 10,825 samples (56.13%) in urban areas. These results suggest a strong association between rainfall factors and physicochemical alterations, as well as the risk of greater microbial contamination of water for human consumption.

Key words: physical–chemical factors, rainfall, water diseases, water microbiology, water quality

HIGHLIGHTS

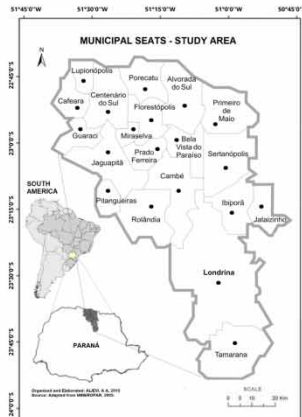
- Water samples collected in rural areas were contaminated by coliforms.
- This study explores the correlation amount of rain and bacterial contamination in rural and urban areas.
- Physicochemical parameters of water samples deviated from the national standards.
- There is a necessity to enhance the quality of water provided by public systems.

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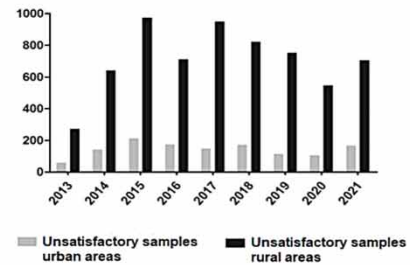
GRAPHICAL ABSTRACT

Water quality monitoring in southern Brazil and assessment of risk factors related to contamination by coliforms and *Escherichia coli*

The aim of this study was to understand the risk factors for human health due to water contamination by total coliforms and *E. coli* from various sources in rural and urban areas, in Southern Brazil



- ✓ Methodology Colilert (IDEXX ©- 9223-A)
- ✓ Nephelometric Method (2130 B)
- ✓ Potentiometry Technique (4500-F)
- ✓ DPD Colorimetric Method (4500- Cl G)



- ✓ Water samples collected in rural areas were contaminated by coliforms;
- ✓ Correlation amount of rain and bacterial contamination in rural and urban areas;
- ✓ Physicochemical parameters of water samples deviated from the national standards;
- ✓ Need to improve the quality of water supplied by public systems.

INTRODUCTION

The public provisioning of potable water is indispensable for daily life and the economic development of the population and is also crucial for the maintenance of public health (Gorai & Kumar 2013; Kumari *et al.* 2014; Uddin *et al.* 2018; Bux *et al.* 2022).

Water quality and availability are affected by human activities and socioeconomic development (Javed *et al.* 2017; Uddin *et al.* 2018). In the absence of water distribution through public supply systems and the availability of basic sanitation resources, the local population seeks alternative solutions, such as capturing water *in natura*, with underground water resources sometimes being the only possible drinking water supply (Hirata *et al.* 2019).

Untreated water sources are vulnerable to microbial contamination. The risk of exposure to microbial pathogens is significant in rural and peri-urban areas and is compounded by misinformation and limited financial resources for periodic monitoring (Wescoat *et al.* 2007; Stallard *et al.* 2021). The Brazilian government sets minimum standards for the quality of water for human consumption as well as standards for monitoring the quality of potable water. Currently, the standard for clean potable water stipulates the absence of any coliform bacteria (measured as total coliforms, TCs), including the absence of *Escherichia coli* (EC) in a 100-mL sample of water, the maximum concentration of fluoride 1.5 mg/L, the maximum turbidity of 5 uT, and the concentration of residual chlorine between 0.2 and 2.0 mg/L (Brasil 2016).

Waterborne diseases are transmitted by water contaminated by viruses, bacteria, and parasites and are eliminated through the feces of infected animals or individuals. The most common route of transmission is the oral route, in which the pathogens are ingested with water, pass through the gastrointestinal tract, and are eliminated through the feces, completing the cycle of infection (Ríos-Tobón *et al.* 2017).

Despite the obvious importance of water potability in southern Brazil, there are few epidemiological studies in which the correlation between water quality parameters (physicochemical and microbiological), rainfall, and residual chlorine has been investigated. Accordingly, the aim of this study was to understand the risk factors for human health due to water contamination by TCs and *E. coli* from various sources in rural and urban areas.

METHODS

Study design

The data utilized in this study were derived from analysis of water samples obtained from the Brazilian National Water Quality Surveillance Program for Human Consumption. The samples represent water sources in 21 municipalities (Figure 1) located in the north of Paraná, southern Brazil, with a population of approximately 850,000 (IBGE 2017).

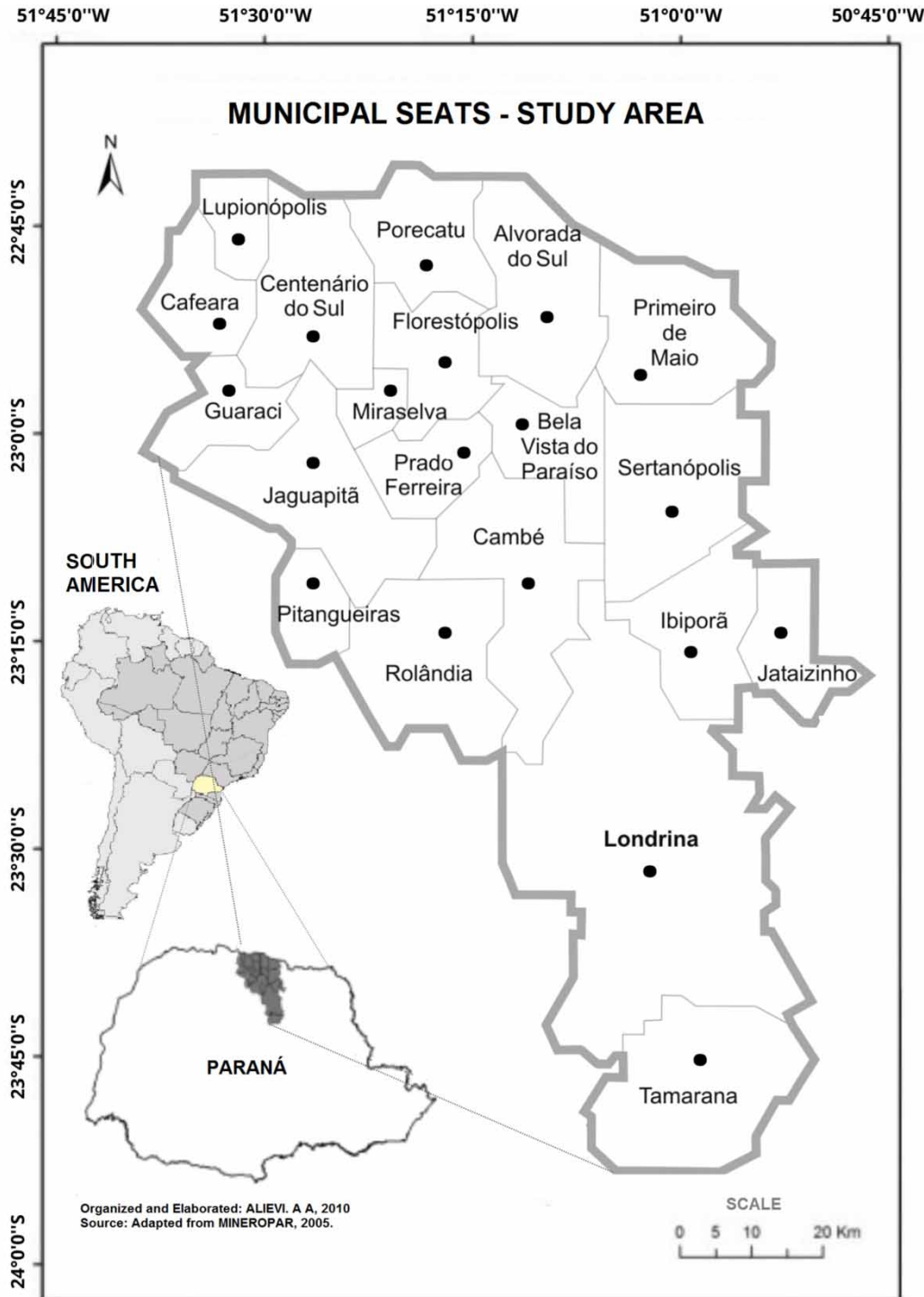


Figure 1 | The municipal seats where water samples were collected in southern Brazil, Paraná.

Characterization of the study

The collection points were delimited in accordance with the Brazilian National Guidelines for the Sampling Plan for the Surveillance of Water Quality for Human Consumption, which were determined based on population. In addition, data from the Laboratory Environment Manager (GAL) system were evaluated according to the Ministry of Health protocols. In extracting the GAL data, a threshold of annual entries was considered, 2013–2021. The data were grouped according to the frequency of annual water surveys carried out by municipalities. The potability standards (both physicochemical and microbiological) that were considered in this analysis are listed in Table 1.

Physicochemical analysis

In the physicochemical analysis, turbidity tests, and fluoride quantification, water samples were collected in sterilized 100-mL flasks. The nephelometric method (2130 B) was used according to the 23rd Edition of the Standard Methods for the Examination of Water and Wastewater (SWWM) and the potentiometry technique (4500-F C) with an ion-selective fluoride electrode. This study was conducted in accordance with the standard methodologies of the American Public Health Association (APHA 2017). Free residual chlorine was exclusively quantified in the field at the time of collection. The applied methodology was in accordance with the SWWM using the standard technical procedure 4500-Cl G. DPD *Colorimetric Method*.

Microbiological analysis

Water samples were collected in sterilized 100-mL flasks containing 10 mg of sodium thiosulfate and stored at 4 °C until sample processing. To determine the presence of TC bacteria and *E. coli*, the chromogenic/enzymatic substrate methodology Colilert – 9223 A was used in accordance with the 23rd Edition of the SWWM. The analysis is based on the reaction of the enzyme-substrate and is suitable for both detection and quantification. TCs and *E. coli* were quantified as the most probable number (MPN) according to the Colilert manual.

Statistical analysis

Indicator factors were also associated with this study. Groups are expressed as odds ratios (ORs) based on the number of cases with unsatisfactory results related to unmet potability parameters based on national legislation. Multivariate logistic regression analysis was performed, and ORs were calculated from the prediction model (Menard 2002). The level of significance was considered as $\alpha = 5\%$. Statistical analyses were performed using the R software (version 4.1.1). The statistical significance of the variables in this model does not make the model predictive.

RESULTS AND DISCUSSION

Disparities in water quality in rural and urban areas

The collected samples were analyzed in terms of whether they satisfactorily met the water quality standards issued by the Brazilian Government. Of the total satisfactory samples, 10,289 (52%) were collected from urban areas and 1,709 (9%) from rural areas. In contrast, the number of samples considered unsatisfactory for the potability standard in urban areas was 1,688 (9%). In rural areas, where water collection is predominantly *in natura* – that is, without any treatment – the

Table 1 | Potability standards of water quality variables

Water quality variables	Potability standards		
	SAA	SAC	SAI
Free residual chlorine (mg/L)	0.2–2.0	0.2–2.0	0.2–2.0
Fluoride (mg/L)	1.5	1.5	1.5
Turbidity (uT)	To 5.0	To 5.0	To 5.0
TCs (present/absent)	Up to one positive sample ^b per SAA	Up to one positive sample ^b per SAC	Absent ^a
<i>Escherichia coli</i> (present/absent)	Absent ^a	Absent ^a	Absent ^a

SAA, water supply system; SAC, collective alternative solution; SAI, individual alternative solution.

^aOrdinance GM/MS No. 888/2021.

^bIn SAA and SAC that supply up to 20,000 inhabitants.

high rate of poor water quality was reflected in the fact that 6,001 samples (30%) were considered unsatisfactory or unfit for human consumption.

The sampling rate influenced the parameters analyzed in this study because the water supply system was superior in relation to the Collective Alternative Solution and the Individual Alternative Solution corresponding to the sampling planning requirements in force in the national documents (Brasil 2016). In this analysis, the percentage of unsatisfactory samples in rural areas was 30% (6,001 samples), which overlaps with the rate of 9% (1,688 samples) in urban areas. In Brazil, access to drinking water and sewage systems is inconsistent in rural and urban areas. Consequently, vulnerable populations are exposed to higher rates of contaminated water from alternative water sources, such as clandestine connections in networks or wells, leading to diseases related to inadequate sanitation (Brasil 2020).

The establishment of social tariffs in Brazil would make it possible for individuals living in areas with poor water quality to access better-regulated water sources, which would lead to improved social equity without negatively impacting the financial stability of the country. This is an especially important option, given the current debate in Brazil over the legality of cutting off water supply to non-paying users (Grott *et al.* 2018).

Authors such as Moe & Rheingans (2006) raised the important issue of maintaining water quality in water distribution systems, especially in developing countries, where there are resource limitations such as the infrastructure of sets of pipes and the use of disinfectant properly. The main problems, in addition to the vulnerability of microbiological contamination of the water, are centered on the high demand for urbanization, causing the discontinuity of the quality that the distribution networks offer illegal connections to the distribution systems in poor neighborhoods.

Assessing microbial contamination in urban and rural drinking water sources

Of the 19,687 samples analyzed in this study, 11,574 (60%) were collected from urban areas and 7,710 (40%) from rural areas. In urban areas, the great majority of samples (10,825, 93.52%) were collected from trestle-hydrometers, compared with 376 (3.24%) samples from a source/springs/mine, 163 (1.40%) from deep tube wells, 161 (1.39%) from artesian wells, 26 (0.22%) from shallow wells, 18 (0.15%) from groundwater wells, and 5 samples (0.04%) from cisterns. On the other hand, in rural areas, springs and artesian wells were the predominant collection points, with 3,041 samples (39.44%) collected from springs, 2,118 (27.47%) from artesian wells, 752 (9.75%) from shallow wells, 846 (10.97%) from a trestle-hydrometer site, 523 (6.78%) from groundwater wells, 401 (5.20%) from deep tube wells, and 29 (0.39%) from cisterns.

We evaluated the ratio of the incidence of microorganisms present in drinking water to the sample origin and verified the prevalence of bacteriological contamination in water sources with no treatment, supporting the hypothesis of a deficit in water quality. These results are largely consistent with those of previous studies in which high levels of coliform bacteria, including *E. coli*, were found in groundwater wells (Richardson *et al.* 2009; Motta *et al.* 2014; Atherholt *et al.* 2015).

Effect of water supply form on water quality parameters

Of the 19,687 samples analyzed in this study, 3,417 (16.63%) were obtained from a spring. Of these samples, 2,920 (85%) were contaminated with coliform bacteria including 2,012 samples (58%), in which *E. coli* was detected. Turbidity above the regulation standard was found in 252 (7%) of the water samples from springs, and 10 samples (0.29%) contained fluoride above the maximum permitted value. In the spring water samples, rainfall and contamination were associated in only 591 (17%) of the samples.

In the case of water sourced from groundwater extraction, 2,279 (11.57%) samples were obtained from artesian wells, of which 1,442 (62%) were contaminated by coliform bacteria and 599 (26%) by *E. coli*. In terms of turbidity and fluoride, 43 (1.88%) and 56 (2.45%) of the samples presented values above the maximum allowed value, respectively. Rainfall was correlated with contamination in 371 (16%) of the samples.

Only 541 samples (2.74%) were collected from groundwater wells, and 481 of these samples (89%) were contaminated by coliforms and 284 (52%) by *E. coli*. In 21 of the samples (3.88%), turbidity was above the recommended standard, and rainfall was a factor in 128 (24%) of the samples with microbial contamination.

Of the 564 samples collected from the deep tubular wells, representing only 2.86% of all samples, 279 (50%) were contaminated by coliforms and 92 (16%) by *E. coli*. The level of turbidity was found to exceed the standard in nine samples (1.59%) and fluoride exceeded the maximum allowed level in three (0.53%) of the samples. Among the samples collected from the deep tubular wells, 136 (24%) showed an association between rainfall and contamination.

From shallow wells, 778 samples (3.95% of all samples) were collected. Of these, 559 (71%) had coliform bacteria and 419 (54%) *E. coli*. Turbidity exceeded the standard in 64 samples (8.22%), and only one sample (0.1%) contained fluoride above the maximum level. In 145 of the samples (19%), rainfall was found to be a factor in contaminated samples.

The largest proportion of water samples was collected from trestle-hydrometers: 11,671 (59.28%) of the total samples in this study. Of these, 1,310 (11%) were found to contain coliforms but only 263 (2%) of the samples contained *E. coli*. Turbidity exceeded the standard in 56 (0.47%) of the samples, and 36 samples (0.30%) contained fluoride at a concentration that exceeded the regulation maximum. In 998 (8.55%) of the samples, rainfall was a factor in the contamination of the samples (Figure 2).

The contamination of potable water by coliform bacteria, including *E. coli*, can occur in previously treated water as well as in water from alternative sources in both rural and urban areas, posing a risk to human health. In the determination of coliforms, a differentiation is made between those of fecal and non-fecal origin. Despite the recognition that the TC group includes coliform species of environmental origin and, therefore, is probably not specific for fecal contamination (Fewtrell & Bartram 2001).

Historically, in Brazil, there have been sanitary deficits, affecting the most vulnerable populations that lack access to treated drinking water as well as proper wastewater disposal. These sanitary deficits cause deaths from waterborne diseases, such as diarrhea, cholera, typhoid fever, and hepatitis (Bertoncini & Cavassin 2019). In our study, we showed that microbiological parameters such as the presence of coliforms ($p < 0.05$) and *E. coli* ($p < 0.05$) represented a greater number of unsatisfactory samples for the parameters guided by Brazilian legislation. Microorganism-contaminated water is one of the main causes of intestinal dysfunction, making it essential to monitor the microbiological quality of water for human consumption (Edberg *et al.* 2000; Uprety *et al.* 2020).

Current Brazilian legislation requires that, in suspected outbreaks of waterborne diseases, analytical tests should be carried out, including an assessment of the turbidity pattern, levels of residual disinfectant agent (chlorine), and the presence of

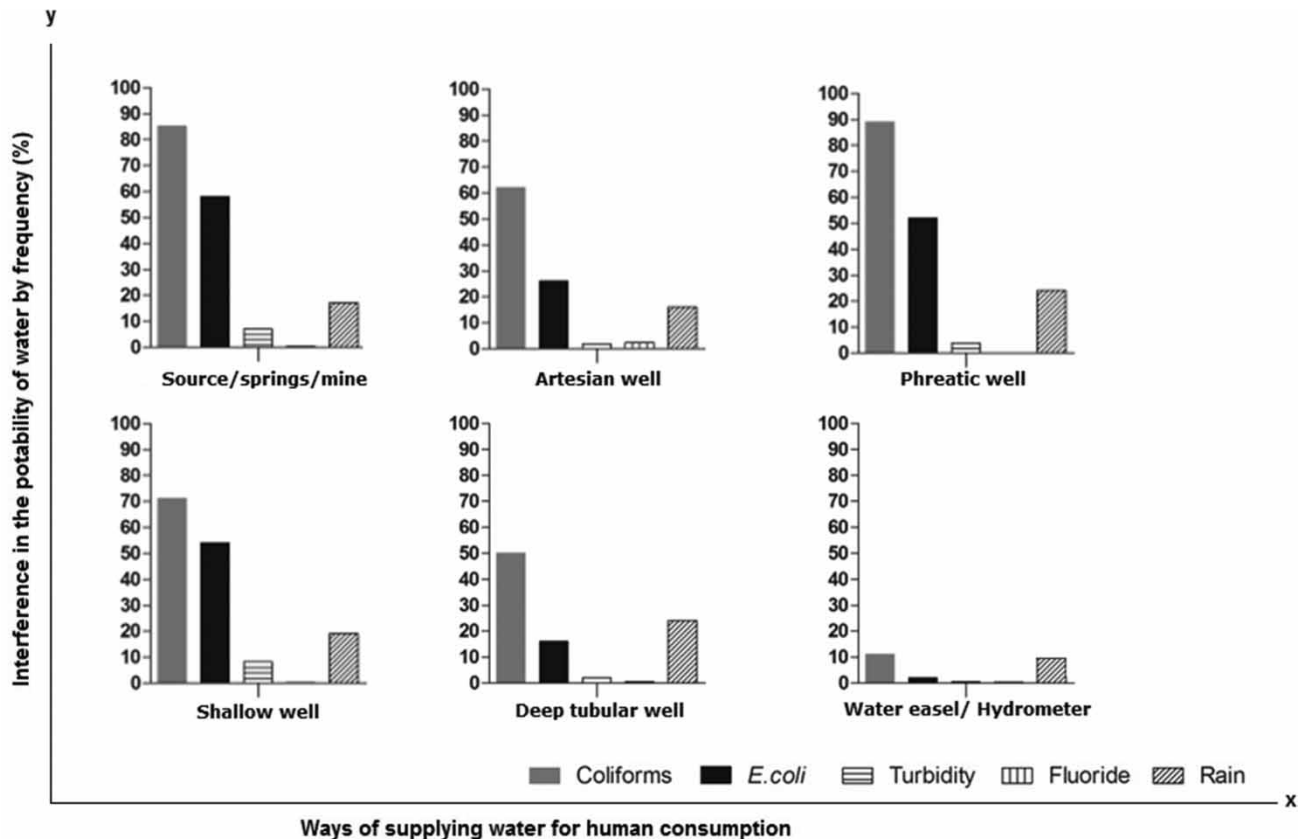


Figure 2 | Percentage of parameter interference in water collection sources for human consumption (2013–2021).

E. coli, to determine whether the water is safe for human consumption (Brasil 2021). According to the current water quality standard, bacteriological indicators are linked to TCs as an indicator of the integrity of the distribution system and *E. coli* as an indicator of fecal contamination. The increased number of drinking water samples showing microbial contamination should serve as an alert for the municipalities covered in this study.

According to the Ministry of Health, in Brazil, a total of 40,238,250 cases of acute diarrheal diseases (ADDs) were reported during the period from 2013 to 2021 (Table 2). In the same period, the region of this study recorded 121,882 cases of ADDs (Brasil 2023). Despite these data being aggravating, we cannot state that only water contamination can be corroborated for these cases, as diarrheagenic diseases can be bacterial, viral, parasitic, or even caused by toxins.

Water contamination in rural areas: TC bacteria and *E. coli*

We compared the rate of the contamination of potable water from different forms of supply (Table 3) and compared treated and non-treated sources.

Of the total samples of potable water collected in rural areas, 5,979 (77.54%) were found to be contaminated; approximately 3,431 (44.50%) tested positive for the presence of *E. coli*. In addition, the presence of rainwater affected approximately 1,616 (20.95%) of the samples that did not comply with potability regulations.

The water quality of samples from the different sources was found to decrease in the following order: deep tubular wells (OR: 1.36; CI: 1.15–1.83) > artesian wells (OR: 2.99; CI: 2.27–3.3) > groundwater wells (OR: 13.07; CI: 9.61–18.84) > shallow wells (OR: 17.59; CI: 12.8–24.38). Our results are in agreement with the findings of Kilungo *et al.* (2018), in which closed wells showed lower levels of TCs, *E. coli*, and turbidity than did open wells. The quantification of bacteria that indicate water quality can vary significantly, depending on factors such as the geological configuration of the well, the number of times the well is sampled, and the analytical method for generating the data (Atherholt *et al.* 2015; Atherholt *et al.* 2017).

Table 2 | Cases of ADDs in Brazil (2013–2021)

Year	Number of cases of ADD (per year), in Brazil
2013	4,419,092
2014	4,885,544
2015	4,260,404
2016	4,801,442
2017	4,910,833
2018	4,970,505
2019	5,581,305
2020	3,169,235
2021	3,239,890
Total: 40,238,250 cases of ADD in Brazil	
In 21 municipalities of this study (2013–2021): 121,882 cases	

ADD, acute diarrheal diseases.

Table 3 | Risk of contamination from alternative sources to public supply

Origins	Interference in water potability (OR)	P(Likelihood-ratio (LR)-test)
Shallow well	17.59 (13.37–23.13)	<0.001
Source/springs/mine	15.57 (14.07–17.24)	<0.001
Water well	13.07 (9.77–17.49)	<0.001
Artesian well	2.99 (2.75–3.24)	<0.001
Deep tube well	1.36 (1.16–1.59)	0.002

Conditions that affect potable water quality include climate (heavy rainfall and long periods of rain), hydrogeological environment (groundwater exposure, soil recharge rates, and runoff flow), whether the well was appropriately located, proximity to septic tank systems, land use, the use of manure in the soil, wastewater products, and the presence or absence of water quality monitoring (Charrois 2010; Latchmore *et al.* 2020).

Another category of critical factors that determine the likelihood of contamination is the structural condition of the well, including the model of the well, depth, age and condition, damage to the piping, distance from cesspools, the presence or lack of sanitary protection, and the existence of holes at the collection point, which would allow the entry of chemical, physical, and biological contaminants through percolation or flow (Oliveira 2017; Grott *et al.* 2018).

Regarding the quality of water from springs (OR: 15.57; CI: 8.75–12.96), this type of water source was found to be more vulnerable to microbiological contamination, compared to other types of sources. The springs analyzed in previous studies showed contamination by microorganisms, such as TCs and *E. coli*, similar to what was found with alternative sources of supply (Mormul *et al.* 2006; Rocha *et al.* 2006; Rheinheimer *et al.* 2010; Cavalcante 2014; Agrizzi *et al.* 2018).

In a similar study conducted in the north of Paraná, the authors found genes that encode *E. coli* in 51 (17.29%) of the samples analyzed, and water was identified as a vehicle for the transmission of strains. Thus, microbial contamination has the potential to cause failures in water distribution systems (Cestari *et al.* 2016). In a meta-analysis study, the presence of microorganisms that indicate poor water quality was correlated with the occurrence of diarrhea (Ercumen *et al.* 2014).

In a prospective study carried out by Moe *et al.* (1991), the authors correlated the prevalence of diarrheal diseases with the quality of water for human consumption using microbiological indicators of water quality, indicating highly contaminated water as an important source of exposure to contamination fecal and diarrheal pathogens. However, the relationship between drinking water storage and diarrheal diseases found conflicting results (VanDerslice & Briscoe 1993), considering that changes in the quality of drinking water during domestic storage may be more an indicator of domestic hygiene than a risk factor for diarrheal diseases.

Studies carried out by Loyola *et al.* (2020) demonstrate the contamination of fecal indicator bacteria in water for human consumption, reflecting on the health of children related to diarrheal disease. The study has some limitations regarding the imprecision about the source of contamination being exclusively of water origin, with the possibility of other origins arising contamination by enteric pathogens, such as the variability of sanitary conditions. Other current studies encounter the same difficulty, although improving the quality of the water source can have a substantial impact on exposure to diarrheal diseases (Navab-Daneshmand *et al.* 2018; Khan 2020).

Microbial contamination of water in urban areas

Water treated at treatment plants accounts for the largest portion of drinking water sources in urban regions. In Brazil, conventional water treatment has successive stages: coagulation, flocculation, sedimentation by filtration, and disinfection, which is carried out by means of Water Treatment Stations (WTS) (Richter & de Azevedo Netto 2021). In urban areas, only 1,268 (10.95%) of the samples showed contamination in the form of TCs, and *E. coli* was detected in 293 (2.53%) of the samples analyzed in this area, with the presence of rainy periods associated with 1,081 (9.33%) of the contaminated samples.

In urban areas, 10,892 samples (90.94%) were collected from trestle-hydrometer sites (OR: 0.05; CI: 0.2–0.28), which, in comparison to other forms of water supply, are less likely to be contaminated. Water quality parameters of samples from taps (OR: 0.15; CI: 0.02–1.18) showed a greater chance of interfering with water quality when compared to easels and hydrometers. Water distribution systems undergo conventional drinking water treatment processes, such as filtration, sedimentation, disinfection, and flocculation, to improve water quality (Loubet *et al.* 2016).

The presence of coliforms, including *E. coli*, in the water supply and distribution system persisted for years in this study. The long-term deterioration of water quality in the study region is an important finding and is indicative of problems at different stages of the distribution system from the source to the end user. (De Moel *et al.* 2006; Liu *et al.* 2017).

Water distribution systems in public treatment plants can be contaminated by microorganisms gaining access through damaged pipes, the formation of biofilms and recrudescence of bacterial growth, as well as a decrease in the level of chlorination in the distribution system (Hallam *et al.* 2001; Artiola *et al.* 2012).

Physicochemical water quality standards in water for human consumption

In the urban and rural areas, turbidity above the maximum allowed value was observed in approximately 445 (2.30%) samples, which generated unsatisfactory results regarding water quality. In the present study, turbidity is considered a

negative effect on water quality and can cause failure in the quality of drinking water (OR: 26.81; CI: 18.82–38.18). In general, turbidity affects various forms of water supply and is associated with the presence of particles and microorganisms in the water, potentially leading to ineffective disinfection of the water and ensuing health risks.

In 106 (0.54%) of the samples analyzed in this study, fluoride levels were found to be below the value stipulated in the national water quality regulations of Brazil (1.5 mg/L) for effective disinfection. The antimicrobial activity of fluoride in caries is evidence of its effect on bacterial metabolism (Marquis 1995). In this study, fluoridation was significantly correlated ($p < 0.001$) with the non-impedance of water quality. As demonstrated in previous studies, the low fluoride content in water is associated with very mild, mild, and moderate dental fluorosis in children (Yu *et al.* 2018). The harmful impacts of long-term exposure to contaminants in drinking water can be ascertained using health risk assessment methods (Selinus *et al.* 2005; Ayoob & Gupta 2006; Radhika & Sai Charan 2017; Kaur *et al.* 2020; Mohammadpour *et al.* 2022).

From this perspective, the data presented in this study highlight the need for the improved management of regional water resources in order to provide safe drinking water. Due to the large number of samples, there is a possibility of underestimating the real risk; therefore, the use of probabilistic analyses, such as simulation studies, provides a more accurate assessment of the risk to human health (Fernández-Macias *et al.* 2020).

Chlorine, as a disinfectant, was detected in water samples from treatment plants in only 846 (4.38%) of the samples analyzed in rural areas compared with 10,825 (56.13%) of the samples in urban areas. The results of the present study demonstrate how water sources with chlorination levels that do not meet the national standard (OR: 18.58; CI: 17.15–20.13) are more likely to result in unsatisfactory results in terms of microbial contamination; as chlorine level diminishes, the microbial count increases.

CONCLUSIONS

The risk factors influencing water contamination by coliform bacteria, including *E. coli*, in potable water supply systems in the municipalities of northern Paraná from 2013 to 2021, are mainly related to alternative forms of supply. The results of this study underscore the need to take action to improve potable water sources – with a particular focus on rural areas – through the application of continuous disinfection, in addition to improving the structural conditions of wells. Rainfall is a risk factor that impacts water quality, promotes increased turbidity and microbiological contamination, and adversely affects water treatment. Fluoridation, in general, limits microbial growth; however, the results of our study demonstrate that fluoride levels in drinking water sources vary, depending on the maintenance and stability of the supply system. The results showed concentrations below the acceptable levels for the protection of oral health, indicating the need to improve the operational control of fluoridation in treatment plants, along with continuous monitoring through local health surveillance. Insufficient concentrations of free residual chlorine can increase microbiological contamination in water distribution systems, and when adequate disinfectant concentrations are maintained, the incidence of microbiological indicators can be reduced. Therefore, surveillance actions should be intensified, together with water supply companies, through the adoption of congruent disinfection. Water quality monitoring is of great importance for public health; the incidence of outbreaks and waterborne diseases should be investigated in future studies, and actions taken to monitor water quality and enforce the standards for potability.

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DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST

The authors declare there is no conflict.

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