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Parasitological, microbiological, and antimicrobial resistance profiles of raw and drinking water in a tourist city in the tri-border region of South America

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ABSTRACT

Despite the large amounts of freshwater available in Brazil, the deterioration of surface water can represent a risk of waterborne disease for national and international tourists. The main goal of this study was to assess the quality of drinking water in the triple border region of Brazil before and after being treated in water treatment plants (WTPs) and in Municipal Early Childhood Education Centers (MECECs), in terms of parasitological, microbiological, and physical–chemical aspects. Different water samples were monitored: raw water (RW), treated water (TW), and tap water from the MECECs, giving 60 samples in total, to investigate the presence of *Giardia* and *Cryptosporidium*, microbiological indicators, *Pseudomonas aeruginosa*, and antimicrobial resistance profiles using conventional microbiological assays and parasitological, immunological, and molecular techniques. The results obtained were compared with the reference values recommended by the legislation of drinking water in Brazil. For the first time, contamination by *Cryptosporidium* and *Giardia* was demonstrated in RW used to supply WTPs, in TW of Foz do Iguaçu, and in water destined for consumption by children. A total of 52 bacterial isolates were obtained, with high percentages of multidrug resistance to antibiotics, including a carbapenem-resistant profile, highlighting the need to improve quality control standards.

Key words: Brazil, *Cryptosporidium*, fecal indicator bacteria, *Giardia*, multidrug resistance, water matrix

HIGHLIGHTS

- Waterborne pathogens were monitored in different water treatment plant phases.
- *Cryptosporidium* and *Giardia* were detected in raw and treated water.
- Contamination by pathogens and fecal bacteria was found in the distribution system.
- There was a heterogeneity of antimicrobial resistance patterns among bacterial isolates.
- A carbapenem-resistant profile was identified for *Pseudomonas aeruginosa* and *Escherichia coli*.

GRAPHICAL ABSTRACT

1. INTRODUCTION

One of the main current leading causes of disease and death worldwide is related to safe and affordable drinking water, and there is a pressing need for the implementation of measures to achieve its universal and equitable access for all humanity (United Nations 2020).

Brazil is considered the most abundant natural freshwater reservoir on Earth. However, the country has several serious economic, social, and environmental concerns related to the low quality of springs and a growth in environmental disasters, such as dam breaks, which compromise the quality of water, ecosystems, and fauna (Spilk 2015; Sánchez *et al.* 2018).

Foz do Iguaçu is located in a region known as the Tri-Border Area (TBA), which straddles the national boundaries of Brazil, Paraguay, and Argentina. It is notable for its privileged location (the confluence of the Paraná and Iguaçu Rivers) and huge availability of water (Cury & Fraga 2013). In addition, according to the Ministry of Tourism (2019), it is the third most visited destination in Brazil, linked to tourism around the Iguaçu Waterfalls, and is an important location for the use of water in electricity generation (Itaipu Binational) (Brazil. Ministério do Turismo do Brasil 2019).

Despite the large amount of freshwater available in the region, recurrent anthropogenic activities, mainly agricultural and wastewater disposal, allied to an ageing water treatment and distribution system, are major factors linked to the deterioration of water quality worldwide, exposing Brazilian and international tourist populations to an increased risk of waterborne diseases (USEPA 2002; Spilk 2015; Efstratiou *et al.* 2017).

Thus, the continuous monitoring of drinking water is crucial, and stringent criteria must be obeyed to guarantee safe water. Procedures for examining the microbiological content of water samples (fecal indicator bacteria, FIB) are routinely adopted by Brazil and other countries, although these are inactivated or removed more quickly from aquatic environments than pathogenic protozoa, such as *Cryptosporidium* and *Giardia*, which survive for longer periods (Brazil. Ministério da Saúde 2017; Hassan *et al.* 2021).

The characteristics of the protozoa *Cryptosporidium* and *Giardia* are ease of transmission, the fact they are immediately infectious upon excretion, and their resistance to chlorine and its derivatives, which associated with low infective doses, increases the probability of causing infection (Efstratiou *et al.* 2017; Hassan *et al.* 2021).

Both protozoa have already been detected in surface waters in Brazil, especially *Giardia* in the southern and southeastern regions of the country, while giardiasis is considered endemic (Coelho *et al.* 2017).

Although this disease is widely distributed throughout the country, six Brazilian states have a prevalence rate of higher than 30%, including Paraná (Coelho *et al.* 2017), where the city of Foz do Iguaçu, the subject of this study, is located. A recent study conducted with 178 children attending four Municipal Early Childhood Education Centers (MECECs) in Foz do Iguaçu, aiming to evaluate the prevalence of intestinal parasitosis, revealed that *Giardia duodenalis* was the most frequently detected protozoa (Ferreira 2017).

The fact that there are no data regarding the environmental epidemiology of protozoa in raw and treated water in Foz do Iguaçu, together with the prevalence data mentioned above, were important motivating factors behind the investigation of drinking water quality in school environments, as this is considered a major public health concern (WHO 2015; Waideman *et al.* 2020). Another important goal of this study was the evaluation of antimicrobial resistance phenomena, focusing on *Enterococcus* spp., *Pseudomonas aeruginosa*, and *Escherichia coli*, and the comparison of the antibiotic resistance profile of the isolated strains in water.

2. METHODS

2.1. Study design and sampling site characteristics

The present study was carried out in the city of Foz do Iguaçu, which is located in the triple border region (Brazil, Argentina, and Paraguay) in the extreme west of the state of Paraná. The work was submitted to Sanepar (the Paraná sanitation company) (CA 577/2017) and the Municipal Education Secretary of Foz do Iguaçu (Process No. 34979/2017) for approval and the signing of the Terms of Consent for the study.

Sanepar is responsible for the city's water and sanitation service. It has two water catchment sources from two water treatment plants (WTPs), 171 and 172, which are located around 10 km apart. The first treatment unit, WTP 171, with a decanter, has a water collection source, which is the Tamanduá River, and has an operating capacity of 900 m^3/h. Historically, the average turbidity of the raw water (RW) varies by period from 20 to 50 uT.

The second treatment unit, WTP 172, produces potable water for 70% of the city and has an operating capacity of 3,600 m^3/h. Its water collection source is the Paraná River in the region of the Itaipu Power Plant. It has a direct filtration system, and the average turbidity is 2.0–4.0 uT.

Foz do Iguaçu has about 8,000 students of preschool age who are enrolled in 37 MECECs. Water for these establishments is supplied by both WTPs in the city. For this study, six MECECs serving 1,200 children were selected, which are defined based on having previously participated in studies on the prevalence of intestinal parasites in preschool-age children in the city (Ferreira 2017).

Water samples were harvested monthly, for 6 months, from November 2017 to April 2018, from WTP 171 and 172, at the point of water collection prior to abstraction by the treatment system (RW) and at the outlet of the reservoir or inlet of the distribution system (TW). Water samples from the first tap after the hydrometer of each MECEC were also taken, before the reservoir, to verify contamination by protozoa and bacteria during the distribution of the TW from each WTP.

The study was divided into three major groups as follows: RW, TW and tap water from the MECECs, with a total of 120, 1,200 and 3,600 L of water processed and analyzed from each site, respectively, comprising a total of 60 samples.

All the water samples were duly identified by date, time, place of collection, residual chlorine content and turbidity, which were measured at the time of collection.

2.2. Detection of *Giardia* cysts and *Cryptosporidium* oocysts

For the investigation of pathogenic protozoa, total volumes of 10 L of RW and 100 L of TW and MECEC tap water were collected from each sample site in decontaminated polyethylene gallon containers, which were previously treated with Tween 80 (0.1%) surfactant solution.

After the collection, samples were submitted to the membrane filtration technique (47 mm diameter, 3 μm Millipore®) for the concentration of cysts and oocysts of *Giardia* and *Cryptosporidium* present in the samples, by vacuum filtration, with a pump adjusted to a flow of 0.4–4.0 L/min. Throughout the monitoring period, the entire volume of water collected from all sites was filtered. The elution and concentration of the protozoa was conducted according to Cantúsio-Neto *et al.* (2010), with minor modifications in the centrifugation step (1,500×g for 15 min).

Aliquots of 10 μL of RW and 20 μL of TW and MECEC water from each sediment were examined by a direct immunofluorescence assay (IFA) with monoclonal anti-*Cryptosporidium* and anti-*Giardia* antibodies conjugated with fluorescein isothiocyanate (FITC), (Merifluor™, Meridian, Bioscience) and the incorporation of the vital dye DAPI, 4.6′-diamidine-2-phenylindol (Sigma®), into the protozoa nuclear material. All slides were read under an immunofluorescence microscope (Zeiss®), and the confirmation of the protozoa morphology was carried out simultaneously with the FITC reading and using specific filters for DAPI, and by differential interference contrast microscopy (DIC) through the adoption of criteria stipulated by the United States Environmental Protection Agency (2012). The estimation of the number of cysts and oocysts present per liter of water examined was calculated using the following equation:

$$X = N/K \times S/A$$

where X is the concentration of cysts and oocysts/L; N is the number of oocysts or cysts detected in the slide well; K is the volume of the examined sediment in the sample (10 μL for RW and 20 μL for TW); S is the total volume of the sediment obtained (μL); A is the filtered volume of the sample (L).

For TW samples considered positive by IFA, DNA extraction was performed using a ZR Fungal/Bacterial DNA kit (Zymo Research®) and a DNA isolation kit DNeasyPowerSoil® (Qiagen, Germany) for *Giardia* and *Cryptosporidium*, respectively, following the manufacturer's instructions.

A 530 bp fragment of the triose phosphate isomerase (*TPI*) gene was amplified by nested-PCR to detect *G. duodenalis* (Sulaiman *et al.* 2003). For *Cryptosporidium* spp., a nested-PCR targeting the *18S rRNA* gene was performed (Xiao *et al.* 1999). The PCR products were separated on 1.5% electrophoresis grade agarose gel (GIBCO BRL, Life Technologies, Inc., Grand Island, NY) and stained with GelRed™. Images were obtained using an image capture system (UVP Life Science Software, CA, USA).

Oocysts of *C. parvum* (Iowa strain) obtained from Waterborne® Inc., New Orleans, Louisiana, and *G. duodenalis* cysts from the purified fecal samples of howler monkeys were used as internal positive controls for PCR. Ultrapure water was used as a negative control in all reactions.

2.3. Physical–chemical, microbiological, and antimicrobial resistant profile analysis

The physical–chemical parameters were analyzed on the day of collection with the aid of a multiparametric probe (Hidrolab DS5X) for turbidity (uT) and free residual chlorine content (mg/L).

For microbiological analysis, RW, TW and MECEC water samples were harvested in previously sterilized flasks for the analysis of FIB and the verification of network integrity in accordance with Standard Methods for the Examination of Water and Wastewater (APHA 2017).

For the detection of total coliform and *E. coli*, the Colilert® substrate (Idexx, Laboratories, Inc., Maine, USA) was used. Briefly, the blister pack present in the kit was added to 100 mL of each sample, and the content was homogenized and incubated at 35 °C for 24 h. Quantitative results were then obtained by verifying the staining (yellowish) and fluorescence under a UV light, which enabled the quantification and simultaneous detection of total coliforms and *E. coli*, respectively.

The isolation of enterococci was performed using the Enterolert® substrate following the manufacturer's specifications. The investigation of pathogenic bacteria involved the analysis of *P. aeruginosa* due to its importance and the possibility that it might exhibit an opportunistic character in infections, as well as broad resistance to antibiotics used in clinics. The Pseudalert® substrate (Idexx, USA) was used to achieve this.

For the FIB and *P. aeruginosa*, the results were expressed as MPN/100 mL. For heterotrophic bacteria isolation count, the results were expressed in CFU/mL (APHA 2017).

The isolated bacteria were identified using the MALDI-TOF method (bioMérieux), applying mass spectrometry to the bacteria obtained from water samples (Singhal *et al.* 2015). *E. coli*, *Enterococcus* sp., and *P. aeruginosa* isolates were analyzed for antimicrobial susceptibility testing by the diffusion disc technique according to the Clinical and Laboratory Standards Institute (CLSI 2011), and the results were expressed as susceptible (S), intermediate (I), and resistant (R).

The results obtained were compared with the recommended physical–chemical, microbiological, and parasitological limits determined by Health Surveillance (HV), Annex XX of Consolidation Ordinance N° 5/2017, data from Ordinance N°. 2.914/2011, the current national legislation during the execution of the study (Brazil. Ministério da Saúde 2017).

2.4. Statistical analysis

The physical–chemical, microbiological, and parasitological variables were compared using the Student's *t*-test and the Mann–Whitney test. Correlation tests were also performed using Pearson's or Spearman's Correlation. These tests were applied to the independent samples of RW and TW and from the MECECs.

For the correlation tests, confidence levels of 95% were defined. Thus, the following ranges of values were considered: 0–0.3 (weak correlation); 0.3–0.7 (moderate correlation); 0.7–1.0 (strong correlation).

Statistical analysis was performed using BioEstat software version 5.0 with a significance level of 5% ($p<0.05$).

3. RESULTS

3.1. Occurrence of *Giardia* and *Cryptosporidium* in water samples

Contamination by pathogenic protozoa was detected in 11.6% (7/60) of the samples analyzed by IFA. Of this total, the presence of *Giardia* spp. was detected in 57.1% (4/7) samples and *Cryptosporidium* spp. in 42.9% (3/7) of samples.

Of the RW, 33.0% of the samples (4/12) were positive for one of the protozoa in both the monitored rivers. *Giardia* spp. cysts were detected only in the Tamanduá River, while *Cryptosporidium* spp. oocysts were identified only in the Paraná River, which were used for water abstraction by WTP 172 (Table 1).

Contamination by both protozoa was not detected in drinking water delivered by WTP 171. However, in TW from WTP 172, *Giardia* spp. and *Cryptosporidium* spp. (Figure 1) were identified once (Table 1). *Cryptosporidium* oocysts were not detected in any of the MECEC water samples, while *Giardia* cysts were detected in 2.8% of samples (n=1). A single oo(cyst) was detected in well slides by IFA, where amorphous and empty (oo)cyst have also been observed by DIC. For the TW samples considered positive on direct immunofluorescence microscopy (n=3), and submitted to the molecular assay, the amplification of DNA was negative.

Statistical correlations between *Cryptosporidium* spp. and *Giardia* spp. and turbidity were not observed in the RW samples used for water abstraction from WTP 171 and 172 or in MECEC water supplied by WTP 171.

3.2. Monitoring of physical–chemical, microbiological, and antimicrobial resistance profiles

The analysis of the physical–chemical parameters revealed that the samples of TW from the WTPs and the MECECs exhibited acceptable values for chlorine and unacceptable values for turbidity according to the national legislation (Table 2).

In microbiological analyses, the highest impact of fecal contamination was detected in the Tamanduá River used for water abstraction by WTP 171 (Table 2). Expressive counts of the pathogenic bacteria *P. aeruginosa* were also reported at the same site, in comparison with the surface water from the Paraná River from WTP 172. However, a deterioration of water quality in

Table 1 | Positivity for *Cryptosporidium* spp. and *Giardia* spp. and concentration of oocysts and cysts per liter of RW and TW from WTPs and MECECs in Foz do Iguaçu, Paraná, Brazil

	Protozoa parasites[+]		
Water source	***Cryptosporidium* spp.[++] (oocysts/L)**	***Giardia* spp.[++] (cysts/L)**	**Month/year of detection**
RW 171*	ND	9.0	January/2018
	ND	11.0	April/2018
RW 172*	9.0	ND	February/2018
	8.5	ND	March/2018
TW 171**	ND	ND	–
TW 172**	ND	1.58	November/2017
	0.31	ND	February/2018
MECEC 171***	ND	0.7	January/2018
MECEC 172***	ND	ND	–

*RW, raw water (10 L); **TW, treated water from WTPs (100 L); ***MECEC water (100 L); ND, not detected; [+]Microscopical identification based on direct immunofluorescence microscopy; [++]DAPI analysis, negative.

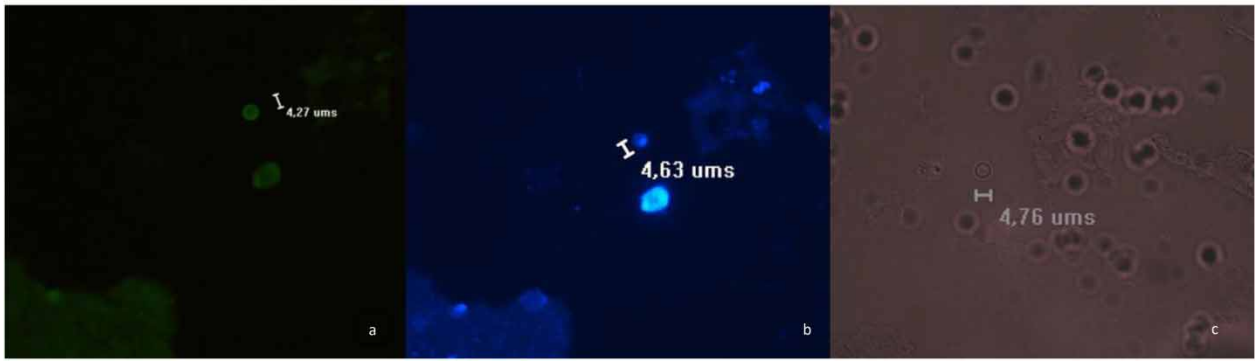

Figure 1 | *Cryptosporidium* spp. oocyst visualized in a TW sample from WTP 172: (a) FITC demonstrating characteristic bright apple green fluorescence, typical size, and shape; (b) DAPI; (c) DIC. Magnification 40×.

Table 2 | Microbiological and physical–chemical analysis of RW and TW from WTPs and MECECs in Foz do Iguaçu, Paraná, Brazil

	Fecal indicator bacteria					Parameters	
Water source	**Total coliforms***	***Escherichia coli****	***Enterococcus* spp.***	***P. aeruginosa****	**Heterotrophic bacteria****	**Turbidity*****	**Chlorine*****
RW 171	>2.500 ± 0.0	1.555 ± 481^{+}	1.063 ± 923	1.187 ± 909	1.196 ± 1.555	29.0 ± 23.5^{+}	0.0 ± 0.0
RW 172	2.052 ± 1.001	1.4 ± 1.0	778 ± 1.097	2.0 ± 1.4	31.3 ± 40.3	20.0 ± 14.3	0.0 ± 0.0
TW 171	<1.0 ± 0.0	<1.0 ± 0.0	1.3 ± 1.0	<1.0 ± 0.0	<1.0 ± 0.0	1.0±0.4	1.5 ± 0.2
TW 172	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	1.2 ± 0.4^{+}	1.3 ± 0.5^{+}	1.4 ± 0.2^{+}
MECEC 171	<1.0 ± 0.0	<1.0 ± 0.0	1.3±1.0	<1.0 ± 0.0	1.3 ± 1.0	1.0 ± 0.2	1.2 ± 0.2
MECEC 172	2.0 ± 3.1^{+}	1.1 ± 0.4	<1.0 ± 0.0	1.4 ± 2.0^{+}	3.5 ± 12.2	1.2 ± 0.5^{+}	1.0 ± 0.2

RW, raw water; TW, treated water; MECECs, Municipal Early Childhood Education Centers.
Data presented as mean±standard deviation.
*Maximum allowable value (MAV): ≤1.0 most probable number (MPN)/100 mL or absence.
**Less than 500 CFU/mL of heterotrophic bacteria is recommended.
***Maximum allowable value for turbidity: 0.5 uT for a rapid water filtration system and minimum of 02 mg/L of free chlorine residual.
$^{+}$indicates differences between elements in line ($p<0.05$).

the RW of WTP 172 was evidenced when monitoring another FIB (*Enterococcus* sp.) (Table 2). Samples of TW from WTP 171 and the MECEC supplied by this WTP exhibited contamination by *Enterococcus* spp.

In MECEC water provided by WTP 172, the presence of *E. coli* and *P. aeruginosa* was detected, showing that drinking water consumed by children was unsuitable for consumption based on the maximum allowable value (Table 2).

A strong correlation was observed between turbidity and *E. coli* in RW from WTP 171 (r=0.9545; p=0.003). A relationship was also found between turbidity, chlorine, and heterotrophic bacteria of TW from WTP 172 (r=0.8105; 0.8428; p=0.0504; 0.0351), respectively.

In the MECEC water samples supplied by WTP 172, moderate correlations between turbidity and total coliforms and *P. aeruginosa* (r=0.4918 and 0.4566; p=0.0146 and 0.0249, respectively) were detected.

From the total number of water samples (RW and TW), 52 bacterial isolates were obtained, 33 of which belonged to the Enterobacteriaceae family. Of these, nine were classified as *E. coli* (Table 3). The other 24 bacteria were isolated from RW samples and were classified as: *Salmonella* spp. (26%), *Klebsiella* spp. (18%), *Enterobacter* spp. (12%), *Citrobacter* spp. (12%), *Serratia* spp. (12%), *Acinetobacter* spp. (4%), *Hafnia* (4%), and *Proteus* spp. (4%).

Concerning the *E. coli* isolates, a high percentage of multidrug resistance to antibiotics was observed in RW from WTP 171 and 172 (Table 3). Additionally, 20% of the isolates in RW from WTP 171 exhibited a resistance profile for both imipenem and meropenem. For TW from WTP 172, one isolate was identified with a resistance profile to three drug classes in an antibiogram (Table 3).

Enterococcus spp. was isolated in 10 samples, two of which were from TW destined for human consumption from WTP 171. The primary enterococcal isolates were *Enterococcus faecalis* (50.0%) (Table 4). The resistance profile of these isolates

Table 3 | Antimicrobial susceptibility profiles of *E. coli* isolated in samples of RW and TW in Foz do Iguaçu, Paraná, Brazil

			Antimicrobial agent no. (%)											
Water source	**No. of isolates**	**Susceptibility***	**AMK**	**AMC**	**AMP**	**CPM**	**FOX**	**CAZ**	**CIP**	**CHL**	**GEN**	**IPM**	**MEM**	**SXT**
RW 171	5	S	2 (40)	1 (20)	1 (20)	5 (100)	1 (20)	4 (80)	5 (100)	3 (60)	1 (20)	4 (80)	4 (80)	1 (20)
		I	3 (60)	1 (20)	–	–	–	1 (20)	–	1 (20)	–	–	–	1 (20)
		R	–	**3 (60)**	**4 (80)**	–	**4 (80)**	–	–	**1 (20)**	**4 (80)**	**1 (20)**	**1 (20)**	**3 (60)**
RW 172	3	S	1 (33)	2 (67)	2 (67)	3 (100)	2 (67)	2 (67)	3 (100)	3 (100)	–	3 (100)	3 (100)	3 (100)
		I	–	–	–	–	–	1 (33)	–	–	1 (33)	–	–	–
		R	**2 (67)**	**1 (33)**	**1 (33)**	–	**1 (33)**	–	–	–	**2 (67)**	–	–	–
TW 172	1	S	–	1 (100)	–	1 (100)	–	1 (100)	1 (100)	–	–	1 (100)	1 (100)	–
		I	1 (100)	–	–	–	–	–	–	1 (100)	–	–	–	–
		R	–	–	**1 (100)**	–	**1 (100)**	–	–	–	**1 (100)**	–	–	–

RW, raw water; TW, treated water; *S, susceptible; I , intermediate; R, resistant.
AMK, amikacin; AMC, amoxicillin–clavulanic acid; AMP, ampicillin; CPM, cefepime; FOX, cefoxitin; CAZ, ceftazidime; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim–sulfamethoxazole.
Bold values refers to the most important results, i.e. lines that show which isolates were resistant to antimicrobials.

Table 4 | Antibiogram profile and *Enterococcus* isolate species identified from different water samples

Water source	Month/year	Antimicrobial resistance profile (R)	Number of isolates	Identified species
RW 171	November/17	–	1	*E. faecium*
RW 171	December/17	CLI	1	*E. faecalis*
RW 172	December/17	CLI	1	*E. faecalis*
RW 171	January/18	CLI; CHL; ERY; GEN	1	*E. faecalis*
RW 172	February/18	CLI; SXT	1	*E. durans*
RW 171	February/18	CLI	1	*E. faecalis*
RW 171	March/18	CLI	1	*E. faecalis*
RW 171	April/18	CLI	1	*E. faecium*
TW 171	April/18	CLI	1	*E. faecium*
MECEC 171	April/18	CLI	1	*E. faecium*

RW, raw water; TW, treated water; MECEC, Municipal Early Childhood Education Centers.
Antimicrobial agents tested: AMP, ampicillin; CLI, clindamycin; CHL, chloramphenicol; CIP, ciprofloxacin; DX, doxycycline; ERY, erythromycin; GEN, gentamicin; LVX, levofloxacin; LNZ, linezolid; NOR, norfloxacin; PEN, penicillin; TE, tetracycline; TEC, teicoplanin; SXT, trimethoprim–sulfamethoxazole; VA, vancomycin.

showed that 90% were resistant to clindamycin and one of these was also resistant to trimethoprim–sulfamethoxazole. One isolate of *E. faecalis* exhibited resistance to four antimicrobial agents (Table 4).

For *P. aeruginosa*, nine isolates were obtained, most of which (88.8%) came from RW samples: of the five isolates from the Tamanduá River (WTP 171), 80% showed multidrug resistance to seven antibiotic drug classes, including gentamicin and amikacin (Table 5).

From RW from the Paraná River (WTP 172), 67 and 33% showed a profile of resistance to meropenem and imipenem, respectively. One bacterial isolate of *P. aeruginosa* was detected in MECEC TW supplied by WTP 172. For this water matrix, however, the isolate was shown to be sensitive to both antibiotics. In contrast, the same isolate was considered resistant to three other antibiotics (Table 5).

4. DISCUSSION

While Foz do Iguaçu is one of the Brazilian cities most visited by international tourists, no data relating to a wide variety of drinking water parameters have been available until the present study. Contamination by waterborne protozoa and an

Table 5 | Antimicrobial profile of *P. aeruginosa* isolated from RW and TW from MECECs in Foz do Iguaçu

			Antimicrobial agent no. (%)											
Water source	No. of isolates	Susceptibility*	CPM	MEM	SXT	CIP	IPM	CAZ	GEN	VA	AMC	FOX	AMK	AMP
RW 171	5	S	5 (100)	4 (80)	1 (20)	5 (100)	5 (100)	3 (60)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)
		I	–	–	–	–	–	–	–	–	–	–	–	–
		R	–	**1 (20)**	**4 (80)**	–	–	**2 (40)**	**4 (80)**	**4 (80)**	**4 (80)**	**4 (80)**	**4 (80)**	**4 (80)**
RW 172	3	S	3 (100)	1 (33)	–	3 (100)	2 (67)	2 (67)	1 (33)	–	–	–	–	–
		I	–	–	–	–	–	–	–	–	–	–	–	–
		R	–	**2 (67)**	**3 (100)**	–	**1 (33)**	**1 (33)**	**2 (67)**	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
MECEC 172	1	S	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	–	–	–	1 (100)
		I	–	–	–	–	–	–	–	–	–	–	–	–
		R	–	–	–	–	–	–	–	–	**1 (100)**	**1 (100)**	**1 (100)**	–

RW, raw water; TW, treated water; *S, susceptible; I, intermediate; R, resistant.
AMK, amikacin; AMC, amoxicillin–clavulanic acid; AMP, ampicillin; CPM, cefepime; FOX, cefoxitin; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim–sulfamethoxazole; VA, vancomycin.
Bold values refers to the most important results, i.e. lines that show which isolates were resistant to antimicrobials.

antimicrobial resistance profile was evidenced in many RW samples from the city and also in several TW samples, which is a worrying sign.

Brazil is the leading producer of reports into the isolation and investigation of the presence of *Cryptosporidium* and *Giardia* in different water matrices in Latin America and, together with Colombia, is the only country with legislation that addresses protozoa in water (Rosado-García *et al.* 2017). Despite such advances, it should be noted that research into both protozoa in TW is not covered by Brazilian legislation (updated recently in 2021), which only applies to RW in certain situations.

The identification of *Cryptosporidium* spp. and *Giardia* spp. through the parasitological analysis of RW sources in the present study is, therefore, similar to data obtained from other studies where their widespread distribution was detected (Efstratiou *et al.* 2017; Araújo *et al.* 2018; Plutzer *et al.* 2018). The presence of agricultural, anthropogenic, and industrial activities in areas surrounding the water catchment sites of Foz do Iguaçu is potentially linked to water contamination: WTP 171 is located close to the urbanized region of the city and is continuously used for recreation, while in WTP 172 there is a frequent presence of excreta and animals wandering loose.

The results of the present study revealed a lower concentration of protozoa, especially for *Giardia* cysts in RW, than other studies carried out in Brazil (Cantúsio-Neto *et al.* 2010; Coelho *et al.* 2017), which may also imply a lower chance of large quantities of protozoa being captured and entering the water treatment system. However, in 33.3% of the months sampled, protozoa were detected in finished water from WTP 172, which produces drinking water for the majority of the population of the city. These results suggest that the water treatment provided by this WTP may not be effective at controlling both pathogens.

While the numbers of cysts and oocysts present per liter of TW found in the present study may be considered low, it is known that small fractions of oocysts present in TW have been responsible for massive outbreaks in the USA and England, representing a particular danger to individuals with immunological impairments (Mac Kenzie *et al.* 1994; Puleston *et al.* 2014).

However, the data from the present study should be interpreted with caution: while a direct IFA is considered the reference method for the detection of both protozoa through water samples, with the advantage of being quantitative and allowing discrimination between live and dead organisms, it cannot provide information about infective potential or the circulating species or genotypes (USEPA 2012).

With respect to negative amplification results, DNA of samples submitted to PCR detection can be critical when there are reaction inhibitors and a low number of (oo)cysts, which can cause false-negative results (Araújo *et al.* 2018).

It is also important to emphasize that both protozoa were detected in TW from WTP 172, which is considered suitable for human consumption in accordance with the maximum allowable *E. coli* value established by the Brazilian legislation. Although not detected in the TW from WTP 171, fecal contamination was evidenced at this site when a complementary indicator (*Enterococcus* sp.) was investigated.

These results also highlight the importance of inserting at least two FIB to guarantee safe water, a fact also observed by Waideman *et al.* (2020), in which several samples from kitchen faucets and drinking fountains from children's public schools were only considered unsuitable for human consumption when enterococci data contamination was evaluated.

As for microbiological investigation, the fluctuations in bacterial growth observed over the monitoring period, with concentrations greater than 1,000 MPN/mL for *E. coli*, *P. aeruginosa* and enterococci found in RW of WTP 171 and lower mean concentrations in RW of WTP 172, can also be associated with the drag and runoff of nearby water.

In Brazil, the presence of total coliforms and *E. coli* is used to predict water quality. Specifically, Brazilian legislation determines that monitoring of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts in RW should be carried out when an annual geometric mean greater than or equal to 1.000 *E. coli*/100 mL is identified (Brazil. Ministério da Saúde 2017).

In the present study, the mean limit stipulated by national legislation for *E. coli* was attained after 6 months of monitoring of RW from WTP 171, demonstrating the need to monitor the presence of protozoan parasites.

Regarding the physical–chemical and microbiological parameters, a strong correlation was observed between turbidity and *E. coli* in the samples of RW from WTP 171, as well as between turbidity, chlorine and heterotrophic bacteria in TW samples from WTP 172.

These results are unsurprising as fecal and heterotrophic bacteria are usually associated with pollution, organic matter, and consequently with high turbidity levels in water from natural sources (Bessa *et al.* 2014; Bortoloti *et al.* 2018).

However, the correlation with heterotrophic bacteria observed in TW in our study once again emphasizes a worrying situation, as it reflects the overall conditions of the treatment used by the WTP.

In the present study, the WTPs used a rapid water filtration system, so the turbidity values should be lower than 0.5 uT in 95% of samples (Brazil. Ministério da Saúde 2017). For the proper control and monitoring of protozoa in TW, a maximum turbidity limit less than or equal to 0.3 uT is suggested and is considered ideal for significantly improving parasite removal, especially *Cryptosporidium* spp. oocysts (Brazil. Ministério da Saúde 2017).

Another important finding evidenced through our monitoring study was that contamination by pathogens or microorganisms might also have occurred during the distribution network system: on one occasion, *Giardia* spp. cysts were detected in TW from WTP 171 provided for consumption by children in MECECs.

Similarly, the detection of total coliforms and *E. coli* (contradicting legislation) and *P. aeruginosa* in the water samples of the MECECs supplied by WTP 172 highlighted this hypothesis, as all the parameters analyzed at the end of the treatment of the water were in accordance with current legislation. Moderate correlations between turbidity and total coliforms and *P. aeruginosa* were detected in MECEC water samples supplied by the same WTP.

Taken together, these results lead us to infer that drinking water from MECECs can represent a risk for consumers, especially as these institutions work mainly with children, whose immune system is still developing and reflects the urgent need for frequent monitoring and improvements in water quality for the protection of public health.

Factors that can influence water contamination in the distribution system are large networks running throughout the city, and the composition of pipes, especially those made of iron, since they are subject to corrosion and can act as risk amplification sites if not properly sealed and if periodic maintenance is not carried out (USEPA 2002; WHO 2004).

Waterborne diseases due to the contamination of the network and distribution systems are not infrequent and have been reported in Scandinavia, the United Kingdom, and the USA (WHO 2004).

In Brazil, the contamination of the distribution network by waterborne protozoa oocysts was considered an important source of outbreak of *Cyclospora cayetanensis* in Antonina in the state of Paraná in 2001 (Moura *et al.* 2002). On that occasion, the piping network was considered a significant risk factor associated with the outbreak, since the water distribution network was over 50 years old and made of cast iron, which causes leakage, leading to the repair and replacement of the network.

With regard to antimicrobial resistance to *E. coli*, *P. aeruginosa*, and *Enterococcus* sp. (leading causative agents of severe healthcare-acquired infections with limited antimicrobial treatment options) in the present study, an extensive heterogeneity of resistance patterns was found among the isolates.

The resistance of *E. coli* detected in RW to the antimicrobial's ampicillin, cefoxitin, gentamicin, and trimethoprim–sulfamethoxazole is a similar finding to those published in other studies, which demonstrates that bacteria resistant to antimicrobials can be present and released into the environment from human and agricultural sources (Bessa *et al.* 2014; Barbosa-Vasconcelos *et al.* 2018).

In addition, our results confirmed that the water treatment applied did not remove all fecal organisms, as *E. coli* was found in TW from source 172, and was considered resistant to ampicillin, cefoxitin, and gentamicin.

Furthermore, multidrug resistant isolates from the opportunistic bacteria *P. aeruginosa* were detected in different RW samples, including carbapenem-resistant isolates, the increased prevalence of which is of global significance in relation to meropenem and imipenem, and the difficulty involved in its control (Schiavano *et al.* 2017). The only isolate detected in TW from the MECECs, meanwhile, was found to be susceptible to both antimicrobial agents and resistant to three other important antibiotics.

Moreover, the high levels of *E. coli* and enterococci and the remarkable variety of antimicrobial resistance profiles observed in the present study corroborate the idea that water supplies may have a preponderant set of antimicrobial resistant determinants, which are easily distributed and can reach other environments and hosts (Bessa *et al.* 2014; Bortoloti *et al.* 2018).

In conclusion, the purpose of assessing water quality in the present study was related to public health protection. Indeed, the criteria adopted aim to support actions that, if properly implemented among the population, will ensure the safety of the water supply by eliminating or reducing the minimum concentration of constituents known to be harmful and dangerous to human health.

5. CONCLUSIONS

The presence of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts in samples of TW reveals the limited effectiveness of conventional treatment applied in WTPs in the city and highlights the potential impact and possible risks associated with the water supply.

The emerging global concern around antimicrobial agents in different matrices, such as those of recreational or drinking water, was frequently detected in the TBA of an important tourist region, as was the presence of high multidrug resistance, especially for *P. aeruginosa*, followed by *E. coli* and *Enterococcus* sp., posing a threat to the autochthonous and visitor population.

The results reinforce the need to ensure compliance with legislation for the potability of water, ensuring its safe supply, avoiding possible water-based outbreaks and negative impacts on public health.

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DATA AVAILABILITY STATEMENT

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

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