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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

The presence of antibiotics and multidrug-resistant *Staphylococcus aureus* reservoir in a low-order stream spring in central Brazil

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Abstract

The disposal of industrial effluents strongly influences low-order streams, which makes them fragile ecosystems that can be impacted by contamination. In central Brazil, the Extrema River spring targets the dumping of pharmaceutical products from the surrounding industries. So, this work aimed to investigate the presence of antibiotics in Extrema River spring samples and the isolation of *Staphylococcus aureus*, a potential multidrug-resistant bacteria, verifying the antimicrobial resistance profile of these isolates. Three campaigns were carried out in different locals (P1–P3) between October and December 2021, in the dry and rainy seasons. The high-performance liquid chromatography-tandem mass spectrometry (LCMS) approach indicated the presence of sulfamethoxazole (≥ 1 ng/L), metronidazole (< 0.5 ng/L), and chloramphenicol (< 5 ng/L) in the water samples in November (rainy season). *S. aureus* was isolated in P1 ($n = 128$), P2 ($n = 168$), and P3 ($n = 36$), with greater resistance to trimethoprim-sulfamethoxazole (90%), clindamycin (70%), and gentamicin (60%). The presence of antibiotics in the Extrema River spring may cause *S. aureus* antibiotic resistance development. The presence of antibiotics and the high percentage of isolated multidrug-resistant *S. aureus* in the Extrema River spring cause concern and indicate the clandestine dumping of effluents from nearby pharmaceutical industries. Since preserving the springs of low-order streams is important for the environment and public health, we encourage monitoring the wastewater from Extrema River's nearby pharmaceutical industries and preserving the spring of this river.

Keywords Cerrado · Ecotoxicity · Emerging pollutants · One health · Water quality

Introduction

The Cerrado biome is the second most extensive vegetation formation on the South American continent. Its area represents one-fourth of Brazil's entire land surface [1]. It has been considered one of the most important savannas in the world due to the richness of plant and animal species and water resources [2]. The Cerrado's biodiversity has suffered with the expansion of Brazilian agriculture in the last 50 years, which has caused changes in its soil areas and water bodies [3, 4]. In this anthropization process, industrial activity intensified and guaranteed market in different sectors [5]. As a result, industries have discharged effluents directly into aquatic ecosystems, which alters the runoff volume and water temperature, impairing its integrity and future sustainability [6]. Water pollution has been a global concern, caused by industry and agriculture due to the significant

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growth of emerging pollutants predicted to increase not less than double until 2035 [7].

The city of Anápolis has 396,526 inhabitants and is located in central Brazil (Goiás State). The city's economy revolves around the pharmaceutical, transformation, and automobile industries concentrated in the Agroindustrial District of Anápolis (DAIA). DAIA was created in 1976 and produces various types of waste [8]. Part of this industrial waste is disposed of in the Extrema River, which belongs to the Antas River watershed, and its spring is close to the DAIA. Values above the allowed for Fe and Cr and the genotoxic potential of water samples from the Extrema River have already been demonstrated [9]. Low-order rivers, like the Extrema River, are vulnerable to deforestation, agriculture, and urbanization, leading to eutrophication and hypoxia of ecosystems downstream [10, 11]. Springs are ecotones between groundwater, surface water, and the adjacent soil ecosystem, fed by a continuous flow of groundwater with a thermally stable habitat with diverse biota [12]. Springs and their terrestrial surroundings are considered hotspots for the biodiversity of the regions [13]. They are threatened by multiple anthropogenic stressors, such as capture, habitat degradation, and aquatic contaminants [14].

Antibiotics are part of persistent contaminants in aquatic environments through industrial, domestic, or veterinary disposal, whose most prolonged half-life reaches 1800 days [15, 16]. Antibiotic classes have already been recorded in rivers in China [17, 18], Canada [19], the USA [20], and Brazil [21, 22]. The indiscriminate and excessive use of antibiotics has caused an increase in multidrug-resistant bacteria [23]. The relationship between bacterial resistance and the occurrence of antibiotics in surface waters has been reported as harmful to public health, compromising the effectiveness of antimicrobial therapy, as pathogenic microorganisms are becoming resistant to most antibiotics [24]. The spread of antibiotic-resistant bacteria has been classified as one of the three threats to public health in the *twenty-first* century by the World Health Organization (WHO) [25].

Staphylococcus aureus is a highly resistant Gram-positive coccus to broad-spectrum antimicrobials. *S. aureus* has been reported to be resistant to methicillin, penicillins, cephalosporins, and carbapenems and tends to develop resistance to quinolones, aminoglycosides, and macrolides [26, 27]. *S. aureus* is an opportunistic pathogen that causes infections and intoxications in humans and animals, mainly in nosocomial environments [28]. However, staphylococcal infections could evolve to serious systemic infections and death, especially when associated with antimicrobial resistance (AMR) [29]. Initially, outbreaks of antimicrobial-resistant *S. aureus* infections were associated mainly with compromised patients exposed to hospital environments. However, since the late 90s, with the emergence of new more aggressive community-associated isolates, these infections are no longer limited to these settings. Nowadays, it is common to observe outbreaks of *S. aureus* infections among young healthy individuals with close contact and sharing common facilities [30].

S. aureus waterborne transmission routes have not been traditionally associated with infections. However, the potential for *S. aureus* to be spread via aquatic environments is continually evaluated. This microorganism has been identified in freshwater [31, 32], seawater [30, 33–36], sub-catchment water system [37], drinking water [38, 39], and wastewater [40–45]. Additionally, it has been demonstrated that people shed their colonizing organisms into seawater and, therefore, can be sources of potentially pathogenic *S. aureus* in recreational marine waters [30]. These observations suggest the persistence of *S. aureus* in the aquatic environment and the water as a possible route for *S. aureus* transmission.

In addition to the risk of transmission of *S. aureus* by water, the ESKAPE pathogens (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter bau-mannii*, *Pseudomonas aeruginosa*, and *Enterobacter*), that cause AMR crisis faced by hospitals globally, can accumulate AMR genes primarily due to horizontal gene transfer (HGT) aided by plasmids and mobile genetic elements [46, 47]. The origins of the AMR genes are environmental bacteria, mainly soil ones, which have co-evolved with antimicrobial-producing organisms for millennia [48]. There is a temporal lag between the clinical use of a drug and the arrival of relevant mobile AMR genes in human pathogen populations [49]. So, identifying environmental reservoirs of multidrug-resistant bacteria alert to the urgent need for environmental monitoring policies [47]. Thus, the objective of this study was to investigate the presence of antibiotics in the Extrema River's spring, due to its close proximity to many pharmaceutical industries, and to isolate *S. aureus*, verifying the AMR profile of these isolates.

Methods

Sampling points

The samples were collected in surface water from the Extrema River spring in Anápolis, central Brazil (Goiás State). Three collection points near the spring were sampled (P1, P2, and P3), as follows: (P1) 16°23'8"S 48°56'38"W, (P2) 16°23'8"S 48°56'37"W, and (P3) 16°23'8"S 48°56'36"W (Fig. 1). The collection area has gallery forest phytophysiognomy, forest vegetation accompanying streams, and small river courses forming closed corridors over water courses, typical of the Brazilian Cerrado. In this region, two other phytophysiognomies are predominant, savanna stricto sensu, and dry forest [50]. Many pharmaceutical industries near the spring area (~ 5 km) have potential

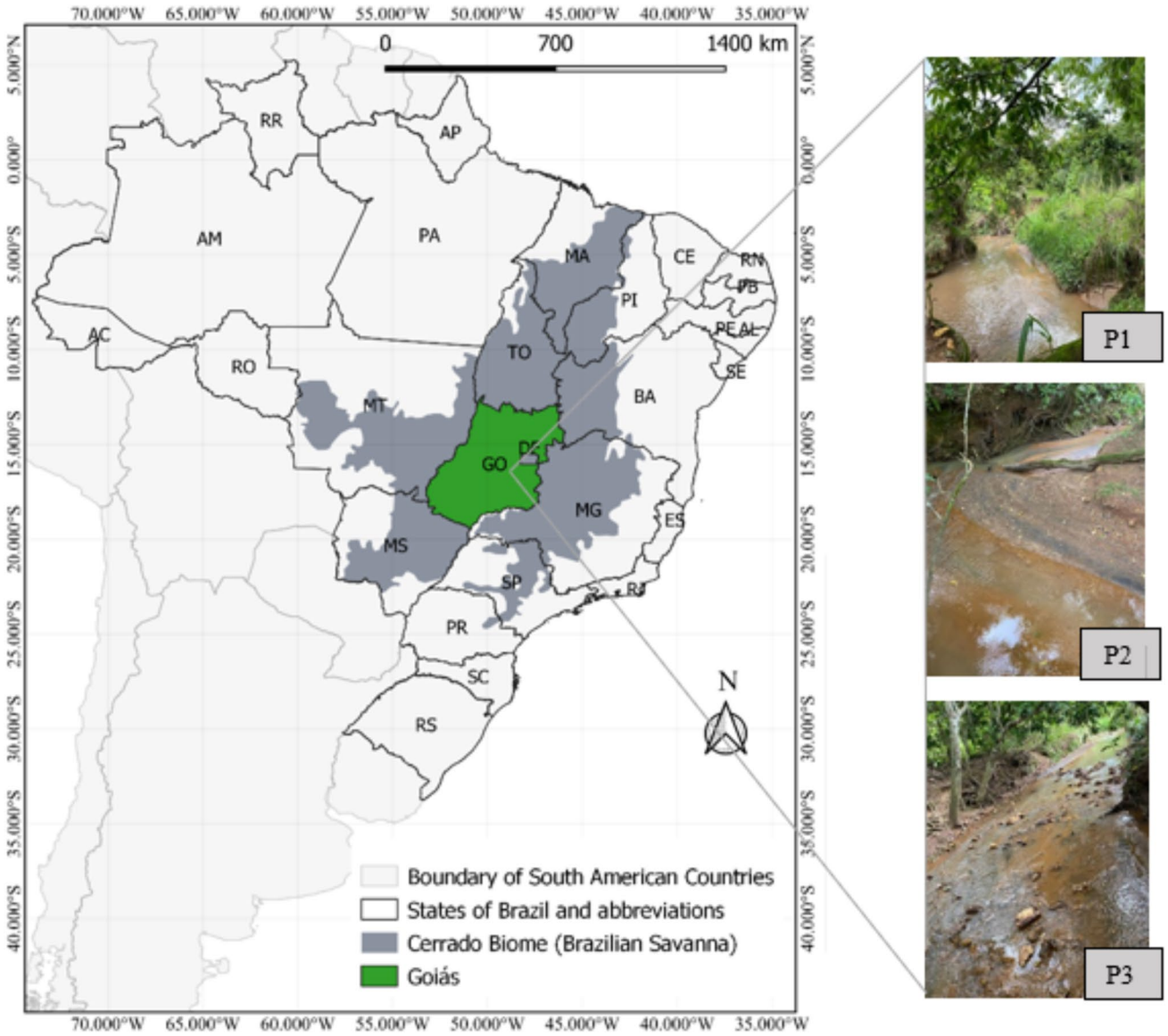


Fig. 1 Localization and images of the three points sampled (P1, P2, and P3) of the Extrema River in the city of Anápolis, Goiás, Central Brazil

clandestine wastewater runoff. The region's climate is Cwb (tropical altitude, with dry winters and hot, humid summers), according to the Köppen classification [51]. The samples were collected between October (dry season), November, and December (rainy season) in 2021. A monthly collection was carried out. The water quality parameters of the Extrema River are shown in Supplementary Information (Supp. Table S1). The water (2 liters at each point) was collected in sterile amber glass bottles, sealed with a stopper and lid to transport the material at 4 °C. In the laboratory, samples were immediately filtered for *S. aureus* isolation. The samples intended for high-performance liquid chromatography coupled with mass spectrometry (LCMS) were immediately stored in an ultra-freezer at −80 °C (ColdLab®, Piracicaba, SP, Brazil).

Sample preparation for antibiotic identification

Before analyzing the samples by LCMS, the samples were pretreated as follows: samples were filtered through Millex® 0.45 µm polyvinylidene fluoride (PVDF) membrane filters (Merck KGaA, Darmstadt, Germany). A total of 10 mL of each sample were first lyophilized. The powder residue was resolubilized in methanol three times at 0.5 mL with homogenization, totaling 1.5 mL. This suspension was transferred to a clean microtube and vacuum dried in the SpeedVac. After drying, it was resuspended in 100 µL of mobile phase, followed by vortexing for 5 min, centrifuging at 14000 rpm for 10 min at 8 °C. Finally, 60 µL of the supernatant was transferred to the insert and taken to the chromatographic system for injection.

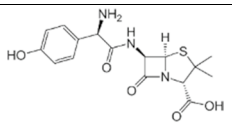
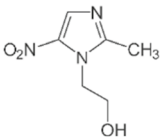
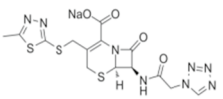
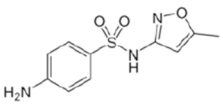
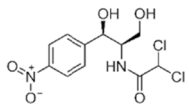
High-performance liquid chromatography-tandem mass spectrometry (LCMS) conditions

Chromatographic separations were carried out using an ExionLCTM chromatographic system (Sciex, Singapore) composed of a pump with a quaternary solvent manager (AC Pump), an automatic injector (AC Autosampler), and a chromatographic column oven (AC column oven). A Kinetex C8 (150 × 4.6 mm, 5 µm particle size) column with phenomenex guard cartridges (4 × 3 mm) at 35 °C was utilized as a stationary phase. The mobile phase was a mixture of acetonitrile, methanol, and ammonium formate 10 mM containing formic acid 0.2% (5:15:80, v/v/v) on isocratic elution mode at a flow rate of 0.8 mL.min^{-1}, being 20 min of the total chromatographic run. In the injection, the samples were kept at 15 °C, and 20 µL were injected. The tandem mass analysis was performed by a 4500 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer (Sciex, Singapore) equipped with a turbo Ion Spray source. The temperature of the electrospray source was 650 °C. Mass spectrometry (MS) analysis was performed in positive and negative ion modes, 4500 V or −4500 V, respectively. Curtain gas (CUR) was 10 psi, and ion source gas pressures (GS1 and GS2) were 50 psi. MS parameters dependent on compounds include ionization mode, declustering potential (DP), collision energy (CE), and monitored ions (Table 1). These parameters were optimized by infusion of individual standard solutions of each compound at concentrations of 500 µg/L. The software used to control the system functions was Analyst® 1.7.2 (Sciex, Singapore). Only semi-quantitative identification of antibiotics was carried out in this work, that is, the presence above the limit of detection (LoD, Supp. Fig. S1). The LoD was obtained through the lowest analyte concentration that could be determined. To reach this parameter, injections of increasingly lower antibiotic concentrations were performed until a signal/noise ratio of at least 3:1 was obtained. Considering that the samples were concentrated at 100× before injection in the LCMS, the concentration of the antibiotics in the river would be 100× lower than the LoD.

Isolation of *Staphylococcus aureus* from surface water

Membrane filtration was used to recover *S. aureus* from surface water samples from the Extrema River, following the methodology of Goldstein et al. (2012) [44] with modifications. Briefly, 10 mL of each triplicate sample was vacuum filtered through a cellulose nitrate filter membrane, with a pore size of 0.45 µm and a diameter of 47 mm (GVS North America, Sanford, USA). The membranes were placed on Baird Parker agar base (KASVI®, São José dos Pinhais, PR, Brazil) and incubated at 37 °C for 24 h. After this period, the amounts of black colonies with presumptive halos of *S. aureus* were counted. Then, confirmatory catalase,

Table 1 Mass spectrometry (MS) parameters for antibiotic analysis

Compound	Structure	Mode	Precursor ion (m/z)	Product ion (m/z)	DP (V)	CE (V)
Amoxicillin (AMX)		[M+H]	366	114	59	23
Metronidazole (MTZ)		[M+H]	172	128	51	19
Cefazolin (CFZ)		[M+H]	455	323	56	17
Sulfamethoxazole (SX)		[M+H]	254	156	58	23
Chloramphenicol (CHL)		[M-H]	321	157	-66	-21

coagulase, and gram stain tests were performed (phenotypic confirmation).

Phenotypic confirmation of *Staphylococcus aureus* isolates

S. aureus isolates were phenotypically confirmed by catalase- [52] and coagulase-positive (Staphclin latex kit, Laborclin®, Pinhais, PR, Brazil) [53] reactions and Gram-positive staining [54].

Selection of multidrug-resistant *Staphyloccocus aureus* isolates and antibiotic resistance profile

Colonies of *S. aureus* were selected from each sampling point and streaked on MRSA Chromogenic agar (Laborclin®, Pinhais, PR, Brazil) and incubated at 37 °C for 24 h [55]. Blue-green colonies were presumptive of MRSA ($n = 20$ from the three sampled points). These colonies were stored in brain heart infusion (BHI) broth (KASVI®, São José dos Pinhais, PR, Brazil) and added with 15% glycerol at −80 °C in an ultra-freezer (ColdLab®, Piracicaba, SP, Brazil). *S. aureus* ATCC 25923 and phosphate-buffered saline were used as positive and negative controls, respectively, for quality control and isolation process assurance. Antibiotic susceptibility tests were performed by the disk diffusion method, according to Tsai et al. (2020) [56] and CLSI M100 [57], testing nine different antibiotics (Laborclin®, Pinhais, PR, Brazil) (Supp. Table S2). Multidrug resistance was defined by an *S. aureus* isolate that grew in the presence of at least three different classes of antibiotics [58]. Cultures of these isolates were deposited in the Elisa F. L. C. Bailão (EFLCB) working collection (collection of microorganism cultures of the State University of Goiás [CCM-UEG], Central Campus) at the Biotechnology Laboratory (LaBiotec).

Genotypic confirmation of *Staphylococcus aureus* isolates by polymerase chain reaction (PCR)

DNA extraction was performed according to the manufacturer's protocol (Zymo, CA, USA). The extracted nucleic acid was stored at −80 °C until further analysis. *S. aureus nuc* gene was used as a specific target using the following primers: forward 5'-GCGATTGATGGTGATACGGTT-3' and reverse 5'-AGCCAAGCCTTGACGAACTAAAGC-3' (amplicon = 279 bp) [59]. The following cycle conditions were used: 1× cycle for the initial denaturation step at 94 °C for 5 min; 40× cycles for the denaturation step at 94 °C for 1 min; annealing step at 54 °C for 30 s; extension step at 72 °C for 30 s; 1× cycle for final extension step at 72 °C for 10 min. The *nuc* amplicon was visualized in a 1.5% agarose gel. A 100 bp ladder (invitrogen by ThermoFisher Scientific, Waltham, Massachusetts, EUA) was added to compare the fragment amplified in the gel.

Statistical analysis

For the antibiotic resistance parameters of *S. aureus*, the mean ± standard deviation was calculated for each group using BioEstat 5.3 software [60]. For Pearson correlations between dissolved oxygen (DO) and the number of *S. aureus* isolates, pH and the number of *S. aureus* isolates, and DO and pH were used in past software 4.06b version [61].

Results

Antibiotic detection in Extrema River spring

It was possible to confirm the presence of sulfamethoxazole (≥ 1 ng/L) at P3 in the November campaign (Supp. Fig. S2). Some antibiotics are suggested to be present also in P3 in the November campaign but appear below the established LoD, metronidazole (< 0.5 ng/L), and chloramphenicol (< 5 ng/L) (Supp. Fig. S3).

Multidrug-resistant *Staphylococcus aureus* detection in Extrema River spring

From P1, P2, and P3 samples, 128, 168, and 36 *S. aureus* colonies were isolated, respectively (Fig. 2). The smaller number of *S. aureus* isolates in P3 in the three campaigns could be correlated with the lower dissolved oxygen (DO) quantified in P3 ($r = 0.870$, $p = 0.002$) in the three campaigns. Moreover, DO and pH were positively correlated in this study ($r = 0.780$, $p = 0.013$). The isolates EFLCB 010-029 were genotypically confirmed as *S. aureus* since the *nuc* gene was amplified in all samples (Supp. Fig. S4). Of the *S. aureus* colonies isolated, there was a large number of multidrug-resistance to antibiotics (P1 $n = 88.8\%$, P2 $n = 33.3\%$, P3 = 66.6%) (Fig. 2). In the evaluation of the AMR profile, 90% of *S. aureus* isolates showed resistance to trimethoprim-sulfamethoxazole, 70% to clindamycin, and 60% to gentamicin. The isolates from P1 showed a high AMR profile (Table 2).

Discussion

Low-order streams (1st to 3rd order) dominate a riverside landscape for the function and health of the river network, being fragile ecosystems that are impacted by the discharge of effluents [62]. Improper disposal of effluents brings negative impacts to One Health. Brazil is the only country in

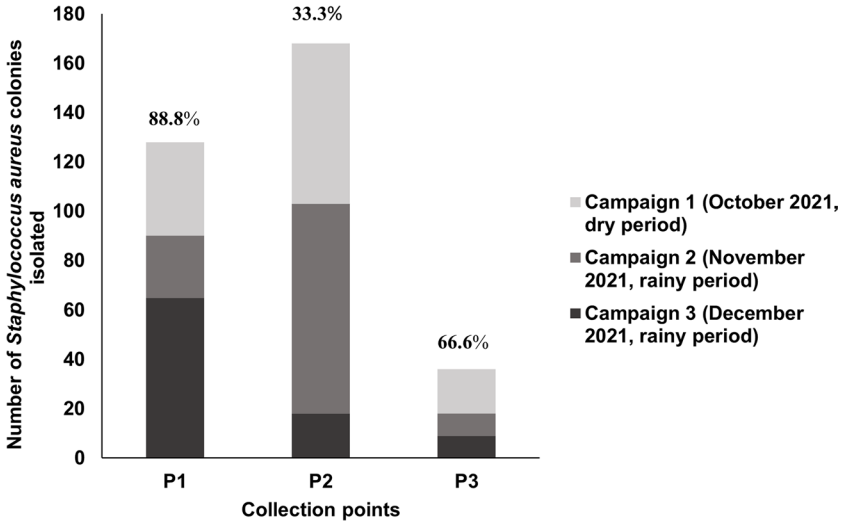


Fig. 2 The number of *Staphylococcus aureus* colonies isolated by sampling point from the Extrema River spring, central Brazil. The number above the bars considers the percentage of multidrug-resistant *S. aureus* in each collection point. Multidrug resistance was defined by *S. aureus* isolate that grew in the presence of at least three different classes of antibiotics**:** chloramphenicol 30 µg, ciprofloxacin 5 µg, clindamycin 2 µg, erythromycin 15 µg, gentamicin 10 µg, rifampicin 5 µg, trimethoprim**-**sulfamethoxazole 25 µg, tetracycline 30 µg, and cefoxitin 30 µg

South America with specific legislation on the ecotoxicological assessment of effluents, resolutions n° 357/2005 and n° 430/2011 of the National Council for the Environment (CONAMA) [63]. However, many Brazilian states, such as Goiás, do not have their own legislation that would complement federal legislation. In this sense, much still needs to

Table 2 Antibiotic resistance profile of ***Staphylococcus aureus*** isolates from the Extrema River surface water, Central Brazil

S. aureus **code: EFLCB***	**CHL**	**CIP**	**CLI**	**ERY**	**GEN**	**RIF**	**SXT**	**TET**	**FOX**
P1									
010	R	R	R	R	R	R	R	R	S
011	R	R	R	R	R	R	R	R	S
012	R	R	R	R	R	R	R	R	S
013	S	S	S	S	S	S	S	S	S
014	R	R	R	R	R	R	R	R	S
015	S	S	S	S	S	S	S	S	S
P2									
016	S	S	R	S	R	S	R	S	S
017	S	S	R	S	R	S	R	R	S
018	S	S	R	S	S	S	R	S	S
019	R	S	S	S	S	S	R	S	S
020	S	S	R	S	R	S	R	S	S
021	S	S	R	R	R	S	R	S	S
022	S	S	S	R	S	S	R	S	S
023	S	S	S	S	S	S	R	S	S
P3									
024	S	S	S	S	S	S	R	S	S
025	S	S	R	R	S	R	R	R	S
026	I	R	R	R	R	R	R	R	S
027	S	S	R	S	R	S	R	S	S
028	S	S	R	R	R	R	R	R	S
029	S	S	R	R	R	R	R	R	S

*Elisa F. L. C. Bailão (collection of cultures of microorganisms from the State University of Goiás, Central Campus). Values correspond to triplicate mean ± standard deviation of inhibition halos (mm). *R*, bacterial resistance; *I*, intermediate sensitivity; *S*, bacterial sensitivity. Antibiotics evaluated: chloramphenicol 30 µg (CHL), ciprofloxacin 5 µg (CIP), clindamycin 2 µg (CLI), erythromycin 15 µg (ERY), gentamicin 10 µg (GEN), rifampicin 5 µg (RIF), trimethoprim-sulfamethoxazole 25 µg (SXT), tetracycline 30 µg (TET), and cefoxitin 30 µg (FOX)

be done to control and monitor the emission of effluents in Brazilian water resources. In this work, we first investigated the presence of antibiotics in the Extrema River spring, a low-order stream close to pharmaceutical industries (DAIA). DAIA has a sewage treatment plant inaugurated in 2003 to treat the industrial effluents from 78 industries. However, nowadays, there are more than 150 industries, and not all pre-treat effluents, which overload the system with a large volume of effluents and cause recurrent leaks [8]. In the November campaign, antibiotics in the Extrema River spring at P3 were observed, namely sulfamethoxazole (≥ 1 ng/L), metronidazole (< 0.5 ng/L), and chloramphenicol (< 5 ng/L). It is important to highlight that P3 is the nearest point from DAIA. In Cerrado, November is considered the month with more volume of rain after a 5-month drought. Maybe the pharmaceutical industries clandestinely discharge more effluents in November, relying on the dilutional property of rainfall. Additionally, the presence of antibiotics in the streams indicates the persistence of degradation, continuous discharge, input in large volumes, and incomplete removal during septic treatment [64, 65]. The half-life of sulfamethoxazole and chloramphenicol in environmental water is 3.1–30 days and 60 days, respectively [66, 67].

Pharmaceutical contaminants have already been reported in low-order streams in China [68], India [69, 70], Japan [71], France [72], the UK [73], and the USA [74]. In international studies, the most detected antibiotic is sulfamethoxazole, appearing in 96% of the analyzes. The antibiotic with the highest concentration is also sulfamethoxazole (> 20,000 ng/l), while the lowest concentration is metronidazole (1800 ng/l). Additionally, 53% of the studies use wastewater as a source, while only 18% of the papers use river water as a source. Therefore, increasing the water analysis in rivers and in other aquatic matrices is very interesting for the scientific community [75]. In Brazil, antibiotics were detected in surface water in the states of Amazonas, Maranhão (including sulfamethoxazole–22–120 ng/L) [76], Minas Gerais [77, 78], Rio Grande do Sul (including sulfamethoxazole > 1.0 ng/L [79], < 300 ng/L [22], < 200 ng/L [80]) [22, 79–81], and São Paulo (including sulfamethoxazole < 5 ng/L [82], 0.78–106 ng/L [21]) [21, 82, 83]. To the best of our knowledge, it is the first time that the presence of antibiotics in central Brazil freshwater has been described. However, genes encoding resistance to β-lactams, macrolides, quinolones, fluoroquinolones, tetracyclines, and sulfonamides were recently reported in another central Brazil stream (João Leite River) [84].

The most recent study by Wilkinson et al. (2022) [85], reviewed by Bouzas-Monroy et al. (2022) [86], pointed out that of 1052 river sites monitored in 104 countries, 43.5% (461 sites) contained concentrations of active pharmaceutical ingredients of concern, with approximately 34.1% of the 137 sampling campaigns having at least one site where concentrations were of ecotoxicological concern. Thus, for the conservation of freshwater springs, the Arthington document (2021) [87] points out that a more strategic, integrated, and collaborative global effort is needed between countries through the following solutions: inventory, assessment, and research of contaminated areas; ecosystem restoration and rehabilitation; design and management of protected areas; science and sociological governance. The restoration of springs and biodiversity protection depends on a reliable scientific database that evaluates the ecological results of natural processes and management actions.

The occurrence of antibiotics in the aquatic environment poses a global threat to One Health. First of all, antibiotics can cause acute and chronic toxicity toward aquatic organisms and affect the evolution of the bacterial community structure, which plays a significant role in the ecosystem [65]. Additionally, the presence of antibiotics in freshwater is of special concern because increasing concentrations of discarded antibiotics can force the appearance of some extremely multidrug-resistant strains, not just as the consequence of mutations, but also by HGT, which poses a serious public health problem [88]. *S. aureus* was isolated from the three sampled points (P1, P2, and P3) in the Extrema River spring. The smaller number of *S. aureus* isolates in P3 in the three campaigns could be correlated with the lower DO quantified in P3 in the three campaigns. It has been demonstrated that *S. aureus* is affected by a decrease in dissolved oxygen equal to or below 7 mg/L, because it utilizes a lot of oxygen to survive [89]. Moreover, DO and pH were positively correlated in this study. As pH decreases, hydrogen ions and oxygen react with water, which results in a decrease in the DO [90]. A total of 65% of the isolates presented resistance to at least three classes of antibiotics. Additionally, all the *S. aureus* isolates are resistant to trimethoprim-sulfamethoxazole in P2 (November) and P3 (December), a drug produced in DAIA and identified in the Extrema River spring, suggesting that the clandestine discharge of antibiotics from industrial effluents in the region may cause *S. aureus* AMR development. This data is worrying, as *S. aureus* is a pathogen of significant concern for hospitals and the community. Treating *S. aureus* infections is becoming challenging, especially considering the emergence of antibiotic-resistant strains [91].

The lack of estimates of human exposure to environmental bacteria, generally antibiotic-resistant bacteria, is a gap that makes it difficult to assess the effects of exposure. Streams and rivers can be important routes for disseminating these antibiotic-resistant bacteria, mainly because people usually use freshwater for recreational activities, ingestion, and irrigation. People have been observed using the Extrema River water for recreational purposes, which could be a source of acquiring and disseminating multidrug-resistant *S. aureus* strains. Some studies have pointed to the presence

of AMR microorganisms and AMR genes in aquatic ecosystems impacted by human activities [25, 92–94]. Thus, surveillance and study of microorganisms in impacted freshwater environments can provide information on how microbial communities change because of human activities.

Conclusions

Our data indicate that the Extrema River's spring is receiving clandestine effluents from pharmaceutical industries. So, it needs restoration and intense environmental surveillance for biodiversity and public health protection. Microbial communities contribute significantly to the quality of surface waters. Moreover, the impacts of land use and industrial effluent disposal on bacteria promote the development of multidrug-resistant strains. Thus, antibiotics in this ecosystem disrupt environmental and public health. This study encourages policies to monitor emerging pollutants in the Extrema River's spring, which can be helpful since the safe amounts of disposal of these compounds are not yet legally regulated.

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Data Availability Data and materials not included in the manuscript are available under request.

Declarations

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