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Resistance profile of *Escherichia coli* strains in micro-basins with different soil use and occupation, in the Northwest of Rio Grande do Sul, Brazil

Perfil de resistência de cepas de *Escherichia coli* em microbacias com diferentes usos e ocupação do solo, no Noroeste do Rio Grande do Sul, Brasil

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ABSTRACT

Escherichia coli, a highly adaptive microorganism, has gained scientific interest due to its rapid mutation and resistance to antimicrobial treatments. The primary sources that contribute to its rapid spread and the emergence of infections remain hotly debated. Thus, the objective of this research was to provide a baseline, as the first study in the region that analyzes the microbiological quality of water in micro-basins with different characteristics of land use and occupation. Intending to identify whether there is resistance in the isolated strains of *E. coli* and what their possible origins are, the study provides a behavior modeling of the studied area. To this end, three sampling campaigns were carried out, from which these microorganisms were isolated and identified by polymerase chain reaction, and were subsequently subjected to susceptibility tests with nine antibacterials widely used in the treatment of infections caused by this bacterial species. The results revealed that the watershed receiving urban sanitary effluents presented the highest contamination levels, with persistent multidrug-resistant strains throughout the collection period. Among all strains, 80% were resistant to ampicillin, while 33.3% showed resistance to cefazolin. Levofloxacin was the most effective antibiotic, with 100% susceptibility. Micro-basins with predominant agricultural and pasture activities demonstrated less resistance than urban-impacted areas. This highlights the urgent need for constant monitoring and further studies to uncover the main determinants of this urgent ecological and public health problem.

Keywords: bacterial resistance; water contamination; sanitary effluents.

RESUMO

Escherichia coli, um microrganismo altamente adaptativo, tem ganhado interesse científico devido à sua rápida mutação e resistência a tratamentos antimicrobianos. As fontes primárias que contribuem para sua rápida disseminação e surgimento de infecções permanecem muito debatidas. Assim, o objetivo desta pesquisa foi de prover uma linha de base, como o primeiro estudo na região que analisa a qualidade microbiológica da água em microbacias com diferentes características de uso e ocupação do solo. Com o intuito de identificar se há resistência nas cepas isoladas de *E. coli* e quais suas possíveis origens, o estudo fornece um modelo do comportamento da área estudada. Para tanto, foram realizadas três campanhas de amostragem, das quais esses microrganismos foram isolados e identificados por reação em cadeia da polimerase, sendo posteriormente submetidos a testes de suscetibilidade com nove antibacterianos amplamente utilizados no tratamento de infecções causadas por essa espécie bacteriana. Os resultados revelaram que a bacia hidrográfica receptora de efluentes sanitários urbanos apresentou os maiores níveis de contaminação, com cepas multirresistentes persistentes durante todo o período de coleta. Entre todas as cepas, 80% foram resistentes à ampicilina, enquanto 33,3% mostraram resistência à cefazolina. Levofloxacino foi o antibiótico mais eficaz, com 100% de suscetibilidade. Microbacias com atividades agrícolas e de pastagem predominantes demonstraram menos resistência do que áreas impactadas por áreas urbanas. Isso destaca a necessidade urgente de monitoramento constante e mais estudos para descobrir os principais determinantes deste urgente problema ecológico e de saúde pública.

Palavras-chave: resistência bacteriana; contaminação da água; efluentes sanitários.

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Introduction

Water quality is a basic pillar of survival on Earth. Despite being a condition of existence for humanity, much of this resource is contaminated and in inadequate conditions for specific uses (Li and Wu, 2019). Therefore, it is pertinent that periodic studies are carried out that evaluate and identify possible contaminants with potential toxicity.

One of the biggest problems and active contaminants of water are the microorganisms that come into contact with water sources through the discharge of effluents (domestic, industrial, agro-industrial, etc.) without prior treatment. Thus, the environmental pressure suffered by bacteria, due to the indiscriminate use of antibiotics, promotes the selection of more resistant pathogens, complicating the conventional forms of treatment, both in the clinical and environmental areas (CDC, 2019).

In this sense, about 86% of the microorganisms that cause infections in humans in Brazil, by water or food transmission, are bacteria, of which, in the last ten years, *Escherichia coli* was the main agent causing diseases (Brasil, 2023). Thus, it is necessary to evaluate possible resistance profiles of this bacterium that can accentuate public health, social, environmental, and economic problems (Kadri, 2019).

Regarding the location covered by the study, Frederico Westphalen is among the municipalities in the Northwest region of the state of Rio Grande do Sul, Brazil, with the highest percentage of pig farming. According to the Rio Grande do Sul Swine Breeders Association (ACSURS), in 2019, the municipality of Frederico Westphalen raised 658,623 pigs, supported by 54,041 sows. This is also the main economic activity in the municipality of Taquaruçu do Sul (Kuwiatz, 2021).

Currently, veterinary medicine accounts for at least 70% of global antibiotic consumption, with the majority being used in animal production. Among the most widely used classes of antibiotics in this sector are tetracyclines (35.63%), followed by penicillins (12.56%), polypeptides (11.33%), and macrolides (9.33%) (WOAH, 2023). This high level of consumption, combined with the improper management of waste and pollutants generated by animal production activities, urban activities without adequate sanitation, and agro-industrial effluents, can cause several problems to the sources of land and water occupation, altering natural cycles and causing an increase resistance in microorganisms (Cao et al., 2021; Savin et al., 2021). When water quality is compromised, it is possible to verify the intensity of environmental damage by monitoring and analyzing certain indicators, of which *E. coli* is one of the most studied microorganisms (Holcomb and Stewart, 2020; Wen et al., 2020).

According to official data from the World Health Organization (WHO), there was an increase in antimicrobial resistance (AMR) by more than 15% in 2020 compared to 2017 for meropenem and third-generation cephalosporin resistance in bloodstream infections from *E. coli* bloodstream. The average rate of third-generation cephalosporin-resistant *E. coli* causing related bloodstream infections across 76 countries, territories, and areas (CTAs) is 42% (WHO, 2022).

On the other hand, there is a large production of antibiotics worldwide to combat microorganisms that cause diseases. However, the excessive use of these drugs to treat infected patients as well as in animal production leads to large amounts of drug residues that are not metabolized by the organism in sewage treatment plants, rivers, and lakes (Manyi-Loh et al., 2018; Polianciuc et al., 2020; Barathe et al., 2024). This process triggers AMR due to the gradual exposure of these microorganisms to drugs, specifically antibiotics that are widely consumed in animal farming and human health, present in the environment, transmitting this ability to future generations and even to other bacteria (Gupta et al., 2016; Alawi et al., 2022).

According to Murray (2022), an analysis of global data estimated that in 2019, about 4.95 million deaths were associated with bacterial AMR, of which 1.27 million were directly attributed to these pathogenic microorganisms. In addition, among the main pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella spp*., and *Acinetobacter spp.*) of death associated with resistance, *E. coli* had more evidence (WHO, 2022).

Understanding the damage of AMR and the main combinations that contribute to it is crucial for making policy decisions based on local realities, infection prevention and control programs, access to essential antibiotics, and research and development of new treatments (Murray, 2022). Despite the excellent surveillance of Healthcare-Associated Infections (HAIs) by the Brazilian Health Regulatory System (ANVISA), there remains an information gap in Brazil on AMR, especially considering the One Health approach, which establishes the need to monitor not only resistant nosocomial bacteria but also the most common isolates transmitted by the community through other routes (Pillonetto et al., 2021).

Considering the above, this work aimed to isolate and confirm the molecular identity of environmental strains of *E. coli* present in a water body, with different profiles of use and occupation of micro-basins. In addition, to quantify them to analyze the classification of the water body, according to the National Environment Council (CONAMA) Resolution nº 357/2005, outlining the resistance or susceptibility profile of the isolated bacteria, in order to map possible sources of contamination and verify the presence of risk for the population.

Material and Methods

Water sample collection and characterization of the study area

The water collections occurred in the micro-basins of Lajeado Pardo, located between the municipalities of Frederico Westphalen and Taquaruçu do Sul, Northwest region of the state of Rio Grande do Sul, Brazil (IBGE, 2023). Lajeado Pardo has an extension of 5,700 m, and its source at coordinates 27º25’43”S latitude and 53º43’25” longitude, with an average altitude of 488 m, from the source to the point of damming for water capture that supplies the municipalities of Frederico Westphalen and Caiçara (RS), Brazil. Three collection campaigns were carried out, which extended from January to March 2022. At each sampling point, 1 L samples were collected in triplicate and refrigerated during transport at 5°C standard deviation (±) 3°C. The tubes used for collection were pre-sterilized and stored in the absence of light during transport to the laboratory.

The classification strategy for the area was conducted based on geospatial data processed using QGIS software version 3.10.9. This included the use of location maps, sub-basin and micro-basin delimitation, as well as land use and cover maps. Spatial images were obtained from the National Institute for Space Research (INPE, 2022) portal, utilizing the CBERS4A satellite equipped with the WPM camera. These images processed at L4 level, covered RGB spectral bands with a spatial resolution of eight meters. The generation of watercourse and basin delimitation was based on the altimetric model provided by Brazil's geomorphometric database (TOPODATA).

The sub-basin was delimited from the outpouring, connecting the points of highest elevation, based on the contour lines. The classes, represented in Figure 1, used for the characterization of land use and occupation, were forest formation, agriculture and pasture and urban infrastructure, according to the classifications suggested by the Brazilian Institute of Geography and Statistics (IBGE, 2020).

Figures 1A and 1B present the representative map of the micro-basins, where it is possible to observe the collection points located at the outpouring of each micro-basin (A, B, C, D, and E) and a point at the water catchment dam for municipal supply. The micro-basin "E" stands out because sampling occurred during a drought period. Due to water scarcity, samples could not be collected from this micro-basin. As a result, samples were collected only from micro-basins A, B, C, and D.

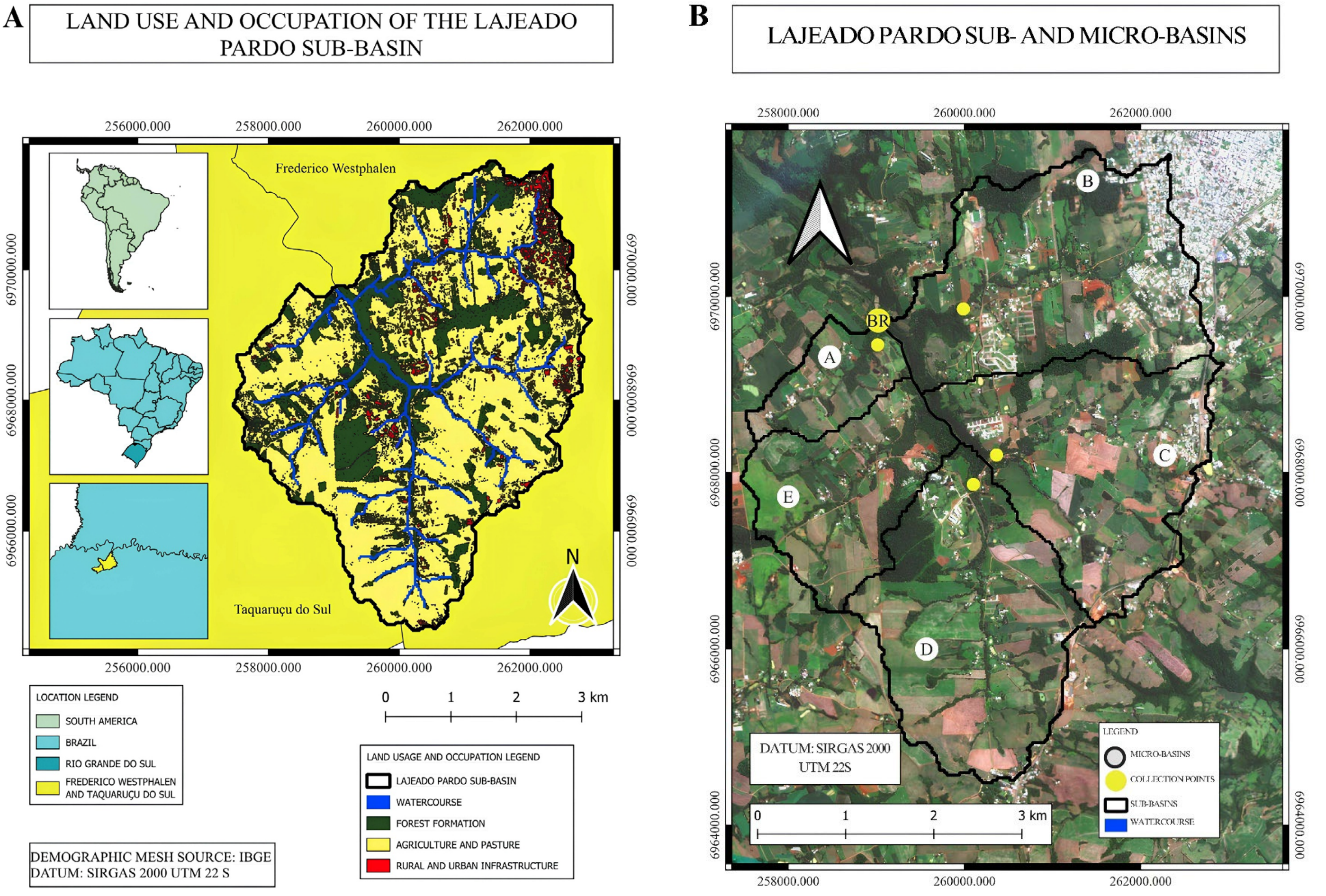


Figure 1 – Location maps of the study area. The location map in Figure 1A displays the land use and cover theme, including the delimitation of micro-basins and the water collection points analyzed in Lajeado Pardo (RS), Brazil. It provides an overview of the study area, highlighting the spatial distribution of land use categories and the geographic positioning of sampling sites. Figure 1B exhibits a detailed map focusing on the delimitation of the micro-basins, emphasizing the specific locations of the collection points where the analyzed water samples were obtained. It allows for a clearer visualization of the hydrological divisions and sampling points within the study area. A, B, C, and D are collection points in each micro-basin and BR is the final collection point at the water body dam.

Microbial cultivation conditions of standard strain

Cultures were stored at -20°C in 80% brain and heart infusion broth, with 20% glycerol (Kouadio-Ngbesso et al., 2019). The standard strain used as a positive control was *E. coli* INCQS P3089 provided by the reference microorganisms laboratory of the National Institute of Quality Control in Health (INCQS), from the Oswaldo Cruz Foundation (FIOCRUZ).

Quantification of *Escherichia coli* and total coliforms

The water samples collected were submitted to simultaneous detection of the enumeration of total coliforms and *E. coli*, using the Colilert method, the standard for water and sewage in Brazil (Brasil, 2013), a method approved by the Standard Methods for Examination of Water and Wastewater (SMEWW) (Rice, 2012).

To compile the data from the small wells and large positive wells and generate the most probable number (MPN) of the Quanti-Tray/2000 system, we used the free software IDEXX Water MNP Generator®, provided by the IDEXX laboratory. When entering the values of small and large positive wells, the software automatically informs the MPN of both total coliforms and *E. coli*, for each sample.

Bacterial isolation methods for *Escherichia coli*

The membrane filter method was applied, with cellulose acetate filters of 47 mm in diameter and pore size of 0.45 μm, by taking a water sample (100 mL) previously homogenized and filtering in a sterile system by negative pressure (vacuum) in order to retain the microorganisms on the membrane. Subsequently, the membrane was aseptically placed on an appropriate selective solid medium (MacConkey) in a Petri dish, which by capillarity, comes into contact with the bacterium. Afterwards, the plates were incubated for 24 hours in a biochemical oxygen demand (BOD) incubator (Solab SL/200/120), at 35–37°C for 18–24 hours (Brasil, 2013). Presumptive *E. coli* isolates were then inoculated into EC medium (44.5±0.2°C for 24 hours) and positive cases were further subcultivated in the same medium until pure colonies were obtained. This procedure ensured the selection of specific microorganisms and standardized the samples for further analyses. For negative control, autoclaved distilled water was used and positive control was performed with reference strain from stock culture, previously described.

Procedures and reagents for polymerase chain reaction

To identify the strains isolated in selective media, the polymerase chain reaction (PCR) methodology was used, aiming to amplify specific deoxyribonucleic acid (DNA) sequences that represent each species or gene studied.

Analysis of specificity, preparation, and dilution of oligonucleotides — primers

The primers for species identification (forward and reverse) available in the literature were analyzed for their specificity, formation of secondary structures, and other parameters for good amplification. For this, we used the Netprimer® software and after analysis, the one that presented the best evaluation was chosen. The product acquired, with 25 nmol mass purified by desalination, was diluted in stock and work solutions. The stock solution was prepared to dilute 10 times the amount of primer in each sample. For the work solution, 10 uL of stock solution were removed, and to this amount, another 100 uL of ultrapure water were added. Thus, the final concentration of the work solution was 1 pmol/uL.

For each thermocycling reaction, the reagent mix and its quantities were performed according to the standard recommended by the manufacturing company Ludwig Biotec, where 12.5 uL of Pre-Mix, 2.5 uL of each primer (forward and reverse), 6.5 uL of ultrapure water and 1 uL were added to a 200 uL microtube (deoxyribonuclease and ribonuclease free). DNA template at an approximate concentration of 5 ng, quantified by ultraviolet (UV) spectrophotometer, at a wavelength of 260 nm.

The Pre-Mix used in the reaction has all the basic reagents for the PCR reaction: reaction buffer tris-hydroxymethyl aminomethane and potassium chloride (KCl), pH 8.4, concentration of 2.0 mM magnesium chloride (MgCl2), 0.2 mM deoxynucleoside triphosphates (dNTP) and 2.5 U of recombinant Thermus aquaticus (Taq) DNA polymerase.

Obtaining bacterial DNA and amplification

The bacterial genetic material (DNA) was obtained by the simple boiling method, considered not only superior in its simplicity, cost, and short handling time but also reliable (Peng et al., 2013; Yamagishi et al., 2016).

The amplification was tested with different curve parameters, including those used by McDaniels et al. (1996), to standardize the reaction before the main experiment. In this specific case, the methodology was adjusted for the reaction, following one initial cycle for denaturation at 95°C for 5 minutes and 40 cycles of three steps: denaturation (94°C for 30 seconds), annealing (55°C for 30 seconds), and extension (72°C for 2.5 minutes). Finally, one more cycle was programmed at 72°C for 5 minutes of extension at the end of the reaction.

For sample loading, 4 μL of amplified DNA was mixed with 2 μL of loading dye, resulting in a final volume of 6 μL per well. The molecular weight marker (ladder) was similarly prepared by mixing 4 μL with 2 μL of dye. Positive and negative controls were included in separate wells to validate the run, considering a value of approximately 10 V/cm between electrodes of the tank. Thus, we used 70 V for 60 minutes. Confirmation of the samples as *E. coli* was obtained by comparing the sample bands with the positive control, which contained DNA extracted from *E. coli* ATCC (the American Type Culture Collection organization). This approach ensured accurate identification by matching the band profiles of the tested samples with the known standard. The primers used for species identification were previously validated in the study by McDaniels et al. (1996).

Sample running in buffer and agarose gel

The Tris-Borate-EDTA (TBE) solution was made with 108 g of trisamino (hydroxymethyl) aminomethane, 55 g of boric acid, 7.5 g of ethylenediaminetetraacetic acid tetrasodium (EDTA), completing up to the level of 1L of deionized water, in a volumetric flask. There was no need to adjust the hydrogen potential (pH), as it remained at 8.0 at the end of the preparation. The preparation followed the amounts of reagents used in standard commercial TBE solutions for the use proposed in this study.

The specific conditions for agarose gel electrophoresis analysis were standardized to ensure precise visualization of the amplified products. The gel was prepared by dissolving 0.2 g of agarose powder in 20 mL of 1X TBE buffer, which was obtained by diluting a 10X concentrated stock solution. The solution was heated until the powder was completely dissolved and, after cooling, poured into the electrophoresis tank to solidify.

Antimicrobial sensitivity tests

From the previously isolated strains, together with the standard strain, we followed the methodology of Bauer (1966), with adjustments, considering updates made by the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST, 2022).

Each strain was tested in three replicates for nine types of antibiotics used for the treatment of *E. coli*, namely: ampicillin (AMP; 10 µg), amoxicillin-clavulanate (AMC; 20/10 µg), chloramphenicol (CLO; 30 µg), gentamicin (GEN; 10 µg), ciprofloxacin (CIP; 5 µg), cefazolin (CFZ; 30 µg), ceftazidime (CAZ; 30 µg), fosfomycin (FOS; 200 µg), and levofloxacin (LVX; 5 µg).

After preparing all reagents and inputs for testing and performing the experiment following the rules of the methodology mentioned above (incubated inverted at 37°C for 16–18 hours), the halos were read with a digital caliper and then compared with the tables of clinical cutoff points (BrCAST, 2022). The 15 strains analyzed were further evaluated for their multidrug resistance (MDR) profiles. Strains were classified as exhibiting MDR if they demonstrated resistance to at least two of the nine antibiotics tested. This approach ensured the identification of strains with significant resistance patterns, highlighting their potential clinical and environmental relevance (Odonkor and Addo, 2018).

Results and Discussion

Determination of land use and occupation in the different micro-basins of the study area

The classifications attributed to land use and occupation, considered according to the previous field analysis, predicted the predominance of agriculture and pasture (AP), forest formations (FF), and urban infrastructures (UI). Table 1 determines the classification of land use and occupation and the percentages of each class by micro-basin (A, B, C, D, and E), as illustrated in Figure 1B.

Table 1 – Percentage of land use and occupation in each micro-basin.

CLASS	A	B	C	D	E
FF	22.87	26.70	19.17	19.99	27.79
AP	74.14	61.66	76.08	76.89	69.50
UI	2.99	11.64	4.74	3.11	2.71

FF: forest formation; AP: agriculture and pasture; UI: urban infrastructure.

In all micro-basins, AP areas were predominant (71.18%), followed by small groups of FF (22.98%) and UI (5.84%), representing averages of the total area analyzed of 23.565.413.43 km² (A- 1.432.097.56; B- 6.613.175.57; C- 5.848.565.32; D- 6.253.511.23; and E- 3.418.063.75). These data demonstrate an FF even smaller than the global trend, which, according to the Food and Agriculture Organization of the United Nations and the United Nations Environment Programme (FAO and UNEP, 2020), occupies 31% of the planet's land area. In micro-basin B, the largest contingent of UI was observed, which accounted for 55.88% of the total calculated in the micro-basin.

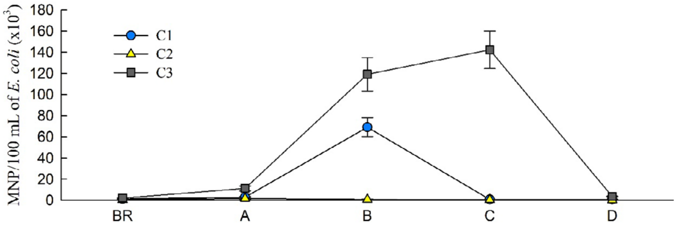
Taking into account the small amount of FF as riparian vegetation, as can be seen in the maps shown previously (Figure 1), water resource contamination may be facilitated due to the low coverage of physical barriers to prevent entry of sediments, chemical and microbiological contaminants (Martins et al., 2021). In this sense, the impacts of riparian vegetation suppression can be observed in the short and long term from the reduction of water quality, posing risks to environmental and human health (Ritter et al., 2015; Silva et al., 2015; Crisigiovanni et al., 2020; Shah et al., 2022).

In this context, effluents resulting from agricultural, livestock, industrial, and domestic activities interact dynamically with the environment as they do not remain static in the places where they are discharged, given that there are no effluent treatment stations in the study area. Its packaging, when carried out, is done individually, with individual systems for packaging domestic effluent. For animal effluents, at most you can count on a manure pit that matures the waste before it is disposed of on agricultural land. Whether by runoff, infiltration, dispersion of waste from these activities, or untreated effluents disposed directly into water resources, contamination can occur (Cycoń et al., 2019; USGS, 2019; FAO, 2023).

Due to this dynamic between contaminants and the environment, the problem of bacterial resistance as an emerging pollutant has grown. Considering that most antibiotics are not completely metabolized in human and animal bodies, part of these drugs come into contact with bacteria present in effluents, water, and soil. Thus, microorganisms acquire resistance due to environmental pressure from constant exposure (Larsson and Flach, 2022).

Microbiological analysis: determination of presence or absence and quantification of *Escherichia coli*

From the qualitative and quantitative analyses, the presence of total coliforms and *E. coli* was observed in all samples analyzed (Figure 2).



*X-axis: collection point within each micro-basin (micro-basin E was not collected, as there was no water during the dry season). *Y-axis: MNP/100 ml of *Escherichia coli* in the three collections (C1: January; C2: February; C3: March).

Figure 2 – Most probable number of *Escherichia coli* in 100 mL, at the five points analyzed in three periodic collections. Collection points in each micro-basin (A, B, C, and D) and final collection point at the water body dam (BR).

The quantified results of the samples examined presented large quantities, mainly of total coliforms.

The CONAMA Resolution nº 357/2005 (Brasil, 2005) legislation classifies the water bodies in Brazil and this classification is important as it frames the rivers in classes according to their intended predominant uses over time. The river where this research was conducted does not have a classification. In this case, the legislation assigns it to class 2. This class allows supply for human consumption after conventional treatment, in addition to primary contact activities, irrigation of vegetables, aquaculture, use in parks, gardens, sports and leisure fields with which the public may have direct contact, according to CONAMA Resolution nº 274/2000 (Brasil, 2000).

Regarding the classification of the water body, CONAMA nº 357/2005 (Brasil, 2005) suggests that at least six samples must be collected during a one-year period, with bimonthly frequency. Therefore, continuous monitoring of the water body is suggested, especially at the collection points of micro-basins B and C, which exceeded the *E. coli* limits for classes 2 and 3 in most of the analyses. In addition to the parameters indicated in this study, it is necessary that the other indicators participating in CONAMA nº 357/2005 also be evaluated for the classification of the water body (Brasil, 2005).

For bathing, according to CONAMA nº 274/2000 (Brasil, 2000), which defines the criteria for this purpose, it can be considered that the water analyzed is unsatisfactory or unfit, considering that of all the samples collected, only 20% had the MPN below 800 *E. coli* per 100 mL. In the third sampling campaign, all points analyzed exceeded 2000 *E. coli* per 100 mL; the micro-basins B and C showed an MPN above 100 thousand per 100 mL, indicating a high degree of contamination. Therefore, all collection points would be inappropriate for recreational activity.

Another study (Flach et al., 2024) that evaluated physical and chemical parameters for the classification of the Lajeado Pardo water body also found that it cannot be identified as class 2, in addition to presenting risks of eutrophication. Furthermore, the parameters for *E. coli* found by Konzen et al. (2024) in another water body present in the region also demonstrated an excessive presence of this microorganism, which indicates risks in case of primary contact.

Isolation and molecular identification of *Escherichia coli*

The pair of primers for amplification of genetic material to identify *E. coli* species has 670 kbp (F- ACCTGCGTTGCGTAAATA; R- GGGCGGGAGAAGTTGATG), as reported by McDaniels et al. (1996). According to the analysis of the Netprimer software, the pair of primers did not present secondary structures such as binding between primers or the primer itself. The oligo repetitions did not occur with more than three bases in sequence, containing a sufficient amount of cytosine and guanine for the reaction. The annealing temperature was adequate to the desired parameters and the binding stability with the template strand was satisfactory.

From the isolation of the strains and the gel electrophoresis run (in five batches), the species were identified according to amplification. Based on Figure 3, it is possible to confirm amplification from the molecular weight standard (MW), Strain 1 (E1), Strain 2 (E2), Strain 3 (E3), and negative control (NC). For the other strains, the same procedure was carried out, in batches of three samples per gel.

All batches showed positive results for environmental isolates in selective bacterial growth media, confirming the presence of *E. coli* in all samples, according to the results observed in the Colilert tests.

Resistance profile of isolated strains to antimicrobials

The drugs for susceptibility tests in this study were chosen to meet the specificity requirements of application for infections caused by *E. coli*, following the Brazilian guidelines (BrCAST, 2022). In addition, the importance of choosing drugs that have the best-defined cutoff points was verified, avoiding possible problems of interpretation and classification regarding the levels of bacterial sensitivity or resistance. Considering the importance of susceptibility tests, an analysis is essential to aggregate the maximum possible classes, facilitating the understanding of which resistance mechanism is active in the strains. In this sense, the strains presented different resistance profiles (Table 2).

Despite belonging to the same species, they were possibly subjected to different degrees of environmental pressure and came into contact with varied enhancers. The selective pressure of use, overuse, and misuse of antimicrobials in humans, animals, and agriculture leads to a gradual increase in antibiotic resistance (Paitan, 2018) which, subsequently, causes treatable bacteria to become untreatable or require application of a last line of antibiotics (Dávalos-Almeyda et al., 2022). Considering the different characteristics of the micro-basins studied, there may be a great influence of the type of use and occupation of these environments on the dynamic profiles of bacterial resistance.

As a general overview, only three isolated strains showed behavior equal to that of the standard strain. Of the isolates from micro-basin B, strain Ec2B showed a similar profile to Ec3B, and strain Ec1B also presented resistance. These microorganisms were isolated from the same sector and this generates an alert for new collections and evaluations to be carried out, mainly at the collection point of micro-basin B, which covers part of the municipality's urban occupation.

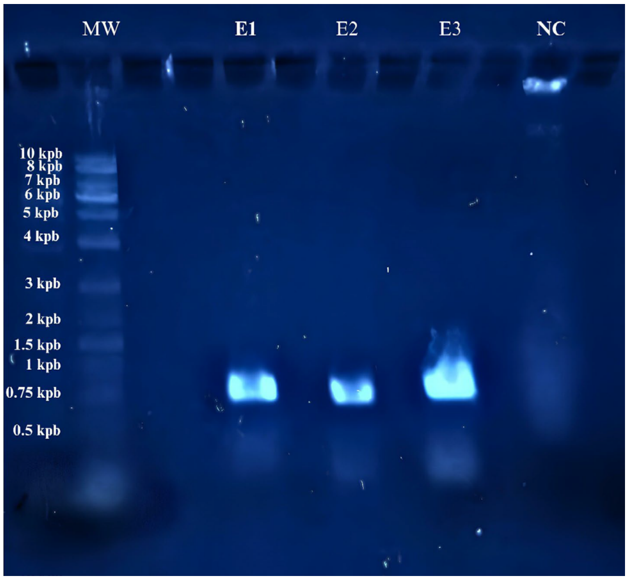


Figure 3 – Gel electrophoresis samples to visualize template strand amplification for species identification.

Table 3 shows the results of the AST where the least effective antibiotic tested against the 15 strains tested was AMP, where 80.0% of the strains were resistant to this drug, followed by CFZ (33.3%), CIP/CLO/FOS (13.3%), and AMC/CAZ (6.7%). No strain was resistant to GEN and LVX.

As in the studies carried out in other countries by Ram et al. (2008) and Kadykalo et al. (2020), *E. coli* isolates were found that cannot be treated with conventional doses of CLO, AMP, AMC, and CIP antibiotics. According to WHO (2020), the resistance rate to CIP, an antibiotic commonly used to treat urinary tract infections, ranged from 8.4 to 92.9% for *E. coli*. AMP, the least efficient drug in this study, is used against gram-positive and gram-negative organisms. This drug can have a synergistic action with aminoglycosides and β-lactamase inhibitors, such as clavulanic acid and sulbactam (Foulds, 1986; Barnhart, 1989). Its clinical indications include infections of the respiratory and urinary tracts, gonorrhea, meningitis, septicemia, and enteric infections.

The most effective drugs found in this work were aminoglycoside gentamicin, fluoroquinolone levofloxacin, and fosfomycin. Gentamicin is a drug not represented in the reports of antibiotic resistance by the WHO (Figure 4), probably because it is a less used drug due to the induction of nephrotoxicity (Balakumar et al., 2010). However, the levofloxacin, despite appearing in the reports, still has one of the lowest resistance rates. This drug, according to Bundrick et al. (2003), admin-

Table 2 – Bacterial resistance profile of isolated strains, characterized as *Escherichia coli*.

STRAIN	CIP	AMP	CLO	GEN	FOS	LVX	CFZ	AMC	CAZ
Standard	S	S	S	S	S	S	S	S	S
Ec1-BR	S	S	S	S	S	S	S	S	S
Ec2-BR	S	R	S	S	S	S	SID	S	S
Ec3-BR	S	R	S	S	S	S	S	S	S
Ec1-A	S	S	S	S	S	S	S	S	S
Ec2-A	S	R	S	S	S	S	SID	S	S
Ec3-A	S	R	S	S	S	S	S	S	S
Ec1-B	S	R	S	S	S	S	SID	S	S
Ec2-B	R	R	R	S	R	S	R	ATU	SID
Ec3-B	SID	R	R	S	R	S	SID	S	S
Ec1-C	S	R	S	S	S	S	S	S	S
Ec2-C	S	R	S	S	S	S	S	S	S
Ec3-C	S	R	S	S	S	S	S	S	S
Ec1-D	S	R	S	S	S	S	S	S	S
Ec2-D	S	S	S	S	S	S	S	S	S
Ec3-D	S	R	S	S	S	S	S	S	S

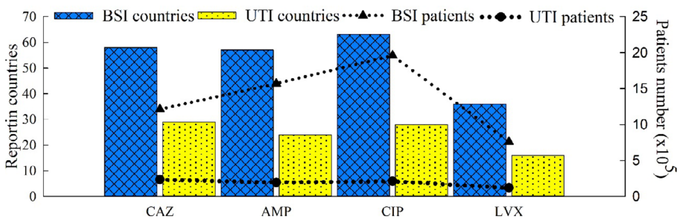
AMP: ampicillin (10 µg); AMC: amoxicillin-clavulanate (20/10 µg); CLO: chloramphenicol (30 µg); GEN: gentamicin (10 µg); CIP: ciprofloxacin (5 µg); CFZ: cefazolin (30 µg); CAZ: ceftazidime (30 µg); FOS: fosfomycin (200 µg); LVX: levofloxacin (5 µg); R: resistant; S: sensitive; SID: sensitive with increased dose; ATU: area of technical uncertainty.

The strains were named by the bacterial species (Ec) and numbered according to the collection site, being BR the water catchment dam and A, B, C, and D the micro-basins, in three collection campaigns.

Table 3 – Antimicrobial susceptibility test with Escherichia coli isolates from raw water samples.

Antibiotic (concentration)	Susceptible isolates n (%)	Resistant isolates* n (%)
CIP (5 µg)	13 (86.7)	2 (13.3)
AMP (10 µg)	4 (20.0)	11 (80.0)
CLO (30 µg)	13 (86.7)	2 (13.3)
GEN (10 µg)	15 (100.0)	0 (0.0)
FOS (200 µg)	13 (86.7)	2 (13.3)
LVX (5 µg)	15 (100.0)	0 (0.0)
CFZ (30 µg)	10 (66.7)	5 (33.3)
AMC (20/10 µg)	14 (93.3)	1 (6.7)
CAZ (30 µg)	14 (93.3)	1 (6.7)

AMP: ampicillin; AMC: amoxicillin-clavulanate; CLO: chloramphenicol; GEN: gentamicin; CIP: ciprofloxacin; CFZ: cefazolin; CAZ: ceftazidime; FOS: fosfomycin; LVX: levofloxacin. * Strains with an SID or ATU profile were considered resistant.



Source: data from WHO report (2021).

BSI: basic systemic infections; UTI: urinary tract infections; CAZ: ceftazidime; AMP: ampicillin; CIP: ciprofloxacin; LVX: levofloxacin.

Figure 4 – Relationship between the number of countries and patients with notifications of basic systemic infections and urinary tract infections due to resistant *Escherichia coli* strains.

istered only once a day, showed the same efficacy as ciprofloxacin administered twice a day in patients infected by *E. coli*, mostly. This suggests greater effectiveness as a therapy for the treatment of infections caused by this bacterium (Xue et al., 2021). Furthermore, according to Sojo-Dorado et al. (2022), fosfomycin is an important drug to consider for selected patients with urinary tract infections, with single dose (Jariyapamornkoon et al., 2024).

In addition, this microorganism with high pathogenic potential is reported in studies from various places in the world, regardless of the countries' degree of economic development (Wright and Perinpanayagam, 1987; Phillips et al., 1988; Astal et al., 2002; Woodford et al., 2007; Porres-Osante et al., 2014; Raman et al., 2018). Therefore, studies that identify and characterize bacterial strains with pathogenic potential need to continue guiding the actions of authorities responsible for population safety.

Thus, it is considered necessary to carry out periodic collections to assess whether these microorganisms persist in the environment, as they may pose a high risk to various species, including humans, if they come into contact with multiresistant organisms. As shown in Figure 4, some of the antibiotics observed as ineffective in this work, according to the report of the WHO (2021), were also reported in several countries around the world. They stand out, mainly resistance to CIP and AMP which, in addition to being among the most reported in different countries, also represent a greater number in patients with urinary tract infections.

Similar to the results found in this work, many other studies (Hadifar et al., 2017; Odonkor and Addo, 2018; Zhanel et al., 2018; Mbelle et al., 2019; Salleh et al., 2022) around the world found in samples collected from surface water, drinking water, and clinical isolates, *E. coli* resistant to the most diverse antibiotics used to treat infections caused by it. In humans, according to Geurtsen et al. (2022) this microorganism is capable of infecting the respiratory tract, gallbladder, kidneys, large and small intestine, prostate, urethra, skin, bloodstream, and brain.

Considering the wide spectrum of infections that *E. coli* can cause, it is relevant to understand the origin and causes of the mutations suffered by this bacterium as well as ways of remediation (Silva et al., 2024). Resistant bacteria not only exist in hospital wastewater but also persist in surface waters, river sediments, and in the organisms themselves present in these environments, even after water decontamination (CDC, 2023). The concept of one health, according to the Center for Disease Control and Prevention, reaffirms the idea that human health is directly linked to the whole environment, because everything is connected. Thus, it is essential to understand that a comprehensive and multisectoral vision is necessary, considering the interactions of man with the environment, in order to mitigate the problems related to global antimicrobial resistance.

Conclusion

The trend of multidrug-resistant *E. coli* strains is global. This research adds information to supply scientific databases on the behavior of a versatile microorganism that is highlighted among health problems worldwide. In this study, a high load of *E. coli* was identified in most collection points. It was noticed that, although the literature shows the strong influence of swine and cattle farming on AMR, the watershed with greater urban influence provided a greater microbiological load and isolation of strains resistant to a greater number of antibiotics.

In this sense, there are serious data gaps regarding the resistance profile of *E. coli* isolated from infected patients in Brazil. Further to this reality is the monitoring of water bodies in relation to their microbiological quality. Therefore, the need to expand research that monitors these pathogens and their behavior is emphasized, outlining their resistance profile distributed in different environments, considering the wide geographic, climatic, and cultural diversity of the Brazilian population.

Authors' contributions

Bones, U.A.: conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing – original draft; writing – review & editing. **Rosa**, G.M.: conceptualization; data curation; formal analysis; funding; acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review & editing. **Flach**, K.A.: conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing – original draft; writing – review & editing. **Costa Junior**, J.A.: funding; acquisition; investigation; methodology; project administration; resources; supervision. **Endres**, N.E.F.: investigation; methodology; software; writing – review & editing.

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