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BACTERIAL ISOLATES AND ANTIMICROBIAL RESISTANCE PROFILE IN ENTEROBACTERIA FROM SURFACE WATER SAMPLES

ISOLADOS BACTERIANOS E PERFIL DE RESISTÊNCIA ANTIMICROBIANA EM ENTEROBACTÉRIAS DE AMOSTRAS DE ÁGUAS SUPERFICIAIS

AISLADOS BACTERIANOS Y PERFIL DE RESISTENCIA ANTIMICROBIANA EN ENTEROBACTERIAS DE MUESTRAS DE AGUAS SUPERFICIALES

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ABSTRACT

Contaminated water is watering whose natural composition has been altered to the point of becoming unsuitable for use, representing, in addition, an important vehicle for the transmission of diseases such as cholera and diarrhea. The objective of this study was to investigate the antimicrobial sensitivity profile of strains of *Enterobacter* spp., *Klebsiella* spp., and *Escherichia coli* isolated from water samples collected from the Meia Ponte River. The samples were cultured, isolated, and observed for bacterial morphology. Bacterial identification was then performed, followed by antimicrobial susceptibility testing. The results were interpreted based on the Brazilian Guide to Antimicrobial Susceptibility Testing. Subsequently, chromosomal and plasmid DNA extractions were performed, and the qPCR technique was applied to detect resistance genes present in the analyzed strains. All 18 bacteria analyzed had morphological characteristics compatible with enterobacteria. In the sensitivity test, all samples were found to be resistant to at least one of the classes of antimicrobials tested, with five strains demonstrating multidrug resistance. The qPCR reaction confirmed the presence of genes related to antimicrobial resistance. The biofilm formation test showed that 22.22% of the samples (4/18) are weak formers. The study's findings reinforce the microbiological risk to which the riverside population is exposed and highlight the urgency of actions aimed at improving basic sanitation, proper disposal of medicines, and raising public awareness about the impacts of environmental contamination by resistant microorganisms. Control, prevention, and health education measures are essential in the face of the advance of multidrug-resistant bacteria in aquatic ecosystems.

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Keywords: Basic Sanitation. Environmental Contamination. Infections. Spread of Resistance. Transmission Vehicle.

RESUMO

A água contaminada é aquela cuja composição natural foi alterada a ponto de se tornar imprópria para uso, representando, além disso, um importante veículo de transmissão de enfermidades, como cólera e diarreias. O presente estudo teve como objetivo investigar o perfil de sensibilidade antimicrobiana em cepas de *Enterobacter* spp., *Klebsiella* spp. e *Escherichia coli* isoladas de amostras de água coletadas no Rio Meia Ponte. As amostras foram cultivadas, isoladas e observadas a morfologia bacteriana. Em seguida, foi realizada a identificação bacteriana, posteriormente, realizou-se o teste de suscetibilidade aos antimicrobianos. A interpretação dos resultados foi conduzida com base no Guia Brasileiro de teste de sensibilidade aos antimicrobianos. Posteriormente, foram realizadas extrações de DNA cromossomal e plasmidial, sendo aplicada a técnica de qPCR para detecção dos genes de resistência presentes nas cepas analisadas. Todas as 18 bactérias analisadas apresentaram características morfológicas compatíveis com enterobactérias. No teste de sensibilidade, observou-se que todas as amostras apresentaram resistência a pelo menos uma das classes de antimicrobianos testados, sendo que cinco cepas demonstraram multidroga resistência. A reação de qPCR confirmou a presença de genes relacionados à resistência antimicrobiana. O teste para formação de biofilme demonstrou que 22,22% das amostras (4/18) são formadoras fracas. Os achados do estudo reforçam o risco microbiológico a que a população ribeirinha está exposta e evidenciam a urgência de ações voltadas à melhoria do saneamento básico, ao descarte adequado de medicamentos e à conscientização da população sobre os impactos da contaminação ambiental por microrganismos resistentes. Medidas de controle, prevenção e educação sanitária se fazem imprescindíveis frente ao avanço de bactérias multirresistentes em ecossistemas aquáticos.

Palavras-chave: Contaminação Ambiental. Disseminação da Resistência. Infecções. Saneamento Básico. Veículo de Transmissão.

RESUMEN

El agua contaminada es aquella cuya composición natural há sido alterada hasta el punto de resultar inadecuada para el consumo, además de constituir un importante vehículo de transmisión de enfermedades como el cólera y la diarrea. El presente estudio tuvo como objetivo investigar el perfil de sensibilidad antimicrobiana en cepas de *Enterobacter* spp., *Klebsiella* spp. y *Escherichia coli* aisladas de muestras de agua recogidas en el río Meia Ponte. Las muestras se cultivaron, aislaron y se observó la morfología bacteriana. A continuación, se realizó la identificación bacteriana y, posteriormente, se llevó a cabo la prueba de susceptibilidad a los antimicrobianos. La interpretación de los resultados se realizó basándose en la Guía Brasileña de pruebas de sensibilidad a los antimicrobianos. Posteriormente, se realizaron extracciones de ADN cromosómico y plasmídico, aplicando la técnica de qPCR para la detección de los genes de resistencia presentes en las cepas analizadas. Las 18 bacterias analizadas presentaron características morfológicas compatibles con enterobacterias. En la prueba de sensibilidad, se observó que todas las muestras presentaban resistencia al menos a una de las clases de antimicrobianos probados, y cinco cepas mostraron resistencia a múltiples fármacos. La reacción de qPCR confirmó la presencia de genes relacionados con la resistencia antimicrobiana. La prueba de formación de biofilm demostró que el 22,22 % de las muestras (4/18) son formadoras

débiles. Los resultados del estudio refuerzan el riesgo microbiológico al que está expuesta la población ribereña y ponen de manifiesto la urgencia de adoptar medidas destinadas a mejorar el saneamiento básico, la eliminación adecuada de medicamentos y la sensibilización de la población sobre los impactos de la contaminación ambiental por microorganismos resistentes. Las medidas de control, prevención y educación sanitaria son imprescindibles ante el avance de las bacterias multirresistentes en los ecosistemas acuáticos.

Palabras clave: Contaminación Ambiental. Diseminación de la Resistencia. Infecciones. Saneamiento Básico. Vehículo de Transmisión.

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INTRODUCTION

Antimicrobial resistance has become one of the biggest public health problems today. The inappropriate use of antibiotics and the incorrect disposal of pharmaceutical and hospital waste have contributed to the emergence and spread of resistant microorganisms in different environments, especially in aquatic ecosystems. These locations act as natural reservoirs of bacteria and resistance genes, favoring the horizontal transfer of genetic elements and increasing the risk of environmental and clinical spread.

Among the most relevant bacteria in this context are those belonging to the Enterobacteriaceae family, such as *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp., widely recognized for their ability to develop and spread resistance to multiple classes of antimicrobials. Genes such as *bla*KPC, *bla*CTX-M, *bla*SHV, and *bla*VIM are commonly associated with the production of β-lactamases and carbapenemases, enzymes that compromise the effectiveness of widely used antibiotics, including those of the latest generation. The detection of these genes in aquatic environments is concerning, as it indicates the presence of environmental reservoirs of resistance that can reach human and animal populations.

In the state of Goiás, the Meia Ponte River is one of the main water bodies, responsible for supplying a large part of the population and maintaining the regional flora and fauna. However, the continuous discharge of domestic, industrial, and hospital effluents has compromised its quality, favoring the presence of chemical contaminants, antibiotic residues, and resistant microorganisms. This reality makes the river a strategic environment for studying the spread of resistant bacteria and their genetic mechanisms.

Given this scenario, the main objective of this study was to bioprospect and identify bacteria of the genera *Escherichia coli, Enterobacter* spp., and *Klebsiella* spp. isolated from water samples from the Meia Ponte River in Goiás, verify the antimicrobial sensitivity profile, and evaluate the occurrence of the resistance genes *bla*KPC, *bla*CTX-M, *bla*SHV, and *bla*VIM. To achieve this purpose, water samples were collected at different points along the river, followed by bacterial isolation, phenotypic and molecular identification, antimicrobial susceptibility testing, and detection of resistance genes by PCR.

The results obtained demonstrated the presence of resistant strains among the three genera analyzed, with varying profiles of resistance and incidence of the genes studied. Samples with the potential to form biofilms were also observed, a characteristic that may contribute to the persistence of these bacteria in the environment and hinder their control. The presence of multidrug-resistant isolates in a regionally important water source reinforces the need for continuous monitoring and actions aimed at preserving water quality and preventing the spread of antimicrobial resistance.

THEORETICAL FRAMEWORK

Water is an essential resource for life and a fundamental right of every human being (WHO/UNICEF, 2005). Federal regulations on the control and monitoring of water quality for human consumption are described in Ordinance MS No. 2914, dated December 12, 2011. According to this regulation, water is considered suitable for consumption if it is potable, i.e., free of contaminants that could compromise human health, meeting the criteria defined by the ordinance. Thus, it can be used safely for drinking, food preparation, and personal hygiene.

The state of Goiás, located in the Midwest Region, is home to approximately seven million residents spread across 246 municipalities, with about 49.3% of households having adequate sewage systems (IBGE, 2022). According to data from the National Sanitation Information System (SNIS), the state presents a mixed scenario, in which drinking water supply has above-average coverage, with approximately 88% of the population, however, regarding sewage collection and treatment, the data is less favorable, covering about 58% and 42% respectively (SNIS, 2023).

This situation clearly highlights the need for urgent public action in the sanitation sector, which is essential to improve quality of life, reduce hospitalizations, and even promote gains in

education and income (Exame, 2024). In the state of Goiás, the Meia Ponte River is considered one of the main tributaries, benefiting a large part of the population and playing an important role for the region's flora and fauna. However, the improper disposal of chemical components, antibiotics, and other contaminants contributes to water pollution and the development of antimicrobial resistance (Oliveira, 2022).

The development of antimicrobials is considered one of the greatest advances in medicine in the 20th century, as it enabled the effective treatment and control of numerous infectious diseases, contributing significantly to increased life expectancy globally. However, at the beginning of the 21st century, antimicrobial consumption jumped by 35%, and increasingly indiscriminate use over the years has resulted in the emergence of resistant bacteria (Zonghui Jian et al, 2021). According to the World Health Organization (WHO), antimicrobial resistance is among the ten greatest threats to global public health, with an estimated economic loss of $100 trillion and the deaths of ten million people worldwide by 2050, highlighting the seriousness of the situation (WHO, 2019).

Antimicrobial resistance is defined as the ability acquired by certain bacteria to survive and multiply even in the presence of antimicrobial agents. This resistance may be intrinsic in nature, resulting from the absence of specific targets or the presence of structures that confer natural resistance to the antimicrobial agent. Alternatively, it may be acquired through the horizontal transfer of genes located on the chromosome or in plasmids, which promote mutations that confer drug resistance (Huemer et al, 2020).

In Gram-negative bacteria, the presence of the outer membrane represents a significant barrier to the action of various antimicrobials. This is because many of these agents need to cross this structure to reach their intracellular targets. Thus, changes in the composition or integrity of the membrane can compromise the effectiveness of drugs, contributing to the emergence of resistance mechanisms (Breijyeh et al., 2020).

Enterobacteria are a family of Gram-negative, facultative anaerobic, glucose- fermenting, oxidase-negative bacilli, widely distributed in the gastrointestinal tract, as well as in aquatic and hospital environments (Murray et al., 2021). Among the most relevant genera are *Escherichia coli* (*E. coli*), *Enterobacter* spp., and *Klebsiella* spp. *E. coli*, when in its pathogenic form, can cause a range of diseases, such as gastroenteritis, extraintestinal infections, and respiratory tract infections (Murray, 2021).

Enterobacter spp. are commonly found in aquatic environments, soils, and the

gastrointestinal tract of humans and animals. Although some species are considered commensal, others behave as opportunistic pathogens, especially in hospital environments, and are associated with urinary tract, respiratory, and bloodstream infections (Silva; Sousa; Vieira, 2020). *Klebsiella pneumoniae* is an opportunistic bacterium associated with hospital infections such as pneumonia, septicemia, and urinary tract infections. However, it has also been isolated in aquatic environments, which poses a risk of environmental spread of resistance genes (Lameira, 2022; Oliveira, 2023).

Enterobacteria have multiple mechanisms for developing antimicrobial resistance through genetic mutations and the acquisition of mobile genes, such as plasmids and integrons (Murray et al., 2021). In *Enterobacter* spp., in addition to the production of β-lactamases, changes in the permeability of the outer membrane and the presence of efflux pumps also contribute to the multidrug-resistant phenotype of these strains, representing a growing challenge in both public health and environmental microbiology (Reis et al., 2022). In the case of *Klebsiella* spp., resistance to carbapenems stands out, conferred by genes such as *bla*KPC, *bla*NDM, and *bla*OXA-48, with enzymes capable of inactivating most beta-lactam antibiotics (Murray et al., 2021).

The occurrence of these species in contaminated water is concerning, as water bodies act as reservoirs and pathways for the spread of resistance genes, which poses a challenge to public health (Lameira, 2022; Oliveira, 2023). The ingestion of contaminated water can lead to bacterial infections and gastroenteritis, which, often due to the presence of resistance genes a n d factors, are aggravated by delays in detection and implementation of effective treatments (Oliveira, 2023). Thus, there is a need for further research to better understand the resistance mechanisms associated with Enterobacteriaceae, such as those carried out in the present study, to develop more efficient monitoring, prevention, and control strategies.

METHODOLOGY

Facilities Where the Methodological Procedures Were Performed

All methodological procedures were conducted at the Microorganism Biotechnology Laboratory (LBMic) located at the Institute of Tropical Pathology and Public Health (IPTSP) of the Federal University of Goiás (UFG), utilizing available equipment and materials.

Study Area

This project serves as a continuation of a previous study focused on the isolation, identification, and resistance profile of *Escherichia coli* strains from the Meia Ponte River, Goiás. Raw water samples were collected at four strategic upstream points in the Meia Ponte River basin - Collection Point 1 (16°54'16.3" S, 49°07’37.8” W) , Collection Point 2 (16°44’27.7” S, 49°08’35.3”W) , Collection Point 3 (16°39’26.0” S, 49°12’27.1” W), and Collection Point 4 (16°36’36.7” S, 49°16’58.8” W) - during the river's flood season. These sampling locations were chosen based on the potential concentration of contamination factors, such as the presence of industries and butcher shops near the riverbank.

Conservation and Morphological Identification

The samples previously stored in glycerol were cultured in BHI (Brain Heart Infusion) broth to verify their viability. For each test tube, 200 µL of the suspension was added, which remained in agitation in the shaker for 24 hours. Gram staining was then performed using the LB® staining kit (crystal violet and fuchsin) to confirm the isolation and observe the bacterial morphology. To perform this step, the bacteria were previously inoculated in nutrient agar and incubated for 24 hours.

Identification by Maldi-Tof

The strains were identified by Maldi-tof using Bruker Maldi Biotyper (Bruker®) equipment. The method allowed microbial identification by analyzing the protein profile of the samples, comparing the spectra obtained with a reference database in the software.

Antimicrobial Sensitivity Test (AST)

The choice of antimicrobials used in this procedure was in accordance with (BrCAST, 2025): Amoxicillin + clavulanic acid (10 µg), Cefepime (30 µg), Meropenem (10 µg), Aztreonam (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Azithromycin (20 µg). The test was responsible for evaluating bacterial resistance against the main antimicrobials of different classes: penicillins,

cephalosporins, carbapenems, monobactams, fluoroquinolones, aminoglycosides, and macrolides. It was performed by inoculating the samples in a Petri dish with Muller-Hinton agar and adding the antimicrobials in disc form. The results were evaluated according to BrCast (2025) (Brazilian Committee for Antimicrobial Sensitivity Testing).

Chromosomal DNA Extraction

For DNA extraction, bacterial strains were pre-cultured in 5 mL of BHI broth at 30 °C} for 24 hours. Chromosomal DNA extraction followed a modified protocol by Sambrook et al. (1989), involving sequential steps of centrifugation, resuspension in TE buffer/lysozyme, incubation with SDS/Proteinase K, treatment with NaCl and $NaCl^2$ (TAB) solutions, organic extraction with chlorophyll, precipitation with isopropanol at 20 °C, and final washing with ice-cold 70% ethanol.

Plasmid DNA Extraction

The Plasmid DNA extraction utilized the Pharmacia® FLEXIPREP kit protocol, involving centrifugation, lysis with Solutions I and II, neutralization with Solution III, and precipitation with isopropanol.

Concentration of DNA Extracted from Samples

Both chromosomal and plasmid DNA extracts were confirmed by 1.5% agarose gel electrophoresis at 60 amps and visualized under UV light, with their concentrations quantified using a NanoDrop ND-100 spectrophotometer at 260 nm.

Identification of Resistance Genes by qPCR

The extracted DNA samples were used for qPCR to identify key resistance genes. Chromosomal DNA was tested for *bla*CTX-M and *bla*KPC, while plasmid DNA was tested for *bla*VIM and *bla*SHV. These genes were selected due to their high prevalence in Enterobacteriaceae and epidemiological relevance, with ***bla***KPC and *bla*VIM being crucial as

they encode carbapenemase enzymes. Oligonucleotide sequences and thermal cycling conditions are detailed in Table 1.

Table 1. Specifications of the oligonucleotides used in this study.

Target Gene	Oligonucleotide	Primer Author	Thermal Cycle Conditions
*bla*SHV	F: 5'- CGGTGGTAACGGCGCAGTG - 3'	Santos, 2020	Initial denaturation – 95 ºC – 5 min 95 ºC – 15 sec 60 ºC – 60 sec (40x) Final extension – 95 ºC – 10 min
	R: 5' - CGATGTTACGCAGCAGGGCA - 3'		
*bla*CTX-M	F:5'-GGCGGCTCCATCGGTGTG-3'		
	R:5'-AGGCCGGCTTGTGGACAC-3'		
*bla*KPC	F:5'-GGCGGCTCCATCGGTGTG-3'		
	R:5'-AGGCCGGCTTGCTGGACAC-3'		
*bla*VIM	F:5'-GTTATGCCGCACCCACCCC–3'		
	R:5'-CAGATTGCCGATGGTGTTTGGT-3'		

Legend: F: forward primer R: reverse primer.
Source: author.

qPCR

The detection of the presence or absence of resistance genes was performed using real-time PCR (qPCR) of the Sybr Green type on an Applied one Step® instrument. The reaction employed MartexMix real-time PCR – SYBR Green/ROX, following the manufacturer's standards. The final reaction volume was 13 µL, consisting of 7 µL of MartexMix PCR, 1 µL of the extracted DNA sample (concentration 10-20 ng/ µL), 1 µL each of the forward and reverse primers (10 mol/ µL), and 3 µL of ultrapure, DNA/RNA-free water. The thermal cycling protocol included an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds, concluding with a dissociation step. Negative controls were executed using sterile water.

Biofilm Formation

Biofilm formation was assessed via a protocol involving several key steps. Samples were initially inoculated onto nutrient agar plates and incubated for 24 hours for bacterial growth. Colonies were then suspended in a sterile saline solution and subsequently transferred to BHI (Brain Heart Infusion) broth in microplates. After 24-hour incubation period, non-adherent cells were removed by discarding the liquid content and performing three washes with Milli-Q water. The attached biofilm was then fixed by adding 200 µL of ethanol to each well, incubating for 20

minutes at room temperature, and subsequently staining with crystal violet. Following another triple wash with Milli-Q water, the plates were air-dried for 20 minutes at room temperature. Quantification was achieved by adding 200 µL of 33% acetic acid solution to each well, incubating for 20 minutes, and reading the absorbance in a microplate reader at a wavelength of 650 nm. Results were expressed as the average of absorbances obtained in triplicate.

RESULT AND DISCUSSION

At the end of the study, 19 strains that had been previously preserved in glycerol and characterized by biochemical and phenotyping tests performed by the LBMic laboratory were analyzed. These procedures allowed the identification of the genera *Shiguella* spp., *Salmonella* spp., and *Klebsiella* spp., and these results were used as a starting point for the experimental stages developed in this study.

Gram staining confirmed the morphology of Gram-negative coccobacilli, which were discarded due to cross-contamination. However, after analysis by MALDI-TOF, a discrepancy was found between the results, since the spectrometry technique identified that the species analyzed belong to the *Enterobacteriaceae* family, namely *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp. Thus, the accuracy rate of the tests performed previously was limited to 33.3% (6/18) of the samples and was only able to identify the genus, while MALDI-TOF allowed the species of each sample to be identified. The contrast in results between the two identification techniques is illustrated in Table 2.

Table 2. Phenotyping x Maldi-tof results

Samples	Phenotyping	MALDI-TOF
1	*Salmonella* spp.	*Klebsiella pneumoniae*
2	*Salmonella* spp.	*Enterobacter kobei*
3	*Salmonella* spp.	*Enterobacter roggenkampii*
4	*Salmonella* spp.	*Enterobacter bugandensis*
5	*Klebsiella* spp.	*Klebsiella oxytoca*
6	*Klebsiella* spp.	*Klebsiella pneumoniae*
7	*Klebsiella* spp.	*Klebsiella pneumoniae*
8	*Salmonella* spp.	*Enterobacter clocae*
9	*Shiguella* spp.	*Escherichia coli*
10	*Salmonella* spp.	*Escherichia coli*
11	*Klebsiella* spp.	*Escherichia coli*
12	*Shiguella* spp.	*Escherichia coli*

13	*Salmonella* spp.	*Enterobacter bugandensis*
14	*Shiguella* spp.	*Escherichia coli*
15	*Klebsiella* spp.	*Klebsiella pneumoniae*
16	*Klebsiella* spp.	*Escherichia coli*
17	*Klebsiella* spp.	*Klebsiella pneumoniae*
18	*Klebsiella* spp.	*Klebsiella pneumoniae*

Source: author

About TSA, among the main results, 94.4% (17/18) showed resistance to at least two different antimicrobials, 94.4% (17/18) showed resistance to penicillin, and 22.2% of the samples (4/18) were resistant to all antimicrobials tested, all of which were representatives of the genus *Enterobacter* spp. The results are illustrated in Table 3.

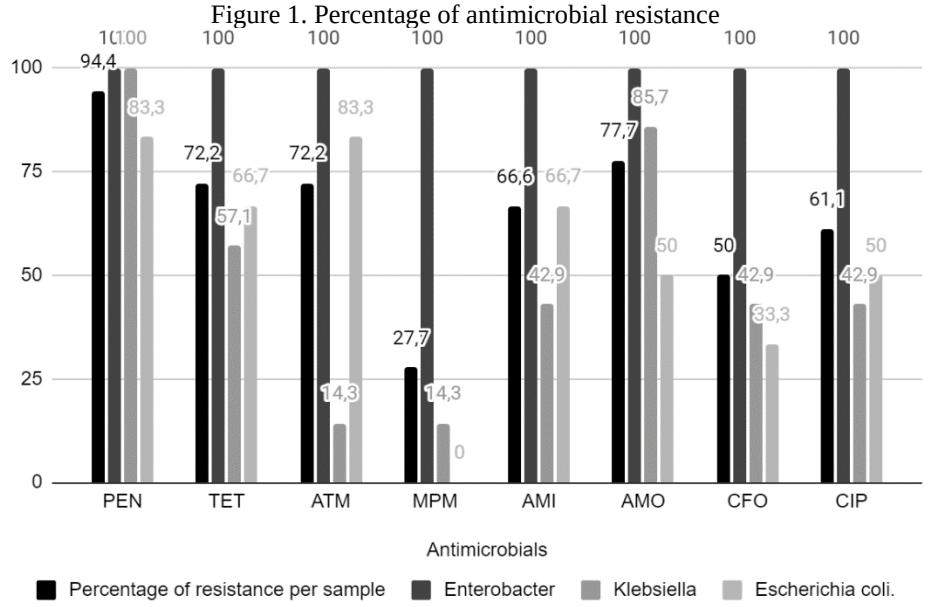
Table 3. Antimicrobial susceptibility test results

Sample	Gender Identification	PEN	TET	ATM	MPM	AMI	AMO	CFO	CIP
1	*Klebsiella*	R	R	R	S	S	R	S	S
2	*Enterobacter*	R	R	R	R	R	R	R	R
3	*Enterobacter*	R	R	R	S	R	R	R	R
4	*Enterobacter*	R	R	R	R	R	R	R	R
5	*Klebsiella*	R	R	I	I	R	R	R	R
6	*Klebsiella*	R	S	S	S	S	R	S	S
7	*Klebsiella*	R	R	S	R	R	R	S	S
8	*Enterobacter*	R	R	R	R	R	R	R	R
9	*Escherichia coli*	R	R	R	S	R	R	R	R
10	*Escherichia coli*	R	R	R	S	R	S	S	R
11	*Escherichia coli*	R	R	R	S	R	R	R	R
12	*Escherichia coli*	R	R	R	S	S	R	S	S
13	*Enterobacter*	R	R	R	R	R	R	R	R
14	*Escherichia coli*	S	S	R	S	R	S	S	S
15	*Klebsiella*	R	S	S	S	S	R	S	S
16	*Escherichia coli*	R	S	S	S	S	S	S	S
17	*Klebsiella*	R	R	I	S	R	R	R	R
18	*Klebsiella*	R	S	S	S	S	S	R	R

Legend: PEN: penicillin; TET: tetracycline; ATM: aztreonam; MPM: meropenem; AMI: amikacin; AMO: amoxicillin; CFO: cefoxitin; CIP: ciprofloxacin. Source: author

After testing the sensitivity of the antimicrobials, an assessment of the sensitivity and resistance profiles of the isolates was performed, and the percentage of resistance of the *Klebsiella* spp., *Enterobacter* spp, and *Escherichia coli* strains to the tested antimicrobials was identified. The results are shown in Figure 1.

Figure 1. Percentage of antimicrobial resistance



Legend: PEN: penicillin; TET: tetracycline; ATM: aztreonam; MPM: meropenem; AMI: amikacin; AMO: amoxicillin; CFO: cefoxitin; CIP: ciprofloxacin. Results interpreted according to Brcast 2025.
Source: author.

The qPCR assay was performed using DNA obtained from chromosomal and plasmid extractions. During the chromosomal extraction process, two samples were discarded due to the low amount of DNA recovered. In subsequent analyses, all valid samples, both plasmid and chromosomal, showed positive amplification for the four genes tested, indicating amplification in 100% of the samples analyzed.

Finally, in the evaluation of biofilm formation, only four samples (22.2%) showed absorbance < 0.120, which, according to the protocol used, configures them as weak formers. The others resulted in non-formers, as they had absorbances below 0.060. The averages obtained after the triplicates of the experiments for each sample are described below in Table 4.

Table 4. Absorbance results for the biofilm formation test

Sample	Average absorbance			
	Test 01	Test 02	Test 03	Total
1	0,0525	0,0481	0,0565	0,0523
2	0,0471	0,0475	0,05	0,0482
3	0,0661	0,0673	0,0683	0,0672
4	0,0696	0,0681	0,0605	0,066
5	0,059	0,0351	0,0468	0,0469
6	0,0655	0,0723	0,0811	0,0729
7	0,0408	0,0343	0,0491	0,0414
8	0,0383	0,034	0,0431	0,0384
9	0,042	0,046	0,0436	0,0438
10	0,0403	0,0378	0,0398	0,0393
11	0,0373	0,0316	0,0348	0,0345
12	0,0641	0,0395	0,0446	0,0494
13	0,0555	0,0403	0,0415	0,0457
14	0,0691	0,066	0,0986	0,0779
15	0,0388	0,0391	0,0441	0,0406
16	0,0453	0,0413	0,0438	0,0434
17	0,0571	0,0584	0,0512	0,0555
18	0,0556	0,0609	0,0621	0,0595

Source: author

According to the review conducted by Arbefeville et al. (2024), although traditional biochemical methods are still relevant and widely used in clinical microbiology laboratories, these procedures have significant limitations, especially in terms of processing time and accuracy in species-level identification. In contrast, more modern approaches, such as MALDI-TOF MS mass spectrometry, provide greater reliability and considerably reduced response times in the identification of pure colonies. These observations corroborate the findings of the present study and are illustrated in Table 2, indicating that the limitations of conventional methods are not restricted to the clinical setting but also extend to the analysis of isolates of environmental origin.

A study published in 2023 by Oliveira et al. revealed the Meia Ponte River in Goiás as a reservoir of multidrug-resistant bacteria, with 30.54% of the 203 isolated strains belonging to the *Enterobacteriaceae* family. The research corroborated these data and reinforced a warning regarding public health. In addition, the presence of *E. coli* in water resources acts as an indicator of fecal coliforms, which symbolizes the impact of urban pollution in the region.

The results illustrated in both Table 3 and Figure 1 partially contrast with the study by Zhang et al. (2024), in which 73.1% of isolates were resistant to three or more classes of

antibiotics, but the highest prevalence of multidrug resistance was observed in *E. coli* (51.4%) and *K. pneumoniae* (43.1%), while *Enterobacter cloacae* accounted for only 5.4% of cases.

In contrast, all isolates that showed resistance to all antimicrobials tested in the present study belong to the genus *Enterobacter* spp. This divergence may be related to environmental differences and distinct selective pressures, since the samples in the present study were obtained from rural aquatic environments, whereas the study by Zhang et al. (2024) investigated isolates of clinical origin, which may explain the predominance of multidrug-resistant *E. coli* and *K. pneumoniae*, pathogens most often associated with hospital infections. In both studies, however, meropenem had the lowest resistance rate, reinforcing its role as one of the few therapeutic options still effective against strains producing extended-spectrum β-lactamases (ESBL).

According to the review article by Cho et al. (2023), studies on the presence of ESBL-producing Enterobacteriaceae in South America are still scarce. However, most of them were conducted in Brazil and detected a widespread occurrence of these bacteria (including *E. coli*, *Klebsiella* spp., and *Enterobacter* spp.) in aquatic environments and resistance genes, with emphasis on *bla*CTX-M, followed by *bla*SHV, *bla*TEM, *bla*KPC, and *bla*OXA. These results are also consistent with those found in the present study, since 100% of the isolates analyzed amplified for the *bla*CTX-M, *bla*SHV, and *bla*KPC genes, which reinforces the importance of further investigations into a scenario that is expanding worldwide.

In this same review, it was found that several studies conducted in South America identified that rivers and streams located in urban areas, often exposed to domestic and industrial effluents, are more prone to contamination by ESBL- producing bacteria. This fact is also consistent with those presented, given the geographical location of the Meia Ponte River and the various sources of pollution to which it is subject, which have a direct impact on the health of the population.

The biofilm formation test, although limited by the small sample size, presented results consistent with the findings of recent studies. Sabença et al. (2023) observed that most β-lactamase-producing *Klebsiella* spp. isolates showed a weak biofilm formation phenotype, indicating that adhesion capacity is not necessarily related to antimicrobial resistance. Similarly, Barilli et al. (2024), when investigating *Escherichia coli* strains isolated from commercially available meat, found that 51.6% of the samples were resistant to multiple drugs; however, only about 25% of the strains produced biofilm, with most classified as weak producers.

Furthermore, the study conducted by Reshmi Debbarma et al. (2024), although primarily focused on evaluating nitrogen removal capacity, shows that the Enterobacter cloacae CAUCoF_BF_01 strain has the potential to form biofilms, associated with the production of specific extracellular compounds, such as polyhydroxybutyrate (PHB). In addition, the authors demonstrate that the metabolic activity of this strain—including phenotypic expression related to biofilm formation—is significantly influenced by environmental factors such as carbon/nitrogen ratio, temperature, pH, and oxygenation conditions. These findings indicate that biofilm formation is not a static characteristic, but rather a phenomenon dependent on environmental conditions, which may contribute to the interpretation of the results obtained in the present study and reinforces the need for further investigation to elucidate the mechanisms involved in greater depth.

CONCLUSION

The results obtained are in line with the objectives initially planned. The comparison between the identification methods showed that conventional phenotyping had limited accuracy (33.3%), while MALDI-TOF MS proved to be a highly effective and accurate tool for characterizing bacterial species, highlighting its potential to improve environmental microbiological investigations and ensure greater reliability of the results obtained.

A correlation was found between antimicrobial resistance and the presence of β-lactamase genes, although the mechanisms of resistance are diverse and not limited to this issue. In addition, the low potential for biofilm formation suggests that other mechanisms, such as conjugative plasmids and mobile elements, may play a greater role in the spread of resistance in this environment. Future research should be conducted to further investigate the development of these multidrug-resistant strains, as well as to investigate possible mechanisms that contribute to the spread of these genes, especially in aquatic environments.

Overall, the study shows that the role of the Meia Ponte River as a reservoir for multidrug-resistant bacteria is already an alarming reality that may have direct implications for the local population. Thus, the importance of broad and integrated approaches that aim to mobilize not only the responsible public agencies but also the resident in general is emphasized. There is a need for greater awareness of the correct disposal of medicines, reinforcement of basic sanitation resources, and policies aimed at minimizing damage and risks so that the population can enjoy

greater safety and quality of life.

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