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BIVALVE MOLLUSKS AS INDICATORS OF ENVIRONMENTAL POLLUTION IN THE AMAZON RIVER, BRAZIL

MOLUSCOS BIVALVES COMO INDICADORES DE POLUIÇÃO AMBIENTAL NO RIO AMAZONAS, BRASIL

MOLUSCOS BIVALVOS COMO INDICADORES DE CONTAMINACIÓN AMBIENTAL EN EL RÍO AMAZONAS, BRASIL

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Winnie Karen de Farias Lima Pires[1], Luciana Sampaio Lima[2], Jô de Farias Lima[3], Dália dos Prazeres Rodrigues[4], Aldo Aparecido Proietti Junior[5], Maurício José Cordeiro de Souza[6], Vânia Tie Koga Ferreira[7], Rosemary Ferreira de Andrade[8], Raimundo Nonato Picanço Souto[9], Rubens Alex de Oliveira Menezes[10]

ABSTRACT

Bivalve mollusks play an essential role in the maintenance of two aquatic ecosystems and can act as bioindicators of environmental pollution due to their capacity to accumulate pollutants. In the state of Amapá, the exploration of these organisms is poorly studied, especially in relation to microbiological contamination and antimicrobial resistance. This study aims to analyze the occurrence of Escherichia coli and other enterobacteria in two species of water mollusks (Prisodon obliquus and Triplodon corrugatus) collected in the Amazon River, in Macapá (AP), as well as to evaluate their antimicrobial resistance profile. This is a transversal study carried out between October 2022 and March 2023. Foram colletadas 40 samples on three points along the banks of the Amazon River. Microbiological and molecular analyzes will include bacterial isolation, antimicrobial susceptibility tests using the disk diffusion method, and detection of resistance and virulence genes by PCR. Foram

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[1] Postgraduate Program in Health Sciences. Universidade Federal do Amapá. Amapá, Brazil. E-mail: minniekaren@hotmail.com

[2] Special Laboratory of Applied Microbiology. Universidade Federal do Amapá. Amapá, Brazil. E-mail: sampaioluciana@yahoo.com.br

[3] Aquaculture and Fisheries Nucleus. Embrapa Amapá. Amapá, Brazil. E-mail: jo.lima@embrapa.br

[4] National Reference Laboratory for Bacterial Enteroinfections. Fundação Osvaldo Cruz. Rio de Janeiro, Brazil. E-mail: daliarodrigues@yahoo.com.br

[5] Special Laboratory of Applied Microbiology. Universidade Federal do Amapá. Amapá, Brazil. E-mail: aldo.proietti@unifap.br

[6] Postgraduate Program in Health Sciences. Universidade Federal do Amapá. Amapá, Brazil. E-mail: malacovis@hotmail.com

[7] Postgraduate Program in Health Sciences. Universidade Federal do Amapá. Amapá, Brazil. E-mail: vaniatie@gmail.com

[8] Postgraduate Program in Health Sciences. Universidade Federal do Amapá. Amapá, Brazil. E-mail: rosemary.unifap@gmail.com

[9] Postgraduate Program in Health Sciences. Universidade Federal do Amapá. Amapá, Brazil. E-mail: rnpsouto@unifap.br

[10] Postgraduate Program in Health Sciences. Universidade Federal do Amapá. Amapá, Brazil. E-mail: rubens.alex@unifap.br

62 bacterial isolates were identified, including 31 from E. coli, 18 from Klebsiella pneumoniae and others belonging to Enterobacter, Citrobacter and Serratia. The strains present high resistance to streptomycin (100%), nalidixic acid (93.5%) and ceftazidime (90.3%). The blaCMY gene, associated with cephalosporin resistance, was identified in E. coli samples. The presence of multiresistant bacteria in mollusks from the waters of the Amazon River represents a potential public health risk and evidence of the need for integrated environmental surveillance and antimicrobial resistance control actions based on a One Health approach.

Keywords: *Escherichia coli*. Antimicrobial Resistance. Bivalve Mollusks. Public Health. Amazon.

RESUMO

Os moluscos bivalves exercem papel essencial na manutenção dos ecossistemas aquáticos e podem atuar como bioindicadores de poluição ambiental devido à sua capacidade de acumular contaminantes. No estado do Amapá, a exploração desses organismos é pouco estudada, especialmente em relação à contaminação microbiológica e à resistência antimicrobiana. Este estudo teve objetivo de analisar a ocorrência de *Escherichia coli* e outras enterobactérias em duas espécies de moluscos de água doce (*Prisodon obliquus* e *Triplodon corrugatus*) coletadas no rio Amazonas, em Macapá (AP), bem como avaliar seu perfil de resistência antimicrobiana. Trata-se de um estudo transversal realizado entre outubro de 2022 a março de 2023. Foram coletadas 40 amostras em três pontos da orla do rio Amazonas. As análises microbiológicas e moleculares incluíram isolamento bacteriano, testes de suscetibilidade a antimicrobianos pelo método de difusão em disco e detecção de genes de resistência e virulência por PCR. Foram identificados 62 isolados bacterianos, sendo 31 de *E. coli*, 18 de *Klebsiella pneumoniae* e outros pertencentes a *Enterobacter*, *Citrobacter* e *Serratia*. As cepas apresentaram elevada resistência a estreptomicina (100%), ácido nalidíxico (93,5%) e ceftazidima (90,3%). O gene blaCMY, associado à resistência a cefalosporinas, foi identificado em amostras de *E. coli*. A presença de bactérias multirresistentes em moluscos de água doce do rio Amazonas representa risco potencial à saúde pública e evidencia a necessidade de ações integradas de vigilância ambiental e controle da resistência antimicrobiana sob a abordagem One Health.

Palavras-chave: *Escherichia coli*. Resistência Antimicrobiana. Moluscos Bivalves. Saúde Pública. Amazônia.

RESUMEN

Los moluscos bivalvos ejercen un papel esencial en la manutención de los ecosistemas acuáticos y pueden actuar como bioindicadores de contaminación ambiental debido a su capacidad de acumular contaminantes. En el estado de Amapá, la exploración de varios organismos está poco estudiada, especialmente en relación con la contaminación microbiológica y la resistencia antimicrobiana. Este estudio tiene como objetivo analizar la ocorrência de Escherichia coli y otras enterobacterias en dos especies de moluscos de agua doce (Prisodon obliquus y Triplodon corrugatus) coletadas en el río Amazonas, en Macapá (AP), bem como disponible su perfil de resistencia antimicrobiana. Trata-se de un estudio transversal realizado entre octubre de 2022 y marzo de 2023. Foram coletadas 40 amostras em três pontos da orla do rio Amazonas. Como análisis microbiológicos y moleculares incluyen aislamiento bacteriano, testículos de suscetibilidad a antimicrobianos mediante método de difusión en disco y detección de genes de resistencia y virulencia por PCR. Foram identificó 62 bacterias aisladas, entre ellas 31 de E. coli, 18 de Klebsiella pneumoniae y otros pertenecientes a Enterobacter, Citrobacter y Serratia. Como cepas presentan elevada resistencia a estreptomicina (100%), ácido nalidíxico (93,5%) y ceftazidima (90,3%). El gen blaCMY, asociado a la resistencia a las cefalosporinas, fue identificado en nuestras muestras de E. coli. La presencia de bacterias multirresistentes en moluscos de agua doce del río Amazonas representa un riesgo potencial para la salud pública y evidencia la necesidad de acciones integradas de vigilancia ambiental y control de la resistencia antimicrobiana para abordar One Health.

Palavras-chave: *Escherichia coli*. Resistencia Antimicrobiana. Moluscos Bivalvos. Salud Pública. Amazonia.

1 INTRODUCTION

Bivalves play a pivotal role in maintaining the health and balance of aquatic ecosystems. These organisms, characterized by their two-shelled structure, are found in various habitats, including marine, brackish, and freshwater environments. They serve as crucial players in nutrient cycling, effectively filtering organic matter suspended in the water column. Bivalves exhibit benthic habits, often partially or entirely buried in sandy bottoms. Their presence and activities contribute significantly to essential ecosystem services, as evidenced by studies by [1]. Because they can accumulate contaminants within their tissues, bivalves have emerged as valuable bioindicators of environmental pollution. This characteristic makes them particularly useful for assessing water quality and detecting the presence of pollutants [2].

In South America, the Hybrid family is highly diverse, including 64 species of freshwater mussels across six genera. Among these species are *Prisodon obliquus* Schumacher (1817) and *Triplodon corrugatus* (Lamarck 1819), which thrive in muddy, clay, or sandy-silt substrates and are typically found in shallow marginal areas. These mussel species, along with others, have historically been plentiful in the Lower Amazon region. Despite their abundance, their consumption remains low and is limited to specific groups, such as riverside residents with higher education and purchasing power, as well as tourists visiting the region [3]. The author suggests that the low consumption can be attributed to two main factors: a lack of widespread information about these mussels' occurrence and nutritional value, and cultural factors influencing dietary habits.

Amapá, a Brazilian state located at the northernmost tip of the country, has a 598-kilometer coastline and contains a diverse hydrographic basin with various aquatic ecosystems. These ecosystems include marine, estuarine, lake, and river environments, with 40% of the rivers belonging to the Amazon Basin and the remaining 60% to the South Atlantic Basin [4]. The state is home to the Amazon River, the largest river in the world by volume of water. In addition to its natural beauty, the river supports economic activities such as fishing and provides drinking water as well as a sewage network. However, challenges in environmental management arise due to the lack of sewage treatment and improper waste disposal [5,6].

Enterobacteriaceae are a common group of microorganisms found in various environments. They are known for their ability to ferment glucose, reduce nitrate to nitrite, and their incapacity to form spores. These bacteria are often present in the gastrointestinal tract of various organisms and can cause enteric diseases. Moreover, Enterobacteriaceae play a crucial role in spreading antimicrobial resistance genes, increasing the risk of severe infections in pathogenic organisms.

Escherichia coli is widely used to monitor antimicrobial resistance in both Gram-negative and Gram-positive bacteria. Aquatic environments can facilitate the transfer of genetic material, including antibiotic-resistance genes, between non-pathogenic bacteria. This transfer of resistance genes to pathogenic bacteria is a significant concern [7]. In response to the potential health risks from consuming bivalves containing resistant bacteria, the Brazilian government has mandated the monitoring of Escherichia coli in these organisms through Ordinance N° 884/2023 issued by the Ministry of Agriculture, Livestock, and Supply [8].

The study's main goal is to examine how common Escherichia coli (*E. coli*) and other pathogens are in two species of freshwater mussels from the Amazon River in Macapá, Amapá, and analyze their resistance to antibiotics. The study also aims to measure the level of pollution in the mussels and raise awareness about the potential health risks of multi-drug-resistant bacteria to both human and animal health. Additionally, the study emphasizes the importance of better policies regarding antibiotic use, medication disposal, and pollution control to reduce these risks.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

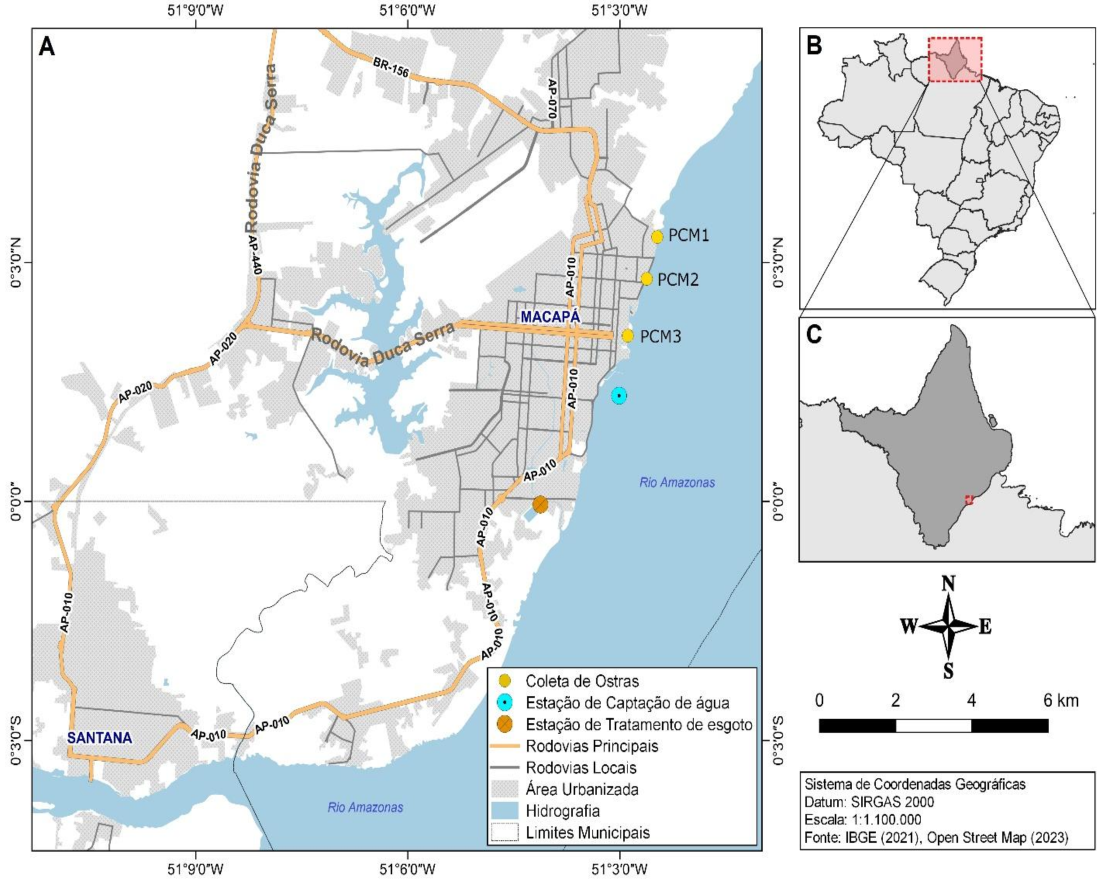
This study was conducted between October 2022 and March 2023. *Prisodon obliquus* (Schumacher, 1817) and *Triplodon corrugatus* (Lamarck 1819) (Figs. 1a-b) were manually from the Amazon River coast in the Macapá metropolitan area, specifically from three designated sampling points: PCM1, PCM2, and PCM3. The geographical coordinates of each sampling point are provided in Fig. 1**.**

The three sampling points (PCM1, PCM2, and PCM3) share similar characteristics, including the presence of oysters, significant human and animal interaction through artisanal fishing, recreational activities, and leisure, and the use of these waters as a direct and domestic water source. The seeding of cultures and the isolation of bacterial colonies were performed in the Applied Microbiology Special Laboratory (LEMA) at the Federal University of Amapá (UNIFAP).

The identification of bacteria, antimicrobial susceptibility testing, and virulence and resistance gene detection were conducted at the National Reference Laboratory for Bacterial Enteric Infections (LABENT) at FIOCRUZ.

Figure 1

Geographic map of oyster sampling points PCL1 (W 51°2'53,38" - N 0°2'4,88"), PCL2 (W 51°2'37,25" - N 0°2'47,69") and PCL3 (W 51°2'28,32" - N 0°3'19,22"). Location of the state of Amapá in gray (B) and in red, north of Brazil (A)



Source: Authors.

2.2 SAMPLE COLLECTION

A total of 40 freshwater mussels were captured and transported in isothermal boxes to the Applied Microbiology Special Laboratory (LEMA) at UNIFAP, being 24 *P. obliquus* and 16 *T. corrugatus*. In the laboratory, they underwent biometrics and taxonomic identification based on the pictorial key suggested by [9] (Figure 2). Upon arrival, the oysters were thoroughly washed in running water using brushes to remove any dirt or debris. The shells were then aseptically opened with sterile forceps to access the internal contents [10].

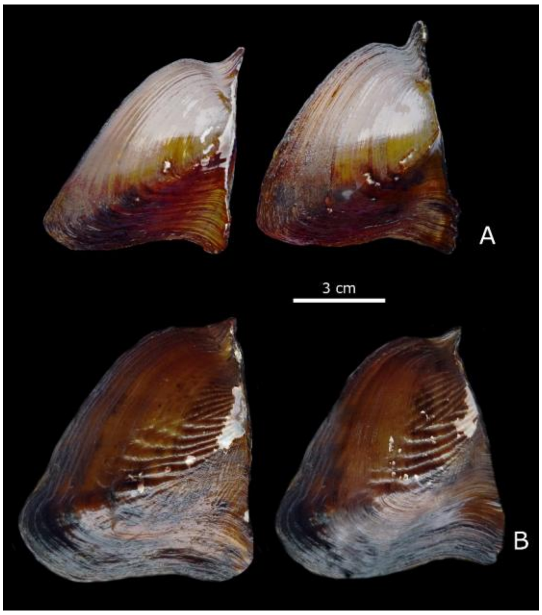
The widespread presence of *Escherichia coli* in food has raised concerns regarding food safety and public health due to the emergence of antimicrobial resistance among this bacterium. This resistance is particularly prevalent against quinolones and beta-lactam antibiotics, raising the risk of treatment failures and the spread of resistant bacteria [11]. While some *Escherichia coli* strains reside harmlessly in the intestinal microbiota, others harbour virulence factors such as fimbrial and afimbrial adhesins, toxins, and invasive properties, enabling them to cause diarrheal diseases, including intestinal infections [12]. The ability of *Escherichia coli* to transfer resistance genes to other pathogenic bacteria further complicates the issue.

Monitoring antibiotic resistance in commensal bacteria like *Escherichia coli* offers several advantages: High Prevalence: *Escherichia coli* is ubiquitous in food and the environment, making it a suitable indicator of resistance trends. Easy Isolation: *Escherichia coli* is relatively easy to isolate and culture compared to many pathogenic bacteria, facilitating laboratory testing. Correlation with Pathogenic Resistance: The resistance patterns observed in *Escherichia coli* often mirror those found in pathogenic bacteria, providing valuable insights into the overall resistance landscape [7].

Therefore, monitoring *Escherichia coli* for antibiotic resistance serves as a crucial tool for assessing food safety risks and informing public health interventions. By tracking resistance trends in this sentinel bacterium, we can proactively identify and address emerging threats to human health. Bivalve mollusks, such as clams, oysters, and mussels, can act as reservoirs of various pathogenic and resistant bacteria, posing a significant threat to human health through food borne illnesses. These bacteria can accumulate in bivalves due to their filtering activities, which draw in and concentrate microorganisms from the surrounding water [13]

Figure 2

Freshwater mussels specimens sampled from the Amazon River coast in the Macapá-AP. A - Prisodon obliquus (SCHUMACHER 1817) and B - Triplodon corrugatus Lamarck 1819)



Source: Authors.

2.3 SOWING IN CULTURE AND IDENTIFICATION

The freshwater mussels' internal content, weighing a total of 25 g, was extracted and added to Peptone Saline for pre-enrichment. The mixture was then placed in a bacteriological oven at 37°C for 18-24 hours. Aliquots of 100 µl from this culture were inoculated onto MacConkey Agar plates supplemented with Ciprofloxacin (10 µg/mL) [11]. Suspicious colonies were further screened on Triple Sugar Iron Agar (TSI) and stored on Nutrient Agar for additional analysis.

2.4 SUSCEPTIBILITY TO ANTIMICROBIALS

The susceptibility of *E. coli* isolates to antimicrobials was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MH) (Sigma-Aldrich, Inc. St. Louis, MO, USA©) as recommended in (CLSI 2023). The *E. coli* isolates were tested for susceptibility to Nalidixic Acid (NAL), Cefoxitin (FOX), Ceftazidime (CAZ), Chloramphenicol (CHL), Imipenem (IMP), Streptomycin (SRT), Levofloxacin (LEV), Gentamicin (GEN), Nitrofurantoin (NIT), Sulfamethoxazole-trimethoprim (SXT), and Tetracycline (TCY). The Multiple Antimicrobial Resistance Index (MAR) was calculated using the following equation:

$$MAR = \frac{numberofresistantantibiotics}{totalnumberofantibioticstest} \qquad (1)$$

In which values > 0.2 indicate the ability of this microorganism to be a reservoir of resistance [14].

2.5 VIRULENCE MARKERS AND RESISTANCE GENES

For the molecular component of the study, bacterial DNA was extracted using the PureLink® commercial kit (Invitrogen) according to the manufacturer's instructions. Virulence genes were detected using multiplex polymerase chain reaction (PCR) following protocol [15]. Resistance genes were detected by PCR, targeting genes associated with resistance to fluoroquinolones and cephalosporins, mediated or not by mobile genetic elements. Among the quinolone resistance genes, *qnrA*, *qnrB*, and *qnrS* were investigated in multiplex reactions, while cephalosporin resistance genes (*blaCMY)* were detected in simplex reactions. PCR products were subjected to electrophoresis on 2% agarose gels (Sigma Aldrich) stained with ethidium bromide and visualized on an ImageQuant transilluminator. The results were interpreted based on band patterns.

3 RESULTS

In total, 62 bacterial isolates were identified using biochemical and PCR tests. Table 2 shows 47 samples were identified as Enterobacteriaceae, while 15 samples did not grow on specific Enterobacteriaceae media. Of these, 31 isolates were identified as Escherichia coli. These were recovered from samples collected at the following points: PCM1 (12 isolates), PCM2 (9 isolates), and PCM3 (10 isolates). Additionally, 18 isolates of *Klebsiella pneumoniae* were identified, with eight from PCM1, one from PCM2, and nine from PCM3. Eight isolates of Enterobacter sp. were also detected, with three from PCM1, two from PCM2, and three from PCM3. *Citrobacter freundii* and *Serratia sp*. were identified once each, both from PCM1, while *Chromobacterium violaceum* was found once at PCM3.

Table 1

Enterobacteria present in oysters at collection points

Order	**Samples**	**Points**	***E. coli***	***K. pneumoniae***	***Enterobacter sp.***	***C. freundii***	***Serratia sp.***	***C. violaceum***
1	O1	PCM1	+	-	-	-	-	-
2	O3	PCM1	-	+	-	-	-	-
3	O5	PCM1	+	-	-	-	-	-
4	O7	PCM1	-	+	-	-	-	-
5	O8	PCM1	+	-	-	-	-	-
6	O7	PCM1	-	-	-	-	+	-
-	O14	PCM1	+	+	-	-	-	-
-8	O16	PCM1	-	+	+	-	-	-
9	O18	PCM1	+	-	-	-	-	-
10	O19	PCM1	+	-	-	-	-	-
11	O20	PCM1	-	+	-	-	-	-
12	O21	PCM1	+	+	-	-	-	-
13	O25	PCM1	-	-	-	-	-	+
14	O27	PCM1	-	-	+	-	-	-
15	O28	PCM1	+	-	-	-	-	-
16	O29	PCM1	-	-	+	-	-	-
17	O30	PCM1	+	-	-	-	-	-
18	O31	PCM1	+	-	-	-	-	-
19	O32	PCM1	+	+	-	-	-	-
20	O33	PCM1	+	+	-	-	-	-
21	O34	PCM2	+	-	-	-	-	-
22	O35	PCM2	+	-	-	-	-	-
23	O36	PCM2	+	-	-	-	-	-
24	O37	PCM2	+	-	-	-	-	-

25	O38	PCM2	+	-	-	+	-	-
26	O39	PCM2	+	-	-	-	-	-
27	O40	PCM2	-	-	+	-	-	-
28	O41	PCM2	+	-	-	-	-	-
29	O42	PCM2	-	+	-	-	-	-
30	O43	PCM2	+	-	-	-	-	-
31	O44	PCM2	+	-	-	-	-	-
32	O45	PCM2	+	-	+	-	-	-
33	O46	PCM3	+	-	+	-	-	-
34	O47	PCM3	+	-	-	-	-	-
35	O48	PCM3	+	-	-	-	-	-
36	O49	PCM3	+	+	-	-	-	-
37	O50	PCM3	+	-	-	-	-	-
38	O51	PCM3	+	+	-	-	-	-
39	O52	PCM3	+	+	-	-	-	-
40	O53	PCM3	-	+	-	-	-	-
41	O54	PCM3	-	+	-	-	+	-
42	O55	PCM3	-	+	-	-	-	-
43	O56	PCM3	+	-	+	-	-	-
-44	O57	PCM3	+	+	-	-	-	-
45	O58	PCM3	+	-	-	-	-	-
46	O59	PCM3	-	+	+	-	-	-
47	O60	PCM3	-	+	-	-	-	-
	Total		31	18	8	1	2	1

Source: Authors.

*PCM: Macapá collection point. As shown in Table 3, among the 31 *Escherichia coli* isolates, the highest frequency of resistance was observed for antibiotics belonging to the fluoroquinolone, cephalosporin, aminoglycoside, sulfonamide, and tetracycline classes. The isolates were also tested for virulence factors and resistance genes associated with cephalosporins and fluoroquinolones. The *E. coli* strains exhibited high resistance rates to Streptomycin (STR) (100.0%), Nalidixic Acid (NAL) (93.5%), Ceftazidime (CAZ) (90.3%), Sulfamethoxazole-trimethoprim (SXT) (87.1%), Levofloxacin (LEV) (87.1%), and Tetracycline (TCY) (74.2%). Conversely, they showed susceptibility to Imipenem (IMP) (93.5%), Chloramphenicol (CHL) (90.3%), Cefoxitin (FOX) (83.9%), Nitrofurantoin (NIT) (77.4%), and Gentamicin (GEN) (67.7%).

Table 2

Susceptibility profile of Escherichia coli isolates in oysters in Macapá

Collection points		PCM1PCM2PCM3Total							
Antimicrobials (sgl)	Class	Susceptibility (n/%)							
		S	R	S	R	S	R	S	R
Nalidixic acid (NAL)	Quinolones	-	12	1	8	1	9	2 (6.5%)	29 (93.5%)
Cefoxitin (FOX)	ß-lactams	12	-	7	2	7	3	26 (83.9%)	5 (16.1%)
Ceftazidime (CAZ)	ß-lactams	2	10	-	9	1	9	3 (9.7%)	28 (90.3%)
Chloramphenicol (CHL)	Amphenicols	11	1	9	-	8	2	28 (90.3%)	3 (9.7%)

Streptomycin (STR)	Aminoglycosides	-	12	-	9	-	10	-	31 (100%)
Gentamicin (GEN)	Aminoglycosides	7	5	7	2	7	3	21 (67.7%)	10 (32.3%)
Imipenem (IPM)	ß-lactams	12	-	8	1	9	1	29 (93.5%)	2 (6.5%)
Levofloxacin (LEV)	Fluoroxacin	1	11	2	7	1	9	4 (12.9%)	27 (87.1%)
Nitrofurantoin (NIT)	Nitrofurans	9	3	7	2	8	2	24 (77.4%)	7 (22.6%)
Sulfamethoxazole-trimethoprim (SXT)	Sulfonamides	1	11	1	8	2	8	4 (12.9%)	27 (87.1%)
Tetracycline (TCY)	Tetracyclines	2	10	5	4	1	9	8 (25.8%)	23 (74.2%)

Source: Authors
Data collect. S= Sensitive; R= Resistant

4 DISCUSSION

Studies have identified aquatic ecosystems, including rivers and surface waters, as reservoirs of antimicrobial-resistant bacteria. Enterobacteriaceae with multiple antimicrobial resistance have been observed in these environments, often associated with the food consumption of their host organisms. *Escherichia coli* and *Klebsiella pneumoniae* are the most commonly studied species in epidemiological investigations of antimicrobial resistance due to their high survival and dissemination rates [11]. In this study, *E. coli* was the most frequently isolated bacterium from water and its aquatic organisms, shrimp and oysters, suggesting a direct link to fecal contamination.

According to [11], the wide diversity of *E. coli* strains in aquatic environments, their virulence factors associated with human pathogenicity, and their ability to transfer and disseminate genetic material have made them a focus of research worldwide. Another significant finding of this study was the presence of strains of several other Enterobacteriaceae, such as *K. pneumoniae* and *Enterobacter spp.*, capable of causing severe foodborne infections with serious economic consequences and the potential for lethal outcomes.

According to [16], the genus *Klebsiella* has undergone a remarkable diversification in the past decade, giving rise to organisms capable of exchanging genetic elements and undergoing mutations that confer antimicrobial resistance and a hypervirulent profile. This has led to the emergence of multidrug-resistant and hypervirulent (MDR-hv) bacterial clones, as well as strains that are simultaneously hypervirulent and resistant to multiple antibiotics.

In a study conducted by [17], a high level of sulfamethoxazole-trimethoprim (SXT) contamination was documented in the surface waters of the Thap Put District, Phang Nga Province, southern Thailand. This result is consistent with the authors' earlier study, which revealed high SXT resistance in marine organisms. The widespread contamination presents a significant ecological risk, although this outcome contradicts findings from other research on seafood isolates, including marine and freshwater fish, from the same region [18].

Sulfamethoxazole-trimethoprim is commonly used in human medicine to treat respiratory and urinary tract infections. By contrast, a study in the Amazon estuary, situated in the northern Brazilian state of Pará, found that all E. coli isolates tested for susceptibility to imipenem, chloramphenicol, and SXT were sensitive to these antibiotics [19]. Ceftazidime (CAZ) was one of the most resistant cephalosporins evaluated in this study. Public water sources in the central-west region of São Paulo state exhibited resistance to cephalosporins of various generations. CAZ is one of the most widely used antibiotics today. Antimicrobial-resistant Enterobacteriaceae have been frequently detected in humans and animals in various environments. The circulation of these bacteria between ecological environments is highly complex due to the multiple reservoirs and different transmission routes [20].

The most significant resistance mechanism that compromises the efficacy of extended-spectrum cephalosporins (Ceftazidime - CAZ) in members of the Enterobacteriaceae family is based on the production of plasmid-mediated enzymes, known as extended-spectrum β-lactamases (ESBLs). These enzymes inactivate the aforementioned antibiotic compounds by disrupting their β-lactam rings. Streptomycin (STR), an aminoglycoside antibiotic used to treat serious bacterial infections, particularly those caused by Gram-negative bacteria, has also been found to exhibit resistance in surface waters worldwide. Sulfonamides, another class of antimicrobials with resistance reported in this study, share a similar mechanism of action to aminoglycosides, acting as barriers to folic acid production, which is essential for bacterial growth and replication. These antibiotics are commonly used against intestinal infections caused by the bacterium *E. coli.* Tetracyclines are bacteriostatic, possessing a broad spectrum of action and acting to inhibit protein synthesis [21].

The high level of cephalosporin resistance observed in this study raises significant public health concerns. Not only is there a potential for bivalve consumption to cause foodborne outbreaks, but there is also concern about the extent of water contamination to which human populations may be exposed. These mollusks can serve as vectors for antibiotic-resistant pathogens that can be indirectly transmitted to humans, posing serious threats to public health. The presence of antimicrobial residues in aquatic environments can have detrimental consequences for non-target microorganisms, contaminate food-producing animals and drinking water supplies, and contribute to the rise of antimicrobial resistance [17].

Due to their filter-feeding habit, bivalve mollusks can accumulate a high concentration of pathogenic and resistant bacteria, playing a significant role in the human-animal-food-environment interface. These bacteria can be transmitted through the consumption of bivalves, as they filter water from the environments they inhabit. Consequently, the consumption of contaminated bivalves can lead to outbreaks of infections and food poisoning [13]. [22] Delve into the virulence factors characteristic of extraintestinal pathogenic *E. coli,* highlighting the role of various adhesins and toxins in compromising the host's defense system and hindering nutrient acquisition. These toxins and adhesins often serve as carriers for mobile genetic elements called pathogenicity islands, which facilitate genetic mutation among commensal and pathogenic strains. Importantly, extended-spectrum β-lactamase (ESBL)-producing E. coli exhibit resistance to most third- and fourth-generation cephalosporins, which are classified by the WHO as the highest priority critically important antimicrobials.

The eagg gene emerged as the most prevalent virulence gene in a study of mollusks along the coastal margins of Lake Timsah in Ismailia Province, Egypt. Freshwater mussel's samples harbored virulent and multidrug-resistant *E. coli* strains. Similarly, multidrug-resistant *E. coli* isolates were recovered from fresh mollusk species in Mumbai, India [23].

The *blaCMY* gene, found in PCM2, confers resistance to cephalosporins, including ceftazidime (CAZ), which are widely used in both human and veterinary medicine. This gene encodes a β-lactamase enzyme capable of hydrolyzing β-lactam antibiotics, including cephalosporins and penicillins, rendering them ineffective. Our understanding of antimicrobial resistance (AMR) in oysters and coastal waters remains inadequate, with limited comparative data from other aquatic animals. Globally, studies on the prevalence of E. coli and its antimicrobial susceptibility in aquatic organisms like oysters are scarce. In the Brazilian Amazon, particularly in Amapá, there is no documented information on this occurrence. This study, therefore, represents the first to address this critical topic, which is essential for understanding the future of AMR and its implications for public health.

5 CONCLUSION

The identification of multidrug resistance (MDR) factors, resistance markers, and virulence factors in freshwater mussels from the Amazon River raises serious concerns about the potential health risks associated with their consumption. This is particularly alarming given that the water supply for the city of Macapá is sourced from the same location where these freshwater mussels are harvested. The direct use of river water by the local population for fishing and domestic purposes further amplifies the potential for exposure to these resistant bacteria. Understanding the interconnectedness between bacterial species, AMR genotypes, and mobile genetic elements (plasmids) is crucial for comprehending the selection and dissemination of AMR, particularly in a One Health perspective. The high AMR rates observed in the majority of isolates can inform policy formulation to better control the rapid development of resistant bacteria contamination in Amapá. An integrated One Health approach encompassing human, animal, plant, and environmental health is essential to effectively address the multifaceted aspects of health and well-being.

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