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Comparative Assessment of the Microbiological Quality of Water and Antimicrobial Resistance of *Aeromonas* spp. in a Nile Tilapia Farming System in a Reservoir and an Excavated Pond

Avaliação Comparativa da Qualidade Microbiológica da Água e da Resistência Antimicrobiana de *Aeromonas* spp. em um Sistema de Cultivo de Tilápia do Nilo em Reservatório e Viveiro Escavado

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Abstract

This study comparatively evaluated the microbiological water quality in two aquaculture environments: a net-cage system installed in the Pedra do Cavalo Reservoir (Paraguaçu River, Bahia) and an associated excavated pond used for Nile tilapia (*Oreochromis niloticus*) production. Samples were collected over a 12-month period at bimonthly intervals, totaling 27 samples from the river (P1–P3) and pond (P4) sites. Counts of mesophilic heterotrophic bacteria, coliforms, *Enterococcus*, and *Aeromonas* were performed, along with antimicrobial resistance and virulence analyses of *Aeromonas* isolates. No significant differences were observed for mesophilic bacteria and *Enterococcus* among sampling points ($p > 0.05$), whereas total and thermotolerant coliforms were significantly higher in the pond ($p < 0.05$), indicating greater fecal contamination in this environment. *Aeromonas* species were detected in 33.3% of the samples, with *A. schubertii*, *A. hydrophila*, and *A. sobria* identified. Among the 12 confirmed isolates, 91.7% exhibited multidrug resistance, with MAR indices ranging from 0.62 to 1.00. Enzymatic activities associated with virulence, including amylase (41.67%; 5/12), lipase (41.67%; 5/12), and caseinase (41.67%; 5/12), were observed. These findings indicate that, although microbiological parameters were generally within regulatory limits,

aquaculture environments, particularly pond systems, may act as reservoirs of antimicrobial-resistant and potentially pathogenic bacteria. The results highlight the influence of environmental conditions and anthropogenic factors on microbial dynamics and reinforce the need for continuous monitoring and improved management practices in aquaculture systems.

Keywords: Coliforms. Net-Pen. Antimicrobial Resistance. *Aeromonas hydrophila*.

Resumo

Este estudo comparou a qualidade microbiológica da água em dois ambientes aquícolas: um sistema de cultivo em tanques-rede instalado no Reservatório de Pedra do Cavalo (Rio Paraguaçu, Bahia) e um viveiro escavado associado à produção de tilápia do Nilo (*Oreochromis niloticus*). As amostras foram coletadas ao longo de 12 meses, em intervalos bimestrais, totalizando 27 amostras provenientes dos pontos no rio (P1–P3) e do viveiro (P4). Foram realizadas contagens de bactérias heterotróficas mesófilas, coliformes, *Enterococcus* e *Aeromonas*, além da análise de resistência antimicrobiana e de fatores de virulência dos isolados de *Aeromonas*. Não foram observadas diferenças significativas ($p > 0,05$) entre os pontos amostrais para bactérias mesófilas e *Enterococcus,* enquanto coliformes totais e termotolerantes apresentaram valores significativamente maiores no viveiro ($p < 0,05$), indicando maior contaminação fecal nesse ambiente. Espécies de *Aeromonas* foram detectadas em 33,3% das amostras, com identificação de *A. schubertii*, *A. hydrophila* e *A. sobria*. Entre os 12 isolados confirmados, 91,7% apresentaram perfil de multirresistência, com índice MAR variando de 0,62 a 1,00. Atividades enzimáticas associadas à virulência, como amilase (41,67%; 5/12), lipase (41,67%; 5/12) e caseinase (41,67%; 5/12), também foram observadas. Os resultados indicam que, embora os parâmetros microbiológicos estejam, em geral, dentro dos limites estabelecidos pela legislação, os ambientes aquícolas, especialmente os viveiros escavados, podem atuar como reservatórios de bactérias potencialmente patogênicas e resistentes a antimicrobianos. Esses achados reforçam a influência das condições ambientais e de fatores antrópicos na dinâmica microbiana e destacam a necessidade de monitoramento contínuo e de práticas de manejo mais sustentáveis na aquicultura.

Palavras-chave: Coliformes. Tanque-Rede. Resistência Antimicrobiana. *Aeromonas hydrophila*.

1 Introduction

Aquaculture has been expanding rapidly as an alternative to meet the growing global demand for aquatic protein, especially amid stagnant or declining marine catches. In this context, maintaining water quality in farming systems is essential for the health and performance of farmed organisms, as well as for the preservation of receiving ecosystems (Yusoff *et al*., 2024).

Although attention is often focused on physicochemical parameters such as dissolved oxygen, ammonia, nitrite, temperature, and pH, the microbiological quality of water is equally important, particularly in intensive systems such as net-cage and pond-based aquaculture. Despite its relevance, microbiological monitoring is still often underestimated, even though it has direct implications for fish health, environmental balance, and food safety (Bentzon-Tilia; Sonnenschein; Gram, 2016).

Intensive aquaculture systems are characterized by high organic loads from uneaten feed and feces, which can alter the water's microbial profile, favor the proliferation of opportunistic or pathogenic microorganisms, and contribute to the dissemination of antimicrobial-resistant bacteria

(Yusoff *et al*., 2024).

Coliform bacteria are widely used as indicators of fecal contamination (Wen *et al.*, 2024), particularly *Escherichia coli*, which inhabits the intestinal tract of humans and warm-blooded animals (Bag *et al*., 2024). Enterococci (*Enterococcus* spp.) are also relevant indicators, commonly found in soil, food, and aquatic environments, and recognized as opportunistic pathogens (Pereira *et al*., 2023).

In aquaculture environments, non-enteric bacteria such as *Aeromonas* have been widely reported as fish pathogens and potential zoonotic agents. These microorganisms are capable of producing virulence factors, including hemolysins, proteases, and lipases, and may develop resistance to multiple classes of antimicrobials (Semwal; Kumar; Kumar, 2023). Their occurrence is influenced by factors such as high stocking density, inadequate sanitary management, poor water quality, and antimicrobial use.

These conditions favor not only the emergence but also the spread of resistant bacteria in aquatic ecosystems, which may act as reservoirs of resistance genes. Such genes can be transferred horizontally among bacterial populations, increasing the risk of interspecies transmission and representing an emerging public health concern (Mohammed *et al*., 2025). This scenario reinforces the importance of monitoring strategies aligned with the One Health approach, which integrates animal, human, and environmental health.

In parallel, antibiotic resistance genes and other emerging contaminants have been increasingly detected in aquatic environments, sharing similar sources and pathways of dissemination. Despite advances in monitoring, knowledge gaps remain regarding their control and management (Gogoi *et al*., 2018; Mohammed *et al*., 2025).

In Brazil, studies addressing the microbiological impacts of intensive fish farming systems in reservoirs and rivers remain limited, particularly regarding antimicrobial resistance and the microbiological quality of adjacent waters. In this context, comparative analyses across distinct aquaculture environments may provide relevant insights into microbial dynamics and the dissemination of resistance.

Therefore, this study aimed to comparatively assess the microbiological water quality of two aquaculture-related environments: a natural reservoir influenced by net-cage aquaculture and a controlled excavated pond system, both associated with Nile tilapia (*Oreochromis niloticus*) production in the Pedra do Cavalo Reservoir (Paraguaçu River, Bahia). The analysis included enumerating mesophilic heterotrophic bacteria, coliforms, *Enterococcus*, and *Aeromonas*, as well as characterizing the antimicrobial resistance and virulence factors of *Aeromonas* isolates.

2 Materials and Methods

The study was conducted at a fish farm located in the Pedra do Cavalo Reservoir, in the municipality of Cabaceiras do Paraguaçu. The Paraguaçu Basin, which supplies the Pedra do Cavalo Dam reservoir, covers 53,650 km², with the reservoir occupying 186 km² and encompassing several municipalities in Bahia, such as Cachoeira, São Félix, Muritiba, Governador Mangabeira, and Conceição da Feira (Silva *et al.*, 2015).

The aquaculture system studied has the fry and fattening phases in net pens, with post-larvae reared in excavated ponds. Samples were collected at four points: point P1, located upstream of the fish farm's net-pens, near Porto Castro Alves; point P2, located in the fish farm area, near the net-pens; point P3, located downstream of the net-pens, near Kekeu's Kiosk; and point P4, in one of the excavated ponds of the fish farm.

Samples were collected over a 12-month period, at bimonthly intervals, resulting in six sampling campaigns. During each campaign, samples were collected alternately from the river points (P1, P2, and P3) and the pond (P4), resulting in more samples from the river than from the pond. In total, 27 samples were obtained, including 21 from the river and 6 from the pond. The sampling design was defined to cover seasonal variation over a one-year period and to allow comparison between distinct aquaculture-related environments (reservoir and pond). The number of samples was established based on logistical feasibility and previous studies with similar designs, ensuring representative temporal and spatial coverage of the study area.

At each point, approximately 900 mL of water was collected at depths of 50-100 cm. Sampling was conducted from September 2023 to August 2024, in the morning. The collected material was analyzed at the Food and Environmental Microbiology Laboratory (LABMAA) at UFRB. The microbiological quality of the water was monitored by quantifying aerobic mesophilic heterotrophic bacteria, total coliforms, thermotolerant coliforms, *Enterococcus*, and *Aeromonas*.

The quantification of mesophilic heterotrophic bacteria was performed by duplicate plating on standard count agar (PCA) medium (Silva *et al.*, 2010). For the determination of the Most Probable Number (MPN) of total coliforms and thermotolerant coliforms, the multiple tube technique was used with a series of five tubes and dilutions up to 10^{-4}. The analyses were performed in three distinct stages: presumptive test, confirmatory test, and biochemical test, as described by Silva *et al.* (2010).

For the quantification of enterococci, the MPN/100 mL method was also used, with growth in Azide Dextrose Broth containing bromocresol purple as the pH indicator, incubated at 35 ºC for 24 h. Positive tubes (turbid and yellow) were inoculated into M-*Enterococcus* agar culture medium and incubated at 35 ºC for 48 h. Then, five colonies from each plate showing enterococcal characteristics (light red or dark red) were removed and inoculated onto Brain Heart Infusion (BHI) agar for subsequent species-level identification. For the biochemical identification of the isolates,

morphotintorial tests (Gram staining) and biochemical tests were performed: catalase, arginine decarboxylase, motility, growth at 10 ºC and 45 ºC, growth in hypersaline medium 6.5% NaCl, pH 9.6, growth in 0.04% tellurite and fermentation of carbohydrates (arabinose, lactose, raffinose, mannitol and sorbitol) (Silva *et al*., 2010).

For the *Aeromonas* Presence/Absence test, 10 mL of water was inoculated into a test tube containing 10 mL of Trypticase Soy Broth with 30 µg/mL ampicillin, and incubated at 28 °C for 24 h. After this period, the culture was streaked onto *Pseudomonas/Aeromonas* selective agar (GSP) medium with 10 µg/mL ampicillin and incubated at 28 °C for 24 h in a microaerophilic environment. After this time, five characteristic *Aeromonas* colonies (colonies with a yellow color and transparent halo due to starch hydrolysis) were selected from each plate and subjected to biochemical tests: oxidase, catalase, glucose, sucrose, arabinose, indole, VP, and TSI, according to Palumbo et al. (2001).

The antimicrobial susceptibility of *Aeromonas* strains was determined by the disk diffusion technique, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020). Eight antimicrobials were tested: gentamicin (30 µg), cephalothin (30 µg), cefoxitin (30 µg), aztreonam (30 µg), chloramphenicol (30 µg), nitrofurantoin (100 µg), sulfamethoxazole/trimethoprim (25 µg), and tetracycline (30 µg). For the test, a loopful of overnight cultures on TSA agar was transferred to 9 mL tubes of 0.85% saline solution until a bacterial density of 0.08 to 0.10 (1.5 x 10^8 CFU/mL) was reached using a spectrophotometer (Spectrum SP – 1105 model) at 625 nm, equivalent 0.5 McFarland standard. Then, a swab was dipped into the culture and spread uniformly on the surface of Mueller-Hinton agar, incubated at 28 °C for 24 h, and the diameters of the inhibition halos were measured according to CLSI criteria (CLSI, 2013).

The Multiple Antimicrobial Resistance (MAR) index was calculated to determine multiple resistance, according to Krumperman (1983), using the formula: $a/b \times 100$, where: a = the number of antimicrobials to which the isolate was resistant, and b = the number of antimicrobials to which the isolate was exposed. Bacteria with a MAR index > 0.2 were considered resistant to multiple antibiotics (Adeyemi *et al*., 2022), and isolates that showed resistance to 3 or more classes of antibiotics were also considered MDR (multidrug-resistant bacteria) (CLSI, 2020).

The virulence phenotypic factors (lipase, gelatinase, caseinase, phospholipase, amylase, and hemolytic activity) in *Aeromonas* strains were analyzed using the methods described by Cabrera Rodríguez *et al*. (2008), Hongping *et al*. (2011), and Leanovich *et al*. (2025).

For the microbiological counts, data were first $\log_{10}$ transformed. The assumptions of normality and homoscedasticity were evaluated using the Shapiro-Wilk and Bartlett tests, respectively. Since the data did not meet the parametric assumptions, the non-parametric Kruskal-Wallis test (non-parametric) was applied to evaluate the differences in microbial loads among the sampling points (P1,

P2, P3, and P4). When significant differences were detected, Dunn's post-hoc test with Bonferroni adjustment was used for pairwise comparisons. A significance level of $p < 0.05$ was adopted for all statistical tests. All analyses were performed using RStudio (Version 2026.01.1+403).

This study did not involve human participants or experimental animals. The samples consisted exclusively of environmental water, and therefore, according to current regulations, ethical approval by a research ethics committee was not required.

3 Results and Discussion

Mesophilic aerobic bacteria showed no significant differences among sampling points ($p = 0.191$), although slightly higher values were observed in the pond (P4) compared to river sites (Table 1).

Table 1 - Counts of mesophilic bacteria, coliforms, and *Enterococcus* in water samples from the Paraguaçu River (P1, P2, P3) and the pond (P4)

Microorganisms	P1	P2	P3	P4	*p*-value
Mesophilic (CFU/100 mL)	5.28±0.95	4.96±0.32	5.01±0.59	6.01±1.20	0.191
Total coliforms (MPN/100 mL)	0.00±0.00^{b}	0.43±0.95^{ab}	0.00±0.00^{b}	2.76±1.56^{a}	0.001
Thermotolerant coliforms (MPN/100 mL)	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	1.62±1.85^{a}	0.010
Enterococcus (MPN/100 mL)	0.00±0.00	0.00±0.00	0.00±0.00	0.51±1.24	0.321

Values are expressed as Mean ± Standard Deviation of Log10-transformed data. Different lowercase letters in the same row indicate statistically significant differences between sampling points, as determined by the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction ($p < 0.05$). CFU: Colony Forming Units. MPN: Most Probable Number.
Source: research data.

Total and thermotolerant coliforms differed significantly among sampling points ($p < 0.05$), with higher counts in the pond (P4), indicating greater fecal contamination in this environment. Peak values were recorded during specific sampling periods, particularly in January 2024, when thermotolerant coliform levels exceeded the limits established by CONAMA Resolution No. 357/2005 (Brasil, 2005). Although Brazilian legislation does not establish specific limits for total coliforms in aquaculture waters, their presence is widely recognized as indicative of recent fecal contamination (Conte *et al.*, 2004).

Enterococcus counts did not differ significantly among sampling points ($p = 0.321$), although detection was limited to the pond (P4), suggesting localized contamination.

The higher microbial loads observed in the pond may be associated with organic matter accumulation, lower water renewal, and elevated temperatures, which favor microbial proliferation in intensive aquaculture systems (Silva Júnior; Pereira, 2021). In contrast, river sites (P1–P3) remained within acceptable microbiological limits, likely due to greater dilution capacity and water

flow.

The results of the microbiological analysis of isolated microorganisms are presented in Table 2. The pond (P4) showed the highest microbial diversity, with the presence of *E. coli*, *Enterobacter cloacae*, *A. schubertii* and, *A. hydrophila*, while only *Aeromonas* species were detected at the river sampling points (P1–P3). Notably, *A. schubertii* was identified at all sampling locations.

Table 2 - Percentage of bacteria isolated according to the sampling point

Sampling Points	Isolated Species				
	E. coli **n (%)**	***E. cloaceae*** **n (%)**	***A. schubertii*** **n (%)**	***A. hydrophila*** **n (%)**	***A. sobria*** **n (%)**
Point P1	-	-	1 (8.3)	-	-
Point P2	-	-	1 (8.3)	-	-
Point P3	-	-	1 (8.3)	1 (8.3)	1 (8.3)
Point P4	3 (25.0)	3 (25.0)	1 (8.3)	2 (16.7)	-

n = número de isolados.
Source: research data.

Aeromonas was detected in 33.3% of the samples, with a higher occurrence in river sites (25%) than at pond sites (17%). A total of 21 suspected isolates were obtained, of which 12 were confirmed at the species level. The highest incidence occurred during the rainy months, suggesting that precipitation and surface runoff may contribute to the transport of organic matter and fecal contamination into the aquatic environment. Similar patterns have been reported in aquaculture systems, where increased *Aeromonas* occurrence is associated with seasonal rainfall (Silva *et al*., 2019).

Point P3, located downstream of the fish farm and near an area of intense human activity, showed the greatest diversity of *Aeromonas* species (Table 2). This finding suggests a strong anthropogenic influence, likely related to recreational use, waste disposal, and domestic sewage input, which may favor the introduction and dissemination of these microorganisms.

The identification of potentially pathogenic species, such as *A. schubertii, A. hydrophila*, and *A. sobria,* underscores the sanitary relevance of these environments. These species are widely recognized as pathogens of aquatic organisms and opportunistic agents in humans, being associated with infections ranging from mild gastroenteritis to severe systemic conditions (Ren et al., 2019; Zhang *et al*., 2024).

The occurrence of *A. hydrophila* and *A. sobria* at P3 and P4 is particularly relevant, as these species exhibit high transmission capacity and are commonly associated with stressed aquaculture systems. Environmental imbalances, including elevated temperature, organic load, and reduced water quality, can promote bacterial proliferation and increase host susceptibility (Araújo *et al*., 2020).

Overall, the presence and diversity of *Aeromonas* in areas under anthropogenic pressure highlight not only the risk of infection for aquatic organisms and humans, but also the potential for

dissemination of antimicrobial resistance. These environments may act as reservoirs of resistance genes, reinforcing the importance of monitoring microbial communities in aquaculture systems within a One Health framework (Kumar; Rathore, 2024).

Multidrug resistance (MDR) phenotypes were observed in 91.7% of *Aeromonas* strains, with MAR indices ranging from 0.62 to 1.00 (Table 3). Additionally, 17% of the isolates showed resistance to five antimicrobial classes and 8.3% to six classes, indicating a high level of resistance. The highest resistance frequencies were observed for β-lactams (cephalothin and cefoxitin), amphenicol (chloramphenicol), nitrofuran (nitrofurantoin), and sulfonamide (sulfamethoxazole/trimethoprim).

Table 3 - Antimicrobial resistance profile (MDR) and Multiple Resistance Index (MAR) of *Aeromonas* isolated from water samples in the Paraguaçu River and the excavated pond.

Origin	Microorganisms	Antibiotics	Number of antimicrobials	MAR Index
Point P1	*A. schubertii*	CFL, CFO, ATM, CLO, NIT, SUT	6	0.75
	A. schubertii	CFL, CFO, ATM, CLO, NIT, SUT, TET	7	0.87
	A. schubertii	CFL, CFO, ATM, CLO, NIT, SUT	6	0.75
Point P2	*A. hydrophila*	CFL, CFO, ATM*, CLO, NIT, SUT	5	0.62
	A. schubertii	CFL, CFO, ATM, CLO, NIT, SUT	6	0.75
	A. schubertii	-	-	-
Point P3	*A. schubertii*	CFL, CFO, CLO, NIT, SUT	5	0.62
	A. sobria	CFL, CFO, ATM, CLO, NIT, SUT	6	0.75
	A. schubertii	GEN, CFL, CFO, ATM, CLO, NIT, SUT, TET	8	1.00
Point P4	*A. hydrophila*	CFL, CFO, CLO, NIT, SUT, TET	6	0.75
	A. hydrophila	CFL, CFO, ATM, CLO, NIT, SUT	6	0.75
	A. schubertii	CFL, CFO, ATM, CLO, NIT, SUT	6	0.75

*Intermediate resistance. β-lactams: CFL: Cefalothin (30 µg). CFO: Cefoxitin (30 µg). ATM: Aztreonam (30 µg). Amphenicol: CLO: Chloramphenicol (30 µg). Nitrofuran: NIT: Nitrofurantoin (100 µg). Sulfonamide: SUT: Sulfazotrim (25 µg). Tetracycline: TET: Tetracycline (30 µg). Aminoglycoside: GEN: Gentamicin (30 µg). MAR: Multiple Resistance Index.
Source: research data.

Despite limited use of these antimicrobials in local aquaculture, the high levels of resistance observed suggest that environmental contamination plays a key role in the selection of resistant strains. Aquatic environments under anthropogenic influence are recognized as important reservoirs of antimicrobial resistance genes, receiving inputs from domestic sewage, urban runoff, and agricultural effluents that may contain antibiotic residues and resistant bacteria (Karkman *et al*., 2018).

In addition, co-selection and cross-resistance mechanisms, often mediated by mobile genetic elements such as plasmids, contribute to the persistence of multidrug-resistant phenotypes even in the absence of direct antimicrobial exposure (Seiler; Berendonk, 2012). These processes may explain the high resistance rates observed in both river and pond isolates, reinforcing the role of aeromonas as a reservoir and disseminator of resistance determinants in aquatic systems.

The ability of *Aeromonas* spp. to form biofilms further enhances their persistence in aquatic

environments and increases tolerance to antimicrobial agents (Wang *et al*., 2022). This characteristic poses an additional challenge controlling these microorganisms in aquaculture systems.

Similar findings have been reported in other Brazilian aquatic environments. Evangelista-Barreto *et al.* (2010) observed that 60% of *Aeromonas* isolates from the Cocó River exhibited resistance to at least one antimicrobial, while more recent studies have highlighted the occurrence of resistant species such as *A. hydrophila* in fish intended for human consumption, emphasizing the potential for dissemination along the food chain (Carusi *et al*., 2024).

Overall, the high frequency of MDR phenotypes observed in this study underscores the role of aquaculture-related environments as hotspots of antimicrobial resistance, reinforcing the need for continuous monitoring and integrated management strategies within a One Health framework.

The virulence profiles of *Aeromonas* strains are presented in Table 4. Overall, enzymatic activities for amylase, lipase, and caseinase were observed in 41.67% (5/12) of the isolates, while phospholipase activity was detected in 16.67% (2/12). None of the strains exhibited gelatinase activity.

Table 4 - Virulence factors of *Aeromonas* strains isolated from water samples in the Paraguaçu River and the excavated pond

Source of Isolation	Species	Number of Positive Isolates					
		AA (n/%)	GA (n/%)	PA (n/%)	HA (n/%)	LA (n/%)	CA (n/%)
Point P1	*A. schubertii* (n = 3)	1 (33.3)	0	0	3 (100)	2 (66.7)	3 (100)
Point P2	*A. schubertii* (n = 3)	1 (33.3)	0	1 (33.3)	3 (100)	1 (33.3)	2 (66.7)
Point P3	*A. hydrophila* (n = 1)	0	0	0	1 (100)	0	0
	A. sobria (n = 1)	0	0	0	1 (100)	0	0
	A. schubertii (n = 1)	0	0	1 (33.3)	1 (100)	1 (100)	1 (100)
Point P4	*A. hydrophila* (n = 2)	2 (100)	0	0	2 (100)	2 (100)	1 (50)
	A. schubertii (n =1)	1 (100)	0	0	1 (100)	0	0

AA: Amylase activity. GA: Gelatinase activity. PA: Phospholipase activity. HA: hemolytic activity. LA: Lipase activity. CA: Caseinase activity. Values expressed as number of positive isolates (n) and percentage (%).
Source: research data.

When analyzed by environment, all isolates from the pond (P4; 3/3) exhibited amylolytic activity, with lipase detected in 66.67% (2/3) and caseinase in 33.33% (1/3) of the strains. No phospholipase or gelatinase activity was observed in pond isolates. In contrast, river isolates (P1–P3; n = 9) showed lower frequencies of amylase (22.22%; 2/9) and phospholipase (22.22%; 2/9), but higher caseinolytic activity (66.67%; 6/9). Gelatinase activity was not detected in any of the isolates. These differences suggest distinct patterns of enzymatic expression between environments, possibly associated with local physicochemical conditions and selective pressures.

Hemolytic activity also differed between environments. All pond isolates (3/3) exhibited gamma hemolysis, indicating the absence of detectable hemolysins. In contrast, 44.44% (4/9) of river

isolates showed alpha hemolysis, while 55.56% (5/9) were classified as gamma hemolytic. Notably, alpha-hemolytic strains were exclusively detected in river samples, suggesting that environmental variability and anthropogenic influence may modulate toxin expression.

The detection of enzymatic activities such as lipase, amylase, and caseinase reinforces the pathogenic potential of *Aeromonas* spp., as these factors are associated with host tissue degradation, nutrient acquisition, and colonization (Sreedharan; Philip; Singh, 2012; Kishk; Moustafa; Kirrella, 2020).

In particular, lipolytic activity is relevant, as lipases are associated with alterations in host cell membrane integrity and may facilitate toxin entry and the establishment of infection (Pattanayak *et al.*, 2020). Although no β-hemolytic activity was observed, the presence of alpha hemolysis in river isolates suggests potential cytotoxic effects influenced by environmental conditions (Che *et al*., 2024).

Overall, these findings indicate that *Aeromonas* strains from both environments harbor important virulence-related traits, with distinct expression patterns reflecting differences in environmental conditions and selective pressures.

4 Conclusion

The results of this study indicate that the evaluated waters generally complied with the limits established by CONAMA Resolution No. 357/2005 for thermotolerant coliforms, suggesting acceptable sanitary conditions under this parameter. The absence of *Enterococcus* spp. throughout the monitoring period further supports the microbiological stability of the system.

However, the detection of *Aeromonas* species at all sampling points, particularly *A. schubertii* and *A. hydrophila*, reveals the presence of opportunistic microorganisms with pathogenic potential. Their higher occurrence during the rainy season highlights the influence of environmental and seasonal factors on microbial dynamics.

The high frequency of antimicrobial resistance (91.7%), including multidrug-resistant strains, underscores the role of aquaculture environments as potential reservoirs and dissemination routes of resistance determinants.

These findings emphasize that, even when conventional microbiological indicators are within regulatory limits, relevant microbiological risks may persist. Therefore, the adoption of preventive measures, including biosecurity practices, rational antimicrobial use, and continuous monitoring, is essential to ensure animal health, food safety, and environmental sustainability, in accordance with the One Health approach.

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