



## *Aeromonas* sp. in freshwater fish and antimicrobial resistance: emerging pathogen

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**ABSTRACT:** The bacteria *Aeromonas* sp. are naturally reported in aquatic ecosystems and possess pathogenic potential, being considered as emerging pathogens in humans and animals. They also cause considerable losses in fish farming and, through water, can contaminate numerous foods. This study quantified and analyzed the antimicrobial resistance profile of *Aeromonas* sp. in fish. A total of 72 samples of two fish varieties (leather fish, *Pseudoplatystoma fasciatum* x *Leiarius marmoratus* and round fish, *Colossoma macropomum* x *Piaractus mesopotamicus* and *Colossoma macropomum* x *Piaractus brachypomus*) were purchased from two types of sources (fresh and frozen) and three commercial establishments (supermarket, market, and fishmonger). The 55 isolated *Aeromonas* cultures were evaluated for their antimicrobial resistance profile by the disc diffusion method. Upon quantification, the count of *Aeromonas* sp. ranged from 4.22 to 6.00 Log CFU/g; ten different species, including *A. eucrenophila*, *A. hydrophila*, *A. caviae*, *A. media*, *A. jandaei*, *A. veronii* bv. *sobria*, *A. trota*, *A. schubertii*, *A. veronii* bv. *veronii*, and *A. shigelloides*, were identified. Among the 55 isolates, 64.45% showed resistance to Ampicillin-sulbactam, and 75% were sensitive to gentamicin and ciprofloxacin. It was concluded that 100% of the evaluated samples were contaminated by *Aeromonas* sp., which may present a risk to consumer health since bacteria can be etiological agents of Foodborne Diseases. The antimicrobial resistance profile showed resistance to ampicillin and multi-resistance to different classes of antimicrobials, demonstrating problems with choosing an antimicrobial for treatment of any disease.

**Key words:** public health, contamination, pathogenic bacteria.

## Quantificação de *Aeromonas* sp. em peixes de água doce e resistência a antimicrobianos: patógeno emergente

**RESUMO:** *Aeromonas* sp. são bactérias presentes naturalmente no ecossistema aquático e apresentam potencial patogênico, sendo considerados patógenos emergentes em humanos e animais. Ainda, causam perdas consideráveis em piscicultura e, através da água, podem também contaminar inúmeros alimentos. Diante disso o objetivo do trabalho foi quantificar e analisar o perfil de resistência aos antimicrobianos de bactérias do gênero *Aeromonas* sp. em peixes. Um total de 72 amostras foram adquiridas, sendo duas variedades de peixes (peixe de couro *Pseudoplatystoma fasciatum* x *Leiarius marmoratus* e os peixes redondos *Colossoma macropomum* x *Piaractus mesopotamicus* e *Colossoma macropomum* x *Piaractus brachypomus*), dois tipos (fresco e congelado) e de três estabelecimentos comerciais (supermercado, feira e peixaria). As 55 culturas isoladas de *Aeromonas* foram avaliadas quanto ao perfil de resistência aos antimicrobianos pelo método de difusão em disco. Os valores encontrados na quantificação de *Aeromonas* sp. variaram de 4,22 a 6,00 Log UFC/g, e foram identificadas dez espécies diferentes, sendo *A. eucrenophila*, *A. hydrophila*, *A. caviae*, *A. media*, *A. jandaei*, *A. veronii* bv. *sobria*, *A. trota*, *A. schubertii*, *A. veronii* bv. *veronii* e *A. shigelloides*. Entre os 55 isolados, 64,45% apresentaram resistência a Ampicilina-sulbactam, 75% foram sensíveis à gentamicina e ciprofloxacina. Concluiu-se que 100% das amostras avaliadas estavam contaminadas por *Aeromonas* sp. podendo apresentar risco à saúde do consumidor visto que estas podem ser agentes etiológicos de Doenças Transmitidas por Alimentos (DTAs). O perfil de resistência aos antimicrobianos comprovou a resistência a ampicilina e apresentou multirresistência a diferentes classes de antimicrobianos, demonstrando um problema relacionado à opção de antimicrobiano para tratamento no caso do desenvolvimento de alguma doença.

**Palavras-chave:** saúde pública, contaminação, bactérias patogênicas.

## INTRODUCTION

Fish consumption has increased worldwide in recent years because of its high

nutritional value (FAO, 2020). Nutrients available in fish include proteins, essential amino acids, essential fats such as omega 3, vitamins, and minerals (BARIK, 2017). This nutritional composition can

vary due to several factors, such as place of farming and species.

In the Brazilian Midwest, mainly aquaculture fish are consumed because of their availability in the region. Round fish, which include “pacu” (*Piaractus mesopotamicus*), “tambaqui” (*Colossoma macropomum*), and its hybrids “tambacu” (*Colossoma macropomum* x *Piaractus mesopotamicus*) and “tambatinga” (*Colossoma macropomum* x *Piaractus brachypomus*), and leather fish such as the Amazonian “pintado” (*Pseudoplatystoma fasciatum* x *Leiarius marmoratus*) are widely sold in local shops.

Bacteria are known to be naturally present in the microbiota of fish and their aquatic environment and can cause spoilage and/or pathogenic illness. The most commonly found bacteria in freshwater fish vary according to the microbiota of the water; *Aeromonas* sp. is considered one of the dominant genera (CHATTOPADHYAY & ADHIKARI, 2014).

Fish affected by *Aeromonas* sp. present a high mortality rate (MORAES & MARTINS, 2004) due to hemorrhages from the rupture of blood vessels in the fins, which cause ulcerations, anemia, sepsis, and death as observed by BOIJINK & BRANDÃO (2001) in species of “jundiá” (*Rhamdia quelen*). Since *Aeromonas* sp. is a common pathogen in aquatic environments and causes high fish mortality, it causes economic losses and is an obstacle for fish farming (LEÃO et al., 2020).

Apart from high density, the incidence of *Aeromonas* sp. in aquaculture and other environments is related to stress factors such as changes in environmental conditions, temperature oscillation, inadequate harvest, irregular transport, and factors related to the inadequate forms of commercialization (BARCELLOS et al., 2008).

*Aeromonas* sp. are considered emerging pathogens that cause disease in humans and are capable of infecting immunocompromised and immunocompetent patients. Of the identified *Aeromonas* species, *A. hydrophila*, *A. sobria*, and *A. caviae* are the most important for causing diseases such as intestinal infections, gastroenteritis, and septicemia in humans and animals (JANDA & ABBOTT, 2010; BHOWMICK & BHATTACHARJEE, 2018; LEÃO et al., 2020).

The pathogenicity of *Aeromonas* sp. represents a risk to public health and can cause great losses in the productive sector, augmented by the irrational administration of antimicrobials. The indiscriminate use of antimicrobials during fish rearing causes the emergence of resistance to these drugs. In addition, antimicrobial resistance

is an ecological problem characterized by complex interactions involving diverse microbial populations that affect the health of humans, animals, and the environment (COLLIGNON & MCEWEN, 2019).

One Health is particularly relevant where they include food safety, zoonosis control and combating antimicrobial resistance (WHO, 2017). Therefore, monitoring the incidence of *Aeromonas* sp. in fish, as well as its resistance to antimicrobials, is necessary. Thus, this study quantified and analyzed the antimicrobial resistance profile of bacteria of the genus *Aeromonas* sp. in fish.

## MATERIALS AND METHODS

### Experimental design

After preliminary evaluation of the most consumed fish species, the following fish were selected for this study: leather fish (Amazonian “pintado” (*Pseudoplatystoma fasciatum* x *Leiarius marmoratus*)), the round fish (tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*)), and “tambatinga” (*Colossoma macropomum* x *Piaractus brachypomus*)).

Samples of both types of fish were acquired from three types of established commercial locations, in both fresh and frozen form.

Statistical treatment was determined by 3 points of sale (market, fishmonger, and supermarket), 2 forms of commercialization (frozen and fresh), and 2 varieties of fish (“pintado” and round), consisting of 6 repetitions.

The fresh samples were collected approximately one hour before analyses, kept in their commercial packages, immediately packed in an isothermal box containing recyclable ice, and transported to the Food Microbiology Laboratory of the Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso (IFMT). The time between collection of fresh fish and the beginning of the analysis did not exceed 2 hours, being compliant with the recommendations of ISO 7218:2007 (ISO, 2013). The frozen samples were collected a day before and were stored under refrigeration for thawing (SILVA et al., 2017).

### Quantification of *Aeromonas* sp.

To quantify *Aeromonas*, the methodology described by RALL et al. (1998) was used, where a 25 g sample was transferred to 225 ml of 0.1% peptone salt solution, and then serial dilutions were made until a dilution of  $10^{-3}$  was achieved. For counting, surface plating was performed on each

plate containing amido-ampicillin agar (SAA) and was homogenized using a drigalski loop and then incubated in an inverted microbiological incubator at 28 °C for 24 hours.

#### *Isolation of colonies and presumptive identification of the genus Aeromonas*

After the incubation period, the plates were read and 10 characteristic colonies were selected, with 5 colonies from each of the two plates selected in the count. Colonies were transferred using a nickel chromium needle to trypticase soy agar (TSA) and incubated in an incubator at 28 °C for 24 hours. After inoculation in TSA and incubation, the inocula were plated on Triple Sugar-Iron Agar (TSI) and incubated at 28 °C for 24 hours. The cultures that presented an acid reaction (fermentation of carbohydrates), with or without gas and hydrogen sulfide (H<sub>2</sub>S) production, were submitted to the following biochemical tests for genus identification: catalase, oxidase, and gram staining tests. The cultures that were catalase positive, oxidase positive, and gram negative were presumed to be of the genus *Aeromonas* (RALL et al., 1998).

#### *Aeromonas species identification and biochemical classification*

To identify *Aeromonas* species, tests for indole production, hydrolysis of esculin, gas production from glucose, Voges-Proskauer, motility, and carbohydrate fermentation (arabinose, sucrose, and mannitol) were performed (SILVA et al., 2017). The biochemical properties referring to the results of the tests were classified according to the characteristic of each species (Table 1).

#### *Antimicrobial resistance (ATM)*

The isolated *Aeromonas* species, identified by biochemical classification, were kept refrigerated until further analysis. For antimicrobial resistance analysis, the cultures were replicated, and fresh cultures with incubation times of 24h were used.

The antimicrobial resistance profile was evaluated using the disk diffusion method. The zone of inhibition was measured using a ruler with the naked eye with the plate positioned approximately 30 cm away (BAUER et al., 1966).

To investigate the sensitivity of the species to antimicrobials, the classes considered were sulfonamides, phenicols, penicillins, cephalosporins, aminoglycosides, quinolones, and tetracyclines. The Antimicrobial Sensitivity Score (ASR) parameters, *in vitro*, followed the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2010,

2018). Ten antimicrobials purchased commercially from Laborelin were used: Ampicillin Sulbactam 20 µg - ASB, Amoxicillin 30 µg - AMC, Cefepime 30 µg - CPM, Cefoxitin 30 µg - CFO, Ciprofloxacin 5 µg - CIP, Chloramphenicol 30 µg - CLO, Gentamicin 10 µg - GEN, Levofloxacin 5 µg - LVX, Sulfazotrim 25 µg - SUT, and Tetracycline 30 µg - TET. Any strain which could develop in the presence of 3 or more classes of antimicrobials was defined as multidrug resistant (SCHWARZ et al., 2010).

#### *Statistical analysis*

The experiment was carried out in an entirely randomized design (DIC) in a double factorial scheme considering the points of sale as statistical treatment (Market, fishmonger, and supermarket). The factorials considered were the interactions between type and origin, variety and type, and variety and origin.

The Shapiro-wilk normality test was applied to all data. The variables that presented normality in the test ( $P > 0.05$ ) were submitted to a double factorial analysis by the Tukey comparison test. For differentiation of the means of the statistical treatments, the Scott-Knott comparison test was used. Analysis was undertaken by using the statistical software R version 4.0.3 (R CORE TEAM, 2019).

## RESULT AND DISCUSSION

#### *Quantification of Aeromonas sp.*

The mean values of *Aeromonas* sp. ranged from 4.22 to 6.00 Log<sub>10</sub> CFU/g, shown as an expressive count in 100% of the samples (Table 2). Regardless of the type and point of commercialization, contamination in the round fish samples was significantly greater in comparison to that in samples of the leather fish (Tables 3 and 4). This may be explained by the fact that removal of scales from the round fish requires greater handling, which could make it more susceptible to contamination and consequent multiplication of *Aeromonas* species.

The presence of *Aeromonas* in fish is mainly linked to the aquatic environment (JANDA & ABBOTT, 2010). However, contamination is aggravated by inadequate cooling, freezing, and handling practices and can be avoided through proper conservation and good manufacturing practices (BEAZ-HIDALGO & FIGUERAS, 2013). Furthermore, *Aeromonas* is able to grow at 5 °C (PRAVEEN et al., 2016). The ability of these bacteria to propagate at low temperatures affects the quality of fish, decreasing its shelf life and leading to economic losses in the fish processing companies.

Table 1 - Biochemical properties of *Aeromonas* species.

Species	Gram	Oxidase	Catalase	Motility	VPz	Indol	Hydrolysis of esculin	Glucose gas	Arabinose	Sucrose	Mannitol	Inositol
<i>A. hydrophila</i>	-	+	+	+	+	+	+	+	+	+	+	-
<i>A. caviae</i>	-	+	+	+	-	+	+	-	+	+	+	-
<i>A. sobria</i>	-	+	+	+	v	+	-	+	-	+	+	-
<i>A. media</i>	-	+	+	-	-	v	+	-	+	+	+	n.a
<i>A. eucrenophila</i>	-	+	+	+	-	n.a	+	+	v	-	+	-
<i>A. veronii</i> <i>bv.</i> <i>sobria</i>	-	+	+	+	+	+	-	+	-	+	+	-
<i>A. veronii</i> <i>bv. veronii</i>	-	+	+	+	+	+	+	+	-	+	+	-
<i>A. jandaei</i>	-	+	+	+	+	+	-	+	-	-	+	-
<i>A. schubertii</i>	-	+	+	+	-	-	-	-	-	+	-	-
<i>A. trotta</i>	-	+	+	+	-	+	-	+	-	-	+	-
<i>A. shigelloides</i>	-	+	+	+	+	n.a	n.a	-	n.a	n.a	n.a	n.a

Adapted: RALL et al., 1998; JANDA; ABBOTT, 2003; 2010.

Legend: v - variable; n.a - not detected; - negative; + positive; VP - Voges-Proskauer.

NEYTS et al. (2000), reported that 72% of fish samples from retail and supermarkets were contaminated, and the count of *Aeromonas* ranged from 2.28 to 5.38 Log<sub>10</sub> CFU/g, which is consistent with the results of the current study. In contrast, CASTRO-ESCARPULLI et al. (2003) reported a lower percentage of *Aeromonas* than that in this study, with only 33% of frozen tilapia samples collected in Mexican markets showing contamination. The contamination of fishes by *Aeromonas* is independent of the region and place of commercialization as verified; however, when the fishes are frozen, they may show less contamination than fresh fishes due to the fact that low temperatures suppress microbial growth. In addition, freezing can cause the formation of crystals in the bacteria, leading to their death, which may explain the lower amount of bacteria in frozen fish.

Similar studies were conducted by SANTOS et al. (2019), where they identified the presence of *Aeromonas* sp. in samples of “Tambaqui” commercialized in fairs (100% of contaminated samples) and supermarkets (86.7%). SILVA et al. (2010) identified the presence of *Aeromonas* in fish samples commercialized in open markets (50%) in the city of São Paulo and NAGAR et al. (2011), identified fish samples contaminated by *Aeromonas* commercialized in different locations in Turkey. These studies indicated that the presence

of *Aeromonas* is a public health concern, since the presence of pathogenic species in food can directly affect human health.

A variety of pathogenic species of *Aeromonas* has been identified in several studies similar to this study. For example, SARRIAGUZMÁN et al. (2014) reported the presence *A. hydrophila* (15%) in fresh fish and PESSOA et al. (2020) observed greater dominance of *A. hydrophila* species (41%) followed by *A. caviae* (18%) in “Tambaqui”. The prevalence of *A. hydrophila* (28%), *A. veronii* *bv. sobria* (25%), and *A. veronii* *bv. veronii* (46%) was also observed in vacuum-packed milkfish under modified atmosphere (SIMON et al., 2016).

The identification of *Aeromonas* in a variety of foods has been increasing, as evidenced by several researchers, who concluded that its presence in foods can lead to economic losses and adversely affect consumer health (CALLISTER & EGGER, 1987; PEREIRA et al, 2004; HIRSCH et al., 2006; SILVA et al., 2010; YÜCEL & BALCI, 2010; LANZARIN et al., 2011; NAGAR et al., 2011; SUÁREZ & HERRERA, 2011; DAR et al., 2015; ALHAZMI, 2015; PRAVEEN et al, 2016; ABD-EL-MALEK, 2017; SANTOS et al., 2019; WU et al., 2019a; AZZAM-SAYUTI et al., 2021).

It is known that each bacterium has a specific behavior depending on the intrinsic and

Table 2 - Analysis of *Aeromonas* sp. in samples of fresh and frozen fish marketed in the city of Cuiabá, Mato Grosso, Brazil.

Statistical treatments	Colony forming units per gram of sample (CFU/g) of <i>Aeromonas</i> sp. in the varieties studied. Mean $\pm$ standard deviation expressed in log <sub>10</sub>
T1 (round, fresh, market);	4.49 $\pm$ 0.10 <sup>c</sup>
T2 (round, fresh, fair);	4.94 $\pm$ 0.70 <sup>b</sup>
T3 (round, fresh, fishmonger);	5.79 $\pm$ 0.32 <sup>a</sup>
T4 (leather, fresh, fishmonger);	5.72 $\pm$ 0.38 <sup>a</sup>
T5 (leather, fresh, fair);	5.07 $\pm$ 0.32 <sup>b</sup>
T6 (leather, fresh, market)	5.07 $\pm$ 0.32 <sup>b</sup>
T7 (round, frozen, fair);	4.83 $\pm$ 0.79 <sup>b</sup>
T8 (leather, frozen, fair);	4.56 $\pm$ 0.70 <sup>c</sup>
T9 (round, frozen, market);	6.00 $\pm$ 0.25 <sup>a</sup>
T10 (leather, frozen, market);	4.32 $\pm$ 0.30 <sup>c</sup>
T11 (leather, frozen, fishmonger);	4.22 $\pm$ 0.78 <sup>c</sup>
T12 (round, frozen, fish market)	5.31 $\pm$ 0.52 <sup>a</sup>

Different letters in the same column show significant difference at 5% level ( $P < 0.05$ ) by Scott-Knott test.

extrinsic conditions to which they are exposed, and *Aeromonas* sp. can characteristically grow at both high and low temperatures. Therefore, proper conservation and good manufacturing practices are essential to minimize contamination and ensure product quality.

#### Identification of species

A total of 55 isolates of *Aeromonas* spp. were obtained in this study, of which 35 and 20 were isolated from fresh fish and frozen fish, respectively. Of the identified species, *A. caviae* (31%), *A. hydrophila* (24%), and *A. eucrenophila* (20%) showed the highest prevalence (Table 5). The first two species are of great importance for public health, and can cause serious damage to consumer health, such as dysentery, blood and mucus in the feces, abdominal pain, fever, and vomiting, especially in the elderly and immunocompromised people (ÜNÜVAR, 2018). In general, studies have reported the isolation of these three main *Aeromonas* species (*A. hydrophila*, *A.*

*veronii* *bv. sobria* and *A. caviae*) from patients with food-related diseases (RADU et al., 2003; SIMON et al., 2016).

*Aeromonas* sp. is considered an emerging microorganism, where cases of outbreaks and incidences by *Aeromonas* species have been reported worldwide (GUERRA et al., 2007; ZHANG et al., 2012; WU et al., 2014; TSHETEN et al., 2016; SILVA et al., 2017; AZZAM-SAYUTI et al., 2021; WU et al., 2019a), demonstrating it to be a global public health concern.

*A. eucrenophila* was the third most frequent bacterium in this study, representing 20% of the isolates (Table 5). Although, there are not many reports on the identification of this species in food, some isolates have been observed in freshwater fish and in water supply samples (HIRSCH et al., 2006; NAGAR et al., 2011). *A. eucrenophila* is commonly isolated from freshwater and infected fish (SCHUBERT & HEGAZI, 1988), and is considered to be a fish pathogen and responsible for causing diarrhea in humans (SINGH & SANYAL, 1999).

Table 3 - *Aeromonas* sp. count (Log<sub>10</sub> CFU/g) in marketed frozen and fresh fish.

Variety	Frozen	Fresh
Leather	4.37 <sup>bb</sup>	5.28 <sup>aa</sup>
Round	5.39 <sup>aa</sup>	5.08 <sup>aa</sup>

Different lowercase letters in the same row show significant differences for species. Different capital letters in the same column show significant differences for variety; Tukey's test ( $P < 0.05$ ).

Table 4 - *Aeromonas* sp. count (Log<sub>10</sub> CFU/g) in fish from different commercialization sites.

Type of fish	Fair	Marketplace	Fish shop
Leather	4.82 <sup>B</sup>	4.68 <sup>B</sup>	4.98 <sup>B</sup>
Round	4.89 <sup>A</sup>	5.25 <sup>A</sup>	5.56 <sup>A</sup>

Different capital letters in the same row show significant differences for species, Tukey test ( $P < 0.05$ ).

We also observed that majority of the most prevalent *Aeromonas* sp. species isolates were identified in fresh fish samples (Table 5). After the fish is captured, the deterioration process is very fast (PRABHAKAR et al., 2020). Hence, it is important to immediately preserve them on ice to inhibit microbial growth. In addition, inadequate marketing conditions, where fish are kept uncovered on ice exposed to the environment, contribute significantly to high *Aeromonas* sp. counts in fresh fish.

Evaluation of the species of fish indicated that round fish presented the highest number of *Aeromonas* sp., with a total of 80%, while leather fish presented only 20% of the isolates. Furthermore, *A. caviae* and *A. hydrophila* isolates were the most frequent in round fish (Table 6). Round fish tend to involve greater handling and consequently showed a higher microbial load. Studies have shown that fish are subject to microorganism-induced changes in physicochemical properties and deterioration owing to inadequate handling, transport and/or storage conditions (WU et al., 2019b).

The supermarket, fishmonger, and market establishments showed 29%, 35%, and

36% of *Aeromonas* sp., respectively, demonstrating a diversity of species among the evaluated sites (Table 7). Although, there was no quantitative difference between the number of species isolated from the various establishments (Table 4), there was variability in the species identified among the different establishments. Notably, *A. hydrophila* and *A. caviae* were identified in greater numbers in the fish market samples. The prevalence of bacteria of the genus *Aeromonas* varies substantially according to the sampling site (KOLDA et al., 2020), fish species, and production system (WAMALA et al., 2018).

#### ATM profile

A total of 55 *Aeromonas* sp. isolates were evaluated for susceptibility to antimicrobials, and all isolates showed sensitivity to all antimicrobials, ranging from 51% to 75% (Table 10). Further, all showed resistance below 39% for all antimicrobials, ranging from 25% to 38%, except ampicillin-sulbactam, for which 65% of the isolates showed resistance in this study (Tables 8 and 9). This could be due to the intrinsic characteristic of resistance to this class of antibiotics.

Table 5 - *Aeromonas* species distributions in fresh and frozen fish samples.

Species	-----Isolated-----		
	Fresh Fish	Frozen Fish	TOTAL
<i>A. eucrenophila</i>	9	2	11 (20%)
<i>A. hydrophila</i>	7	6	13 (23.64%)
<i>A. caviae</i>	11	6	17 (30.9%)
<i>A. media</i>	3	1	4 (7.27%)
<i>A. jandaei</i>	2	0	2 (3.64%)
<i>A. veronii</i> bv. <i>sobria</i>	1	1	2 (3.64%)
<i>A. trolta</i>	1	0	1 (1.82%)
<i>A. schubertii</i>	0	1	1 (1.82%)
<i>A. veronii</i> bv. <i>veronii</i>	0	3	3 (5.45%)
<i>A. shigelloides</i>	1	0	1 (1.82%)
Total	35 (64%)	20 (36%)	55 (100%)

Table 6 - *Aeromonas* species distributions in round and leather fish samples.

Species	-----Isolated-----		
	Round	Leather	TOTAL
<i>A. eucrenophila</i>	8	3	11 (20%)
<i>A. hydrophila</i>	10	3	13 (23.64%)
<i>A. caviae</i>	14	3	17 (30.9%)
<i>A. media</i>	4	0	4 (7.27%)
<i>A. jandaei</i>	2	0	2 (3.64%)
<i>A. veronii</i> bv. <i>sobria</i>	2	0	2 (3.64%)
<i>A. trotta</i>	1	0	1 (1.82%)
<i>A. schubertii</i>	1	0	1 (1.82%)
<i>A. veronii</i> bv. <i>veronii</i>	2	1	3 (5.45%)
<i>A. shigelloides</i>	0	1	1 (1.82%)
Total	44 (80%)	11 (20%)	55 (100%)

It is known that *Aeromonas* sp. are resistant to ampicillin and susceptible to amoxicillin-clavulanic acid, and cefazolin, with the degree differing among species (CLSI, 2010), a fact that aids in the species identification of this microorganism. Considering the resistance and susceptibility of *Aeromonas* to these antimicrobials, it is suggested to avoid these drugs in the treatment of cases of contamination by *Aeromonas*. BANDEIRA JUNIOR et al. (2018) investigated the use of phytochemicals in the treatment of fish in ponds and obtained good results in the control of *Aeromonas* sp. with the proposed replacement of the antibiotics florfenicol and oxytetracycline.

AZZAM-SAYUTI et al. (2021) showed that all *Aeromonas* sp. isolates from freshwater fishes in Peninsular Malaysia were resistant to the

antimicrobial ampicillin. While SANTOS et al. (2019), observed that 98%, 91%, 41%, 14%, and 5% of isolates from “Tambaqui” samples marketed in the city of São Luiz - MA showed resistance to ampicillin, cefoxitin, sulfatrimethoprim, ciprofloxacin, and gentamicin and cefepime, respectively. *Aeromonas* strains are described as being resistant to ampicillin and other antimicrobials of the penicillin class, carbenicillin and ticarcillin (PEIXOTO et al., 2012). The resistance to these antimicrobial classes does not allow effective treatment in an epidemiological outbreak.

The results of the above studies varied from those of the current study, where the resistance to ampicillin (65%), cefoxitin (38%), and sulfazotrim (29%) was lower and that to ciprofloxacin (25%), gentamicin (24%), and cefepime (29%) was higher. Despite the

Table 7 - Distribution of *Aeromonas* species in fish samples commercialized in fairs, markets, and fishmongers.

Species	-----Isolated-----			
	Marketplace	Fair	Fish shop	TOTAL
<i>A. eucrenophila</i>	6	2	3	11 (20%)
<i>A. hydrophila</i>	2	4	7	13 (23.64%)
<i>A. caviae</i>	5	5	7	17 (30.9%)
<i>A. media</i>	1	3	0	4 (7.27%)
<i>A. jandaei</i>	1	0	1	2 (3.64%)
<i>A. veronii</i> bv. <i>sobria</i>	0	2	0	2 (3.64%)
<i>A. trotta</i>	0	0	1	1 (1.82%)
<i>A. schubertii</i>	0	1	0	1 (1.82%)
<i>A. veronii</i> bv. <i>veronii</i>	0	3	0	3 (5.45%)
<i>A. shigelloides</i>	1	0	0	1 (1.82%)
Total	16 (29%)	20 (36%)	19 (35%)	55 (100%)

Table 8 - Antibiogram of the 55 *Aeromonas* sp. isolates from fish samples commercialized in the city of Cuiabá, Mato Grosso, Brazil.

Antimicrobials	Sensitive	Intermediate	Resistant
----- <i>Aeromonas</i> sp.-----			
-----Sulphonamides-----			
Sulfazotrim	37 (67%)	2 (4%)	16 (29%)
-----Phenicols-----			
Chloramphenicol	40 (73%)	1 (2%)	14 (25%)
-----Penicillins-----			
Ampicillin-sulbactam	14 (25%)	5 (9%)	36 (65%)
Amoxicillin-clavulanic acid	28 (51%)	8 (15%)	19 (35%)
-----Cephalosporins-----			
Cefepime	39 (71%)	0 (0%)	16 (29%)
Cefoxitin	33 (60%)	1 (2%)	21 (38%)
-----Aminoglycosides-----			
Gentamicin	41 (75%)	1 (2%)	13 (24%)
-----Quinolones-----			
Levofloxacin	40 (73%)	0 (0%)	15 (27%)
Ciprofloxacin	41 (75%)	0 (0%)	14 (25%)
-----Tetracyclines-----			
Tetracycline	32 (58%)	5 (9%)	18 (33%)

differences in percentages, they were still positive for resistance, which shows that these antimicrobials should not be used to combat *Aeromonas*. Furthermore, the difference in results could be due to several factors, such as species, conservation method, and origin. According to GRILO et al. (2020), natural environments, especially aquatic ecosystems are ideal bases for the development and dissemination of antimicrobial resistance.

*Aeromonas* sp. isolates showed 75% sensitivity to the antimicrobials gentamicin (aminoglycosides class) and ciprofloxacin (quinolones class). These results suggested that these drugs may be beneficial in the treatment of infection by this bacterium. *Aeromonas* showed a resistance of 24% to gentamicin, 25% to ciprofloxacin, and 75% sensitivity for both antimicrobials. This is contradictory to the results by CHEN et al (2021), who observed that *Aeromonas* sp. isolates showed no resistance to ciprofloxacin and gentamicin. While SCARANO et al. (2018) reported that more than 90% of the isolated strains of *Aeromonas* showed susceptibility to gentamicin and chloramphenicol. The sensitivity to these antimicrobials occurs due to the low use of these in combating *Aeromonas*. Therefore, reducing the overuse of antimicrobials is especially important to decrease the occurrence and spread of antimicrobial-resistant bacteria (FRANZ et al., 2018) and prevent sensitive bacteria from becoming resistant.

Among the 55 strains evaluated, 27.3% showed multidrug resistance to three or more classes of antimicrobials (Table 9); these were *A. caviae*(n=6), *A. eucrenophila*(n=4), *A. veronii* bv. *veronii* (n=1), *A. media* (n=2), *A. jandaei* (n=1), and *A. trola* (n=1). The multidrug resistance of these bacteria is a unique health issue, and can cause harm in all spheres, environmental, animal health, and humans, since *A. caviae* are known to cause diseases in humans and animals, while *A. jandaei* and *A. veronii* are the species frequently associated with fish diseases (JANDA; ABBOTT, 2010; AZZAM-SAYUTI et al., 2021).

*A. trola* is an exceptional case because it showed resistance to all antimicrobials in this study. It is considered a rare species because it has been rarely associated with any type of incidence or outbreak; however, in a recent study, FERNANDEZ-BRAVO & FIGUERAS (2020), presented a new clinical case associated with *A. trola*, where a 69-year-old patient with diarrheal syndrome was admitted to a hospital. In addition, the antimicrobial resistance pattern showed that the strain was susceptible to ampicillin, penicillins in combination with beta-lactamase inhibitors, quinolones, and aminoglycosides among others. The authors of the study also described the multidrug resistance of *Aeromonas* sp. isolates from shrimp (*Litopenaeus vannamei*), where they were resistant to ampicillin, clindamycin, nalidixin, tetracycline, cephalothin, erythromycin, and trimethoprim-sulfamethoxazole

Table 9 - Antimicrobial resistance of the 55 *Aeromonas* species cultures isolated from freshwater fish.

----- <i>Aeromonas</i> species-----										
Classes	<i>A. caviae</i> (n=15)	<i>A. eucrenophila</i> (n=11)	<i>A. veronii</i> <i>bv. veronii</i> (n=5)	<i>A. hydrophila</i> (n=13)	<i>A. media</i> (n=4)	<i>A. jandaei</i> (n=2)	<i>A. veronii</i> <i>bv. sobria</i> (n=2)	<i>A. trota</i> (1)	<i>A. shigelloides</i> (n=1)	<i>A. schubertii</i> (n=1)
-----Resistant-----										
-----Sulphonamides-----										
Sulfazotrim	5 (33%)	6 (55%)	1 (20%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
-----Phenicol-----										
Chloramphenicol	5 (33%)	4 (36%)	1 (20%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
-----Penicillins-----										
Ampicillin-sulbactam	9 (60%)	6 (55%)	5 (100%)	7 (54%)	3 (75%)	2 (100%)	1 (50%)	1 (100%)	1 (100%)	1 (100%)
Amoxicillin	6 (40%)	5 (45%)	2 (40%)	2 (15%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
-----Cephalosporins-----										
Cefepime	6 (40%)	4 (36%)	2 (40%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Cefoxitin	7 (47%)	6 (55%)	2 (40%)	1 (8%)	3 (75%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
-----Aminoglycosides-----										
Gentamicin	4 (27%)	4 (36%)	1 (20%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
-----Quinolones-----										
Levofloxacin	6 (40%)	4 (36%)	1 (20%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Ciprofloxacin	5 (33%)	4 (36%)	1 (20%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
-----Tetracyclines-----										
Tetracycline	7 (47%)	5 (45%)	1 (20%)	0 (0%)	2 (50%)	2 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)

(DE SILVA et al., 2018). DAHANAYAKE et al. (2019) reported the presence of *A. hydrophila*, *A. media*, *A. veronii*, and *A. caviae* in mussels (*Ruditapes philippinarum*) and the isolates were reported as strains resistant to ampicillin, cephalothin, rifampin, oxytetracycline, colistin sulfate, nalidixic acid, and piperacillin and most were multidrug resistant.

Multidrug resistance is defined as the condition where the microorganism is resistant to at least one agent from three or more classes of antimicrobials. Although, the name of certain microorganisms describes resistance to a single antimicrobial agent (e.g. ampicillin-resistant *Aeromonas*), these pathogens are often resistant to most antimicrobials (ANVISA, 2015).

It is known that *Aeromonas* is sensitive to heat treatment and resistant to the ampicillin of the

penicillin class; therefore, this class will not be adequate for the treatment of a contamination by this bacterium (MELAS et al., 1999; PEREIRA et al., 2004).

It is worth mentioning that the lack of inspection by the competent bodies in establishments that commercialize fish exposes the population to public health risks. Besides this exposure, the concern with the inappropriate use of antimicrobials for the control of *Aeromonas* is related to the presence of these compounds in the environment, since there are not enough studies demonstrating their degradation with the use of heat treatment. Therefore, further studies will be necessary to verify and determine the probable classes to be used to combat diseases caused by *Aeromonas* species, and if the heat treatment influences the presence of antimicrobials in the food.

Table 10 - Antimicrobial sensitivity of 55 *Aeromonas* species cultures isolated from freshwater fish.

----- <i>Aeromonas</i> species-----										
Classes	<i>A. caviae</i> (n=15)	<i>A. eucreno phila</i> (n=11)	<i>A. veronii</i> <i>bv. veronii</i> (n=5)	<i>A. hydrophila</i> (n=13)	<i>A. media</i> (n=4)	<i>A. jandaiei</i> (n=2)	<i>A. veronii</i> <i>bv. sobria</i> (n=2)	<i>A. trota</i> (1)	<i>A. shigelloides</i> (n=1)	<i>A. schubertii</i> (n=1)
-----Sensitive (N=%)-----										
-----Sulphonamides-----										
Sulfazotrim	9 (60%)	5 (46%)	4 (80%)	12 (92%)	2 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
-----Phenicolos-----										
Chloramphenicol	10 (67%)	6 (55%)	4 (80%)	13 (100%)	2 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
-----Penicillins-----										
Ampicillin-sulbactam	4 (27%)	4 (36%)	0 (0%)	5 (39%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Amoxicillin	9 (60%)	5 (46%)	2 (40%)	7 (54%)	2 (50%)	0 (0%)	1 (50%)	0 (0%)	1 (100%)	1 (100%)
-----Cephalosporins-----										
Cefepime	9 (60%)	7 (64%)	3 (60%)	13 (100%)	2 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
Cefoxitin	8 (53%)	5 (46%)	3 (60%)	11 (85%)	1 (25%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
-----Aminoglycosides-----										
Gentamicin	11 (73%)	6 (55%)	4 (80%)	13 (100%)	2 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
-----Quinolones-----										
Levofloxacin	9 (60%)	7 (64%)	4 (80%)	13 (100%)	2 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
Ciprofloxacin	10 (67%)	7 (64%)	4 (80%)	13 (100%)	2 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)
-----Tetracyclines-----										
Tetracycline	5 (33%)	6 (55%)	3 (60%)	12 (92%)	2 (50%)	0 (0%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)

## CONCLUSION

This study concluded that the occurrence of *Aeromonas* sp. is independent of its commercialization origin; however, the variety and form of preservation are points to be evaluated since frozen “pintado” had the lowest incidence of *Aeromonas*. Antimicrobial resistance is a problem when it comes to combating *Aeromonas* because, as shown in this study, this bacterium is resistant to ampicillin and is multidrug-resistant, which makes it difficult to choose appropriate antimicrobials for treatment if this pathogen infects patients or contaminates the production system.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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