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The Occurrence of Bacteria of the Genus *Aeromonas* spp. in *Oreochromis niloticus* (Tilapia) and in the Water of Amateur Sport Fish Ponds and Sensitiveness to Antimicrobials

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Abstract

Since amateur fishing in fishponds has been on the increase in Brazil, there is a great concern on the microbiological quality of fish. One hundred and thirty-eight samples were collected during the dry and rainy periods in ten fish farms. *Aeromonas* spp. counts and tests for sensitiveness to antimicrobials were performed, coupled to the physical and chemical analyses of water. Tests revealed that 70% of samples were contaminated by *Aeromonas* spp., with water averaging 2.92 Log CFU/100 mL during the rainy period and 3.16 Log CFU/100 mL during the dry one. Fish contaminated by *Aeromonas* spp. averaged 2.58 Log CFU/100 mL during the rainy period and 3.53 Log CFU/100 mL during the dry one. *Aeromonas* spp. samples were multi-resistant to 2 or 8 antimicrobials in 62.5% of the samples. Ampicillin was the antimicrobial with the highest resistance percentage rate. Results showed that fish bred in amateur fish farms constituted a health risk for the population.

Keywords

Public Health, *Oreochromis niloticus*, Microbiological Quality, Antimicrobial Resistance, Fish Farms

1. Introduction

Fish farming is currently one of the most relevant food sources, with high growth rates. Fish production has increased significantly in Brazil and worldwide. The wave of amateur and recreational fishing on fish farms is definitely on the increase, with the Nile tilapia featuring the most farmed fresh water fish [1]

According to IBAMA Ordinance 136/1998, a fish farm may be defined as a company which establishes fish tanks or ponds for the commercial exploitation of amateur fishing [2].

Fish farming is a profitable activity in the country. However, several environmental issues, such as an increase in the population growth of specific bacteria of natural water microbiota, wastes from feeding, and a possible proliferation of microorganisms resistant to antimicrobials used indiscriminately, have been detected. Several bacteria related to fish pathology have been reported, among which may be mentioned *Aeromonas* spp., a pathogenic bacterium harmful to fish and humans [3].

Aeromonas spp. strains are renowned for their increased capacity to acquire and exchange resistance genes with antimicrobials. In fact, there is a strong relationship between aquaculture, *Aeromonas* spp. diversity and resistance to antimicrobials. Robust link clues exist between prophylactic and systemic use of antimicrobials in aquaculture and the spread of resistance against antimicrobials [4].

The genus *Aeromonas* has pathogenic strains for aquatic animals. They have been pinpointed in the literature as the main etiological agents, causing infection in fish and jeopardizing other aquatic animals, with great economic loss for Brazilian fish farms. Several bacteria of the genus have been identified as pathogenic to public health. Epidemiological data have shown that food consumption, contact with and intake of contaminated water are the main transmission means of the pathogen and are described as the main etiological agents in human gastroenteritis [5] [6]. However, ANVISA's RDC 12/2001 does not establish any limits for the microorganism in food [7]

Aeromonas spp. has been described as an opportunist pathogen worldwide, especially in developing countries, associated with traveller's diarrhea. Although these pathogens mainly inhabit aquatic environments, they may be found in animals, and have been related to several human and animal infections [8]. Reports on human contamination by several species, particularly *Aeromonas hydrophila, A. caviae* and *A. veronii biovar sobria*, are extant worldwide. The former is associated to watery diarrhea and dysentery [9]. New infection cases have been reported in 2010, caused by wounds and by gastroenteritis, associated to two new species, *A. sanarellii* and *A. taiwanensis* [10].

It has been proved that bacteria of the genus *Aeromonas* are pathogenic microorganisms which harm humans and animals. Their importance to public health is due to their high virulence and resistance to antimicrobials, observed in an increasing number of cases, in Brazil and worldwide, underscoring the occurrence of cases of gastroenteritis caused by the microorganisms, vectored by contaminated food and water. Since few research works on *Aeromonas* have been undertaken in amateur fishing ponds in the state of Bahia, Brazil, studies on the occurrence of *Aeromonas* spp. and their sensitiveness to antimicrobial agents in water and in fish retrieved from fishing ponds in the towns of Amélia Rodrigues, Ubaíra, Amargosa, São Felipe, Conceição do Almeida, Dom Macedo

Costa, Santo Antônio de Jesus, Ubaíra and in the island of Itaparica will be greatly appreciated by the scientific community.

2. Materials and Methods

The amateur sporting fish ponds selected for current research lie in the towns of Amélia Rodrigues, Amargosa, São Felipe, Conceição do Almeida, Dom Macedo Costa, Santo Antônio de Jesus, Ubaíra and in the island of Itaparica, all in the state of Bahia, Brazil. Main criterion for selection consisted of fishing ponds with *Oreochromis niloticus* adults since the Nile tilapia is the most farmed fresh water fish in the state of Bahia [1].

Sample collection occurred between March and November 2017 during two periods, namely, the dry and rainy periods. For this purpose, 500-mL water samples from the water surface were collected from four equidistant sites of each pond, in up to three ponds in each fish farm, totaling 48 samples, from 60 fishing ponds. Sterilized flasks were dipped in the water, opened, filled with water, conditioned in isothermal boxes with ice, and transported to the Laboratory of Animal Parasitology and Microbiology of the Universidade Federal do Recôncavo da Bahia. Microorganisms were identified and counted within 24 h of incubation within a single stage.

Two fish samples, collected weekly, were retrieved from up to three ponds, totaling 90 fish samples. Fish were caught by fishing lines or nets used on the fish farms. They were slaughtered by thermal shock (thermonarcosis) [11] and samples were conditioned in refrigerated thermal boxes and transported to the laboratory.

The fish were eviscerated and 25 g of each sample were weighed, to which were added 225 mL of peptone water 0.1% for the first dilution. Serial dilutions were performed until dilution 10^{-6} [9]. The fish were prepared for processing following methodologies by Silva *et al.* [12] and Apha [13]. All procedures were undertaken for each sample retrieved from different fish farms.

Aeromonas Isolation Agar (Base), supplemented by *Aeromonas* Selective Supplement, was employed to analyze *Aeromonas* spp. Since the medium contains ampicillin, the former becomes selective. Ampicillin is employed for the selective and differential isolation of *Aeromonas hydrophila* from clinical and environmental samples. In fact, it is the medium employed for fast and simultaneous identification of the species *Aeromonas, Plesiomonas, Proteus* and *Enterobacteriaceae*, or rather, it is employed as an overall medium to investigate enteric diseases.

The pour plate method was employed to count the microorganisms *Aeromonas* spp. [9] [12]. Serial dilution, up to dilution 10^4, was performed after the preparation of the sample. The incubation process occurred posterior to serial dilution. Plating was done in triplicate. Plates were inverted for incubation and colonies with a dark green color, opaque, featuring dark hubs, were positive for *Aeromonas* ssp. *Aeromonas* spp. isolates were counted with a colony counter (Phoenix counter CE 550A) from plates with 25 - 250 colonies [9] [12] [13].

Identified *Aeromonas* spp. colonies, up to five per sample, were seeded on brain heart infusion broth culture for conservation in nutrient agar and glycerol [9] [12] [13] [14] [15].

Resistance profile against antibacterial agents was assessed by disc diffusion method in Muller-Hinton agar. Thirteen antimicrobials were tested. A bacterial suspension was prepared from 3 to 4 colonies of each strain under analysis and homogenized in a 100% sterile physiological solution [9]. Tested antimicrobials contained the following active principles: Amikacin (10 µg), Amoxicillin + Clavulanate (20 × 10 µg), Ampicillin (10 µg), Cephalothin (30 µg), Cefepime (30 µg), Cephoxytin (30 µg), Cephtazidim (30 µg), Cefuroxime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Sulfazotrim (20 µg) and Meropenem (10 µg) [9] [12].

Color, pH and turbidity were the parameters for the physical and chemical analyses of water. They were evaluated by previously calibrated digital bench equipments, namely colorimeter, pH-meter and turbidity-meter, respectively. Dissolved oxygen (DO) and Biochemical Oxygen Demand (BOD) were also assessed [9] [12].

All results were matched with current Brazilian legislation, namely, Resolution 12, published on 2nd January 2001 by ANVISA, dealing with microbiological standards for animal-derived food [7], and Resolution 357, published in 2005 by CONAMA, dealing with physical, chemical and microbiological standards of water [16].

Data were processed and analyzed by program R for statistical analysis. All quality variables underwent Shapiro-Wilks data normality test. Descriptive and analytic statistics were performed, such as means, medians, standard deviation, maximum and minimum, percentage distribution and Spearman's coefficient of correlation. Significance levels were ($p < 0.01^{**}$) and ($p < 0.05^{*}$), whilst ANOVA (analysis of variance) compared the microbiological profile of the fish farms [17].

Current project was submitted to the Committee for Ethics in the Use of Animals in Experiments (CEUA) of the Universidade Federal do Recôncavo da Bahia (UFRB). Sample collection was undertaken after approval (N. 23007.005912/2017-60).

3. Results

Table 1 shows results of colony forming units in Log CFU/100mL of contamination by *Aeromonas* spp. in fish pond water under analysis. Forty-eight samples were contaminated by *Aeromonas* spp., ranging between 1.17 and 4.47, mean 2.92 Log CFU/100 mL during the rainy season and 3.16 Log CFU/100 mL during the dry season (**Table 1** and **Figure 1(b)**). Further, 70% of the 90 fish samples were contaminated by *Aeromonas* spp. ranging between < 1 and 5.28, mean 2.58 Log CFU/100 mL during the rainy season and 3.53 Log CFU/100 mL during the dry season (**Table 1** and **Figure 1(a)**).

Table 1. Minimum and maximum rates, mean, standard deviation, analysis of variance ANOVA (AV) and Shapiro-Wilks normality test (W) for *Aeromonas* spp. in water and fish assessed in ponds on fish farms in towns of the state of Bahia and on the Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

	DESCRIPTIVE STATISTICS	RAINY PERIOD	DRY PERIOD		
		Aeromonas spp. Log CFU/g^{-1}	*Aeromonas* spp. Log CFU/g^{-1}	W	AV
WATER	Minimum	1.17	<1	0.92**	0.076
	Maximum	4.40	4.47		
	Mean	2.92	3.16		
	Standard deviation	2.08	0.86		
FISH	Minimum	<1	<1	0.89**	0.004**
	Maximum	4.47	5.28		
	Mean	2.58	3.53		
	Standard deviation	1.48	1.48		

**significant at 1% probability by Shapiro-Wilks test. ^{ns} not significant at 5% probability.

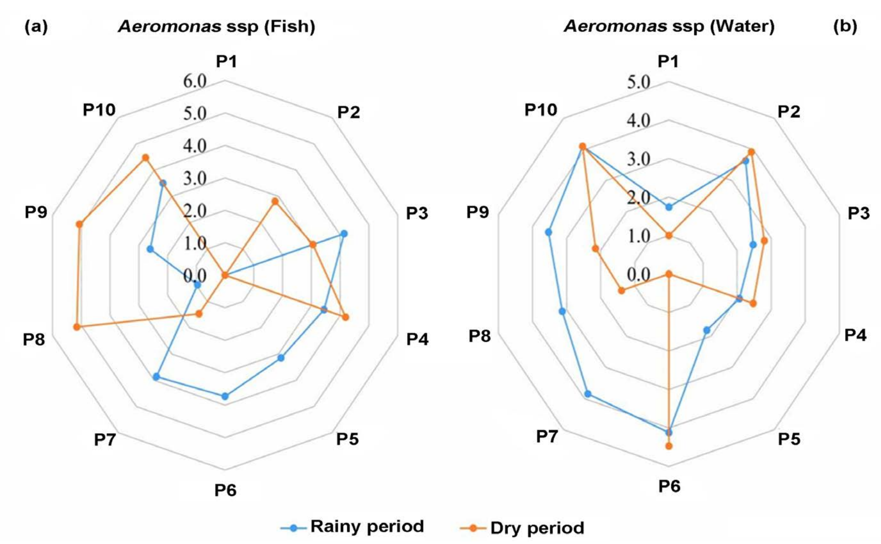

Figure 1. (a) (b): Microbiological analyses. Mean of results for *Aeromonas* spp. in Log, Most Probable Number (Log MPN/100 mL) in water and fish of fish ponds on fish farms in towns of Bahia and Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

Further, pH rates ranged between 5.00 and 7.11, mean 6.07 during the rainy season and 6.04 during the dry season. Consequently, 87% (42/48) of samples complied with standards. Color also complied with current legislation, whilst DO reached 87.5% (43/48) of samples in the two periods and failed to comply with legislation. In current analysis, BOD reached 93.8% (45/48) of samples, or rather, it failed to comply with limits (up to 5 mg/L O_2) established by CONAMA [16].

ANOVA statistical assessment showed a possible significant variation among fishponds for *Aeromonas* spp. in fish ($p > 0.05$). The Shapiro-Wilks normality test demonstrated lack of normal distribution (Table 1). After the elaboration of a matrix for Spearman's correlation, parameters with significance $p \leq 0.01$ and $p \leq 0.05$ were chosen. Figure 2 shows variations of each parameter.

Figure 2 reveals that most correlations were not significant, even though there were significant correlations of greatness ranging between weak and strong. The greatest positive and significant correlations ($p < 0.01$) occurred between counts of total coliforms for fish and color and turbidity counts ($r = 0.62^{**}$) and for pH and DO ($r = 0.61^{**}$). The above demonstrated that a level increase or decrease of any variable triggered a proportional increase or decrease of the other. Physical and chemical factors (pH and DO) also showed a negative correlation with significance rate at $p \leq 0.01$ ($r = 0.47^{**}$), featuring an inversely proportional relationship. In other words, an increase in one of the variables triggered a decrease in the other. In current study, Spearman's correlations were not significant between *Aeromonas* spp. in the water and in fish, although there were positive correlations at significance levels $p \leq 0.01$ between BOD and *Aeromonas* spp. in fish ($r = 0.51^{**}$).

Aeromonas spp. isolates from the fish ponds under analysis revealed high resistance rates against the 13 antimicrobials tested. Resistance and multi-resistance percentages against antibiotics indicate threats against human health and therefore urgency to discover alternative effective antibiotics. The sensitiveness and multi-resistance profile of 109 isolates included the evaluation of 13 antibiotics by the disc diffusion method. In fact, they represent different classes of antibiotics usually employed in the treatment of infections in animals and humans. Twenty-seven out of the 109 isolates showed resistance against 1 antimicrobial, whilst 45 isolates proved to be multi-resistant against 2 to 8 antimicrobials. The interpretation of the sensitivity profile complied with CLSI M45-A (2006) by halo measuring (Table 2 and Table 3; Figure 3 and Figure 4).

Highest resistance levels to antimicrobials occurred for ampicillin (49.5%), cefalotin (38.5%), cefoxitin (26.4%), cefuroxime (21.97) amoxicillin/clavulanic acid and sulfamethoxazole + trimethoprim (20.8%). Reduced resistance levels and greater efficiency against *Aeromonas* spp. isolates (<10%) were detected for ciprofloxacin and meropenem (1.09%), amikacin (2.2%), gentamicin (3.29%), cefepime (6.59%) and imipenem (7.69%). All isolates were sensitive to ceftazidime (100%). Results showed that 70.3% of samples had an intermediate resistance to at least one antimicrobial under analçysis (Table 2 and Table 3).

In the case of the 45 (49.45%) multi-resistant isolates, 42.2% (19/45) were resistant to 2 drugs; 44.44% (11/45) were resistant to 3 drugs; 22.22% (10/45) were resistant to 4 drugs; 8.88% (4/45) were resistant to 5 drugs and 2.3% (1/45) were multi-resistant to 8 antimicrobials. Multiple Resistance to Antimicrobials (MRAM) index ranged between 15.38% (2/13) and 61.53% (8/13) of antimicrobials (Figure 3 and Figure 4). Fish farms P2, P5, P7, P8 and P9 out of the ten evaluated had a higher percentage rate of samples which were resistant to antimicrobials. Highest percentage (75%) occurred in P9.

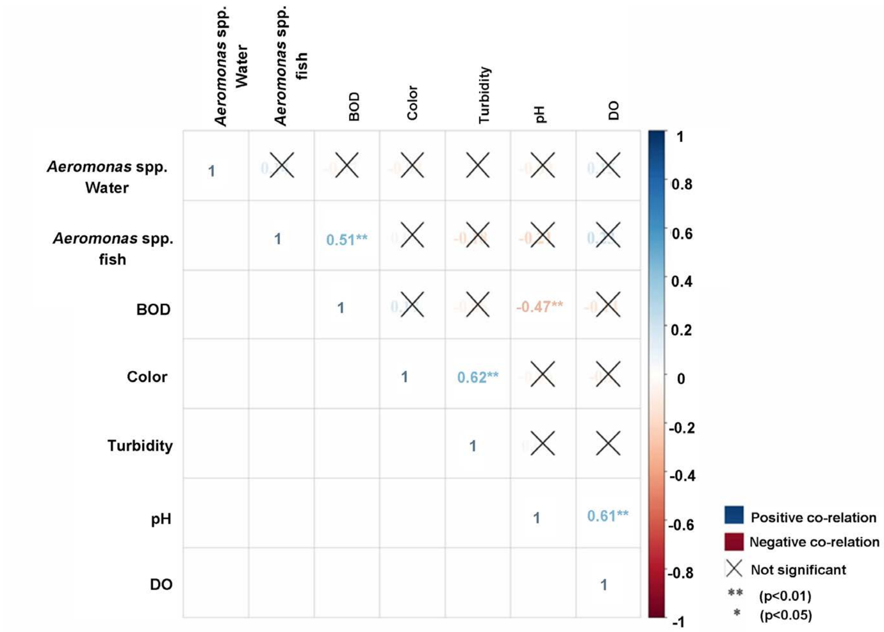

Figure 2. Statistical analyses. Spearman Co-relationship of Colony Forming Units (Log CFU) in water and fish from fish ponds on fish farms in towns of Bahia and Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

Table 2. Antibiotics tested—frequency of resistant isolates, with intermediary resistance and sensitive (%) in water and fish samples in towns of Bahia and Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

Fish farm	N	Tested antibiotics																	
		AMI			AMP			AMC			CPM			CFO			CAZ		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
P1	6	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100	0	0	100
P2	11	18.3	27.2	54.5	63.6	0	36.4	27.3	9.1	63.6	0	0	100	9.1	0	90.9	0	0	100
P3	16	0	6.2	93.8	37.5	6.2	56.3	18.7	25	56.3	0	0	100	0	0	100	0	0	100
P4	12	-	-	-	58.4	8.3	33.3	8.3	25	66.7	0	0	100	41.6	8.3	50.1	0	0	100
P5	2	-	-	-	50	0	50	0	0	100	0	0	100	50	0	50	0	0	100
P6	8	-	-	-	50	0	50	12.5	12.5	75	0	0	100	37.5	12.5	50	0	0	100
P7	7	0	0	100	42.8	0	57.2	42.8	14.2	43	0	0	100	42.8	28.6	28.6	0	0	100
P8	11	-	-	-	63.6	0	36.4	18.2	0	81.8	0	0	100	36.3	18.2	45.5	0	0	100
P9	8	0	0	100	25	0	75	12.5	0	87.5	0	0	100	50	0	50	0	0	100
P10	10	0	0	100	20	0	80	10	30	60	0	0	100	30	10	60	0	0	100

Fish farm (Prop), Number of samples (N), Amikacin (AMI), Ampicillin (AMP), Amoxillin + Clavulanic acid (AMC), Cefepime (CPM), Cefoxitina (CFO), Cefitazidina (CAZ). Frequency (%) of resistant isolates (R), with intermediary resistance (I) and sensitiveness (S).

4. Discussion

Fish have an important role in human diet and a per capita rise in fish consumption has been reported in Brazil and worldwide. Nevertheless, intensive industrial and agricultural growth may contaminate natural and artificial aquatic environments. It may affect not only the health of fish but triggers concern on food safety when fish is consumed by humans. It is a well-known fact that fish and

Table 3. Continuation: Antibiotics tested—frequency of resistant isolates, with intermediary resistance and sensitive (%), in water and fish samples in towns of Bahia and Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

Fish farm	N	CONTINUATION: Tested Antibiotics																				
		CFL			CRX			CIP			GEN			IPM			SUT			MER		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
P1	6	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
P2	11	9.1	18.1	72.8	0	18.2	81.8	9.1	0	90.9	9.1	0	90.9	27.2	0	72.8	18.2	0	81.8	9.1	0	90.9
P3	16	6.2	18.7	75.1	6.2	25	68.8	0	0	100	6.2	0	93.8	12.4	0	87.6	6.2	6.2	87.6	0	0	100
P4	12	58.3	16.6	25.1	25	16.6	58.4	0	0	100	0	0	100	8.3	0	91.7	33.3	8.3	58.4	0	8.3	91.7
P5	2	0	50	50	100	0	0	0	0	100	0	0	100	0	0	100	50	0	50	0	0	100
P6	8	50	25	25	37.5	12.5	50	0	0	100	0	0	100	0	0	100	12.5	25	62.5	0	0	100
P7	7	42.9	14.2	42.9	14.2	0	85.8	0	14.2	85.8	0	0	100	0	0	100	14.2	0	85.8	0	0	100
P8	11	72.7	18.2	9.1	27.2	0	72.8	0	0	100	9.1	0	90.9	9.1	18.2	72.7	18.2	0	81.8	0	9.1	90.9
P9	8	75	12.5	12.5	62.5	0	37.5	0	0	100	0	0	100	0	0	100	37.5	0	62.5	0	0	100
P10	10	50	20	30	20	30	50	0	0	100	0	0	100	10	0	90	40	30	30	0	10	90

Fish farms (Prop), Number of samples (N), Cefalotin (CFL), Cefuroxine (CRX), Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IPM), Sulfamethoxazole = Trimitopim (SUT), Meropenem (MER) Frequency (%) of resistant isolates (R), with intermediary resistance (I) and sensitivity (S).

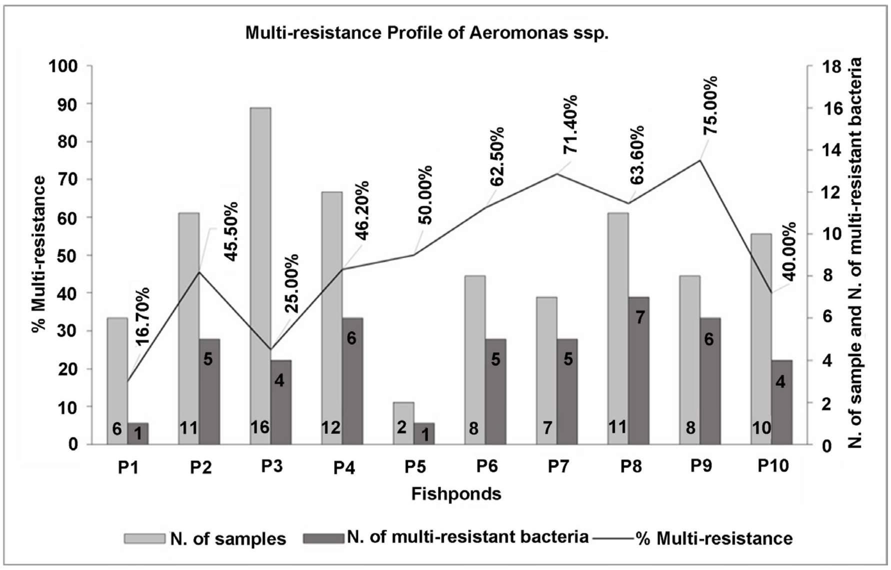

Figure 3. Multi-resistance profile of *Aeromonas* sp. isolates of water and fish samples from fish ponds on fish farms in towns of Bahia and Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

fish products are frequently associated with diseases in humans [18].

Current study has detected high percentage levels of *Aeromonas* spp. bacteria during the two periods under analysis, with the rainy season as the most dangerous. Significant contamination during the rainy season is due to soil leaching, or rather, the accumulation of wastes from homothermal animals, such as dogs, cattle, fowls, close to the fish ponds. These animals were seen by current

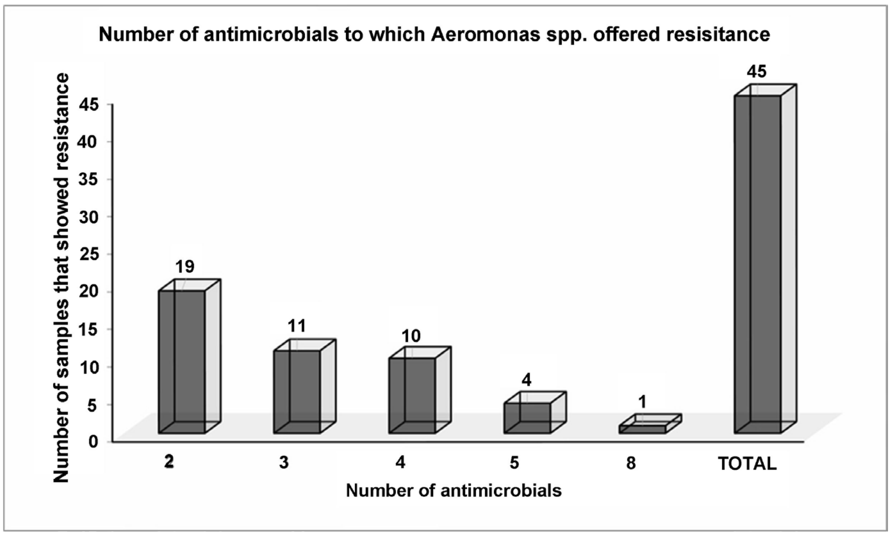

Figure 4. Multi-resistance profile of *Aeromonas* spp. isolates. Number of antimicrobials to which they offered resistance in water and fish samples in towns of Bahia and Island of Itaparica, state of Bahia, Brazil, between March and November 2017.

researchers when they were collecting the samples. Contamination also occurs due to cesspits and sewerage issues from small inns on the farms and other similar types of human activity lacking a sewage system. Data have been corroborated by other researches which also described contamination of fish ponds by *Aeromonas* spp. [9] [19] [20].

Water contamination is particularly high during the rainy season and alters the physical and chemical characteristics of water and the microbiological quality of fish. Increase in rain water and a greater load of dirt in the water are the causes of high pathogen rates. Natural factors, such as rock dissolution, photosynthesis and household sewerage affect pH. Increase in rain volume causes a rise in pH through the dilution of dissolved compounds [21].

There has been an increase in the number of contamination cases in animal-derived food by *Aeromonas* spp. in fresh or sea water. According to Resolution by CONAMA 375/05 [16] on the quality of fresh water for fish farming and fishing activities (class 2), thermotolerant coliforms should not exceed 1000 (3 log) in 80% or more in at least 6 samples collected during the year [16]. Although official parameters for *Aeromonas* spp. are lacking, coliform rates in the water of the fish farms analyzed are a real public health issue [22].

Health legislation in several countries determines limits in the quantity of *Aeromonas* spp. in drinking and mineral water. Bacteria have been frequently detected in public supply water and even in bottled mineral water [22] [23] [24]. Ribeiro *et al.* [25] state that limits for *Aeromonas* spp. in mineral water at the source and in bottled water were provisionally determined in Italy, in 1997, or rather, 10 UFC/100 mL and 100 UFC/100 mL respectively.

According to ANVISA'S RDC n. 12, published on the 2nd January 2001, the microbiological standards for "*in natura*" and thawed fish are: positive staphylococci coagulase/g. from 10^3 NMP/100 ml^{-1} (maximum 3 Log CFU/g) and

Salmonella spp. (absence in 25 g) [7]. Although Brazilian legislation fails to give standards, high *Aeromonas* spp. levels indicate deficient hygiene and sanitary conditions, coupled to pathogenic bacteria with harmful species, such as *Aeromonas hydrophila, A. caviae*, *A. veronii biovar sobria* and *A. hydrophila,* associated with aqueous diarrhea and dysentery [9].

Data from the Ministry of Health have shown that animal-derived food, such as fish, triggers outbreaks of Food and Water Borne Diseases (FWBD). Water is the natural habitat of these bacteria and an important source of food contamination. *Aeromonas* spp. in food is a predominant feature in fish consumption, even though it has been studied in swine, chickens and humans. It may occur in the excrement of infected animals and sick people who handle food [24] [26] [27]. Neyts *et al.* [28] detected *Aeromonas* spp. in 72% of fish and shrimps. Data corroborate those in current analysis.

The above data demonstrate that the microbiological composition of fish farming water is associated with several bacteria found in fish, actually corroborate studies by Lorenzon *et al.* [19], Santos *et al.* [29], Osman & El-khateeb [30], Stratev & Odeyemi [31], Pinheiro *et al.* [32] and Huicab-Pech *et al.* [33].

It has also been perceived that the fish farms analyzed either lacked proper water management or there was no management at all, with the consequent water and fish contamination. Lack of water management may cause disease and even death in fish. Due to faulty knowledge and inadequate man power, producers employ antimicrobials indiscriminately, causing the proliferation of resistant or multi-resistant bacteria to antimicrobials [19]. According to Souza [34], there is very scanty information on fish farming management and its consequences on water quality and on the health of fish in fish farms.

Aeromonas spp. is usually isolated from patients suffering from traveller's diarrhea. Hofe *et al.* [35] detected the pathogen as the cause of traveller's diarrhea in 18 (2%) out of 863 patients. A study on the outbreak of diarrhea in the town of São Bento do Una PE Brazil, in 2004, revealed that 114 (19.5%) out of the 2170 registered cases were caused by *Aeromonas* spp. [36].

There was a high positive correlation for DO and pH and for pH and BOD, which have a direct relationship with the maintenance of water life, in the case of aerobic respiration processes (as in DO) and in the maintenance of an environment that favors important chemical reactions for life (as in pH). There was also an expected positive correlation between color and turbidity, since increase in water turbidity is caused by suspended matter, such as clay, silt, organic and inorganic matter, soluble organic compounds. Increase in turbidity increases apparent color [37].

The co-relationship between pH and BOD revealed negative correlation at $p \leq 0.01$, where a pH increase is a decrease of BOD in water, perhaps caused by water acidification through a fermentation process produced by bacterial growth and evidenced in current study [38] [39]. The above has been corroborated by the negative analysis of data pairing between pH and *Aeromonas* ssp. In other words, when the later increase, pH decreases at significance level $p \leq 0.05$. Similar data were also reported by other authors [40].

Positive correlations at significance level $p \le 0.01$ between *Aeromonas* spp. in fish and BOD revealed that the latter increases when *Aeromonas* spp. increases. BOD results in current assay indicate contamination in fish pond water and explain the high levels of these microorganisms in water and fish. In fact, BOD indicates organic matter in water and is defined as the concentration of dissolved oxygen required to stabilize levels of organic matter in water [41].

Baron *et al.* [42] registered that *Aeromonas* spp. bacteria acquire the mechanisms of antimicrobial resistance and may accompany the dissemination of antimicrobial resistance in water. The literature has few interpretation criteria for *Aeromonas* spp. in antimicrobial susceptibility tests. EUCAST has no epidemiological rate for *Aeromonas* spp. to interpret Minimum Inhibitory Concentrations (MIC). The only interpretation criteria available are the clinical breakpoints of CLSI guidelines adapted from *Enterobacteriaceae*.

Several research works have shown the importance of *Aeromonas* spp. resistant to antimicrobials from fish farming and suggest that high multi-resistant levels indicate the possibility of horizontal diffusion of resistant genes. They also suggest that, since *Aeromonas* spp. are reported in the environment, the exchange of plasmids among them may be facilitated, with an increase in the frequency of multi-resistant samples [9] [43] [44].

Bacteria of the genus *Aeromonas* are pathogenic for humans and are a risk to their health. Results in current assay reveal that evaluated fish farms have high *Aeromonas* spp. contamination rates. In fact, water is an important disseminator of *Aeromonas* spp. Food, especially animal-derived, should be well monitored for the prevention of infections [43] [44].

Hirsch *et al.* [45] underscored that 43% of analyzed isolates had multiple resistance to antimicrobials (MRAM) for two or more out of the nine drugs under analysis. Data indicate imminent risk due to isolation of samples which are potentially pathogenic to humans and to the isolates' multi-resistant profile.

Aeromonas strains are known for their increasing capacity to acquire and exchange resistant genes to antimicrobials. There is a strong co-relationship between fish farming, *Aeromonas* diversity and resistance to antimicrobials. There are robust clues for bonds between prophylactic and systemic use of antimicrobials in fish farming and the spread of resistance against antimicrobials. In fact, the above factors may influence results since the indiscriminate use of antimicrobials has been detected in several fish farms [4] [44].

The contamination in fish and water reported in current assay is a potential infection vector by *Aeromonas* spp. The indiscriminate and unauthorized use of antimicrobials directly affects the development of bacteria with a multi-resistance profile against antimicrobials, such as the contamination of the environment. *Aeromonas* spp. are frequently isolated in animal-derived food and in fish farms with high resistance percentage to several antimicrobials of groups A, B and C, registered in current study and in others by Silva *et al.* [9], Peixoto *et al.* [27], Baron *et al.* [42], Hirsch *et al.* [45] and Nagar *et al.* [4].

Ahmed *et al.* [46] insist that *Aeromonas* spp. are known to be intrinsically sensitive to all antimicrobials against non-fastidious gram-negative bacilli, except for several β-lactam antibiotics, due to the production of induced multiple chromosomically-codified β-lactamases.

Current assay demonstrates that all strains were resistant to amoxicillin, carbenicillin and ampicillin. Data complied with results in current study since the antimicrobials tested, such as ampicillin, amoxicillin/clavulanic acid, cefalotin, cefoxitin, cefuroxime and sulfamethoxazole + trimethoprim, showed the highest resistance percentage. Further, ceftazidime, ciprofloxacin, meropenem, amikacin, gentamicin, cefepime and imipenem were the strongest among all the tested antimicrobials. All isolates were sensitive to ceftazidime. The above data were corroborated by results of several antimicrobials tested by Silva *et al.* [9] and Carneiro *et al.* [47].

According to Silva *et al.* [9] and Silva *et al.* [44] resistance to ampicillin is expected in all species of *Aeromonas* ssp., since it is employed as a selective factor in culture media. The resistance of *Aeromonas* ssp. bacteria is due to the fact that they are the natural producers of β-lactamase or induce the enzyme's activity. Bacteria had the lowest percentage rates of resistance to ceftazidime, ciprofloxacin and meropenem. These active principles should be selected to control the pathology in the case of disease caused by *Aeromonas* spp. in the fish farms evaluated.

Consequently, the presence of *Aeromonas* spp. in food, especially animal-derived food, should be thoroughly investigated to lessen the number of diseases caused by infections related to the genus. Owners of fish farms should also be made aware of the dangers caused by the indiscriminate use of antimicrobials. They should be convinced on the preservation of the environment and the proper management of fish ponds. Lack of information and of qualified manpower contributes towards hygiene and sanitary conditions of fish. In fact, they are factors directly related to the increase of resistant bacteria.

Indexes are a powerful tool in decision taking. However, technological efficiency of alternative water uses and aspects related to environmental impacts (positive or negative), social developments and economic factors should be taken into account. When the possibilities in the application of the described sustainability indexes are discovered, new possibilities of intervention will be perceived. They actually foreground the formulation of new strategies for efficient water management.

Research and studies on sustainability indexes systems have been prepared and implemented for the management of water resources. The investigation of case studies and the analysis of index systems employed are a great help to improve management tools.

A set of universal and unanimously accepted indexes does not exist since indexes describe a process of control linked to specificities and to local conditions. Indexes should be defined or adapted to each local situation, taking into account the characteristics, priorities and specific interests for their better effective application, reliability and political and social acceptance, taking into consideration the regional integrating and multi-dimensional aspects.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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