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MICROBIOLOGY

Resistance of *Aeromonas hydrophila* isolates to antimicrobials and sanitizers

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ABSTRACT: In recent decades, *Aeromonas hydrophila* has emerged as a foodborne bacterial pathogen of public health concern, especially as it exhibits resistance to the major chemical sanitizers commonly used in the food industry. Meanwhile, this pathogen may be spread from diseased fish flesh through the contamination of equipment contact surfaces during food processing, thus posing a food safety risk. Thise determined the susceptibility profiles of retail fish-borne *A. hydrophila* isolates to 24 common antibiotics and five major sanitizers used in the food industry. The polymerase chain reaction technique was used to confirm all *A. hydrophila* isolates to the species level, and the agar diffusion method was applied to determine the antimicrobial susceptibility profiles. All isolates were confirmed to be *A. hydrophila* species. This bacterium was observed to have resistance to multiple antibiotics, with the highest resistance index being for those of the beta-lactam class. Additionally, the isolates showed high resistance to four of the five chemical sanitizers tested, with the highest resistance rate being toward sodium hypochlorite. The results suggested that *A. hydrophila* isolates with multiple resistance to the antimicrobials and main sanitizers used in the food industry can be found in retail fish sold in the Cuiabá region of Mato Grosso, Brazil.
Key words: fish, microorganisms, pathogen, antibiogram, susceptibility to sanitizers.

Resistência de isolados de *Aeromonas hydrophila* a antimicrobianos e sanitizantes

RESUMO: *Aeromonas hydrophila* emergiu nas últimas décadas como um patógeno humano relevante. O fato desse patógeno alimentar emergente apresentar resistência antimicrobiana é um desafio considerável para as Agências de Saúde devido à resistência desta espécie aos principais sanitizantes químicos comumente utilizados na indústria alimentícia em que representa um risco à segurança dos alimentos, uma vez que pode contribuir para a disseminação desta bactéria na carne de peixes e levar à contaminação da superfície de contato dos equipamentos durante o processamento dos alimentos. Este estudo teve como objetivo determinar os padrões de suscetibilidade de isolados de *A. hydrophila* de peixes comercializados no varejo a 24 antibióticos comuns e cinco principais sanitizantes utilizados nas indústrias alimentícias. Neste estudo, todos os isolados de *A. hydrophila* foram confirmados em nível de espécie pela técnica de Reação em Cadeia da Polimerase (PCR). A técnica de difusão em ágar foi utilizada para determinar o perfil de suscetibilidade antimicrobiana frente aos 24 antibióticos testados e para avaliar a suscetibilidade aos principais sanitizantes utilizados na indústria alimentícia. A partir dos resultados, todos os isolados foram confirmados como sendo da espécie *A. hydrophila* pela técnica de PCR molecular. Observou-se *A. hydrophila* com perfil de resistência a múltiplos antibióticos, em que os da classe dos Beta-Lactâmicos foram os antimicrobianos com maior índice de resistência. Além disso, a suscetibilidade aos sanitizantes apresentou alta resistência em quatro dos cinco sanitizantes testados, sendo o hipoclorito de sódio foi o sanitizante químico com maior índice de resistência entre os isolados deste estudo. Os resultados sugerem que isolados de *A. hydrophila* com perfil de resistência a antimicrobianos e aos principais sanitizantes utilizados na indústria alimentícia podem ser encontrados em peixes comercializados no varejo da região de Cuiabá/Mato Grosso.
Palavra-chave: peixe, micro-organismos, patógeno, antibiograma, suscetibilidade a sanitizantes.

INTRODUCTION

Fish constitutes an important food in the human diet. However, they may contain *Aeromonas hydrophila*, a bacterial species found ubiquitously in aquatic environments (CHATTOPADHYAY & ADHIKARI, 2014). This human pathogen represents a serious health risk owing to its production of enterotoxins, hemolysins, and cytotoxins as well as other metabolites related to its pathogenicity and virulence (DASKALOV, 2006; IGBINOSA et al., 2012; STRATEV &; ODEYEMI, 2016; PESSOA et al., 2019; FERNÁNDEZ-BRAVO & FIGUERAS, 2020). More importantly, it has shown resistance to different antimicrobials (PRAVEEN et al., 2016). Although several studies have reported the presence of *Aeromonas* in various food sources, including retail fish (ABD-EL-MALEK, 2017; PASTRO et al., 2019; SANTOS et al., 2019), there is no specific legislation that sets standards for this microorganism in Brazil, resulting in a potential health risk to consumers.

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Antimicrobial resistance in emerging pathogens is a potential threat to human health and has become one of the biggest challenges to public health systems worldwide (MCEWEN & COLLIGNON, 2018). Most antimicrobials are common to veterinary and human use, which increases the potential of resistance development in pathogens that infect humans and animals and intensifies the risk of spread through food contamination (EMA, 2014; STRATEV & ODEYEMI, 2016).

The infective dose of *A. hydrophila* is not yet fully understood (PARK et al., 2021). According to GONZÁLEZ-SERRANO et al. (2002), an efficient cooking process can inactivate this pathogen, but insufficient cooking and cross-contamination will pose a potential health risk, primarily in immunosuppressed individuals, children, and the elderly.

Pathogens that exhibit resistance to antimicrobials also exhibit resistance to sanitizers (ROZMAN et al., 2021). The effectiveness of sanitizers, especially against gram-negative bacteria, is crucial in food manufacturing and processing units (ODEYEMI et al., 2022), and their ineffectiveness can represent a threat to food safety and consumer health (GONÇALVES, 2011; SHENG & WANG, 2021).

In Brazil, only two antimicrobials for treating infections in aquatic animals are regulated (SINDAN, 2021). According to the normative instruction number 26 declared by the Ministry of Agriculture, Livestock and Supply (MAPA) on July 9, 2009, the use of beta-lactams, tetracyclines, sulfonamides, quinolones, and amphenicols as additives for enhancing animal performance or as animal food preservatives is prohibited (BRASIL, 2009). The indiscriminate use of antibiotics in fish farming has favored the development of resistance genes through selective pressure and is one of the main factors responsible for the development and spread of multidrug-resistant bacteria (MUZIASARI et al., 2016).

To reduce the microbial load that can contaminate food, the industry uses sanitizers, which are defined as substances that contain one or more active ingredients with biocidal activity against harmful microorganisms (ROZMAN et al., 2021) at safe levels, are not harmful to health, and are intended for use on surfaces, objects, and environments (KUAYE, 2017). Chlorinated compounds, iodinated compounds, quaternary ammonium compounds, and peracetic acid are among the main groups of sanitizers used in the food industry (ANDRADE, 2008; KUAYE, 2017).

The resistance of *A. hydrophila* to antimicrobials and the main sanitizers used in the food industry makes this microorganism a target of study and has stimulated the search for better performing sanitizers. It is likely that fish sold in the retail environment in Brazil are contaminated with *A. hydrophila*, considering the non-application of good handling and sanitation practices in an adequate manner. In view of this, the study determined the resistance of retail fish-borne *A. hydrophila* isolates to 24 common antibiotics and five main sanitizers used in the food industry.

MATERIALS AND METHODS

Samples

In this study, 19 *A. hydrophila* isolates obtained from the Food Microbiology Laboratory of the Federal Institute of Education, Science and Technology of Mato Grosso (IFMT) Campus Cuiabá Bela Vista were used. The bacteria were from a previous study by SILVA (2023), having being isolated from samples of the Amazonian Pintado (*Pseudoplatystoma fasciatum* × *Leiarius marmoratus*), tambacu (*Colossoma macropomum* × *Piaractus mesopotamicus*), and tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) acquired from a supermarket, fair, and fish market in the city of Cuiabá, Mato Grosso, Brazil. The bacterial isolates cultured on tilted trypticase soy agar (TSA) in test tubes were stored at 4 °C refrigeration until the time of this study. The cultures were recultured daily for maintenance of the strains according to the method described by SILVA et al. (2017).

Molecular characterization of the A. hydrophila isolates

Young colonies of *A. hydrophila* on TSA were suspended in sterile water and boiled for 10 min in a dry bath (thermoblock) according to the methods described by PARK et al. (2021) and LAU et al. (2020). Then, each suspension was centrifuged at 24,104 × *g* for 5 min, following which the supernatant containing total genomic DNA was collected and quantified using a NanoDrop spectrophotometer. An aliquot of the extracted DNA was used for molecular identification of the isolate and the remaining was stored at –20 °C.

Molecular identification was performed using the polymerase chain reaction (PCR) technique. The 16S rRNA gene was targeted using 625 bp species-specific forward (5′-GAAAGGTTGATGCCTAATACGTA-3′) and reverse (5′-CGTGCTGGCAACAAAGGACAG-3′) primers (EL-GHAREEB et al., 2019; PARK et al.,

2021). The conventional PCR mixture consisted of 3 µL of genomic DNA (50–150 ng) and 5 µL of 5× FIREPol Master Mix Ready To Load – 250 rxn (12.5 mM, composed of magnesium chloride, DNA Taq polymerase, reaction equilibration buffer, and sufficient dinucleotides for gene amplification; Solis BioDyne), 0.5 µL of each primer at 10 µM (Synthesis Biotechnology), and sterile water for making up the final volume to 25 µL.

According to the method described by EL-GHAREEB et al. (2019), the following PCR program was used: denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, hybridization of primers at 50 °C for 40 s, and extension at 72 °C for 1.5 min; and a final extension at 72 °C for 5 min. After electrophoresis of the amplified products on a 1.5% agarose gel (100 V, 30 min), the bands were visualized using a UV LTB-HE transilluminator (Loccus Biotechnology). For the standard positive control bands, a commercial 100 bp DNA ladder (Sinapse Inc.) with fragments of known molecular weights was used.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the *A. hydrophila* isolates was performed using the agar diffusion method according to the technique described by BAUER et al. (1966), where the bacterial colonies were inoculated into Mueller–Hinton agar and overlaid atop a disc impregnated with the specific antibiotic (Laborclin). In total, the following 24 commercial antibiotics previously defined by the Clinical and Laboratory Standards Institute (CLSI, 2020) and scientific literature (HAFEZ et al., 2018; RAMADAN et al., 2018; GUFE et al., 2019; ROGES et al., 2020) were tested: combination beta-lactams (ampicillin/sulbactam, 10 µg), aminoglycosides (amikacin, 30 µg; streptomycin, 10 µg), beta-lactams (amoxicillin, 10 µg; ampicillin, 2 µg), 1st generation cephalosporins (cephalothin, 30 µg), 2nd generation cephalosporins (cefoxitin, 30 µg; cefuroxime, 30 µg), 3rd generation cephalosporins (cefotaxime, 30 µg; ceftazidime, 30 µg; ceftriaxone, 30 µg), 4th generation cephalosporins (cefepime, 30 µg), phenicols (chloramphenicol, 30 µg), sulfonamides/inhibitors of folic acid metabolism (sulfazotrim, 25 µg), lipopeptides (polymyxin B, 300 IU), macrolides (erythromycin, 15 µg), nitrofurans (nitrofurantoin, 300 µg), penicillins and beta-lactamase inhibitors (piperacillin/tazobactam, 30/6 µg; penicillin G, 10 U), quinolones/fluoroquinolones (nalidixic acid, 30 µg; ciprofloxacin, 5 µg; levofloxacin, 5 µg), and tetracyclines (doxycycline, 30 µg; tetracycline, 30 µg). Antimicrobial resistance was classified as sensitive, intermediate, or resistant on the basis of the zone of inhibition (in millimeters) measured on each disc and the standard table for *Enterobacteriaceae* (CLSI, 2020), which establishes the cut-off points of the inhibition halos.

For the antimicrobial susceptibility test, each bacterial suspension was first prepared to a turbidity of 0.5 (McFarland scale) in sterile 0.85% saline solution. Then, the suspension was inoculated into Mueller–Hinton agar medium, and the mixture was overlaid atop each antibiotic-impregnated disc. Incubation was carried out in a bacteriological oven at 35 °C (±2 °C) for 18 – 24 h.

After 18–24 h growth of the isolate, the diameter (in millimeters) of the inhibition halo was measured using a meter stick and interpreted using the standard table for classification of the level of strain sensitivity to the antimicrobial tested. As no halo was formed for some of the antibiotics tested (ampicillin, cephalothin, erythromycin, penicillin G, and polymyxin B), they could only be classified as resistant.

Sanitizer susceptibility testing

The susceptibility profile of *A. hydrophila* to sanitizers was determined using the method described by WANJA et al. (2020), with modifications. The following commercial sanitizers commonly applied in the food industry were tested: 300 $mg \cdot L^{-1}$ peracetic acid, 70% alcohol, 700 $mg \cdot L^{-1}$ quaternary ammonium, 200 $mg \cdot L^{-1}$ sodium hypochlorite, and 0.3% hydrogen peroxide (YUAN et al., 2020). These were diluted according to the manufacturers' instructions for use and the concentrations cited in the literature. The same steps and conditions applied to the agar diffusion test for antibiotics was applied to that for the sanitizers, starting with the prior preparation of the sanitizer dilutions and of the bacterial suspensions to a turbidity of 0.5 in sterile 0.85% saline solution. After inoculation of the bacterial suspension into Mueller–Hinton agar, the mixture was overlaid atop sterile monodiscs (Laborclin) soaked in the respective sanitizers. Following 18–24 h incubation, the inhibition halos were measured. Results were interpreted according to the criteria described by KUAYE (2017), and the isolates with no halo formation were considered resistant to the sanitizer applied.

Statistical analysis

The data of this study were tabulated, typed, and saved on an electronic spreadsheet. Statistical analysis was conducted using R Core Team software (FOX & WEISBERG, 2020; LENTH, 2020; R CORE 15 TEAM, 2020; JAMOVI PROJECT, 2021), where

descriptive analysis was performed. Because of the non-normality of the data (Shapiro–Wilk test), the Wilcoxon non-parametric test was used.

RESULTS AND DISCUSSION

All 19 *A. hydrophila* isolates were confirmed to the species level by means of the molecular PCR technique, using species-specific primers directed against the 16S rRNA gene. High levels of *A. hydrophila* resistance to various antimicrobial agents were found in this study (Table 1), especially to ampicillin (> 89%, n = 17), cephalothin (> 84%, n = 16), ampicillin sulbactam (> 78%, n = 15), penicillin G (> 73%, n = 14), and cefuroxime and erythromycin (> 52%, n = 10), all of which are not regulated for the treatment of aquatic animal infections (SINDAN, 2021). In contrast, the levels of resistance to piperacillin tazobactam (< 6%, n = 1) and tetracycline (< 10.5%, n = 2) were low.

Multidrug resistance, characterized as resistance to at least one antibiotic in three or more classes (JEONG et al., 2007), was evidenced in this study, as the *A. hydrophila* isolates recovered from

Table 1 - Antimicrobial susceptibility of Aeromonas hydrophila isolates (n = 19) recovered from retail fish.

Class	--------Antimicrobial/Symbol-------			---Sensitive---		--Intermediate--		---Resistant---	
				N	%	N	%	N	%
Aminoglycosides		Amikacin	AMI/30 µg	15	78.9	0	0.0	4	21.1
		Streptomycin	EST/10 µg	10	52.6	3	15.8	6	31.6
Beta-lactams		Amoxicillin	AMC/10 µg	9	47.4	4	21.1	6	31.6
		Ampicillin	AMP 2 µg	-	-	-	-	17	89.4
	Beta-lactam combination agent	Ampicillin / sulbactam	ASB/10 µg	2	10.5	2	10.5	15	78.9
	Penicillin and beta-lactamase inhibitors	Penicillin G	PEN/10 U	-	-	-	-	14	73.2
		Piperacillin / tazobactam	PIP/30/6 µg	18	94.7	0	0.0	1	5.30
Cephalosporins	1st generation cephalosporin	Cephalothin	CFL/30 µg	-	-	-	-	16	84.2
	2nd generation cephalosporin	Cefoxitin	CFO/30 µg	11	57.9	0	0.0	8	42.1
		Cefuroxime	CRX/30 µg	8	42.1	1	5.3	10	52.6
	3rd generation cephalosporin	Cefotaxime	CTX/30 µg	8	42.1	3	15.8	8	42.1
		Ceftazidime	CAZ/30 µg	14	73.7	1	5.3	4	21.1
		Ceftriaxone	CRO/30 µg	8	42.1	3	15.8	8	42.1
	4th generation cephalosporin	Cefepime	FEP/30 µg	15	78.9	0	0.0	4	21.1
Phenicols		Chloramphenicol	CLO/30 µg	13	68.4	3	15.8	3	15.8
Sulfonamides		Sulfazotrim	SUT 25 µg	12	63.2	1	5.3	6	31.6
Lipopeptides		Polymyxin B	POL 300 U	-	-	-	-	4	21.05
Macrolides		Erythromycin	ERI/15 µg	-	-	-	-	10	52.4
Nitrofurans		Nitrofurantoin	NIT/300 µg	11	57.9	1	5.3	7	36.8
Quinolones / fluoroquinolones		Nalidixic acid	NAL/ 30 µg	13	68.4	2	10.5	4	21.1
		Ciprofloxacin	CIP/ 5 µg	15	78.9	0	0.0	4	21.1
		Levofloxacin	LVX/5 µg	14	73.7	2	10.5	3	15.8
Tetracyclines		Doxycycline	DOX/30 µg	15	78.9	0	0.0	4	21.1
		Tetracycline	TET/30 µg	17	89.5	0	0.0	2	10.5

Legend - no classification; N: number of isolates classified as susceptible, intermediate, and resistant with regard to the antimicrobials tested; %: susceptibility index.

retail fish showed resistance to at least one antibiotic in all the classes investigated. This phenomenon may be attributed to the indiscriminate use of antimicrobials in aquaculture, genetic mutations, or the horizontal dissemination of resistance genes (LAU et al., 2020).

The high resistance to beta-lactams (ampicillin, cephalothin, ampicillin sulbactam, penicillin G, and cefuroxime) is in accordance with the known intrinsic resistance of *Aeromonas* species to this class of antibiotics. Production of the chromosomal enzyme beta-lactamase is recognized as a common feature among species of genus *Aeromonas* and contributes to their resistance to beta-lactams (ROSSOLINI et al., 1996). This intrinsic resistance is related to the chemical instability of the beta-lactam ring in the antibiotic structure, making the drugs susceptible to hydrolysis through bacterial beta-lactamase activity (ZDANOWICZ et al., 2020).

In the isolation of *Aeromonas*, the use of ampicillin is recommended (MCMAHON & WILSON, 2001) owing to the intrinsic resistance of the species to this beta-lactam antibiotic, which facilitates selection of the bacterium among other bacteria present in the medium that are inhibited by the antimicrobial agent. The findings of this study confirm this common characteristic of the *Aeromonas* species and is in agreement with the findings of other studies (DAHDOUH et al., 2016; RAMADAN et al., 2018; WU et al., 2019).

Results obtained regarding *A. hydrophila* resistance to the cephalosporins were similar to those of other studies (RAMADAN et al., 2018; SANTOS et al., 2019; ZAHER et al., 2021). This drug resistance is mainly due to the degradation activities of extended-spectrum beta-lactamases and ampC (a chromosomal cephalosporinase), the main beta-lactamases involved in bacterial resistance to cephalosporins, which may be inherent to the bacteria or even acquired, resulting in the inability of the antibiotics to reach their site of action (MACHADO et al., 2019).

The resistance and multidrug resistance profiles of *Aeromonas* species from freshwater fish are characterized mainly by the residues from the aquaculture practice itself, where resistance genes are spread throughout the environment and resistance determinants are transferred from terrestrial animals to bacteria and human pathogens (CABELO, 2006; FAUZI et al., 2021; NHINH et al., 2021). The indiscriminate use of antimicrobials in aquatic animals and the consequent contamination of the environment are primarily responsible for the spread of resistant strains in fish (KIMERA et al., 2020). The high level of antibiotic resistance in *A. hydrophila* reported in this study suggested that the indiscriminate use of antimicrobials in humans and animals has contributed to the development and dissemination of resistance genes along the food chain. It also indicated that unregulated antimicrobials are being misused and overused in fish farming, given that only oxytetracycline and florfenicol are approved for use in Brazil (CARVALHO & SANTOS, 2016; BUENO et al., 2017; SIDAN, 2021).

It is worth noting that some authors have suggested that the beta-lactamase gene is present in the diverse microbiota in the aquatic environments where these fish are raised, representing a serious problem in view of the high enzymatic potential to hydrolyze beta-lactam antibiotics (ZDANOWICZ et al., 2020). This poses a threat to human health and potentiates the risk of occurrence of antimicrobial resistance (EMA, 2014; STRATEV & ODEYEMI, 2016).

With regard to the resistance of the *A. hydrophila* isolates to the main industrial sanitizers at the concentrations tested in this study (Table 2), the bacteria showed the lowest percentage (15.78%) of resistance to 3% hydrogen peroxide, which was the most effective of the tested sanitizers. However, it is important to note that the classification of sanitizer sensitivity is complex. The resistance rate obtained in this study can be justified by the fact that *A. hydrophila* produces catalase, an important cell-detoxifying enzyme that is responsible for converting hydrogen peroxide (a toxic metabolite) into water and oxygen molecules (KUAYE, 2017). As a strong oxidant, hydrogen peroxide is commonly used as a bactericide and sporicide, with low toxicity and residual effect (GERMANO & GERMANO, 2015). Its sanitizing effectiveness against *A. hydrophila* was evidenced in this study for more than 80% of the isolates.

In contrast, a high rate of resistance to 200 $mg \cdot L^{-1}$ sodium hypochlorite was observed in more than 90% of the isolates. Chlorine compounds, including sodium hypochlorite, are generally among the most commonly used sanitizers in the food industry. These chemicals have good sanitizing efficacy at low concentrations, are not affected by water hardness, and have high effectiveness against a wide spectrum of bacteria, notwithstanding the fact that they are the cheapest among most sanitizers (ANDRADE, 2008; KUAYE, 2017). However, at the recommended concentration, this active ingredient was not sufficient for inhibiting the growth of the *A. hydrophila* isolates in this study.

The chemical sanitizers based on peracetic acid and quaternary ammonium, as well as alcohol,

Table 2 - Radius (in millimeters) of the zone of inhibition of various sanitizers tested against *Aeromonas hydrophila* isolates.

Isolate	Peracetic acid 300 mg·L^{-1}	Alcohol 70%	Quaternary ammonium 700 mg·L^{-1}	Sodium hypochlorite 200 mg·L^{-1}	Hydrogen peroxide 3%
1	0	0	4	0	26
2	0	8	9	0	25
3	0	4	0	0	0
4	0	8	0	0	26
5	0	0	0	0	20
6	0	0	0	0	26
7	0	0	0	8	29
8	0	0	15	0	0
9	0	0	7	0	22
10	8	0	10	0	28
11	9	0	10	0	23
12	0	0	0	0	24
13	0	0	0	0	20
14	0	0	0	0	28
15	0	0	0	0	23
16	0	0	0	0	22
17	0	0	8	0	25
18	0	0	0	0	27
19	0	0	0	0	0
N	17	16	12	18	3
%	89.47	84.21	63.15	94.73	15.78

The sanitizers tested followed the usual concentrations cited in the literature and were prepared according to the manufacturers' instructions.
Legend - N: number of resistant isolates; %, resistance index.

were ineffective against the *A. hydrophila* isolates at the concentrations tested. More than 80% of the isolates were resistant to 300 mg·L^{-1} peracetic acid and 70% alcohol, whereas more than 60% were resistant to 700 mg·L^{-1} quaternary ammonium. The resistance to disinfectants is related to several cellular mechanisms — internal and external to the bacterial cell — that had resulted from phenotypic and genotypic adaptations (ROZMAN et al, 2021).

Although, there are several aspects related to the mechanism of bacterial resistance to sanitizers, the most common ones are the restricted permeability of the bacterial cell wall, enzymatic degradation, expression of efflux systems, biofilm formation, and changes in the target sites (LAMBERT, 2002; CHAPMAN, 2003). Efflux pumps comprise the main pathways of resistance to sanitizers, and the exposure of microorganisms to inhibitory and subinhibitory concentrations of antimicrobials contributes to the development of resistance (WEBBER & PIDDOCK, 2003; GNANADHAS et al., 2013).

In nature, gram-negative bacteria tend to be more resistant than gram-positive bacteria to antimicrobials owing to the complexity of their cell wall. According to MAILLARD (2002), the biocidal activity of sanitizers can vary significantly among different types of microorganisms and even among different strains of the same species.

Despite its importance, bacterial resistance to sanitizing agents has not been treated with interest and due attention by the academic community. The indiscriminate use of sanitizing agents can significantly decrease their effectiveness against clinically important microorganisms (ROZMAN et al., 2021). This outcome poses a serious threat to food safety, especially coupled with the fact that foodborne outbreaks can become highly recurrent if sanitizer resistance becomes established, especially in the food industry (CARLIE et al., 2020). Therefore, results of this study suggest the need for further research on the factors that affect the performance of sanitizing agents in the fish processing environment, aiming the ultimate goals of selecting the most efficient sanitizer to be applied in the sanitization process and corroborating the safety of this food type.

CONCLUSION

The *A. hydrophila* isolates in this study showed multiple resistance to 24 antimicrobials from several classes, especially to ampicillin, cephalothin, ampicillin sulbactam, penicillin, erythromycin, and cefuroxime. In contrast, piperacillin tazobactam and tetracycline were the antimicrobials with the highest percentages of susceptible strains. As determined from the resistance index, 200 $mg \cdot L^{-1}$ sodium hypochlorite was the least effective sanitizer against the isolates, whereas 3% hydrogen peroxide was the most effective.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

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

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