UNOESC
Fazendo parte da sua vida

Evidência
BIOCIÊNCIAS, SAÚDE E INOVAÇÃO
evidence: bioscience, health and innovation

Evidência: Biociências, Saúde e Inovação - ISSN: 1519-5287 | eISSN 2236-6059

DOI: https://doi.org/10.18593/evid.33034

Bioscience

PRESENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF SALMONELLA SPP. ISOLATED FROM FISH FARMS

Presença e perfil de susceptibilidade antimicrobiana de salmonella spp. isoladas de pisciculturas

Renata Pires de Araújo[1], Fabiana Gomes da Silva Dantas[1], Adriana Araújo de Almeida-Apolonio[2], Monyque Palagano da Rocha[1], Bruno do Amaral Crispim[1], Alexeia Barufatti[1], Dalia dos Prazeres Rodrigues[3], João Víctor de Andrade dos Santos[1], & Kelly Mari Pires de Oliveira[1]

[1] Universidade Federal da Grande Dourados (UFGD) – Dourados, MS, Brazil; [2] Universidade Estadual de Mato Grosso do Sul (UEMS) – Dourados, MS, Brazil; [3] Fundação Oswaldo Cruz (FIOCRUZ) –Rio de Janeiro, RJ, Brazil

How to cite: Araújo, R. P. de, Dantas, F. G. da S., de Almeida-Apolonio, A. A. de, da Rocha, M. P. da, Crispim, B. A., Barufatti, A., Rodrigues, D. dos P., dos Santos, J. V. de A. dos, & de Oliveira, K. M. P. de. Presence and antimicrobial susceptibility profile of *Salmonella* spp. isolated from fish farms. *Evid 24;* 1-10. https://doi.org/10.18593/evid.33034

Araújo, R. P. A. de
pires_araujo@hotmail.com
https://orcid.org/0000-0002-5013-0829

Dantas, F.G. da S.
fabianasilva@ufgd.edu.br
https://orcid.org/0000-0003-1651-9213

Almeida-Apolonio, A. A. de
araujo.a@hotmail.com
https://orcid.org/0000-0002-3836-8519

Rocha, M. P. da
monyque_da_rocha@hotmail.com
https://orcid.org/0000-0003-3610-180X

Crispim, B. do A.
brunocrispim.bio@gmail.com
https://orcid.org/0000-0003-0606-4905

Barufatti, A.
barufattialexeia@gmail.com
https://orcid.org/0000-0001-8789-2117

Rodrigues, D. dos P.
daliarodrigues@yahoo.com.br
https://orcid.org/0000-0003-1101-5518

Santos, J. V. de A. dos
victorandrade.j.s@gmail.com
https://orcid.org/0000-0002-4860-4394

Oliveira, K. M. P. de*
kellyoliveira@ufgd.edu.br
https://orcid.org/0000-0002-9897-7770

* Corresponding author: Rodovia Dourados – Itahum, Km 12, Cidade Universitária, CEP 79804-970, Dourados, MS, Brasil.

ABSTRACT: *Salmonella* spp. are important foodborne pathogens often associated with infectious outbreaks caused by consuming contaminated food. Although these microorganisms are widely distributed in several animal production chains, there are few records of their occurrence in aquaculture. This study aimed to evaluate the presence and antimicrobial resistance of *Salmonella* spp. isolated from fish farms. Samples were collected from three fish farms, covering water, fish, and epilithic biofilm. *Salmonella* isolates were identified by PCR, and serotyping was performed using the slide agglutination method, according to the Kaufman-White system. Susceptibility to antimicrobial agents was assessed using the Kirby and Bauer disk diffusion method. The presence of class 1 and 2 integrons was determined by PCR. Ninety samples were examined, of which 13 (14.44%) were positive for S. *enterica*, of which 5 strains were isolated from lagoon water, 3 from fish, and 5 from epilithic biofilm. Among the samples, the serotypes S. Minnesota, S. Panamá, and S. Anatum were identified. Antimicrobial resistance rates were highest for sulfonamides (92.30%), trimethoprim (84.61%), tetracycline (46.15%), and streptomycin (46.15%). Multiple antibiotic resistance was confirmed in 84.61% of the isolates, with 100% presenting integron class 1 and 7.69% integron class 2. This study demonstrated a high prevalence of multiresistant bacteria in fish farms, which reinforces public health concerns due to the use of antibiotics and the risk associated with food from farmed freshwater fish. Given these results, the need for new external research into control and prevention strategies for *Salmonella* spp. is evident. in aquaculture, as well as greater regulation in fish production chains.

Keywords: Aquaculture, Serotyping, Integrons, Antibiotic resistance, Public health.

RESUMO: *Salmonella* spp. são importantes patógenos de origem alimentar que estão frequentemente associados a surtos infecciosos causados pelo consumo de alimentos contaminados. Embora esses microrganismos estejam amplamente distribuídos em diversas cadeias produtivas animais, existem poucos registros de sua ocorrência na aquicultura. Este estudo teve como objetivo avaliar a presença e resistência antimicrobiana de *Salmonella* spp. isoladas de pisciculturas. Amostras foram coletadas de três pisciculturas, abrangendo água, peixe e biofilme epilítico. Os isolados de *Salmonella* foram identificados por PCR, e a sorotipagem foi realizada pelo método de aglutinação em lâmina, de acordo com o sistema Kaufman-White. A suscetibilidade aos agentes antimicrobianos foi avaliada utilizando o método de difusão em disco de Kirby e Bauer. A presença de integrons das classes 1 e 2 foi determinada por PCR. Noventa amostras foram examinadas, destas, 13 (14,44%) foram positivas para S. *enterica*, das quais 5 cepas foram isoladas de água de lagoa, 3 cepas de peixes e 5 cepas de biofilme epilítico. Entre as amostras foram identificados os sorotipos S. Minnesota, S. Panamá e S. Anatum. As taxas de resistência aos antimicrobianos foram mais elevadas para sulfonamidas (92,30%), trimetoprima (84,61%), tetraciclina (46,15%) e estreptomicina (46,15%). A resistência múltipla a antibióticos foi confirmada em 84,61% dos isolados, com 100% deles apresentando integron classe 1 e 7,69% integron classe 2. Este estudo evidenciou uma alta prevalência de bactérias multirresistentes em pisciculturas, o que reforça as preocupações relacionadas à saúde pública devido ao uso indiscriminado de antibióticos e o risco associado aos alimentos provenientes de peixes de criadouros de água doce. Diante desses resultados é evidente a necessidade de novas pesquisas voltadas para estratégias de controle e prevenção de *Salmonella* spp. na aquicultura, bem como de maior regulação nas cadeias produtivas de peixes.

Palavras-chave: Aquicultura, Sorotipagem, Integrons, Resistência a antibióticos, Saúde pública.

Submitted: 16/08/2023 | **Accepted:** 20/10/2023 | **Published:** 02/07/2024
Editor: Marcos Freitas Cordeiro

INTRODUCTION

Animal-derived meat, rich in nutrients such as protein, lipids, and minerals, is desirable for humans. However, it is susceptible to contamination during the procedures of production (Byun et al., 2022; Xu et al., 2020). Similar to other sectors of livestock production, fish production uses intensive and semi-intensive practices, characterized by high stock density and volume; heavy use of formulated feeds containing antibiotics, antifungals, and other pharmaceuticals; heavy use of pesticides and disinfectants; consortium with other animals; use of tank fertilization such as manure use; use of agricultural by-products in fish feed; introduction of drugs to control or prevent pathogens; and use of growth promoters (Cabello & Godfrey, 2016; Fernandes et al., 2018; Kawsar et al., 2022; Sapkota et al., 2008). Current aquaculture practices could thus lead to exposure to chemicals and biological agents. Freshwater products, in particular, are reported as vehicles for foodborne transmission of pathogens, such as *Salmonella* (Fernandes et al., 2018, 2021; Sapkota et al., 2008).

Salmonella spp., members of the Enterobacteriaceae family, are Gram-negative, rod-shaped bacteria that cause salmonellosis. *Salmonella* spp. are categorized into two species (*Salmonella enterica* and S. bongori), six subspecies, and >2600 serotypes (Issenhuth-Jeanjean et al., 2014; Jajere, 2019). Different serotypes are responsible for subclinical, clinical, and severe infections in farm and pet animals and typhoid fever and gastroenteritis in humans worldwide, including in developed and industrialized countries (Evangelopoulou et al., 2015; Kurtz et al., 2017; Weinberger & Keller, 2005). *Salmonella enterica* is an important human and animal pathogen, commonly transmitted to humans through contaminated food and water, direct contact with animals, or, more indirectly, through environmental pathways (Eng et al., 2015; Jajere, 2019; Michael & Schwarz, 2016). Food animals such as swine, poultry, and cattle are prime sources of *Salmonella* infections (Eng et al., 2015; Miranda et al., 2017). The incidence of salmonellosis caused by fish consumption has become a concern for public health agencies in several countries in recognition of significant increases in the consumption of aquaculture products (Fernandes et al., 2018; Zhang et al., 2015). Further, aquatic foods are often raw, increasing pathogen exposure risk (Fernandes et al., 2018). Thus, *Salmonella* remains a formidable public health challenge with reported increases in incidence and antibiotic resistance and thereby may play an important role in the dissemination of antimicrobial resistant isolates throughout the food chain (Centers for Disease Control and Prevention [CDC], 2013; Cummings et al., 2012; Eng et al., 2015). Food animals represent the major reservoir for the transmission of antimicrobial resistant *Salmonella* into humans. In addition, the incorporation of resistance genes in human bacteria threatens the efficiency of human antibacterials (Gómez-Aldapa et al., 2017; Hassena et al., 2021).

Genetic elements such as integrons have contributed to the rapid transmission of drug resistance in bacterial pathogens, especially among members of the Enterobacteriaceae family. Integrons are mobile DNA elements capable of detecting and excision genes, particularly those responsible for antibiotic resistance (Malek et al., 2015). The continuous change in the distribution and antimicrobial resistance profiles of *Salmonella* serotypes in food of animal origin poses a challenge for salmonellosis control. This is further exacerbated by the increasing global trade of food products of animal origin, which facilitates the dissemination of new and antimicrobial resistant serotypes. Therefore, continuous surveillance of the distribution and antimicrobial resistance profiles of *Salmonella* serotypes is crucial to identify the sources of infection, detect outbreaks, and implement prevention and control measures (Mechesso et al., 2020). From a One Health perspective, aquaculture has contributed significantly to the emergence and dissemination of antimicrobial resistant bacteria and resistance genes (Martins et al., 2019). Thus, the present study aimed to evaluate the presence and antimicrobial resistance profile of *Salmonella* spp. isolated from fish farms.

MATERIAL AND METHODS

Sample Collection

Three fish farms, located in the region Dourados, Mato Grosso do Sul, Brazil (Fish farm 1 – Fish, Lambari (*Astyanax*

lacustris) were raised in monoculture); (Fish farm 2 – Fish were raised in polyculture systems, with four native species in the same earth pond: Tambaqui (*Colossoma macropomum*), Pacu (*Piaractus mesopotamicus*), Dourado (*Salminus brasiliensis*) and Patinga (*Piaractus mesopotamicus*); (Fish farm 3 – Fish, Nile Tilapia (*Oreochromis niloticus*), were raised in monoculture) were evaluated in this study.

Ninety samples were collected, including water (48), fish (24), and epilithic biofilm (18). Water samples were collected from fish tanks in sterile bottles, submerging containers to 20 cm. Fish samples were randomly selected from tanks with the aid of a fishing net. Fish were anesthetized with eugenol (50 mg/L) and placed in sterile polypropylene bags. Epilithic community samples were obtained using Polyethylene terephthalate (PET) slides, installed 30 days before collection. Epilithic slides were placed in a sterile polypropylene bag on the day of collection. After collection, the samples were placed in polystyrene boxes containing ice; temperatures were between 4 and 8°C during transportation. The samples were delivered and analyzed in the laboratory within eight hours. Study protocols were approved by the Ethics Commission on the Use of Animal Use from the Federal University of Grande Dourados (CEUA-UFGD) under process number 20/2018.

Salmonella Isolation and Identification

The detection of *Salmonella* was based on standard methods (International Standard Organization [ISO], 2007). For the pre-enrichment step with buffered peptone water (BPW, Oxoid, Basingstoke, United Kingdom), the samples were processed using different methods according to their specificities. For water samples, aliquots of 25 mL of water samples were added to 225 mL of BPW. Fish samples were placed in sterile plastic bags and homogenized and rinsed with 250 mL of BPW following the protocol described in ISO 17604:2015 (ISO, 2015). To analyze the epilithic biofilm, the slides were scraped with a sterile scalpel, and the material was conditioned with 250 mL of BPW. The flasks of different samples (water, fish, and epilithic biofilm) containing BPW were incubated at 37 °C for 18-24 h.

After that, 0.1 mL of pre-enriched sample was added to 10 mL of Rappaport-Vassiliadis (RV) broth (Merck KGaA, Darmstadt, Germany), and another 1 mL of the same sample was transferred to 10 mL of tetrathionate (TT) broth (Merck KGaA, Darmstadt, Germany). Broth cultures were incubated at 42 °C and 37 °C, respectively, for 24 h. Following incubation, a loopful of RV and TT enrichment broth culture was streaked onto xylose-lysine-tergitol agar (XLD) (Merck KGaA, Darmstadt, Germany) plates. Plates were incubated at 37 °C for 24 h. Isolated colonies giving typical reactions were purified by streaking onto nutrient agar plates (Merck KGaA, Darmstadt, Germany) and subjected to biochemical tests.

Serotyping of Salmonella

The serotyping used slide agglutination, according to Kauffmann–White. All isolates were serotyped at the Brazilian National Reference Laboratory of Enterobacteria of the Oswaldo Cruz Foundation, Brazilian Ministry of Health.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility was evaluated using Kirby and Bauer disk diffusion for 14 antimicrobial agents, following the Clinical Laboratory Standards Institute guidelines (2012). Antimicrobials evaluated were ampicillin 10 µg (AMP), amoxicillin-clavulanic acid 20/10 µg (AMC), cefotaxime 30 µg (CTX), ceftazidime 30 µg (CAZ), cefoxitin 30 µg (FOX), ceftriaxone 30 µg (CRO), chloramphenicol 30 µg (C), tetracycline 30 µg (TE), nalidixic acid 30 µg (NA), ciprofloxacin 5 µg (CIP), gentamicin 10 µg (CN), streptomycin 10 µg (S), trimethoprim 5 µg (W) and sulfonamide 300 µg (SX) (Oxoid, Basingstoke, United Kingdom). *Escherichia coli* (ATCC 25922) was used as a quality control strain. Diameters of zones of inhibition around discs were measured and compared against recommendations of the CLSI to classify the strains as resistant, intermediate, or sensitive to a specific antibiotic (Clinical and Laboratory Standards Institute [CLSI], 2019).

Salmonella isolates resistant to three or more classes of antibiotics were defined as multidrug resistant (MDR) according to the definition of the National Antimicrobial Resistance Monitoring System (2014). The production of AmpC or extended-spectrum ⍺-lactamases (ESBL) was also evaluated using the combined disk method (Jarlier et al., 1988).

PCR Screening for Integrons

DNA was extracted and the presence of class 1 and 2 integrons was examined by PCR for integrase gene, *intI1*, with primers: F – CAGTGGACATAAGCCTGTTC and R – CCCGACGCATAGACTGTA, and integrase gene intI2, F – TTGCGAGTATCCATAACCTG and R – TTACCTGCACTGGATTAAG according to Corrêa et al. (2014).

RESULTS

Salmonella Isolation and Serotyping

Salmonella spp. were isolated from the three fish farms. Fish Farms 1 and 2 showed the presence of *Salmonella* spp. only on epilithic biofilms. In contrast, *Salmonella* was found in fish farm 3 in pond water and fish (Table 1). *Salmonella* spp. was isolated from 13 (14.44%) of the total 90 samples examined − pond water (n = 5), fish (n = 3), and epilithic biofilm (n = 5) samples.

The 11 isolates were classified into three distinct S. *enterica* serotypes: Anatum (n = 1), Minnesota (n = 6), and Panama (n = 4). Two isolates did not have the serotype identified (*Salmonella enterica* subsp. *enterica*). The distribution of *Salmonella* serotypes in the different samples and fish farms is presented in Table 1.

Table 1
Place, source, serotypes, resistance patterns, and presence of class 1 and 2 integrons in Salmonella from fish farms.

Fish Farm	Samples	Serotype	Antibiotic Resistance pattern	Number of Antibiotics (Classes)	Integron Class
1	Epilithic biofilm	Anatum	AMC+C+TE+W+SX	5 (5)	1
	Epilithic biofilm	Panama	AMP+AMC+TE+SX	4 (3)	1
	Epilithic biofilm	Panama	S+W+SX a	3 (3)	1
	Epilithic biofilm	Panama	S+W+SX a	3 (3)	1
2	Epilithic biofilm	Unidentified	W+SX	2 (2)	1
3	Pond water	Minnesota	AMC+TE+S+W+SX	5 (4)	1
	Pond water	Minnesota	CAZ+W+SX	3 (3)	1
	Pond water	Minnesota	C+ TE+NA+ W+SX	5 (5)	1
	Pond water	Unidentified	C+TE+W+SX	4 (4)	1
	Pond water	Panama	CAZ+C+TE+W+SX	5 (5)	1
	Fish	Minnesota	S+SX	2 (2)	1
	Fish	Minnesota	S+W+SX a	3 (3)	1
	Fish	Minnesota	AMP+AMC+S+W+SX	5 (3)	1 and 2

AMP: ampicillin, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FOX: cefocitin, CRO: ceftriaxone, C: chloramphenicol, TE: tetracycline, NA: nalidixic acid, CIP: ciprofloxacin, CN: gentamicin, S: streptomycin, W: trimethoprim, SX: sulfonamide. aPredominant MDR pattern

Antibiotic Susceptibility in S. Enterica

Antibiotic resistance among *Salmonella* isolated from pond water, fish, and epilithic biofilm samples showed that all isolates were susceptible to cefotaxime, cefoxitin, ceftriaxone, and gentamicin (Table 2). A high proportion of isolates (30.76%) showed intermediate resistance to ciprofloxacin. The highest rates of resistance were detected for sulfonamides (92.30%), trimethoprim (84.61%), and tetracycline and streptomycin (46.15%). Eleven isolates (84.61%) were considered MDR (Table 1). One isolate was identified in fish (33.33%) and one in epilithic biofilm (20%) with a resistance profile of up to two classes of antimicrobials. Isolates showing resistance profiles of three

to five classes of antimicrobials tested were present in all samples in different quantitative, being pond water with five isolates (100%), two fish isolates (66.66%), and four epilithic biofilm isolates (80%). The predominant MDR pattern was resistance to streptomycin, trimethoprim, and sulfonamides (S+W+SX). None of the *Salmonella* isolates had an Amp C or ESBL phenotype.

Table 2
Antimicrobial resistance pattern of Salmonella isolates [n = 13] obtained from fish farms.

Antimicrobial agents	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	9 (69.23)	1 (7.69)	3 (23.07)
Amoxicillin-Clavulanic Acid	9 (69.23)	1 (7.69)	3 (23.07)
Cefotaxime	13 (100)	0 (0)	0 (0)
Ceftazidime	11 (84.61)	0 (0)	2 (15.38)
Cefoxitin	13 (100)	0 (0)	0 (0)
Ceftriaxone	13 (100)	0 (0)	0 (0)
Chloramphenicol	8 (61.53)	1 (7.69)	4 (30.76)
Tetracycline	7 (53.84)	0 (0)	6 (46.15)
Nalidixic Acid	9 (69.23)	2 (15.38)	2 (15.38)
Ciprofloxacin	9 (69.23)	4 (30.76)	0 (0)
Gentamicin	13 (100)	0 (0)	0 (0)
Streptomycin	0 (0)	7 (53.84)	6 (46.15)
Trimethoprim	2 (15.38)	0 (0)	11 (84.61)
Sulfonamide	1 (7.69)	0 (0)	12 (92.3)

Incidence of Class 1 and Class 2 Integrons in S. Enterica Isolates

The class 1 integrase gene, *intI1*, was identified by PCR in all isolates of *Salmonella* and showed resistance to two or more classes of antibiotics. Among 13 tested *Salmonella* isolates, only one isolate (7.69%) displayed class 2 integron, intI2, serotype Minnesota (Table 1).

DISCUSSION

The occurrence of *Salmonella* in fish, seafood, and fish farms has been documented in several countries worldwide (Amagliani et al., 2012; Budiati et al., 2016; Dib et al., 2018; European Food Safety Authority [EFSA], 2010; Santos et al., 2019; Zhang et al., 2015). The presence of this pathogen in all fish farms studied to date suggests fecal contamination since these bacteria are natural inhabitants of human and animal intestines. They are not naturally present in the aquatic environment or the normal microbiota of aquatic animals (Dib et al., 2018). Although *Salmonella* is not part of the fish microbiota, fish are asymptomatic carriers that can pose a risk to humans when consumed contaminated and also pose a risk in serving as reservoirs for antibiotic resistant *Salmonella* (Fernandes et al., 2018). Thus, the presence of these microorganisms in fish farms is likely due to fecal pollution of surface water from human and animal sources, organic material, and overstocking of fish (Amagliani et al., 2012).

All serotypes (Anatum, Minnesota, and Panama) isolated in this study have been identified in poultry, swine, fish, seafood, and other livestock animals. The unique serotype found in more than one fish farm was Panama. This serotype usually causes gastrointestinal infection in humans. However, it is more widely known for causing invasive disease and colonizing extraintestinal sites. This serotype has been isolated from diverse sources, including human and non-human, such as chicken, fish, pork, and water (Carneiro et al., 2019; Fernandes et al., 2018; Pulford et al., 2019; Santos et al.,

2019). The Minnesota serotype was found only in fish samples in this study. This serotype is a foodborne pathogen mainly associated with the poultry supply chain, where it colonizes the gastrointestinal tract of poultry and consequently can be disseminated to the environment, humans, and other animals (Moura et al., 2017). Conversely, serotype Anatum was found only in epilithic biofilm samples. This serotype constituted the most significant proportion of isolates among non-human sources, and it is a serotype regarding foodborne illnesses. It has caused outbreaks involving various food sources (Fernandes et al., 2018; Ferrari et al., 2019).

Santos et al. (2019) suggest fish feed may be a gateway to *Salmonella* in fish farms. Products of poultry chains are used in aquaculture as fertilizer in culture tanks to stimulate the production of algae (Fernandes et al., 2018; Food and Agriculture Organization [FAO], 2010), and meat meal, bone meal, blood meal, feather meal, and poultry viscera meal are common proteins used as feed ingredients for fish (Furuya & Furuya, 2010). Therefore, *Salmonella* serotypes found in poultry can be transmitted to fish farms by these by-products.

The higher occurrence of *Salmonella* at Fish Farm 3 may be due to the use of poultry litter as fertilizer in culture tanks and high density stock ponds. Fish become potential vehicles for *Salmonella* transmission to animals, humans, and environments (Cabello et al., 2013; Iwamoto et al., 2010). In this study, as well as in Budiati et al. (2016) and Santos et al. (2019) cold-blooded animals, such as fish, are possible hosts and passive *Salmonella* transporters that can excrete bacteria without apparent symptoms or clinical manifestations.

Salmonella in fish farms may present an emerging risk to public health. Aquatic food products are often consumed raw or prepared in a way that does not kill bacteria, thus increasing the risk of infection. Santos et al. (2019) reported several cases of *Salmonella* detected in fish slaughterhouses audited by the Brazilian Federal Service of Inspection. According to technical standards for microbiological analysis of food in Brazil, when a batch of products (fresh, frozen meat, or processed products) is positive for *Salmonella*, it cannot be marketed. It must be discarded, and producers are not reimbursed when disposal occurs for sanitary reasons (Brazil, 2001). Further, *Salmonella* presence on fish surfaces facilitates cross-contamination during fish processing (Amagliani et al., 2012). An important factor regarding *Salmonella* cross-contamination is that microorganisms remain viable on food contact surfaces for significant periods. This viability is due to the formation of biofilms, a bacterial mode of survival that protects bacteria from stressful environmental conditions, such as drying and cleaning (Fernandes et al., 2018).

The occurrence of *Salmonella* in epilithic biofilm from fish farms demonstrated that the bacterium can form biofilm on a wide variety of contact surfaces. Cells comprising biofilm can survive long-term with resistance to stress, such as desiccation and antibiotics (Fernandes et al., 2018). This ability is critical because it allows *Salmonella* to adapt to multiple conditions, such as soil and aquatic environments, and survives long enough for efficient passage into new hosts (Amagliani et al., 2012; Budiati et al., 2016). Cabello et al. (2013) suggested that biofilm epilithons of aquacultural structures represent conditions in aquatic environments that favor horizontal gene transfer.

Another risk factor to public health is the widespread use of antimicrobial drugs in fish farming and the related risk of the emergence and spread of resistance among human pathogens (Serrano, 2005; Zhang et al., 2015). In animal husbandry, including aquaculture, antibiotics are widely used prophylactically and metaphylactically. Also, their use as growth promoters in subtherapeutic doses has contributed to promoting the development of resistance (Cabello et al., 2013; Serrano, 2005). Differences in levels of antimicrobial resistance and decreased susceptibility may be due to the frequent use of antimicrobials. Increased antimicrobial use in fish farms may create a selective pressure for higher levels of antimicrobial resistance.

Antimicrobial agents tested in the present study are commonly used in aquaculture; several are also used in human medicine and classified by the World Health Organization as critically important for human use (Heuer et al., 2009). Among the antimicrobials listed are sulfonamides, tetracycline, and streptomycin, for which isolates showed greater resistance. The high incidence of resistance to sulfonamides, tetracycline, trimethoprim, and streptomycin observed in the present study might be associated with their frequent use in aquaculture feed and their use present at therapeutic or subtherapeutic levels to prevent bacteriosis

(Zhang et al., 2015). In Brazil, oxytetracycline is approved for use in aquaculture, and tetracycline is among the most sold and used veterinary antimicrobial agents (European Medicines Agency [EMA], 2015).

However, in Brazil, the use of unregistered antibacterials ("off label") for fish farming is a relatively common practice, although illegal. Sulfonamides and fluoroquinolones are among the most used (Leal et al., 2017). This use could explain resistance to sulfonamide in 92.30% of isolates and reduced susceptibility to fluoroquinolones. The emergence of ciprofloxacin resistance in isolates is of public health significance because quinolones are the first choice drugs for the treatment of infections caused by *Salmonella* spp. in humans (Zhang et al., 2015). In addition, sulfonamide resistance in *Salmonella* isolates could be linked to the presence of class 1 integron. Class 1 integron possess two conserved sequences (5'CS and 3'CS) separated by a variable region including the gene cassettes integrated with antibiotic resistant genes. The 3′-conserved segment (3'CS) possesses genes qacEΔ1 and sul1, encoding resistance to quaternary ammonium salts and sulfonamide, respectively (Deng et al., 2015).

Integrons are DNA elements capable of capturing and mobilizing exogenously functional gene cassettes, permitting rapid adaptation to selective pressure (Deng et al., 2015). The high frequency of MDR among Intl1 positive isolates is consistent with an association between the presence of class 1 integron and emerging MDR in *Salmonella*. Class 1 integrons are the most commonly reported among *Salmonella* clinical isolates with variable prevalence among countries and sources (Argüello et al., 2018). These integrons are the most common type identified in MDR *Salmonella* and play a critical role in disseminating resistance genes among pathogens (Kaushik et al., 2018). In this sense, the high number of MDR *Salmonella* strains and class 1 integrons from fish farms appears to be an emerging problem (Lintzmaia et al., 2021). A wide variety of food products and environmental sources can transmit *Salmonella*. Class 1 integrons are associated with a variety of resistance gene cassettes, which confer resistance to antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, which were used as first-line drugs for the treatment of salmonellosis (Mthembu et al., 2019). The most frequent resistance patterns to streptomycin, trimethoprim, and sulfonamides in this work may be associated with class 1 integrons trimethoprim resistance determinants are also detected frequently and trimethoprim + sulfamethoxazole have been frequently used therapeutic combination (Deng et al., 2015).

In this study, the presence of class 2 integrons was also detected, which are also an important vehicle for transmitting of resistance genes in S. *enterica* strains. However, they are reported to have low occurrence and prevalence compared to class 1 integrons, and their presence in *Salmonella* isolates is reported only in a few studies (Argüello et al., 2018; Deng et al., 2015; Kaushik et al., 2018). Integrons can be spread horizontally and the susceptibility of *Salmonella* to antibiotics does not have a homogeneous distribution (neither geographical nor temporal). In this way, these data provide additional information about global mobility and genomic plasticity, contributing to strain's persistence along the food chain.

Thus, the occurrence of resistance to antimicrobials in *Salmonella* severely limits therapeutic options. Further, antimicrobial resistance can spread among bacterial populations. To support the current knowledge regarding the epidemiological distribution of MDR strains between the food-animal-environmental interface, our results provide valuable information on the distribution of multidrug-resistant S. *enterica* serotypes in food-producing animal settings. These considerations suggest that excessive aquacultural use of antimicrobials may have significant effects on animal and human health and the environment more broadly, and, therefore, the use of antimicrobial agents in animals should be controlled or avoided to prevent the spread of drug resistance. A One Health approach including multiple interventions is necessary to understand better, prevent, and control *Salmonella* and its infections.

CONCLUSIONS

This study provides data of the occurrence *Salmonella* in fish farms and shows an emerging problem for possible human foodborne infections associated with aquaculture. *Salmonella* was detected in fish farms, water, fish, and epilithic biofilm. Many of the *Salmonella* isolates were

resistant to multiple antimicrobials and multidrug resistance. These considerations suggest that excessive aquacultural use of antimicrobials may have significant effects on animal and human health and the environment more broadly, and, therefore, the use of antimicrobial agents in animals should be controlled or avoided to prevent the spread of drug resistance. A One Health approach including multiple interventions is necessary to understand better, prevent, and control *Salmonella* and its infections and ensure the safety of our food supplies.

ACKNOWLEDGMENTS

This study was supported by the Federal University of Grande Dourados – UFGD, the National Reference Laboratory for Enteric Diseases – Oswaldo Cruz Institute (FIOCRUZ), and the National Council for Scientific and Technological Development (CNPq).

REFERENCES

Amagliani, G., Brandi, G., & Schiavano, G. F. (2012). Incidence and role of Salmonella in seafood safety. *Food Research International*, *45*(2), 780-788. https://doi.org/10.1016/j.foodres.2011.06.022

Argüello, H., Guerra, B., Rodríguez, I., Rubio, P., & Carvajal, A. (2018). Characterization of Antimicrobial Resistance Determinants and Class 1 and Class 2 Integrons in Salmonella enterica spp., Multidrug-Resistant Isolates from Pigs. *Genes*, *9*(5). https://doi.org/10.3390/genes9050256

Brazil. (2001, February 1). *Resolução nº 12, de 02 de janeiro de 2001*. Dispõe sobre os princípios gerais para o estabelecimento de critérios e padrões microbiológicos para alimentos. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2001/res0012_02_01_2001.html

Budiati, T., Rusul, G., Wan-Abdullah, W. N., Chuah, L.-O., Ahmad, R., & Thong, K. L. (2016). Genetic Relatedness of Salmonella Serovars Isolated from Catfish (Clarias gariepinus) and Tilapia (Tilapia mossambica) Obtained from Wet Markets and Ponds in Penang, Malaysia. *Journal of Food Protection*, *79*(4), 659-665. https://doi.org/10.4315/0362-028X.JFP-15-372

Byun, K.-H., Na, K. W., Ashrafudoulla, M., Choi, M. W., Han, S. H., Kang, I., Park, S. H., & Ha, S.-D. (2022). Combination treatment of peroxyacetic acid or lactic acid with UV-C to control Salmonella Enteritidis biofilms on food contact surface and chicken skin. *Food Microbiology*, *102*, 103906. https://doi.org/10.1016/j.fm.2021.103906

Cabello, F. C., & Godfrey, H. P. (2016). Even therapeutic antimicrobial use in animal husbandry may generate environmental hazards to human health. *Environmental Microbiology*, *18*(2), 311-313. https://doi.org/10.1111/1462-2920.13247

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., & Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology*, *15*(7), 1917-1942. https://doi.org/10.1111/1462-2920.12134

Carneiro, M. R. P., Berto, L. H., Oliveira, J. G. S., Santos, A. F. M., Jain, S., Rodrigues, D. P., & Fracalanzza, S. E. L. (2019). *Salmonella* Panama: Genetic Diversity of the Isolates Collected from Human and Non-human Sources. *Revista Da Sociedade Brasileira de Medicina Tropical*, *52*, e20180285. https://doi.org/10.1590/0037-8682-0285-2018

Centers for Disease Control and Prevention [CDC]. (2013). *An atlas of* Salmonella *in the United States, 1968-2011: Laboratory-based Enteric Disease Surveillance*. US Department of Health and Human Services. https://www.cdc.gov/salmonella/pdf/salmonella-atlas-508c.pdf

Clinical and Laboratory Standards Institute [CLSI]. (2019). *Clinical and Laboratory Standards Institute. CLSI performance standards for antimicrobial susceptibility testing* (29th suppl.). CLSI.

Corrêa, F. E. L., Dantas, F. G. S., Grisolia, A. B., Crispim, B. A., & Oliveira, K. M. P. (2014). Identification of class 1 and 2 integrons from clinical and environmental Salmonella isolates. *Journal of Infection in Developing Countries*, *8*(12), 1518-1524. https://doi.org/10.3855/jidc.4734

Cummings, K. J., Warnick, L. D., Davis, M. A., Eckmann, K., Gröhn, Y. T., Hoelzer, K., MacDonald, K., Root, T. P., Siler, J. D., McGuire, S. M., Wiedmann, M., Wright, E. M., Zansky, S. M., & Besser, T. E. (2012). Farm Animal Contact as Risk Factor for Transmission of Bovine-associated Salmonella Subtypes. *Emerging Infectious Diseases*, *18*(12), 1929-1936. https://doi.org/10.3201/eid1812.110831

Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., Chen, D., Bian, H., Li, Y., & Yu, G. (2015). Resistance integrons: Class 1, 2 and 3 integrons. *Annals of Clinical Microbiology and Antimicrobials*, *14*, 45. https://doi.org/10.1186/s12941-015-0100-6

Dib, A. L., Agabou, A., Chahed, A., Kurekci, C., Moreno, E., Espigares, M., & Espigares, E. (2018). Isolation, molecular characterization and antimicrobial resistance of enterobacteriaceae isolated from fish and seafood. *Food Control*, *88*, 54-60. https://doi.org/10.1016/j.foodcont.2018.01.005

Eng, S.-K., Pusparajah, P., Ab Mutalib, N.-S., Ser, H.-L., Chan, K.-G., & Lee, L.-H. (2015). Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, *8*(3), 284-293. https://doi.org/10.1080/21553769.2015.1051243

European Food Safety Authority [EFSA]. (2010). The Community Summary Report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in the European Union in 2008. *EFSA Journal*, *8*(1), 1496. https://doi.org/10.2903/j.efsa.2010.1496

European Medicines Agency [EMA]. (2015). *European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013. (EMA/387934/2015)*. https://www.ema.europa.eu/en/documents/report/fifth-esvac-report-sales- veterinary-antimicrobial-agents-26-european-union/ european-economic-area-countries-2013_en.pdf

Evangelopoulou, G., Kritas, S., Christodoulopoulos, G., & Burriel, A. R. (2015). The commercial impact of pig Salmonella spp. Infections in border-free markets during an economic recession. *Veterinary World*, *8*(3), 257-272. https://doi.org/10.14202/vetworld.2015.257-272

Fernandes, D. V. G. S., Carvalho, R. C. T., Castro, V. S., Cunha-Neto, A., Muller, B., Carvalho, F. T., Prazeres Rodrigues, D., Vieira, B. S., & Souza Figueiredo, E. E. (2021). Salmonella in the processing line of farmed Tambatinga (Colossoma macropomum x Piaractus brachypomus) in Mato Grosso, Brazil: Serotypes of occurrence and antimicrobial profile. *Tropical Animal Health and Production*, *53*(1), 146. https://doi.org/10.1007/s11250-021-02584-8

Fernandes, D. V. G. S., Castro, V. S., Cunha Neto, A., & Figueiredo, E. E. S. (2018). *Salmonella* spp. in the fish production chain: A review. *Ciência Rural*, *48*, e20180141. https://doi.org/10.1590/0103-8478cr20180141

Ferrari, R. G., Rosario, D. K. A., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E. S., & Conte-Junior, C. A. (2019). Worldwide Epidemiology of Salmonella Serovars in Animal-Based Foods: A Meta-analysis. *Applied and Environmental Microbiology*, *85*(14), e00591-19. https://doi.org/10.1128/AEM.00591-19

Food and Agriculture Organization [FAO] (Ed.). (2010). *Report of the FAO Expert Workshop on the Application of Biosecurity Measures to Control Salmonella Contamination in Sustainable Aquaculture: Mangalore, India, 19 - 21 January 2010*. Food And Agriculture Organization of The United Nations.

Furuya, W. M., & Furuya, V. R. B. (2010). Nutritional innovations on amino acids supplementation in Nile tilapia diets. *Revista Brasileira de Zootecnia*, *39*, 88-94. https://doi.org/10.1590/S1516-35982010001300010

Gómez-Aldapa, C. A., Gutiérrez-Alcántara, E. J., Torres-Vitela, M. R., Rangel-Vargas, E., Villarruel-López, A., & Castro-Rosas, J. (2017). Prevalence and behavior of multidrug-resistant Salmonella strains on raw whole and cut nopalitos (Opuntia ficus-indica L.) and on nopalitos salads. *Journal of the Science of Food and Agriculture*, *97*(12), 4117-4123. https://doi.org/10.1002/jsfa.8279

Hassena, A. B., Haendiges, J., Zormati, S., Guermazi, S., Gdoura, R., Gonzalez-Escalona, N., & Siala, M. (2021). Virulence and resistance genes profiles and clonal relationships of non-typhoidal food-borne Salmonella strains isolated in Tunisia by whole genome sequencing. *International Journal of Food Microbiology*, *337*, 108941. https://doi.org/10.1016/j.ijfoodmicro.2020.108941

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., & Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *49*(8), 1248-1253. https://doi.org/10.1086/605667

International Standard Organization [ISO]. (2007). *ISO 6579:2002/amd.1:2007(E): Microbiology – General guidance for the detection of Salmonella*.

International Standard Organization [ISO]. (2015). *ISO 17604:2015 (E) Microbiology of the food chain – Carcass sampling for microbiological analysis*.

Issenhuth-Jeanjean, S., Roggentin, P., Mikoleit, M., Guibourdenche, M., Pinna, E., Nair, S., Fields, P. I., & Weill, F.-X. (2014). Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. *Research in Microbiology*, *165*(7), 526-530. https://doi.org/10.1016/j.resmic.2014.07.004

Iwamoto, M., Ayers, T., Mahon, B. E., & Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews*, *23*(2), 399–411. https://doi.org/10.1128/CMR.00059-09

Jajere, S. M. (2019). A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary World*, *12*(4), 504-521. https://doi.org/10.14202/vetworld.2019.504-521

Jarlier, V., Nicolas, M. H., Fournier, G., & Philippon, A. (1988). Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. *Reviews of Infectious Diseases*, *10*(4), 867-878. https://doi.org/10.1093/clinids/10.4.867

Kaushik, M., Kumar, S., Kapoor, R. K., Virdi, J. S., & Gulati, P. (2018). Integrons in Enterobacteriaceae: Diversity, distribution and epidemiology. *International Journal of Antimicrobial Agents*, *51*(2), 167-176. https://doi.org/10.1016/j.ijantimicag.2017.10.004

Kawsar, M. A., Alam, M. T., Pandit, D., Rahman, M. M., Mia, M., Talukdar, A., & Sumon, T. A. (2022). Status of disease prevalence, drugs and antibiotics usage in pond-based aquaculture at Narsingdi district, Bangladesh: A major public health concern and strategic appraisal for mitigation. *Heliyon*, *8*(3), e09060. https://doi.org/10.1016/j.heliyon.2022.e09060

Kurtz, J. R., Goggins, J. A., & McLachlan, J. B. (2017). Salmonella infection: Interplay between the bacteria and host immune system. *Immunology Letters*, *190*, 42-50. https://doi.org/10.1016/j.imlet.2017.07.006

Leal, C. A. G., Oliveira, T. F., & Figueiredo, H. C. P. (2017). Uso de antibacterianos na piscicultura: Erros, acertos e risco—Parte 2. *Panorama Da Aquicultura*, *27*(162), 24. https://panoramadaaquicultura.com.br/antibacterianos-na-piscicultura-erros-acertos-e-riscos-parte-2-2/

Lintzmaia, D. J. H., Rebelatto, I. S., Santos, A. R., Costa, S. B. D., Ritter, D. O., & Lanzarin, M. (2021). Bactérias emergentes na qualidade nutricional do pescado / Emerging bacteria in the nutritional quality of fish. *Brazilian Journal of Development*, *7*(10), 95657-95662. https://doi.org/10.34117/bjdv7n10-063

Malek, M. M., Amer, F. A., Allam, A. A., El-Sokkary, R. H., Gheith, T., & Arafa, M. A. (2015). Occurrence of classes I and II integrons in Enterobacteriaceae collected from Zagazig University Hospitals, Egypt. *Frontiers in Microbiology*, *6*, 601. https://doi.org/10.3389/fmicb.2015.00601

Martins, A. F. M., Pinheiro, T. L., Imperatori, A., Freire, S. M., Sá-Freire, L., Moreira, B. M., & Bonelli, R. R. (2019). Plesiomonas shigelloides: A notable carrier of acquired antimicrobial resistance in small aquaculture farms. *Aquaculture*, *500*, 514-520. https://doi.org/10.1016/j.aquaculture.2018.10.040

Mechesso, A. F., Moon, D. C., Kim, S.-J., Song, H.-J., Kang, H. Y., Na, S. H., Choi, J.-H., Kim, H.-Y., Yoon, S.-S., & Lim, S.-K. (2020). Nationwide surveillance on serotype distribution and antimicrobial resistance profiles of non-typhoidal Salmonella serovars isolated from food-producing animals in South Korea. *International Journal of Food Microbiology*, *335*, 108893. https://doi.org/10.1016/j.ijfoodmicro.2020.108893

Michael, G. B., & Schwarz, S. (2016). Antimicrobial resistance in zoonotic nontyphoidal Salmonella: An alarming trend? *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *22*(12), 968-974. https://doi.org/10.1016/j.cmi.2016.07.033

Miranda, A. L., Cordeiro, S. M., Reis, J. N., Cardoso, L. G., & Guimarães, A. G. (2017). Phenotypic and genotypic characterization of *Salmonella* spp. Isolated from foods and clinical samples in Brazil. *Anais Da Academia Brasileira de Ciências*, *89*, 1143-1153. https://doi.org/10.1590/0001-3765201720160449

Moura, Q., Fernandes, M. R., Cerdeira, L., Ienne, S., Souza, T. A., Negrão, F. J., & Lincopan, N. (2017). Draft genome sequence of a multidrug-resistant CMY-2-producing Salmonella enterica subsp. Enterica serovar Minnesota ST3088 isolated from chicken meat. *Journal of Global Antimicrobial Resistance*, *8*, 67-69. https://doi.org/10.1016/j.jgar.2016.10.011

Mthembu, T. P., Zishiri, O. T., & El Zowalaty, M. E. (2019). Detection and Molecular Identification of Salmonella Virulence Genes in Livestock Production Systems in South Africa. *Pathogens (Basel, Switzerland)*, *8*(3), 124. https://doi.org/10.3390/pathogens8030124

Pulford, C. V., Perez-Sepulveda, B. M., Rodwell, E. V., Weill, F.-X., Baker, K. S., & Hinton, J. C. D. (2019). Salmonella enterica Serovar Panama, an Understudied Serovar Responsible for Extraintestinal Salmonellosis Worldwide. *Infection and Immunity*, *87*(9), e00273-19. https://doi.org/10.1128/IAI.00273-19

Santos, R. R., Xavier, R. G. C., Oliveira, T. F., Leite, R. C., Figueiredo, H. C. P., & Leal, C. A. G. (2019). Occurrence, genetic diversity, and control of Salmonella enterica in native Brazilian farmed fish. *Aquaculture*, *501*, 304-312. https://doi.org/10.1016/j.aquaculture.2018.11.034

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., & Lawrence, R. (2008). Aquaculture practices and potential human health risks: Current knowledge and future priorities. *Environment International*, *34*(8), 1215-1226. https://doi.org/10.1016/j.envint.2008.04.009

Serrano, P. (2005). *Responsible use of antibiotics in aquaculture*. https://api.semanticscholar.org/CorpusID:85792575

Weinberger, M., & Keller, N. (2005). Recent trends in the epidemiology of non-typhoid Salmonella and antimicrobial resistance: The Israeli experience and worldwide review. *Current Opinion in Infectious Diseases*, *18*(6), 513-521. https://doi.org/10.1097/01.qco.0000186851.33844.b2

Xu, Z., Wang, M., Zhou, C., Gu, G., Liang, J., Hou, X., Wang, M., & Wei, P. (2020). Prevalence and antimicrobial resistance of retail-meat-borne Salmonella in southern China during the years 2009-2016: The diversity of contamination and the resistance evolution of multidrug-resistant isolates. *International Journal of Food Microbiology*, *333*, 108790. https://doi.org/10.1016/j.ijfoodmicro.2020.108790

Zhang, J., Yang, X., Kuang, D., Shi, X., Xiao, W., Zhang, J., Gu, Z., Xu, X., & Meng, J. (2015). Prevalence of antimicrobial resistance of non-typhoidal Salmonella serovars in retail aquaculture products. *International Journal of Food Microbiology*, *210*, 47-52. https://doi.org/10.1016/j.ijfoodmicro.2015.04.019