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Frequency of pathogens in routine bacteriological diagnosis in fish and their antimicrobial resistance

Frequência de patógenos na rotina de diagnóstico bacteriológico em peixes e sua resistência a antimicrobianos

Arthur Roberto da Costa[1]; Roberta Torres Chideroli[2]; Larissa Melo Chicoski[3]; Diego Candido de Abreu[3]; Leonardo Mantovani Favero[1]; Natália Amoroso Ferrari[1]; Raffaella Menegheti Mainardi[4]; Vanessa Gomes da Silva[4]; Ulisses Padua Pereira[5]*

Highlights

MDR profile was detected in 17.24% of the isolates;
Aeromonas spp. showed 55.1% overall resistance to tetracycline;
Some bacteria were resistant to all the Brazilian aquaculture-registered antibiotics;

Abstract

Aquaculture is one of the sectors of animal husbandry with the fastest growth rate. However, the increase in the sector's production chain without proper management can result in factors that favor the development of diseases, especially infectious diseases caused by bacteria. Many factors, such as agriculture or industry resides, improper use of antibiotics in animals or humans, have contributed to increased environmental pressure and the appearance of antibiotic-resistant bacteria, while residues from these drugs can remain in the carcasses and in water a risk to public and environmental health. From that, we identified the bacterial genus/species and their bacterial resistance to antibiotics from samples received from fish disease outbreaks for bacteriosis diagnosis between January 2017 and October 2020. Isolated bacteria were subjected to the Kirby and Bauer sensitivity test for five classes of antibiotics (penicillins, fluoroquinolones, aminoglycosides, amphenicols, and tetracyclines). Of the 181 analyzed outbreaks, 232 bacteria were isolated, including *Streptococcus* spp., *Aeromonas* spp., *Edwardsiella* spp.,

[1] Master Students in the Postgraduate Program in Animal Science, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: arthurrc94@gmail.com; leonardo_mfavero@yahoo.com; naamfer@gmail.com
[2] Post Doctorate Student in the Postgraduate Program in Animal Science, UEL, Londrina, PR, Brazil. E-mail: ro.vetuel@gmail.com
[3] Veterinary Infectious Diseases Intern, Department of Preventive Veterinary Medicine, UEL, Londrina, PR, Brazil. E-mail: lari.cap@gmail.com; c.diego01@gmail.com
[4] PhD Students in the Postgraduate Program in Animal Science, UEL, Londrina, PR, Brazil. E-mail: raffamm@hotmail.com; vgsilva.vg@gmail.com
[5] Professor PhD, Department of Preventive Veterinary Medicine, UEL, Londrina, PR, Brazil. E-mail: upaduapereira@uel.br
* Author for correspondence

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Plesiomonas shigelloides, Pseudomonas aeruginosa, Chromobacterium violaceum, Flavobacterium spp., *Citrobacter* spp., *Enterococcus* spp., *Vibrio* spp., *Enterobacter* spp., *Chryseobacterium meningosepticum.* Of the 232 bacteria, 40 strains were classified as multidrug resistant (MDR), with *Plesiomonas shigelloides, Aeromonas* spp., and *Edwardsiella* spp. representing more than half of this number (22/total). With several bacteria demonstrating resistance to Brazilian aquaculture-legalized drugs (tetracycline and florfenicol), it is mandatory to research, not only for alternatives to the use of antibiotics, but also for other drugs effective against the main circulating bacterial pathogens. In addition, vigilance over the occurrence of resistant bacteria is necessary, considering the appearance of zoonotic bacteria with multi-resistant characteristics, becoming a public health concern.
Key words: Antimicrobial resistance. Multidrug resistant. Pathogens. Aquaculture.

Resumo

Aquicultura é um dos setores da produção animal com o mais rápido crescimento. Muitos fatores, como resíduos industriais e/ou de agricultura e o uso indevido de antibióticos em animais ou humanos têm contribuído para aumentar a pressão ambiental e o aparecimento de bactérias resistentes a antibióticos contribuindo para os resíduos dessas drogas permanecerem nas carcaças e na água, o que é um risco para a saúde pública / ambiental. A partir disso, foram identificados o gênero / espécie de bactérias e sua resistência bacteriana aos antibióticos de amostras recebidas de surtos de doenças em peixes para diagnóstico bacteriológico no período entre janeiro de 2017 e outubro de 2020. As bactérias isoladas foram submetidas ao teste de sensibilidade de Kirby e Bauer para cinco classes de antibióticos (penicilinas, fluoroquinolonas, aminoglicosídeos, anfenicóis e tetraciclinas). Nos 181 surtos analisados, 232 bactérias foram isoladas, sendo estas *Streptococcus* spp., *Aeromonas* spp., *Edwardsiella* spp., *Plesiomonas shigelloides, Pseudomonas aeruginosa, Chromobacterium violaceum, Flavobacterium* spp., *Citrobacter* spp., *Enterococcus* spp., *Vibrio* spp., *Enterobacter* spp., *Chryseobacterium meningosepticum* dentre outras. Destas 232 bactérias, 40 cepas foram classificadas como multirresistentes (MDR), com *Plesiomonas shigelloides, Aeromonas* spp. e *Edwardsiella* spp. representando mais da metade destas (22 / total). Com várias bactérias demonstrando resistência aos medicamentos legalizados na aquicultura brasileira (tetraciclina e florfenicol), torna-se obrigatória a pesquisa, não apenas de alternativas ao uso de antibióticos, mas também de outros medicamentos eficazes contra os principais patógenos circulantes. Além disso, a vigilância sobre a ocorrência de cepas resistentes é necessária levando-se em consideração o aparecimento de bactérias zoonóticas com essa característica, tornando-se um ponto de interesse à saúde pública.
Palavras-chave: Resistência a antimicrobianos. Multirresistente. Patógenos. Aquicultura.

Introduction

The rapid growth of the world population, combined with the demand for healthier food, has highlighted aquaculture as among the key sectors of animal production. The total estimated production of global aquaculture surpasses 80 million tons, reaching USD 250 billion and growing more than 3% per year (Food and Agriculture Organization [FAO], 2020). This significant growth is caused by an intensified production, with systems characterized by a high density of animals, a stress factor that can lead to infectious bacterial diseases as a consequence (Gabriel & Akinrotimi, 2011).

Although it is not recommended, antimicrobials are frequently used as a food additive to prevent the appearance of bacterial diseases, particularly after stressful situations, such as handling and transport, besides using them as medicine during outbreaks (Lulijwa, Rupia, & Alfaro, 2020). Nevertheless, the use of antibiotics in animal production must be directed carefully and in emergencies, such as massive outbreaks, to avoid problems related to residues in food, water environmental pollution, and the increase in antimicrobial resistance (Monteiro, Garcia, & Pilarski, 2018)

The antimicrobial resistance (AMR) crisis is an important topic of investigation concerning both human and animal health (Mobarki, Almerabi, & Hattan, 2019); the indiscriminate use and permanence of antimicrobials in the environment leads to selective pressure on the environmental microbiome, with subsequent transfer of resistant strains via horizontal resistance gene transfer to more pathogenic organisms, such as *Edwardsiella ictaluri,* which is detrimental to the catfish industry in the USA (Abdelhamed et al., 2018). The unrestricted use of antimicrobials for disease control and prophylaxis in aquaculture has contributed to the emergence of resistance to single and multiple classes of antibiotics significant against fish pathogens, as well as the emergence of multidrug-resistant bacteria (MDR), which are resistant to at least one drug in three classes of antibiotics (Basak, Singh, & Rajurkar, 2016; Magiorakos et al., 2012; Miller & Harbottle, 2018).

To attenuate this situation, which is critical in the One Health prism, many studies have been conducted to find an alternative to the use of antimicrobials, such as probiotics, genetic selection for disease resistance, and phytogenic and immunostimulants (Kavitha, Raja, & Perumal, 2018; Mian et al., 2020; Suphoronski et al., 2019; Vianna et al., 2020); but for the most part, the use of antibiotics is still prevalent in aquaculture. Another way to decrease the use of antimicrobials is to implement biosecurity measures, using reliable sources of stocks, immediate detection of pathogens with appropriate diagnostic tools, correct disinfection, and mainly perform the screening of pathogens to block entry routes (Bera et al., 2018)

In this study, we analyzed the data on the resistance to antimicrobials of bacteria isolated from aquaculture fish for bacterial diagnostics in the period between January 01, 2017 and October 31, 2020.

Materials and Methods

Fish collected for diagnostic purposes throughout the country were transferred to our laboratory. During the necropsy procedure, small portions of the spleen, liver, brain, and kidney, the organs most affected by bacteriosis in fish (Marcusso et al., 2015), were streaked on 5% sheep blood agar and incubated at 28 °C for 48-72 h. Bacterial colonies were identified by Gram staining, and assessed for the presence of catalase and oxidase; additionally, the Bactray I and III commercial identification kit was used for identifying gram-negative bacteria (Laborclin®). The antibiotic susceptibility test was performed using the Kirby and Bauer methodology according to the standardization of the Clinical and Laboratory Standards Institute [CLSI] (2019) with four classes of antibiotics: the penicillins (penicillin (10U) and amoxicillin (10μg)), fluoroquinolone (enrofloxacin (5μg), aminoglycosides (gentamicin (10μg) and streptomycin (10μg), florfenicol (30μg) as an amphenicol, and tetracycline (30μg). The antibiotics were selected according to producer's request and laboratory's availability. In some cases, chloramphenicol, sulfamethoxazole/ trimethoprim, ceftiofur, amikacin, and neomycin were used as substitutes. To better analyze all the information, a table was created with all the disease outbreaks and isolated bacteria (Supplementary Table 1). This table also discusses the fish species (with common name), location of the farm, and antibiotic resistance profile of the isolated bacteria.

Table 1
Number of isolated bacterial species analyzed per year

Isolated Bacteria	2017	2018	2019	2020	TOTAL
Aeromonas spp.	6	12	13	18	**49**
Chromobacterium violaceum	-	-	4	6	**10**
Chryseobacterium meningosepticum	-	-	-	1	**1**
Citrobacter spp.	-	3	1	2	**6**
Edwardsiella spp.	7	2	2	12	**23**
Enterobacter spp.	-	-	-	2	**2**
Enterococcus spp.	-	-	4	-	**4**
Flavobacterium spp.	3	2	3	-	**8**
Plesiomonas shigelloides	3	7	3	8	**21**
Pseudomonas aeruginosa	5	2	-	3	**10**
Streptococcus spp.	22	33	9	17	**81**
Vibrio spp.	-	1	1	2	**4**
Unidentified Gram-negative bacteria	2	2	1	8	**13**
TOTAL	**48**	**64**	**41**	**79**	**232**

Results and Discussion

Among the 181 outbreaks analyzed, most were from farmed tilapias (*Oreochromis niloticus*) with 170 cases (93.92%). This is expected because tilapia is the largest produced and technified fish species in the country. In native fish species, the second major aquaculture group in Brazil, multiple outbreaks were analyzed: five in pintados (*Pseudoplatystoma corruscans*), one in acará (*Geophagus brasiliensis*), one in matrinxã (*Brycon* spp.), one in pirarucu (*Arapaima gigas*), one in tambacu (*Colossoma macropomum* ♀ × *Piaractus mesopotamicus* ♂), one tambatinga (*Colossoma macropomum* ♀ × *Piaractus brachypomus* ♂), and one traíra (*Hoplias* spp.), which complemented our data.

Regarding the geographical location of the outbreaks, the majority emanated from Paraná, with 148 cases (81.77%), 15 from São Paulo, three from Bahia, three from Mato Grosso do Sul, three from Santa Catarina, three from Tocantins, two from Minas Gerais, two from Pernambuco, one from Rio Grande do Norte, and one in Goiás (Supplementary table 1). Paraná state is responsible for the vast majority of Brazilian aquaculture; however, it is important to obtain epidemiological data from different regions. Unfortunately, in this study, the number of fish from other states was not significant because of the costs and difficulty of transporting these animals to the laboratory for processing the samples.

Bacterial isolation

A total of 232 bacteria were isolated from 181 disease outbreaks during the study period (Table 1). Among these identified isolates, 81 were *Streptococcus* spp. (34.91%), 49 *Aeromonas* spp. (21.12%), 23 *Edwardsiella* spp. (9.91%), 21 *Plesiomonas shigelloides* (9.05%), 10 *Pseudomonas aeruginosa* (4.31%), 10 *Chromobacterium violaceum* (4.31%), eight *Flavobacterium* spp. (3.45%), six *Citrobacter* spp. (2.59%), four *Enterococcus* spp. (1.72%), four *Vibrio* spp. (1.72%), two *Enterobacter* spp. (0.86%), and one *Chryseobacterium meningosepticum* (0.43%). In addition, 13 Gram-negative bacteria (5.60%) were isolated, but could not be identified with the Bactray commercial identification kit (Laborclin®), resulting in blank codifications.

The genus *Streptococcus* includes a few main pathogens in the fish industry, *S. agalactiae, S. iniae, S. dysgalactiae, S. parauberis,* and *S. ictaluri. Streptococcosis* is generally a summer-disease; however, recently, some strains have been associated with low temperatures (Marcusso et al., 2015). Affected fish show ascites, lethargy, skin darkness, hemorrhages in the skin, exophthalmia, erratic swimming, and acute and elevated mortality (Chideroli et al., 2017; Figueiredo, Nobrega, Leal, Pereira, & Mian, 2012). In addition, Streptococcus agalactiae serotype III, detected in this study in fish from the states of Bahia and Tocantins, is a zoonotic foodborne pathogen that causes meningitis in healthy adults after the consumption of raw fish (Ip et al., 2016).

Motile *Aeromonas* septicemia is caused by diverse species of *Aeromonas,* mainly *A. hydrophila, A. veronii, A. dhakensis, A. jandaei, A. sobria,* and *A. caviae.* These bacteria are normally present in water and infect fish when there is a change in the environment (stress due to handling, high levels of toxic ammonia, injuries due to cannibalism, and low levels of oxygen). When analyzing our data, we observed a gradual increase in the incidence of isolated pathogenic *Aeromonas* spp., a signal of the growing relevance of this pathogen in Brazilian aquaculture (Table 1). The clinical signs are similar to those of streptococcosis disease: ascites, skin darkness, hemorrhagic spots on the body surface, moderate exophthalmia, and erratic swimming (Monir, Yussof, Mohamad, & Ina-Salwany, 2020). The same pattern of a water-natural bacterium causing moderate exophthalmia and skin lesions was also observed in *Plesiomonas shigelloides* (Behera et al., 2018) and *Pseudomonas aeruginosa* (Algammal et al., 2020).

Edwardsiella is a genus of epidemiological importance in the catfish industry in the USA (Griffin et al., 2020). These bacteria cause septicemia in various fish species, with the presence of hepatic congestion, ascites, spiraling swimming, and red spots in the ventral area of infected fish (Leung, Wang, Yang, & Siame, 2019); moreover, *E. tarda* is considered a human opportunistic pathogen that causes gastroenteritis and gas gangrene in immunosuppressed fish (Mohanty & Sahoo, 2007). The number of *Edwardsiella* spp. isolated in 2020 was greater than the total number isolated between 2017 and 2019, highlighting the possible propagation of this pathogen in fish farming nationwide (Table 1). Similarly, the disease vibriosis caused by *Vibrio* spp. (the most common species being *V. anguillarum, V. cholerae, V. salmonicida,* and *V. vulnificus*), generally presents similar symptoms in juveniles, but with a higher morbidity than mortality (Sumithra et al., 2019).

Flavobacterium is a bacterium found in fresh water, mostly known for columnaris disease, affecting fry and juveniles of various fish species, with characteristic skin lesions (dirty-white/yellowish-areas, with an "eaten" aspect) (Loch & Faisal, 2015). These bacteria are probably underdiagnosed because of the difficulty in isolating them in common media (Gao & Gaunt, 2016). *Chryseobacterium meningosepticum* is a previously classified *Flavobacterium* that was isolated in this study on a fibrinous lesion in the ovary of a reproducer (Vandamme, Bernardet, Segers, Kersters, & Holmes, 1994).

Finally, *Citrobacter* spp., *Enterococcus* spp., and *Enterobacter* spp. are common in the intestinal microbiome of tilapia and are usually secondary pathogens due to stress and parasitosis in farmed fish (Arumugam, Stalin & Rebecca, 2017; Molinari et al., 2003). *Enterococcus* spp. have also been studied in relation to their importance as probiotics because of their potential to boost the immune system (Safari, Adel, Lazado, Marlowe, & Caipang, 2016).

Antimicrobial resistance

Detailed results on the antimicrobial resistance profiles of the bacterial isolates are shown in Table 2. The overall resistance to the antibiotics tested was as follows: penicillin 58.59%, amoxicillin 49.11%, streptomycin 39.65%, tetracycline 38.36%, gentamicin 31.03%, enrofloxacin 11.93%, and florfenicol 9.48%.

The resistance to the substitute antibiotics was as follows: chloramphenicol: 0 resistances in 31 uses; sulfamethoxazole/trimethoprim, three resistances in 12 uses, ceftiofur: zero resistance in five uses, amikacin: two resistances in eight uses, and neomycin: one resistance in five uses (Table 3).

Resistance to penicillin antibiotic group (penicillin and amoxicillin) was very high, with some bacteria reaching nearly 100% resistance (*Aeromonas* spp., *Chromobacterium violaceum, Chryseobacterium meningosepticum, Citrobacter* spp., *Enterobacter* spp., *Pseudomonas aeruginosa,* and *Vibrio* spp.) (Table 2). Penicillin was the first antibiotic discovered and used by Alexander Fleming in 1928. Its unrestricted use provides selective pressure in most bacteria, making it one of the most resistant antibiotics (Gaynes, 2017; Palma, Tilocca, & Roncada, 2020). However, it is still important in treating human infections (such as streptococcosis and syphilis); hence, it is important to evaluate their resistance status (Camargos, Fischer, Mocelin, Dias, & Ruvinsky,

Table 2
Antibiotics resistance on the isolated bacteria

Bacteria	Samples	MDR	Pen	Amo	Enr	Tet	Gen	Str	Flo
Aeromonas spp.	49	7	91.8	87.5	8.5	55.1	4.5	12.2	6.1
Chromobacterium violaceum	10	2	100.0	90.0	10.0	70.0	10.0	10.0	10.0
Chryseobacterium meningosepticum	1	0	100.0	100.0	0.0	100.0	0.0	0.0	0.0
Citrobacter spp.	6	3	100.0	66.7	0.0	66.7	0.0	33.3	33.3
Edwardsiella spp.	23	6	71.4	42.9	4.8	39.1	9.5	23.8	21.7
Enterobacter spp.	2	1	100.0	100.0	0.0	50.0	0.0	50.0	100.0
Enterococcus spp.	4	2	25.0	0.0	75.0	100.0	50.0	50.0	0.0
Flavobacterium spp.	8	2	75.0	33.3	0.0	25.0	37.5	12.5	12.5
Plesiomonas shigelloides	21	9	95.2	85.7	20.0	66.7	4.8	38.1	9.5
Pseudomonas aeruginosa	10	0	57.1	57.1	28.6	30.0	25.0	0.0	20.0
Streptococcus spp.	81	4	11.1	7.4	11.7	9.9	74.6	74.1	1.2
Vibrio spp.	4	0	100.0	75.0	0.0	75.0	0.0	0.0	0.0
Unidentified Gram negative bacteria	13	4	76.9	76.9	15.4	46.2	27.3	30.8	23.1
TOTAL	232	40	58.6	49.1	11.9	38.4	31.0	39.7	9.5

Pen: Penicillin, Amo: Amoxicillin, Enr: Enrofloxacin, Tet: Tetracycline, Gen: Gentamicin, Str: Streptomycin, Flo: Florfenicol
MDR: Multidrug Resistant;
*Resistance to an antibiotic is expressed as percentage (%) of the samples tested.

2006). Nine of the 81 strains of *Streptococcus* spp. were resistant to penicillin, including one strain of *Streptococcus agalactiae* serotype III, a zoonotic pathogen (Chideroli et al., 2017).

Although there was 39.65% resistance to streptomycin and 31.03% resistance to gentamicin, a class of aminoglycosides, most of this non-susceptible profile came from *Streptococcus* and *Enterococcus* spp., which are recognized as resistant to these antibiotics (Krause, Serio, Kane, & Connolly, 2016). When considering only Gram-negative bacteria in this study, we found 20.55% resistance to streptomycin and 11.4% resistance to gentamicin, considering them, in some cases, as alternatives for treating those bacterial group. Meanwhile, we must be aware that strains of *Pseudomonas* spp. and *Citrobacter* spp. already exhibit resistance mechanisms that inactivate these aminoglycosides (Doi, Wachino, & Arakawa, 2016).

Tetracycline and florfenicol are antibiotics released from aquaculture in Brazil (Sindicato Nacional da Indústria de Produtos para Saúde Animal [SINDAN], 2020). The florfenicol resistance was rather low (9.48%), being the less resisted antibiotic in this study, with only *Enterobacter* spp presenting more than 50% resistance. Therefore, we can conclude that its use is still effective in aquaculture, and thus, is the most suitable for combating outbreaks.

Tetracycline is one of the most commonly used antibiotics in veterinary medicine; therefore, the resistance values found are not surprising (Pereira-Maia et al.,

Table 3
Antibiotics used as substitutes in some cases against isolated bacterial pathogens

Antibiotic	Times used	Sensible	Resistant
Chloramphenicol	31	31 (5 *Aeromonas*, 2 *Edwardsiella*, 1 *Flavobacterium*, 1 MDR *Flavobacterium* 1 MDR *Plesiomonas*, 2 *Pseudomonas*, 18 *Streptococcus*, 1 Unidentified Gam negative)	0
Sulfamethoxazole/ trimethoprim	12	9 (2 *Aeromonas*, 3 *Pseudomonas*, 4 *Streptococcus*)	3 (1 *Edwardsiella*, 1 MDR *Edwardsiella*, 1 Unidentified Gram negative)
Ceftiofur	5	5 (1 *Edwardsiella*, 1 MDR *Edwardsiella*, 3 *Pseudomonas*)	0
Amikacin	8	6 (1 *Aeromonas*, 1 MDR *Flavobacterium*, 1 *Flavobacterium*, 3 *Pseudomonas*)	2 (1 *Edwardsiella*, 1 MDR *Edwardsiella*)
Neomicin	5	4 (1 *Edwardsiella*, 3 *Pseudomonas*)	1 (1 MDR *Edwardsiella*)

MDR: Multidrug resistant; this status indicates that the strain tested was resistant to antibiotics of at least three different classes.

2010). Some fish farmers, through personal communication, reported that tetracycline drug is their medication of choice during the increase in fish mortality. It is noted in this study that the overall resistance of isolated bacteria to those antibiotics increased during the period, specially to tetracycline (Figure 1).

Due to this irresponsible use of antibiotics, several bacteria are being characterized because of their resistance to antimicrobial drugs. Interestingly, even though the average resistance of *Aeromonas* spp. to tetracycline was 55.1%, all the strains isolated from the state of São Paulo (7) were resistant to the drug, indicating that this antimicrobial is mostly ineffective when treating motile *Aeromonas septicemia* in this state. MDR bacteria were detected, with 40 strains (17.24%) resistant to at least one drug from three different classes of antimicrobials (Magiorakos et al., 2012). *Plesiomonas shigelloides*, *Aeromonas* spp., and *Edwardsiella* spp. represent more than half (22/total) of these MDR bacteria, and are known for their ability to obtain, carry, and transfer plasmids with antimicrobial resistance genes, which makes their presence in aquatic environments a risk to public health (Abdelhamed et al., 2018; Freitas et al., 2018; Martins et al., 2019). Remarkable findings of the AMR analysis include the presence of *Edwardsiella* spp. and one *Streptococcus* spp., both isolated in 2017 from tilapias in Paraná. *Streptococcus* was resistant to all seven antibiotics tested, and *Edwardsiella* was sensitive only to enrofloxacin.

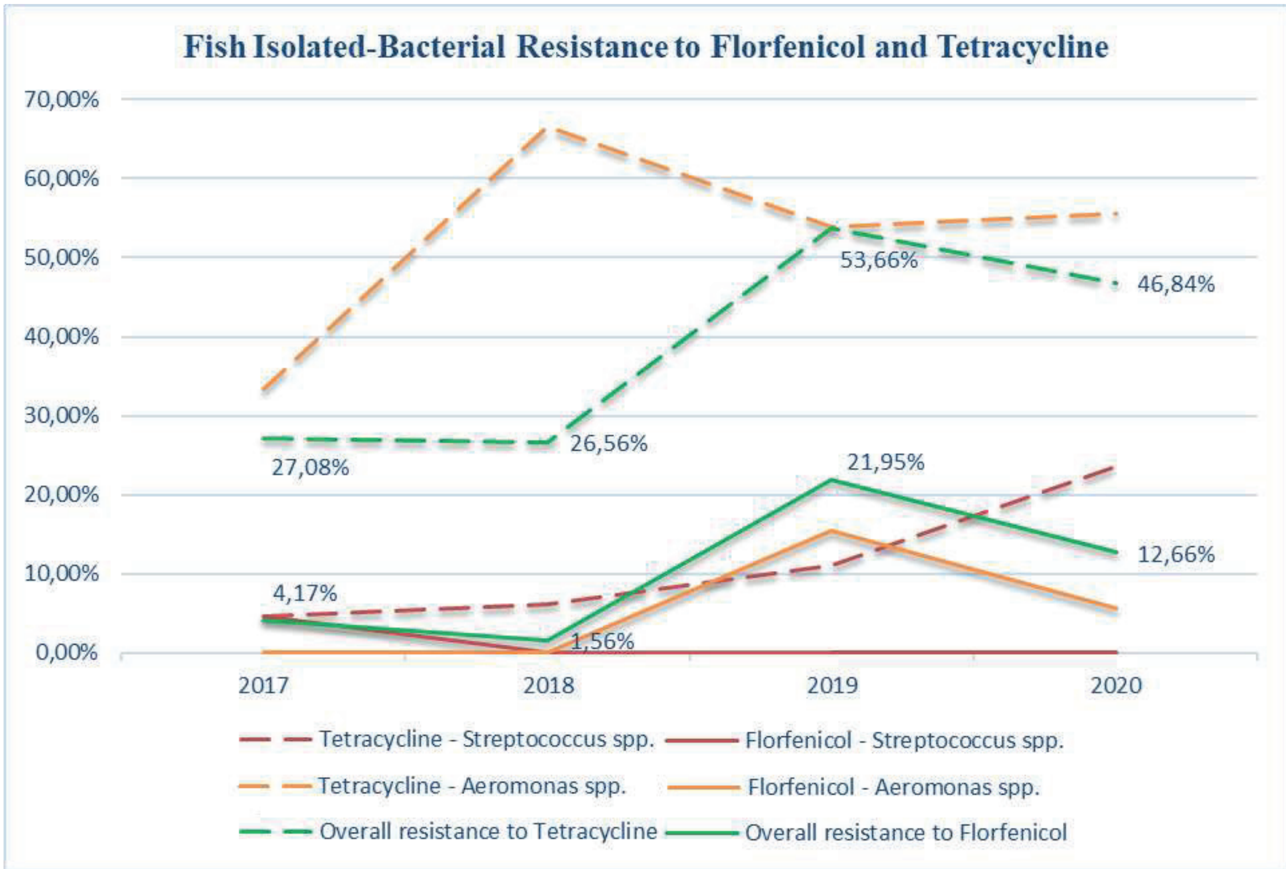

Figure 1. Resistance ratio to florfenicol and tetracycline in the bacteria isolated in the period 2017-2020.

A total of 15 bacterial strains (three *Aeromonas,* one *Citrobacter,* four *Edwardsiella,* one *Enterobacter,* one *Flavobacterium,* two Plesiomonas, one Streptococcus, and two unidentified Gram-negative bacteria) showed resistance to both florfenicol and tetracycline, the only permitted antibiotics for use in aquaculture in Brazil. Hence, it is mandatory to research, not only for alternatives to using antibiotics, but also for other drugs effective against the main circulating pathogens. In this context, the importance is even greater when considering the use of antimicrobials prohibited in fish farming when producers see no effect with tetracycline/florfenicol treatment. In the USA, in addition to the drugs distributed in Brazil, sulfadimethoxine/ormetoprim has also been approved for use (Food and Drug Administration [FDA], 2020). Moreover, Seo et al. (2020), when researching new potential drugs, stated a 100% survival rate of *Paralichthys olivaceus* infected with *Streptococcus* spp., when treated with intramuscular (i.m) doses of ceftiofur sodium.

In this study, we also found a good susceptibility rate for ceftiofur (Table 3), despite performing few tests with this drug; however, it has the disadvantage that it needs to be injected i.m., precluding their use in farms with thousands of fish. Another drug that showed prominent results in this study was enrofloxacin, with an average resistance of 11.93% and only *Enterococcus* spp., a natural-bacterium from the intestine of tilapia, exhibited more than 50% resistance. Hence, enrofloxacin can be considered a good drug for aquaculture use, with a low harmful effect on the intestinal microbiome.

Public health practices and improved surveillance with a description of the antimicrobial susceptibility profile contribute to the successful source tracking of multidrug-resistant bacteria (Ryu et al., 2019). Thus, studies that emphasize the problems associated with antimicrobial resistance should be performed for this purpose, thereby improving the rational use of antimicrobials for human and animal health.

Final Considerations

In this study, the isolated aquaculture pathogens showed resistance to tetracycline and florfenicol, along with other antibiotics. These bacteria are present in water and can multiply and transfer antibiotic resistance genes to other pathogens in the environment, threatening both human and animal health. Accordingly, it is crucial to be aware of the resistance profile of aquaculture pathogens to guide the use of antimicrobials. Therefore, further studies with alternative methods, and even drugs, are needed for the treatment and prevention of bacteriosis in aquaculture. Also, sequencing of resistant strains found in Brazilian fish should be made, to create a database for surveillance of resistance in aquaculture.

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