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Original Article

Antimicrobial susceptibility profile of bacteria of the genus *Aeromonas* and *Escherichia coli* isolated from samples of yellow hake (*Cynoscion acoupa*)

Perfil de suscetibilidade a antimicrobianos de bactérias do gênero *Aeromonas* e *Escherichia coli* isoladas de amostras de pescada amarela (*Cynoscion acoupa*)

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Abstract

The aim of this study was to evaluate the antimicrobial susceptibility profile of *Aeromonas* sp., and *Escherichia coli* isolated from samples of yellow hake (*Cynoscion acoupa*). We analyzed 53 *Aeromonas* spp. and four *E. coli* isolates. We observed increased resistance of *E. coli* to levofloxacin and sulfa-trimethoprim as well as resistance of *Aeromonas* spp. to ampicillin, amoxicillin-clavulanate, cefuroxime, and cefotaxime. The multiple antimicrobial resistance(MAR) index indicated multidrug resistance in 90.54% (n=48) of *Aeromonas* spp. isolates and in 50% (n=2) of *E. coli* isolates. One strain of *Aeromonas* spp. was resistant to all 11tested antimicrobials (MAR index = 1.00). *In vitro*,piperacillin + tazobactam was the most effective antimicrobial for *E. coli*,and cefepime and levofloxacin were the most effective antimicrobials for *Aeromonas* spp. Therefore, in case of illnesses caused by these microorganisms, these antimicrobials should be used. The multidrug resistance of *Aeromonas* spp. and *E. coli* in this study is elevated. This is worrisome considering the increase in bacteria resistant to multiple drugs, reducing the options for successful clinical antimicrobial use.

Keywords: antibiogram, fish, trade, multiresistance.

Resumo

O trabalho teve como objetivo avaliar o perfil de suscetibilidade a antimicrobianos de bactérias do gênero *Aeromonas* e *Escherichia coli* isoladas de amostras de pescada amarela (*Cynoscion acoupa*). Foram analisados 53 isolados de *Aeromonas* spp. e quatro isolados de *Escherichia coli*. Foi verificada maior resistência de *E. coli* à levofloxacina e sulfa-trimetoprim; assim como houve resistência antimicrobiana dos isolados de *Aeromonas* spp. à ampicilina, amoxicilina-clavulanato, cefuroxima e cefotaxima. Os valores encontrados para o índice MAR, evidenciam multirresistência em 90,54% (n=48) dos isolados de *Aeromonas* spp. e em 50% (n=2) dos isolados de *E. coli*. Uma cepa de *Aeromonas* spp. apresentou resistência a todos os onze antimicrobianos testados (MAR = 1,00). A pesquisa mostrou que, no tratamento de infecções causadas por *E. coli*, a piperacilina-tazobactam é a mais eficaz *in vitro*. Para *Aeromonas* spp., os antimicrobianos mais eficazes *in vitro* foram cefepime e levofloxacina. Dessa forma, em caso de enfermidades ocasionadas por esse micro-organismo, seriam esses os antimicrobianos de eleição para o tratamento das infecções. A multirresistência dos isolados de *Aeromonas* spp. e *E. coli* foi considerada elevada. Este é um dado importante visto que o aumento do aparecimento de bactérias resistentes a múltiplas drogas é preocupante, diminuindo as opções de uso de antimicrobianos com sucesso clínico.

Palavras-chave: antibiograma, peixe, comércio, multirresistência.

1. Introduction

The yellow hake (*Cynoscion acoupa*) is a marine fish species with a wide geographical distribution along all regions of the Brazilian coast. In the state of Maranhão, Brazil, the yellow hake represents 10% of the fishing production. It has significant commercial value not only for its meat but also for its swim bladder, which is highly valued even on

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the international market (Almeida et al., 2016; Tavares et al., 2023).The high demand for this fish raises concerns regarding its microbiological quality when sold at markets and open-air fairs (Machado et al., 2015; Oliveira et al., 2020). Fish can potentially carry pathogenic microorganisms, including coagulase-positive *Staphylococcus*, coliforms, *Vibrio parahaemolyticus*, *Escherichia coli*, *Salmonella* spp., *Listeria* spp., and *Aeromonas* spp., among others. The presence of these microorganisms compromising the quality and freshness of the product and could lead to foodborne diseases (Abrantes et al., 2022; Makowiecky, 2023; Soares and Gonçalves, 2012), whichoccurs due to the presence ofapathogen or its toxin(s) (Flores and Melo, 2015). This contamination may be related to the development of pathogenic microorganisms with resistance profiles to different antimicrobials (Machado et al., 2015). The occurrence of bacteria with resistance to antimicrobials is worrisome. Over the years, bacterial resistance to antimicrobials has grown, mainly due to the indiscriminate use of these drugs. Antimicrobial resistance refers to the ability of bacteria, fungi, viruses, or parasites to develop mechanisms to combat the action of antibiotics, antifungals, and other agents. This process occurs naturally; however, it has been accelerated by the improper and excessive use of antimicrobials (Repik et al., 2022).

Some laboratory methods can be used to determine the *in vitro* sensitivity of bacteria to antimicrobials, including the disk diffusion test (CLSI, 2015, 2019). This is one of the simplest, most reliable, and most widely used susceptibility methods by microbiology laboratories. Through this method, the diameters of inhibition zones are measured in millimeters and interpreted according to astandard table for antimicrobial susceptibility tests and classified as resistant, intermediate, or sensitive (NCCLS, 2003).

In the present study, we aimed to evaluate the antimicrobial susceptibility profile of *Aeromonas* sp. and *E. coli* isolated from yellow hake (*C. acoupa*) samples sold in the city of São Luís, Maranhão, Brazil.

2. Methodology

We analyzed the antimicrobial susceptibility profile of 53 isolates of *Aeromonas* spp. and four isolates of *E. coli* from yellow hake sold in São Luís, Maranhão, Brazil, in March 2017, according to the methodology of the American Public Health Association (APHA) (Vanderzant and Splitt-Stoesser, 1992).We performed the disk diffusion method according to the CLSI, testing the following antimicrobials: ampicillin 10µg (AMP), amikacin 30 µg (AMI), amoxicillin-clavulanate 20/10 µg (AMC), cefepime 30 µg (CPM), cefoxitin 30 µg (CFO), cefotaxime 30 µg (CTX), cefuroxime 30 µg (CRX), levofloxacin 5 µg (LVX), gentamicin 120 µg (GEN), piperacillin-tazobactam 100 µg (PPT), and sulfamethoxazole-trimethoprim 25 µg (SUT). We chose these antimicrobials because they are commonly used in human and veterinary medicine. The results were classified as resistant, intermediate resistance, or sensitive (NCCLS, 2003).

We calculated the multiple antimicrobial resistance (MAR) index as the ratio of a/b, where “a”is the number of antimicrobials to which the isolate was resistant, and “b”is the number of antimicrobials to which the isolate was exposed. A value >0.17 indicates multidrug resistance (Krumperman, 1983).

3. Results

The antimicrobial susceptibility profile of the *E. coli* isolates is shown in Table 1. In general, the *E. coli* isolates exhibited resistance to 9out of the 11 tested antimicrobials. We observed the highest resistance for levofloxacin (50.00%) and sulfamethoxazole-trimethoprim (50.00%). Theseisolates also showed sensitivity to 10 out of the 11 tested antimicrobials, with piperacillin-tazobactam being 100% effective. Additionally, the isolates demonstrated intermediate resistance to six of the tested antimicrobials.

We also observed antimicrobial resistance in *Aeromonas* spp. (Table 2). Besides the 92.45% resistance to ampicillin, we observed high percentages of resistance for cefuroxime (81.13%), amoxicillin-clavulanate (64.15%), and cefotaxime (52.83%). Cefepime and levofloxacin were the most effective antimicrobials (both 75.47%).

The MAR index ranged from 0.18 to 1.00 in *Aeromonas* spp. and *E. coli* isolates, indicating multidrug resistance in 90.54% (n=48) of *Aeromonas* spp. isolates and 50.00% (n=2) of *E. coli* isolates. One *Aeromonas* spp. isolate was resistant to all 11 tested antimicrobials (MAR = 1.00) (Table 3).

4. Discussion

E. coli isolates showed sensitivity to piperacillin-tazobactam, cefepime, cefoxitin, and cefotaxime, making these antimicrobials suitable for the treatment of infections caused by *E. coli*. Piperacillin-tazobactam was the most effective. Similarly to ourdata, Cordeiro (2017) observed that all isolates were sensitive to the antimicrobials cefepime, cefotaxime, levofloxacin, piperacillin-tazobactam, and sulfamethoxazole-trimethoprim when evaluating the antimicrobial susceptibility of *E. coli* isolated from salmon sashimi (prepared from *Salmo salar*).

Ogur (2022) determined the load of pathogenic bacteria of 14 species of marine fish obtained from two suppliers in Turkey. All samples were unacceptable according to the critical limit of *E. coli*. The load of pathogenic bacteria in the analyzed marine fish was quite high, and they were unsafe in terms of microbiological quality.

According to Melo et al. (2012), piperacillin-tazobactam exertsbactericidal activity by inhibiting septum formation and cell wall synthesis. It is indicated for the treatment of urinary tract infections, peritonitis, hemolytic uremic syndrome, and gastroenteritis in humans caused by *E. coli*.

Cordeiro (2017) also found *E. coli*isolates resistant to sulfamethoxazole-trimethoprim. The improper and indiscriminateuse of antimicrobials contributes to increase bacterial resistance (Cardoso et al., 2015). In this case, the resistance of *E. coli* isolates to sulfamethoxazole-trimethoprim may be associated with the persistence of this antimicrobial in the aquatic environment and/or its indiscriminate use in Brazil.

Table 1. Antimicrobial susceptibility profile of *E. coli* isolates from yellow hake (*Cynoscion acoupa*) sold in São Luís - MA, 2017.

Antimicrobials	Number of isolates (N)			
	Resistant	Intermediate resistance	Sensitive	Total
ampicillin	1 (25%)	3 (75%)	0	4
amikacin	1 (25%)	1 (25%)	2 (50%)	4
amoxicillin-clavulanate	1 (25%)	1 (25%)	2 (50%)	4
gentamicin	1 (25%)	1 (25%)	2 (50%)	4
cefuroxime	1 (25%)	1 (25%)	2 (50%)	4
cefepime	0	1(25%)	3 (75%)	4
cefoxitin	1 (25%)	0	3 (75%)	4
cefotaxime	1 (25%)	0	3 (75%)	4
levofloxacin	2 (50%)	0	2 (50%)	4
piperacillin-tazobactam	0	0	4 (100%)	4
sulfamethoxazole-trimethoprim	2 (50%)	0	2 (50%)	4

N (%): Number of bacteria resistant to antimicrobials and the respective percentage.

Table 2. Antimicrobial susceptibility profile of *Aeromonas* sp. isolates from yellow hake (*Cynoscion acoupa*) sold in São Luís - MA, 2017.

Antimicrobials	Number of isolates (N)			
	Resistant	Intermediate resistance	Sensitive	Total
ampicillin	49 (92.45%)	1 (1.89%)	3 (5.66%)	53
amikacin	17 (32.08%)	1 (1.89%)	35 (66.04%)	53
amoxicillin-clavulanate	34 (64.15%)	6 (11.32%)	13 (24.53%)	53
gentamicin	13 (24.53%)	3 (5.66%)	37 (69.81%)	53
cefuroxime	43 (81.13%)	1 (1.89%)	9 (16.98%)	53
cefepime	12 (22.64%)	1 (1.89%)	40 (75.47%)	53
cefoxitin	25 (47.17%)	3 (5.66%)	25 (47.17%)	53
cefotaxime	28 (52.83%)	4 (7.55%)	21 (39.62%)	53
levofloxacin	5 (9.43%)	8 (15.09%)	40 (75.47%)	53
piperacillin-tazobactam	10 (18.87%)	8 (15.09%)	35 (66.04%)	53
sulfamethoxazole-trimethoprim	14(26.42%)	7 (13.21%)	32 (60.38%)	53

N (%): Number of bacteria resistant to antimicrobials and the respective percentage.

Table 3. Multiple Antimicrobial Resistance (MAR) index values of *Aeromonas* sp. and *E. coli* isolates from yellow hake (*Cynoscion acoupa*), 2017.

Isolates N (%)	Antimicrobials (n)									
	2	3	4	5	6	7	8	9	10	11
Aeromonas sp.	13 (24.52%)	13 (24.52%)	3 (5.66%)	2 (3.77%)	2 (3.77%)	1 (1.89%)	7 (13.20%)	1 (1.89%)	5 (9.43%)	1 (1.89%)
E. coli	1 (25%)	-	-	-	-	-	1 (25%)	-	-	-
MAR index	0.18	0.27	0.36	0.45	0.54	0.63	0.73	0.81	0.91	1.00

n: Number of antimicrobials, N (%): Number of bacteria resistant to “n” antimicrobials and the respective percentage.

Dias et al. (2010) and Machado et al. (2015) found antimicrobial resistance to ampicillin in *E. coli* isolated from red snapper, mackerel, and mussels. Antimicrobial resistance to ampicillin in *E. coli* isolated from yellow hake is likely explained by the fact that ampicillin resistance is genetic (chromosomal or via plasmids). According to Lima et al. (2006), if this resistance is encoded by plasmids, itcan be disseminated to phylogenetically distinct bacterial species that may or may not be pathogenic.

According to Peixoto et al. (2012), information on antimicrobial resistance in bacteria from food is essential to indicate a drug that is efficient in treating patients infected by bacteria. Among the species of the genus *Aeromonas*, *Aeromonas hydrophila* is an important pathogen for fish and public health, as it can develop under refrigeration and produce exotoxins. We found that cefepime and levofloxacin were the most effective antimicrobials against *Aeromonas* spp., while ampicillin, amoxicillin-clavulanate, cefuroxime, and cefotaxime were the least effective antimicrobials and should not be indicated for the treatment of diseases caused by *Aeromonas* spp.

According to Anvisa (Brasil, 2017), intermediate resistance allows for the use of an antimicrobial for the treatment of infections. In this case, the antimicrobial reaches adequate concentrations and can be used in situations where there is no contraindication for the use of higher doses.

The resistance of *E. coli* and *Aeromonas* spp. isolates can be explained by the indiscriminate use of antimicrobials in food and/or aquaculture (fish and shrimp farming), which can reach the marine aquatic environment. Resistance may vary according to severalparameters: proximity to areas that adopt the use of antimicrobials (fish farms or hospital sewage released in coastal zones and estuaries), therainy season, and water polluted with industrial sewage (Machado et al., 2015).The difference in resistance profiles can also be justified by the characteristics of the habitat in which yellow hake is found and the frequency that it is found and captured in such environments. Yellow hake marketed in São Luís,Maranhão, Brazil, comes from various municipalities along the coast of this state, including Raposa, Cedral, Cururupu, and Humberto de Campos, among others. Almeida et al. (2016) affirmed that yellow hake is a demersal (species that spends most of its time on sandy, muddy, and rocky substrates) and pelagic (species that swims freely in the water column) species. It has a coastal habit and can be captured in mangrove regions or regions with depths ranging from 1 to 35 m. Thus, the species may have come into contact with drug residues in the marine environment.

Multiple resistance to antimicrobials has been described in bacteria with potential pathogenicity for fish cultivated in semi-intensive systems such as carp (*Cyprinus carpio*). Isolates obtained from carp also showed multiple resistance to the tested drugs, with three isolates resistant to all tested antimicrobials (Amarante et al., 2018). Sebastião et al. (2022) recovered seven *Aeromonas* spp. strains from tambaqui (*Colossoma macropomum*). These seven isolates showed resistance to ampicillin, erythromycin, and sulfonamide.

Evangelista-Barreto et al. (2010) analyzed the MAR index for *Aeromonas caviae*, *Aeromonas sobria*, and *Aeromonasveronii*. *A. caviae* presented the highest index, being resistant to four out of the eight tested antimicrobials. Cordeiro et al. (2020) also observed multiresistance of *Aeromonas* sp., *E. coli* and *Salmonella* sp. isolates from salmon samples. Hirsch et al. (2006) analyzed the MAR index in *Aeromonas* spp. isolated from tilapia and stated that the study of multiresistant isolates is important, becausethese microorganisms can be transmitted through contaminated foods, and there is the possibility of transferring resistance genes to the intestinal microbiota of consumers.

Bacteria have biological systems that allow the exchange/sharing of antibiotic-resistance genes among microorganisms that become resistant or multiresistant, leading to diseases in humans that are difficult to treat (Leal et al., 2017). Gastroenteritis is the most common form of human infection caused by *Aeromonas* spp. However, septicemia, hemolytic uremic syndrome, peritonitis, wounds, respiratory infections, and cutaneous pustules are other common clinical signs. In fish, the disease can cause exophthalmia, fin erosion, hemorrhagic septicemia, and even death of the animal (Silva, 2010).

Machado et al. (2015) analyzed multiresistance in *E. coli* isolates from red snapper and mackerel marketed in Fortaleza, Ceará, Brazil, and observed that 68.7% of the isolates had a multiresistance profile. The authors associated the results with the constant handling of other fish and contact with various utensils in the same environment (cross-contamination), contributing to the transfer of multiresistant bacteria between handled fish.

5. Conclusion

We observed resistance of *Aeromonas* spp. and *E. coli* isolates to different antimicrobials, especially ampicillin. *E. coli* isolates showed greater sensitivity to piperacillin-tazobactam, making this antimicrobial more suitable for the treatment of infections caused by this bacterium. *Aeromonas* spp. isolates were more sensitive to cefepime and levofloxacin, making these the preferred antimicrobials for the treatment of *Aeromonas* spp. infections. We also observed high multiresistance of the isolates, which is worrisome considering the emergence of bacteria resistant to multiple drugs, reducing the options for successful clinical antimicrobial use.

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