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Aeromonas Isolated from Lagoon Tilapias in Côte D'Ivoire: Diversity, Distribution and Potential Virulence

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Abstract *Aeromonas* is a common bacterium in aquatic habitats and recognised as an occasional fish pathogen. The present study aimed at determining the prevalence and distribution of *Aeromonas* in tilapias from the Ebrié Lagoon (Côte d'Ivoire). The extracellular virulence factors and antibiogram of isolates were also examined. The bacteriological analysis of 512 tilapias showed a prevalence of 10.93%. Out of 87 isolated strains, 6 species were identified by Malditof, 3 of which were dominant, *Aeromonas jandaei* (24%), *A. caviae* (23%), *A. hydrophila* (22%) and 3 less represented, *A. sobria* (13%), *A. veronii* (10%), *A. trota* (8%) *Aeromonas jandaei* and *A. hydrophila* were more frequent in the gut than in the gills. All isolates produced gelatinase and nuclease, but adherence to host cells and the ability to produce haemolysins, lipases and proteases varied from one isolate to another. Of the antibiotics tested, 100% of isolates were susceptible to aztreonam, 98% to ceftazidime and 93% to cefepime. In contrast, high resistance was observed with ampicillin (98%), amoxicillin-clavulanic acid (93%) and cefalotin (62%). These characteristics reveal the potentially pathogenic status of the isolates, which could constitute a threat to human beings.

Keywords: *Aeromonas, potential virulence; antibiotic resistance*

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1. Introduction

Aeromonas are bacteria of the Aeromonadaceae family, most commonly found in freshwater and estuarine environments in association with aquatic animals. They also colonise meat and dairy products and can be isolated from almost any environmental niche. 36 species of *Aeromonas* have been reported and divided into two phenotypically distinct groups [1]. The first group includes the single species *Aeromonas salmonicida*, which is psychrophilic, while the second group is composed of mesophilic species of which *Aeromonas hydrophila* is the representative species. Motile *Aeromonas* species, in particular *A. hydrophila, A. veronii, A. caviae, A. jandaei, A. dhakensis* and *A. bestiarum* can infect both animals and humans [2,3]. Recognised as an occasional pathogen of fish, some authors consider *Aeromonas* to be the leading cause of fish mortality [4]. As such, the prevalence and distribution of this bacterial genus in aquatic environments is the subject of increasing scientific interest. Several studies have shown that most *Aeromonas* isolates from sick fish including carp, catfish and tilapia are *A. hydrophila, A. caviae and A. veronii* [5,6,7].

In Côte d'Ivoire, tilapia represents an important part of the lagoon fishery catch and is one of the most popular fish [8,9]. Unfortunately, the microbial pollution of the Ebrié Lagoon favours the contamination of fish products by pathogens such as *Aeromonas* [10,11]. Furthermore, little data is available on the microbiological quality of lagoon tilapia caught and sold on the markets. To prevent human contamination by handling or consumption of these products, it was necessary to carry out this study, which has the following objectives (i) to determine the prevalence of *Aeromonas* and its distribution in tilapia fish, (ii) to characterize the potential virulence of the isolates and (iii) to assess their susceptibility to some common antibiotics.

2. Materials and Methods

2.1. Study Environment

The fish samples for this study came from two sites in the Ebrié lagoon system where fishing activities are more or less intense: Aghien lagoon (5°22'N to 5°26'N and 3°49'W to 3°55'W) and Biétri bay (5°15'N to 5°18'N and 3°58'W to 4°00'W). These environments are characterized by a high level of anthropogenic impact, resulting from agricultural and livestock activities in the case of the Aghien lagoon and industrial activities in the case of Biétri Bay.

2.2. Sampling

Fish samples were captured with nets and aseptically identified on site. Tilapia specimens were individually placed in sterile bags and stored in a cooler containing cold accumulators. Finally, the samples were transported to laboratory of the Chemistry and Environmental Microbiology Unit of Institut Pasteur de Cote d'Ivoire within 3 hours of capture for microbiological analysis.

2.3. Isolation and Identification of *Aeromonas*

Each fish sample was cleaned with cotton wool lightly soaked in 70% alcohol to avoid any external contamination and then dissected using aseptic techniques according to [12]. The gills, gut and liver were removed and 1g of each organ was placed in a zip bag containing 9 mL of EPA broth. The organ plus broth was homogenized in a stomacher for 10 minutes before being placed at 37°C for 24 hours. Isolation of presumptive strains was performed by plating the enriched broth on M-Aeromonas selective Agar Base medium (Havelaar), supplemented with ampicillin (10mg/L). Yellow colonies (1-2mm) presumptive of *Aeromonas* were subjected to 3 orientation tests (fresh state, Gram, oxidase), the glucose fermentation test and the O129 sensitivity test according to [13]. Gram-negative bacilli with monotrich polar mobility, possessing a cytochrome oxidase with glucose fermentation and resistant to compound O129 were identified as belonging to the genus *Aeromonas*. The establishment of biochemical characters was carried out using the Api 20E gallery. Confirmation at the specific level of the different isolates was carried out by MALDITOF-MS (Biomerieux), using a reference strain *Escherichia coli* ATCC 8739 for calibration and validation of the results.

2.4. Phenotypic Determination of Virulence

The haemolytic activity of *Aeromonas* strains, the casein hydrolysis test, and DNAse production were demonstrated on different agars according to the technique described by [14]. Gelatinase production was performed according to the method of [15] and lipase production was performed by plating on lipid agar poured into Petri dishes [16]. The ability of *Aeromonas* to adhere to host cells was demonstrated by the production of polysaccharide inter cellular adhesin (PIA) on Congo Red Agar medium following the technique of [17].

2.5. Antibiotic Resistance Test

After confirmation of the biochemical tests, the *Aeromonas* strains were tested for resistance to certain antibiotics used in veterinary and human medicine according to the recommendations of the Antibiogram Committee of the French Microbiology Society [18]. These tests were performed using the Müller-Hinton agar disc diffusion technique [19]. The antibiotic molecules used to show the resistance profile of *Aeromonas* strains were: Ampicillin (AMP, 10µg), Amoxicillin-clavulanic acid (AMC, 21-10µg), Cefalotin (CF, 30µg), Cefepime (FEP, 30µg), Ceftazidime (CAZ, 30µg), Aztreonam (ATM, 30µg), Ciprofloxacin (CIP, 5µg), Levofloxacin (LVX, 5µg), Trimethoprim-sulfamethoxazole (SXT, 25µg) and Tetracycline (TET, 30µg).

3. Results

3.1. Prevalence of *Aeromonas* Strains in Tilapia Samples

A total of 512 tilapia samples were collected and analyzed. Fifty-six (56) specimens carried at least one *Aeromonas* species, a prevalence of 10.93%. The total number of *Aeromonas* isolated was 87. Species identification yielded 6 motile taxa of which the most dominant were *Aeromonas jandaei* (24%), *A. caviae* (23%), and *A. hydrophila* (22%) (Figure 1). The 3 other less represented species are *A. sobria*, *A. veronii* and *A. trota* with respectively 13%, 10% and 8%.

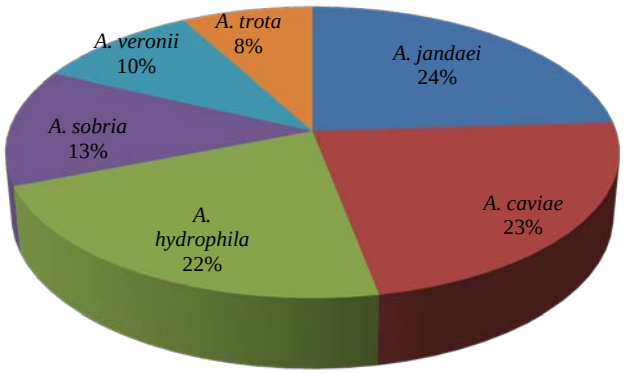

Figure 1. Proportion of *Aeromonas* species isolated from tilapias

3.2. Distribution of *Aeromonas* by Organ

The distribution of *Aeromonas* in the different organs of the fish showed a level in the intestine (42.5%). In addition, in the liver and gills 30% and 27% respectively were observed. *Aeromonas hydrophila*, *A. jandaei*, *A. sobria* and *A. trota* were the most isolated in the gut compared to the other organs with 58%, 47.6%, 45.5% and 43% respectively. While *A. caviae* (45%), was most encountered in the liver. The species *A. veronii* was the most isolated in fish gills (44.5%) (Figure 2).

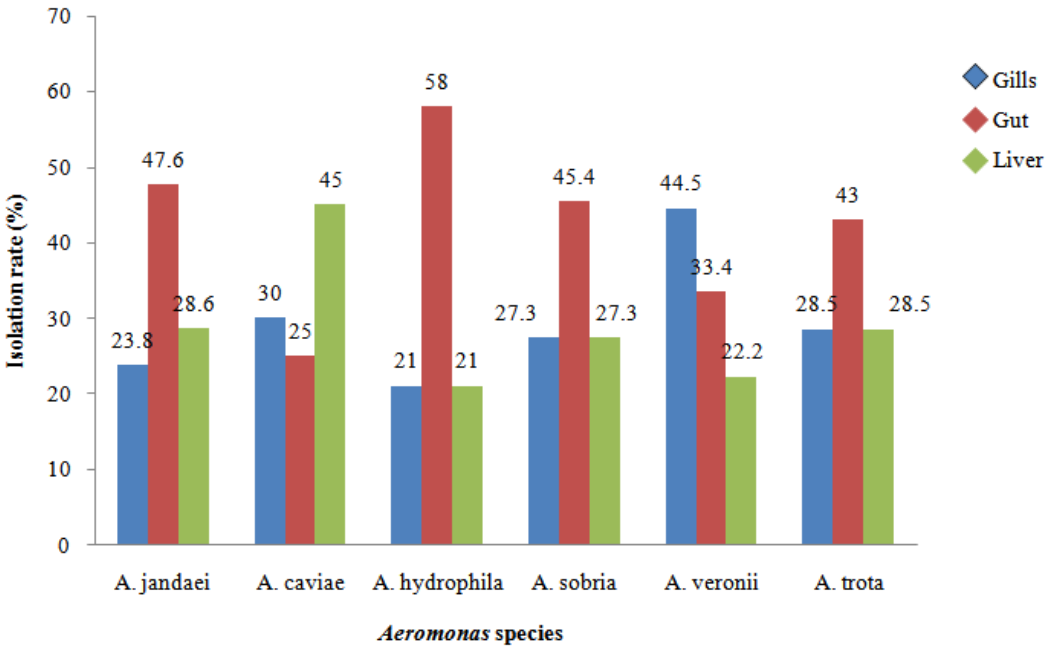

Figure 2. Distribution of *Aeromonas* species according to the organs of tilapias

3.3. Virulence Factors

After 24 hours of incubation of cultures at 37°C, the observations made are shown in Figure 3. The presence of a clear halo reflecting complete lysis of red blood cells around the colonies indicated β-haemolysis. Casein hydrolysis, DNA degradation and lipase production were also observed by the presence of a clear halo around the cultures in respective media. Gelatinase activity resulted in liquefaction of the medium, despite cooling to 4°C for 1 hour, and AIP-producing *Aeromonas* strains gave black colonies with rough surfaces, in contrast to non-producing strains that gave red colonies with smooth surfaces.

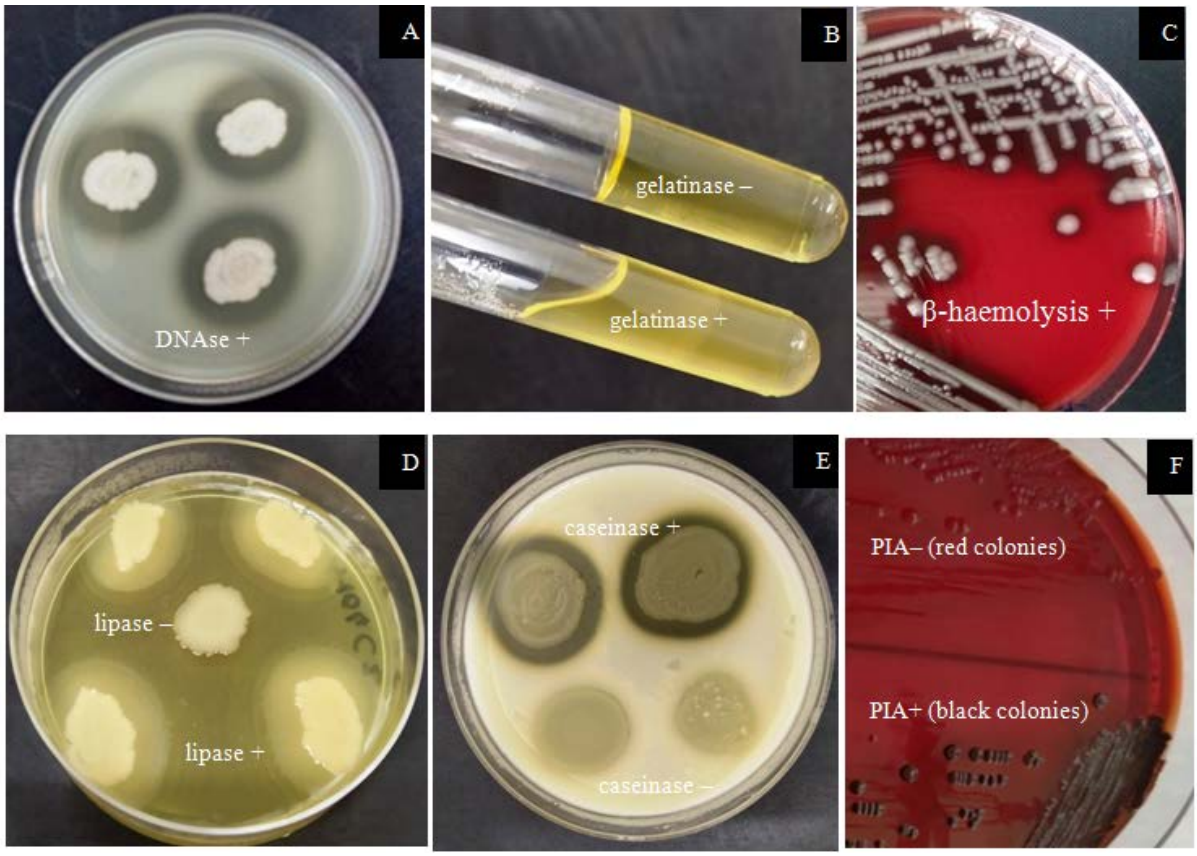

Figure 3. Phenotypic virulence factors in *Aeromonas* isolated from tilapia (A = Detection of DNAase production on DNA agar; B = Detection of gelatinase production in tube agar; C = Detection of hemolysin production on blood agar; D = Detection of lipase production on lipid agar; E = Detection of caseinase production on milk agar; F = Detection of PIA production on Congo Red agar)

Table 1 shows that all the *Aeromonas* strains isolated and identified produced DNAse and gelatinase. Similarly, haemolytic and lipase activities were also observed in all isolates at high levels ranging from 81 to 100%. However, protease production and the ability of isolates to produce AIPs varied from species to species with rates ranging from 10 to 89%.

Table 1. Prevalence Of Virulence Factors In *Aeromonas* Isolated From Tilapia

	Percentage of *Aeromonas* producing virulence factors						Total
Virulence tests	*A. jandaei*	*A. caviae*	*A. hydrophila*	*A. sobria*	*A. veronii*	*A. trota*	
DNAse	100	100	100	100	100	100	100
Haemolysin	81	95	89,5	91	100	85,7	90
Lipase	71	75	89,5	63,6	100	85,7	78,2
Caseinase	57	80	84,2	72,7	89	43	72,4
Gelatinase	100	100	100	100	100	100	100
PIA production	10	55	58	54,5	77,8	14,3	43,7

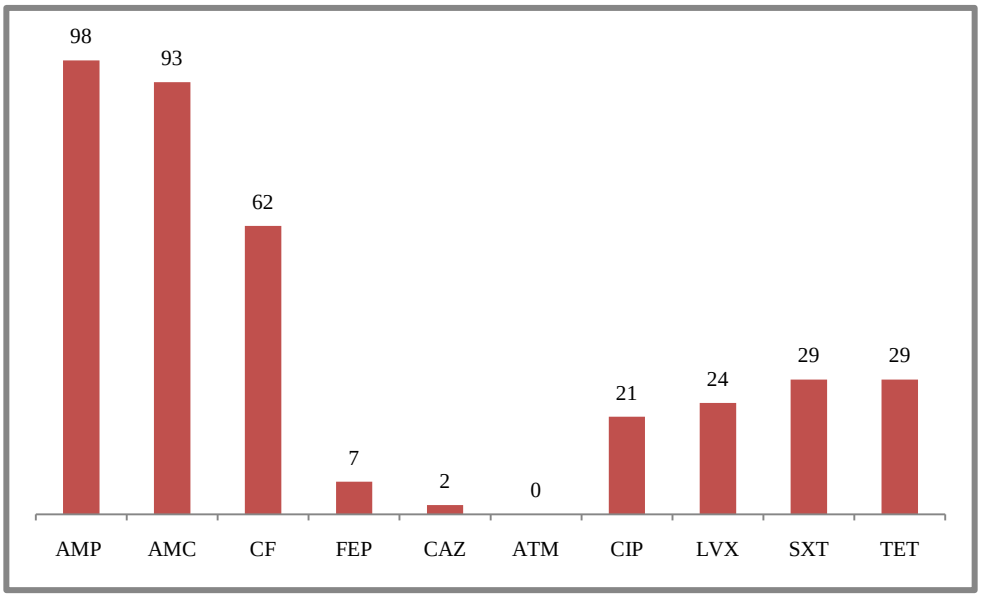

Figure 4. Antibiotic resistance profiles of *Aeromonas* isolated from tilapia fish (Profiles are shown as % Resistant. AMP = Ampicillin; AMC = Amoxicillin-clavulanic acid; CEF = Cefalotin; FEP = Cefepime; CAZ = Ceftazidime; ATM = Aztreonam; CIP = Ciprofloxacin; LVX = Levofloxacin; SXT = Trimethoprim-sulfamethoxazole; TET = Tetracycline)

3.4. Antibiotic Resistance of Isolated Strains

The resistance study was conducted on the 87 *Aeromonas* isolates using 10 antibiotics belonging to different families (Figure 4). The isolates were largely resistant to ampicillin and amoxicillin-clavulanic acid with rates of 98% and 93% respectively. *Aeromonas* also showed relatively high resistance to cephalotin (62%). On the other hand, a good sensitivity of the strains was observed to aztreonam (100%), ceftazidime (98%) and cefepime (93%). Resistance to fluoroquinolones was 24% and 21% for levofloxacin and ciprofloxacin respectively. Trimethoprim-sulfamethoxazole and tetracycline showed an identical resistance of 29%.

4. Discussion

In the present study, 6 motile species belonging to the genus *Aeromonas* were identified in the fish samples: *Aeromonas jandaei, A. caviae, A. hydrophila, A. sobria, A. veronii* and *A. trota*. [20] also reported 6 species belonging to the same bacterial genus in the shellfish (*Patinopecten yessoensis*) from Korea, with a majority of species common to the present study. [4] documented a result of 7 *Aeromonas* species in ornamental fish, including *A. schubertii* not present in our study. The high species diversity of *Aeromonas* in the fish samples is thought to reflect the lagoon habitat, fed by untreated domestic and industrial wastewater and bacteria from the surrounding leached soil.

Our results revealed that the 3 dominant species were *A. jandaei* (24%), *A. caviae* (23%) and *A. hydrophila* (22%), while the least represented were *A. sobria* (13%), *A. veronii* (10%) and *A. trota* (8%). It was generally assumed that diseases caused by motile *Aeromonas* in freshwater and brackish fish were mainly related to *A. hydrophila*, while other species were probably overlooked. In our study, two other predominant species *A. jandaei* and *A. caviae* were identified. The species *A. jandaei*, considered as a new fish pathogen associated with epizootic ulcerative syndrome (EUS) were implicated in Nile tilapia mortalities by [21]. Recently in a study on understanding an epidemic in Arapaimidae fish, *A. hydrophila* in association with *A. jandaei* were isolated from dead or dying fish [22]. Experimental co-infection

with these two species established, according to Koch's postulate, the existence of a synergy of action between these bacterial species.

Concerning the distribution of *Aeromonas* in fish organs, *A. jandaei* and *A. hydrophila* were frequently found in intestinal samples and to a lesser extent in the gills. This may be related to the fact that *Aeromonas* is a symbiont of the fish digestive tract and in addition the gills are the main entry route for most ichthyopathogenic bacteria [23].

In the present study identifying some virulence factors associated with the pathogenicity of *Aeromonas* species, 90% of isolates produced β-haemolysis. A similar result of 91.4% was observed in Egypt with *Aeromonas* isolated from tilapia [24], whereas the haemolysin production observed in *Aeromonas* isolated from ornamental fish was only 78.95% [4]. In the present study all species produced haemolysin with a high proportion for A. veronii, whereas the literature reports that A. caviae is consistently non-haemolytic and non-enterotoxic [25]. A similar result in Norway on 31 *Aeromonas* isolated from food and water showed that *A. caviae*, *A. hydrophila*, *A. schubertii* and *A. veronii bv veronii* all secreted cytotoxins [26]. The β-hemolysin produced by *Aeromonas* is thought to be an important virulence factor, responsible for pore formation in infected cells as well as intra-abdominal accumulation of ascites fluid in the host [27].

Our results show that all isolates produced gelatinase and nuclease, also 72.4% were able to hydrolyse casein. [28] made a similar finding with *A. hydrophila* strains from tilapia, while a study in Côte d'Ivoire by [29] reported less proteolytic activity (68.9%) in strains isolated from edible frogs. According to [23], proteases play a major role in the pathogenicity of ichthyopathogenic *Aeromonas* species. These enzymes cause physical destruction of the cell architecture, which is expressed by induction of oedema, necrotic lesions and even cases of septic shock in humans [27]. The health risk is especially real for highly pathogenic strains that may produce toxins homologous to Shiga-toxin.

The *Aeromonas* in the present study showed phenotypic lipase activity in 78.2% of isolates. Specifically, 100% of *A. veronii* and 90% of *A. hydrophila* isolates produced lipase, whereas this activity ranged from 64% to 86% for the other species in our data. In work in Korea, comparable levels of extracellular virulence factors of 79% were observed among *Aeromonas* isolated from fish [30]. In comparison to these results, [14] showed high lipase production with 100% of *Aeromonas* isolates. The lipases described in *Aeromonas* exhibit lecithinase and cytotoxic activities, playing a significant role in virulence [31].

About half of the isolates (43.7%) obtained in this study showed potential for adhesion to host cells. This infectivity, considered as a virulence factor, has already been reported by [32] for all *Aeromonas* tested in an experimental study in freshwater fish. This ability is thought to be due to the lateral flagella, which are considered to be an adaptation to living on surfaces. Indeed, studies conducted by [33] have established that lateral flagella are actively involved in the attachment, penetration and colonisation of the mucosa of the digestive tract during chronic dysentery. Furthermore, microorganisms aggregated in biofilms are able to resist antimicrobials, biocides and disinfectants compared to those that are planktonic [34].

The susceptibility tests performed in this study revealed that *Aeromonas* isolated from fish are generally resistant to cephalotin (C1G). Similar results have been reported with very high β-lactam resistances of 98.5 to 100% observed in *Aeromonas* isolates [35]. These resistances to penicillins and first generation cephalosporins are associated with the natural β-lactamase production observed in *Aeromonas* [36]. In addition, full susceptibility to aztreonam, high susceptibility to cefepime and ceftazidime and good efficacy of fluoroquinolones against *Aeromonas* have been noted. Tetracyclines are one of the most widely used antimicrobial classes in human and veterinary medicine and our results show that 71% of *Aeromonas* isolates were susceptible. However, study led by [20] on seafood in Korea documents high rates of tetracycline resistance of up to 78.3% on motile *Aeromonas*. The relative resistance observed in our study is probably due to a phenomenon of resistant bacteria selection in the aquatic environment as a consequence of the misuse of tetracyclines. Indeed, resistance genes linked to selection pressure, carried by hospital and livestock farm effluents, domestic and industrial wastewater, are found in the waters of the Ebrié lagoon where they are likely to be transmitted to bacteria carried by fish [37]. This represents a risk to human health, as the bacteria can be transmitted to humans through the food chain.

5. Conclusion

This study revealed that tilapias from the Ebrié lagoon have a high diversity of *Aeromonas* and that most of the isolated strains had the ability to adhere to host cells to colonize them, produced various toxins and enzymes that contribute significantly to virulence. In addition, relative resistance to several antibiotics was observed in the majority of isolates. These characteristics reveal the potentially pathogenic status of the isolates in our study and the risk of their transmission to humans via the food chain. This is a public health problem associated with an economic problem for the fish farming industry, to which particular attention must be paid.

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