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Research article

Antimicrobial resistance and the prevalence of integron in Aeromonas *hydrophila* from hemorrhagic diseased *Pangasius* catfish of the Mekong Delta

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Abstract

Antimicrobial resistance from bacterial pathogens is becoming a health concern in many countries. The purpose of this investigation was to assess antimicrobial resistance and find out the prevalence of class 1 integrons from *A. hydrophila* that led to hemorrhagic disease in intensively farmed Tra catfish in the Mekong Delta, Vietnam. The research showed that a total of 74 isolates were isolated and identified as *A. hydrophila* by the API 20E kit and using the amplification products of the aerolysin gene for confirmation. The susceptibility of all isolates was tested against fifteen antimicrobial agents using the disk diffusion method. Our study indicated that most strains were resistant to the following antibiotics: trimethoprim-sulfamethoxazole, ampicillin, cefalexin, tetracycline, amoxicillin, chloramphenicol, florfenicol, neomycin, and gentamycin. In addition, the investigation also indicated that most *A. hydrophila* isolates displayed multiple antibiotic resistance phenotypes. The MAR (multiple antibiotic resistance) index was high, ranging from 0.40 to 0.66 for *A. hydrophila* isolates, which indicated that these isolates were exposed to high risk sources of contamination where antibiotics were commonly used. Among all isolates, 12.16% (9/74 isolates) of class 1 integrons were determined by polymerase chain reaction.

Keywords: *Aeromonas hydrophila,* Antimicrobial resistance, Class 1 integrons, Mekong Delta, *Pangasianodon hypophthalmus*

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INTRODUCTION

Striped catfish, or *Pangasius* (Tra) catfish (*Pangasianodon hypophthalmus)*, is one of the most popular cultured freshwater fish in the Mekong Delta, Vietnam. According to VASEP (2022), Tra catfish production reached 1.7 million tons and accounted for 26% of Vietnam's total seafood export value. In recent years, however, the intensification of culturing this fish at very high stocking densities has led the farmers to face multiple problems and significant economic loss due to serious disease outbreaks (De Silva and Phuong, 2011). Within the diseases of striped catfish, a hemorrhagic disease caused by *Aeromonas hydrophila* is the most common and damaging disease (Tu et al., 2008; Crumlish et al., 2012). Currently, antibiotics are still used to control and treat infectious bacterial pathogens, including *A. hydrophila*, by most of the producers in the Mekong Delta (Tran et al., 2015). However, many studies showed that the misuse, overuse, or incorrect dosage of antimicrobials used for a long time had led to antimicrobial resistance bacteria in aquaculture (Nawaz et al., 2008; Pridgeon et al., 2011). Now, antimicrobial resistance is a big concern for community health because antimicrobial resistance bacterial pathogens can transfer antimicrobial-resistant determinants to different bacterial pathogens (Davies and Davies, 2010).

An integron is a mobile genetic element that has the ability to recognize and capture one or more cassette genes – commonly antimicrobial resistance genes (Cambray et al., 2010). Hence, integron-carrying bacteria often display multi-antibiotic resistance (Collis and Hall, 1995). Until now, scientists have discovered about 10 classes of integrons (Correia et al., 2003). Numerous studies have been published in Vietnam on antimicrobial resistance and the transmission of antimicrobial resistance genes of bacterial pathogens in striped catfish culture aquatic environments (Tu et al., 2009; Nguyen et al., 2014). However, phenotypic characteristics, the antimicrobial resistance, and class 1 integrons in *A. hydrophila* which there is still no information. Therefore, it is necessary to investigate antimicrobial resistance and determine the prevalence of class 1 integrons in *A. hydrophila*.

MATERIALS AND METHODS

Hemorrhagic diseased fish for isolation of bacteria

Striped catfish samples (80 samples) were randomly collected in order to isolate *A. hydrophila* from hemorrhagic diseased fish in seven provinces of the Mekong Delta, Vietnam (Figure 1), such as: An Giang (12 samples), Dong Thap (12 samples), Can Tho (12 samples), Tien Giang (11 samples), Vinh Long (11 samples), Tra Vinh (11 samples), and Ben Tre (11 samples).

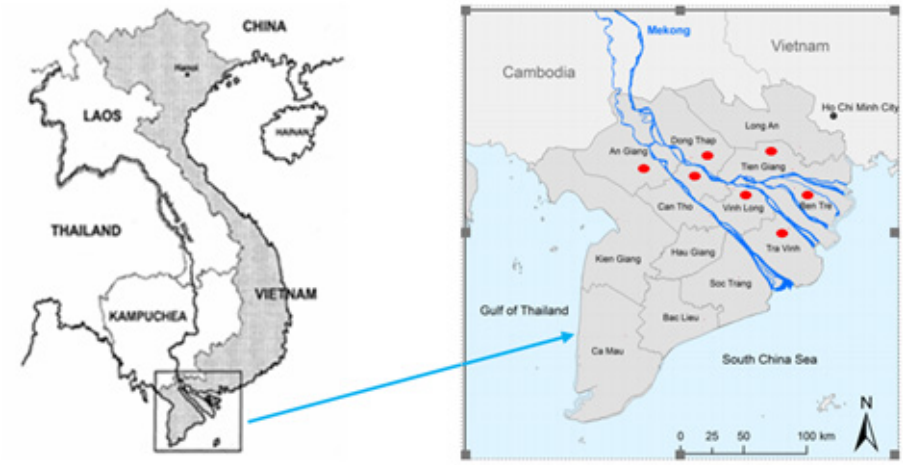

Figure 1 Sampling locations of Tra catfish in the Mekong Delta (● : sampling locations).

In this study, three intensive catfish farms (pond areas ranged from 0.4 to 1.5 ha; depths were generally 3.5–4.5 m) in each province were chosen for sample collection. This work was performed from January 2020 to April 2021. The diseased fish caught 3–4 fish per pond but remained alive or moribund. The body weights of fish ranged from 40 to 550 g per fish, with external and internal signs such as a red, swollen vent, ascitic fluid with a pink to yellow color, and the head, mouth, and base fins with hemorrhagic spots (Figure 2).

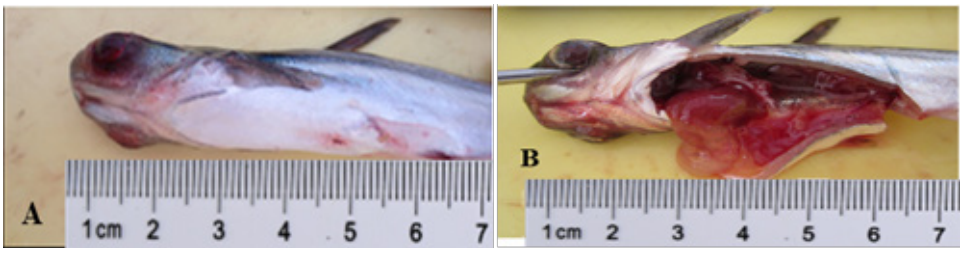

Figure 2 A. hydrophila-caused hemorrhagic disease in Pangasius catfish, (A); Disease fish with hemorrhagic spots in the fins, eye area and around the anus, (B); The fish body cavity contains a lot of red fluid

Bacterial isolation and identification

Isolation of *A. hydrophila*

From the kidney, liver, and spleen of diseased fish, the *A. hydrophila* isolates were aseptically isolated and streaked onto tryptic soy agar (TSA) and incubated at 30°C for 24-48 hours (Dang et al., 2021). The presumptive colonies (light or creamy yellow) were tested for basic physiochemical characteristics such as microscopic cell observation, bacterial motility, Gram staining, oxidase, and catalase reactions, and glucose fermentation/oxidation ability (Barrow and Feltham, 1993; Buller, 2004). For further analysis, presumptive colonies were also confirmed by a commercial API 20E kit according to the manufacturer's recommendation, and read after 24 hours. The pure bacterial isolates were kept at -80°C in tryptic soy broth (TSB) with 20% glycerol.

Identification of *A. hydrophila*

Bacterial DNA extraction

Bacterial DNA was extracted according to standard techniques (Sambrook et al., 1989) with some modifications. In brief, pure bacteria were cultured in TSB and shaking at 120 cycles per minute. Then, the bacterial cell biomass was dissolved in lysis buffer (1 M Tris-HCl, 0.5 mM EDTA, 5 M NaCl, 10% SDS, pH 8.0), and incubated at room temperature for 10 minutes. The solution was centrifuged at 13,000 x g for 5 minutes, and the supernatant (600-800 µl) was transferred into fresh tubes. After adding an equivalent amount of 95% ethanol to the tubes. The pellet was washed by adding 500 µl 70% ethanol and centrifuged for 5 minutes at 13,000 x g. Finally, the pellet was dissolved in 100 µl 0.1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The extracted DNA was verified for purity, quantitated, and stored at -20°C until use and further analysis.

PCR for confirmation of A. hydrophila

The primers AeroFd: 5'-CCAAGGGGTCTGTGGCGACA-3' and AeroRs: 5'-TTTCACCGGTAACAGGATTG-3' for amplification of the 209-bp aerolysin gene of *A. hydrophila* were used in our study (Pollard et al., 1990). The PCR reaction mixture (25 µL) includes 1X PCR buffer (3.5 µl); 2.5 mM $MgCl_2$ (2 µl); 2.5 mM each dNTP mixture (4 µl); 2.5U/µl *Taq* DNA polymerase (0.25 µl) (Fermantas, USA); 0.4 µM each primer (1 µl), 0.5 µl DMSO 10% (dimethyl sulfoxide, Merk), 2 µl DNA template (40 ng), and 10.75 µl ddH_2O (sterile double-distilled water). The thermocycling profile was conducted under the following conditions: 94°C initial denaturation for 4 minutes, followed by 30 cycles of 95°C denaturation for 30 seconds, 60°C primer annealing for 45 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. The PCR also included a negative control (no DNA template), and *A. hydrophila* ATCC 7699 was used as a positive control. PCR products were also sent to Macrogen (Korea) for aerolysin gene sequencing.

Testing for antimicrobial susceptibility

Antimicrobial resistance of *A. hydrophila* was tested by the Kirby-Bauer disk diffusion method (Bauer et al., 1966) on TSA with 15 antibiotics (Oxoid, England). The antibiotic disks used in this study: amoxicillin (AMO/10µg), cefotaxime (CTX/30µg), cefalexin (CFL/30µg), ampicillin (AMP/10µg), chloramphenicol (CHL/30µg), florfenicol (FFC/30µg), ciprofloxacin (CIP/5µg), enrofloxacin (ENR/5µg), norfloxacin (NOR/5µg), doxycycline (DOX/30µg), gentamicin (GEN/10µg), tetracycline (TET/30µg), streptomycin (STR/10µg), neomycin (NEO/30µg) and trimethoprim-sulfamethoxazole (SXT/1,25/23,75µg). Single bacterial colonies were suspended in 0.85% saline solution, and the turbidity matched the McFarland No. 3 standard (bioMerieux, France), and streaked on a TSA plate. Plates were incubated for 24 hours at 30°C before being read. Using interpretative standards recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2012), the isolates were considered resistant (R) and susceptible (S) based on the inhibition zone (mm). As a quality control, the reference strain *Escherichia coli* ATCC 25922 was used.

The MAR index determination

The MAR index is calculated as a/b, where a is the number of antibiotics to which the isolate was resistant and b is the total number of antibiotics for which the isolate was tested. A MAR index value greater than 0.2 is seen when the isolate is exposed to high-risk sources of Tra catfish contamination and antibiotic use is prevalent. In contrast, this value is observed if antibiotics are rarely or never used (Krumperman, 1983).

Detection of class 1 integrons

The specific primer pair HS463a: 5'-CTGGATTTCGATCAGCACG-3' and HS464: 5'-ACATGCGTGTAAATCATCGTCG-3' was used to amplify 470-bp fragments of the integrase gene (*intI1*) of class 1 integrons (Barlow et al., 2004). Polymerase chain reactions were performed with the following conditions: 94°C initial denaturation for 5 minutes; followed by 30 cycles of 94°C denaturation for 30 seconds, 55°C annealing for 30 seconds, and 72°C extension for 45 seconds, and final extension at 72°C for 10 minutes (Barlow et al., 2004). The PCR also included a negative control (no DNA template). PCR products were sent to Macrogen (Korea) for sequencing of the integrase gene (*intI1*) of class 1 integrons.

Statistical analysis

Descriptive statistics were used to determine the prevalence of *A. hydrophila* in the study area, MAR index values, and antimicrobial resistance patterns. BLAST analysis tools were employed to confirm species level, and *intI1* gene of class 1 integrons (https://blast.ncbi.nlm.nih.gov).

A. hydrophila isolation

Seventy-four *A. hydrophila* strains were isolated from the spleen, kidney, and liver of hemorrhagic fish in different intensive farms of the Mekong Delta: An Giang (8 isolates), Dong Thap (10 isolates), Can Tho (8 isolates), Tien Giang (12 isolates), Vinh Long (15 isolates), Tra Vinh (8 isolates), and Ben Tre (13 isolates). A list of *A. hydrophila* isolates and their distribution in our research were summarized in Table 1.

Table 1 Bacterial sources and distribution of *A. hydrophila* in the Mekong Delta

Geographical origin	Farm	Fish samples	No of isolates
An Giang	3	12	8
Dong Thap	3	12	10
Can Tho	3	12	8
Tien Giang	3	11	12
Vinh Long	3	11	15
Tra Vinh	3	11	8
Ben Tre	3	11	13
Total	27	80	74

Morphological and biochemical characteristics of *A. hydrophila* isolates

After 24 hours of development on the TSA plate at 30°C, the colonies of all isolates were round-shaped with a diameter of 1-3 mm, lightly convex, and yellowish. All the isolates in the study had identical biochemical test results. They were Gram-negative, rod-shaped, catalase-positive, and had motility ability. All isolates were positive for arginine dihydrolase, gelatin hydrolysis, citrate utilization, H_2S production, the Voges-Proskauer test, the indole test, glucose oxidation, sucrose oxidation, mannitol oxidation, amygdalin oxidation, and negative for lysine decarboxylase, ornithine decarboxylase, urease, inositol oxidation, sorbitol oxidation, rhamnose oxidation, melibiose oxidation, and arabinose oxidation.

PCR confirmation of *A. hydrophila*

The result showed that the aerolysin gene of all bacterial strains was amplified to the expected product size of 209 bp (Figure 3). The BLAST analysis results after aerolysin gene sequencing showed a 99.38% similarity to *A. hydrophila* (CP050012.1) in GenBank.

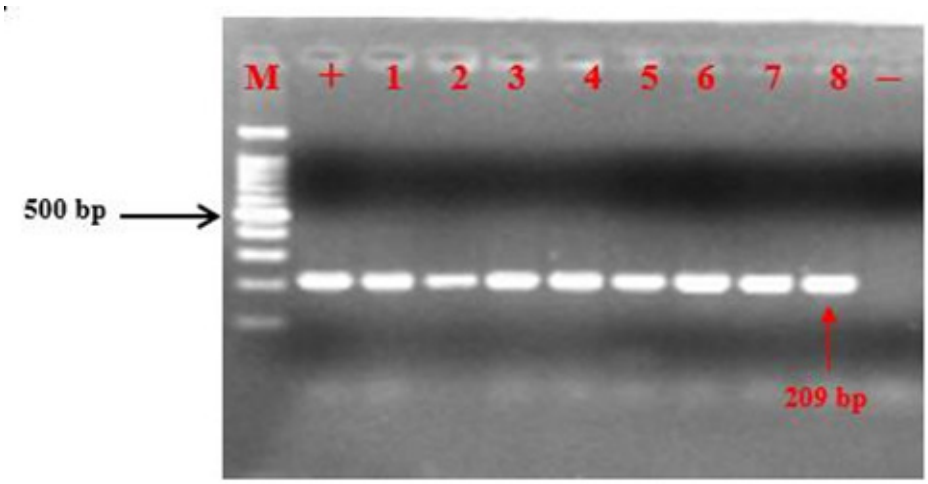

Figure 3 Figure 3 The result of amplification products electrophoresis from A. *hydrophila* isolates. Positive reactions produced 209 bp DNA fragments (arrow). M: 100 bp DNA ladder (Fermentas); +: Positive control (A. *hydrophila* ATCC7699); Lane 1-8: products of aerolysin gene amplification of striped catfish A. *hydrophila* isolates 1A3, 7A3, 9A3, 10A3, 13A3, 1A4, 2A4, and 3A4, respectively; –: negative control

Antimicrobial resistance in *A. hydrophila*

The susceptibility of seventy-four *A. hydrophila* isolates was tested for 15 antibiotics, and the frequency of resistance to the various antibacterial agents for all bacteria was presented in Figure 4. Generally, most *A. hydrophila* isolates were resistant to 15 antibiotics tested in this study. For instance, 100% of bacteria in the research are resistant to trimethoprim-sulfamethoxazole and antibiotics belong to β-lactam such as ampicillin, amoxicillin, and cefalexin (except cefotaxime). Resistance rates to tetracycline, chloramphenicol and florfenicol were found in 93.24%, 63.51%, and 62.16% of the isolates,

respectively. Meanwhile, resistance to other antibiotics such as neomycin, gentamycin, enrofloxacin, streptomycin, norfloxacin, ciprofloxacin, and doxycycline ranged from 10 to 54%. However, bacteria were still highly susceptible to doxycycline, ciprofloxacin, cefotaxime, and norfloxacin (from 60% to 72%). The MAR index values of *A. hydrophila* strains fluctuated from 0.40 (Tien Giang province) to 0.66 (Dong Thap province), with an average of 0.50, demonstrating the high-risk sources of contamination that originated from Tra catfish farm, where antimicrobials were commonly used (Tran *et al.*, 2015) in the Mekong Delta (MAR index values in each province are not shown in detail).

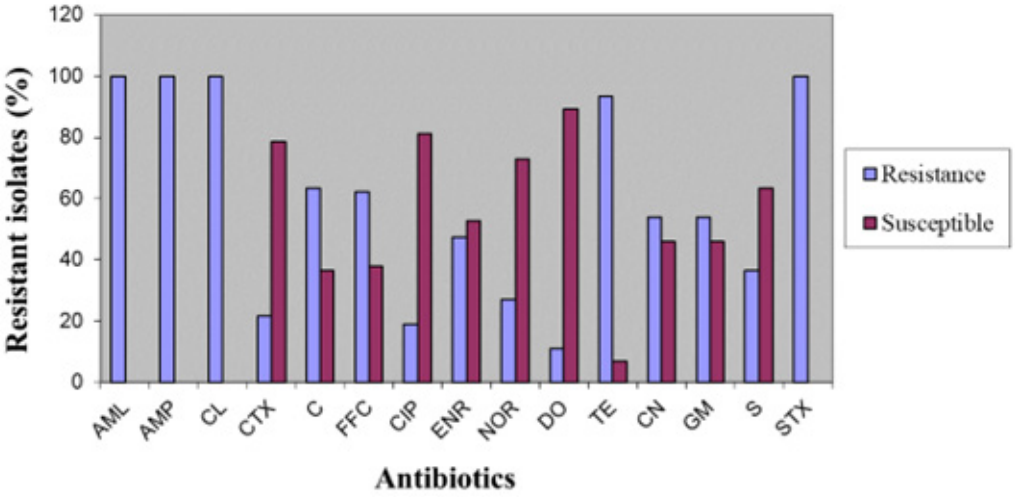

Figure 4 Occurrence of resistance to antibiotics for 74 isolates obtained from striped catfish in the Mekong, Delta. AML stands for amoxicillin, CL: cefalexin, AMP: ampicillin, CTX: cefotaxime, FFC: florfenicol, C: chloramphenicol, CIP: ciprofloxacin, ENR: enrofloxacin, DO: doxycycline, NOR: norfloxacin, TE: tetracycline, CN: neomycin, GM: gentamycin, S: streptomycin, and SXT: trimethoprim-sulfamethoxazole

Multiple antibiotic resistance and resistant phenotypes

Of the seventy-four isolates tested, only one isolate was still sensitive to eleven antimicrobial agents (1.35%). All *A. hydrophila* strains were resistant to at least four antimicrobials (Figure 5). Generally, Figure 5 also indicated that the majority of isolates were found to be resistant to five to thirteen antimicrobial agents. The highest frequency was resistance to seven, ten, and eleven antimicrobial agents (16.22%), the resistance rate to nine antibacterial agents was 12.16%, and five and eight antibacterial agents were 9.46%. Meanwhile, the lowest frequency was resistance to four, fourteen, and fifteen antibacterial agents at 1.35%.

In addition, many different multiple antibiotic resistance phenotypes were recorded from 74 bacterial isolates in the study (data not shown). However, the pattern of AMP-AML-CL-TE-SXT was the most popular pattern, which occurred in 9.46% (seven isolates out of seventy-four) of multiple antimicrobial resistance in *A. hydrophila*. The common multiple antibiotic resistance phenotypes of these bacteria were presented in Table 2.

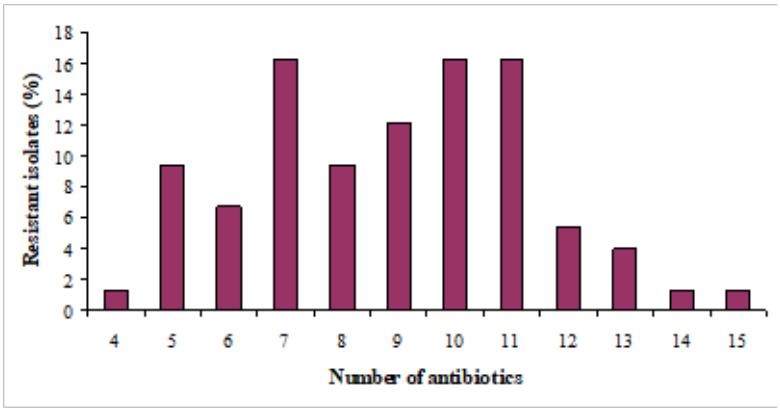

Figure 5 Multiple antibiotic resistance of *A. hydrophila* isolates

Table 2 Some common multiple antibiotic resistance phenotypes of *A. hydrophila* strains

Multiple antibiotic resistance phenotypes	Number of strains	Percentage (%)
AMP-AML-CL-TE-SXT	7	13.73
AMP-AML-CL-C-FFC-TE-SXT	6	11.76
AMP-AML-CL-C-FFC-ENR-TE-CN-S-GM-SXT	3	5.88
AMP-AML-CL-FFC-TE-SXT	2	3.92
AMP-AML-CL-TE-CN-SXT	2	3.92
AMP-AML-CL-C-FFC-ENR-TE-SXT	2	3.92
AMP-AML-CL-TE-CN-S-SXT	2	3.92
AMP-AML-CL-C-FFC-ENR-TE-CN- GM-SXT	2	3.92
AMP-AML-CL-ENR-TE-CN-GM-SXT	2	3.92
AMP-AML-CL-C-FFC-NOR-TE- CN-S-GM-SXT	2	3.92
AMP-AML-CL-C-FFC-TE-CN-GM-SXT	2	3.92
AMP-AML-CL-CTX-C-FFC-CIP-ENR-NOR-TE-SXT	2	3.92

AML stands for amoxicillin, CL: cefalexin, AMP: ampicillin, CTX: cefotaxime, FFC: florfenicol, C: chloramphenicol, CIP: ciprofloxacin, ENR: enrofloxacin, DO: doxycycline, NOR: norfloxacin, TE: tetracycline, CN: neomycin, GM: gentamycin, S: streptomycin, and SXT: trimethoprim-sulfamethoxazole.

The prevalence of integrons in *A. hydrophila*

Seventy-four antibiotic-resistant *A. hydrophila* strains were determined by the occurrence of class 1 integrons in this research. The results indicated that 9/74 isolates (12.16%) with class 1 integrons were carried out (Figure 6). The BLAST results for the integrase gene (*intI1*) sequencing revealed a 97.54% similarity to the *intI1* gene (LC705341.1) in GenBank. Generally, 100% class 1 integron-positive *A. hydrophila* strains were also resistant to trimethoprim-sulfamethoxazole.

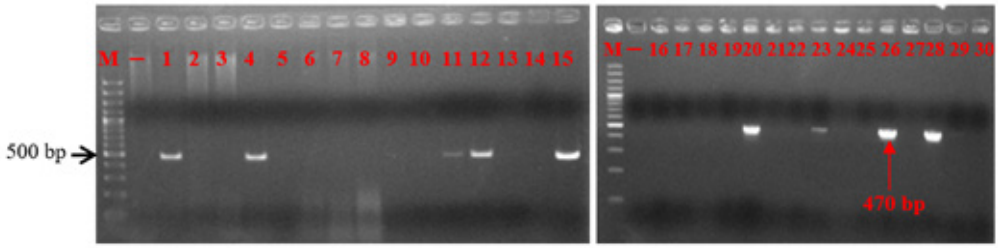

Figure 6 The occurrence of the IntI1 gene in *A. hydrophila* isolates. The positive reaction produced a 470 bp DNA fragment (arrow); M 100-bp DNA ladder; Lane –: negative control; Lane 1, 4, 11, 12, 15, 20, 23, 26, and 28: products of IntI1 gene amplification of *A. hydrophila* isolates 1A3, 4A3, 11A3, 12A3, 15A3, 1A4, 2A4, 3A4, and 1A4, respectively.

Furthermore, seven bacterial isolates with class 1 integrons displayed multiple antibiotic resistance phenotypes (Table 3).

Table 3 Multiple antibiotic resistance phenotypes of *A. hydrophila* isolates containing class 1 integrons

Isolate code	Multiple antibiotic resistance phenotypes	*IntI* gene
1A3	AML-AMP-CL-CTX-C-FFC-TE-S-GM-SXT	+
4A3	AML-AMP-CL-TE-CN-S-SXT	+
11A3	AML-AMP-CL-C-FFC-CIP-TE-GM-SXT	+
12A3	AML-AMP-CL-CTX-C-FFC-CIP-ENR-NOR-TE-SXT	+
15A3	AML-AMP-CL-TE-SXT	+
$1A_4$	AML-AMP-CL-TE-SXT	+
2A4	AMP-AMO-CFL-CTX-FFC-TET-NEO-STR-GEN-SXT	+
3A4	AML-AMP-CL-TE-SXT	+
5A4	AMP-AMO-CFL-TET-NEO-SXT	+

+: positive for amplification of the *Int1* gene. (AML stands for amoxicillin, CL: cefalexin, AMP: ampicillin, CTX: cefotaxime, FFC: florfenicol, C: chloramphenicol, CIP: ciprofloxacin, ENR: enrofloxacin, DO: doxycycline, NOR: norfloxacin, TE: tetracycline, CN: neomycin, GM: gentamycin, S: streptomycin, and SXT: trimethoprim-sulfamethoxazole).

DISCUSSION

In Vietnam, antibiotics are commonly used in aquaculture. Tran et al. (2015) discovered that farmers used a total of 24 different antimicrobials to treat bacillary necrosis and motile aeromonad septicaemia or hemorrhagic disease in Pangasius catfish. The impact of antimicrobial use in aquaculture environments has been reported in recent years (Cabello, 2006). The occurrence and spread of antimicrobial resistance in bacteria have been a global problem for public health (Marshall and Levy, 2011; Stalder et al., 2012). Many investigations into the antibiotic resistance of bacteria due to the widespread use and overuse of antibiotics have been carried out (Sarter et al., 2007; Lukkana et al., 2012; Nawaz et al., 2012). This study found that most *A. hydrophila* strains were resistant to numerous antibiotics, including amoxicillin, cefalexin, ampicillin, tetracycline, chloramphenicol, florfenicol, neomycin, gentamycin, and trimethoprim-sulfamethoxazole (Figure 4). The emergence of antibacterial resistance among fish pathogens reduces the effectiveness of antibiotic therapy in aquaculture (Barza, 2002; Marshall and Levy, 2011). Besides, Vietnam also

has one of the highest rates of AMR in Asia, due, in part, to the overuse of antimicrobial drugs, both in the animal health sector and in humans in both hospitals and the community (Torumkuney et al., 2022).

In the study, high resistance to amoxicillin, ampicillin, and cefalexin was found in *A. hydrophila* isolates, with the exception of cefotaxime (38%) (Figure 4). The findings of the research were in agreement with the report of Nguyen (2014), where the authors found that more than 80% to 94% of total *A. hydrophila* originated from striped catfish cultured in the aquatic environment of the Mekong Delta and were resistant to amoxicillin and ampicillin. The high occurrence of resistance to β-lactams can be explained by *A. hydrophila* possessing an intrinsic resistance to these antibiotics (Akinbowale et al., 2007) and the presence of β-lactamase genes found among *Aeromonas* spp. (Walsh et al., 1995; Rossolini et al., 1996). Therefore, in the future, it will be necessary to strictly control this group of antibiotics because resistant bacteria can transmit antibiotic genes.

Resistances to aminoglycosides such as neomycin, gentamycin, and streptomycin ranged from 47% to 54% (Figure 4). In general, these results were higher than those of previous studies (Verner-Jeffreys et al., 2009; Matyar et al., 2010). This rate, however, was higher than resistance to quinolones such as ciprofloxacin (32%), and norfloxacin (40%). In Vietnam, quinolones are prohibited or limited for use in aquaculture (MARD, 2014). Some publications indicated that *Aeromonas* spp. had low resistance to ciprofloxacin (Hatha et al., 2005; Akinbowale et al., 2006). Therefore, the high resistance rate to these antibiotics found in striped catfish *A. hydrophila* isolates may be due to their frequent use in aquaculture in recent years.

In aquaculture, trimethoprim-sulfamethoxazole (SXT) was widely used to treat Gram-negative bacterial disease (Serrano, 2005). This research showed that 100% of *A. hydrophila* isolates were resistant to SXT (Figure 4). This rate was significantly higher compared with several results of previous studies (Sarter et al., 2007). The research of Pham et al. (2011) indicated that the incidence of *A. hydrophila* isolates resistant to SXT was 32.8%. In Australia, just 1 out of 129 strains of *Pseudomonas* spp. isolated from 9 culture areas was resistant to SXT (Akinbowale et al., 2007). A recent study in Vietnam indicated that 61% of *Aeromonas* spp. isolated from the stripped catfish culture water environment were resistant to SXT (Nguyen et al., 2014). The previous study revealed that *Aeromonas* spp. isolated from India, Turkey, and the UK were found to be resistant to SXT by 64.9% to 67% (Vivekanandhan et al., 2002; Matyar et al., 2010). Thus, increasing the rate of antibiotic resistance of *A. hydrophila* to SXT will reduce the effectiveness of this antibiotic in the treatment of infectious diseases.

In aquaculture, the tetracycline group of antibiotics has been commonly used in Vietnam and many other countries around the world, including Vietnam (Tu et al., 2008). As a consequence, the occurrence of antibiotic-resistant bacteria with the tetracycline group of antibiotics was inevitable and reported by the previous authors (Sarter et al., 2007; Watkinson et al., 2007; Chen et al., 2010). In our study, *A. hydrophila* was highly resistant to tetracycline (93.24%) (Figure 4). On the contrary, the low resistance of the bacteria to doxycycline (28%) was observed in the research (Figure 3). *A. hydrophila*, which originated from the USA's catfish ponds, was resistant to tetracycline (43% resistance)

and oxytetracycline (62% resistance) (McPhearson et al., 1991). In the study of Akinbowale et al. (2006), it was shown that bacteria originating from Australia's aquaculture sources were also resistant to tetracycline and oxytetracycline at 18% and 20%, respectively. Another study by Jacobs and Chenia (2007) found that *Aeromonas* spp. collected from aquaculture systems in South Africa had a 78% rate of resistance to tetracyclines. The report by Nguyen et al. (2014) indicated that *Aeromonas* spp. had resistance to tetracycline (34.8%) and doxycycline (15.2%). Moreover, many studies found that tetracycline resistance determinants were plasmid-borne resistance genes in bacteria and caused disease in aquatic animals (Schmidt et al., 2001). Hence, farmers should not use tetracycline as a treatment for hemorrhagic disease in catfish.

Remarkably, our investigations indicated that *A. hydrophila* isolates were highly resistant to antibiotics belonging to the phenicol group, including chloramphenicol (63.51%) and florfenicol (62.16%) (Figure 4). Chloramphenicol has been banned in animal husbandry and aquaculture in many countries in the world, including Vietnam (MARD, 2014) because it causes bone marrow regression (Dowling, 2006). However, the findings showed that the resistance percentages of *A. hydrophila* to these antibiotics were still significantly higher in comparison to many reported studies (Vivekanandhan et al., 2002; Akinbowale et al., 2007). The research of Pham et al. (2011) indicated that 3.28% of the *A. hydrophila* isolates derived from diseased striped catfish were resistant to chloramphenicol. Chloramphenicol resistance was found in 56% of the isolated *Aeromonas* spp. from ornamental fish and their carriage water (Verner-Jeffreys et al., 2009). The publication of Miranda and Rojas (2007) demonstrated that 100% of the florfenicol-resistant bacteria in Chilean salmon farms were also resistant to chloramphenicol. Because resistant bacteria can spread antibiotic genes, it will be vital to closely regulate this class of medicines in the future.

Multiple antibiotic resistance has been observed in Gram-positive and Gram-negative bacteria all over the world (White et al., 2001; Alekshun and Levy, 2007). The study indicated that 100% *A. hydrophila* isolates displayed resistance to multiple antibiotics (Figure 5). According to McNicol et al. (1980), 57% of *A. hydrophila* isolates in the natural environment in Bangladesh showed multiple antibiotic resistance. In aquatic animals, 14 strains (100%) of *A. hydrophila*-caused fish disease in India displayed multiple antibiotic resistance (Kaskhedikar and Chhabra, 2010). Additionally, the investigation by Lukkana et al. (2012) in Thailand found that the multiple antibiotic resistance phenomenon of *A. hydrophila* also occurred (94%) in Tilapia. In the future, more new solutions for the prevention and treatment of fish diseases, such as herbal remedies or probiotics, are needed to alleviate the pressure on the use of antibiotics in aquaculture and contribute to safe food production for consumers and communities.

Integrons were commonly found in Gram-negative bacteria and were linked to multi-antibiotic resistance in *Enterobacteriaceae* bacteria (White et al., 2001). The occurrence of class 1 integrons in this study was detected in 9 out of 74 isolates (12.16%) tested (Figure 6). However, this rate was lower than those in the other studies (Mukherjee and Chakraborty, 2006; Nawaz et al., 2009; Kadlec et al., 2011; Kha, 2012). According to Ndi and Barton (2012), class 1 integrons are present in 23% of *Pseudomonas* spp. isolated from salmon

and mud in Australia. For *Aeromonas* spp., the presence of class 1 integrons was recorded around the world (Kadlec et al., 2011; Schmidt et al., 2001). In 2001, class 1 integrons were discovered in *A. salmonicida* in Holland, Norway, Scotland, France, Japan, and the United States with a ratio of 55.3% (L'abée-Lund and Sørum). Furthermore, the presence of this integron was also reported in 46% of *A. hydrophila*, which caused disease in Tilapia in Thailand (Lukkana et al., 2012), and 43.5% of common carp in Mexico (Sarria-Guzmán et al., 2014). Especially, all class 1 integron-positive strains were resistant to SXT (Table 3). In Thailand, according to Lukkana et al. (2012), cassette genes *dfrA1* and *dfrA12* bind with class 1 integrons in *A. hydrophila* causing disease in Tilapia that have resistant phenotype towards SXT. The class 1 integrons were found to provide more information related to the multiple antibiotic resistance phenomenon of *A. hydrophila* in the study. However, future studies are needed to elucidate this relationship.

CONCLUSIONS

This study discovered that *A. hydrophila* has significant antimicrobial resistance in the Mekong Delta. The occurrence of class 1 integrons was detected in hemorrhagic diseased fish due to *A. hydrophila*. The occurrence of class 1 integrons in the findings indicated that catfish aquaculture may play a role as a reservoir of antibiotic resistance determinants in aquaculture, and these bacteria may transfer their antimicrobial resistance genes to other bacteria. To our knowledge, this is the first report on class 1 integrons detected in *A. hydrophila* causing hemorrhagic disease in Tra catfish in the Mekong Delta farms.

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AUTHOR CONTRIBUTIONS

Quach Van Cao Thi: Conceptualization, performed experiments, draft, and editing preparation, and wrote the manuscript.
Nguyen Bao Trung: performed experiments, sample collection, statistical analysis, writing, and editing of the manuscript.
Tu Thanh Dung: conceptualization, interpretation of the study, and writing manuscript.

CONFLICT OF INTEREST

We have no conflict of interest.

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