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Research article

The antibiotic susceptibility of *Aeromonas schubertii* isolated from snakehead in An Giang province of the Mekong Delta

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Abstract

Antibiotic resistance and the transmission of resistance genes among bacteria in aquaculture are attracting world attention. The investigation was conducted to evaluate the antibiotic susceptibility of *Aeromonas schubertii*, which causes intensively cultured internal white spot disease in snakehead from An Giang province of the Mekong Delta. The findings isolated 29 bacterial strains from the kidney, spleen, and liver of diseased snakehead fish. Based on the morphological, biochemical, and 16S rRNA gene sequencing results, two strains of As9 and As11 showed a similarity of 98.71% and 97.07% to *A. schubertii* strains ZL-1 and GZ210812, respectively. The disc diffusion method was used to conduct antibiograms of the strains for 12 antibiotics. The research indicated that the bacteria were susceptible to the antibiotics ceftazidime, cefalexin, and doxycycline at rates of 86%, 59%, and 55%, respectively. Meanwhile, bacteria were highly resistant to amoxicillin (93%), flumequine (83%), ciprofloxacin (76%), rifampicin (66%), erythromycin (62%), colistin (59%), florfenicol (59%), and novobiocin (41%). Interestingly, 100% of the bacterial strains in the study demonstrated a multidrug-resistant phenotype, resistant to at least two antibiotics or more.

Keywords: *Aeromonas schubertii,* An Giang province, Antibiotic diagram, Mekong Delta, Snakehead.

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INTRODUCTION

Snakehead (*Channa striata*) is an aquatic animal that is commonly farmed in some provinces of the Mekong Delta, such as An Giang, Dong Thap, Vinh Long (Le Xuan Sinh and Do Minh Chung, 2009), and some other Asian countries, such as India, China, and Indonesia (Talwar and Jhingran, 1991; Gustiano et al., 2021; Rahayu et al., 2021; Arma et al., 2022). Snakehead fish production increased sharply in the decade 2006–2016, from 132.2 ha to 552.9 ha and from 15.9 thousand tons to 85.6 thousand tons (Mai Thanh Lam et al., 2018). Snakehead fish can live in low-oxygen environments (Mollah et al., 2009) and tolerate adverse environmental conditions (Purnamawati et al., 2017; Purnamawati et al., 2019). In addition, snakehead fish meat is delicious, has high nutritional value, and has medicinal uses (Sinh et al., 2014; Romadhoni et al., 2016; Rahman et al., 2018). Research by Le Xuan Sinh et al. (2009) indicated that snakehead fish can be reared in many different models, such as earthen ponds, nets, and cages. In the past years, in order to increase productivity and output, snakehead fish have been reared under the model of intensive farming with high density (Le Xuan Sinh et al., 2009). This may be one of the main causes of disease outbreaks, which greatly affect the production and economic efficiency of snakehead fish farming in the Mekong Delta (Pham Minh Duc et al., 2012).

Aeromonas species commonly cause disease in farmed fish and significant economic losses to fish farmers worldwide (Turska-Szewczuk et al., 2013; Alghabshi et al., 2018; Soto-Rodriguez et al., 2018; Li et al., 2020; Xue et al., 2022). *A. schubertii* was first discovered to cause disease in humans (Hickman-Brenner et al., 1988). By 2010, *A. schubertii* was thought to be the causative agent of the mass mortality of snakehead fish in farms, causing great loss to farmers and serious damage to the Chinese economy (Chen et al., 2012). Currently, this bacterium has been reported to occur in a number of aquatic animals, such as the hybrid snakehead *Channa maculata* × *C. argus* (Liu et al., 2012), the whiteleg shrimp *Penaeus vannamei* (Cao et al., 2015), the Nile tilapia *Oreochromis niloticus* (Liu et al., 2018), and the snakehead *Channa maculata* (Liu et al., 2019).

In the Mekong Delta, *A. schubertii* was first identified as the causative agent of internal white nodules on intensively cultured snakehead in An Giang and Dong Thap provinces (Dang Thi Hoang Oanh and Nguyen Trong Nghia, 2016). Recently, *A. schubertii* was also identified to cause this disease in snakehead fish, which were farmed intensively in the four provinces of Tra Vinh, An Giang, Dong Thap, and Dong Nai (Tu Thanh Dung et al., 2019). *A. schubertii*-infected fish displayed typical signs, such as the appearance of small white nodules on the internal organs (Dang Thi Hoang Oanh and Nguyen Trong Nghia, 2016; Tu Thanh Dung et al., 2019). According to Tu Thanh Dung et al. (2019), the disease often occurs in fingerlings, accounting for over 50%.

Currently, vaccines are still the most effective method to prevent infectious diseases in aquatic animals (Ma et al., 2019; Mondal and Thomas, 2022; Du et al., 2022). However, there has not been any vaccine against *A. schubertii* on snakehead fish raised in Vietnam, except for the vaccine against the infectious disease Bacillus Necrosis Pangasius in *Pangasianodon hypophthalmus* (Su et al., 2021; Tu Thanh Dung et al., 2022). Untill now, antibiotics are still used mainly in aquaculture in many countries (Miranda et al., 2018; Chowdhury et al., 2022). However, the phenomenon of bacteria resistant to antibiotics makes treatment more difficult and expensive (Prestinaci et al., 2015; Serwecinska, 2020; Yuan et al., 2022). Research results by Pham et al. (2023) illustrated that *A. schubertii* isolated from diseased snakeheads in Vinh Long province was resistant to many antibiotics. So far, however, the study on the antibiotic susceptibility of this bacterium in diseased snakehead fish in An Giang province has not been carried out. Therefore, the goal of this study was to provide the latest information on *A. schubertii*'s susceptibility to various antibiotics, thereby contributing to the selection of appropriate antibiotics to treat disease and reducing bacterial resistance to antibiotics in snakehead fish in the future.

MATERIALS AND METHODS

Sample collection of diseased snakehead fish

The diseased snakehead fish sample (weighing 500–700 g) was collected from ponds (12 ponds) in three districts of Tri Ton, Thoai Son, and Long Xuyen of An Giang province to isolate *A. schubertii* (Figure 1). Diseased fish (moribund) with typical signs such as lethargy swimming and white nodules in the liver, kidney, and spleen were randomly collected in ponds ranging in area from 1,500 to 2,000 m^2. Samples were collected several times from 3–4 fish per pond during the period from April 2023 to June 2023.

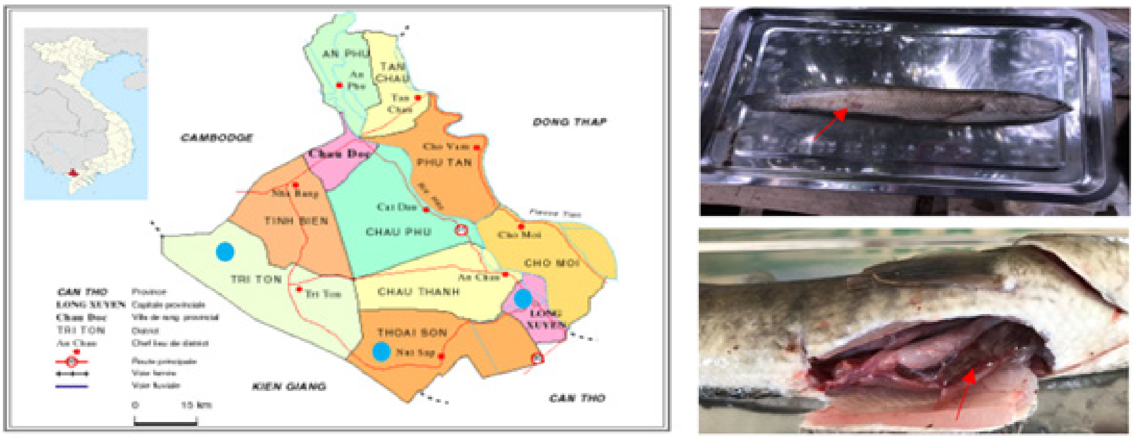

Figure 1 Location of diseased fish sample collection (light blue circle) and diseased fish for *A. schubertii* isolation

Bacterial isolation

A. schubertii bacteria was isolated on TSA medium (HiMedia, India) according to the manuals of Frerichs and Millar (1993) and Tu Thanh Dung et al. (2019). Briefly, the fish was cleaned of fish slime and aseptically dissected. Bacteria were isolated from the kidney, liver, and spleen of diseased fish and incubated at 28–30^{o}C for 24 hours. Bacterial strains were cultured several times until pure and stored in 20% (v/v) glycerol at -80^{o}C.

Bacterial identification

Bacterial strains after being purified will be checked for morphological characteristics (color, shape, and size of colonies), basic physiology, and biochemical characteristics, such as Gram stain, spore staining, motility, oxidase, and catalase reactions (Buller, 2014). The bacteria were then tested using the API 20E kit (Biomérieux, France). In addition, bacteria were also identified by biomolecular methods and sequenced with the 16S rRNA gene with primers 27F: 5'-AGAGTTTGATCCTGGCTC-3' and 1492R: 5'-TACGGTTACCTTGTTACGACT-3' (Heuer et al., 1997).

PCR reaction components include: 1X PCR buffer; 1.5 mM MgCl2; 150 µM dNTPs; 2U *Taq* DNA polymerase; 20 pmol of forward primer (27F) and reverse primer (1492R) and 20–40 ng of bacterial DNA. The thermal cycle performed the PCR reaction, consisting initial denaturation stages at 94°C for 5 minutes, then 35 cycles including denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute, extension at 72°C for 2 minutes and the final elongation at 72°C for 10 minutes. The amplified DNA product is 1,500 bp in size. Bacteria were randomly selected for sequencing at Macrogen, Korea (www.macrogen.com).

Antibiogram

By using the Kirby-Bauer disc diffusion method on Muller-Hinton agar (MHA, Merck), antibiotic susceptibility was assessed (Bauer et al., 1966). Twelve antibiotics were used to perform the antibiogram: amoxicillin (AMO/25 µg), cefalexin (CEL/30 µg), ceftazidime (CEF/30 µg), florfenicol (FLO/30 µg), ciprofloxacin (CIP/5 µg), flumequine (FLU/30 µg), doxycycline (DOX/30 µg), tetracycline (TET/30 µg), erythromycin (ERY/15 µg), rifampicin (RIF/30 µg), novobiocin (NOV/5 µg) and colistin (COL/10 µg) (Oxoid, UK).

In brief, single bacterial colonies were suspended in 0.85% saline solution, and the turbidity matched the 0.5 McFarland standard (bioMerieux, France), and streaked on a TSA plate. Then, the bacterial solution was spread on the surface of the MHA agar. Finally, the antibiotic discs were placed on the agar. After 24–48 hours of incubation at 28°C, the inhibition zone diameter was measured and followed the standards of the Clinical and Laboratory Standards Institute (CLSI, 2015; CLSI, 2020) to determine the resistance (R), intermediate (I), and susceptibility (S) of bacteria to antibiotics. The reference strain *Escherichia coli* ATCC 25922 was used as quality control.

Multiple antibiotic resistance was considered for isolates that were resistant to at least two antibiotics (Sarter et al., 2007). The multi-resistance index (MAR) of each bacterial strain was determined as follows: MAR = X/Y, where X = total antibiotic resistance and Y = total number of antibiotics used to perform the antibiogram (Krumperman, 1983). A MAR value > 0.2 demonstrates that antibiotics are frequently used, while a MAR value ≤ 0.2 shows that antibiotics are rarely or never used in snakehead ponds.

Data analysis

Descriptive statistics were used to determine MAR index values, and antimicrobial resistance. The sequence similarity of bacterial strains was compared with sequences in the National Center for Biotechnology Information (NCBI) data bank using the BLASTn program. The bacterial sequences will be compared with each other (multialignment) using the

CLUTAL W program (Thompson et al., 1997). The phylogenetic tree showing the genetic relationships between bacterial strains was constructed by using MEGA6 (Molecular Evolutionary Gentics Analysis) software based on the Neighbor-joining algorithm (Saitou and Nei, 1987) with bootstrap values of 1,000 replications (Tamura, 2013).

RESULTS

Isolation of bacteria

A total of 29 bacterial strains originated from the liver, kidney, and spleen of internal white spot disease fish on TSA medium. The tested results displayed that most of the colonies of bacterial strains isolated on TSA medium had pale yellow colonies, convexity, whole cover, and tiny round colonies with colony sizes ranging from 0.5–1 mm after 36–48 hours of incubation at 28°C (Figure 2A). All bacterial strains were motile, non-spore-forming, Gram-negative, and short rod-shaped (Figure 2B), catalase and oxidase-positive reactions (Figure 2C). In addition, the results also showed that all bacterial strains were capable of fermenting glucose under both aerobic and anaerobic conditions (Figure 2D).

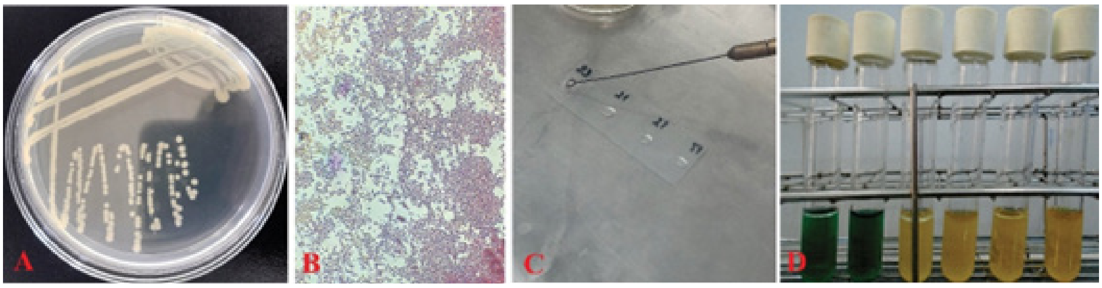

Figure 2 Characteristics of *A. schubertii* (isolate As 9) derived from diseased snakehead fish A. Bacterial colonies grow on TSA medium; B. Gram staining (100X); C. Catalase activity; D. Oxidation/Fermentation reaction

The API 20E kit indicated that isolate As9 and As11 were positive for gelatin, and glucose and negative for ortho-nitrophenyl galactosidase, arginine, lysine, Voges-Proskauer, ornithine, citrate, H_2S, urease, tryptophane deaminase, indole, inositol, mannitol, saccharose, amygdalin sorbitol, rhamnose, melibiose, and arabinose. The morphological, and biochemical characteristics of strain As9 and reference strain *A. schubertii* ATCC 43700 (Buller, 2014) are detailed

Table 1 Morphological, and biochemical characteristics of strain As9 and As11

Phenotypic characteristics	Isolate As 9	Isolate As 11	*A. schubertii* ATCC 43700*
Gram stain	Gram negative	Gram negative	Gram negative
Shape	Short-rod	Short-rod	Short-rod
Motility	+	+	+
Oxidase reaction	+	+	+
Catalase reaction	+	+	+
Oxidation/Fermentation	+/+	+/+	+/+
Orthnitrophenyl galactosidase (ONPG)	-	-	-
Arginine	-	-	+
Lysine	-	-	+
Ornithin	-	-	-
Citrate utilization	-	-	+
H_2S production	-	-	-
Urease	-	-	-
Tryptophane deaminase	-	-	
Indole production	-	-	-
Voges–Proskauer reaction	-	-	-
Gelatin	+	+	+
Glucose	+	+	+
Mannitol	-	-	-
Inositol	-	-	
Sorbitol	-	-	-
Rhamnose	-	-	-
Sucrose	-	-	-
Melibiose	-	-	-
Amygdalin	-	-	
Arabinose	-	-	-

*+: positive, -: negative, * Buller (2014)*

Identification results by PCR technique

All isolates were able to amplify the 16S rDNA gene fragment at size of 1,500 bp by PCR (Figure 3). in Table 1.

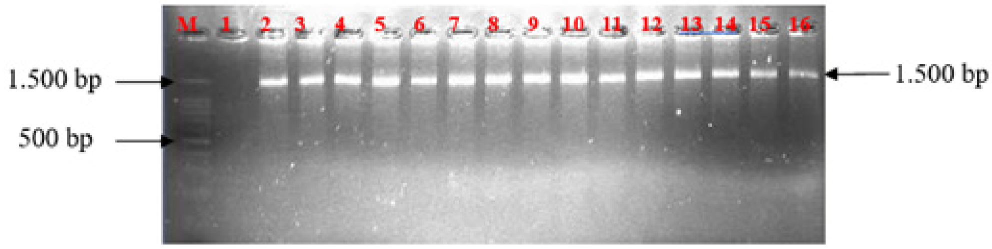

Figure 3 Results of amplification of 16S rRNA gene segments of representative bacterial strains by PCR

Sequencing results showed that the strain As9 was 98.71% homologous to *A. schubertii* strain ZL-1 (MK410038.1), *A. schubertii* strain Gui210820 (OP328816.1), and *A. schubertii* strain CDC 2446-81 (NR_037014. 2). Meanwhile, strain As11 homologous to *A. schubertii* strain GZ210812 (ON606290.1), *A. schubertii* strain CUVETASC03 (ON007120.1), and *A. schubertii* strain WL-1 (JQ303345.1) were 97.07%, 97.06%, and 97.06%, respectively. The phylogenetic tree displayed that two strains of As9 and As11 were distributed in the same group (Figure 4). Strain As9 is closely related to *A. schubertii* strain ZL-1 (MK410038.1) and *A. schubertii* strain Gui210820 (OP328816.1), while strain As11 is closely related to *A. schubertii* strain CDC 2446-81 (NR_037014.2).

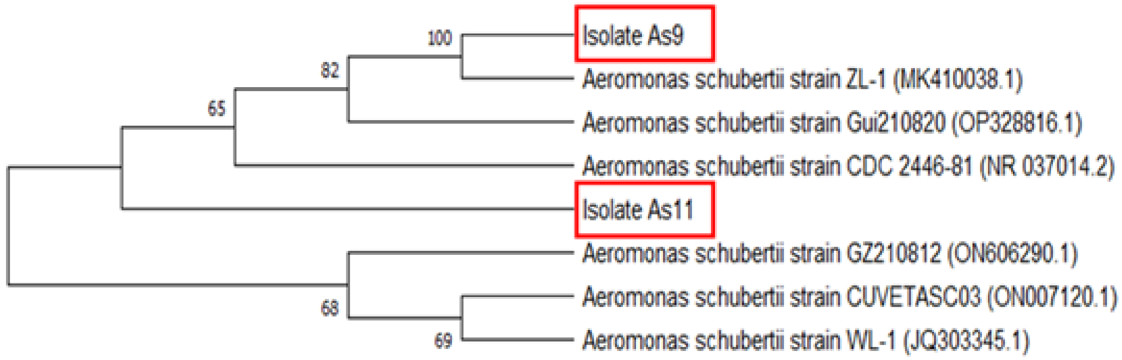

Figure 4 Phylogenetic tree of two isolates based on partial 16S rRNA gene sequences (bootstrap values are given at branching points)

Antibiogram results

Antibiogram results revealed that the percentage of isolates susceptible to ceftazidime, cefalexin, and doxycycline antibiotics was 86%, 59%, and 55%, respectively (Figure 5). Meanwhile, bacteria were highly resistant to amoxicillin (93%), flumequine (83%), ciprofloxacin (76%), rifampicin (66%), erythromycin (62%), colistin (59%), florfenicol (59%), and novobiocin (41%). Therefore, the antibiogram analysis results indicated the lowest resistance to the antibiotic ceftazidime (3%) and low susceptibility to the antibiotics colistin (3%) and amoxicillin (3%) (Figure 5).

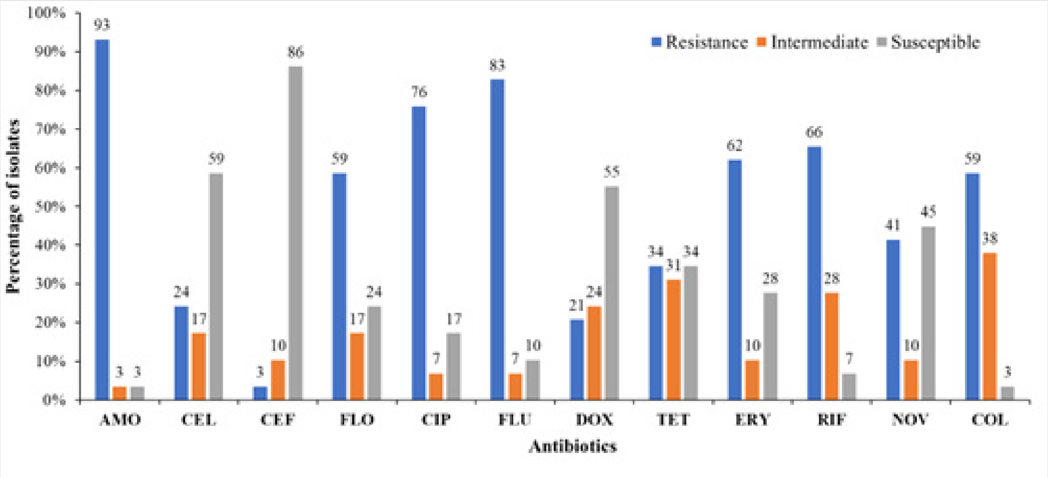

Figure 5 Percentage of bacterial strains susceptibility to antibiotics
AMO: amoxicillin, CEL: cefalexin, CEF: ceftazidime, FLO: florfenicol, CIP: ciprofloxacin, FLU: flumequine, DOX: doxycycline, TET: tetracycline, ERY: erythromycin, RIF: rifampicin, NOV: novobiocin, COL: colistin.

Multidrug resistance of bacterial strains

The findings showed that 100% of bacterial strains in the study were multi-drug resistant, of which the most resistant bacteria were 10 antibiotics (10%) and the least resistant were two antibiotics (3%). In addition, the isolates with multi-resistance to 6 and 7 antibiotics accounted for the highest rate (21%), followed by multi-resistant to 8 antibiotics (14%), multi-resistant to 4 and 5 antibiotics (10%), and the lowest, multi-resistance to 2 and 9 antibiotics (3%) (Figure 6).

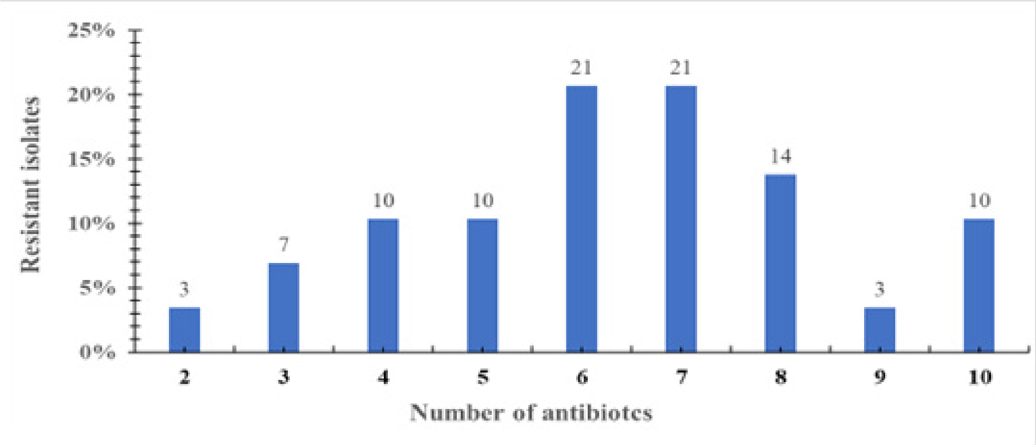

Figure 6 Rate of multidrug resistance in bacterial isolates.

Multidrug-resistant phenotypes of bacteria

The study identified 28 different multi-resistance phenotypes from 29 bacterial isolates for 12 tested antibiotics (Table 2). The analysis results showed that there are two most common multi-resistant phenotypes of bacteria: AMO-CEL-CIP-FLU-DOX-TET-ERY-RIF-NOV-COL and AMO-CEL-CIP-FLU-DOX-TET-ERY-RIF-NOV-COL, accounting for 7% (Table 2).

Table 2 Bacterial multi-resistant phenotypes

Bacterial multi-resistant phenotypes	Number of bacterial strains	Ratio (%)
AMO-ERY	1	3
AMO-CEL-CIP	1	3
AMO-FLU-RIF	1	3
AMO-FLU-TET-COL	1	3
AMO-FLU-ERY-COL	1	3
AMO-FLU-NOV-COL	1	3
AMO-FLO-CIP-FLU-RIF	1	3
AMO-CIP-FLU-RIF-COL	1	3
FLU-TET-RIF-NOV-FLO	1	3
AMO-CEL-FLO-CIP-FLU-ERY	1	3
AMO-CEL-FLO-RIF-NOV-COL	1	3
AMO-CAF-FLO-CIP-FLU-RIF	1	3
AMO-FLO-CIP-FLU-RIF-NOV	1	3
AMO-FLO-CIP-FLU-RIF-COL	1	3
AMO-CIP-FLU-ERY-NOV-COL	1	3
AMO-CEL-FLU-CIP-ERY-RIF-COL	1	3
AMO-CAF-CIP-FLU-ERY-NOV-COL	1	3
AMO-FLO-CIP-FLU-ERY-RIF-NOV	1	3
AMO-FLO-CIP-TET-ERY-NOV-COL	1	3
AMO-CIP-FLQ-DOX-TET-ERY-RIF	1	3
CIP-FLO-FLU-DOX-TET-ERY-COL	1	3
AMO-FLO-CIP-FLU-DOX-TET-ERY-RIF	1	3
AMO-FLO-CIP-FLU-DOX-ERY-RIF-NOV	1	3
AMO-FLO-CIP-FLU-ERY-RIF-NOV-COL	1	3
AMO-CIP-FLU-DOX-TET-ERY-RIF-COL	1	3
AMO-FLO-CIP-FLU-TET-ERY-RIF-NOV-COL	1	3
AMO-CEL-FLO-CIP-FLU-TET-ERY-RIF-NOV-COL	1	3
AMO-CEL-CIP-FLU-DOX-TET-ERY-RIF-NOV-COL	1	3
AMO-CEL-CIP-FLU-DOX-TET-ERY-RIF-NOV-COL	2	7

AMO: amoxicillin, CEL: cefalexin, CEF: ceftazidime, FLO: florfenicol, CIP: ciprofloxacin, FLU: flumequine, DOX: doxycycline, TET: tetracycline, ERY: erythromycin, RIF: rifampicin, NOV: novobiocin and COL: colistin.

Multidrug resistance index of bacterial strains

The results revealed that 96.55% of bacterial strains had a multi-resistance index higher than 0.2 (the MAR index ranged from 0.25–0.83), except for the strain As58, which had a multi-resistance index less than 0.2 (Figure 7). This result indicated that antibiotics are frequently used in snakehead fish ponds.

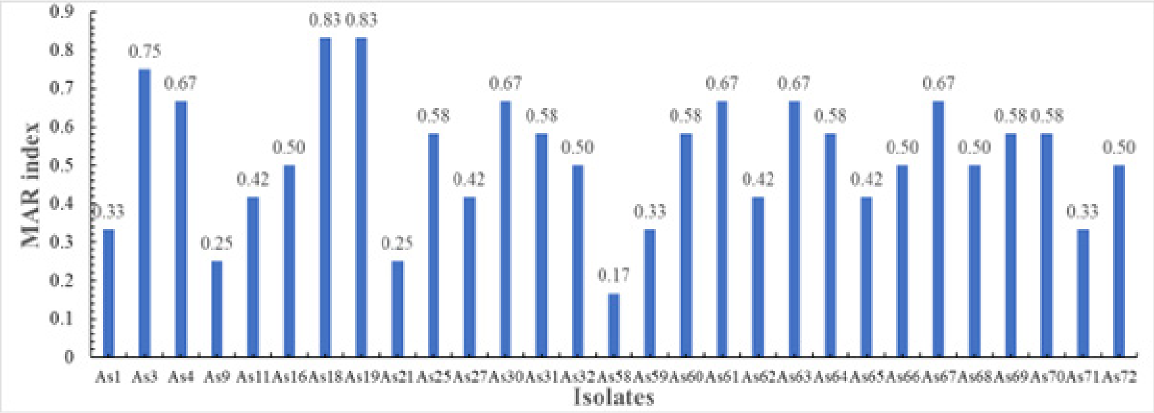

Figure 7 Rate of multidrug resistance in bacterial isolates.

DISCUSSION

Bacterial drug resistance has been reported in many areas, such as food, veterinary animals, and humans (Hashempour-Baltork et al., 2019; Samtiya et al., 2022; Kim and Ahn, 2022; Urban-Chmiel et al., 2022; Xu et al., 2022). Besides, the resistance of bacteria in aquatic animals has also been recorded in many countries (Schar et al., 2021; Okeke et al., 2022). In the current research, all isolates were resistant to antibiotics to different levels (ranging from 41 to 93%), with some bacteria being highly resistant to antibiotics such as amoxicillin (93%), flumequine (83%), ciprofloxacin (76%), rifampicin (66%), erythromycin (62%), colistin (59%), florfenicol (59%), and novobiocin (41%; Figure 5). Antibiotic-resistant *Aeromonas* bacteria are reported to be common in aquaculture (Dhanapala et al., 2021). The resistance of bacteria in aquatic animals is considered a major threat because many studies show that resistant bacteria all carry resistance genes and can transmit resistance genes to pathogenic bacteria through horizontal gene transfer processes such as transformation, transduction, and conjugation (Tu Thanh Dung et al., 2009; Hong et al., 2018; Jia et al., 2022; Yuan et al., 2023). In particular, this process is very dangerous for *Aeromonas* bacteria such as *A. hydrophila*, *A. caviae*, *A. veronii* biovar *sobria*, *A. jandaei*, *A. trota*, and *A. schubertii* because these bacterial species have the potential to cause disease in humans (Hickman-Brenner et al., 1988; Janda et al., 1994; Martins et al., 2002; Janda and Abbott, 2010; Ugarte-Torres et al., 2018; Pessoa et al., 2022; Grave et al., 2022). Therefore, to clarify this issue, studies on the gene exchange process between *A. schubertii* bacteria and other bacteria in ponds are necessary. Besides, antibiotic susceptibility can be influenced by isolated bacterial strains and different factors such as water sources, farming practices, and geographical locations. However, within the scope of our research, this work only concentrates on *A. schubertii* strains isolated from diseased fish. Hence, it is necessary to collect a large number of representative samples from the above sources to evaluate the antibiotic sensitivity of bacteria more accurately. Moreover, the disc diffusion method's limitations, such as its restricted antibiotic range and qualitative measurement, must be taken into account. In the future study, the MIC approach are essential for a more precise and trustworthy resistance evaluation.

For β-lactam antibiotics, the isolates in this study were highly resistant to amoxicillin (93%) (Figure 5). The rate of bacteria resistant to amoxicillin is lower than in the studies of Doan Thi Minh Chau et al. (2018) and Pham et al. (2023). This group of authors recorded that the rate of amoxicillin resistance of bacteria *A. schubertii* deriving from diseased snakehead fish in Tra Vinh and Vinh Long provinces was 100% and 96%, respectively. Research by Algammal et al. (2020) revealed that 100% of *A. hydrophila* isolated from tilapia, *Oreochromis niloticus*, in Egypt were resistant to amoxicillin. Meanwhile, the rate of bacteria resistant to cefalexin was 24%, and the rate of bacteria only resistant to ceftazidime was 3%. This result is in line with the study of Pham et al. (2023), which reported relatively low bacterial resistance to cefalexin and ceftazidime with rates of 12% and 4%, respectively. However, the study of Doan Thi Minh Chau et al. (2018) indicated that the bacteria were completely resistant to cefalexin (100%). The genus *Aeromonas* is considered to be naturally resistant to β-lactam antibiotics (Wen-Chen et al., 1998; Saavedra et al., 2004; Chen et al., 2012). This is because the β-lactam rings are chemically unstable, which is rapidly hydrolyzed by the enzyme β-lactamase produced by *Aeromonas* (Goni-Urriza et al., 2000). The high prevalence of bacteria resistant to β-lactam antibiotics in the study suggests that the β-lactamase gene may be widespread in the gene pool of this bacterium (Piotrowska et al., 2017; Bush and Bradford, 2020; Santosaningsih et al., 2023). Pham et al. (2023) sequenced the genomes of two strains of *A. schubertii*, including strain WL1483 and LF1708. The results revealed that gene clusters encoding for β-lactamase were found in both genomes, including *BlrA* (β-lactam response regulator transcription factor), *BlrB* (β-lactam sensor histidine kinase), and class D beta-lactamase genes. Hence, it is necessary to identify β-lactamase genes to elucidate this issue in the future.

Florfenicol is one of the antibiotics commonly used in snakehead fish farming models in the Mekong Delta, Vietnam (Nguyen Quoc Thinh et al., 2020). Previous studies have shown that many different bacterial species causing diseases in humans, veterinary animals, and aquatic animals have been resistant to florfenicol, such as *Vibrio cholerae* (Das et al., 2020), *Escherichia coli* (Li et al., 2020), *K. pneumoniae* (Lu et al., 2018), *Salmonella* (Nair et al., 2018; Nahar et al., 2018), *Yersinia enterocolitica* (von Altrock et al., 2010), *Proteus* (Sanches et al., 2023), and *Leclercia adecarboxylata* (Ying et al., 2019). The antibiogram analysis results in this study demonstrated that 59% of the bacteria were resistant to florfenicol (Figure 5). This rate is lower than the study of Doan Thi Minh Chau et al. (2018), which was 79.17%, while Pham et al. (2023) was 92%. Research by Revina et al. (2017) depicted that *Aeromonas* spp. isolated from *Salmo trutta* salmon were resistant to florfenicol (4.55%). Research by Dang et al. (2022) indicated that 20% of *A. hydrophila* derived from freshwater fish in the Red River Delta, Vietnam, were resistant to florfenicol. Meanwhile, the study of Thaotumpitak et al. (2023) showed that 100% of strains of *A. hydrophila* that originated from caged tilapia in Thailand were susceptible to florfenicol.

For quinolone antibiotics, the study revealed that the isolates were highly resistant to ciprofloxacin and flumequine, with a rate of 76% and 83%, respectively (Figure 5). So, the rate of bacteria resistant to flumequine in this report is higher than that of Doan Thi Minh Chau et al. (2018) (54.17%), but lower than that of Pham et al. (2023), which is 88%. Meanwhile, the study of Quach Van Cao Thi et al. (2023) indicated that *A. hydrophila* isolates deriving from hemorrhagic pangasius had a ciprofloxacin resistance rate of 20%. Previous studies have shown that the rate of bacterial resistance to quinolone antibiotics is usually less than 25% (Miranda and Zemelman, 2001; Miranda and Zemelman, 2002; Akinbowale et al., 2006). Research by Chen et al. (2012) exhibited that *A. schubertii* strain ZS20100725 collected from snakehead fish *Channa maculata* (Lacepede) in China was sensitive to ciprofloxacin. Similarly, a study by Liu and Li (2012) showed that strains of *A. schubertii* originated from snakehead fish, *Ophiocephalus argus* (Cantor), in China were also susceptible to ciprofloxacin. El-Ghareeb et al. (2019) indicated that 92.59% of *A. hydrophila* obtained from *Tilapia nilotica* and *Mugil cephalus* in Egypt were susceptible to ciprofloxacin. Meanwhile, the study by Algammal et al. (2020) showed that 100% of *A. hydrophila* collected from tilapia, *Oreochromis niloticus*, were susceptible to ciprofloxacin.

Antibiotics belonging to the tetracycline group have a broad spectrum of activity, so they are widely used in aquaculture (Bondad-Reantaso et al., 2023). Therefore, the emergence of bacterial strains resistant to this group has been reported in many previous studies (Akinbowale et al., 2007; Kim et al., 2011; Li et al., 2022). In this study, the results showed that bacteria resistant to tetracycline and doxycycline were 34% and 21%, respectively (Figure 5), which is higher than those reported by Pham et al. (2023), which are 16% and 4%. However, the study by Carnahan et al. (1989) revealed that *A. schubertii* was still susceptible to tetracycline. Research by Doan Thi Minh Chau et al. (2018) exhibited that *A. schubertii* obtained from diseased snakehead fish in Tra Vinh province was highly susceptible to doxycycline (83.33%). However, the study results also showed that bacteria were highly resistant to oxytetracycline (79.17%) and tetracycline (58.33 %). Meanwhile, the study by Algammal et al. (2020) demonstrated that *A. hydrophila*, which originated from tilapia, *Oreochromis niloticus*, in Egypt, was resistant to tetracycline with a rate of 90.1%. According to Li et al. (2022), 49% of *Aeromonas* spp. derived from *Pelteobagrus fulvidraco* and hybrid snakehead (*Channa maculata* x *Channa argus*) in China are resistant to tetracycline.

For macrolide antibiotics, the antibiogram results demonstrated that the isolates in the study were resistant to over 50%, specifically erythromycin (83%), and rifampicin (66%) (Figure 5). Hence, the rate of bacteria resistant to erythromycin in the study was higher than that of Doan Thi Minh Chau et al. (2018) (70.83%), but lower than that reported by Pham et al. (2023), which was 84%. However, the findings of Doan Thi Minh Chau et al. (2018) revealed that 100% of the bacteria were resistant to rifampicin. Research results by Vivekanandhan et al. (2002) indicated that *A. hydrophila* derived from fish and shrimp in India was 100% resistant to rifampicin and more than 95% resistant to erythromycin. The review results of Stratev and Odeyemi (2016) showed that *A. hydrophila* isolated from aquatic animals was resistant to erythromycin with a rate ranging from 43–100%, while the bacteria was resistant to rifampicin with a rate less than 50%. The results of Pham et al. (2023) found that 84% of the bacteria were resistant to rifampicin. Meanwhile, the study by Algammal et al. (2020) exhibited that *A. hydrophila*, which originated from the tilapia *Oreochromis niloticus* in Egypt, was resistant to erythromycin at a rate of 39.3%.

The findings found a high percentage of bacteria resistant to aminoglycoside antibiotics, specifically novobiocin (41%; Figure 5). This result is lower than the study of Doan Thi Minh Chau et al. (2018), which was

62.5%, and the report by Pham et al. (2023), which was 48%. Belem-Costa et al. (2007) showed that 100% of strains of *A. hydrophila* derived from pacu, *Piaractus mesopotamicus* (Holmberg, 1887), and tilapia, *Oreochromis niloticus* (Linnaeus, 1758), were resistant to novobiocin. Similar results were also reported in the study by Odeyemi and Ahmad (2017), showing that *Aeromonas* species originated from different water sources in Melaka, Malaysia, were completely resistant to novobiocin. Rahman et al. (2021) showed that 20% of *A. hydrophila* isolated from *Clarias batrachus* was resistant to novobiocin. Meanwhile, the study by Wei et al. (2015) showed that *A. hydrophila* from red tilapia (*Oreochromis* sp.) in Kelantan, Malaysia, was resistant to novobiocin at a rate of 40%.

Colistin, a polymyxin antibiotic, was introduced into clinical practice in the 1960s but was replaced shortly after in the 1970s due to its toxicity (Li et al., 2006). However, many studies have shown that *Aeromonas* spp. are resistant to this antibiotic (Tuo et al., 2018; Gonzalez-Avila et al., 2021). The findings showed that bacteria were highly resistant to colistin (59%; Figure 5). This investigation is higher than that reported by Pham et al. (2023), which is 28%. However, the antibiogram results of Doan Thi Minh Chau et al. (2018) showed that 24 strains of *A. schubertii* were sensitive to colistin at a rate of 79.17%. The study by Kaskhedikar and Chhabra (2009) showed that 100% of *A. hydrophila* from retail chicken samples in India were resistant to colistin. Bacteria are resistant to colistin because they have *mcr* genes (mobile colistin resistance) such as *mcr-1*, *mcr-3*, and *mcr-5* (Tuo et al., 2018; Gonzalez-Avila et al., 2021). Research by Thaotumpitak et al. (2023) found the *mcr-3* gene in a strain of *A. hydrophila* isolated from caged red tilapia in Thailand. Therefore, further studies need to be performed to identify these resistance genes and shed light on this issue.

The phenomenon of bacterial multidrug resistance in aquaculture has been reported in many previous studies (Resende et al., 2012; Scarano et al., 2018; Koudou et al., 2020; Ha et al., 2023; Pham et al., 2023). In this investigation, 100% of bacteria exhibited multidrug resistance (Figure 6). This result is in line with the study by Nguyen et al. (2014), which showed that the percentage of *Pseudomonas* spp. and *Aeromonas* spp. isolates from catfish ponds in the Mekong Delta illustrated multidrug resistance of 96.6% and 61.9%, respectively. Tartor et al. (2021) reported that 87.2% of *A. hydrophila* isolates from diseased tilapia (*Oreochromis niloticus*) in Egypt were multi-resistant to tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin, ampicillin, florfenicol, and erythromycin. Similarly, the study by Eid et al. (2022) showed that 90% of *Aeromonas* spp. collected from fish and multi-drug-resistant water samples. Research by Woo et al. (2022) showed 18.6% of *A. hydrophila* and 24.2% of *A. veronii* isolated from several multi-drug resistant aquatic animals. Research by Quach Van Cao Thi et al. (2023) indicated that 100% of strains of *A. hydrophila* isolated from multidrug-resistant hemorrhagic disease catfish. Recently, a study by Sherif and Kassab (2023) also presented that eight strains of *A. hydrophila* isolated from tilapia were also multi-resistant to antibiotics such as tetracycline, trimethoprim, and sulfamethoxazole.

This investigation demonstrated that 96.55% of bacteria had a MAR index greater than 0.2, of which the lowest MAR index was 0.17 and the highest was 0.83 (Figure 7). This result showed that bacterial strains were obtained from areas with high and frequent use of antibiotics in snakehead fish ponds. Hence, the MAR index of the bacterial strains in the study is consistent with the previous research results (Sarter et al., 2007; Matyar et al., 2010; Su et al., 2011). The report of Laith and Najiah (2013) showed that multi-resistant strains of *A. hydrophia* have a MAR index ranging from 0.1 to 0.5. In the Mekong Delta, research by Nguyen et al. (2014) on strains of *Pseudomonas* spp. and *Aeromonas* spp. isolates from catfish ponds also had multi-resistance indexes of 0.457 and 0.293, respectively. Similarly, the MAR index of pathogenic bacteria in salmon farmed in Turkey was also determined to range from 0.2 to 0.6 (Türe and Alp, 2016). The findings are agree with previous investigations showing that antibiotics are commonly used in aquaculture in the Mekong Delta (Truong Quoc Phu and Tran Kim Tinh, 2012; Nguyen Quoc Thinh et al., 2014). Research by Le Minh Long et al. (2014) indicated that there are 19 types of antibiotic-containing products used in pangasius ponds in Dong Thap province. Meanwhile, the investigation results of Phu et al. (2015) revealed that there are 24 different antibiotics used in fish ponds, and most of the antibiotics are often combined together to prevent and treat diseases in pangasius in the Mekong Delta.

CONCLUSIONS

Based on the morphological, biochemical, and 16S rRNA gene sequencing results, the findings isolated *A. schubertii* strains from the liver, kidney, and spleen of a white spot disease snakehead intensively cultured in An Giang province. Two strains of As9 and As11 are homologous to *A. schubertii* strains ZL-1 and GZ210812 at rates of 98.71% and 97.07%, respectively. The isolated bacteria in the study were highly resistant to amoxicillin, flumequine, ciprofloxacin, rifampicin, erythromycin, colistin, florfenicol and novobiocin, and susceptible to ceftazidime, cefalexin and doxycycline. In particular, 100% of the bacterial strains in the study displayed various multidrug-resistant phenotypes, resistant to at least two antibiotics or more.

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