ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGENS CAUSING HEMORRHAGIC DISEASE IN ASIAN SWAMP EEL (*Monopterus albus* Zuiew, 1793) IN THE MEKONG DELTA

Tu Thanh Dung[1], Nguyen Si Thanh[2], Le Minh Khoi [1], Nguyen Bao Trung [1,*]

ABSTRACT

Several types of diseases occur during Asian swamp eel (*Monopterus albus*) culture, the most prevalent being bacterial hemorrhagic disease. In the Mekong Delta, farming Asian swamp eel (*M. albus*) is severely affected by a highly fatal hemorrhagic disease that causes massive economic losses. This study aimed to determine the causative agent of hemorrhagic disease in Asian swamp eels. Several clinical signs were observed, including red swollen vents, head edema, abdomen and/or internal organ hemorrhage. A total of 52 bacterial isolates were recovered on Tryptone Soya Agar from different diseased eel farms in the Mekong Delta, including Hau Giang, Can Tho, An Giang, Vinh Long, and Bac Lieu provinces. A bacterial morphology examination showed that the isolates were Gram-negative, short rod-shaped, 0.1 - 0.3 mm colony size with creamy yellow color after 24 hours incubation at 28°C. Furthermore, an assessment of the biochemical identification system indicated the presence of motile *Aeromonas* spp. In addition, the 16S rRNA gene sequencing results confirmed that the majority of the isolates were *Aeromonas veronii*. A challenge test was carried out to determine the pathogenicity of *A. veronii* isolates collected from the eels. The calculated LD_{50} value of the CT07 isolate was $1.51x10^4$ CFU/eel after a 14-day injection in eels with clinical signs similar to those observed in diseased eels on the farms. Moribund eels were also sampled for histopathological examination. Moreover, antibiotic susceptibility testing of 19 isolates was performed using eight antibiotics in this study.

Keywords: *Aeromonas veronii, Asian swamp eel, challenge test, hemorrhagic disease, antibiotic susceptibility.*

Received: 12 June 2023; revised: 8 August 2023; accepted: 18 August 2023

1. INTRODUCTION

The Asian swamp eel (*Monopterus albus*) is a commercially crucial cultured freshwater fish in China and Southeast Asian countries, including Vietnam [1]. In Vietnam, seafood is considered a key economic sector, particularly in the Mekong Delta. Scientists are especially interested in the eel since it has the ability to change females [2]. Along with that development, farmers also face a big problem of disease in Asian swamp eels. In particular, a group of bacteria, *Aeromonas* spp., was commonly recorded as the main pathogen in freshwater fish in the Mekong Delta.

Aeromonas veronii has caused significant losses in many cultured freshwater and marine fish species worldwide. It has infected almost all freshwater and some brackish water fish species and caused mass mortalities in severe production and economic losses [3]. *Aeromonas* spp. isolated from EUS-infected fish was found virulent in fish on artificial infection studies [4]. Among different motile *Aeromonas* species, *A. veronii* has recently emerged as one of the important fish pathogens causing infections in catfish, *Ictalurus punctatus* [5], tilapia, *Oreochromis niloticus* [6], European seabass, *Dicentrarchus labrax* [7], goldfish,

[1] College of Aquaculture and Fisheries, Can Tho University
[2] Advanced Program in Aquaculture, Course 44, College of Aquaculture and Fisheries, Can Tho University
*Email: nbtrung@ctu.edu.vn

Carassius auratus [8], common carp, *Cyprinus carpio* [9]. However, there have been no specific studies on bacterial hemorrhagic diseases in Asian swamp eels in the Mekong Delta. Due to the diversity of pathogens, the prevention and treatment of diseases in aquatic animals is only effective when the causative agent of the disease is accurately identified. To find out more information on the causes of hemorrhagic disease in swamp eels, the study entitled "Isolation and characterization of bacterial pathogens causing hemorrhagic disease in Asian swamp eel (*Monopterus albus* Zuiew, 1793) in the Mekong Delta)" was carried out.

2. MATERIALS AND METHODS

2.1. Sample collection

Asian swamp eels with clinical signs such as hemorrhage at internal organs and/or head edema were collected from eel farms in the Mekong Delta, including Hau Giang, Can Tho, Vinh Long, and Bac Lieu provinces from April 2021 to May 2023. The eel samples were collected directly at the farm and delivered to the laboratory at the Faculty of Aquatic Pathology (CTU) for further analysis.

2.2. Bacterial isolates

The liver and kidney of eel samples were streaked onto tryptone soy agar (TSA, Merck), glutamate starch phenol red (GSP, Merck), and incubated at 28°C for bacterial isolation. After 24 hours of incubation, the predominant colonies were subcultured until pure colonies were obtained. Then, the isolates were preserved at -80°C in tryptone soya broth (TSB, Merck) containing 25% glycerol for further study.

2.3. Morphological and biochemical identification

The obtained colonies were examined for morphological features, including color, Gram staining, motile, oxidase, catalase, and oxidative-fermentative (O/F) tests [10]. Biochemical characterization of the isolates was performed using the API® 20 E (Biomerieux, France) according to the manufacturer's instructions.

2.4. 16S rRNA sequencing and phylogenetic analysis

The isolated colonies were species-identified using 16S rRNA sequences. The bacterial 16S rRNA amplification was conducted using the universal primers 27F (5′-AGAGTTTGATCCTGG CTCAG-3′) and 1492R (5′-GGTTACCTTGTTACG ACTT-3′). The multiple alignments of the 16S rRNA sequences achieved in this study and their closely related species retrieved from GenBank were conducted using the Clustal W method, and the phylogenetic tree was performed using the Neighbor-Joining method with the bootstrap set to 10,000 replicas in MEGA 7.0 software [11].

2.5. Experimental determination of the bacterial virulence

All isolates were evaluated for their capacity to cause disease in eels using 10^6 and 10^5 CFU/mL injection doses. The CT07 strain was selected for further investigation due to its potential to cause eel death (data not shown). Healthy eels (6 - 9 g) were handled in accordance with the Animal Ethics Committee of Can Tho University. The eels were randomly arranged at a density of 10 eels/tank (35 L) with a water column around 10 cm high, aeration, and nylon rope as substrates.

The pathogenic bacteria strain CT07 was recovered from glycerol stocks and streaked directly onto TSA. After 24 hours, a pure colony of each isolate was cultured in 10 mL of TSB overnight at 28°C, 200 rpm. To determine the 50% lethal dose of pathogenic bacteria based on a previous study [12], five groups of healthy eels (n = 150) were injected intraperitoneally with the bacterial strain at concentrations of $7.73x10^2$, $7.73x10^3$, $7.73x10^4$, $7.73x10^5$ CFU/eel. In contrast, the control group was injected with sterile physiological saline (0.9% NaCl). Thereafter, cumulative mortality was monitored for the next 14 days. The liver and kidney of moribund eels were streaked onto TSA medium for bacterial isolation, and eel tissues (liver, kidney, and intestine) were collected for histopathology examination. These re-isolated strains were

biochemically compared to the CT07 strain used in the challenge.

2.6. Histopathological analysis

Internal organs (liver, kidney, and intestine) were collected from moribund eels and fixed in a 10% NBF (Neutral buffered formalin) solution. After 24 hours, the samples were rinsed with running water, transferred to 75 - 100% ethanol for dehydration, cleared with xylene, and finally infiltrated with paraffin wax. The standard paraffin embedding procedure was applied to the section with a thickness of 5 µm, and the slices were stained with hematoxylin and eosin (H&E) methods. Light microscopy (ECLIPSE E200, Nikon) was utilized to detect pathological changes in stained histological sections.

2.7. Investigation of antibiotic susceptibility

Antibiotic susceptibility of 19 bacterial isolates was carried out using the disc diffusion method under the Clinical and Laboratory Standards Institute guidelines [13]. A total of 8 antibiotics, including amoxicillin/clavulanic (AMX/30 µg), cefotaxime (CTX/30 µg), cephalexin (CL/30 µg), colistin sulfate (CT/50 µg), doxycycline (DO/30 µg), florfenicol (FFC/30 µg), rifampicin (RIF/30 µg), trimethoprim/sulfamethoxazole (SXT/1.25/ 23.75 µg), were employed to evaluate bacterial susceptibility. The inhibition zones of diameter were classified as (S) susceptible, (I) intermediate, and (R) resistant [13].

3. RESULTS AND DISCUSSION

3.1. Clinical symptoms and sample collection

The clinical signs of infected Asian swamp eels are shown in figure 1, mainly as a hemorrhage at internal organs and/or head edema. In the nursing stage, abdominal bleeding was the primary clinical symptom (Figure 1.A). In the adult stage, the body of infected eels appeared with sores and/or head edema (Figure 1.B-C).

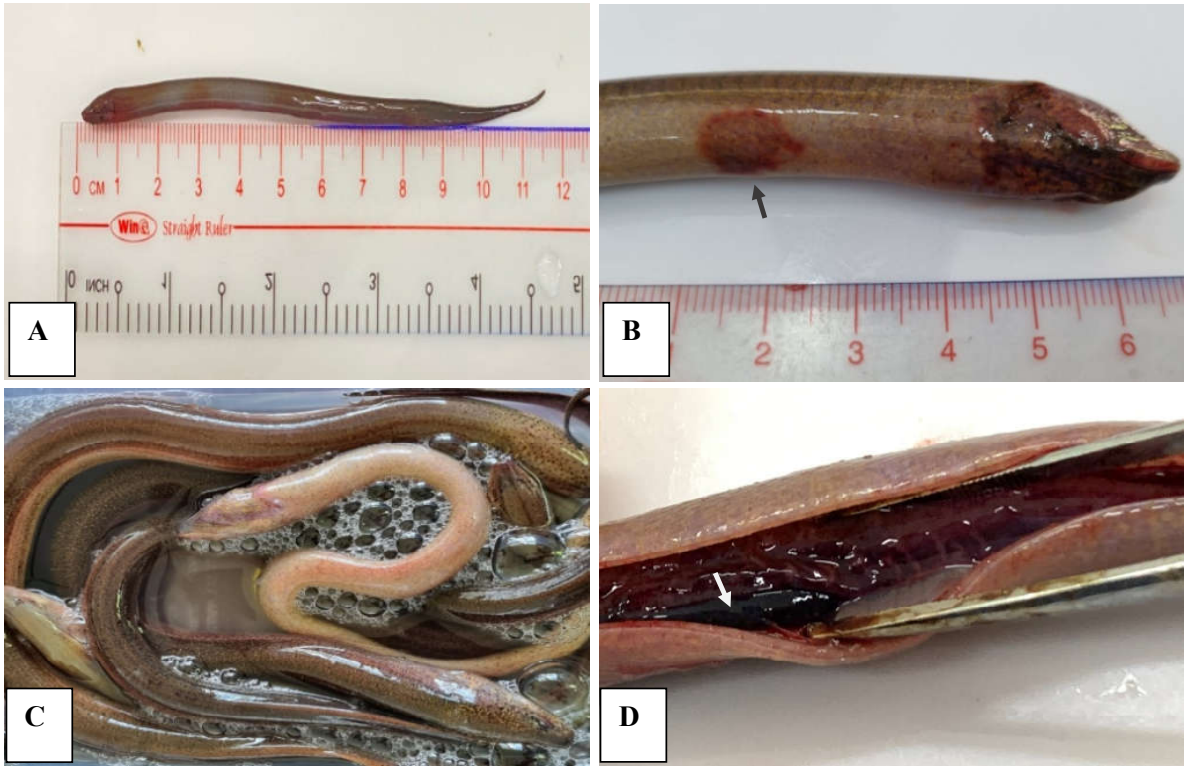

Figure 1. Clinical symptoms of infected Asian swamp eel

(A) Abdominal bleeding in the nursing stage; (B, C) eel with ulceration and edema of the head in the adult stage; (D) clinical sign of internal organ hemorrhage in diseased eels

The results of the bacterial isolation from diseased eels are shown in Table 1. A total of 158 diseased eels were used, and 52 isolates of *Aeromonas* spp. were obtained from the eel liver and kidney.

Table 1. Summarised results of *Aeromonas* spp. isolation from Asian swamp eels infected with hemorrhagic disease

Time	Location	Number of eels sampled	Number of *Aeromonas* spp. isolates
April to June 2021	An Giang province	7	2
June to October 2022	Vinh Long province	21	7
March 2022 to May 2023	Hau Giang province	92	32
April to December 2022	Can Tho city	28	9
May 2023	Bac Lieu province	10	2
Total		158	52

3.2. Bacterial identification

All isolates grew well on TSA after 24 hours incubation at 28°C. The colonies were round, slightly raised, creamy yellow on TSA (Figure 2.A) and yellow on GSP agar (Figure 2.B). The bacterial isolates were capable of causing β-hemolysis on BA medium supplemented with 5% sheep blood (Figure 2.C). The morphological characteristics of the isolated bacteria showed that they were motile, giving positive reactions to catalase, oxidase, oxidizing, and fermenting (O/F) glucose. Gram-staining showed that the bacterial cells stained red/pink and appeared short rod-shaped, indicating that the strain was Gram-negative (Figure 2.D).

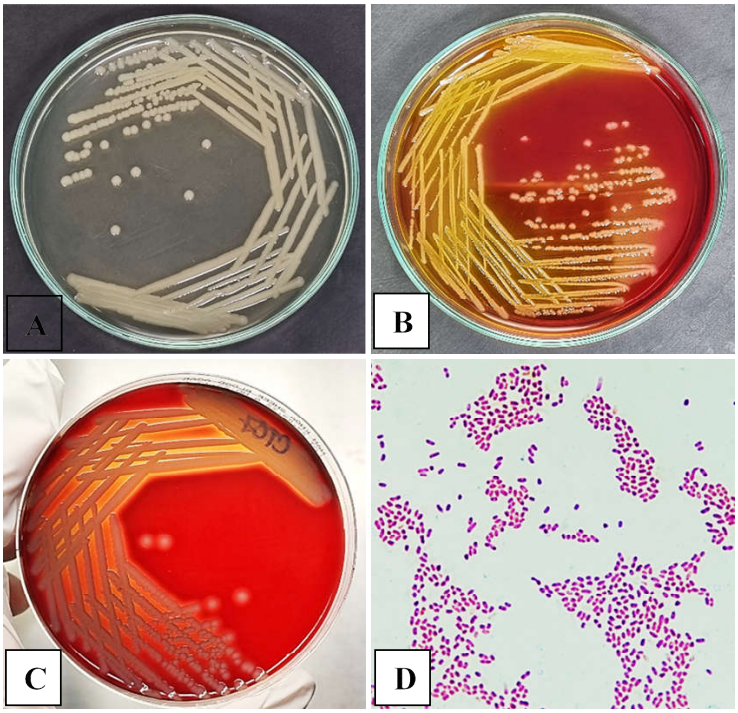

Figure 2. Characterization of the isolated bacteria

(A) Colonies on TSA; (B) Colonies on GSP; (C) Colonies on BA medium supplemented with 5% sheep blood; (D) Gram-stained bacterial cells (100X).

The physiological and biochemical characteristics of 52 isolates are presented in Table 2. According to the biochemical results using the API 20E kit, the bacterial isolates collected from hemorrhagic eels exhibited biochemical features compatible with *Aeromonas* spp., as described by previous studies [14], [15]. The isolated bacteria were phenotypically identical except for the ADH, ODC, TDA, VP, AMY, and ARA reactions (Table 2).

Table 2. Results of physiological and biochemical characteristics of isolated bacteria

Identification test (s)	*Aeromonas veronii* ATCC 35604^T [14]	*Aeromonas veronii* [15]	*Aeromonas* spp. (This study)
Gram	-	-	-
Shape	Rod-shaped	Rod-shaped	Rod-shaped
Motility	Motile	Motile	Motile
Oxidase	+	+	+
Catalase	+	+	+
O/F test	+/+	+/+	+/+
ONPG	+	+	+
ADH	-	+	+
LDC	+	+	+
ODC	+	-	-
CIT	+	+	+
H_2S	-	-	-
URE	-	-	-
TDA	+	-	+
IND	+	+	+
VP	+	-	+
GEL	+	+	+
GLU	+	+	+
MAN	+	+	+
INO	-	-	-
SOR	-	-	-
RHA	-	-	-
SAC	+	+	+
MEL	-	-	-
AMY	+	-	+
ARA	-	-	+

Notes: (+) positive; (-) negative.

In addition to the API 20E examination, 16S rRNA sequencing and phylogenetic analysis are used for species identification. The BLASTn results showed that the CT07 strain was 99.7%

identical to *Aeromonas veronii* strain HN1903 (Accession: MT990643.1) in the NCBI database. After multiple alignments of highly similar sequences, the phylogenetic results indicate that the 16S rRNA gene sequence of *A. veronii* forms a distinct cluster (Figure 3). As a result, the strain was designated as *A. veronii* based on its morphological, physiological, biochemical, and molecular properties.

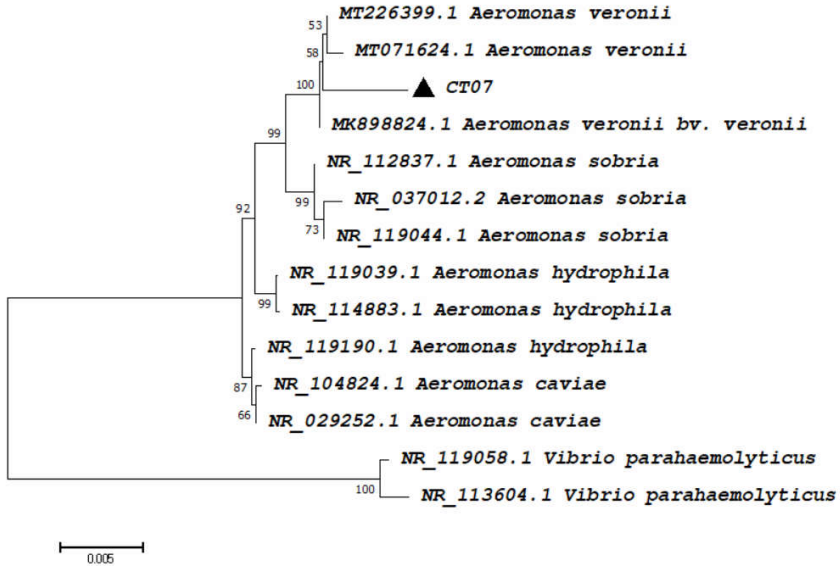

Figure 3. Phylogenetic tree between isolate (CT07) and other reference strains based on 16S rRNA gene sequences. At branching nodes, the bootstrap support values are provided

Motile *Aeromonas* species are diverse and widely distributed in freshwater environments [16]. Previous studies reported that *A. veronii* was an opportunistic bacterium that caused high mortality in aquatic animals [17], [18]. To date, several freshwater species were infected by *A. veronii,* such as channel catfish, *Ictalurus punctatus* [5], tilapia, *Oreochromis niloticus* [6], European seabass, *Dicentrarchus labrax* [7], goldfish, *Carassius auratus* [8], common carp, *Cyprinus carpio* [9]. In this study, the clinical symptoms (Figure 1) were remarkably comparable to those observed in a previous study on Asian swamp eels infected with *A. veronii* in China, including red swollen vents, head edema, abnormal swimming, abdomen hemorrhage and hemorrhage at internal organs [19].

3.3. Pathogenicity of *Aeromonas veronii* in Asian swamp eels

Figure 4. Clinical signs of eels post-challenge with strain CT07. (A) surface hemorrhage, (B) abdomen hemorrhage, (C) head edema, (D) red swollen vents

To calculate the lethal dose of the CT07 strain in Asian swamp eels, the challenge experiment was conducted at doses of $7.73x10^2$, $7.73x10^3$, $7.73x10^4$, $7.73x10^5$ CFU/eel (with abbreviated codes as CT07-2, CT07-3, CT07-4, and CT07-5, respectively) and treatment was injected with physiological saline (0.9% NaCl) as a control treatment.

The challenged eels exhibited typical symptoms of diseased eels similar to those observed at the farms, including lethargic swimming, anorexia, loss of mucus, abdominal hemorrhage, head edema, red swollen vents, liver, and renal hemorrhage (Figure 4), which were similar to previous study report [19]. Previous studies showed that *Aeromonas* spp. infection was the most dangerous causative agent in several aquatic animals [15], [20].

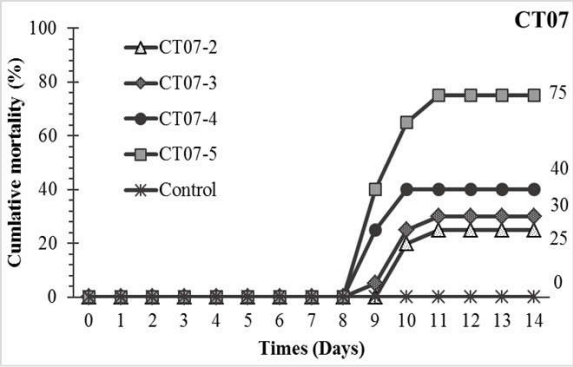

Figure 5. Cumulative mortality (%) of healthy Asian swamp eel injected with CT07

All challenge treatments exhibited mortality during the 14-day experimental monitoring, whereas no mortality was noted in the control group. In particular, the eels began to show signs of lethargy swimming and slow response to noise on the first day. At all bacterial challenge treatments, the mortality started on day 8, where the CT07-6 treatment had the highest mortality. Afterward, the cumulative mortality percentage increased dramatically in all treatments, and the eels ceased dying on day 12 post the injection (Figure 5). The challenge results showed that the calculated LD_{50} value was 1.51×10^5 CFU/mL and lower than the previous study of 1.52×10^7 CFU/mL [19]. Therefore, farmers should be aware of the presence of *Aeromonas* spp. in the Asian swamp eel hatchery and have suitable treatments to prepare a pond before stocking. Similarly, acute mortality was also found in the experimental challenge of red tilapia (*Oreochromis* sp.) with *A. veronii* [21]. Furthermore, *A. veronii* severely threatens freshwater species and could harm fish health [22]. The discrepancies in the clinical signs and pathological changes in the infected fish might be associated with the kind and degree of stress exerted on the fish, the level of bacterial virulence, the genetic disease resistance of the fish species, and different strains of the bacteria [19].

3.4. Histopathology of infected eels

Histological observations at the liver, kidney, and intestine showed that hemorrhage appeared on all three organs when the eels were infected with *A. veronii* (Figure 6). In the liver, congestion was recorded in the blood vessels and capillaries, followed by the degeneration of hepatocytes and the concentration of inflammatory cells (Figure 6.A-B). In addition, the hemorrhage was presented in the renal tissue, and proximal tubules were observed with necrosis leading to loss of tubular structure. Concentrations of inflammatory cells, melano-macrophages, and necrotic tissues around the hemorrhagic zone were also recorded (Figure 6.C-D). The mucous membranes in the intestine were shed and the intestinal villi were collapsed, leading to a disruption in the absorption of nutrients. Red blood cells hemorrhagic from the inside of the intestinal villi and connective tissue layer slough into the intestinal lumen, accompanied by sloughing of mucous-secreting cells and intestinal microvilli (Figure 6.E-F). In this study, the histological structure of eels infected with *A. veronii* was similar to previous studies on other species [15], [23]. Moreover, all internal organs (especially intestinal tissue) of cultured eels infected with *A. veronii* in China were also observed [19]. Multi-organ hemorrhage of infected eels is suggested to be related to the hemolytic virulence of *Aeromonas* species [15], and the bacteria hemolysis was confirmed when grown on blood agar plates. The signs of coagulation necrosis and renal structural disorder

in the previous study were similar to this study [19]. In addition, fragmentation, shrinkage, and fading of kidney cell nuclei were also observed. (Figure 6.D). Pathological signs were seen in renal tissue-specific to *A. veronii* infection.

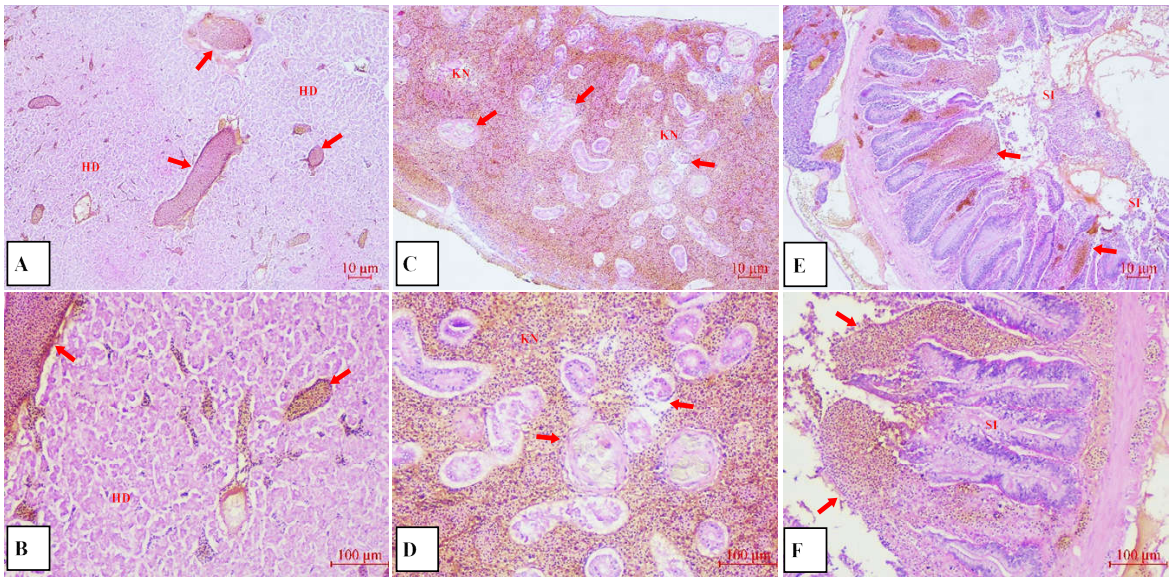

Figure 6. Histopathological changes of the liver, kidney, and intestine from the infected eels ***(A, B): liver of diseased eel with hepatocyte degeneration (HD) and congestion in the blood vessels (red arrow); (C, D): kidney of diseased eel observed inflammatory kidney tissue (KN) and tubular coagulation necrosis (red arrow); (E, F): intestine of diseased eel with the shedding of the mucous membrane (SI) and hemorrhage from the connective tissue (red arrow).***

3.5. Antibiotic susceptibility

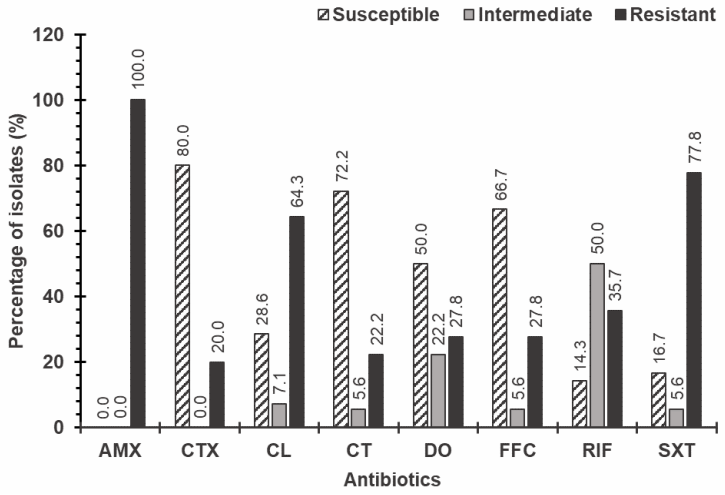

Figure 7. Antibiotic susceptibility evaluations on isolates

Amoxicillin/clavulanic (AMX/30 µg), cefotaxime (CTX/30 µg), cephalexin (CL/30 µg), colistin sulfate (CT/50 µg), doxycycline (DO/30 µg), florfenicol (FFC/30 µg), rifampicin (RIF/30 µg), trimethoprim/sulfamethoxazole (SXT/1.25/23.75 µg).

The antibiotic susceptibility data of 19 *A. veronii* isolates against eight antibiotics are shown in figure 7. The results revealed that the majority of the isolates were susceptible to cefotaxime

(72.22%) and colistin sulfate (80%). In contrast, *A. veronii* isolates exhibited a more significant percentage of resistance to cephalexin (64.28%), trimethoprim/sulfamethoxazole (77.77%), and amoxicillin/clavulanic (100%). The previous study suggested that the first option of treatment for *Aeromonas* infections is cephalosporins, aminoglycosides, quinolones, tetracyclines (excluding tetracyclines), phenicols, nitrofurans, and sulfonamides [19]. In addition, *A. veronii* was also found highly susceptible to cefotaxime, nevertheless resistant to two other medicines, ampicillin and nalidixic acid [24].

Overall, the principle of utilizing drugs should not only consider drug sensitivity but also strictly comply with regulations for aquatic products. Therefore, we recommend florfenicol and cefotaxime to control hemorrhagic disease in Asian swamp eels.

4. CONCLUSION

The main pathogen causing hemorrhagic disease in diseased Asian swamp eels in the Mekong Delta was confirmed to be *Aeromonas veronii* by morphological, biochemical identification, and 16S rRNA sequencing. The experimental injection challenge study was performed and fulfilled Koch's postulates with the LD_{50} of $1.51x10^5$ CFU/eel. The pathological changes were noted in the liver, kidney, and intestines of Asian swamp eels infected with *A. veronii*. Antibiotic sensitivity testing showed that the isolated bacteria were susceptible to several antibiotics, and we recommend florfenicol and cefotaxime to control hemorrhagic disease in Asian swamp eels.

ACKNOWLEDGMENT

This study was funded by the Department of Science and Technology of Hau Giang province, Vietnam. We appreciate the eel farmers in Hau Giang, Can Tho, An Giang, Vinh Long, and Bac Lieu for their enthusiastic collaboration, information exchange, and sample collecting during the study.

REFERENCES

1. R. Zhou, H. Cheng, Q. Zhang, Y. Guo, R.K. Cooper, T.R. Tiersch (2002). SRY-related genes in the genome of the rice field eel (*Monopterus albus*). *Genet. Sel. Evol.*, 34, 1-9. https://doi.org/ 10.1186/ 1297-9686-34-1-129.

2. L. H. Nguyen (2010). *Eel farming profession*. Agricultural Publishing House.

3. B. C. Das, D. V. Haridas, N. Jacob, A. Ramakrishnan, A.A.P. H, R.K. V. J, D. Pillai (2021). Outbreaks of epizootic ulcerative syndrome in Kerala, India following episodes of flooding. *Dis. Aquat. Organ.*, 143, 189–193. https://doi.org/10.3354/ DAO03567.

4. M. M. Rahman(2004). Studies on the distribution, virulence properties and detection of a newly identified species *Aeromonas* sp. *T8 group*. https:// ci. nii.ac.jp/ naid/ 500000259142 (accessed September 19, 2023).

5. T. D. Hoai, T. T. Trang, N. Van Tuyen, N. T. H. Giang, K. Van Van (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture,* 513, 734425. https:// doi.org/10.1016/ J.AQUACULTURE.2019.734425.

6. M. A. Hassan, E. A. Noureldin, M. A. Mahmoud, N.A. Fita (2017). Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA, Egypt. *J. Aquat. Res.*, 43, 161–167. https://doi.org/10.1016/ j.ejar.2017.06.001.

7. M. Smyrli, A. Prapas, G. Rigos, C. Kokkari, M. Pavlidis, P. Katharios (2017). *Aeromonas veronii* infection associated with high morbidity and mortality in farmed European seabass *Dicentrarchus labrax* in the Aegean Sea, Greece. *Fish Pathol.*, 52, 68 - 81. https://doi.org/10.3147/ jsfp.52.68.

8. S.S. Shameena, K. Kumar, S. Kumar, S. Kumar, G. Rathore (2020). Virulence characteristics of *Aeromonas veronii* biovars isolated from infected freshwater goldfish (*Carassius auratus*). *Aquaculture.* 518, 734819. https://doi.org/10.1016/ j.aquaculture.2019.734819.

9. C. Ran, C. Qin, M. Xie, J. Zhang, J. Li, Y. Xie, Y. Wang, S. Li, L. Liu, X. Fu, Q. Lin, N. Li, M.

R. Liles, Z. Zhou (2018). *Aeromonas veronii* and aerolysin are important for the pathogenesis of motile aeromonad septicemia in cyprinid fish. *Environ. Microbiol.* 20, 3442 - 3456. https:// doi. org/ 10. 1111/ 1462 - 2920. 14390.

10. G. N. Frerichs, S. D. Millar (1993). Manual for the isolation and identification of fish bacterial pathogens, na.

11. S. Kumar, G. Stecher, K. Tamura (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.,* 33, 1870 - 1874. https://doi.org/10.1093/ MOLBEV/ MSW054.

12. L.J. Reed, H. Muench (1938). A simple method of estimating fifty percent endpoints. *Am. J. Epidemiol.*, 27, 493 - 497. https://doi.org/ 10.1093/ oxfordjournals.aje.a118408.

13. CLSI (2020). Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals, 3rd Edition, Clinical and Laboratory Standards Institute.

14. H. T. Dong, C. Techatanakitarnan, P. Jindakittikul, A. Thaiprayoon, S. Taengphu, W. Charoensapsri, P. Khunrae, T. Rattanarojpong, S. Senapin (2017). *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). *J. Fish Dis.,* 40, 1395–1403. https://doi.org/10.1111/ jfd.12617.

15. H. T. Dong, C. Techatanakitarnan, P. Jindakittikul, A. Thaiprayoon, S. Taengphu, W. Charoensapsri, P. Khunrae, T. Rattanarojpong, S. Senapin (2017). *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). *J. Fish Dis.,* 40, 1395–1403. https://doi.org/10.1111/ jfd.12617.

16. D. A. Allen, B. Austin, R.R. Colwell (1983). Numerical Taxonomy of Bacterial Isolates Associated with a Freshwater Fishery. *Microbiology.* 129, 2043–2062. https://doi.org/10. 1099/00221287-129-7-2043.

17. A. Gholamhosseini, V. Taghadosi, N. Shiry, M. Akhlaghi, H. Sharifiyazdi, S. Soltanian, N. Ahmadi (2018). First isolation and identification of *Aeromonas veronii* and *Chryseobacterium joostei* from reared sturgeons in Fars province, Iran, in: *Vet. Res.* Forum, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, p. 113.

18. H. H. Mohammed, E. Peatman (2018). Winter kill in intensively stocked channel catfish (*Ictalurus punctatus*): Coinfection with *Aeromonas veronii*, *Streptococcus parauberis* and *Shewanella putrefaciens*. *J. Fish Dis.*, 41, 1339–1347. https:// doi.org/10.1111/jfd.12827.

19. L. Xia, P. Han, X. Cheng, Y. Li, C. Zheng, H. Yuan, W. Zhang, Q. Xu (2019). *Aeromonas veronii* caused disease and pathological changes in Asian swamp eel *Monopterus albus*. *Aquac. Res.,* 50, 2978–2985. https://doi.org/10.1111/are.14253.

20. B. Austin, D. A. Austin (2016). Bacterial fish pathogens: Disease of farmed and wild fish, sixth edition. *Springer Netherlands*. https://doi. org/ 10.1007/978-3-319-32674-0.

21. H. T. Dong, V. V. Nguyen, H. D. Le, P. Sangsuriya, S. Jitrakorn, V. Saksmerprome, S. Senapin, C. Rodkhum (2015). Naturally concurrent infections of bacterial and viral pathogens in disease outbreaks in cultured Nile tilapia (*Oreochromis niloticus*) farms. *Aquaculture,* 448, 427 – 435. https://doi.org/10.1016/j.aquaculture. 2015.06.027.

22. T. Peepim, H. T. Dong, S. Senapin, P. Khunrae, T. Rattanarojpong (2016). Epr3 is a conserved immunogenic protein among *Aeromonas* species and able to induce antibody response in Nile tilapia. *Aquaculture,* 464, 399–409. https:// doi.org/10.1016/j.aquaculture.2016.07.022.

23. Z. Han, J. Sun, B. Jiang, X. Hu, A. Lv, L. Chen, Y. Guo (2021). Concurrent infections of *Aeromonas veronii* and *Vibrio cholerae* in koi carp (*Cyprinus carpio* var. koi). *Aquaculture,* 535, 736395. https://doi.org/10.1016/j.aquaculture. 2021. 736395.

24. J. Vila, F. Marco, L. Soler, M. Chacon, M. J. Figueras (2002). In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii* biotype *sobria*. *J. Antimicrob. Chemother.*, 49, 701 - 702. https:// doi.org/10.1093/jac/ 49.4.701.