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Molecular and virulence profiling of *Aeromonas veronii* from diseased Nile tilapia in Central Java, Indonesia

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Abstract. *Mulia DS, Nisa Z, Suwarsito, Purbomartono C, Olga, Muslimin B, Setyawan A. 2025. Molecular and virulence profiling of* Aeromonas veronii *from diseased Nile tilapia in Central Java, Indonesia. Biodiversitas 26: 3647-3657.* Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) is a widely cultivated species with promising economic potential; however, bacterial infections pose a significant barrier to successful production. Molecular characterization is essential for accurately identifying pathogenic bacteria involved in disease outbreaks. This study aimed to determine the molecular and virulence of *Aeromonas veronii* isolated from diseased Nile tilapia in Central Java, Indonesia. Diseased fish were collected using a purposive sampling technique. Molecular identification was conducted using markers for 16S rRNA, *gyrB*, and ten virulence-associated genes, including *aerA/haem*, *alt*, *ast*, *act*, *flaA*, *lafA*, *fstA*, *ahp*, *ela*, and *lip*. Antibiotic susceptibility testing was performed using eight antibiotics: bacitracin (10 µg), penicillin (10 µg), amoxicillin (25 µg), ciprofloxacin (5 µg), vancomycin (30 µg), clindamycin (2 µg), tetracycline (30 µg), and chloramphenicol (30 µg). Results revealed that seven isolates were identified as *A. veronii*. All isolates (100%) harbored two significant virulence genes, *aerA/haem* and *ela*, while none of the isolates (0%) tested positive for the other eight virulence genes. The consistent presence of these two virulence factors suggests a high pathogenic potential of the isolates. Antibiotic susceptibility assays indicated that all isolates (100%) were resistant to bacitracin, penicillin, amoxicillin, vancomycin, and clindamycin, but remained sensitive to ciprofloxacin, tetracycline, and chloramphenicol. In conclusion, the present findings support the implementation of targeted diagnostic approaches for *A. veronii* infections. Detecting key virulence factors and multidrug resistance in *A. veronii* highlights its potential as a significant threat to aquatic animal health. Consequently, there is an urgent need to strengthen biosecurity measures, including routine microbial monitoring, judicious and responsible use of antibiotics, and improved environmental management practices. Furthermore, developing alternative strategies, such as vaccination, represents a proactive and sustainable long-term solution to enhance the resilience of aquaculture systems.

Keywords: *Aeromonas veronii*, aquaculture pathogen, *gyrB* gene, pathogenicity profiling, Sanger sequencing

INTRODUCTION

Freshwater aquaculture is a rapidly expanding sector with substantial economic potential, and several species, including Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)), are widely cultivated due to their adaptability and high market demand (Anantasuk et al. 2024). In Indonesia, Nile tilapia production reached 11,368,542.35 tons in 2023, representing a 0.875% increase from the previous year (Central Bureau of Statistics 2024). Despite this growth, disease outbreaks remain a significant obstacle to sustainable cultivation. Among the most prevalent bacterial diseases is Motile *Aeromonas* Septicemia (MAS), caused by various *Aeromonas* spp. (Pessoa et al. 2019; El-Sharaby et al. 2021).

Aeromonas spp. are Gram-negative, oxidase-positive, facultative anaerobic bacteria commonly found in aquatic environments (Yazdanpanah-Goharrizi et al. 2020; Mailafia et al. 2021). While these bacteria are part of the natural microbiota under stable conditions (Pessoa et al. 2019), they act as opportunistic pathogens under stress or immunocompromised conditions, potentially causing mortality rates up to 100% in freshwater fish (Shameena et al. 2020; Pereira et al. 2022; Matter et al. 2024). Some species also infect marine organisms (Cunningham et al. 2019; Hossain et al. 2019), and zoonotic risks to humans (Yuwono et al. 2021).

Clinical signs of MAS disease include hemorrhagic lesions, fin erosion, ascites, necrosis, and exophthalmia (El-Son et al. 2019; Elgohary et al. 2020). The number of recognized *Aeromonas* species has grown significantly, from 24 to 36 in 2020 (Fernández-Bravo and Figueras 2020). Molecular identification using 16S rRNA, *gyrB*, and *rpoD* genes has proven effective in species differentiation (Lee et al. 2023). In Indonesia, *A. veronii* bv *veronii* has been isolated from diseased catfish and gourami based on

16S rRNA sequencing (Mulia et al. 2023, 2024). Based on *gyrB* sequencing, five species of *Aeromonas* were found to cause MAS disease signs in Nile tilapia (*Orechromis* sp.), catfish (*Clarias batrachus* (Linnaeus, 1758)), and striped catfish (*Pangasianodon hypopthalmus* (Sauvage, 1878)) in Malaysia, namely *A. dhakensis, A. veronii, A. hydrophila, A. caviae*, and *A. jandaei* (Azzam-Sayuti et al. 2021). Using the 16S rRNA and *gyrB* gene sequences along with Multilocus Sequence Typing (MLST) analysis, bacteria were identified from diseased Nile tilapia in Thailand, namely *A. hydrophila* and *A. veronii* (Anantasuk et al. 2024).

Pathogenicity in *Aeromonas* is closely linked to the expression of virulence genes, which mediate adhesion, invasion, and toxin production (Awan et al. 2018; Khor et al. 2018; Fernández-Bravo and Figueras 2020). In a study conducted in Egypt, *A. veronii* isolated from diseased Nile tilapia were found to harbor several virulence genes, including *alt, act*, *aer, lip*, and *fla* (Youssef et al. 2022). Meanwhile, isolates obtained from diseased *Channa argus* (Cantor, 1842) in China carried a broader range of virulence genes: *aer, act, alt, fla, ascV, aexT*, and *ela* (Sun et al. 2025).

Regular bacterial identification is essential to monitor microbial biodiversity's evolution and assess virulence potential changes. Such efforts are crucial for developing effective control strategies and selecting appropriate anti-*Aeromonas* agents or antibiotics. Moreover, identifying novel, highly virulent strains may offer promising candidates for vaccine development. Despite extensive research on *Aeromonas* spp., data on *A. veronii* infecting Nile tilapia in Central Java remain limited, particularly regarding *gyrB*-based phylogenetic characterization and virulence gene diversity. Profiling key virulence genes such as *aer*, *alt*, and *ela* is vital for evaluating the pathogenic potential of *A. veronii*, as these genes serve as important indicators of disease severity in aquaculture systems. Additional virulence genes of interest include *ast*, *act*, *flaA*, *lafA*, *fstA*, *ahp*, and *lip*, which may also contribute to pathogenic mechanisms. Based on this background, the present study aimed to determine the molecular and virulence gene profile of *A. veronii* isolated from diseased Nile tilapia in Central Java, Indonesia.

MATERIALS AND METHODS

Sample collection

The study was conducted from January to April 2025. Nile tilapia (*O. niloticus*) samples were collected from an aquaculture ponds in Purwosari Village, Baturraden Sub-district, Banyumas District, Central Java Province, Indonesia (Figure 1). Diseased fish were obtained using a purposive sampling method. Sampling was performed from three separate tilapia culture ponds, with ten visibly diseased fish selected from each pond. The fish were carefully netted using a sterilized scoop and immediately transferred into containers filled with water from the original pond to minimize stress. Euthanasia was performed by immersing the fish in water containing 100 ppm of clove oil until they exhibited no response to external stimuli, as indicated by the absence of opercular and caudal fin reflexes. Before dissection, the surface of each fish was disinfected by wiping it with 70% ethanol to eliminate surface contaminants. The fish were then individually wrapped in sterile, labeled plastic bags and transported promptly to the laboratory for further analysis. In the laboratory, a necropsy was conducted under aseptic conditions. Each fish was dissected using sterile instruments to prevent cross-contamination. Bacterial inoculation was performed by swabbing lesions and kidney tissues and streaking the samples onto Glutamate Starch Phenyl (GSP) agar medium for bacterial isolation. The management, conditions, and procedures of experiment in this study were approved by the Ethical Clearance Commission of Universitas Gadjah Mada (approval # certificate: 00137/04/LPPTI/|/201).

Isolation of bacteria

Bacteria were isolated from the kidney and ulcer of diseased Nile tilapia and cultured on GSP medium (Merck, Darmstadt, Germany) at 37°C for 24 h. Furthermore, a single colony was grown in the Tryptone Soya Broth (TSB) medium (Merck, Darmstadt, Germany). The isolates were stored in TSB medium with 20% glycerol at -20°C for further assay.

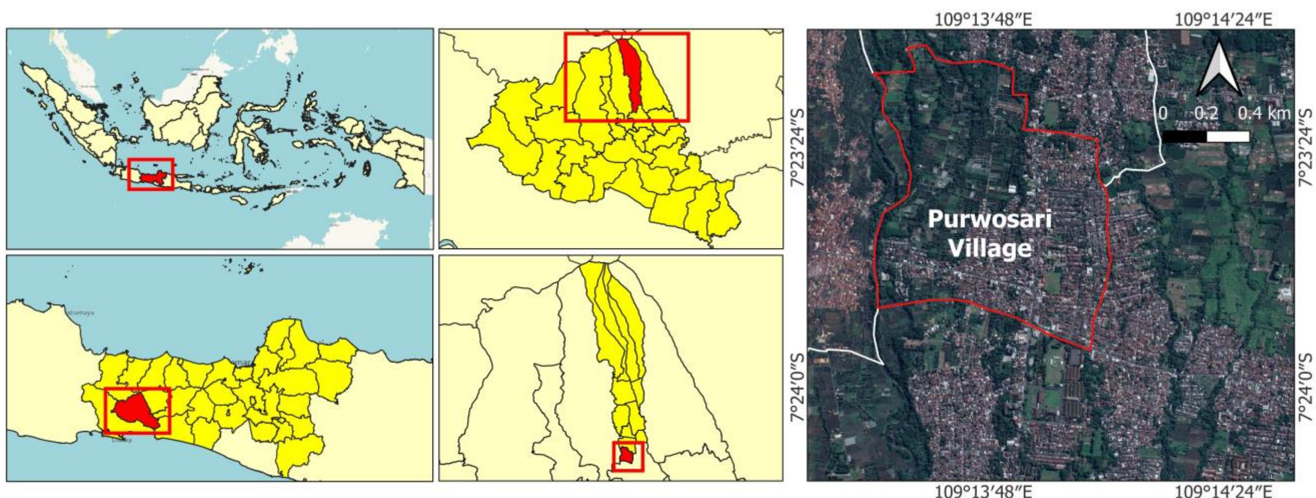

Figure 1. Map of sampling location of diseased Nile tilapia in Purwosari Village, Banyumas District, Central Java, Indonesia

Table 1. Virulence genes primer sets used in this study

Genes	Gene product	Primer sequence	PCR conditions	Product size (bp)	Reference
aerA/ haem	Aerolysin/ hemolysin	F: CCT ATG GCC TGA GCG AGA AG R: CCA GTT CCA GTC CCA CCA CT	Pre denaturation: 95°C 3 min, 35 cycles: Denaturation: 94°C 1 min, Annealing: 56°C 1 min, Extension: 72°C 1 min, Final extension: 72°C 5 min	431	Soler et al. (2002)
alt	Heat-labile cytotonic enterotoxin	F: TGA CCC AGT CCT GGC ACG GC R: GGT GAT CGA TCA CCA CCA GC	Pre denaturation: 95°C 5 min, 25 cycles: Denaturation: 95°C 25 s, Annealing: 56°C 30 s, Extension: 72°C 1 min, Final extension: 70°C 5 min	442	Sen and Rodgers (2004)
ast	Heat stable cytotonic enterotoxin	F: TCT CCA ATG CTT CCC TTC ACT R: GTG TAG GGA TTG AAG AAG CCG	Pre denaturation: 95°C 5 min, 25 cycles: Denaturation: 95°C 25 s, Annealing: 56°C 30 s, Extension:72°C 1 min, Final extension: 70°C 5 min	331	Sen and Rodgers (2004)
act	Cytotoxic heat-labile enterotoxin	F: ATC GTC AGC GAC AGC TTC TT R: CTC ATC CCT TGG CTT GTT GT	Pre denaturation: 94°C 5 min, 35 cycles: Denaturation: 94°C 30 s, Annealing: 55°C 30 s, Extension: 72°C 1 min, Final extension: 72°C 5 min	500	Fu et al. (2014)
flaA	Polar flagellum	F: TCC AAC CGT YTG ACC TC R: GMY TGG TTG CGR ATG GT	Pre denaturation: 95°C 5 min, 25 cycles: Denaturation: 95°C 25 s, Annealing: 56°C 30 s, Extension: 72°C 1 min, Final extension: 70°C 5 min	608	Sen and Rodgers (2004)
lafA	Lateral flagellum	F: CCA ACT T(T/C)G C(C/T)T C(T/C) (C/A)TGA CC R: TCT TGG TCA T(G/A)T TGG TGC T(C/T)	Pre denaturation: 94°C 5 min, 35 cycles: Denaturation: 94°C 2 min, Annealing: 50°C 1 min, Extension: 72°C 2 min, Final extension:74°C 5 min	736	Aguilera-Arreola et al. (2005)
fstA	Ferric siderophore receptor	F: CGC TCG CCC ATC CCC CTC TG R: GCC CCT TGC ACC CCC ACC ATT	Pre denaturation: 92°C 3 min, 30 cycles: Denaturation: 92°C 1 min, Annealing: 55°C 1 min, Extension: 72°C 1 min Final extension: 72°C 5 min	452	Beaz-Hidalgo et al. (2008)
ahp	Serine protease	F: ATT GGA TCC CTG CCT ATC GCT TCA GTT CA R: GCT AAG CTT GCA TCC GTG CCG TAT TCC	Pre denaturation: 94°C 5 min, 35 cycles: Denaturation: 94°C 30 s, Annealing: 55°C 30 s, Extension: 72°C 1 min, Final extension: 72°C 5 min	911	Hu et al. (2012)
ela	Elastase	F: ACA CGG TCA AGG AGA TCAA C R: CGC TGG TGT TGG CCA GCA GG	Pre denaturation: 94°C 5 min, 35 cycles: Denaturation: 94°C 30 s, Annealing: 55°C 30 s, Extension: 72°C 1 min, Final extension: 72°C 5 min	513	Sen and Rodgers (2004)
lip	Lipase	F: ATC TTC TCC GAC TGG TTC GG R: CCG TGC CAG GAC TGG GTC TT	Pre denaturation: 94°C 5 min, 35 cycles: Denaturation: 94°C 30 s, Annealing: 55°C 30 s, Extension: 72°C 1 min, Final extension: 72°C 5 min	382	Sen and Rodgers (2004)

DNA extraction

Bacteria genomic DNA was extracted using High Pure PCR Preparation Kit (Roche, 11796828001, Roche Diagnostics Corporation, Indiana, USA). A bacterial culture of around 1 mL in TSB media was centrifuged for 2 minutes at 13,000 × g after incubating at 37°C for 24 hours. The extracted bacteria DNA was stored at -20°C for further assay.

PCR amplification of 16S rRNA genes

Characterization of bacteria by 16S rRNA was carried out using oligonucleotide universal primers of 27F and 1492R as shown in Table 1. The total PCR volume was 25 µL, containing 13 µL Mytaq HS Red Mix (2× PCR Master Mix) (Bioline, Meridian Life Science, Memphis, UK), 1 µL forward primer, 1 µL reverse primer, 1 µL DNA template (20 ng), and 9 µL Nuclease-Free Water (NFW). The PCR was carried out with an initial denaturation at 95°C for 3 min and 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 90 s, and final extension at 72°C for 5 min. Subsequently, PCR product was subjected to electrophoresis with 1% agarose gel in 1× Tris-acetate EDTA (TAE) buffer before sequencing (1st BASE Laboratories Malaysia).

PCR amplification of *gyrB* genes

Genotypic characterization of bacteria was carried out using the DNA *gyrase* B subunit *(gyrB*). Based on previous methods, specific primers used to amplify gyrB were approximately 1100 bp in size (Yáñez et al. 2003). Two primers were used, namely (forward: 5’-TCC GGC GGT CTG CAC GGC GT-3′ and reverse: 5’-TTG TCC GGG TTG TAC TCG TC-3′). In the PCR protocols, 12.5 µL of exTEN 2× PCR mastermix was added to a final volume of 25 µL for all PCR experiments, 0.4 µM of each forward (First Base Laboratories, Kuala Lumpur, Malaysia) as well as the reverse primer and 2.5 µL (10 to 100 ng) of DNA template. Using an Eppendorf Mastercycler Nexus Thermal Cycler (Eppendorf, Hamburg, Germany), the mixture was subjected to 35 amplification cycles, which included denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 min. The PCR products were then analyzed by electrophoresis on a 1.0% agarose gel in 1× Tris-acetate EDTA (TAE) buffer before sequencing (1st BASE Laboratories Malaysia).

Sequence analysis and phylogenetics

Sequences were edited and assembled using the program DNA Baser (Wang et al. 2019). The similarity was analyzed by using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignments were carried out using the Clustal W Program (Tamura et al. 2013). Furthermore, phylogenetic trees were constructed using the maximum likelihood MEGA™ 11.0 package (The Biodesign Institute, USA) by bootstrap analysis with 1000 replications (Kumar et al. 2018).

Detection of virulence genes

The genes encoding aerolysin/hemolysin (*aerA/haem*), heat-labile cytotonic enterotoxin (*alt*), heat-stable cytotonic enterotoxin (*ast*), cytotoxic enterotoxin (*act*), flagella (*flaA*), lateral flagellum (*lafA*), ferric siderophore receptor (*fstA*), serine protease (*ahp*), elastase (*ela*) and lipase (*lip*) were amplified using PCR (Table 1). The total PCR volume of 25 µL contained 13 µL Mytaq HS Red Mix (2× PCR Master Mix, Bioline, Meridian Life Science, Memphis, UK), 1 µL forward primer, 1 µL reverse primer, 1 µL DNA sample (20 ng), and 9 µL Nuclease-Free Water (NFW). The PCR product was subjected to electrophoresis on a 1.5% agarose gel.

Antimicrobial testing

Antibiotic susceptibility test was carried out using Bacitracin 10 µg, Penicillin 10 µg, Amoxicillin 25 µg, Ciprofloxacin 5 µg, Vancomycin 30 µg, Clindamycin 2 µg, Tetracycline 30 µg, and Chloramphenicol 30 µg discs. Bacteria were inoculated on TSA medium in a continuous streak and then incubated at 37℃ for 24 hours. The diameter of the inhibition zone (in mm) around the disc was measured and determined as sensitive, intermediate, and resistant based on the latest edition of CLSI M100 (CLSI 2024).

RESULTS AND DISCUSSION

Clinical signs of sample fish

The fish sample examined in this study was diseased Nile tilapia exhibiting both external and internal clinical signs. External clinical signs included skin depigmentation, hemorrhagic, erosion, extensive skin lesions, and exophthalmia. Internal clinical signs were characterized by pale gills, blackish-red of the kidney, pale-red liver, and ascites (Figure 2). Previous research has documented clinical signs in tilapia infected with *A. veronii*, including skin darkening, hemorrhagic septicemia, and fin necrosis (El-Wafai et al. 2020). Gourami infected with *A. veronii* bv *veronii* exhibited clinical signs such as skin depigmentation, extensive skin lesions, hemorrhagic fin necrosis, and discoloration of internal organs, including pale to reddish-brown kidneys and livers, along with ascites (Mulia et al. 2024). In crucian carp (*Carassius auratus* subsp. *gibelio* (Bloch, 1782)), infection with *A. veronii* was associated with abdominal distension, congestion at the fin base, and branchial ischemia (Chen et al. 2019). Results from the Koch’s postulates test demonstrated that the bacterial isolates induced a mortality rate ranging from 80% to 100%. These findings confirm the pathogenic nature of all isolates, which possessed virulence factors characteristic of *Aeromonas* infections in Nile tilapia.

Molecular character of pathogenic bacteria in Nile tilapia

Bacterial isolates were obtained from lesions on the body surface and kidney tissues of diseased Nile tilapia. Amplification of the 16S rRNA gene from the seven isolates yielded DNA fragments of approximately 1500 base pairs (bp) in length (Figure 3). Similarly, amplification of the *gyrB* gene produced fragments of approximately 1100 bp for all seven isolates (Figure 4). Phylogenetic analyses showed that the seven isolates of bacteria were *A. veronii* (Figure 5).

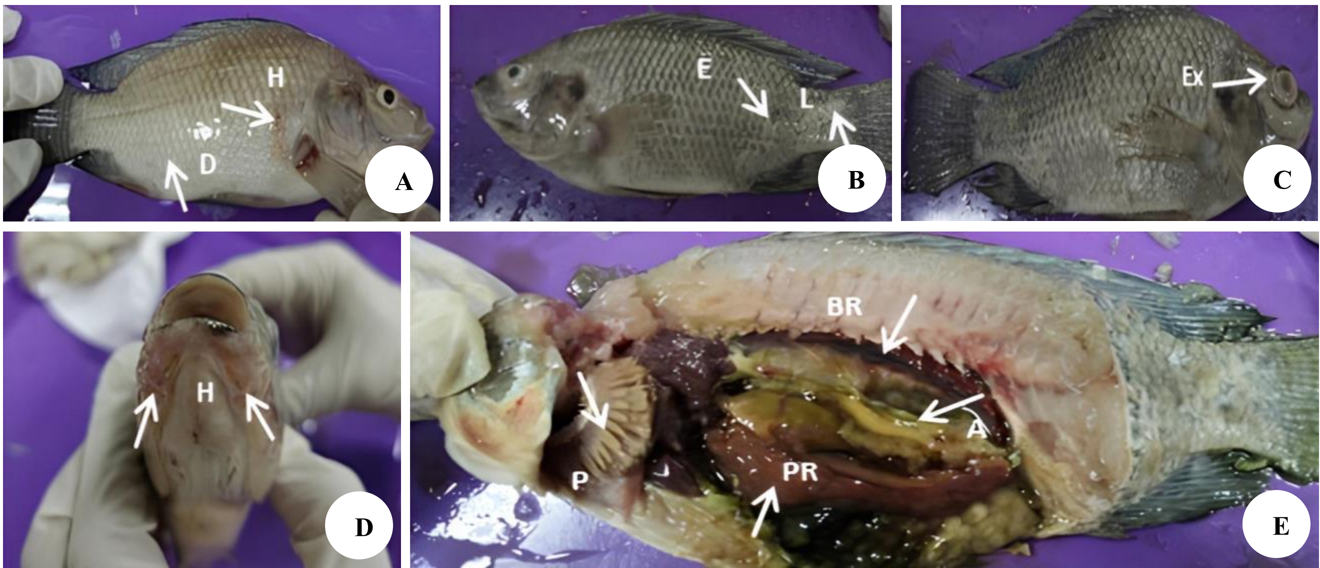

Figure 2. Clinical signs of Nile tilapia. A. D: Depigmentation of the skin, H: Hemorrhagic, B. E: Erotion, L: Extensive skin lesion, C. Ex: Exopthalmia, D. H: Hemorrhagic, E. P: Pale in gills, BR: Blackish red of the kidney, PR: Pale red liver, A: Ascites

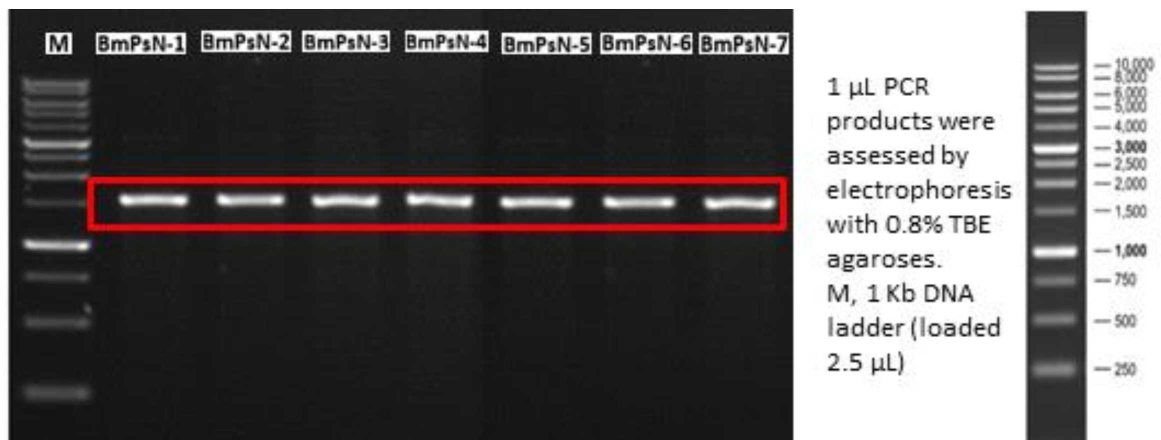

Figure 3. Results of amplification of bacterial DNA isolated with 16S rRNA primers. Arrow: 1500 bp, Marker: 1 kb

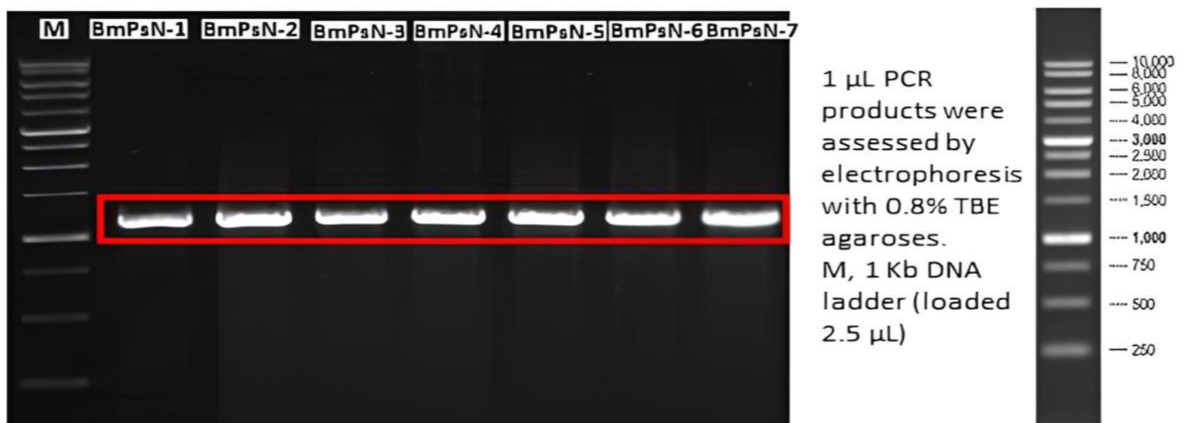

Figure 4. Results of amplification of bacterial DNA isolated with *gyrB* primers. Arrow: 1100 bp, Marker: 1 kb

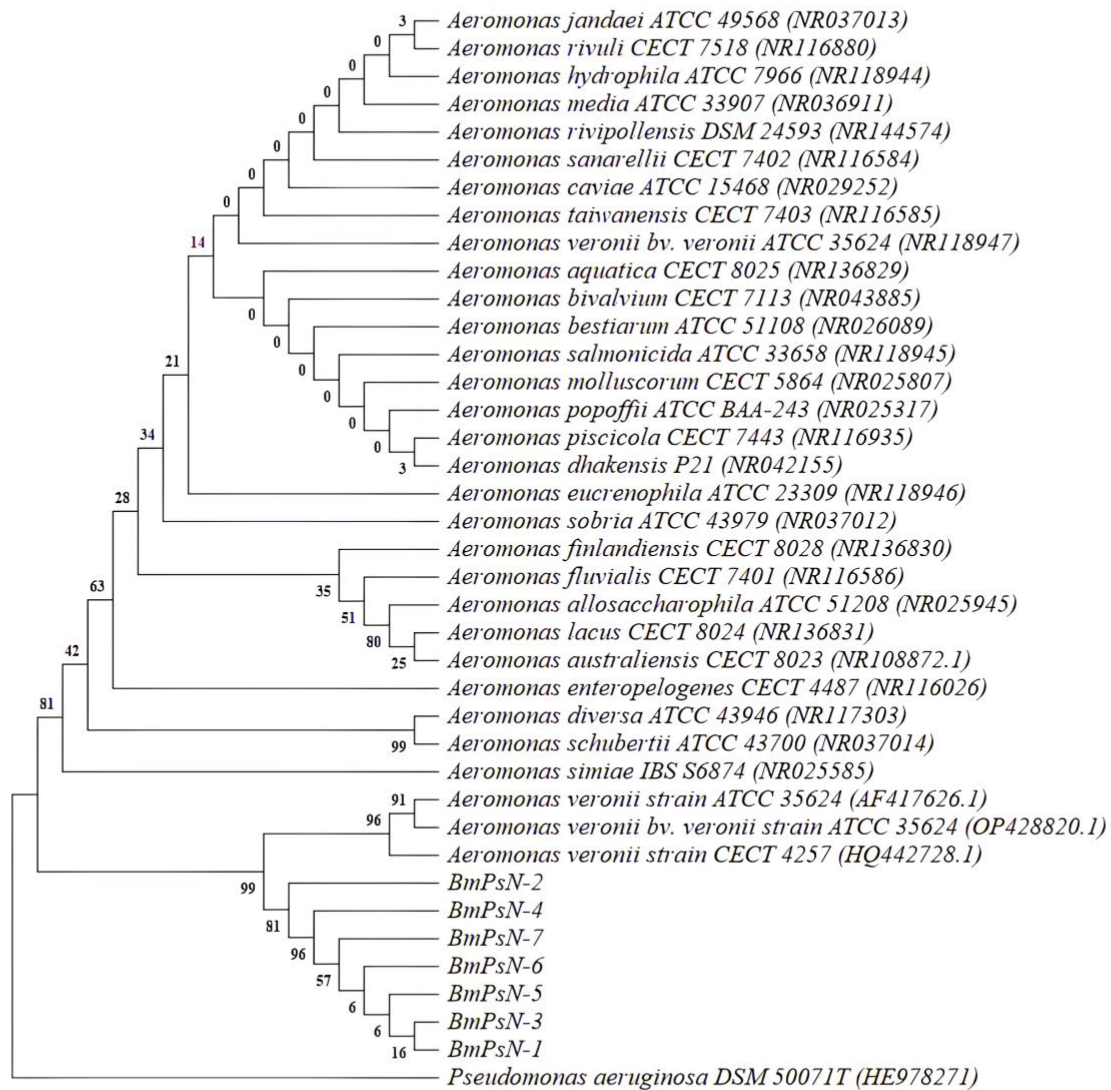

Figure 5. Phylogenetic tree constructed from the *gyrB* sequences from seven isolates of *Aeromonas veronii* strain BmPsN-1, BmPsN-2, BmPsN-3, BmPsN-4, BmPsN-5, BmPsN-6, and BmPsN-7 and other *Aeromonas* species (class of Gammaproteobacteria). *Pseudomonas aeruginosa* was used as an outgroup. The topology was obtained by maximum likelihood with bootstraps of 1000 replications. The scale bar signifies 0.02 substitutions per nucleotide position (Knuc)

Previous research has identified bacterial pathogens in Nile tilapia through molecular techniques targeting the 16S rRNA gene, including the identification of *A. veronii* bv *veronii* strain ATCC 35624 (El-Wafai et al. 2020). In addition to the 16S rRNA gene, other studies have utilized the *gyrB* gene for more precise identification of *Aeromonas* species (Chen et al. 2019). The use of single housekeeping genes such as *gyrB* or *rpoD* has been shown to yield higher phylogenetic resolution and more accurate species-level classification within the *Aeromonas* genus (Azzam-Sayuti et al. 2021).

Housekeeping genes like *gyrB* encode essential proteins involved in fundamental cellular processes and are frequently employed in bacterial taxonomy due to their evolutionary stability and discriminatory power, which surpasses that of the 16S rRNA gene (Zhong et al. 2019). Azzam-Sayuti et al. (2021) applied the *gyrB* gene to characterize pathogenic *Aeromonas* isolates from freshwater fish in Malaysia, identifying *A. veronii* as the most prevalent species (22%), followed by *A. hydrophila* (20%), *A. caviae* (8%), and *A. jandaei* (7%). Similarly, phylogenetic analysis based on *gyrB* gene sequences conducted by Dhanapala et al. (2021) in Sri Lanka revealed that *A. veronii* was the dominant species (75.8%) among isolates collected from ornamental freshwater fish and aquaculture environments, followed by *A. hydrophila* (9.3%), *A. caviae* (5%), *A. jandaei* (4.3%), *A. dhakensis* (3.7%), *A. sobria* (0.6%), *A. media* (0.6%), and *A. popoffii* (0.6%). Moreover, the simultaneous application of both the 16S rRNA and *gyrB* genes has been demonstrated to enhance the reliability and accuracy of *Aeromonas* species identification (Chen and Hu 2023). Supporting this, Gao et al. (2024) reported that *A. veronii* is widely distributed in aquatic environments and is responsible for infections in a broad range of aquatic animals, including *Macrobrachium rosenbergii* (De Man, 1879) in China.

Detection of virulence genes of pathogenic bacteria in Nile tilapia

A total of ten virulence-associated genes were tested in this study, including *aerA/haem, alt, ast, act, flaA, lafA, fstA*, *ahp, ela*, and *lip*. Among these, only two virulence genes, *aerA/haem* and *ela*, were successfully detected in all *A. veronii* isolates, resulting in a 20% detection rate of virulence genes in each isolate (Table 2). Specifically, 100% of the isolates harbored both *aerA/haem* and *ela*. In contrast, none of the isolates (0%) tested positive for the remaining genes, including *alt, ast, act, flaA, lafA, fstA, ahp,* and *lip*. In contrast, Azzam-Sayuti et al. (2021) reported a broader distribution of virulence genes, where 50% of *Aeromonas* isolates carried at least half of the virulence genes tested, including *aer, ahp, alt, hly, lip, fla, ela*, and *act*, while 2% of the isolates lacked all virulence genes. Previous studies have also indicated a high prevalence of the *ela* gene among *Aeromonas* strains, with detection rates ranging from 62% to 86% (Khor et al. 2015). The variation in virulence gene presence among *Aeromonas* species is attributed to their heterogeneous nature, dynamic genome structure, and mechanisms such as horizontal gene transfer, environmental adaptation, and selective and evolutionary pressures. These factors collectively influence the acquisition, maintenance, or loss of specific virulence determinants across different strains (Zhong et al. 2019; Majeed et al. 2023).

Aeromonas spp. are known to produce a wide array of toxins, including hemolysin, aerolysin, and cytotoxic enterotoxins, which contribute significantly to host tissue damage and disease severity (Sherif and AbuLeila 2022). Among these, aerolysin is a hemolytic extracellular protein encoded by the *aerA* gene and is considered one of the most critical virulence factors in *Aeromonas* spp. due to its direct involvement in pathogenesis (Sarkar et al. 2021). Pathogenic *Aeromonas* species also express virulence factors such as hemolysins, lipases, and proteases, all contributing to bacterial invasiveness and tissue degradation. Hemolysins, in particular, have been extensively characterized in several bacterial species, including *A. hydrophila* (Wang et al. 2024), and have also been detected in *A. veronii* (Gao et al. 2024). The *aerA* (aerolysin) and *haem* (hemolysin) genes are directly implicated in the lysis of host cells, including erythrocytes and epithelial cells. Aerolysin specifically binds to glycosylphosphatidylinositol (GPI)-anchored protein receptors on the surface of host cell membranes, leading to pore formation, membrane disruption, and systemic tissue damage (Fernández-Bravo and Figueras 2020; Sheikh et al. 2023). In addition, the *ela* gene in *Aeromonas* encodes elastase, a proteolytic enzyme that degrades elastin, a major component of connective tissue. This facilitates bacterial invasion by breaking down the extracellular matrix, thereby accelerating the spread of infection and intensifying tissue damage, including lesions in the skin, muscle, and internal organs (Borah and Srivastava 2025). From an ecological perspective, the presence of *aerA/haem* and *ela* genes highlights the role of aquatic environments as reservoirs of virulence determinants. These genes indicate the potential for latent or endemic infections within aquaculture systems, posing significant risks to fish health and biodiversity (Fernández-Bravo and Figueras 2020).

Previous studies on *A. veronii* bv *veronii* isolates from gourami detected the virulent genes *aer/haem, ela*, and *lafA*, but the genes *alt, ast, act, flaA, fstA, ahp*, and *lip* were not detected (Mulia et al. 2024). Virulent genes detected in *A. veronii* from Crucian Carp (*C. auratus* subsp. *gibelio*) include *aer, alt*, *ahyB, gcaT, lip*, and *ser*, but the genes *act, ast, fla, ahyB*, and *exu* were not detected (Chen et al. 2019). *A. veronii* from freshwater fish contains the genes fla, ela, hly, and *act* (Azzam-Sayuti et al. 2021). Four *A. veronii* bv *veronii* isolates from moribund hybrid catfish were found to contain the *aer/haem, ast, flaA*, and *lafA* genes. However, only two isolates were detected to contain the *alt* and *fstA* genes (Mulia et al. 2023). Compared with previous studies, differences in the virulence genes were detected. Loss of *Aeromonas* virulence genes resulted in reduced virulence in these strains. However, some strains maintain pathogenicity despite this loss, indicating a complex relationship between virulence factors and pathogenic potential (Guan et al. 2022).

Antimicrobial susceptibility test

An antimicrobial susceptibility test was performed on all *A. veronii* isolates to evaluate their sensitivity to selected antibiotics by measuring the diameter of the inhibition zones formed (Rahman et al. 2023). The results demonstrated that several antibiotics effectively inhibited bacterial growth, particularly ciprofloxacin, tetracycline, and chloramphenicol (Figure 6). The test procedures followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2024). However, because CLSI does not provide specific interpretive criteria (breakpoints) for *Aeromonas*, breakpoints for Enterobacterales are commonly used as a reference (Yoon 2022). The findings revealed that bacitracin, penicillin, amoxicillin, vancomycin, and clindamycin exhibited no inhibitory effect on any isolates, indicating that all isolates were resistant to these antibiotics. Conversely, all *A. veronii* isolates were categorized as susceptible to ciprofloxacin, tetracycline, and chloramphenicol according to CLSI (2024) criteria (Table 3).

These findings are partially consistent with previous reports. For example, *A. veronii* bv *veronii* isolated from gourami was reportedly resistant to tetracycline, bacitracin, and gentamicin, but sensitive to chloramphenicol (Mulia et al. 2024). Azzam-Sayuti et al. (2021) reported that *A. veronii* isolates exhibited 19% resistance, 19% intermediate susceptibility, and 63% sensitivity to tetracycline; 0% resistance, 11% intermediate susceptibility, and 89% sensitivity to gentamicin; and 100% sensitivity to chloramphenicol. Meanwhile, Chen et al. (2019) found that this pathogen was sensitive to tetracycline, moderately susceptible to gentamicin, and resistant to chloramphenicol. Similarly, El-Wafai et al. (2020) noted that *A. veronii* bv *veronii* demonstrated intermediate susceptibility to gentamicin, tetracycline resistance, and chloramphenicol. These discrepancies among studies may be attributed to differences in bacterial strains, geographic origin, sample sources, or antibiotic exposure history in aquaculture environments.

Table 2. Detection of virulence genes of *Aeromonas* spp.

Isolates	Species	The presence of virulence genes										Detection rate per isolate (%)
		aerA/haem	*alt*	*ast*	*act*	*flaA*	*lafA*	*fstA*	*ahp*	*ela*	*lip*	
BmPsN-1	*Aeromonas veronii* strain BmPsN-1	+	-	-	-	-	-	-	-	+	-	2 (20)
BmPsN-2	*Aeromonas veronii* strain BmPsN-2	+	-	-	-	-	-	-	-	+	-	2 (20)
BmPsN-3	*Aeromonas veronii* strain BmPsN-3	+	-	-	-	-	-	-	-	+	-	2 (20)
BmPsN-4	*Aeromonas veronii* strain BmPsN-4	+	-	-	-	-	-	-	-	+	-	2 (20)
BmPsN-5	*Aeromonas veronii* strain BmPsN-5	+	-	-	-	-	-	-	-	+	-	2 (20)
BmPsN-6	*Aeromonas veronii* strain BmPsN-6	+	-	-	-	-	-	-	-	+	-	2 (20)
BmPsN-7	*Aeromonas veronii* strain BmPsN-7	+	-	-	-	-	-	-	-	+	-	2 (20)
Total		7 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (100%)	0 (0%)	14

Table 3. Antimicrobial susceptibility test

Type of antibiotics	Isolates						
	BmPsN-1	BmPsN-2	BmPsN-3	BmPsN-4	BmPsN-5	BmPsN-6	BmPsN-7
Amoxicillin (25 µg)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)
Ciprofloxacin (5 µg)	S (30.01±0.36)	S (30.38±1.11)	S (31.67±0.41)	S (27.93±0.67)	S (27.16±0.60)	S (22.76±0.65)	S (26.33±0.35)
Vancomycin (30 µg)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)
Clindamycin (2 µg)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)
Bacitracin (10 µg)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)
Penicillin G (10 µg)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)	R (0.00±0.00)
Tetracyclin (30 µg)	S (21.53±0.66)	S (20.83±1.72)	S (19.33±1.26)	S (21.23±1.07)	S (22.28±0.37)	S (21.52±1.20)	S (20.58±0.13)
Chloramphenicol (30 µg)	S (21.39±1.07)	S (23.25±0.64)	S (22.61±1.13)	S (24.70±0.27)	S (26.03±0.14)	S (24.70±0.44)	S (23.97±0.70)

Note: S: sensitive, I: intermediet, R: resistant, Number: Diameter of inhibition zone (mm), Ciprofloxacin (5 µg), S: ≥21, I: 16-20, R: ≤15, Tetracyclin (30 µg) S: ≥15, I: 12-14, R: ≤11, Chloramphenicol (30 µg) S: ≥18, I: 13-17, R: ≤12 (CLSI 2024)

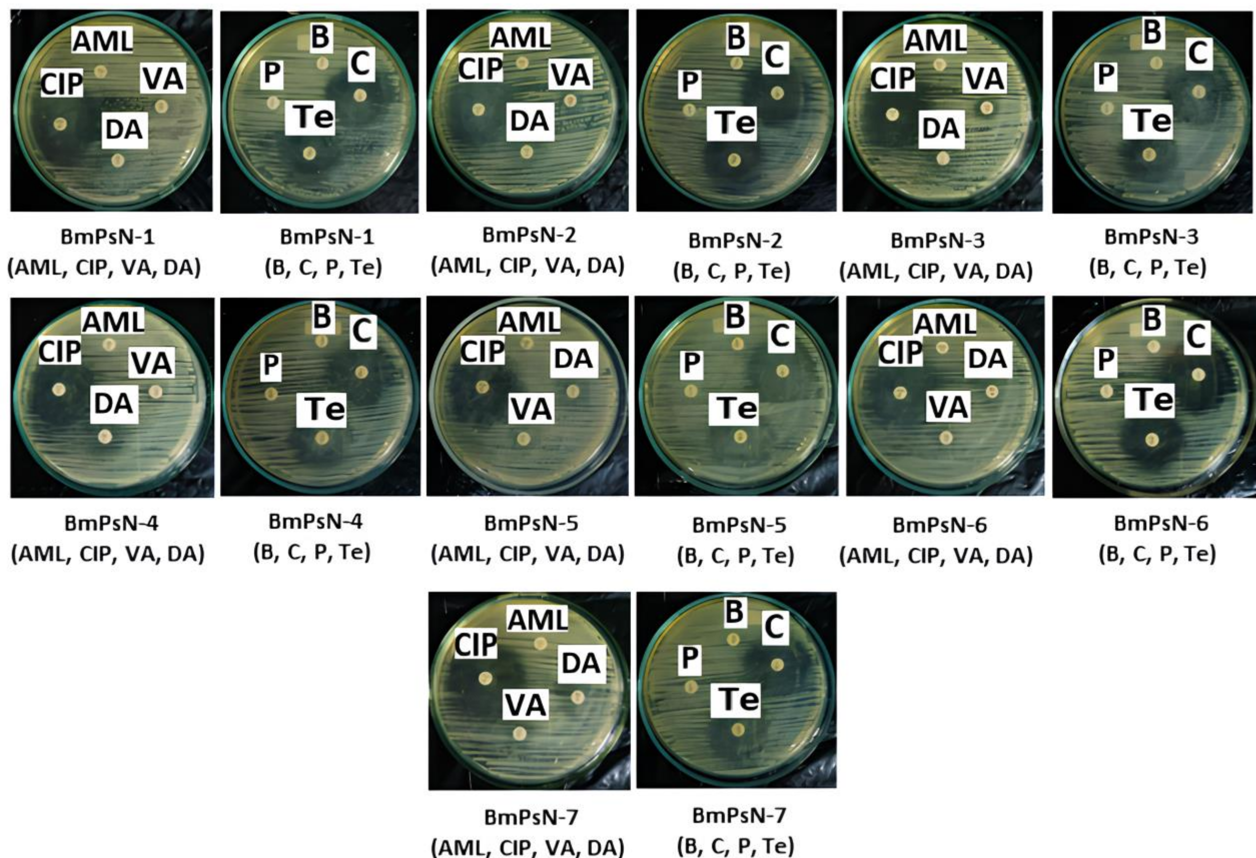

Figure 6. The inhibition zone test was performed for all isolates. AML: Amoxicillin, CIP: Ciprofloxacin, Va: Vancomycin, Da: Clindamycin, B: Bacitracin, P: Penicillin, Te: Tetracyclin, C: Chloramphenicol

Aeromonas spp. in fish have been exposed to high levels of antibiotics during the cultivation process, causing the development of antibiotic resistance (Fauzi et al. 2021). Bacteria adapted by forming cell membranes that prevented the effects of antibiotics and inhibited antibiotics from entering bacterial cells (Ahmed et al. 2024; Wang et al. 2024). Resistance of *Aeromonas* spp. was intrinsic, arising from chromosomal mutations, or extrinsic, acquired through horizontal gene transfer via mobile genetic elements such as plasmids. This transfer occurred through mechanisms such as conjugation, which significantly impacted public health due to multidrug resistance (Bello-López et al. 2019). In addition, some bacteria naturally resist antibiotics despite not interacting directly because bacteria have enzymes that can destroy drugs (Al-Azzawi et al. 2024).

Implications for Nile tilapia health

Detecting virulence genes *aer/haem* and *ela* in *A. veronii* suggests a high pathogenic potential that significantly threatens Nile tilapia health. These genes are associated with cytolytic activity and tissue degradation, contributing to the severity of infections. Furthermore, the observed resistance of *A. veronii* to bacitracin, penicillin, amoxicillin, vancomycin, and clindamycin, combined with its susceptibility to ciprofloxacin, tetracycline, and chloramphenicol, highlights the limited options for effective treatment. The emergence of multidrug-resistant *A. veronii* strains underscores the urgency of implementing responsible antibiotic use and improved biosecurity measures in tilapia farming. Excessive use of antibiotics needs to be reduced to minimize the occurrence and spread of antibiotic-resistant bacteria and to prevent sensitive bacteria from becoming resistant (Franz et al. 2018). Using antibiotics had to follow the correct procedures and doses to avoid negative impacts on fish, aquatic biotas, or the environment. Failure to do so may result in increased morbidity, reduced growth performance, and higher mortality rates, compromising fish welfare and aquaculture sustainability.

In conclusion, *A. veronii* is one of the pathogenic species within the *Aeromonas* genus that is increasingly identified in freshwater aquaculture settings, although its popularity remains lower than that of *A. hydrophila*. This study reports the first *A. veronii* profiling in Nile tilapia in Central Java. The presence of key virulence genes such as *aerA/haem* and *ela* indicates a high potential for pathogenicity and disease outbreaks. Antibiotic susceptibility testing in this study revealed that all seven *A. veronii* isolates were resistant to bacitracin, penicillin, amoxicillin, vancomycin, and clindamycin, while remaining sensitive to ciprofloxacin, tetracycline, and chloramphenicol. This resistance pattern constitutes an essential parameter for evaluating the risks associated with antibiotic application in aquaculture, as it contributes to treatment failure and facilitates the spread of antimicrobial resistance within aquatic ecosystems. The findings of this study highlight the need for targeted diagnostics to detect emerging *A. veronii* infections in tropical aquaculture systems. Enhanced biosecurity management is imperative, particularly through improved cultivation practices, to prevent the dissemination of *A. veronii*, which poses a threat to fish health, reduces aquaculture productivity, and may compromise the genetic diversity of cultured fish populations. Routine monitoring and rapid detection of infections are necessary to ensure prompt and effective control measures, thereby minimizing the risk of widespread disease outbreaks. Regular surveillance of antibiotic resistance patterns should be integrated into aquaculture health management programs. The judicious use of antibiotics, based on sensitivity profiles and correct dosages, must be prioritized to prevent the emergence of new resistance mechanisms that can adversely affect fish welfare and environmental sustainability. In the long term, the development of a specific vaccine against *A. veronii* represents a strategic approach to reduce antibiotic dependence, promote sustainable aquaculture practices, and protect biodiversity within aquatic ecosystems. In addition, environmental management strategies such as improving water quality, reducing fish stocking density, and minimizing physiological stress are crucial in lowering the incidence of opportunistic infections. The implementation of effective filtration and disinfection systems is also recommended to reduce the environmental load of virulent bacteria in aquaculture ponds, ultimately contributing to healthier and more resilient fish farming systems.

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REFERENCES

Aguilera-Arreola MG, Hernández-Rodríguez C, Zúñiga G, Figueras MJ, Castro-Escarpulli G. 2005. *Aeromonas hydrophila* clinical and environmental ecotypes as revealed by genetic diversity and virulence genes. FEMS Microbiol Lett 242 (2): 231-240. DOI: 10.1016/j.femsle.2004.11.011.

Ahmed F, Zhang D, Tang X, Malakar PK. 2024. Targeting spore-forming bacteria: A review on the antimicrobial potential of selenium nanoparticles. Foods 13 (24): 4026. DOI: 10.3390/foods13244026.

Al-Azzawi MK, Hasan NA, Barrak MM. 2024. A review of the development of an understanding of antibiotic interactions, from mechanisms of action to novel resistance and the search for natural alternatives. Eur J Med Genet Clin Biol 1 (6): 78-102. DOI: 10.61796/jmgcb.v1i6.585.

Anantasuk N, Phurahong T, Pumchan A, Hirono I, Unajak S. 2024. Molecular characterization and bivalent vaccine development of *Aeromonas hydrophila* and *Aeromonas veronii* in Nile tilapia (*Oreochromis niloticus*). Aquaculture 590: 741042. DOI: 10.1016/j.aquaculture.2024.741042.

Awan F, Dong Y, Wang N, Liu J, Ma K, Liu Y. 2018. The fight for invincibility: Environmental stress response mechanisms and *Aeromonas hydrophila*. Microb Pathog 116: 135-145. DOI: 10.1016/j.micpath.2018.01.023.

Azzam-Sayuti M, Ina-Salwany MY, Zamri-Saad M, Yusof MT, Annas S, Najihah MY, Liles MR, Monir MS, Zaidi Z, Amal MNA. 2021. The prevalence, putative virulence genes and antibiotic resistance profiles of *Aeromonas* spp. isolated from cultured freshwater fishes in peninsular Malaysia. Aquaculture 540: 736719. DOI: 10.1016/j.aquaculture.2021.736719.

Beaz-Hidalgo R, Magi GE, Balboa S, Barja JL, Romalde JL. 2008. Development of a PCR protocol for the detection of *Aeromonas salmonicida* in fish by amplification of the *fstA* (ferric siderophore receptor) gene. Vet Microbiol 128 (3-4): 386-394. DOI: 10.1016/j.vetmic.2007.10.004.

Bello-López JM, Cabrero-Martínez OA, Ibáñez-Cervantes G, Hernández-Cortez C, Pelcastre-Rodríguez LI, Gonzalez-Avila LU, Castro-Escarpulli G. 2019. Horizontal gene transfer and its association with antibiotic resistance in the genus *Aeromonas* spp. Microorganisms 7 (9): 363. DOI: 10.3390/microorganisms7090363.

Borah A, Srivastava A. 2025. Impact of extracellular enzymes on *Staphylococcus aureus* host tissue adaptation and infection. APMIS 133 (1): e13502. DOI: 10.1111/apm.13502.

Central Bureau of Statistics. 2024. Aquaculture Production and Production Value by Province and Main Commodities 2023. Central Bureau of Statistics. https://www.bps.go.id/. [Indonesian]

Chen F, Sun J, Han Z, Yang X, Xian J-A, Lv A, Hu X, Shi H. 2019. Isolation, identification and characteristics of *Aeromonas veronii* from diseased crucian carp (*Carassius auratus gibelio*). Front Microbiol 10: 2742. DOI: 10.3389/fmicb.2019.02742.

Chen T-N, Hu K. 2023. Identification of *Aeromonas* based on 16S rRNA and *gyrB* gene tandem DNA characteristic sequence. J South Agric 54 (6): 1858-1867. DOI: 10.3969/j.issn.2095-1191.2023.06.027.

CLSI [Clinical and Laboratory Standards Institute]. 2024. M100: Performance Standards for Antimicrobial Susceptibility Testing. 34th eds. Clinical and Laboratory Standards Institute, Malvern, Pennsylvania.

Cunningham FL, Hanson-Dorr KC, Ford L, Middleton DR, Crain A, Durst L, Ware C, Griffin MJ, Mischke CC, Wan X-F, Hanson LA. 2019. Environmental factor(s) and animal vector(s) associated with atypical *Aeromonas hydrophila* abundance and dissemination among channel catfish ponds. J World Aquac Soc 51 (3): 750-762. DOI: 10.1111/jwas.12608.

Dhanapala PM, Kalupahana RS, Kalupahana AW, Wijesekera DPH, Kottawatta SA, Jayasekera NK, Silva-Fletcher A, de S Jagoda SSS. 2021. Characterization and antimicrobial resistance of environmental and clinical *Aeromonas* species isolated from fresh water ornamental fish and associated farming environment in Sri Lanka. Microorganisms 9 (10): 2106. DOI: 10.3390/microorganisms9102106.

Elgohary I, Elatief JIA, Fadel NG, Eissa AE, Mahmoud MA. 2020. Pathological, bacteriological and seasonal prevalence of *Aeromonas hydrophila*, *Vibrio vulnificus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*; infecting *Oreochromis niloticus* in some Egyptian fish farms. Egypt J Aquat Biol Fish 24 (5): 467-482. DOI: 10.21608/ejabf.2020.108585.

El-Sharaby SM, El-Ballal S, Ali M, El-Hady M. 2021. Molecular studies on *Aeromonas* species isolated from freshwater fishes collected from Delta region, Egypt. J Curr Vet Res 3 (2): 8-15. DOI: 10.21608/jcvr.2021.199409.

El-Son MAM, Abdelkhalek NKM, El-Ashram AMM, Zaki VH. 2019. Phenotypic and biochemical detection of *Aeromonas hydrophila* isolated from cultured *Oreochromis niloticus* during disease outbreaks. Intl J Fish Aquac Stud 7 (3): 197-202.

El-Wafai NA, El-Zamik FI, Mahgoub SAM, Atia AMS. 2020. Isolation of *Aeromonas* bacteriophage AvF07 from fish and its application for biological control of multidrug resistant local *Aeromonas veronii* AFs2. Zagazig J Agric Res 47 (1): 179-197. DOI: 10.21608/zjar.2020.70242.

Fauzi NNFNM, Hamdan RH, Mohamed M, Ismail A, Zin AAM, Mohamad NFA. 2021. Prevalence, antibiotic susceptibility, and presence of drug resistance genes in *Aeromonas* spp. isolated from freshwater fish in Kelantan and Terengganu states, Malaysia. Vet World 14 (8): 2064-2072. DOI: 10.14202/vetworld.2021.2064-2072.

Fernández-Bravo A, Figueras MJ. 2020. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. Microorganisms 8 (1): 129. DOI: 10.3390/microorganisms8010129.

Franz CMAP, Besten HMW, Böhnlein C, Gareis M, Zwietering MH, Fusco V. 2018. Microbial food safety in the 21st century: Emerging challenges and foodborne pathogenic bacteria. Trends Food Sci Technol 81: 155-158. DOI: 10.1016/j.tifs.2019.01.009.

Fu GH, Xiao D, Hu K, Yang XL. 2014. Multiplex PCR and ERIC-PCR genotype in virulence genes of *Aeromonas hydrophila* in crucian carp. Marine Fish 36: 549-556.

Gao X, Chen Z, Zhang Z, Qian Q, Chen A, Qin L, Tang X, Jiang Q, Zhang X. 2024. Pathogenicity of *Aeromonas veronii* isolated from diseased *Macrobrachium rosenbergii* and host immune-related gene expression profiles. Microorganisms 12 (4): 694. DOI: 10.3390/microorganisms12040694.

Guan Y, Zhang M, Wang Y, Liu Z, Zhao Z, Wang H, An D, Qian A, Kang Y, Sun W, Shan X. 2022. Functional analysis of ascP in *Aeromonas veronii* TH0426 reveals a key role in the regulation of virulence. J Microbiol 60 (12): 1153-1161. DOI: 10.1007/s12275-022-2373-8.

Hossain S, De Silva BCJ, Wimalasena SHMP, Pathirana HNKS, Dahanayake PS, Heo G-J. 2019. Characterization of virulence determinants and multiple antimicrobial resistance profiles in motile *Aeromonas* spp. isolated from ornamental goldfish (*Carassius auratus*). J Exot Pet Med 29: 51-62. DOI: 10.1053/j.jepm.2018.09.013.

Hu M, Wang N, Pan ZH, Lu CP, Liu YJ. 2012. Identity and virulence properties of *Aeromonas* isolates from diseased fish, healthy controls and water environment in China. Lett Appl Microbiol 55 (3): 224-233. DOI: 10.1111/j.1472-765X.2012.03281.x.

Khor WC, Puah SM, Koh TH, Tan JAMA, Puthucheary SD, Chua KH. 2018. Comparison of clinical isolates of *Aeromonas* from Singapore and Malaysia with regard to molecular identification, virulence, and antimicrobial profiles. Microb Drug Resist 24 (4): 469-478. DOI: 10.1089/mdr.2017.0083.

Khor WC, Puah SM, Tan JAMA, Puthucheary SD, Chua KH. 2015. Phenotypic and genetic diversity of *Aeromonas* species isolated from fresh water lakes in Malaysia. PLoS One 10 (12): e0145933. DOI: 10.1371/journal.pone.0145933.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mo Biol Evol 35: 1547-1549. DOI: 10.1093/molbev/msy096.

Lee H-J, Storesund JE, Lunestad B-T, Hoel S, Lerfall J, Jakobsen AN. 2023. Whole genome sequence analysis of *Aeromonas* spp. isolated from ready-to-eat seafood: Antimicrobial resistance and virulence factors. Front Microbiol 14: 1175304. DOI: 10.3389/fmicb.2023.1175304.

Mailafia S, Nabilah B, Olabode HOK. 2021. Phenotypic characterization of *Aeromonas hydrophila* isolates in fresh water fishes in FCT using Microbact™ GNB 24E identification kit. Open Access Lib J 8 (1): 1-12. DOI: 10.4236/oalib.1107066.

Majeed S, De Silva LADS, Kumarage PM, Heo G-J. 2023. Occurrence of potential virulence determinants in *Aeromonas* spp. isolated from different aquatic environments. J Appl Microbiol 134 (3): lxad031. DOI: 10.1093/jambio/lxad031.

Matter AF, El Asley AM, Abbass AA, Shaheen AA. 2024. Utilizing Outer Membrane Proteins (OMPs) as a vaccine to manage Motile Aeromonas Septicemia (MAS) in Nile tilapia (*Oreochromis niloticus*) with evaluating the vaccine immunoprotective effectiveness. Aquac Intl 32: 7235-7252. DOI: 10.1007/s10499-024-01511-6.

Mulia DS, Nisa Z, Suwarsito, Purbomartono C, Isnansetyo A, Nafiqoh N, Yasin ISM, Azzam-Sayuti M. 2024. Phenotypic and genotypic characteristics of pathogenic bacteria *Aeromonas veronii* bv *veronii* causes disease in gourami (*Osphronemus gouramy*). Biodiversitas 25 (12): 5103-5111. DOI: 10.13057/biodiv/d251248.

Mulia DS, Pratiwi R, Asmara W, Azzam-Sayuti M, Yasin ISM, Isnansetyo A. 2023. Isolation, genetic characterization, and virulence profiling of different *Aeromonas* species recovered from moribund hybrid catfish (*Clarias* spp.). Vet World 16 (9): 1974-1984. DOI: 10.14202/vetworld.2023.1974-1984.

Pereira C, Duarte J, Costa P, Braz M, Almeida A. 2022. Bacteriophages in the control of *Aeromonas* sp. in aquaculture systems: An integrative view. Antibiotics 11 (2): 163. DOI: 10.3390/antibiotics11020163.

Pessoa RBG, de Oliveira WF, Marques DSC, Dos Santos Correia MT, de Carvalho EVMM, Coelho LCBB. 2019. The genus *Aeromonas*: A general approach. Microbial Pathog 130: 81-94. DOI: 10.1016/j.micpath.2019.02.036.

Rahman MM, Sadekuzzaman M, Rahman MA, Siddique MP, Uddin MA, Haque ME, Chowdhury MGA, Khasruzzaman AKM, Rahman MT, Hossain MT, Islam MA. 2023. Complete genome sequence analysis of the multidrug resistant *Aeromonas veronii* isolated for the first time from stinging catfish (Shing fish) in Bangladesh. J Adv Vet Anim Res 10 (3): 570-578. DOI: 10.5455/javar.2023.j711.

Sarkar P, Issac PK, Raju SV, Elumalai P, Arshad A, Arockiaraj J. 2021. Pathogenic bacterial toxins and virulence influences in cultivable fish. Aquac Res 52 (6): 2361-2376. DOI: 10.1111/are.15089.

Sen K, Rodgers M. 2004. Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: A PCR identification. J Appl Microbiol 97 (5): 1077-1086. DOI: 10.1111/j.1365-2672.2004.02398.x.

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

Sheikh HI, Nordina B, Paharuddina N, Liew HJ, Fadhlina A, Abdulrazzak LA, Jalal KCA, Musa N. 2023. Virulence factors and mechanisms of

Aeromonas hydrophila infection in catfish Siluriformes: A review and bibliometric analysis. Desal Water Treat 315: 538-547. DOI: 10.5004/dwt.2023.30019.

Sherif AH, AbuLeila RH. 2022. Prevalence of some pathogenic bacteria in caged-Nile tilapia (*Oreochromis niloticus*) and their possible treatment. Jordan J Biol Sci 15: 239-247. DOI: 10.54319/jjbs/150211.

Soler L, Figueras MJ, Chacón MR, Vila J, Marco F, Martinez-Murcia AJ, Guarro J. 2002. Potential virulence and antimicrobial susceptibility of *Aeromonas popoffii* recovered from freshwater and seawater. FEMS Immunol Med Microbiol 32 (3): 243-247. DOI: 10.1016/S0928-8244(01)00303-0.

Sun Y, Hao Y, Wang Z, Wang H, Gao Y. 2025. Isolation, characterization, and hole genome sequencing analysis of *Aeromonas veronii* from *Channa argus* in China. Vet Res Commun 49 (1): 7. DOI: 10.1007/s11259-024-10594-8.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30 (12): 2725-2729. DOI: 10.1093/molbev/mst197.

Wang D, Dai C, Li Q, Li Y, Liu Z. 2019. Complete mitochondrial genome and phylogenic analysis of *Rhinogobius cliffordpopei* (Perciformes, Gobiidae). Mitochondrial DNA B Resour 4 (2): 2473-2474. DOI: 10.1080/23802359.2019.1637287.

Wang L, Li H, Chen J, Wang Y, Gu Y, Jiu M. 2024. Antibacterial Mechanisms and antivirulence activities of oridonin against pathogenic *Aeromonas hydrophila* AS 1.1801. Microorganisms 12 (2): 415. DOI: 10.3390/microorganisms12020415.

Yáñez MA, Catalán V, Apráiz D, Figueras MJ, Martínez-Murcia AJ. 2003. Phylogenetic analysis of members of the genus *Aeromonas* based on *gyrB* gene sequences. Intl J Syst Evol Microbiol 53 (Pt 3): 875-883. DOI: 10.1099/ijs.0.02443-0.

Yazdanpanah-Goharrizi L, Rokhbakhsh-Zamin F, Zorriehzahra MJ, Kazemipour N, Kheirkhah B. 2020. Isolation, biochemical and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss*. Iran J Fish Sci 19 (5): 2422-2436. DOI: 10.22092/ijfs.2020.122060.

Yoon J. 2022. Focused commentary; About revision of CLSI antimicrobial breakpoints, 2018-2021. J Bacteriol Virol 52 (2): 41-53. DOI: 10.4167/jbv.2022.52.2.041.

Youssef HA, Ayoub HF, Soror EI, Matter AF. 2022. Virulence genes contributing to *Aeromonas veronii* pathogenicity in Nile tilapia (*Oreochromis iloticus*): Approaching the development of live and inactivated vaccines. Aquac Intl 31: 1253-1267. DOI: 10.1007/s10499-022-01023-1.

Yuwono C, Wehrhahn MC, Liu F, Riordan SM, Zhang L. 2021. The Isolation of *Aeromonas* species and other common enteric bacterial pathogens from patients with gastroenteritis in an Australian population. Microorganism 9 (7): 1440. DOI: 10.3390/microorganisms9071440.

Zhong C, Han M, Yang P, Chen C, Yu H, Wang L, Ning K. 2019. Comprehensive analysis reveals the evolution and pathogenicity of *Aeromonas*, viewed from both single isolated species and microbial communities. mSystems 4: e00252-19. DOI: 10.1128/msystems.00252-19.