BIODIVERSITAS
Volume 25, Number 12, December 2024
Pages: 5103-5111

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251248

Phenotypic and genotypic characteristics of pathogenic bacteria *Aeromonas veronii* bv *veronii* causes disease in gourami (*Osphronemus gouramy*)

DINI SISWANI MULIA[1,♥], ZAHROTUN NISA[1], SUWARSITO[2], CAHYONO PURBOMARTONO[2], ALIM ISNANSETYO[3], NUNAK NAFIQOH[4], INA SALWANY MD YASIN[5], MOHAMAD AZZAM-SAYUTI[5]

[1]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Banyumas 53182, Central Java, Indonesia. Tel.: +62-281-636751, Fax.: +62-281-637239, ♥email: dinisiswanimulia@ump.ac.id
[2]Department of Aquaculture, Faculty of Agriculture and Fisheries, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Banyumas 53182, Central Java, Indonesia
[3]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia
[4]National Research and Innovation Agency. Jl. M.H. Thamrin No. 8, Jakarta 10340, Central Jakarta, Indonesia
[5]Laboratory of Aquatic Animal Health and Therapeutics, Institute of Bioscience, Universiti Putra Malaysia. 43400 Serdang, Selangor, Malaysia

Manuscript received: 19 November 2024. Revision accepted: 30 December 2024.

Abstract. *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: 5103-5111.* Gourami (*Osphronemus gouramy*) is a type of freshwater fish with excellent potential, but its cultivation is often disturbed by bacterial diseases caused by *Aeromonas* spp. The presence of virulent genes often influences the pathogenicity of *Aeromonas* spp. and treatment with antibiotics causes bacteria resistance. Therefore, this study aimed to determine the phenotypic and genotypic characteristics of *Aeromonas veronii* bv *veronii* causing disease in gourami (*O. gouramy*). A purposive sampling method was used to obtain specific diseased gourami. Phenotypic characteristics were determined morphologically and biochemically, while genotypic characteristics were based on 16S rDNA and virulent genes. A total of 10 virulent genes were used, including the *aerA/haem*, *alt*, *ast*, *act*, *flaA*, *lafA*, *fstA*, *ahp*, *ela*, and *lip* genes. Subsequently, antibiotic susceptibility tests were carried out using 10 µg gentamycin disk, 10 µg bacitracin, 30 µg tetracycline, and 30 µg chloramphenicol. Data on phenotypic and genotypic identification, the detection of virulence genes, and antimicrobial susceptibility tests were analyzed descriptively and qualitatively. The results showed that isolate BmSG-03 was closely related to *A. veronii* bv *veronii* strain ATCC 35624 with a similarity rate and query of 99.58% and 100%, respectively. The *A. veronii* bv *veronii* BmSG-03 isolate was detected to contain the *aerA/haem*, *lafA*, and *ela* genes, while the *alt*, *ast*, *act*, *flaA*, *fstA*, *ahp*, and *lip* genes were not detected. This isolate was resistant to bacitracin, tetracycline, and gentamycin but susceptible to chloramphenicol. Moreover, further investigations were recommended on genotypic characteristics of *A. veronii bv veronii* resistance gene and other antibiotic tests due to limited reports in Indonesia. For effective disease control, there should be a comprehensive database on the characteristics of the pathogen to obtain more effective and appropriate mitigation efforts.

Keywords: *Aeromonas veronii* bv *veronii*, fish disease, fish species, Gram-negative bacteria, susceptibility to antibiotics

INTRODUCTION

Gourami (*Osphronemus gouramy* Lacepède, 1801) is a type of freshwater fish with excellent potential to be cultivated. Indonesia's gourami production contributes more than 98.4 % of the world's total production (FAO 2023). However, disease is a severe problem contributing to significant losses, including the death of fish (Mulia et al. 2020, 2023a; Semwal et al. 2023). In January-December 2023, a significant number of dead fish ranging from 33.33 to 100% were a result of this disease caused by bacteria infections in various farming groups across Banyumas area. Other causes are fungi, viruses, parasites, stress, and poor water quality (Department of Fisheries and Animal Husbandry of Banyumas Regency 2024). One of the diseases often occurring in gourami is aeromoniasis or Motile *Aeromonas* Septicemia (MAS) caused by *Aeromonas* spp. (Rozi et al. 2018). Previous reports have shown that *Aeromonas* spp. is capable of infecting other fish, causing significant losses (Zhang et al. 2020; Mulia et al. 2021, 2022a, 2023b, 2023c; Abdella et al. 2024). MAS is a severe systemic disease that attacks freshwater, brackish water, and marine fish with high mortality rates (Fernández-Bravo and Figueras 2020).

Aeromonas spp. are rod-shaped, non-spore-forming, Gram-negative, facultative anaerobic bacteria, and opportunistic microorganisms (Pessoa et al. 2019; Dwi et al. 2023; Mulia et al. 2023a). Most of these bacteria are pathogenic to fish, including *A. hydrophila*, *A. salmonicida*, *A. caviae, A. sobria*, *A. veronii*, *A. jandaei*, and *A. dhakensis* (Dong et al. 2017; Mulia et al. 2020, 2021, 2022b, 2023a; Azzam-Sayuti et al. 2021a; Dwi et al. 2023). As effective mitigating efforts control, there is a need to carry out periodic isolation of sick fish to identify the main pathogen, thereby enabling an appropriate and effective solution. Previous studies have molecularly identified bacteria isolated from sick catfish in West Java, Central Java, Special Region of Yogyakarta, and East Java. The results showed that several species and strains of *Aeromonas* spp. were

successfully identified, namely *A. hydrophila* Ah-01, *A. caviae* Ac-01, *A. dhakensis* Ad-01, Ad-02, Ad-03, and *A. veronii* bv *veronii* Av-01, Av-02, Av-03, and Av-04 (Mulia et al. 2023a). An experiment has been conducted in Banyumas on catfish, where several strains of *A. jandaei* species were found to cause fish disease (Mulia et al. 2024).

Aeromonas veronii is among pathogenic bacteria with two biovars, namely *A. veronii* bv *sobria* and *A. veronii* bv *veronii* (Shameena et al. 2020). Sarjito et al. (2018) reported *A. veronii* isolated from catfish samples taken from Demak, Central Java. However, the biovar has not been discovered, including the virulence and resistance potential. The overseas studies isolated *A. veronii* bv *veronii* from Chinese long-snout catfish (*Leiocassis longirostris* Günther, 1864), common carp (*Cyprinus carpio* Linnaeus, 1758), silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844), and marsh frog (*Pelophylax ridibundus* Pallas, 1771), followed by phenotypic and molecular identification with 16S rDNA (Soltani et al. 2016; Khalifa and Bekhet 2017).

Hoai et al. (2019) reported that *A. veronii* caused mass mortality of catfish in Vietnam and nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in India (Raj et al. 2019). *A. veronii* was also reported to attack Nile tilapia in Brazil, according to Dos Santos et al. (2023). Infection of *A. veronii* bv *veronii* causes clinical signs in catfish, namely depigmentation of the skin, hyperemia, hemorrhagic, necrosis, ulcer, abdominal dropsy, abdominal ascites, abscess, pale in kidney, and gills (Algammal et al. 2022; Dos Santos et al. 2023; Mulia et al. 2023a). The ability of *A. veronii* bv *veronii* to infect fish is due to the pathogenicity factor (Azzam-Sayuti et al. 2021b). Several virulent genes possessed by *Aeromonas* spp. include *aer/haem*, *alt*, *ast*, *flaA*, *lafA*, *fstA*, *aspA*, *vasH*, *ascV*, and *aexT* (Aravena-Román et al. 2014; Khor et al. 2015; Mulia et al. 2023a). *Aeromonas* spp. are known to resist certain antibiotics (Azzam-Sayuti et al. 2021b; Mulia et al. 2023a). Differences in bacterial resistance need to be determined to facilitate appropriate and effective disease management. Therefore, this study aimed to determine the phenotypic and genotypic characteristics of the pathogenic bacteria *A. veronii* bv *veronii*, which causes disease in gourami.

MATERIALS AND METHODS

Study period and location

The study was conducted from January to June 2023 and samples of gourami were taken from aquaculture ponds in Singasari Village, Banyumas District, Central Java, Indonesia (Figure 1). Sampling was determined from one gourami cultivation pond and three sick gourami fish were randomly taken. External clinical signs in the fish included frayed fins and tail, hyperemia on the fins and tail, reddish spots (hemorrhagic) on the body, peeling scales, and body depigmentation. Internal clinical signs that appeared were blackish-red kidneys and liver, gastric depigmentation, destroyed stomach, and ascites. The euthanasia method was carried out by soaking the fish in a solution of clove oil, containing 1 mL/L of water. 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).

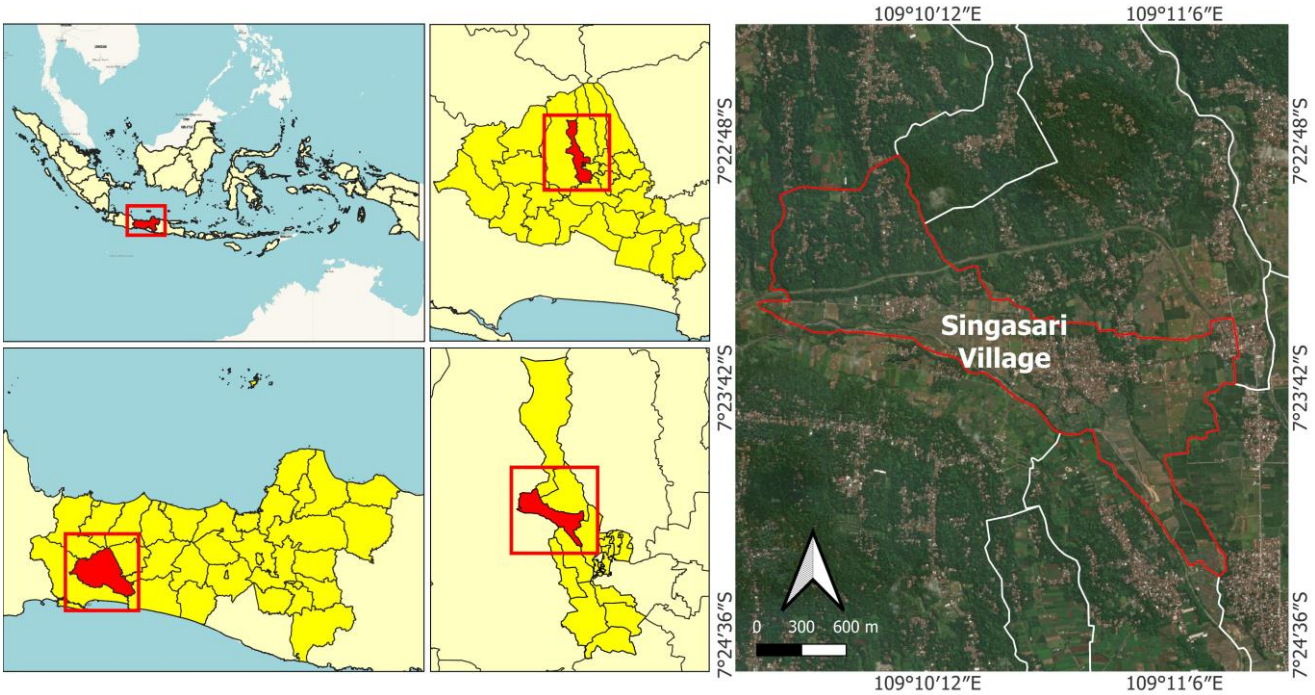

Figure 1. Map of sampling location diseased gourami in Singasari Village, Banyumas District, Central Java, Indonesia

Table 1. Primers sets used in this study

Genes	Gene product	Primer sequence	Product size (bp)	Reference
16S	16 S rRNA gene	F: AGA GTT TGA TCM TGG CTC AG R: TAC GGY TAC CTT GTT ACG ACT T	1500	Isnansetyo and Kamei (2003)
aerA/haem	Aerolysin/hemolysin	F: CCT ATG GCC TGA GCG AGA AG R: CCA GTT CCA GTC CCA CCA CT	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	442	Sen and Rodgers (2004)
ast	Heat-stabile cytotonic enterotoxin	F: TCT CCA ATG CTT CCC TTC ACT R: GTG TAG GGA TTG AAG AAG CCG	331	Sen and Rodgers (2004)
act	Cytotoxic heat-labile enterotoxin	F: ATCGTCAGCGACAGCTTCTT R: CTCATCCCTTGGCTTGTTGT	500	Fu et al. (2014)
flaA	Polar flagellum	F: TCC AAC CGT YTG ACC TC R: GMY TGG TTG CGR ATG GT	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)	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	452	Beaz-Hidalgo et al. (2008)
ahp	Serine protease	F: ATTGGATCCCTGCCTATCGCTTCAGTTCA R: GCTAAGCTTGCATCCGTGCCGTATTCC	911	Hu et al. (2012)
ela	Elastase	F: ACACGGTCAAGGAGATCAAC R: CGCTGGTGTTGGCCAGCAGG	513	Sen and Rodgers (2004)
lip	Lipase	F: ATCTTCTCCGACTGGTTCGG R: CCGTGCCAGGACTGGGTCTT	382	Sen and Rodgers (2004)

Isolation and bacteria culture

Isolates were obtained from the kidney and ulcer of diseased gourami, followed by culture on Glutamate Starch Phenyl (GSP) medium (Merck, Darmstadt, Germany) at 30°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.

Koch's postulate test

Healthy gourami fish with a total length of 10-12 cm were acclimatized for 5 days. A bacteria suspension of 0.1 mL (density of 10^6 CFU mL^{-1}) was injected into 10 gourami fish free of *Aeromonas* spp., carried out in duplicate. Observations were made for 14 days on external and internal clinical signs, including mortality (Mulia et al. 2020).

Bacteria characterization by phenotypic (morphology and biochemical tests)

Phenotypic characterization of bacteria was carried out based on morphology and biochemical tests. The tests carried out were colony morphology, cell morphology with Gram staining, motility, oxidase, catalase, indole production, O/F, Triple Sugar Ion Agar (TSIA)/H_2S production, Methyl Red (MR), Vogest Proskauer (VP), and sugar test (glucose, sucrose, lactose, mannitol, and dextrose) (Mulia et al. 2020).

Bacteria genomic DNA extraction

The bacteria genomic DNA was extracted using High Pure Polymerase Chain Reaction (PCR) Preparation Kit (Roche, 11796828001, Roche Diagnostics Corporation, Indiana, USA). Subsequently, 1 mL of bacteria culture in TSB medium incubated for 24 h at 37°C was centrifuged at 13,000 g for 2 min. The extracted bacteria DNA was stored at -20°C for further assay.

Bacterial characterization by genotypic means (16S rDNA)

Characterization of bacteria by 16S rDNA was carried out using oligonucleotide universal primers of 27F and 1492R, as shown in Table 1. The total PCR volume was 50 µL, containing 26 µL Mytaq HS Red Mix, (2× PCR Master Mix) (Bioline, Meridian Life Science, Memphis, UK), 2 µL forward primer, 2 µL reverse primer, 2 µL DNA template (20 ng), and 18 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 before sequencing (1st BASE Laboratories Malaysia).

Sequence analysis

The DNA sequences were edited and assembled using the program DNA Baser (Wang et al. 2019). The similarity was analyzed using the Basic Local Alignment Search Tool (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST) and multiple sequence arrangements were carried out with the Clustal W Program (Sofi et al. 2021). 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

Detection of virulence genes of *Aeromonas* spp. were amplified by PCR. The total PCR volume of 25 µL contained 13 µL Mytaq HS Red Mix, (2x 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 NFW. The virulence genes of *Aeromonas* spp. detected were *aerA/haem*, *alt*, *ast*, *act*, *flaA*, *lafA*, *fstA*, *ahp*, *ela*, and *lip* (Table 1). The PCR product was subjected to electrophoresis with 1.5% agarose gel.

Antimicrobial susceptibility test

Antibiotic susceptibility test was carried out using 10 µg gentamycin, 10 µg bacitracin, 30 µg tetracycline, and 30 µg chloramphenicol discs. Bacteria were inoculated on TSA medium in a continuous streak and incubated at 30°C 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 CLSI (2020).

Data analysis

Data of phenotypic and genotypic identification, detection of virulence gene, and antimicrobial susceptibility test were analyzed descriptively and qualitatively.

RESULTS AND DISCUSSION

Koch's postulate test

The results of Koch's postulate test showed that the mortality of gourami infected with isolate BmSG-03 reached 100%, with external and internal clinical signs, as presented in Figure 2. External clinical signs included skin depigmentation, extensive skin lesions, hemorrhagic, necrosis of fins. Meanwhile, internal clinical signs were characterized by changes in kidney color to pareddish brown, liver and other organs to pale, and ascites. Previous studies also reported that tilapia infected with *A. veronii* showed clinical signs of skin darkness, hemorrhagic septicemia, and fin necrosis (El-Wafai et al. 2020). The clinical signs that appeared in crucian carp (*Carassius auratus* Linnaeus, 1758). infected with *A. veronii* were abdominal distension, congestion of the fin base, and branchial ischemia (Chen et al. 2019). Clinical signs in this study showed that *A. veronii* bv *veronii* BmSG-03 was pathogenic and had a virulence factor causing *Aeromonas* on gourami.

Phenotypic character of the *A. veronii* bv *veronii* BmSG-03

Table 2 shows the phenotypic characteristics of isolate BmSG-03, which is suspected to be *A. veronii* bv *veronii*. Meanwhile, isolate no. 2 is *A. veronii* bv *veronii* (Janda and Abbot 2010), 3 is *A. veronii* bv *veronii* from fish and human (Aravena-Román et al. 2011), 4 is from moribund hybrid catfish (Mulia et al. 2023a), 5 is from European seabass (*Dicentrarchus labrax*) (Smyrli et al. 2019), and 6 is from Nile tilapia (El-Wafai et al. 2020).

Isolate BmSG-03 grew well on GSP and TSA medium at 30°C, was yellow on GSP medium and white on TSA medium. Further observations showed rod-shaped, Gram-negative, motile, oxidase and catalase negative, produced indole, and O/F positive. TSIA test showed that isolate BmSG-03 could ferment all carbohydrates including ferment sucrose, lactose, mannitol, and dextrose. Additionally, it was found to be methyl red positive, with potential to form acid and gas from glucose but could not produce acetyl methyl carbinol (VP). These phenotypic characteristics were found in *A. veronii* bv *veronii* strains no. 2,3,4,5, although some components data were absent. Some differences occurred in the oxidase and catalase of isolates no. 5 and 6, H_2S production with isolate no. 6, VP with isolate no. 3, 5, and 6. Based on phenotypic characteristics, isolate BmSG-03 showed characters that were consistent with *A. veronii* bv *veronii* (Janda and Abbot 2010; Aravena-Román et al. 2011; Smyrli et al. 2019; Mulia et al. 2023a). *A. veronii* bacteria had biochemical characteristics that showed positive results for motility, indole, glucose, maltose, mannitol, sucrose, and lactose tests. However, there were negative results for MR-VP and arabinose tests (Chen et al. 2019). Variations in phenotypic characteristics were assumed to have clinical significance as a relationship between biotype and enterotoxin production (Aravena-Román et al. 2011).

Genotypic character of the *A. veronii bv veronii* BmSG-03

The results of genotypic characterization with 16S rDNA showed that isolate BmSG-03 was *A. veronii* bv *veronii* strain ATCC 35624 with 100% query, 99.58% identity with Accession number NR_118947 (Mulia et al. 2023a). Similarly, El-Wafai et al. (2020) identified disease-causing bacteria in nile tilapia with a genotypic method using 16S rRNA gene sequences and *A. veronii* bv *veronii* strain ATCC 35624 with 97% identity.

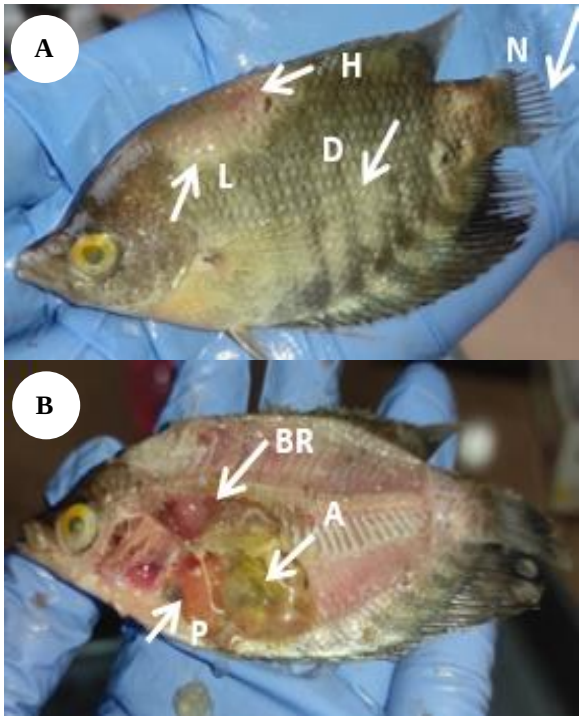

Figure 2. Clinical signs of gourami. A. D: Depigmentation of the skin; L: Extensive skin lesions; H: Hemorrhagic; N: Necrosis of fins; B. BR: Brown reddish in kidney; P: Pale in liver, A: Ascites

Table 2. The phenotypic characters of *Aeromonas veronii bv veronii* BmSG-03 isolated from diseased gourami

Characterization	1	2	3	4	5	6
Colony morphology						
Form	Circular	Circular	Circular	Circular	Circular	Circular
Edge	Even	Even	Even	Even	Even	Even
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Color in TSA	White	White	White	White	White	White
Color in GSP	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Bacterial morphology						
Form	Rod	Rod	Rod	Rod	Rod	Rod
Gram	-	-	-	-	-	-
Motility	+	+	+	+	+	+
Oxydase	-	ND	ND	ND	+	+
Catalase	-	ND	ND	ND	+	+
Indole	+	+	+	ND	+	+
O/F	+	ND	ND	+	ND	ND
H_2S production	-	ND	ND	-	-	+
Methyl Red (MR)	+	ND	ND	ND	ND	ND
Voges Proskauer	-	d	+	-	+	+
D-glucose	+g	+g	+g	+g	+	+g
Sucrose	+	+	+	ND	+	+
Lactose	+g	d	+	+	ND	+
Manitol	+g	+	ND	ND	+	+
Dextrose	+g	ND	ND	ND	ND	ND

Notes: Taxa are identified as: 1. Strains BmSG-03 (data from this study); 2. *A. veronii* bv *veronii* (Janda and Abbot 2010); 3. *A. veronii* bv *veronii* from fish and human (Aravena-Román et al 2011); 4. *A. veronii* bv *veronii* from moribund hybrid catfish (Mulia et al. 2023a); 5. *A. veronii* bv *veronii* from European seabass (Smyrli et al. 2019); 6. *A. veronii* bv *veronii* from Nile tilapia (El-Wafai et al. 2020). Abbreviations: +: >90% of strains positive; -: <10% of strains positive; d: 11-89% of strains positive; ND: No Data available

Phylogenetic tree

The phylogenetic tree results showed that isolate BmSG-03 belonged to *A. veronii* bv *veronii* strain ATCC 35624, as presented in Figure 3. The selection of outgroups in phylogenetic tree construction was based on the correlation of sequences analyzed. The isolate was very closely related and belonged to the same branch. Reading the phylogenetic tree was based on the length and connectedness of the branches and the stated bootstrap values (Hu et al. 2020). *Pseudomonas aeruginosa* was used as outgroups, possessing morphological and molecular characteristics similar to *Aeromonas* spp. (Su et al. 2023).

Detection of virulent genes of *A. veronii* bv *veronii* BmSG-03

Detection of virulent genes in *A. veronii* bv *veronii* BmSG-03 isolate was successfully carried out. Subsequently, 10 virulent genes tested were *aerA/haem*, *alt*, *ast*, *act*, *flaA*, *lafA*, *fstA*, *ahp*, *ela*, and *lip*. The results showed that the *aerA/haem*, *lafA*, and genes were detected, while other genes were not detected in isolate BmSG-03 isolate, as shown in Table 4.

Previous studies reported that *A. veronii* from crucian carp *aer*, *alt*, *ahyB*, *gcaT*, *lip*, and *ser* genes were detected. However, the *act*, *ast*, *fla*, *ahyB*, and *exu* genes were not detected (Chen et al. 2019). *A. veronii* from freshwater fish contained the *fla*, *ela*, *hly*, and *act* genes (Azzam-Sayuti et al. 2021b). Meanwhile, 4 isolates of *A. veronii* bv *veronii* from moribund hybrid catfish were found to contain the *aer/haem*, *ast*, *flaA*, and *lafA* genes. Only two isolates were detected to contain the *alt* and *fstA* genes (Mulia et al. 2023a).

Aerolysin is a hemolytic extracellular product encoded by the aerolysin gene, which plays a significant role in the pathogenicity of *Aeromonas* spp. Specifically, aerolysin is the most critical toxin among potential virulence factors in *Aeromonas* (Dong et al. 2022). Screening of specific cytotoxin and hemolysin genes has been reported to be the most effective method to detect and characterize *Aeromonas* virulence factors (Ahangarzadeh et al. 2022). The *lafA* gene was detected in *A. veronii* bv. *veronii* BmSG-3 isolate. The *lafA* gene was also detected in all *A. veronii* bv. *veronii* isolates from moribund hybrid catfish (Mulia et al. 2023a). However, other studies could not detect the gene from *A. veronii* (Azzam-Sayuti et al. 2021b). The *lafA* gene promoted swarming on solid surfaces, playing a significant role in cell adherence, biofilm formation, and persistence during infection (Liu 2015).

In this study, the *ela* gene was detected in *A. veronii* bv. *veronii* isolates, although the gene was frequently detected in *Aeromonas* isolates, showing dominance in 62% to 86% of strains (Khor et al. 2015). The results showed differences in the virulence genes detected, which affected the level of bacteria pathogenicity in the field. The diversity of virulence genes detected in *Aeromonas* spp. could be due to differences in strains, isolate sources, geographic location, and environment, as stated in previous studies (Abu-Elala et al. 2015).

Table 3. Detection of virulent genes of *Aeromonas veronii* bv. *veronii* BmSG-03

Isolate	Species	Virulence genes									
		aer/haem	***alt***	***ast***	**act**	***flaA***	***lafA***	***fstA***	***ahp***	***ela***	***lip***
BmSG-03	*Aeromonas veronii* bv. *veronii* strain ATCC 35624	+	-	-	-	-	+	-	-	+	-

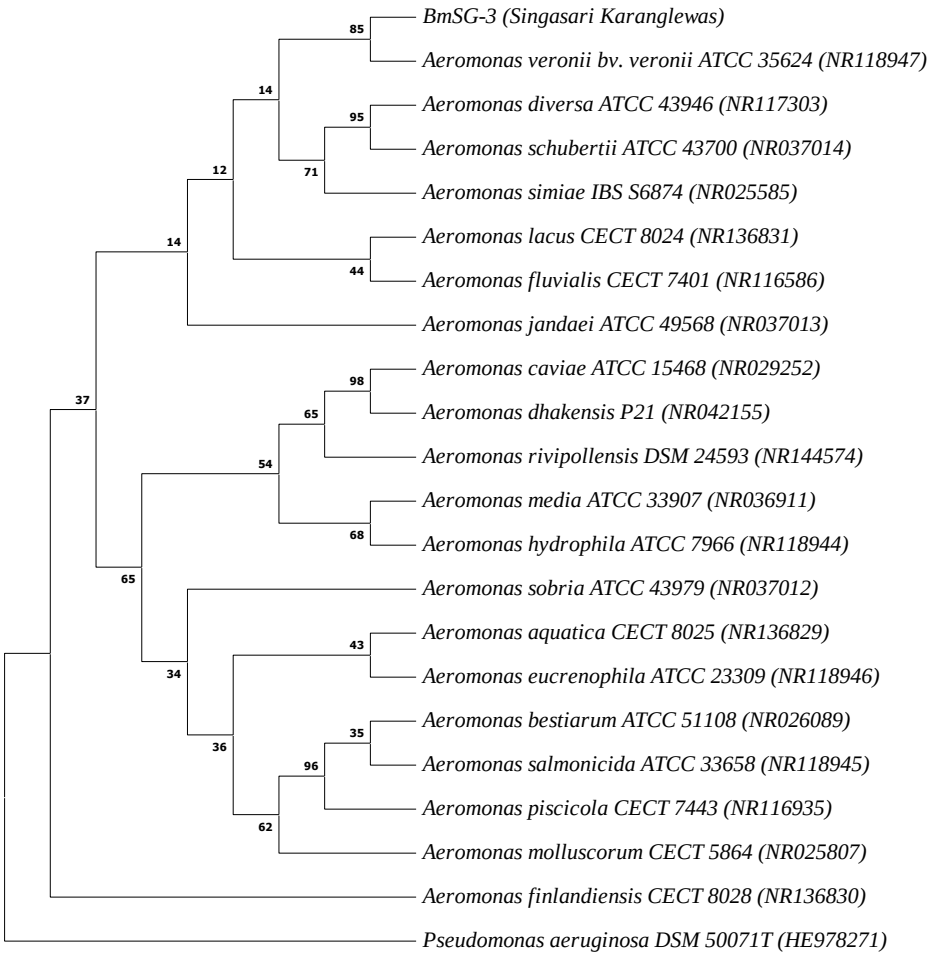

Figure 3. Phylogenetic tree constructed from the 16S rDNA sequences from isolate of *Aeromonas veronii* bv *veronii* (BmSG-03) and other *Aeromonas* species (class of Gammaproteobacteria). *Pseudomonas aeruginosa* was used as an outgroup. The topology was obtained by maximum possibility with bootstraps of 1000 replications. The scale bar signifies 0.02 substitutions per nucleotide position (Knuc)

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed on isolate *A. veronii* bv. *veronii* BmSG-03. Susceptibility testing was conducted to determine the sensitivity of test bacteria to antibiotics through the size of the inhibition zone diameter (Vineetha et al. 2015). Isolate *A. veronii* bv. *veronii* BmSG-03 was resistant to tetracycline, bacitracin, and gentamycin but susceptible to chloramphenicol, as shown in Table 4. Based on previous studies, *Aeromonas* spp. were resistant to gentamycin, tetracycline, and chloramphenicol with 80%, 70%, and 55% resistance (Oladele and Temitope 2016). *A. veronii* bv. *veronii* has resistance to ampicillin, amikacin, gentamicin, rifampicin, penicillin, and nitrofurantoin (Janda and Abbot 2010). *A. veronii* is resistant to tetracycline 19%, intermediate 19%, and susceptible 63%, sensitive 89%, and intermediate 11%, but shows 0% resistance to gentamicin and 100% sensitive to chloramphenicol (Azzam-Sayuti et al. 2021b). *A. veronii* is susceptible to tetracycline, moderately susceptible to gentamycin, and resistant to chloramphenicol (Chen et al. 2019). *A. veronii* bv. *veronii* has intermediate susceptibility to gentamicin and is resistant to tetracycline but susceptible to chloramphenicol (El-Wafai et al. 2020). In another study, Fauzi et al. (2021) obtained 61 isolates from the genus *Aeromonas*, including *A. jandaei*, *A. veronii*, *A. hydrophila*, and *A. sobria*. The results showed that all *Aeromonas* isolates were sensitive to chloramphenicol and nitrofurantoin.

Table 4. Antibiotic susceptibility test of *Aeromonas veronii* bv *veronii* BmSG-03

Antibiotics	Susceptibility	Inhibition zone diameter (mm)
Tetracycline	R	8.27
Bacitracin	R	0.00
Gentamycin	R	6.36
Chloramphenicol	S	26.76

S: susceptible; R: resistant; Number: Inhibition zone diameter (mm)

This resistance result was caused by *Aeromonas* spp. in farmed fish exposed to high levels of antibiotics during the cultivation process, causing antibiotic resistance in bacteria isolates (Fauzi et al. 2021). Generally, bacteria can adapt by forming cell membranes to prevent and inhibit antibiotics from entering bacteria cells (Besung et al. 2019). Furthermore, some bacteria have the natural ability to resist antibiotics without direct interaction due to the ability to destroy drugs with their enzymes (Varela et al. 2021). Variations in sensitivity and resistance patterns can be due to different isolation sources and environmental conditions (Nagar et al. 2011).

Tetracycline is a broad-spectrum antibiotic against Gram-positive and Gram-negative bacteria (Manoharan et al. 2019; Sheykhsaran et al. 2019). Continuous use of tetracycline causes bacteria to control the activity of the antibiotic, similar to bacitracin and gentamicin which is an aminoglycoside antibiotic. Bacteria resistance to gentamicin is due to failure of antibiotic penetration into bacteria cells, low affinity for ribosomes, or inactivation of the drug by enzymes. It is also caused by modification of aminoglycoside-modifying enzymes, which include adenylate, phosphorylate and acetylate enzymes, as well as overexpression of active efflux pump genes and methylation of the 16S rRNA ribosomal subunit (Wang et al. 2022). Bacterial resistance to bacitracin is mediated by BceAB-type transporters, which protect cell wall synthesis by temporarily freeing lipid cycle II intermediates from the inhibitory effects of bacitracin rather than inactivating or importing the antibiotic (Kobras et al. 2020). Chloramphenicol is a broad-spectrum antibiotic capable of inhibiting bacteria protein synthesis by targeting ribosomes. This antibiotic binds to the Peptidyltransferase Center (PTC) of the bacteria ribosome, preventing the formation of peptide bonds essential for protein synthesis (Chen et al. 2021).

This study successfully showed that 3 antibiotics, namely bacitracin, tetracycline, and gentamycin, were ineffective in treating fish infected with *A. veronii* bv. *veronii*, but chloramphenicol could still be used as an antibacterial. However, excessive use of antibiotics must be reduced to minimize the occurrence and spread of antibiotic-resistant bacteria and prevent sensitive bacteria from becoming resistant (Franz et al. 2018). The use of antibiotics must follow the correct procedures and doses to avoid negative impacts on fish, other aquatic biota, or the environment. Using active compounds from natural ingredients is more recommended as anti-*Aeromonas* (Mulia et al. 2023b, 2023c; Purbomartono et al. 2023).

In conclusion, *A. veronii* bv *veronii* is among the species of *Aeromonas* spp., which has recently gained significant attention but is less popular compared to *A. hydrophila*. The species is pathogenic in freshwater fish and has proven to cause disease with signs of *Aeromonas*. This study successfully isolated and identified phenotypically and genotypically (16S rDNA) one isolate of *A. veronii* bv. *veronii* from diseased gourami from the Singasari area, Banyumas, Central Java. The results of Koch's postulate test showed that the pathogenicity of isolate BmSG-03 was very high, with a mortality rate of 100%. Clinical signs that appeared were skin depigmentation, extensive skin lesions, hemorrhagic, necrosis of fins, changes in kidney color to pareddish brown, liver and other organs to pale, as well as ascites. The pathogenicity was attributed to the role of several virulent genes, including *aer/haem*, *lafA*, and *ela*. The species was found to be resistant to tetracycline, bacitracin, and gentamycin but susceptible to chloramphenicol. To improve the understanding of virulence and susceptible antibiotics in *A. veronii* bv. *veronii* in the future, detecting virulent and various resistance genes on antibiotics were considered essential to obtain more complete data on the genotypic characteristics of *A. veronii* bv. *veronii*. Furthermore, the phenotypic and genotypic characteristics of the species from gourami, freshwater, and marine fish, as well as other infected animals, should be compared to obtain the most effective control strategy.

ACKNOWLEDGEMENTS

This study was supported by the PKLN program, Universitas Muhammadiyah Purwokerto, Indonesia (No. A.11-III/6841-S.Pj./LPPM/II/2024).

REFERENCES

Abdella B, Shokrak NM, Abozahra NA, Elshamy YM, Kadira HI, Mohamed RA. 2024. Aquaculture and *Aeromonas hydrophila*: A complex interplay of environmental factors and virulence. Aquac Intl 32: 7671-7681. DOI: 10.1007/s10499-024-01535-y.

Abu-Elala N, Abdelsalam M, Marouf Sh, Setta A. 2015. Comparative analysis of virulence genes, antibiotic resistance and *gyrB*-based phylogeny of motile *Aeromonas* species isolates from nile tilapia and domestic fowl. Lett Appl Microbiol 61 (5): 429-436 DOI: 10.1111/lam.12484.

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.

Ahangarzadeh M, Najafabadi MG, Peyghan R, Houshmand H, Rohani MS, Soltani M. 2022. Detection and distribution of virulence genes in *Aeromonas hydrophila* isolates causing infection in cultured carps. Vet Res Forum 13 (1): 55-60. DOI: 10.30466/vrf.2020.115998.2761.

Algammal AM, Ibrahim RA, Alfifi KJ, Ghabban H, Alghamdi S, Kabrah A, Khafagy AR, Abou-Elela GM, Abu-Elala NM, Donadu MG, El-Tarabili RM. 2022. A first report of molecular typing, virulence traits, and phenotypic and genotypic resistance patterns of newly emerging XDR and MDR *Aeromonas veronii* in *Mugil seheli*. Pathogens 11 (11): 1262. DOI: 10.3390/pathogens11111262.

Aravena-Román M, Chang BJ, Riley TV, Inglis TJJ. 2011. Phenotypic characteristics of human clinical and environmental *Aeromonas* in Western Australia. Pathology 43 (4): 350-356. DOI: 10.1097/PAT.0b013e3283463592.

Aravena-Román M, Inglis TJJ, Riley TV, Chang BJ. 2014. Distribution of 13 virulence genes among clinical and environmental *Aeromonas* spp. in Western Australia. Eur J Clin Microbiol Infect Dis 33 (11): 1889-1895. DOI: 10.1007/s10096-014-2157-0.

Azzam-Sayuti M, Ina-Salwany MY, Zamri-Saad M, Annas S, Yusof MT, Monir MS, Mohamad A, Muhamad-Sofie MHN, Lee JY, Chin YK, Amir-Danial Z, Asyiqin A, Lukman B, Liles MR, Amal MNA. 2021a. Comparative pathogenicity of *Aeromonas* spp. in cultured red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*). Biology 10 (11): 1192. DOI: 10.3390/ biology10111192.

Azzam-Sayuti M, Ina-Salwany MY, Zamri-Saad M, Yusof MT, Annas S, Najihah MY, Liles MR, Monir MS, Zaidi Z, Amal MNA. 2021b. 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.

Besung INK, Suarjana IGK, Gelgel IKTP. 2019. Antibiotic resistance in *Escherichia coli* isolated from laying hens. Buletin Veteriner Udayana 11 (1): 28-32. DOI: 10.24843/bulvet.2019.v11.i01.p05. [Indonesian]

Chen C-W, Pavlova JA, Lukianov DA, Tereshchenkov AG, Makarov GI, Khairullina ZZ, Tashlitsky VN, Paleskava A, Konevega AL, Bogdanov AA, Osterman IA, Sumbatyan NV, Polikanov YS. 2021. Binding and action of Triphenylphosphonium analog of chloramphenicol upon the bacterial ribosome. Antibiotics 10 (4): 390. DOI: 10.3390/antibiotics10040390.

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.

CLSI [Clinical and Laboratory Standards Institute]. 2020. M100: Performance Standards for Antimicrobial Susceptibility Testing. 30th eds. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

Department of Fisheries and Animal Husbandry of Banyumas Regency. 2024. Statistical data on gourami consumption, gourami production, and fish disease incidence. Department of Fisheries and Animal Husbandry of Banyumas Regency, Banyumas. [Indonesian]

Dong HT, Techatanakitarnan C, Jindakittikul P, Thaiprayoon A, Taengphu S, Charoensapsri W, Khunrae P, Rattanarojpong T, Senapin S. 2017. *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in nile tilapia, *Oreochromis niloticus* (L.). J Fish Dis 40 (10): 1395-1403. DOI: 10.1111/jfd.12617.

Dong J, Yan T, Yang Q, Song Y, Cheng B, Zhou S, Liu Y, Ai X. 2022. Palmatine inhibits the pathogenicity of *Aeromonas hydrophila* by reducing Aerolysin expression. Foods 11 (20): 3250. DOI: 10.3390/foods11203250.

Dos Santos SB, Alarcon MF, Ballaben AS, Harakava R, Galetti R, Guimarães MC, Natori MM, Takahashi LS, Ildefonso R, Rozas-Serri M. 2023. First report of *Aeromonas veronii* as an emerging bacterial pathogen of farmed Nile tilapia (*Oreochromis niloticus*) in Brazil. Pathogens 12 (8): 1020. DOI: 10.3390/pathogens12081020.

Dwi NRA, Mulia DS, Suwarsito S, Purbomartono C. 2023. Isolation, characterization and identification of *Aeromonas* sp. on catfish (*Clarias* sp.) in Banyumas Regency, Central Java. Sainteks 20 (2): 189-204. DOI: 10.30595/sainteks.v20i2.19557. [Indonesian]

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: 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.

FAO [Food and Agriculture Organization]. 2023. Fishery statistics global aquaculture production. https://www.fao.org/fishery/ru/culturedspecies/osphronemus_gourami/en.

Franz CMAP, den Besten HMW, Böhnlein C, Gareis M, Zwietering MH, Fusco V. 2018. Reprint of: Microbial food safety in the 21st century: Emerging challenges and foodborne pathogenic bacteria. Trends Food Sci Technol 84: 34-37. 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. Mar Fish 36: 549-556.

Hoai TD, Trang TT, Van Tuyen N, Giang NTH, Van KV. 2019. *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. Aquaculture 513: 734425. DOI: 10.1016/j.aquaculture.2019.734425.

Hu D, Liu B, Wang L, Reeves PR. 2020. Living trees: High-quality reproducible and reusable construction of bacterial phylogenetic trees. Mol Biol Evol 37 (2): 563-575. DOI: 10.1093/molbev/msz241.

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.

Isnansetyo A, Kamei Y. 2003. *Pseudoalteromonas phenolica* sp. nov., a novel marine bacterium that produces phenolic anti-methicillin-resistant *Staphylococcus aureus* substances. Intl J Syst Evol Microbiol 53 (Pt 2): 583-588. DOI: 10.1099/ijs.0.02431-0.

Janda JM, Abbott SL. 2010. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. Clin Microbiol Rev 23 (1): 35-73. DOI: 10.1128/CMR.00039-09.

Khalifa AYZ, Bekhet G. 2017. First isolation and characterization of the pathogenic *Aeromonas veronii* bv. *veronii* associated with ulcerative syndrome in the indigenous *Pelophylax ridibundus* of Al-Ahsaa, Saudi Arabia. Microb Pathog 117: 361-368. DOI: 10.1016/j.micpath.2017.10.019.

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.

Kobras CM, Piepenbreier H, Emenegger J, Sim A, Fritz G, Gebhard S. 2020. BceAB-type antibiotic resistance transporters appear to act by target protection of cell wall synthesis. Antimicrob Agents Chemother 64 (3): e02241-19. DOI: 10.1128/AAC.02241-19.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35 (6): 1547-1549. DOI: 10.1093/molbev/msy096.

Liu D. 2015. *Aeromonas*. In: Tang Y-W, Sussman M, Liu D, Poxton I, Schwartzman J (eds). Molecular Medical Microbiology. 2nd Edition. Academic Press, United States. DOI: 10.1016/B978-0-12-397169-2.00061-5.

Manoharan D, Srinivasan S, Vignesh NR, Senthilvel A. 2019. Tetracyclines: The old, the new and the improved - a short review. Biomed Pharmacol J 16 (3): 1441-1450. DOI: 10.13005/bpj/2722.

Mulia DS, Dwi NR, Suwarsito, Muslimin B. 2024. Molecular characterization of pathogenic *Aeromonas jandaei* bacteria isolated from cultured walking catfish (*Clarias* sp.). Biodiversitas 25 (3): 1185-1193. DOI: 10.13057/biodiv/d250334.

Mulia DS, Isnansetyo A, Pratiwi R, Asmara W. 2020. Molecular characterizations of *Aeromonas caviae* isolated from catfish (*Clarias* sp.). AACL Bioflux 13 (5): 2717-2732.

Mulia DS, Isnansetyo A, Pratiwi R, Asmara W. 2021. Antibiotic resistance of *Aeromonas* spp. isolated from diseased walking catfish (*Clarias* sp.). Biodiversitas 22: 4839-4846. DOI: 10.13057/biodiv/d221116.

Mulia DS, Karim A, Purbomartono C, Isnansetyo A. 2022a. Antibacterial activity of mangrove plant (*Avicennia marina*) to control *Aeromonas hydrophila* infection in African catfish (*Clarias gariepinus*). AACL Bioflux 15 (6): 2900-2909.

Mulia DS, Pratiwi R, Asmara W, Azzam-Sayuti M, Yasin ISM, Isnansetyo A. 2023a. 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.

Mulia DS, Rahayu SD, Suyadi A, Mujahid I, Isnansetyo A. 2023b. Antibacterial activity of mangrove plant extract of *Rhizophora apiculata* in inhibiting the growth of various strains of *Aeromonas hydrophila*. Biodiversitas 24 (9): 4803-4810. DOI: 10.13057/biodiv/d240921.

Mulia DS, Raicha R, Lunggani CP, Erina SN, Wuliandari JR, Purbomartono C, Isnansetyo A. 2023c. Antibacterial activity of ethanol extract of banana, cassava, and pineapple peels against a fish pathogen *Aeromonas hydrophila*. Biodiversitas 24: 481-485. DOI: 10.13057/biodiv/d240155.

Mulia DS, Utomo T, Isnansetyo A. 2022b. The efficacy of *Aeromonas hydrophila* GPl-04 feed-based vaccine on African catfish (*Clarias gariepinus*). Biodiversitas 23: 1505-1510. DOI: 10.13057/biodiv/d230339.

Nagar V, Shashidhar R, Bandekar JR. 2011. Prevalence, characterization, and antimicrobial resistance of *Aeromonas* strains from various retail food products in Mumbai, India. J Food Sci 76 (7): M486-M492. DOI: 10.1111/j.1750-3841.2011.02303.x.

Oladele AC, Temitope OS. 2016. Isolation and characterization of *Aeromonas* species isolat from food and diarrhoeagenic stool in Ibadan Metropolis, Nigeria. Food Sci Qual Manag 51: 20-31.

Pessoa RBG, de Oliveira WF, Marques DSC, Correia MTDS, 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.

Purbomartono C, Isnansetyo A, Murwantoko, Triyanto. 2023. Improving resistance against *Aeromonas hydrophila* and growth performance by oral administration of fucoidan from *Padina boergesenii* Allender & Kraft, 1983 in catfish (*Clarias* sp.). AACL Bioflux 16 (3): 1294-1304.

Raj NS, Swaminathan TR, Dharmaratnam A, Raja SA, Ramraj D, Lal KK. 2019. *Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. Aquaculture 512: 73427. DOI: 10.1016/j.aquaculture.2019.734278.

Rozi R, Rahayu K, Daruti DN, Stella MSP. 2018. Study on characterization, pathogenicity and histopathology of disease caused by *Aeromonas hydrophila* in gouramy (*Osphronemus gouramy*). IOP Conf Ser: Earth Environ Sci 137: 012003. DOI: 10.1088/1755-1315/137/1/012003.

Sarjito S, Haditomo AHC, Desrina D, Ariyati RW, Prayitno SB. 2018. The diversity of causative agent associated with bacterial diseases on catfish (*Clarias gariepinus*) with molecular based from Demak, Indonesia. Omni-Akuatika 14 (2): 100-106. DOI: 10.20884/1.oa.2018.14.2.553. [Indonesian]

Semwal A, Kumar A, Kumar N. 2023. A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. Heliyon 9: e14088. DOI: 10.1016/j.heliyon.2023.e14088.

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.

Sheykhsaran E, Baghi HB, Soroush MH, Ghotaslou R. 2019. An overview of tetracyclines and related resistance mechanisms. Rev Med Microbiol 30 (1): 69-75. DOI: 10.1097/MRM.0000000000000154.

Smyrli M, Triga A, Dourala N, Varvarigos P, Pavlidis M, Quoc VH, Katharios P. 2019. Comparative study on a novel pathogen of European seabass. Diversity of *Aeromonas veronii* in the Aegean Sea. Microorganisms 7 (11): 504. DOI: 10.3390/microorganisms7110504.

Sofi MY, Shafi A, Masoodi KZ. 2021. CLUSTALW software. In: Sofi MY, Shafi A, Masoodi KZ (eds). Bioinformatics for Everyone. Academic Press, United States. DOI: 10.1016/B978-0-323-91128-3.00003-3.

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.

Soltani M, Moghimi SM, Mousavi HE, Abdi K, Soltani E. 2016. Isolation, phenotypic and molecular characterization of motile *Aeromonas* species, the cause of bacterial hemorrhagic septicemia in affected farmed carp in Iran. Iran J Vet Med 10 (3): 209-216. DOI: 10.22059/ijvm.2016.58683.

Su Y, Zheng Y, Han Z, Liang E, Sun J. 2023. Identification and characterization of *Aeromonas veronii* and *Vibrio cholerae* from naturally diseased red swamp crayfish (*Procambarus clarkia*). Aquac Rep 30: 101614. DOI: 10.1016/j.aqrep.2023.101614.

Varela MF, Stephen J, Lekshmi M, Ojha M, Wenzel N, Sanford LM, Hernandez AJ, Parvathi A, Kumar SH. 2021. Bacterial resistance to antimicrobial agents. Antibiotics 10 (5): 593. DOI: 10.3390/antibiotics10050593.

Vineetha N, Vignesh RA, Sridhar D. 2015. Preparation, standardization of antibiotics disc and study of resistance pattern for first-line antibiotics in isolation from clinical samples. Intl J Appl Res 1 (11): 624-631.

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 N, Luo J, Deng F, Huang Y, Zhou H. 2022. Antibiotic combination therapy: A strategy to overcome bacterial resistance to aminoglycoside antibiotics. Front Pharmacol 13: 839808. DOI: 10.3389/fphar.2022.839808.

Zhang H-P, Chen M-Y, Xu Y-X, Xu G-Y, Chen J-R, Wang Y-M, Kang Y-H, Shan X-F, Kong L-C, Ma H-X. 2020. An effective live attenuated vaccine against *Aeromonas veronii* infection in the loach (*Misgurnus anguillicaudatus*). Fish Shellfish Immunol 104: 269-278. DOI: 10.1016/j.fsi.2020.05.027.