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Molecular characterization of pathogenic *Aeromonas jandaei* bacteria isolated from cultured walking catfish (*Clarias* sp.)

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Abstract. *Mulia DS, Dwi NR, Suwarsito, Muslimin B. 2024. Molecular characterization of pathogenic* Aeromonas jandaei *bacteria isolated from cultured walking catfish (*Clarias *sp.). Biodiversitas 25: 1185-1193. Aeromonas* spp. is pathogenic bacteria capable of causing fish disease, including walking catfish (*Clarias* sp.). In bacteria, virulence genes play a significant role in determining pathogenicity, while resistance genes influence resistivity. Therefore, this study aimed to determine molecular characterization of pathogenic *Aeromonas jandaei* bacteria isolated from walking catfish. The purposive sampling method was used to obtain diseased walking catfish from aquaculture ponds in Banyumas District, Central Java Province, Indonesia. The identification of bacteria was molecularly carried out based on 16S rDNA, followed by the detection of virulence genes on *aer/haem*, *alt*, *ast*, *flaA*, *lafA*, and *fstA*, as well as resistance genes on *tet-A*, *strA-strB*, and *qnrA*. The results of molecular analysis showed that three isolates from Singasari, namely BmSL-02, BmSL-04, and BmSL-07, were closely related to *A. jandaei* strain CDC0787-80 with a similarity rate and query coverage of 99.86% and 99%, respectively. Meanwhile, isolates from Cikawung, including BmCL-02, BmCL-05, and BmCL-07 were closely related to *A. jandaei* strain ATCC 49568. A total of six *A. jandaei* isolates had *aer/haem* virulence genes while *alt*, *ast*, and *fstA* were not found. BmSL-04 and BmCL-07 isolates had *flaA* gene, while four isolates including BmSL-02, BmSL-04, BmSL-07, and BmCL-07 had *lafA* gene. Additionally, BmCL-05 had *tetA* resistance gene, while the *strA-strB* and *qnrA* genes were not detected in all isolates. *A. jandaei* isolates were resistant to bacitracin, tetracycline, and gentamycin, but BmSL-04, BmSL-07, BmCL-02, and BmCL-05 were sensitive to chloramphenicol. This study provided valuable information on molecular characteristics of *A. jandaei*, serving as guidance in effective control and monitoring of isolates from Indonesia, specifically Banyumas, Central Java.

Keywords: *Aeromonas*, antibiotics susceptibility, molecular characterization, resistance genes, virulence genes

INTRODUCTION

Freshwater fish cultivation is often hampered by disease problems, with various bacteria identified as pathogens, specifically within genus *Aeromonas* (Austin and Austin 2016; Fowoyo and Achimugu 2019; Pessoa et al. 2019; Mulia et al. 2020, 2022a). The disease caused by these bacteria, such as aeromoniasis or Motile *Aeromonas* Septicemia (MAS), can lead to significant mortality among infected fish populations when not treated quickly and appropriately (Pereira et al. 2022). Previous study has reported several freshwater fish infected with *Aeromonas* spp., including African catfish (*Clarias gariepinus*), walking catfish (*Clarias* sp.), catfish (*Pangasius hypothalamus*), common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*), and eel (*Anguilla japonica*) (Guo et al. 2016; Soltani et al. 2016; Abd El Tawab et al. 2017; Olga et al. 2020; Mulia et al. 2016, 2021, 2022b). Additionally, *Aeromonas* spp. have been reported to infect other aquatic biota (Luo et al. 2018; Cunningham et al. 2019), posing significant health risks to humans (Yuwono et al. 2021).

Aeromonas spp. are characterized as Gram-negative, non-spore forming, facultative anaerobic bacteria, with a rod-shaped morphology, representing opportunistic microorganisms (Janda and Abbot 2010; Pessoa et al. 2019; Mulia et al. 2023a). This genus has a growing diversity of species, with the number increasing from 24 species in 2010 (Janda and Abbott 2010), to 32 in 2017 (Figueras et al. 2017), and reaching 36 species in 2020 (Fernández-Bravo and Figueras 2020). Several *Aeromonas* spp. are pathogenic, including *Aeromonas jandaei* (Dong et al. 2017), which has not been extensively identified in Indonesia. A previous study reported the successful isolation of *A. jandaei* from freshwater fish cultivated in the West Java region (Kusumawaty et al. 2016). This bacteria was first isolated and associated with disease in humans (Carnahan et al. 1991), followed by freshwater crocodiles (*Crocodylus siamensis*) (Pu et al. 2019), and fish, including Nile tilapia, pirarucu (*Arapaima gigas*), *Oreochromis* spp., and catfish (Dong et al. 2017; Assane et al. 2021; Fauzi et al. 2021; Proietti-Junior et al. 2021).

Aeromonas jandaei infection in Nile tilapia (*O. niloticus*) causes the appearance of disease with clinical signs, namely discoloration of skin along with loss of scales, cloudy eyes, hemorrhage, and redness in the skin, fin rot, necrotic liver, hemorrhagic kidney, and yellow exudate

(Assane et al. 2021). Another study reported that climbing perch (*Anabas testudineus*) infected with *A. jandaei* showed clinical signs, namely ulcers, tail-rot, hemorrhagic kidney and liver, decreased appetite and weight loss, abnormal behavior such as irregular swimming, including excessive mucus secretion on the fish body (Mazumder et al. 2021).

Pathogenicity mechanism of *Aeromonas* spp. is complex and is related to virulence genes (Li et al. 2015; Mulia et al. 2020), such as *aer/haem*, *alt*, *ast*, *flaA*, *lafA*, *fstA*, *aspA*, *vasH*, *ascV*, and *aexT* (Beaz-Hidalgo et al. 2013a; Yi et al. 2013; Aravena-Roman et al. 2014; Khor et al. 2015). However, differences in virulence genes are commonly observed, both between strains of a single *Aeromonas* species and among species of *Aeromonas* spp. In a previous study, *A. caviae* strain MD-01 showed the presence of *aer/haem*, *alt*, *ast*, and *flaA* virulence genes were detected, but *lafA* and *fstA* genes were not detected (Mulia et al. 2020). In *A. caviae* strain Ac-01, *aer/haem*, *ast*, *flaA*, *lafA*, and *fstA* genes were detected, while *A. hydrophila* was found to have *aer*/*haem*, *alt*, *ast*, and *flaA* genes (Mulia et al. 2023a).

Aeromonas spp. are resistant to certain antibiotics due to different distribution of resistance genes among strains and species (Mulia et al. 2021). For effective and optimal treatment, there is a need to investigate molecular characterization of *A. jandaei* by detecting virulence and resistance genes. Although there is still limited information, previous investigations have explored the types of virulence and resistance genes possessed by *Aeromonas* spp. for effective and efficient management. Knowledge of the antibiotics susceptibility presented by *A. jandaei* is also essential for appropriate disease management in fish farming. Therefore, this study aimed to determine molecular characterization of pathogenic *A. jandaei* bacteria isolated from walking catfish.

MATERIALS AND METHODS

Study period and location

The study was carried out from November 2022 to May 2023, with samples of diseased walking catfish collected from aquaculture ponds in Singasari and Cikawung villages, Banyumas District, Central Java Province, Indonesia. For analysis, sampling was carried out by determining one catfish cultivation pond, where three diseased walking catfish were randomly selected. For analysis, sampling was conducted by determining one catfish cultivation pond for each place, and three diseased walking catfish were randomly selected. Clinical signs that appeared included skin depigmentation, erosion, lesions on the dorsal, hyperemia, hemorrhagic, ulcer, and abscesses all over the body. The euthanization technique is done by soaking the fish in a clove oil solution of 1 mL/L water.

Isolation and bacteria culture

All isolates were isolated from the kidneys and ulcers of diseased walking catfish by taking one ose each and inoculating it on a Glutamate Starch Phenol (GSP) medium (Merck, Darmstadt, Germany) using a three-quadrant continuous streak. Isolates were grown at 30°C for 24 h, with *Aeromonas* and *Pseudomonas* growing in yellow and red colonies, respectively. Furthermore, a yellow single colony was grown in the Tryptic Soy Broth (TSB) medium (Merck, Darmstadt, Germany) and six isolates were stored in a TSB medium with 20% glycerol at -20°C for further assay.

Bacteria genomic DNA extraction

Bacteria genomic DNA was extracted using a high pure Polymerase Chain Reaction (PCR) preparation kit (Roche, 11796828001, Roche Diagnostics Corporation, Indiana, USA). For each sample, 1 mL of bacteria culture in TSB medium was incubated for 24 h at 30°C and centrifuged at 13,000 g for 2 min. Subsequently, the extracted bacteria DNA was stored at -20°C for further assay.

16S rDNA amplification

The amplification of 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, and 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), followed by similarity assessment through Basic Local Alignment Search Tool (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST). Subsequently, multiple sequence arrangements were carried out using the Clustal W Program. Phylogenetic trees were constructed using the maximum likelihood MEGATM 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. was amplified by PCR with a total volume of 25 µL, containing 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 nuclease-free water (NFW). As shown in Table 1, virulence genes of *Aeromonas* spp. detected were *aerA/haem*, *alt*, *ast*, *flaA*, *lafA*, and *fstA*, while PCR product was subjected to electrophoresis with 1.5% agarose gel.

Table 1. Primers sets used in this study

Genes	Genes product	Primer sequence	Product size (bp)	Reference
16S	16S rDNA	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)
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)
tetA	Tetrasiklin resistent	F: GTA ATT CTG AGC ACT GTC GC R: CTG CCT GGA CAA CAT TGC TT	956	Guardabassi et al. (2000)
strA-strB	Aminoglicoside resistant	F: TTG AAT CGA ACT AAT AT R: CTA GTA TGA CGT CTG TCG	1640	Han et al. (2004)
qnrA	Quinolone resistant	F: TCA GCA AGA GGA TTT CTCA R: GGC AGC ACT ATT ACT CCC A	608	Nawaz et al. (2012)

Detection of resistance genes

Detection of resistance genes of *Aeromonas* spp. was amplified by PCR with a total volume of 25 µL, containing 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 Nuclease-Free Water (NFW). Resistance genes of *Aeromonas* spp. detected were *tet-A, strA-strB,* and *qnrA*, as shown in Table 1, while PCR product was subjected to electrophoresis with 1.5% agarose gel.

Antimicrobial susceptibility test

Antibiotics susceptibility testing was carried out using gentamycin 10 µg disk, bacitracin 10 µg, tetracycline 30 µg, and chloramphenicol 30 µg. Bacteria were inoculated on TSA medium in a continuous streak and incubated at 30°C for 24 hours. Subsequently, diameter of the inhibition zone (in mm) around the disc was measured and determined as Sensitive (S), Intermediate (I), or Resistant (R) based on Clinical and Laboratory Standards Institut (CLSI 2020). For bacitracin R:≤8 mm; I: 9-12 mm; S:≥13 mm, for chloramphenicol R:≤12 mm; I: 13-17 mm; S:≥18 mm, for gentamicyn R:≤12 mm; I: 13-14 mm; S:≥15 mm, for tetracycline R:≤14 mm; I: 15-18 mm; S:≥19 mm (CLSI 2020).

Data analysis

Data from molecular identification, detection of virulence and resistance genes, as well as antimicrobial susceptibility tests were analyzed descriptively and qualitatively.

RESULTS AND DISCUSSION

The amplification results of the 16S rDNA fragment were considered positive when the appearance of a single band matched the size of target DNA fragment in each isolate. As shown in Figure 1, the amplicon DNA bands appeared bright at 1500 bp, indicating successful amplification of the 16S rDNA fragment from bacteria isolates genome using the 16S-F and 16S-R primer pair. The DNA sequence was obtained by arranging the reverse and forward primer readings resulting from the sequencing. This arrangement produced consensus sequence, which was compared with data available in the database using the BLAST program.

The results of BLAST analysis are determined by query coverage and percent identity. Specifically, query coverage represents the percentage of nucleotide lengths consistent with the database in BLAST, with higher values, showing easier analysis due to the same length observed in GenBank. Percent identity or similarity level is a value that shows the correlation of nucleotides or amino acids in the data sequence with database (Rizal et al. 2020). The results of BLAST 16S rDNA analysis of six isolates showed a high level of similarity, namely between 99.44-100%, with query coverage reaching 99-100%, as shown in Table 2. A homology search based on the 16S rRNA gene sequence showed that six isolates were similar to *A. jandaei* strain CDC0787-80 and *A. jandaei* strain ATCC 49568.

Bacteria isolates from Singasari (BmSL-02, BmSL-04, and BmSL-07) were identified as *A. jandaei* strain CDC0787-80, with similarity level of 99.44-99.93% and query coverage of 99-100%. Meanwhile, isolates from Cikawung (BmCL-02, BmCL-05 and BmCL-07) were identified as *A. jandaei* strain ATCC 49568, with a similarity level of 99.86-100% and query coverage of 99-100% (Assane et al. 2021).

Phylogenetic tree

The results of the phylogenetic tree construction showed that the six isolates were closely related to *Aeromonas* spp., as presented in Figure 2. The selection of outgroups in phylogenetic tree construction was based on the correlation of sequences analyzed. Generally, a correlation should be

observed between outgroups and analyzed sequences, with substantial differences. Therefore, *Pseudomonas aeruginosa* was used as outgroups, possessing morphological and molecular characteristics that are similar to *Aeromonas* spp. (Su et al. 2023).

Samples from Singasari (BmSL-02, BmSL-04, BmSL-07) and Cikawung (BmCL-02, BmCL-05, and BmCL-07) were correlated with *A. jandaei* branch, showing a bootstrap value of 98%. The six isolates are very closely related and belong to the same branch, as reading phylogenetic tree was based on the length and connectedness of branches with the bootstrap value stated (Hu et al. 2020). Based on the phylogenetic tree constructed, isolates from Singasari, including BmSL-02, BmSL-04, and BmSL-07 are closely related to *A. jandaei* strain CDC0787-80, while isolates from Cikawung, namely BmCL-02, BmCL-05 and BmCL-07 were correlated with *A. jandaei* strain ATCC 49568.

Aeromonas jandaei, a species of bacteria in the genus *Aeromonas*, was first discovered in 1991 and named after J. Michael Janda, an American microbiologist who has made major contributions to *Aeromonas* species (Mazumder et al. 2021). This rod-shaped, Gram-negative bacteria is commonly found in aquatic environments, including freshwater, seawater, and wastewater, capable of causing infectious diseases in fish and humans (Janda and Abbott 1998). Compared to other *Aeromonas* species, *A. jandaei* shows positive effect in oxidizing mannitol and hydrolyzing esculin, with negative impact in sucrose (Mazumder et al. 2021).

Aeromonas jandaei shows growth capacity at temperatures between 22°C to 35°C, with the potential to survive at pH values ranging from 4.5 to 5.5. Moreover, this species poses significant risk of infections to humans with weakened immune systems, manifesting symptoms such as diarrhea, vomiting, fever, and abdominal pain (Fernández-Bravo and Figueras 2020). *Aeromonas jandaei* also has resistance to several antibiotics, including ampicillin, amikacin, gentamicin, rifampicin, penicillin, and nitrofurantoin (Oladele and Temitope 2016). Previous studies have identified methods for distinguishing *A. jandaei* using *16S rRNA* and *rpoB* gene sequencing. For example, AM-05 isolate obtained from climbing perch (*A. testudineus*) showed close genetic proximity in phylogenetic analysis and potential as a reliable identification marker (Mazumder et al. 2021). Oladele and Temitope (2016) also stated that *A. jandaei* isolated from cabbage and water could be differentiated from other *Aeromonas* spp. using molecular tests such as RAPD analysis and PCR methods through specific primers to amplify DNA fragments from *Aeromonas* isolates.

Virulence of *A. jandaei* was observed by detecting the presence of *aerA/haem, alt, ast, flaA, lafA*, and *fstA* genes. The results showed that *aerA/haem* gene was detected in all isolates, while *flaA* gene was found in BmSL-04 and BmCL-07. Furthermore, *lafA* gene was detected in BmSL-02, BmSL-04, BmSL-07, and BmCL-07, while *alt, ast,* and *fstA* genes were not detected in all isolates, as shown in Table 3.

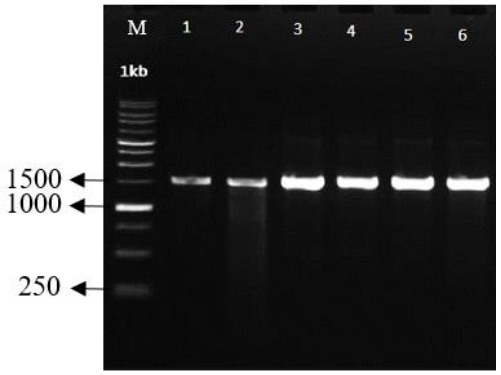

Figure 1. Results of amplification of bacteria DNA isolated with 16S rDNA primers. M: Marker 1 kb, 1: BmSL-02, 2: BmSL-04, 3: BmSL-07, 4: BmCL-02, 5: BmCL-05, 6: BmCL-07

Table 2. BLAST analysis results of 16S rDNA sequences from 6 bacteria isolates

Isolates	Closest comparator	Query (%)	Identity (%)	Accession number
BmSL-02	*A. jandaei* strain CDC0787-80	99	99.86	NR_037013.2
BmSL-04	*A. jandaei* strain CDC0787-80	100	99.93	NR_037013.2
BmSL-07	*A. jandaei* strain CDC0787-80	100	99.44	NR_037013.2
BmCL-02	*A. jandaei* strain ATCC 49568	99	100.00	NR_119040.1
BmCL-05	*A. jandaei* strain ATCC 49568	99	99.93	NR_119040.1
BmCL-07	*A. jandaei* strain ATCC 49568	100	99.86	NR_119040.1

Table 3. Detection of virulent genes of *A. jandaei* isolated from walking catfish (*Clarias* sp.)

Isolates	Species	Virulence genes						Total (%)
		aerA/haem	*alt*	*ast*	*flaA*	*lafA*	*fstA*	
BmSL-02	*A. jandaei* Strain CDC0787-80	+	-	-	-	+	-	2 (33.33)
BmSL-04	*A. jandaei* Strain CDC0787-80	+	-	-	+	+	-	3 (50.00)
BmSL-07	*A. jandaei* Strain CDC0787-80	+	-	-	-	+	-	2 (33.33)
BmCL-02	*A. jandaei* Strain ATCC 49568	+	-	-	-	-	-	1 (16.66)
BmCL-05	*A. jandaei* Strain ATCC 49568	+	-	-	-	-	-	1 (16.66)
BmCL-07	*A. jandaei* Strain ATCC 49568	+	-	-	+	+	-	3 (50.00)

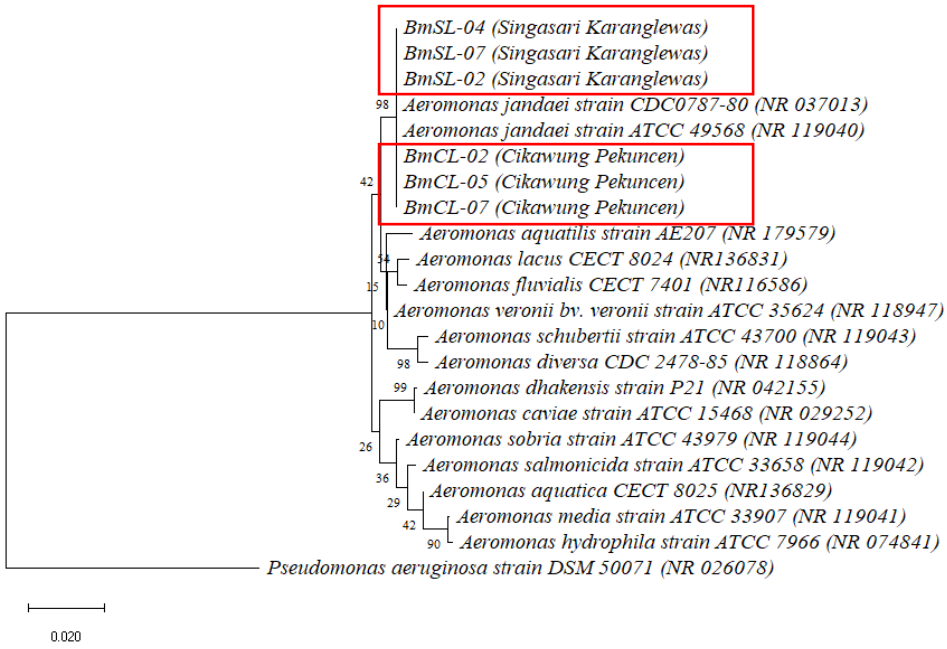

Figure 2. Phylogenetic tree constructed from the 16S rDNA sequences from 6 isolates of *Aeromonas* spp. (BmSL-02, BmSL-04, BmSL-07, BmCL-02, BmCL-05, and BmCL-07) and other *Aeromonas* species (class of Gammaproteobacteria). *Pseudomonas aeruginosa* was used as an outgroup. The topology was obtained by maximum possibility with bootstraps of 1000 replications. The scale bar signifies 0.02 substitutions per nucleotide position (Knuc)

In this study, *A. jandaei* has at least one virulence gene, supporting pathogenicity of bacteria in molecular analysis. Previous investigations have shown that *Aeromonas* spp. originating from various sources contain many virulence genes in various combinations (Králová et al. 2016), with several strains of *A. jandaei* Assane et al. (2021). The results showed that four strains of *A. jandaei* isolated from Nile tilapia, have *aera A, hlyA, eprCAI,* and *nuc*, while *ast, act, ahp*, and *lip* genes were not detected. Meanwhile, *lafB* and alt genes were only detected in one strain, and *ascV* gene was found in two strains. In another study, *A. jandaei* isolated from pirarucu detected *aerA, gcat, lip, DNase*, and *hlyA*, but *alt, act*, and *ser* genes were not found (Proietti-Junior et al. 2021). These results underscore the presence of varying virulence genes, contributing to different levels among *Aeromonas* strains.

Aerolysin is a hemolytic extracellular product encoded by aerolysin gene, playing an important role in pathogenicity of *Aeromonas* spp., particularly *A. jandaei* (Lee et al. 2021). Screening for specific cytotoxin and hemolysin genes has been reported to be the most effective method of detecting and characterizing *Aeromonas* virulence genes (Sonkol et al. 2020). In this study, *flaA* gene was detected in two isolates of *A. jandaei*, namely BmSL-04 and BmCL-07. Another study reported that this gene was also found in *A. dhakensis, A. caviae, A. hydrophila*, and other species (Aravena-Román et al. 2014). Specifically, *Aeromonas* has two types of flagella, namely polar and lateral, which are encoded by *flaA* and *lafA* genes, respectively (Beaz-Hidalgo and Figueras 2013b). Polar flagella increases swimming motility associated with liquid environments, which are essential in bacteria adhesion to epithelial cells (Thormann et al. 2022).

The results showed that the *lafA* gene was found in four isolates of *A. jandaei*, namely BmSL-02, BmSL-04, BmSL-07, and BmCL-07. However, this gene was not detected in *A. hydrophila* (Aguilera-Arreola et al. 2009) and *A. veronii* (Onuk et al. 2013). According to Liu (2015), *lafA* gene promotes swarming movements on solid surfaces, playing an important role in cell adherence, biofilm formation, and persistence during infection. The differences observed in virulence genes are attributed to variation in strain, source of isolates, geographic location, and environment (Abu-Elala et al. 2015), impacting on pathogenicity in the field.

Detection of resistance genes of *A. jandaei*

Detection of resistance to *A. jandaei* was achieved through the identification of *tetA, strA-strB*, and *qnrA* genes in bacteria. The results showed that only *tetA* gene was detected in BmCL-05, while *strA-strB* and *qnrA* were not detected in other isolates, as presented in Table 4.

The *tetA* gene encodes both ribosome-protecting and tetracycline repressor proteins, commonly associated with conjugative and mobilization elements, such as plasmids, transposons, and integrons. Most tet genes encode transport proteins, which pump antibiotics out of bacteria cells and maintain low intercellular concentrations to ensure the normal function of ribosomes (Sheykhsaran et al. 2019). Moreover, *tetA, B, C, D*, and *E* genes often appear in various environmental conditions including activated sludge from waste treatment plants (Guillaume et al. 2000), fish farming ponds (Dang et al. 2007), and surface water (Poppe et al. 2006). Based on the results, not all *Aeromonas* spp. have *strA-strB* and *qnrA* genes (Varela et al. 2016; Wimalasena et al. 2017), as only one showed the presence of *strA-strB* gene (De Silva et al. 2020). In addition, *Aeromonas* spp.

isolated from fish and shrimp do not have the *strA-strB* gene (Deng et al. 2014). Another study investigating ten isolates of *Aeromonas* spp., consisting of four species, namely *A. hydrophila*, *A. caviae*, *A. veronii* bv *veronii*, and *A. dhakensis* do not have the *qnrA* gene (Mulia et al. 2021). Other resistance genes, namely *kpc, ndm, imp, oxa-48,* and *vim* were not detected in *A. jandaei* isolated from pirarucu (*A. gigas*) (Proietti-Junior et al. 2021). These differences in resistance are influenced by variations in strains, source of isolates, geographical location, and environment (Nagar et al. 2011)

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was carried out on six isolates of *A. jandaei* to determine sensitivity to antibiotics such as, tetracycline, gentamicin, chloramphenicol, and bacitracin as determined by the diameter of the inhibition zone formed. A previous study has established that a larger diameter of the inhibition zone shows greater inhibition of bacteria growth (Vineetha et al. 2015). The antibiotics tested included, tetracycline, gentamycin, chloramphenicol and, bacitracin.

All isolates of *A. jandaei* were resistant to bacitracin, tetracycline, and gentamycin, while BmSL-04, BmSL-07, BmCL-02, and BmCL-05 were sensitive to chloramphenicol and BmCL-07 was intermediated, as shown in Table 5. Specifically, BmCL-05 was detected to have *tetA* gene, despite all isolates showing resistance to tetracycline and gentamycin antibiotics in the aminoglycoside group. These results indicated that the absence of resistant genes in isolates did not show antibiotics susceptibility. Similarly, among all isolated *Aeromonas* spp. from wastewater plants, no *tetC* gene was detected, but 75% of isolates were resistant to tetracycline (Igbinosa and Okoh 2012). In a previous study, *A. caviae* MD-03, *A. veronii* bv *veronii* SC-03, and *A. dhakensis* KK-02 were not detected to have *tetA* gene but were resistant to tetracycline. Some isolates *A. hydrophila*, *A. veronii*, and *A. caviae*, did not show the presence of *strA-strB* gene but were resistant to streptomycin (aminoglycoside) (Deng et al. 2014). This phenomenon makes bacteria adapt by forming cell membranes, preventing antibiotics from entering bacteria cells (Besung et al. 2019). In addition, some bacteria are naturally resistant to antibiotics without direct interaction due to the ability to enzymatically degrade drugs (Varela et al. 2021).

Generally, the susceptibility of *Aeromonas* species to antibiotics varies, with resistance rates of 80%, 70%, and 55% to gentamycin, tetracycline, and chloramphenicol, respectively (Oladele and Temitope 2016). *Aeromonas jandaei* has resistance to several antibiotics, such as ampicillin, amikacin, gentamycin, rifampicin, penicillin, and nitrofurantoin (Janda and Abbot 2010). Additionally, two isolates of *A. jandaei* obtained from the Singapore General Hospital have shown 100% resistance to amoxicillin and cephalotaxin, and 50% tetracycline (Khor et al. 2018). In another study, *A. jandaei* isolated from climbing perch (*A. testudineus*) was sensitive to several antibiotics, including ampicillin, tetracycline, gentamycin, and chloramphenicol (Mazumder et al. 2021).

Aeromonas spp. are resistant to tetracycline, gentamycin, and bacitracin due to their ability to develop protective mechanisms. Some *Aeromonas* strains also produce enzymes that inactivate antibiotics, becoming ineffective in inhibiting bacteria growth (Semwal et al. 2023). In this study, the large diameter of the inhibition zone of four *A. jandaei* isolates showed sensitivity to chloramphenicol (Tevyashova 2021). Despite its broad spectrum, chloramphenicol is still effective against several bacteria species by impeding RNA function crucial for protein synthesis in bacteria cells (Giannopoulou et al. 2019). Based on the results, bacitracin, tetracycline, and gentamycin, were not effective in treating fish infected with *A. jandaei*, while chloramphenicol retained its antibacteria efficacy. Appropriate use of antibiotics must adhere to procedures and doses, preventing harm to fish, other aquatic biota, or the environment. Moreover, the use of active compounds from natural ingredients has been recommended as anti-*Aeromonas* (Mulia et al. 2023b, 2023c).

Table 4. Detection of resistance genes for *A. jandaei* isolated from walking catfish (*Clarias* sp.)

Isolates	Species	Resistance genes			Total (%)
		tetA	***strA-strB***	***qnrA***	
BmSL-02	*A. jandaei* Strain CDC0787-80	-	-	-	0
BmSL-04	*A. jandaei* Strain CDC0787-80	-	-	-	0
BmSL-07	*A. jandaei* Strain CDC0787-80	-	-	-	0
BmCL-02	*A. jandaei* Strain ATCC 49568	-	-	-	0
BmCL-05	*A. jandaei* Strain ATCC 49568	+	-	-	1 (33.33)
BmCL-07	*A. jandaei* Strain ATCC 49568	-	-	-	0

Table 5. Antimicrobial susceptibility test

Type of antibiotics	Isolates					
	BmSL-02	**BmSL-04**	**BmSL-07**	**BmCL-02**	**BmCL-05**	**BmCL-07**
Bacitracin	R (0)	R (0)	R (0)	R (0)	R (0)	R (6.06)
Tetracycline	R (10.81)	R (3.68)	R (7.27)	R (9.48)	R (5.54)	R (14.47)
Gentamycin	R (5.39)	R (4.60)	R (4.83)	R (4.77)	R (6.06)	R (9.42)
Chloramphenicol	R (3.34)	S (20.93)	S (20.19)	S (18.76)	S (20.34)	I (17.14)

Note: R: resistant, I: intermediate, S: Sensitive, Number: diameter of inhibition zone (mm)

In conclusion, this study successfully isolated and molecularly identified six isolates of *A. jandaei* from diseased walking catfish from the Singasari and Cikawung areas, Banyumas, Central Java, using 16S rDNA sequencing. The results showed the diversity of virulence genes possessed by *A. jandaei*. All six isolates of *A. jandaei* had *aer/haem* virulence genes but did not possess *alt*, *ast*, and *fstA* genes. BmSL-04 and BmCL-07 had *flaA* gene, and four isolates including BmSL-02, BmSL-04, BmSL-07, and BmCL-07 had *lafA* gene. Additionally, only BmCL-05 had *tetA* gene, while *strA-strB* and *qnrA* genes were not detected in other isolates. All isolates were resistant to bacitracin, tetracycline, and gentamycin, but BmSL-04, BmSL-07, BmCL-02, and BmCL-05 were sensitive to chloramphenicol.

Future studies on virulence and antibiotics susceptibility in *A. jandaei* are essential, requiring the detection of a wider variety of virulence and resistance genes. Additionally, exploring the use of antibiotics will provide more comprehensive data on molecular characterizations of *A. jandaei*. Comparative analyses of molecular characterizations of *A. jandaei* from walking catfish, freshwater fish, and marine fish, are also essential. This is to facilitate the development of appropriate and effective strategies for managing infections caused by *A. jandaei*.

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