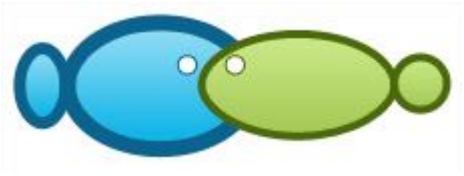

Molecular characterizations of *Aeromonas caviae* isolated from catfish (*Clarias* sp.)

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Abstract. *Aeromonas* spp. are pathogenic bacteria which potentially cause disease to fish, including the catfish. Pathogenicity of *Aeromonas* spp. is determined by virulence factors controlled by the virulent genes, while the effectiveness of antibiotic treatment is influenced by the resistant genes. This study aimed to obtain molecular characterizations of *Aeromonas caviae* isolated from catfish (*Clarias* sp.). The identification of bacteria was molecularly carried out based on 16S rDNA. The virulence factors were detected based on *aer/haem, alt, ast, flaA, lafA*, *fstA* genes and the resistance were evalated based on *tet-A, strA-strB* and *qnrA* genes. The results of molecular analysis showed that MD-01 isolate was closely related to *A. caviae* ATCC 15468 (NR029252) with similarity rate and query of 99.86% and 99%, respectively. This isolate harbored virulence genes of *aer/haem, alt, ast, flaA*, genes, but *lafA* and *fstA* genes were not detected. There were no resistance genes of *tet-A, strA-strB* and *qnrA* found in this isolate. Based on susceptibility to antibiotics, it was resistant to oxytetracycline, ampicillin, erythromycin and chloramphenicol, but susceptible to kanamycin (2.5 µg mL^{-1}) and enrofloxacin (0.05 µg mL^{-1}). This study is the first report on molecular characterization of *A. caviae* from catfish in Indonesia, and gives a significant contribution on the molecular characterization of *A. caviae* for further development of counter measure methods of this pathogenic bacterium.
Key Words: *Aeromonas caviae*, catfish, characterization molecular, susceptibility to antibiotics, virulence genes.

Introduction. Catfish (*Clarias* sp.) is one of potential cultivated-freshwater fishes in Indonesia. It is also the superior product of fishery industry in south and south-east Asian countries, including India and Malaysia, and Nigeria. Catfish has been the main species of cultivated-freshwater fish in United State of America since 1980s (Thomas et al 2013; Dauda et al 2018; Li et al 2018). The catfish aquaculture frequently faces obstacles, such as bacterial disease caused by *Aeromonas* spp., which is popular as pathogenic bacteria for freshwater fish including catfish (Li et al 2013; Guo et al 2016; Abd El Tawab et al 2017; Dong et al 2017; Raji et al 2019). In addition, it can also infect other animals such as chicken, wild waterfowl, bird, reptile, and amphibia (Zhou et al 2011; Tel & Keskİn 2012; Igbinosa 2014; Qi et al 2016; Laviad-Shitrit et al 2018), and cause zoonosis in human (Choi et al 2008; Tang et al 2014).

Aeromonas spp. are rod-shaped, Gram negative, non-spore forming, facultative anaerobic, and opportunistic bacteria (Janda & Abbot 2010; Parker & Shaw 2011; Pessoa et al 2019). *Aeromonas* genus has been continually increasing species number, with 36 species being successfully identified until now (Fernández-Bravo & Figueras 2020). Almost all species belonging to this genus are pathogenic bacteria including *A. caviae*. However, there has been limited research on *A. caviae* identification, particularly related to the information regarding its molecular characterization. This species has been isolated from catfish (*Clarias gariepinus*) (Central Java), eels (*Anguilla* sp.) (Central Sulawesi) and the sediment of Gajah Mungkur Reservoir, Wonogiri, Central Java, by using phenotypic

identification (Sarjito et al 2013; Pujiastuti et al 2016; Syafitrianto et al 2016) instead of molecular identification. The overseas research isolated *A. caviae* from turbout (*Scophthalmus maximus*) larvae, Nile tilapia (*Oreochromis niloticus*), catfish (*Clarias gariepinus*) and phenotypically identified (Ringø & Vadstein 1998; Ashiru et al 2011; Abd El Tawab et al 2017). Moreover, *A. caviae* was isolated from Indian catfish (*Clarias batrachus*) and genotypically identified with molecular technique using 16S rDNA without any details on its molecular characterizations (Thomas et al 2013). Other study reported that *A. caviae* was successfully isolated and genotypically identified from eel (*Anguilla japonica*) followed with virulence genes detection without detection on resistant genes and their relationship with antibiotics susceptibility (Yi et al 2013).

Pathogenicity of *A. caviae* is related to its capability in infecting the host. The infection of *A. caviae* generates the clinical symptom of aeromoniasis on fish such as general symptom due to *Aeromonas* spp., i.e. haemorrhagic septicaemia, as indicated by clinical symptom of hemorrhages on the body, bloody exudate filling the intestine, enlarged liver and spleen, and liquefying kidney (Ogara et al 1998), as well as ulcerations in Indian catfish (*C. batrachus*) (Thomas et al 2013). Its infection also has impact on the histological change of the infected organ.

Clinically, *A. caviae* is one of four species of *Aeromonas* spp. that is able to infect human besides *A. dhakensis*, *A. veronii* and *A. hydrophila* (Fernández-Bravo & Figueras 2020). In human, *A. caviae* causes bacteremia, gastroenteritis, diarrhea, pneumonia, cystitis, and even keratitis in eye relating to contact lens (Rabaan et al 2001; Al-Benwan et al 2007; Dwivedi et al 2008; Chao et al 2013; Li et al 2015).

The capability of *Aeromonas* in infecting the host is influenced by its pathogenicity. The pathogenicity mechanism of this bacterium is quite complex and related to virulence factor (Li et al 2015). It is reported that *Aeromonas* virulence is multifactorial, i.e. structural component, toxins and extracellular products (Beaz-Hidalgo & Figueras 2013). The misuse of antimicrobial (antibiotic) to control virulent bacteria frequently occurs either in its volume or the usage frequency. Such misuse enables the bacterium to face a strong selection pressure of obtaining resistance through various mechanisms, such as genetic mutation and horizontal transfer of resistance genes (Parker & Shaw 2011). Such condition has an impact on the development of bacterial resistance against antibiotic. Therefore, this research investigates the molecular characterization of *A. caviae* from catfish by detecting the virulence and resistance genes relating to antibiotic susceptibility. It is important to collect complete information regarding *A. caviae* isolated from catfish as the basic consideration for accurate and wise control strategies of this bacterium.

Material and Method

Time and place of experimental study. This study was conducted at Laboratory of Fish and Environmental Health, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia in January-September 2019.

Isolation and bacterial culture. From the kidney of diseased catfish taken from aquaculture pond in Magelang, Central Java, Indonesia, MD-01 was isolated before being cultured on glutamate starch phenyl (GSP) medium (Merck) at 30ºC for 24 h. Then, to grow a single colony, tryptic soy broth (TSB) medium (Merck) was used, in which the isolates were stored at 20% glycerol at -80ºC.

Koch's postulate test. After acclimatized for five days, healthy catfish in 5-7 cm of total length, were randomly taken as samples. Dissecting the samples was carried out to obtain the bacteria from the kidney before cultured it on the GSP medium at 30°C for 24 h. To carry out pathogenicity test, bacterial suspension of 0.1 mL (density 10^5 CFU mL^{-1}) was injected to five *Aeromonas* spp. free catfish. Ten days of observation was carried out on external and internal clinical signs, mortality and histopathological observation of kidney, liver and gill.

Histopathogical observation. Prior the infectious injection of *Aeromonas* species, histopathological analysis was carried out on the internal organs of the samples. A solution of 10% formalin was used for the kidney, liver and gill within 24 h before the

samples were cleared, embedded, blocked, cut, dan stained with hematoxylene-eosin (H&E).

Phenotypic identification of Aeromonas spp. This step was carried out by observing the morphological colony, cell and biochemical properties of the bacteria. Tryptic soy agar (TSA) medium (Merck) at 30ºC in 24 h was used to grow the bacteria. The biochemical characteristics consisted of Vogest-Proskauer (VP) test, glucose utilization and gas production, L-arabinose utilization and aesculin hydrolysis.

Bacterial genomic DNA extraction. The extraction of genomic DNA of the bacteria was carried out by using the bacterial DNA kit as instructed in the manual of manufacturer procedure (Promega, Promega Corporation, Madison, USA). As much as one mL of cultured bacteria in TSB medium was incubated at 30°C for 24 h and centrifuged at 13,000 *g* for 2 min. The extracted DNA of the bacteria was then kept in a -20°C storage for further analysis.

16S rDNA amplification. The 16S rDNA was amplified by applying 27F and 1492R oligonucleotide universal primers (Table 1). Within total PCR volume of 25 µL, there were 12 µL of Mytaq HS Red Mix, (2× PCR Master Mix) (Bioline, Meridian Life Science, Memphis, UK), 1 µL of forward primer, 1 µL of reverse primer, 1 µL of DNA template (20 ng), and 10 µL of nuclease-free water (NFW) (Orozova et al 2009). The PCR was carried out by using initial denaturation at 95°C for 3 min, and 30 cycles 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. The PCR product was then given with electrophoresis using 1% agarose gel prior to the sequence (1st BASE Laboratories Malaysia).

Sequence analysis. The DNA sequences were edited and assembled by using the DNA Baser program (Wang et al 2019). The similarity was analysed by using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Clustal W Program (Tamura et al 2013) was used for multiple sequence alignments. The Neighboard-Joining MEGA 7.0.26 package (The Biodesign Institute, USA) were used to construct phylogenetic trees with 1000 replications of bootstrap analysis (Kumar et al 2016).

Detection of virulence genes. The virulence genes were detected by amplifying *Aeromonas* spp. in PCR. In 25 µL of PCR volume, there were 12 µL of Mytaq HS Red Mix, (2x PCR Master Mix, Bioline, Meridian Life Science, Memphis, UK), 1 µL of forward primer, 1 µL of reverse primer, 1 µL of DNA sample (20 ng), and 10 µL of nuclease-free water (NFW) (Orozova et al 2009). This step had detected virulence genes of *Aeromonas* complex consisting of *aerA/haem, alt, ast, flaA, lafA,* and *fstA* (Table 1). Then, the PCR results were proceeded to electrophoresis in 1.5% agarose gel.

Detection of resistance genes. Detecting the resistance genes of *Aeromonas* spp. was emphasized by using the PCR. In 25 µL of PCR volume, there were 12 µL of Mytaq HS Red Mix, (2x PCR Master Mix, Bioline), 1 µL of forward primer, 1 µL of reverse primer, 1 µL of DNA sample (20 ng), and 10 µL of nuclease-free water (NFW) (Orozova et al 2009). The detected resistance genes of *Aeromonas* spp. included *tet-A, strA-strB,* and *qnrA* (Table 1). The PCR products were subjected to electrophoresis with 1.5% agarose gel.

Antibiotic susceptibility test. Evaluation of the antibiotic susceptibility of MD-01 isolate was carried out towards 6 commercial antibiotics i.e. oxytetracycline, enrofloxacin erythromycin, chloramphenicol (Sigma, Sigma Corporate, St. Louis, Missouri, USA), ampicillin, and kanamycin (Wako, Wako Pure Chemical Industries, Osaka, Japan) and the susceptibility was determined based on micro dilution method (NCCLS 1994). Mueller hilton broth (MHB) isolates was injected for further antibiotic susceptibility analysis by using microplate well flat bottom which was incubated at 30ºC for 24 h. Then, addition of 10 µL of resazurin was given to the well of samples and control before being incubated at 30ºC. Observation was carried out after 1 h of incubation.

Data analysis. Descriptive analysis was carried out on the results of the Koch's postulate test, histopathological observation, phenotypic identification, molecular identification, virulence and resistance gene detection, and antibiotic susceptibility test.

Table 1

Primers sets used in this study

Gene	*Gene product*	*Primer sequence*	*Product size (bp)*	*Reference*
16S	16S rRNA gene	F: AGA GTT TGA TCM TGG CTC AG R: TAC GGY TAC CTT GTT ACG ACT T	1500	Isnansetyo & 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 & 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 & Rodgers (2004)
flaA	Polar flagellum	F: TCC AAC CGT YTG ACC TC R: GMY TGG TTG CGR ATG GT	608	Sen & 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	Tetracycline resistant	F: GTA ATT CTG AGC ACT GTC GC R: CTG CCT GGA CAA CAT TGC TT	956	Guardabassi et al (2000)
strA-strB	Aminoglycoside 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)

Results

Koch's postulate test. The results of Koch's postulate test showed the external and internal clinical signs in catfish infected with *Aeromonas* (Figure 1). The external signs included the depigmentation of the skin, abdominal dropsy, and abdominal ascites. The internal signs were characterized with discoloration of kidney to brownish red and other organs to pale red.

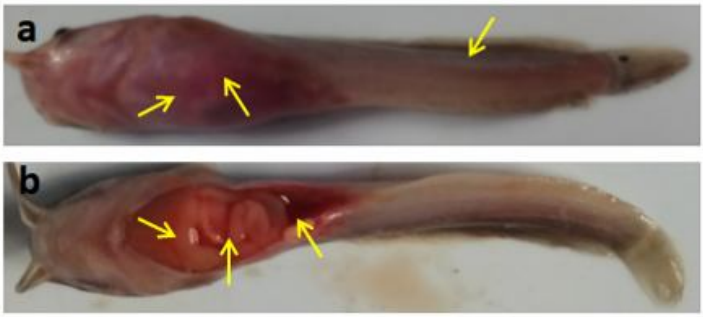

Figure 1. Clinical signs of catfish infected by *A. caviae* MD-01. a. depigmentation of the skin, abdominal dropsy and abdominal ascites; b. soft, brownish red and pale kidney and liver.

Histopathogical observation. The histopathological study showed necrotic degeneration in the kidney tissue, inflammation between tubulus, dilated blood vessels in liver, sinusoid dilating between hepatocytes, inflammation in the gill lamellae of the infected catfish. This condition was different with the control catfish having normal histological view (Figure 2).

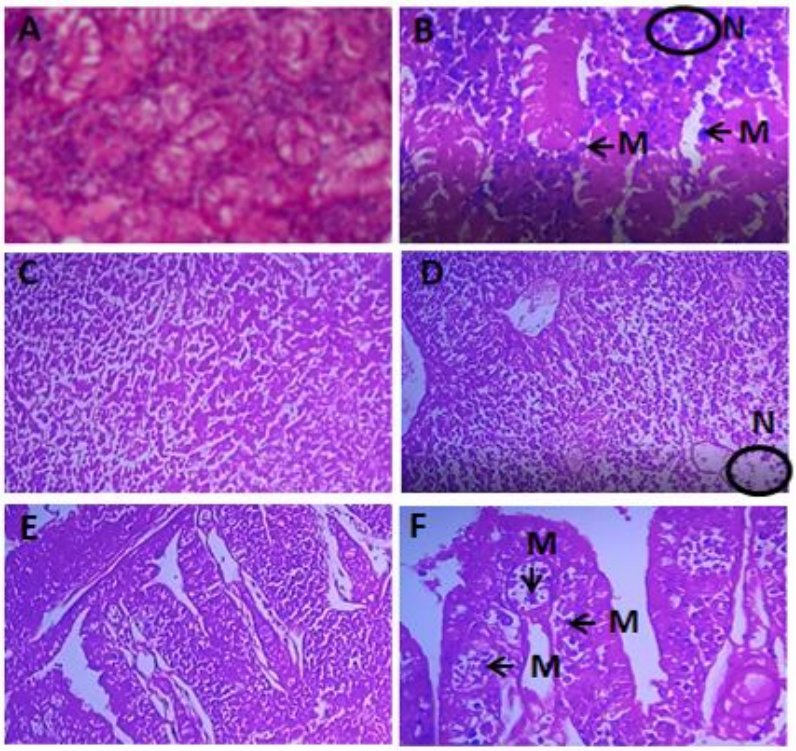

Figure 2. Pathological changes in kidney, liver and gill of catfish. a: Normal kidney in control fish (H&E, x 1000). b: Infected kidney, necrotic in kidney tissue (N), inflammation characterized by macrophage infiltration (M) (H&E, x 1000). c: Normal liver in control fish (H&E, x 100). d: Infected liver, necrotic in liver tissue (N) (H&E, x 100). e: Normal gill in control fish (H&E, x 1000). f: Infected gill, inflammation characterized by macrophage infiltration (M) in lamellae tissues (H&E, x 1000).

Phenotypic characters of the A. caviae MD-01. Table 2 showed the phenotypic characteristics of isolate MD-01 suspected as *A. caviae*, while isolates no 2 was *A. caviae* from *Anguilla* sp. (Syafitrianto et al 2016); 3. *A. caviae* from fish and human (Aravena-Román et al 2011); 4. *A. caviae* (Janda & Abbot 2010); 5. *A. caviae* (Martin-Carnahan & Joseph 2005). Isolate MD-01 1 grew well on GSP and TSA media at 30ºC, was rod-shape, Gram-negative, motile, acid forming (from glucose), able to ferment L-arabinose and hydrolyze aesculin, but could not produce acetylmethyl carbinol (VP). These phenotypic characters were in compliance to the strain of *A. caviae* no. 2,3,4,5 although no data (ND) was available regarding the characters of acid forming (from glucose) on the four strains, for producing acetylmethyl carbinol (VP) on *A. caviae* no. 2, and motility range of 11-89% on *A. caviae* no. 5. It indicated that the phenotypic character of MD-01 isolate matched to *A. caviae*.

Molecular identification of A. caviae MD-01 based on 16S rDNA. Similarity analysis of the sequence of 16S rDNA by using the Blast program showed that the isolates were closed to species *A. caviae* ATCC 15468 (NR029252) with the rates of similarity and query of 99.86% and 99%, respectively.

Phylogenetic tree. Phylogenetic analyses showed that isolates MD-01 was in the clade of *A. caviae* (Figure 3).

Detection of virulence genes. Six virulence genes (*aer/haem, alt, ast, flaA, lafA*, and *fstA*) were amplified by PCR. The results shown that *aer/haem, alt, ast, flaA* genes were detected in *A. caviae* but not *lafA* and *fstA* genes (Figure 4).

Detection of resistance genes. Three resistance genes (*tet-A, strA-strB*, and *qnrA*) were amplified by PCR. The results exhibited that all resistance genes were not detected in *A. caviae* MD-01.

Table 2

The phenotypic characters of *A. caviae* MD-01 isolated from diseased catfish

Characterization	*1*	*2*	*3*	*4*	*5*
Colony morphology					
Form	circular	circular	circular	circular	circular
Edge	even	even	even	even	even
Elevation	convex	convex	convex	convex	convex
Colour in TSA	white	white	white	white	white
Colour in GSP	yellow	yellow	yellow	yellow	yellow
Bacterial morphology					
Form	rod	rod	rod	rod	rod
Gram	-	-	-	-	-
Motility	+	+	+	+	d
Voges Proskauer	-	ND	-	-	-
D-glucose, acid	+	ND	ND	ND	ND
D-glucose, gas	-	-	-	-	-
L-arabinose	+	+	+	+	+
Aesculin	+	+	+	+	+

Taxa are identified as 1. Strains MD-01 (data from this study); 2. *A. caviae* from *Anguilla* sp. (Syafitrianto et al 2016); 3. *A. caviae* from fish and human (Aravena-Roman et al 2011); 4. *A. caviae* (Janda & Abbot 2010); 5. *A. caviae* (Martin-Carnahan & Joseph 2005). Abbreviations: +, >90% of strains positive; –, < 10% of strains positive; d, 11-89% of strains positive. ND, No data available.

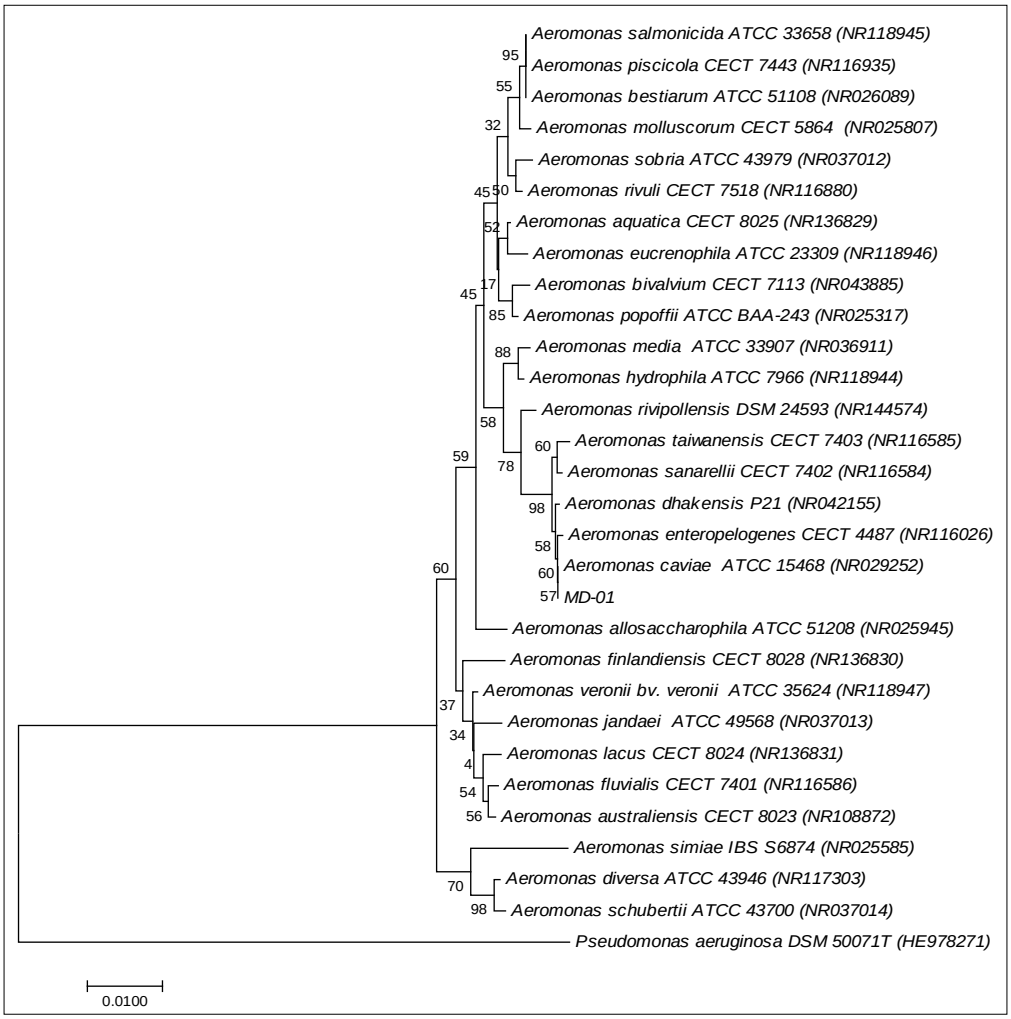

Figure 3. Phylogenetic tree constructed for *A. caviae* and other *Aeromonas* species based on 16S rDNA sequences using the Neighboard-Joining with 1,000 bootstrap analysis. *Pseudomonas aeruginosa* was used as an outgroup. The scale bar represents 0.01 substitutions per nucleotide position (Knuc).

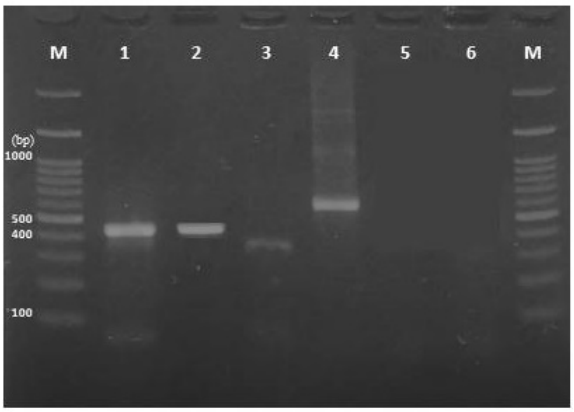

Figure 4. PCR amplification of virulence genes from the genomic DNA of *A. caviae* MD-01 isolate. M, DNA Marker 100 bp; Lane 1, *aer/haem* (431 bp); 2, *alt* (442 bp); 3, *ast* (331 bp); 4, *flaA* (608 bp); 5, *lafA* (736 bp); 6, *fstA* gene (452 bp).

Antibiotic susceptibility test. MIC test on six antibiotics revealed that this bacterium was resistant to oxytetracycline, ampicillin, erythromycin and chloramphenicol. However, it was susceptible to other two antibiotics with MIC value of 2.5 µg mL^{-1} and 0.05 µg mL^{-1} for kanamycin and enrofloxacin, respectively (Table 3).

Table 3

Antibiotic susceptibility test of *A. caviae* MD-01

Antibiotic	*Susceptibility*	*MIC (µg mL^{-1})*
Oxytetracycline	R	-
Ampicillin	R	-
Erythromycin	R	-
Chloramphenicol	R	-
Kanamycin	S	2.5
Enrofloxacin	S	0.05

S = susceptible; R = resistant.

Discussion. This research has successfully isolated and identified *A. caviae* from diseased catfish. In general, pathogenic bacterium dominantly infecting the catfish was *A. hydrophila* (Mulia et al 2011; Abdelhamed et al 2016; Wamala et al 2018). Some other detected species of *Aeromonas* spp. infecting catfish are *A. sobria* (Ashiru et al 2011), *A. salmonicida* (Thomas et al 2013), *A. veronii* (Nawaz et al 2010; Mohammed & Peatmen 2018; Hoai et al 2019) and *A. caviae* (Abd El Tawab et al 2017). Previous researches reported that *A. caviae* was detected in Nile tilapia (Ashiru et al 2011), eel (*Anguilla japonica*) (Yi et al 2013), goldfish (*Carassius auratus*) (Hossain et al 2019), shrimp (*Penaeus monodon*), turtle (*Mauremys reevesii*) (Shakir et al 2012; Deng et al 2014), mussel (*Mytilus galloprovincialis*) (Maravić et al 2013), sediment and surface water (Silva et al 2014), a river before and after treatment at a wastewater treatment plant, the activated sludge of the wastewater treatment plant, and hospital wastewater (Carnelli et al 2017). Another study reported that *A. caviae* was detected in human clinical wound (Aravena-Román et al 2011). In human, this species was more dominantly found rather than other *Aeromonas* species. Kimura et al (2013) reported that 36 strains of *Aeromonas* spp. isolated from patients with *Aeromonas* bacteremia were identified as *A. caviae*, *A. hydrophila* and *A. veronii* biovar *sobria*, i.e. 18, 13 and 5 species, respectively. However, information about *A. caviae* isolated from catfish was still limited regardless its potency as one of the main pathogens for catfish and other freshwater fishes, due to the general characteristics of *Aeromonas* spp. having the ubiquitous distribution and being opportunistic pathogens.

Koch's postulate test showed that this bacterium could infect healthy catfish and generate the aeromoniasis signs. The external signs included depigmentation of the skin, abdominal dropsy and abdominal ascites. The internal signs were characterized with

discoloration of kidney to brownish red and other organs of being pale red. Clinical signs in the form of skin lesion with depigmented also occurred in rainbow trout (*Oncorhynchus mykiss*) infected by *A. caviae*, in addition to other aeromoniasis signs, such as ulcers, inflammation around pectoral fins, hyperemia of the wall of the swim-bladder and petechial hemorrhages on the liver (Řehulka 2002). The results of histopathological assay revealed the damage on kidney, liver and gill of the catfish. Such histological change due to infection of *Aeromonas* spp. was also observed in *Oreochromis niloticus* (Alyahya et al 2018) with signs of hemocyte aggregation in hepatopancreas, degraded hemocytes with cell necrosis and aggregation in gill tissue. Clinical signs and histopathological assay in this study indicated that *A. caviae* was pathogen and had virulence factor that caused aeromonasis on catfish.

Identification of *A. caviae* was done phenotypically and genotypically (molecularly). Based on the phenotypic characteristics, isolate MD-01 exhibited closed characters with *A. caviae* (Martin-Carnahan & Joseph 2005; Janda & Abbot 2010; Aravena-Román et al 2011; Syafitrianto et al 2016). Molecular identification and phylogenetic tree revealed that isolate MD-01 was *A. caviae* ATCC 15468 (NR029252) (Soler et al 2004).

In order to obtain the complete molecular characteristics of *A. caviae*, detection of virulence and resistance genes as well as antibiotic susceptibility were conducted. Distribution of virulence genes on *Aeromonas* spp. determines their pathogenicity potential. All isolates *Aeromonas* spp. at least have one type of virulence gene (Králová et al 2016). This study indicated that *A. caviae* had 4 virulence genes, i.e. *aer*/*haem*, *alt*, *ast*, and *flaA* genes but not *lafA* and *fstA* genes. The *aer*/*haem* (aerolysin/haemolysin) was the gene occurred in *Aeromonas* spp. Previous results documented that *Aeromonas* spp. were characterized with virulence genes of aerolysin and haemolysin. Aerolysin seemed to be representative virulence factors from Aeromonads and detection of *aerA* was proposed as reliable method to identify the pathogenic isolates of *Aeromonas* spp. (Hirono & Aoki 1993; Buckley & Howard 1999). This gene was one of pathogenicity controls in *Aeromonas*. Other experiments also documented that aerolysin was detected on *A. caviae* (Yousr et al 2007; Pablos et al 2011; Aravena-Román et al 2014; Khor et al 2018). However, the aerolysin was not detected (Pollard et al 1990). No aerolysin gene was found on *A. caviae* isolated from chicken (Abu-Elala et al 2015). Haemolysin was also found on *A. caviae* (Wang et al 1996; Chacón et al 2003; Yousr et al 2007).

Other main virulence factors from *Aeromonas* spp. were exotoxin *alt* (cytotonic heat-labile enterotoxin) and *ast* (cytotonic heat-stabile enterotoxin). Former research also successfully detected those genes (Aravena-Román et al 2014), but Ghenghesh et al (2014) and Sen & Rodgers (2004) did not. Correspondingly, Aravena-Román et al (2014) also successfully detected *ast* gene on *A. caviae*, but Sen & Rodgers (2004) and Khor et al (2018) did not. Meanwhile, Králová et al (2016) only detected *ast* gene on one isolate of *A. caviae* out of 18 isolates (5.6%).

This research successfully detected *flaA* gene on *A. caviae* and was parallel to some previous studies (Rabaan et al 2001; Aravena-Román et al 2014; Králová et al 2016; Khor et al 2018). However, *lafA* and *fstA* genes were not detected in this study, which was unlike the research by Aravena-Román et al (2014) that detected *lafA* gene. However, this study was in compliance to the study by Beaz-Hidalgo & Figueras (2013) in which the presence of *fstA* gene on *A. caviae* was not found. Naturally, virulence gene has a role in causing a disease. Virulent bacteria will secrete the compound or material supporting their virulence so that it can weaken or kill the host. It was presumed that some virulence genes cooperated in the virulence of *Aeromonas* spp. including *A. caviae*. Several virulence factors on *Aeromonas* had a role in resolving the response of host immune system and causing the infection (Pablos et al 2009; Pessoa et al 2019). Various virulence factors produced by *A. caviae* indicated their potential to cause the disease on fish, and even human (Abd El Tawab et al 2017). However, the results of this project exhibited the variation on the presence of virulence genes on same bacterial species. The difference in virulence genes detected on *Aeromonas* spp. including *A. caviae* had been previously reported (Abu-Elala et al 2015). It was assumed due to the difference in strain, source of isolates, geographical areas and environment.

The molecular characteristics of *A. caviae* were also viewed based on the presence of its resistance genes. This study reported that *tet-A*, *strA-strB* and *qnrA* genes in *A. caviae* were not detected. Other project also did not find *tet-A* gene in *A. caviae*, instead, it was detected in *A. hydrophila* (Carvalho et al 2012). It was different from the research by Deng et al (2014) that successfully detected *tet-A* gene in *A. caviae*. This study was also parallel to other research in which *strA-strB* gene was not detected in *A. hydrophila*, *A. veronii* and *A. salmonicida*, but successfully found in *A. punctata* (Verner-Jeffreys et al 2009). In other studies, *qnrA* gene was not discovered in *Aeromonas* spp. (Figueira et al 2011; Varela et al 2016; Wimalasena et al 2017) either. The presence of different resistance genes in *Aeromonas* spp. was presumed to be due to the differences in the source and type of isolates, type of species and environmental factors.

This study showed that *A. caviae* was resistant to oxytetracycline, ampicillin, erythromycin, and chloramphenicol, but susceptible to kanamycin and enrofloxacin at 2.5 µg mL^{-1} and 0.05 µg mL^{-1}, respectively. This finding was not in line to Evangelista-Barreto et al (2010) showing that *A. caviae* was susceptible against tetracycline of about 92%. However, this experiment showed that *A. caviae* were resistant to tetracycline of about 14.3% (Deng et al 2014), while other finding documented that *Aeromonas* spp. were resistant to oxytetracycline of about 50% (Čížek et al 2010).

The results show that *A. caviae* was resistant to ampicillin. These findings were parallel to reports by Vila et al (2002) and Chuang et al (2011) investigating *A. caviae* isolate resistant to ampicillin. These results were also in line to the research by Chowdhury et al (2010) studying the resistance of bacteria from mangrove sediment in Malaysia. Their findings revealed that all *Aeromonas* spp. isolates were resistant to b-lactam antibiotics (ampicillin and penicillin). All *Aeromonas* spp. isolated from treated wastewater (100 isolates) exhibited the maximum resistance (100%) to ampicillin and penicillin (Olaniran et al 2015).

In line to the report by Simon et al (2016), it showed that *Aeromonas* spp. were resistant to erythromycin approximately by 39.97%. Dias et al (2012) reported that *Aeromonas* spp. were resistant to erythromycin of about 81-96%. This investigation result was different with that of other experiment finding *A. caviae* being susceptible to chloramphenicol (100%) (Guz & Kozińska 2004; Evangelista-Barreto et al 2010). However, this result was relevant to research by Odeyemi & Ahmad (2017) in which *Aeromonas* spp. were resistant to chloramphenicol approximately by 20.8%. The study of Jacobs & Chenia (2007) showed that *Aeromonas* spp. were resistant to chloramphenicol of about 10.8%.

This work was parallel to investigation by Dias et al (2012) revealing that susceptible isolates of *Aeromonas* spp. to kanamycin was around 49-69%. *Aeromonas* spp. was susceptible to kanamycin of about 76.2% (Khafagy et al 2015). Current finding also complied to Didugu et al (2016) which documented that the isolates of *Aeromonas* spp. were susceptible to enrofloxacin. Furthermore, Ulkhaq & Lusiastuti (2017) investigated that *A. hydrophila* from african catfish (*C. gariepinus*) was susceptible against enrofloxacin. Jung-Schroers et al (2018) also summarized that enrofloxacin was effective to kill *Aeromonas* spp. The difference in resistance or susceptibility was probably due to the variation within the source of isolates, frequency and type of used antimicrobe agents to cure some infections in different geographical areas (Nagar et al 2011).

This research revealed that there were no *strA-strB* and *qnrA* genes in *A. caviae*. Therefore, it was highly logic if this bacterium was susceptible to kanamycin and enrofloxacin. However, despite *tet-A* gene was not found in *A. caviae*, this bacterium was resistant to oxytetracycline. It was caused by the resistance of pathogen against antibiotic as the scientific mechanism for surviving (Fischbach & Walsh 2009). Therefore, the effort of pathogenic bacteria in defeating the antibiotic keeps going on. In addition, the strength of pathogenic bacteria was also affected by the effectivity of antibiotic in inhibiting or diminishing the pathogen. Besides, some bacteria have natural or innate resistance against more than one class of antibiotics (Tenover 2006). The use of exaggerated, inappropriate and unwise antibiotic in controlling infected fishes might cause various mechanisms of antibiotic resistance on pathogen. It was frequently found in different cultured species. As a result, there was increment in the prevalence of

antibiotic resistance (McIntosh et al 2008; Deng et al 2014). The findings in this experiment revealed the presence of a linkage between the absence of resistance genes on *A. caviae* and bacterial susceptibility against certain antibiotic. The *Aeromonas* species of clinical source, food, fish and natural waters exhibited the increase of resistance against antimicrobial (Beaz-Hidalgo & Figueras 2013), which was frequently related to the presence of cellular resistance genes (Piotrowska & Popowska 2014). The resistance of *A. caviae* against some antibiotic has a potency in generating serious threat not only for catfish, but also for other fish and animal, or even human health.

Conclusions. *A. caviae* is one of *Aeromonas* spp. which are not frequently exposed in publications in Indonesia. Oversea publications also did not provide complete information on the molecular characterization of *A. caviae* from catfish. We have shown that isolate MD-01 was able to infect catfish and cause clinical signs of aeromoniasis and histopathology. Phenotypic and genotypic characteristics with 16S rDNA indicated it as *A. caviae*. This research successfully revealed the diversity of virulence (*aer/haem*, *alt*, *ast*, and *flaA*) and resistance (*tet-A*, *strA-strB*, and *qnrA* genes) genes in *A. caviae*. It was susceptible to kanamycin and enrofloxacin but resistant to oxytetracycline, ampicillin, erythromycin and chloramphenicol.

Future progress in the understanding of virulence and susceptible antibiotics in *A. caviae* is important for detecting more various virulence and resistance genes as well as for investigating the more widely use of antibiotics in order to obtain more complete data on molecular characterizations of *A. caviae*. In addition, it is essential to compare the molecular characterizations of *A. caviae* from catfish, freshwater and marine water fishes as well as other animals having a potency to be infected by this bacterium. A comprehensive study is required to obtain accurate, effective and wise strategy to control *A. caviae*.

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