

KERAGAMAN *Aeromonas* spp. PADA LELE (*Clarias* sp.) YANG DIBUDIDAYAKAN DI JAWA, INDONESIA

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INTISARI

Aeromonas spp. merupakan bakteri Gram negatif yang hidup di perairan, dapat menyerang ikan dan biota air lainnya, tidak terkecuali ikan lele. Bakteri ini memiliki tingkat patogenisitas yang tinggi dan beragam. Infeksi *Aeromonas* spp. dapat menyebabkan penyakit aeromoniasis atau *Motile Aeromonas Septicemia* (MAS) dan merupakan salah satu kendala dalam budidaya lele di Indonesia. Selain menyebabkan ikan sakit, serangan bakteri ini dapat menyebabkan kematian massal dalam waktu yang singkat. Tujuan penelitian ini adalah mengetahui keragaman *Aeromonas* spp. pada lele (*Clarias* sp.) yang dibudidayakan di Jawa, Indonesia. Penelitian menggunakan metode survey dan teknik *purposive sampling* untuk menentukan wilayah kolam budidaya lele serta teknik *random purposive sampling* untuk pengambilan sampel lele sakit. Pengambilan sampel dilakukan di kolam budidaya di empat provinsi di Pulau Jawa, yaitu DIY Yogyakarta, Jawa Tengah, Jawa Barat, dan Jawa Timur. Penelitian diawali dengan mengambil sampel lele sakit selanjutnya dilakukan inokulasi bakteri dari organ ginjal pada medium *glutamate starch phenyl* (GSP) secara aseptis. Isolasi dan pemurnian bakteri dilakukan pada medium yang sama. Tujuh puluh empat isolat bakteri berhasil dikumpulkan. Selanjutnya dilakukan uji postulat Koch dan diperoleh 16 isolat patogen. Gejala klinis yang muncul antara lain depigmentasi kulit, erosi, hiperemia, dan hemoragik; abdominal dropsi, asites, abses, dan inflamasi pada bagian perut; lesi, hemoragik, dan pucat pada ginjal; lesi dan pucat pada hati dan insang. Dampak uji postulat Koch juga terjadi pada organ histologis ikan, yaitu nekrosis pada ginjal, hati, dan insang, hemoragik pada ginjal, kongesti pada hati, serta fusi pada lamella insang. Selanjutnya, 16 isolat patogen hasil penelitian ini dan dua isolat koleksi laboratorium dikarakterisasi secara fenotipe (morfologis dan karakteristik biokimiawi) dan genotipe (16S rDNA), diperoleh 10 isolat *Aeromonas*. Sepuluh isolat *Aeromonas* tersebut terdiri dari empat spesies, yaitu *A. dhakensis* (empat isolat, 40%), *A. veronii* bv *veronii* (empat isolat, 40%), *A. caviae* (satu isolat, 10%), dan *A. hydrophila* (satu isolat, 10%). Berdasarkan indeks keragaman Shannon-Wiener yang mencapai 1,1936, *Aeromonas* spp. patogen di Jawa memiliki keragaman spesies sedang. *Aeromonas* spp. patogen



menyebarkan di tiga provinsi, yaitu DIY, Jawa Tengah, dan Jawa Barat. *A. dhakensis* menyebar di DIY (Sleman dan Kulonprogo) dan Jawa Tengah (Magelang dan Kedung Ombo), masing-masing provinsi terdiri dari dua isolat. *A. veronii* bv *veronii* menyebar di DIY (Sleman), Jawa Tengah (Demak), dan Jawa Barat (Bogor), masing-masing satu isolat, kecuali Jawa Barat terdiri dari dua isolat. *A. caviae* berasal dari Jawa Tengah (Magelang), sedangkan *A. hydrophila* dari Jawa Barat (Bogor). *A. dhakensis* merupakan spesies baru yang belum pernah dipublikasikan di Indonesia, bahkan di dunia pun sebagian besar dilaporkan dari manusia dan sebagian kecil spesies ikan, tetapi belum pernah diisolasi dari lele. Selain itu, *A. veronii* bv *veronii* ditemukan sebagai biovar baru di Indonesia. Deteksi gen virulen, yaitu *aerolysin/haemolysin* (*aerA/haem*), *heat-labile cytotoxic enterotoxin* (*alt*), *heat-stable cytotoxic enterotoxin* (*ast*), *polar flagella* (*flaA*), *lateral flagella* (*lafA*), dan *ferric siderophore receptor* (*fstA*) dan uji LD₅₀ dilakukan untuk analisis virulensi *Aeromonas* spp., sedangkan analisis resistensi *Aeromonas* spp. meliputi deteksi gen resisten *tetracycline* (*tetA*), *aminoglycoside* (*strA-strB*), dan *quinolone* (*qnrA*) dan uji resistensi *Aeromonas* spp. berdasarkan *minimum inhibitor concentration* (MIC) dan *minimum bactericidal concentration* (MBC) antibiotik. *Aeromonas* spp. memiliki keragaman gen virulen sedang, berdasarkan indeks keragaman Shannon-Wiener mencapai 1,7490. Setiap isolat minimal memiliki empat jenis gen virulen. Berdasarkan prevalensinya, gen *aerA/haem* dan *flaA* mencapai 100%, *ast* mencapai 90%, *alt* mencapai 80%, *fstA* mencapai 60%, dan *lafA* mencapai 40%. Gen virulen pada *Aeromonas* spp. sebagian besar menyebar mengikuti pola sebaran spesiesnya. Secara umum, lima gen virulen, yaitu *aer/haem*, *alt*, *ast*, *flaA*, dan *lafA* menyebar di tiga provinsi, yaitu DIY, Jawa Tengah, dan Jawa Barat, sedangkan gen *fstA* hanya menyebar di dua provinsi, yaitu DIY dan Jawa Tengah. *Aeromonas* spp. pada lele memiliki virulensi tinggi dengan nilai LD₅₀, yaitu $7,61 \times 10^3$ CFU/mL sampai $1,4907 \times 10^5$ CFU/mL. Sebagian *Aeromonas* spp. memiliki satu gen resisten, dari tiga jenis gen yang dideteksi, yaitu gen *tetA* dan *strA-strB*, tetapi tidak memiliki gen *qnrA*. *Aeromonas* spp. resisten terhadap oksitetrasiklin, ampicilin, dan eritromisin dan sensitif terhadap kanamisin, enrofloksasin, dan kloramfenikol. Antibiotik yang masih efektif digunakan untuk mengobati ikan yang terserang *Aeromonas* spp. adalah kanamisin, enrofloksasin, dan kloramfenikol.

Kata kunci : *Aeromonas* spp., 16S rDNA, fenotipe, gen resisten, gen virulen, genotipe, lele

DIVERSITY of *Aeromonas* spp. IN CATFISH (*Clarias* sp.) CULTIVATED IN JAVA, INDONESIA

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ABSTRACT

Aeromonas spp. is Gram negative bacteria living in waters and able to attack fish as well as other aquatic biota, including catfish. It has high degree of pathogenicity and variety. It can cause aeromoniasis or *Motile Aeromonas Septicemia* (MAS), which is one of the obstacles in catfish cultivation in Indonesia. Beside causing disease to the fish, it can also cause mass mortality within a short time. The objective of this study was to determine the genotype biodiversity of *Aeromonas* spp. pathogens in catfish cultivated in Java, Indonesia. This study used the survey methods and *purposive sampling* techniques to determine the area of catfish cultivation ponds, as well as *random purposive sampling* techniques for diseased catfish sampling. Sampling was carried out in aquaculture ponds of four provinces in Java: Yogyakarta, Central Java, West Java and East Java. The study was started by taking sample of diseased catfish and aseptically inoculating the kidney on *glutamate starch phenyl* (GSP) medium. Isolation and purification of bacteria were carried out on the same medium. Seventy-four bacterial isolates were collected. Furthermore, the Koch Postulate test was carried out and 16 pathogenic isolates were obtained. Subsequent to the Koch postulate test, emerging clinical symptoms included skin depigmentation; erosion, hyperaemia and haemorrhagic; abdominal dropsy, acites, abscesses, and inflammation of the abdomen; lesions, haemorrhagic and pallor of the kidneys; lesions and pallor on the liver on the gills. The impacts of the Koch postulate test is also happened on the observation of the histological organs: necrosis of the kidneys, the liver, and the gill, haemorrhagic of the kidneys, congestion of the liver, and fusion of the gill lamellae. Furthermore, 16 isolates from this study and two isolates from the laboratory collection were characterized phenotypically (morphological and biochemical characteristics) and genotypically (16S rDNA) obtained 10 *Aeromonas* isolates. The ten *Aeromonas* isolates consisted of four species, namely *A. dhakensis* (four isolates, 40%), *A. veronii* bv *veronii* (four isolates, 40%), *A. caviae* (one isolate, 10%), and *A. hydrophila* (one isolate, 10%). Based on the Shannon-Wiener diversity index, which reaches 1.1936, *Aeromonas* spp. pathogens in Java indicate moderate species diversity. *Aeromonas* spp.

pathogens spread in three provinces, namely DIY, Central Java, and West Java. Meanwhile, *A. dhakensis* spreads in DIY (Sleman and Kulonprogo) and Central Java (Magelang and Kedung Ombo), with each province consisting of two isolates. *A. veronii* bv *veronii* spreads in DIY (Sleman), Central Java (Demak), and West Java (Bogor), with each province consisting of one isolate, except two isolates in West Java. *A. caviae* comes from Central Java (Magelang), and *A. hydrophila* comes from West Java (Bogor). *A. dhakensis* is a new species and there has not been any publication about it in Indonesia and even in the world. Most of it has been reported to be found in humans and a small number of fish species, but never in isolated catfish. In addition, *A. veronii* bv *veronii* is found to be a new biovar identified in Indonesia. Detection of virulent genes i.e. *aerolysin/haemolysin* (*aerA/haem*), *heat-labile cytotoxic enterotoxin* (*alt*), *heat-stable cytotoxic enterotoxin* (*ast*), *polar flagella* (*flaA*), *lateral flagella* (*lafA*), and *ferric siderophore receptor* (*fstA*) and LD₅₀ test were carried out in the purpose of virulence analysis for *Aeromonas* spp., meanwhile, the resistance analysis included the detection of resistance genes i.e. *tetracycline* (*tetA*), *aminoglycoside* (*strA-strB*), and *quinolone* (*qnrA*) and resistance test based on the *minimum inhibitor concentration* (MIC) and the *minimum bactericidal concentration* (MBC) of antibiotics. Based on the Shannon-Wiener diversity index, with the value of reaching 1.7490, *Aeromonas* spp. indicates virulent gene diversity. Each isolate has at least four types of virulent genes. Based on the prevalence, the *aerA/haem* and *flaA* genes reached 100%, the *ast* genes reached 90%, the *alt* reached 80%, the *fstA* reached 60%, and the *lafA* reached 40%. Virulent genes in *Aeromonas* spp. are mostly spread in accordance to the distribution pattern of its species. In general, five virulent genes, i.e. *aerA/haem*, *alt*, *ast*, *flaA*, and *lafA*, spread in three provinces, namely DIY, Central Java and West Java. Meanwhile the *fstA* gene only spreads in two provinces, which are Yogyakarta and Central Java. *Aeromonas* spp. in catfish has high virulence with an LD₅₀ value of 7.61 x 10³ CFU/mL to 1.4907 x 10⁵ CFU/mL. Some of *Aeromonas* spp. had one resistance gene out of the three types of detected genes, which were *tetA* and *strA-strB* genes, but did not have the *qnrA* gene. *Aeromonas* spp. was resistant to oxytetracycline, ampicillin and erythromycin and sensitive to kanamycin, enrofloxacin and chloramphenicol. Effective antibiotics to treat fish attacked by *Aeromonas* spp. include kanamycin, enrofloxacin and chloramphenicol.

Keywords: *Aeromonas* spp., 16S rDNA, phenotype, resistance gene, virulent gene, genotype, catfish