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SHORT COMMUNICATION

Resistance Test on *Aeromonas hydrophila* Isolated from African Catfish (*Clarias gariepinus*) against Some Antibiotics Groups

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Aeromonas hydrophila is a type of bacterium causing Motile Aeromonads Septicemia (MAS) disease, which infects fresh water fish, including African catfish, and leads to death as well as huge losses to farmers. This research aims to determine the resistance status of various antibiotic classes in *A. hydrophila* bacteria isolated from African catfish. Bacterial isolates of *A. hydrophila* were taken from the liver and kidneys of infected African catfishes obtained from Parung, Bogor. Characterization of the bacteria was carried out based on colony morphology and biochemical properties. Meanwhile, bacterial resistance test was conducted using antibiotic disks with Kirby-Bauer method. Based on colony morphology and biochemical properties, the characterization results indicated that the bacterial isolates tested were *A. hydrophila*. Further examination of the antibiotic resistance test showed that the bacteria were resistant to penicillin antibiotics and macrolides. Future researches are expected to use molecular identification for *A.hydrophila* bacteria mutant to known the DNA base.

Key word : *Aeromonas hydrophila*, antibiotics, *Clarias gariepinus*, resistance

Bakteri *Aeromonas hydrophila* merupakan bakteri penyebab penyakit *Motile Aeromonads Septicemia* (MAS) yang menginfeksi ikan air tawar, termasuk ikan lele dumbo dan mengakibatkan kematian dan kerugian yang besar. Penelitian ini bertujuan untuk menentukan status resistensi dari berbagai golongan antibiotik pada bakteri *A. hydrophila* yang diisolasi dari ikan lele dumbo. Isolat bakteri *A. hydrophila* diambil dari organ hati dan ginjal ikan lele dumbo sakit yang diperoleh dari wilayah Parung, Bogor. Karakterisasi bakteri dilakukan berdasarkan morfologi koloni dan sifat biokimia serta uji resistensi bakteri menggunakan disk antibiotik dengan metode *Kirby-Bauer.* Hasil karakterisasi bakteri menunjukkan bahwa isolat bakteri yang diuji adalah *A. hydrophila* berdasarkan morfologi koloni dan sifat biokimia. Pemeriksaan lebih lanjut mengenai uji resistensi antibiotik menunjukkan bahwa bakteri *A. hydrophila* resisten terhadap antibiotik golongan penisilin beta-laktam dan makrolida. Penelitian selanjutnya diharapkan dapat menguji secara molekuler untuk mengetahui susunan basa DNA dari bakteri *A. hydrophila* resisten antibiotik.

Kata kunci : *Aeromonas hydrophila*, antibiotik, ikan lele dumbo, resisten

Aeromonas hydrophila is a bacterium that causes Motile Aeromonads Septicemia (MAS), infecting fresh water fish, including African catfish. This disease causes a high mortality rate both on seed stage and adult stage, and leads to a great economic loss (Omeje and Chukwu 2012). The number of deaths caused by this disease reached 173 tons of carps in West Java with a total loss of Rp 126 million suffered by the farmers (in 2005). Janda and Abbott (2010) reported that the incidence of MAS diseases caused the death of 820 tons of carp with total losses reaching 37.5 million dollars.

Bacteria from the genus *Aeromonas*, are opportunistic bacteria that can be isolated from waters, including groundwater, surface water, drinking water, and wastewater. Moreover, these bacteria can also be found in food, cheese, and milk (Sharma *et al*. 2009). Janda and Abbott (2010) have identified twenty-one species in the genus *Aeromonas*, but only several are known pathogen in fish, *A. salmonicida*, *A. hydrophila*, *A. formicans*, and *A. liquefaciens* (Abdel-Raouf and Ibraheem 2008).

A prevention method is commonly applied to prevent bacterial infections in fish by using antibiotics. However, a number of alternatives have been sought to replace the use of antibiotics as antimicrobial agents, such as vaccination, immunostimulants, and probiotics (Guz and Kozinka 2004). because the improper use of antibiotics can cause serious problems, for example increased resistance of bacterial pathogens (Balcazar *et al*. 2006). In previous reports, Costa and Cyrino (2006)

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demonstrated that *A. hydrophila* isolated from *Piaractus mesopotamicus* and *Oreochromis niloticus* is resistant to antibiotics derived from amoxicillin, ampicillin, lincomycin, novobiocin, oxacillin, penicillin, and trimethoprim + sulfametoxasol. Similarly, Dias *et al*. (2012) suggests that *A. hydrophila* bacteria isolated from the ornamental fish are resistant to several types of antibiotics, namely tetracycline, ampicillin, erythromycin, ticarcillin, and carbenicillin. Results of another study also mentioned that the resistance of *A. hydrophila* occurs in isolates derived from carp against ampicillin and penicillin antibiotics (Guz and Kozinka 2004). Unfortunately, the resistance of *A. hydrophila* isolated from African catfish against different classes of antibiotics has not been studied. Therefore, this research aims to determine the status of various classes of antibiotic resistance in *A. hydrophila* bacteria isolated from African catfish.

Isolation and Characterization of Bacterial Isolate. *A. hydrophila* was isolated from the liver and kidneys of African catfish obtained from the diseased area in Parung, Bogor. The bacterial isolates were cultured on Trypticase Soy Broth (TSB) media at 25 mL in a water bath shaker at 160 rpm for 24 h at 29 °C. Further characterization of *A. hydrophila* isolates was conducted based on morphological and biochemical factors in accordance with ISO (7309: 2009), including cell shape, gram stain, motility test (using SIM Medium), oxidation, catalation, and oxidative/fermentative test (OF) using OF Medium. Moreover, bacterial culture isolates were also conducted on Rimmler-Shoots (RS) media, a selective medium for *A. hydrophila*.

Resistance Test. Resistance test on *A. hydrophila* was performed by Kirby-Bauer method using antibiotic discs commercially available. The type of antibiotic used consisted of erythromycin 15 µg (macrolides group), clindamycin 2 µg (lincosamides group), tetracycline 30 µg (tetracycline class), chloramphenicol 30 µg and 50 µg (fenikol group), enrofloxacin 5 µg (fluoroquinolones group), kanamycin 30 µg (aminoglycosides group), penicillin 10 µg and ampicillin 10 µg (beta-lactam penicillin group), as well as rifampicin 5 µg (ansamicyn group). *A. hydrophila* isolates were uniformly distributed in Trypticase Soy Agar (TSA) media in a petri dish, and then placed in the midst of an antibiotic disc plate. Next, they were incubated for 24 h at 29 °C in an incubator. Finally, the level of bacterial resistance to antibiotics was determined by measuring the diameter of clear zone formed and categorized by NCCLS (2002).

Characterization of Bacteria. Characterization of the bacterial isolate based on its colony morphology and biochemical properties showed that the isolates were *A. hydrophila*. (Table 1). This is supported by the observation that the bacterial colony in RS media was yellow with no black spots in the middle. Microscopic observation showed that the bacteria was motile and the results of biochemical tests, furthermore, showed that the bacteria produced catalase and oxidase enzymes that break down the sugar anaerobically and aerobically .

Antibiotic Resistance Test. The results of antibiotic resistance test against *A. hydrophila* bacteria (Table 2) showed that the bacterial isolates were

Table 1. Results of characterization of *A. hydrophila* isolates based on colony morphology and biochemical properties

Parameter	Results	
	A.hydrophila tested	Literature (SNI 7309:2009)
Shape of Colonies	Small Spherical	Not described
Elevation	Convex	Not described
Edges	Smooth	Not described
Color	Beige	Not described
Shape of Cells	Short Stem Cell	Not described
RS Media	+	Not described
Motile	+	+
Catalase	+	+
Oxidase	+	+
Gram	Negative (-)	Negative (-)
OF	Positive O/F	Positive O/F

Table 2. Results of antibiotic resistance test against *A. hydrophil*a bacteria

No.	Antibiotics	Zone Diameter (mm) (NCCLS 2002)			Results of the Research	
		R	I	S	*A. hydrophila* (mm)	Category
1.	Erythromycin 15 µg	≥ 23	14-22	≤ 13	5.4	S
2.	Clindamycin 2 µg	≥ 21	15-20	≤ 14	23	R
3.	Tetracycline 30 µg	≥ 21	15-18	≤ 14	9.5	S
4.	Chloramphenicol 30 µg	≥ 18	13-17	≤ 12	11.4	S
5.	Chloramphenicol 50 µg	≥ 18	13-17	≤ 12	10.4	S
6.	Enrofloxacin 5 µg	≥ 23	17-22	≤ 16	14.1	S
7.	Kanamycin 30 µg	≥ 18	14-17	≤ 13	5.4	S
8.	Penicillin 10 µg	≥ 15	-	≤ 14	16.2	R
9.	Ampicillin 10 µg	≥ 17	14-16	≤ 13	18.3	R
10.	Rifampicin 5 µg	≥ 20	17-19	≤ 16	13.7	S

resistant to clindamycin (a macrolide), as well as penicillin and ampicillin (beta-lactam).

Colony of *A. hydrophila* was yellow with no black spot in the middle. Shotts and Rimler (1973) stated that the colonies of *A. hydrophila* in RS media were round, in convex elevation, flat edges, and yellow. Similarly, the results of a research conducted by Chirila *et al*. (2008) stated that the colonies of *A. hydrophila* were white on Blood Agar, and bluish green on Meitert Istrati media.

Biochemical tests of this bacteria showed that the isolate used in this study is *A. hydrophila*. This is consistent with the results of a research conducted by Jayavignesh *et al*. (2011) which stated that the assay results of biochemical characteristics of *A. hydrophila* are indole (+), MR (-), VP (+), citrate (+), catalase (+), urea (-), oxidase (+), gelatin hydrolysis (+), carbohydrate utilization of lactose (+), glucose (+), and trehalose (+). Tantu *et al*. (2013) adds that the characteristics of *Aeromonas* sp. are Gram negative, oxidase (+), catalase (+), H_2S (+), motile (+), OF (+), and citrate (-).

Antibiotic resistance is one of bacterial properties indicating the nature of immune or resistance to certain antibiotics (Byarugaba 2010). Antibiotic resistance can occur via two processes, namely resistance that occurs due to a spontaneous mutation in the chromosome and resistance that occurs due to plasmid displacement. Resistance on chromosomes is more stable and cannot be moved horizontally into other bacteria, while resistance on plasmids is not stable/easily lost and easily transferred to other bacteria that do not have the gene (Cruz *et al*. 2012).

The mechanism of bacterial resistance to antibiotics depends on the types of bacteria. For Gram negative bacteria, there are several mechanisms of antibiotic resistance used as a means of resistance of bacteria to antibiotics. These mechanisms include several ways. First, resistance occurs by modification in the bacterial cell wall, thus preventing the amount of antibiotics across the cell membrane. Second, resistance occurs by increasing betalactamase production, thereby damaging the structure of beta-lactam. Third, resistance occurs by increasing activity of the efflux pump,so that the bacteria will pump out antibiotics before they can cause any effects. Fourth, resistance occurs by modifying enzymes, so antibiotics cannot interact with receptors. Fifth, resistance occurs by metabolic bypass mechanism, so antibiotics cannot across the cell membran The last one is through ribosomal modification process, thus preventing the merging of antibiotics that inhibit bacterial protein synthesis (Peleg and Hooper 2010).

The resistant mechanism of *A. hydrophila* bacteria to Betalactamase group (ampicillin and penicillin) occurs because *A. hydrophila* bacteria produce Betalactamase enzyme, thus damaging the structure of beta-lactam. The structure of beta-lactam in penicillin group is a structure that inhibits bacterial cell wall synthesis (Lakshmi *et al*. 2014). The results are consistent with the research results by Odeyemi and Ahmad (2015) stating that 53 *Aeromonas* isolated from aquatic environments in Malaysia have 100% resistance to ampicillin and 92.5% resistance to

penicillin. In addition to the fish, 100% isolates of *A. hydrophila* isolated from chickens in India also develop resistance to ampicillin (Kaskhedikar and Chhabra 2009).

The resistance of *A. hydrophila* against lincosamide class of antibiotics (clindamycin) occurs since *A.hydrophila* can modify receptors of antibiotics, so antibiotics cannot bind to the receptors (ribosomes). Leclercq (2002) suggests that bacterial resistance to the lincosamides occurs through three ways, namely modifying the receptors on the ribosome, preventing an increase in the performance of antibiotics after binding to the receptors, and inactivating the antibiotics that move into the bacterial cell. This is supported by the research conducted by Stratev *et al*. (2013) stating that 100% of bacterial isolates derived from *A. hydrophila*, B-hemolytic isolated from fish, Rainbow Trout (*Oncorhynchus mykiss*), has resistance to amikacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, cefazolin, cefotiam, clindamycin, erythromycin, imipenem, linezolid, meropenem, teicoplanin, and vancomycin. Similar results were reported by Igbinosa *et al*. (2013), *i.e*. 82.1% of *Aeromonas* isolates isolated from the river Kat and Tyume in Africa experienced clindamycin resistance. It can be concluded that *A. hydrophila* bacteria isolated from sick African catfish in Bogor can develop resistance against two classes of antibiotics (beta-lactam penicillin and lincosamide).

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