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The Study of Resistance *Aeromonas Hydrophila* to Antibiotics from Aquaculture Systems in Banten Province, Indonesia

Widuri Nayunda Safitri[1], Suherman[2], Toha Tusihadi[2], Siti Hudaidah[1], Yudha Trinoegraha Adiputra[1]*

[1]Department of Fisheries and Marine Science, Faculty of Agriculture, University of Lampung. No.1 Prof.Sumantri Brodjonegoro St., Gedong Meneng, Bandar Lampung, Indonesia, 35145

[2]Balai Pengujian Kesehatan Ikan dan Lingkungan, Raya Carita St., Umbul Tanjung, Cinangka, Serang Regency, Banten Province, Indonesia, 42167

A B S T R A C T

Motile Aeromonas Septicaemia (MAS) is a disease that has the potential to become an epidemic in freshwater cultured fish and is caused by Aeromonas hydrophila. Farmers use antibiotics as treatment and are considered the most effective countermeasures. Antibiotics that are not as recommended can cause bacteria to become resistant. The purpose of this study was to evaluate the resistance of A. hydrophila bacteria to three antibiotics i.e. oxytetracycline, tetracycline, and enrofloxacine. The research was conducted by explorative method. A. hydrophila was isolated from samples of pond water, inlet water, outlet water, pond sediment, catfish (Clarias sp.), and tilapia (Oreochromis niloticus). The samples were taken from fish farm in Banten Province. A. hydrophila isolates were identified by biochemical tests using Vitek 2 compact, then A.hydrophila susceptibility test to three antibiotics was carried out in vitro using the disc diffusion method. A. hydrophila which is known to be resistant to certain antibiotics is continued with the Minimum Inhibitory Concentration (MIC) test. Results of this study, 31 A. hydrophila isolates were obtained, where the percentage of oxytetracycline was 6.4%, tetracycline was 3.2% and enrofloxacin not shown resistance. MIC value of tetracycline and oxytetracycline in the A. hydrophila isolates was 16 µg/mL.

Keywords: *Aeromonas hydrophila*; antibiotics; oxytetracycline; resistance; tetracycline

1. Introduction

Aquaculture activities are a sub-sector that has the potential to expand employment and increase income for the community (Maftuch *et al*., 2018). Freshwater aquaculture is a choice of many fishing activities (Nurhasnawati *et al*., 2016). According to the Ministry of Maritime Affairs and Fisheries in 2021, the number of freshwater fish cultivators in all provinces in Indonesia is estimated to reach 1,392,326. At Banten Province, the number of freshwater fish cultivators reached 35,279 (MMAF, 2021). Freshwater fish have high economic value and marketing is relatively easy to reach (Syamsunarno *et al*., 2017). According to Lumentut *et al*. (2015), freshwater fish farming is dominated by common carp (*Cyprinus carpio*), African catfish (*Clarias gariepinus*), Nile tilapia (*Oreochromis niloticus*), pangasius catfish (*Pangasius* hypopthalamus), and gouramy (*Osphronemus gourami*).

According to Maftuch *et al*. (2018), disease problems are currently main challenges infected and decreased production, water quality and mortality. Fish diseases caused by several types of pathogens such as viruses, parasites, fungi, and bacteria (Luturmas, 2014). One of the pathogenic agents of bacterial disease in cultured fish is *Aeromonas hydrophila* cause of Motile Aeromonas Septicaemia (MAS) disease in freshwater fish (Stratev *et al*., 2016). It is was reported that MAS disease often causes economic losses by reaching a death rate of 80-100% of the total population in a short time (Lukistyowati *et al*., 2012).

Amanu *et al*. (2014) declared *A. hydrophila* can controlled by using antibiotics through injection, immersion or feed. According to the regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia number 1/PERMEN-KP/2019 concerning classification of fish medicine, types of antibiotics that are permitted and most widely used are enrofloxacin, oxytetracycline and tetracycline. Use of antibiotics is not accordance with recommendations, it can result in ability of bacterial cell activity to withstand or stop the destructive effects of antibiotic drugs, which is often called bacterial resistance (Afrianti *et al*., 2011). Antimicrobial resistance is a problem that can threaten health and has the potential to spread antimicrobial resistance in humans (Syafriana *et al*., 2020). The emergence of pathogenic strains in fish that are resistant to antibiotics has been reported by Rahim *et al*. (1984) and Spanggaard *et al*. (1993). Information on the resistance of *A. hydrophila* to antibiotics in freshwater fish farming is still very limited and research resistance is necessary.

2. Materials and Methods

This research was conducted at the Microbiology Laboratory, Fish and Environmental Health Testing Center (BPKIL), Serang Regency. This research was conducted in 2022, July-December. Data collection and data collection will be carried out in African catfish and Nile tilapia aquaculture ponds in

Banten Province (Table 1). This research used explorative method. Samples of ponds, inlet, outlet, sediment, fish bodies were taken from fish farmers in Banten Province. Each sample was identified by biochemical test using Vitek 2Compact. Susceptibility test of *A. hydrophila* to three types of antibiotics was carried out in vitro using the disc diffusion method. *A. hydrophila* isolates known to be resistant to certain antibiotics oxytetracycline, tetracycline, and enrofloxacin were continued with the Minimum Inhabitation Concentration (MIC) test.

Procedures performed in this study included equipment sterilization, sampling, bacterial isolation, bacterial identification, and antimicrobial sensitivity testing using the diffusion method (disc test) and the dilution method (MIC test).

Table. 1 – Sample origin and number of samples.

Origin (City/Regency)	Sample amount	Origin and sample number fish farms				
		Ponds	Inlet	Outlet	Sediment	Fish body
Tangerang City	44	3	1	1	3	3
Tangerang Regency	44	3	1	1	3	3
Tangerang Selatan City	44	3	1	1	3	3
Serang City	44	3	1	1	3	3
Cilegon City	44	3	1	1	3	3
Serang City	44	3	1	1	3	3
Lebak Regency	44	3	1	1	3	3
Pandeglang Regency	44	3	1	1	3	3

3. Results and Discussion

The purpose of this study was to evaluate the resistance of *A. hydrophila* to three antibiotics namely oxytetracycline, tetracycline, and enrofloxacin at regencies and cities in Banten Province. *Aeromonas hydrophila* macroscopically and microscopically (Table 2). Results showed biochemical tests using the Vitek 2 Compact. Based on the results of study 31 isolates were identified as positive for *A. hydrophila*. Observation of the morphology of *A. hydrophila* colonies had a cream color on TSA media, a yellow color on RS media, a round shape, and a convex elevation. This was also conveyed by Anggraini *et al*. (2016), that from the results of the colony morphology in his study it was shown that the bacterial isolates of *A. hydrophila* had yellowish-white (beige) colonies, round shapes, smooth edges, and convex elevations. If observed using a microscope, *A. hydrophila* will have a rod shape and have flagella. According to Wahjuningrum *et al*. (2013), *A. hydrophila* has a colony morphology that is convex elevation, has smooth edges, is cream colored, has rod-shaped cells, and is Gram negative.

Table 2 – *Aeromonas hydrophila* characters in variety agar media.

Test (agar media)		Result
Blood Agar		Beta hemolysis
TSA	Colony:	
	Type	*Circular*
	Edge	Flat
	Elevation	*Convex*
	Color	Grey
RS	Color	Yellow
Gram Color	Cell type	Coccus

Results of *A. hydrophila* disc test were resistant and sensitive (Fig.2) to antibiotics. Measurement of the inhibition zone of the antimicrobial sensitivity test based on Clinical and Laboratory Standards Institute reference (CLSI, 2018), where for tetracycline and oxytetracycline antibiotics have a diameter of ≤11 mm (resistant), 12-14 mm (intermediate), and ≥15 mm (sensitive). Then for this type of antibiotic enrofloxacin has a diameter of ≤15 mm (resistant), 16-20 mm (intermediate), and ≥21 mm (sensitive). Test results from diffusion test on 31 isolates of *A. hydrophila* isolates showed that two isolates were resistant to oxytetracycline, one isolate was resistant to tetracycline, and none was resistant to enrofloxacin (Fig.3).

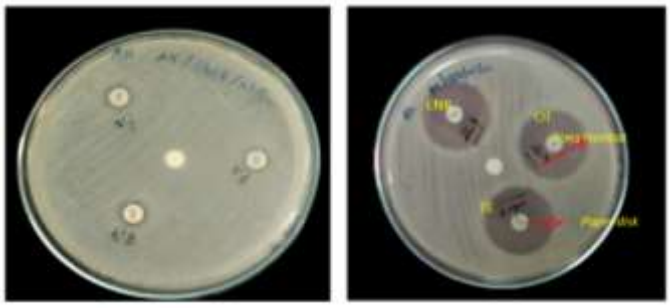

Fig. 2 – Diffusion test results showed resistant (left) and sensitive (right). ENR=enrofloxacin; OT=oxytertracyline; TE=tetracyline.

The use of antibiotics by freshwater fish cultivators in Banten Province was 37.64%. The results of the diffusion test (disc test) of *A. hydrophila* isolates in Banten experienced the highest resistance to oxytetracycline antibiotics of 6.4%, then to tetracycline antibiotics of 3.2% and no resistance to enrofloxacin. Research conducted by Kim *et al*. (2011) also showed that of 16 isolates of *A. salmonicida* highest resistance occurred to oxytetracycline antibiotics by 50% and enrofloxacin antibiotics by 6.25%. The MIC value of oxytetracycline and tetracycline antibiotics in *A. hydrophila* isolates was 16 µg/mL. This is in line with the research of Kim *et al*. (2012) bacteria experienced resistance with an MIC value of 16 µg/mL and this dose was the minimum value that could inhibit bacterial growth.

Table. 3 – Sample origin and number of samples.

No.	Sample code	Origin (City/Regency)	Origin
1.	I.160	Serang City	Fish
2.	I.162	Serang City	Fish
3.	I.163	Serang City	Fish
4.	I.167	Serang City	Fish
5.	AT.1053	Cilegon City	Inlet
6.	L.250	Cilegon City	Sediment
7.	AT.1217	Serang City	Pond
8.	L.585	Pandeglang Regency	Outlet
9.	AT.1443	Pandeglang Regency	Outlet
10.	L.572	Pandeglang Regency	Sediment
11.	AT.1450	Pandeglang Regency	Inlet
12.	AT.1451	Pandeglang Regency	Outlet
13.	I.683	Lebak Regency	Fish
14.	I.685	Lebak Regency	Fish
15.	I.1364	Lebak Regency	Fish
16.	AT.1580	Tangerang Regency	Inlet
17.	AL.304	Tangerang Regency	Inlet
18.	I.512	Tangerang Regency	Fish
19.	L.340	Tangerang Regency	Fish
20.	I.887	Tangerang Regency	Pond
21.	AT.1620	Tangerang Regency	Pond
22.	AT.1572	Tangerang Regency	Inlet
23.	I.891	Tangerang Regency	Pond
24.	AL.253	Tangerang Regency	Outlet
25.	I.888	Tangerang Regency	Fish
26.	I.603	Tangerang Regency	Fish
27.	AT.1298	Tangerang Regency	Pond
28.	I.1478	Tangerang Selatan City	Fish
29.	I.1479	Tangerang Selatan City	Fish
30.	AT.2856	Tangerang Selatan City	Inlet
31.	AT.2858	Tangerang Selatan City	Pond

Results showed oxytetracycline antibiotics were the most common cause of resistance in *A. hydrophila* in Banten Province. Research conducted by Turk & Halis (2016) states that tetracycline class antibiotics are a source of contamination for ecosystems because they are cheap, used in human and animal medicine, as feed additives in production, and are resistant to environmental conditions. Plasmid R contains genes that can cause bacterial cells to be resistant to certain antibiotics, such as sulfonamides, streptomycin, chloramphenicol, kanamycin, and tetracyclines. Resistance factors can occur due to resistance transfer factors which include genes for plasmid replication and determinant factors that can code for enzyme products that inactivate certain drugs or toxic compounds. Mechanism for the occurrence of resistance can be caused by genetic mutations, one species acquiring resistance from another species, or natural resistance to certain types of bacteria.

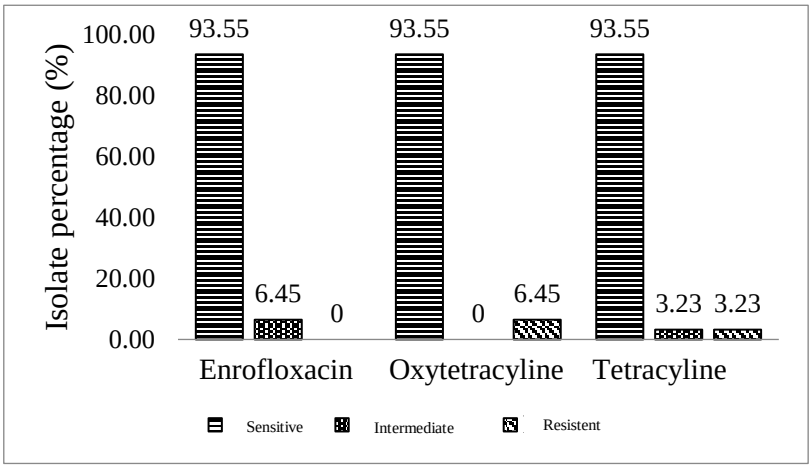

Fig. 3 – Percentage of sensitive, intermediate and resistant *Aeromonas hydrophila* to antibiotics.

Hakimah *et al*. (2021) argued that bacterial resistance occurred due to maintenance, feed management, treatment measures, environment, water sources, transportation processes, storage areas. The relationship between humans, animals and the environment are also a trigger for bacterial resistance. The spread of resistance from animals to the environment can be through urine or faeces which are disposed of directly or reused as fertilizer. Fish that are fed other animal faeces can be one of the triggers for the spread of bacterial resistance (Rousham *et al*., 2018). Bacteria that resistant to antibiotics difficult to eliminated and the ability of antibiotics to kill pathogenic bacteria will depend on concentration and time. The solution that can be done is to limit the use of antibiotics by making strict policies (Guay, 2008).

4. Conclusions

Based on the research results, 31 isolates of *Aeromonas hydrophila* were obtained, where one isolate was resistant to tetracycline and two isolates were resistant to oxytetracycline with MIC value were 16 µg/mL.

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