ASEAN-FEN INTERNATIONAL FISHERIES SYMPOSIUM – 2017

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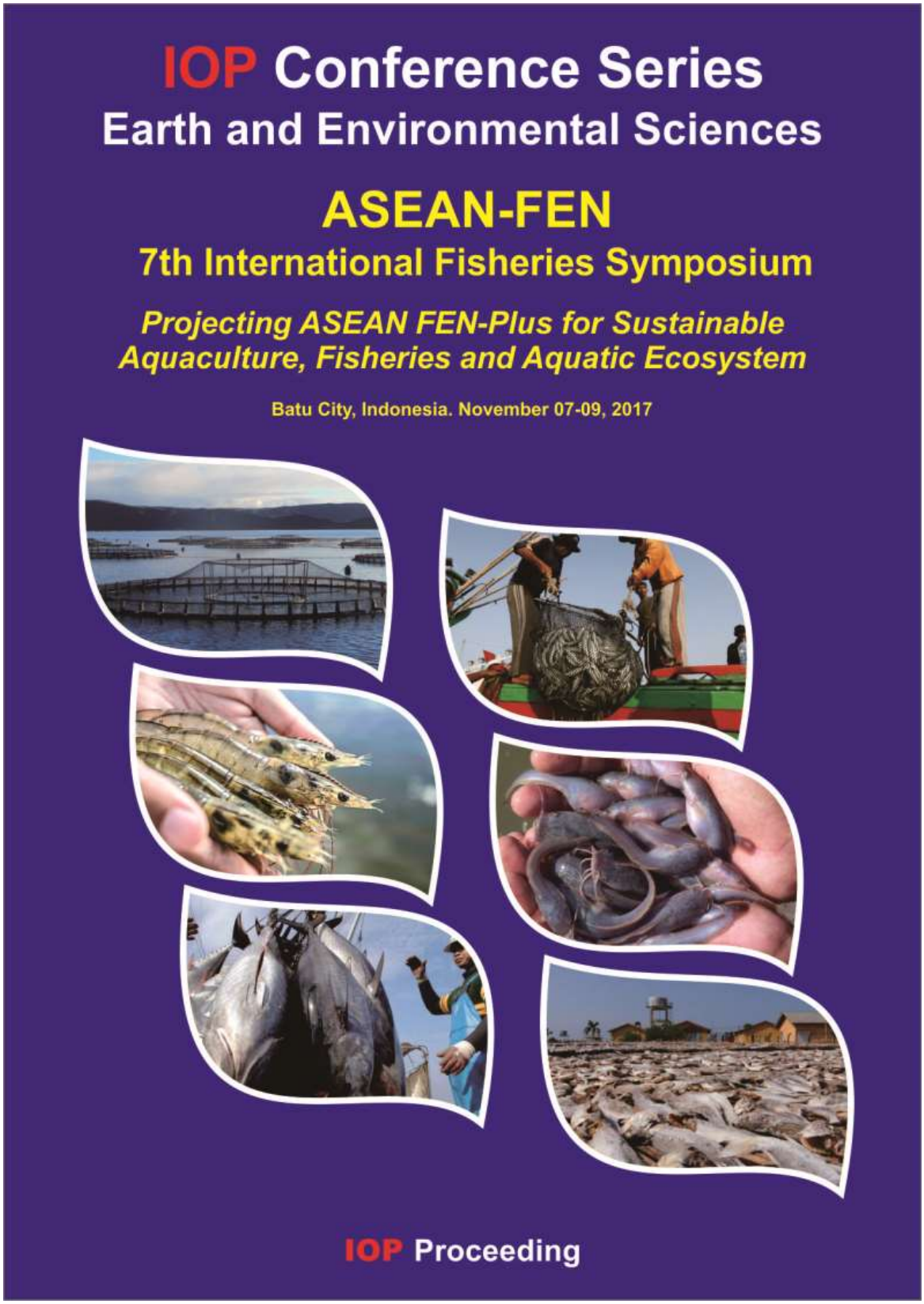

Preface

The 7th ASEAN-FEN International Fisheries Symposium was successfully held in Batu, East Java, Indonesia 7 – 9 November 2017. The conference was hosted by Faculty of Fisheries and Marine Science, Brawijaya University Malang Indonesia. The theme of this symposium was "Projecting ASEAN FEN Plus for Supporting Sustainable Aquaculture, Fisheries and Aquatic Ecosystems", with focus on the advanced innovation to address to the newly emerged issues in aquaculture, fisheries and aquatic ecosystems for the synergies between socioeconomic development and protecting natural resources and the environment**.**

The conference was attended by over 500 researchers from different countries, who presented and discussed the results of their work within the framework of five main areas: 1. Aquaculture, 2. Sustainable fisheries and management, 3. Seafood processing and biotechnology, 4. Aquatic resources, biodiversity and environment, and 5. Fisheries Economic.

ASEAN-FEN IFS 2017 Committee received more than 120 manuscripts from participated universities and research institutes, and 106 manuscripts were accepted for publication. All of the papers were subjected to peer-review by qualified experts in the field selected by the conference committee. The papers selected depended on their quality and their relevancy to the conference.

We would like to thank all the authors who have contributed to this volume and also to the board members, organizing committee, reviewers, speakers, chairpersons, sponsors and all the conference participants for their support to the ASEAN-FEN IFS 2017.

Warm Regards,

Dr.Sc. Asep Awaludin Prihanto, S.Pi., MP.
Chairperson of ASEAN FEN, IFS 2017
Faculty of Fisheries and Marine Science,
Brawijaya University, Malang, Indonesia

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Peer review statement

All papers published in this volume of *IOP Conference Series: Earth and Environmental Science* have been peer reviewed through processes administered by the proceedings Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

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Antimicrobial resistance prevalence of *Aeromonas hydrophila* isolates from motile *Aeromonas septicemia* disease

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Antimicrobial resistance prevalence of *Aeromonas hydrophila* isolates from motile *Aeromonas septicemia* disease

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Abstract. Fish suffer, from bacteria, fungi, virus and parasites or by physical ailments. Gurami (*Osphronemus gouramy*), nila (*Oreochromis niloticus*), carp (*Cyprinus carpio*), catfish (*Clarias* sp.) were the most reported infections caused by *Aeromonas* are bacterial hemorrhagic septicemia or Motile *Aeromonas* Septicemia (MAS). Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms included MAS. However, the use of antibiotics in the long term can cause negative impacts, among others, feared the occurrence of bacterial resistance in certain antibiotics. The results showed five of isolates were sensitive to antibiotics of chloramphenicol, gentamycin, oxytetracycline, cefradoxil and nalidixic acid but resistant to vancomycin colistin sulphate, rifampisin, cephalosporin and novobiocin.

1. Introduction

Motile *Aeromonas septicemia* (MAS) disease can cause death between 80-100% of dumbo catfish seeds in a period of approximately one week [1]. MAS disease has also been reported to have infected freshwater fish in the Banyumas region. The Livestock and Fishery Service Office of Banyumas Area (2005) reported that there were at least 72,000 freshwater fish that were infected with *Aeromonas hydrophyla* in 2003, namely 52,100 gurami and 19,900 catfish; while in 2004 the number amounted to 43,000 freshwater fish, which included 29,900 gurami and 13,100 dumbo catfish.

MAS disease control in cultivation is usually carried out by using antibiotics such as chloramphenicol, novobiocin, gentamicin, oxytetracycline, vancomycin, nalixidic acid, colistin sulphate and several other types of antibiotics. However, the use of antibiotics in the long term can cause negative impacts, among them is the fear of the occurrence of bacterial resistance to certain antibiotics.

The main cause of antibiotic resistance is its inappropriate dosage of use [2]. There have been case reports of bacterial resistance to these chemicals, namely from *Aeromonas caviae*, *Aeromonas sobria*, *Aeromonas bestiarum* and *Aeromonas hydrophyla* which are resistant to tetracycline [3]. The resistance that occurs will cause the bacteria to be resistant to the drug given so that the use of the drug becomes ineffective. Therefore, it is necessary to perform resistance tests of *Aeromonas hydrophyla* isolates against some types of antibiotics to find out which isolates are resistant.

2. Methodology

2.1. Bacteria isolate

Three isolates of *Aeromonas hydrophyla* bacteria were obtained from Sukabumi, West Java; Jepara, Central Java; and Surabaya, East Java.

2.2. Antibiotics

The antibiotics used were 30 (mcg) vancomycin, 30 (mcg) chloramphenicol, 5 (mcg) novobiocin, 10 (mcg) gentamicin, 30 (mcg) oxytetracycline, 10 (mcg) colisin sulphate, 5 (mcg) rifampicin, 5 (mcg) cefixime, 2 (mcg) cefadroxil and 30 (mcg) nalidixic acid.

2.3. Media

The media used in this research was Tryp ticase Soya Agar (TSA), Mueller Hinton Agar (MHA) and NaCl Physiological.

2.4. Research design

This study was conducted in July-August 2017 as an experimental study by observing, measuring and comparing the inhibit zone formed in the resistance test and comparing it with each antibiotic standard [4]. Three isolates of *Aeromonas hydrophyla* collected from Surabaya, Sukabumi and Jepara were used in this study. Each isolate was tested using seven antibiotics namely chloramphenicol, gentamycin, oxytetracycline and nalidixic acid, but resistant to vancomycin, colistin sulphate and novobiocin

2.5. Reidentification of bacteria

The identification of the *Aeromonas hydrophila* bacterial isolate was done by the analytical profile index (API) method by using KIT API.

2.6. Bacterial culture

After the reidentification, the bacterial isolates were cultured in Trypticase Soya Agar (TSA) media to obtain sufficient quantity of bacterial stock. The amount was then determined for the resistance test.

2.7. Production of bacterial suspension

Bacteria turbidity was likened to Mc Farland's cloudy number 1 with the number of bacteria at $3x10^8$ / mL [5]. A reaction tube containing physiological NaCl with a volume of 9 ml was prepared. An amount of 1 ml of bacteria was taken from the bacteria turbidity and then fed into a reaction tube containing physiological NaCl with a volume of 9 ml.

2.8. Resistance test

The resistance test was performed by using diffusion discs or diffusion test discs. Petri dishes contained MHA media were first prepared. An *Aeromonas hydrophyla* suspension of 100 µl was taken using a micropipette and dispersed on the entire surface of the agar plate and flattened using a drigalski to obtain even growth. After 10-15 minutes, the disc paper was placed on the agar medium by using sterile tweezers. The bacteria-planted medium and paper discs were incubated at 30ºC for 18-24 hours [6]. The media was observed and the clear zone that formed around the disc paper was measured using the sliding term. The measurement of the inhibit zone diameter was done by reducing the diameter of the obstacle area by the diameter of the disc paper (6 mm) [7].

2.9. Data analysis

After the data was obtained, namely the clear zone diameter seen around the disk paper that has been overgrown with *Aeromonas hydrophyla* bacteria, the results were then compared to the standard

inhibition zone of each antibiotic according to CLSI (Clinical Laboratory Standards Institute) and descriptively analyzed.

3. Results and discussions

3.1. Reidentification of Bacteria

The results of the identification of *Aeromonas hydrophyla* isolates from Sukabumi, Surabaya and Jepara are presented in table 1.

Table 1. Results identification of isolates *Aeromonas hydrophyla* from Sukabumi(K), Surabaya (S) and Jepara (J).

Physiological character	Isolate			*Aeromonas hydrophyla*
	S	**K**	**J**	
Gram	-	-	-	-
Motility	+	+	+	+
Citrate utilization	-	-	-	V
H_2S	-	-	-	-
Oxidase	+	+	+	+
Indol production	+	+	+	+

The result of examination using KIT API shows that the three isolates identified are *Aeromonas hydrophila* with a 90.16 % isolation for the Sukabumi isolate, 90.05 % for the Surabaya isolate and 88.31 % for the Jepara isolate.

3.2. Resistance test

The results of the resistance tests of each isolate on several antibiotics are presented in table 2.

Table 2. Resistance test result of *Aeromonas hydrophyla* isolate from Sukabumi, Jepara and Surabaya against some antibiotics.

Antibiotic (mcg concentraion)	Isolate(Code)		
	Sukabumi (K)	Jepara (J)	Surabaya (S)
Vancomycin (30)	- (R)	- (R)	- (R)
Chloramphenicol (30)	29,8 (S)	25,8 (S)	27,6 (S)
Novobiocin (5)	8,1 (R)	5,8 (R)	5 (R)
Gentamicin (10)	17,2 (S)	15,1 (S)	14,8 (S)
Oxytetracycline (30)	24,5 (S)	20,2 (S)	24,6 (S)
Colistin Sulphate (10)	9,4 (R)	8 (R)	7,3 (R)
Nalidixic Acid (30)	31,4 (S)	28,1 (S)	28,9 (S)
Rifampicin (5)	- (R)	- (R)	- (R)
Cefixime (5)	- (R)	- (R)	- (R)
Cefadroxil (2)	1,775 (S)	1,7 (S)	1,95(S)

Resistance test results of *Aeromonas hydrophyla* against some antibiotics were marked by the formation of clear zones around the antibiotic dish. Each isolate showed resistance to five antibiotics (prevalence 50 %), with the same type of antibiotic.

Doses of the 10 antibiotics used were standard doses of antibiotics or in general can be considered as effective doses of antibiotics, such as vancomycin 30 mcg, 30 mcg chloramphenicol, novobiocin 5 mcg, 10 mcg gentamicin, oxytetracycline 30 mcg, 10 mcg colistin sulphate, 5mcg rifampicin, cefixime

5mcg, cefadroxil 2mcg and nalidixic acid 30 mcg [4]. When administered correctly, they greatly affect the ability of antibiotics in inhibiting the growth of microorganisms, otherwise it may affect the resistance of these antibiotics [8].

Sensitive results indicate that antibiotics are capable of inhibiting microbes in recommended antibiotic concentrations that can be used for the treatment of microorganism infections. While resistant results indicate that isolates of microorganisms cannot be inhibited by antibiotics at normal doses and are no longer used in treatment [4]. Novobiocin is an antibiotic that has a working mechanism by inhibiting DNA synthesis and tricholic acid in bacterial cell membranes [9]. Test results show a drag zone was seen with a clear color around the antibiotic dish of novobiocin of 8.1 mm in the Sukabumi isolate (K), 5.8 mm in the Jepara isolate (J) and 5 mm in the isolate of Surabaya (S). Based on data from Clinical Laboratory Standards Institute (CLSI) [4], novobiocin antibiotics are categorized as resistant (R) if the inhibit zone formed is less than or equal to (≤) 17 mm. This means that novobiocin antibiotics are resistant to *Aeromonas hydrophyla* bacteria. The cause of resistance to this type of antibiotic is the suspected structural changes of the bacteria that result in these antibiotics not working optimally in carrying out its action and causing bacteria to remain resistant.

Vancomycin is an antibiotic of the polypetides group that has a working mechanism that inhibits the synthesis of bacterial cell walls. Recently increased use of vancomycin has reduced the sensitivity of this antibiotic [7]. Based on the results of the tests, the three isolates of *Aeromonas hydrophyla* namely Sukabumi (K), Jepara (J) and Surabaya (S) do not show any inhibition zone around the vancomicin antibiotics dish (0 mm). This suggests that vancomicin antibiotics are resistant to *Aeromonas hydrophyla* bacteria.

The mechanism of resistance and reduced vancomycin sensitivity is thought to be associated with changes and rearrangement of bacterial cell walls. In addition, the production of excess Penicillin Binding Protein-2 (PBP-2) is also considered an important factor for the expression of resistance to vancomycin. Resistance to vancomycin is mediated by a van A gene specific to glycopeptides. The presence of Van A results in a change in the target terminal of D-alanil-D-alanil or D-alanil-D-serine, which causes its bond with vancomycin to be poor as the critical point for the hydrogen bond is lost. This causes vancomycin to be unable to be bound, resulting in decreased sensitivity [11].

Colistin can inhibit the permeability of Gram negative cell wall bacteria. The cytoplasm of all living cells is limited by the cytoplasmic membrane acting as a barrier to selective permeability, performing an active transport function so that it can control the arrangement of cells. When the integrity of the cytoplasmic function is impaired so that the permeability of the cell wall changes or even becomes damaged, important components, such as proteins, nucleic acids, nucleotides and cells gradually die [11].

The colistin sulphate antibiotic also showed similar activity with vancomycin and novobiocin and is also resistant to all isolated *Aeromonas hydrophyla* isolates. Based on the data obtained, the inhibition zone formed around the antibiotic dish of colistin sulphate was 9.4 mm in the Sukabumi isolate (K), 8 mm in the Jepara isolate (J) and 7.3 mm in the isolate of Surabaya (S). Based on these results, it can be categorized as resistant (R) because it has a zone of <10 mm [4]. Colistin becomes resistant thrugh the modification of the outer membrane structure of LPS, the bacteria is negatively charged so that the LPS phosphate component becomes neutral and weakens the binding of colistin with LPS wall.

The emergence of resistance to an antibiotic can occur through several mechanisms: the bacteria synthesizes an enzyme inactivator or antibiotic destroyer; bacteria change their permeability to drugs; bacteria develop a change of target structure for drugs; or bacteria to develop metabolic path changes directly inhibited by drugs [12].

4. Conclusions

Based on the results of the study, it can be concluded that the isolates of *Aeromonas hydrophila* from Sukabumi, Surabaya and Jepara showed three resistant properties of vancomycin, novobiocin, rifampicin, cephalosporin and colistin sulphate (prevalence 50 %), while it showed sensitive results against 5 other antibiotics.

5. References

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