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Detection of Antibiotic Residue from Shrimp Ponds and Their Environment in East Java Province, Indonesia

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Abstract

Hari Suprapto, L. Sulmartiwi, and Sudarno. 2015. Detection of Antibiotic Residue from Shrimp Ponds and Their Environment in East Java Province, Indonesia. ***Aquacultura Indonesiana, 16 (1): 29-32.*** Indonesia shrimp products are often rejected by foreign countries due to high antibiotic content, whereas a few years ago Japan and European countries are set 0.05 ppb permitted to consume by the people, but now the restriction will become smaller, which is 0.1 ppb. Some farmer have topped using antibiotics but the residues were still found in fish flesh. Possible origin of the antibiotic is from environment because residues are still exist in the mud, water organism which lives in the pond. Samples originating from shrimp, mud, water and other biota were examined. The purpose of this study was to determine the origin of antibiotic present in the cultivation or farming system that does not use antibiotics in shrimp production.: a. Antibiotics obtained from ponds in the Tuban area is 1.8 mg/L (mud), 0.8 mg/L (water) and 2.1 (biota). b. Antibiotics obtained from ponds in Gresik were 0.6 mg/L (mud), 0.3 mg/L (water) and 1.2 (biota). c. Antibiotics obtained from ponds in the area of Situbondo are 0.3 mg/L (mud), 0.1 mg/L (water) and 0.6 (biota). d. a. Antibiotics obtained from ponds in the area are 0.3 mg/L Probolinggo (mud), 0.2 mg/L (water) and 0.6 mg/L (biota).

Keywords : Antibiotic; Environment; Pond; Shrimp

Introduction

Rejection of Indonesian shrimp products by some European countries and Japan occurred few years ago due to high antibiotic content. Previously the residue limit was 0.05 ppb but today 0.001 ppb. The use of vaccine to prevent the disease is recommended rather than the use of antibiotics. Antibiotics should be stopped because it could cause some excesses of bad health and the environment. Initially antibiotic is used to prevent diseases caused by bacteria, especially for the luminous vibriosis in large scale of farming, oxytetracycline, oxolinic acid, chloramphenicol and furazolidone are used through the feed. In the Philippines they used nitrofurans, erythromycin and sulfa drugs in shrimp culture (Baticados *et al*., 1990; Cobello, 2006). On salmon farming in British Columbia, Canada in 2000, antibiotics residues in salmon flesh are 0.1 mg/L (Paone, 2000). Aso reported that crab consumed food remains and salmon faeces contained residue of Further reported that crab eating food remains and feces salmon containing residues Oxytetracycline of 0.8 and 3.8 mg/L. Bjorklund *et al.* (1990) reported that in Finland, although he did not perform measurements, antibiotic residues in wild fish and sediments were detected from the intestine of fish and also a lot of isolated bacteria resistant to antibiotics. *Aeromonas salmonicida* causes furunculosis is also resistant to Romet-30, tetracycline, sulfa drugs, trimethoprim, and oxytetracycline Tribrissen (Paone, 2000). Spanggaard *et al.* (1993) reported that many bacteria in fresh water resistant to antibiotics in Denmark. Nygaard *et al.* (1992) also reported the use of oxytetracycline and oxolinic acid causing bacteria resistant to antibiotics. Furthermore Leano *et al.* (1999) reported that *Vibrio* spp. and *Aeromonas* spp. isolated from fish and shrimp were resistant to streptomycin but sensitive to oxolinic acid. Now a lot of bacteria are resistan to more than one antibiotic. *Vibrio harveyi* and *Vibrio* are the most frequent isolated (Peterson *et al.*, 2002; Tendencia and de la Pena, 2001). The objective of this experiment is to find the sources of antibiotics contaminant in the farm, because according to farmers they had stopped the antibiotics application, but the residue is still found in fish carcasses.

Materials and Methods

Sampling of water, mud and other biota

Water, mud and organism from suspected pond contained antibiotic residues were collected. A total of 100 mL of water was taken and then poured into a dark bottle, sealed and stored at room temperature, 100 g of mud was taken and put into wide-mouthed dark bottles, while other organisms used as the samples were stored at are used as the sample will be stored in the -80°C to avoid spoilage. Prior to the analysis, the biota is tawed at room temperature.

Antibiotics (Chloramphenicol)

Chloramphenicol was analyzed according to Thomson (1966) and Horwitz (1980). Briefly, cuticle and the head taken from 3 pieces of shrimp of each sample then weighted, crushed and mixed with 4 volumes of 0.1 M KH_2PO_4 buffer, pH 4.5 sterile. Half of the buffer is poured to the shrimp in the mixer, blended for 10 sec in a low speed and 10 sec in high speed. The other half is blended for 10 sec of low speed to avoid foaming. The mixture was then centrifuged in 50 mL tubes for 20 min at 10,000 rpm at 4ºC, removed the debris material. Supernatant was transferred into a clean, sterile tube and its volume is measured. Supernatant will be devided in two parts, not diluted and diluted with ratio of 1: 3 with 0.1 M KH_2PO_4 buffer, pH 4.5. Blended and then washed with a half volume of 0.1 M KH_2PO_4 buffer, pH 4.5 for 10 sec in low-speed, stored separately.

Standard curve prepared by using shrimp homogenate without using Chloramphenicol, but using 0.1 N to final concentration of oxytetracycline (OTC) by 0.1, 0.2, 0.4, 0.8 and 1.6 mg/mL OTC. A total of 0.4 mg/mL OTC is used as a control. Each of a total 18 wells for each standard curve and the 18 wells for reference. Zone size was measured and then compared with reference (control). A reference obtained from an average of 90 wells. Rectification zone size obtained from the increase or decrease the difference between the average sizes for the reference zone which same concentration and average size of the grand reference zone. Suspension of spores of *Bacillus cereus* (ATCC No. 11 778) prepared in antibiotic medium A (Nifco, Detroit Michigan) on agar plate. Aliquots (200 mL) was used to test the cylinder is placed in stainless steel, 6 cylinder for each plate, and 3 plates for each test. Nine wells of each shrimp homogenates prepared, as well as references there are 9 wells (0.4 g Chloramphenicol/mL). Zone of inhibition resulting from dilution of shrimp homogenates were then compared with the references above, the results were then calculated by dilution of the homogenate of shrimp that references a result close to the concentration. Total Chloramphenicol in shrimp (g) is calculated by multiplying the concentration of the tested suspension with total volume (mL) of supernatant. Chloramphenicol in the washing water (g) is added to the total homogenate to obtain the number of Chloramphenicol entirely. Amount is then divided by the total weight of shrimp meat to get g Chloramphenicol in shrimp meat.

Results and Discussion

Organic matter and excessive shrimp feed will dissolve in water and will accumulate in pond bottom. Excessive organic material will cause problems in the ecosystem of brackishwater pond ecosystem because the excessive of organic matter is the habitat of several pathogens bacteria and will spend the oxygen. Excessive feeding may cause the elevation of NH_3-N and NO_2 in the pond. Some plants and animals can absorb ammonia in the water; they can serve as a biological filter. Most farmers used biofilter such as mussels, milkfish, tilapia or mullet with density of 3 pieces of fish/m^2 and. *Vibrio* bacteria will thrive in water containing a high organic matter (Austin and Austin, 1988) as well as *Aeromona*s. Organic material causing plankton blooming and interfere growth of fish and shrimp. Water containing high organic matter needs to be reduced by draining the ponds. This pond functions as a reduction of organic matter from water conservancy shrimp because of abundant organic material to be used by plankton to grow and as the main meal of tilapia. Ammonia and some other gases can partially evaporate in the aerated pond. In addition, ~~to~~ the suspended matter will be deposited in these ponds. Biological filter is not able to remove the solids present in the water, but the point is to change harmful substances such as ammonia into harmless substances such as nitrate. For this purpose few bacteria such as *Nitrosomonas* sp. can be added to help the nitrification process in the water. The main biological filter is an autotrophic bacterium, although algae, yeast and protozoa and other small animals sometimes help turn waste from the body of water. Soil has an important role in shrimp aquaculture, and almost all type of land is suitable for shrimp farming. Soil contains high organic matter is not suitable for aquaculture due to the decomposition of organic matter requires a lot of oxygen. The results of the analysis use chromatography presented in the Table 1.

Table 1. showed that in Gresik ponds, mud, water and crabs as ponds organisme contains of antibiotics chloramphenicol. The crabs contains antibiotic much higher than in the mud and water. Initially antibiotic was used to prevent diseases caused by bacteria, such as luminous

Vibriosis shrimp culture. Antibiotics were administered via the feed (medicated feed). In other countries such as the Philippines, antibiotics used in shrimp farming are nitrofurans, erythromycin, and sulfa drugs (Baticados *et al.*, 1990; Primavera *et al.*, 1993) and several other drugs. Uncontrolled use of antibiotics will left the residue in fish and the environment. For example, in fish farming in British Columbia, Canada in 2000, residues of antibiotics in salmon flesh is 0.1 mg/L (Paone, 2000). This residue also found in several places in East Java with range from 1-2 mg/L. In dry season antibiotic concentration in some place is high.

Crab eats feed remaining and fish faeces may contains residues of oxytetracycline 0.8 and 3.8 mg/L. Bjorklund *et al*. (1990) reported that in Finland, although he did not perform measurements of antibiotic residues in wild fish, from sediments and intestine of fish but a lot of resistant antibiotics bacteria can be isolated. *Aeromonas salmonicida* causes furunculosis is also resistant to Romet-30, tetracycline, sulfa drugs, trimethoprim, and oxytetracycline Tribrissen (Paone, 2000). Spanggaard *et al.* (1993) reported that many bacteria in fresh water resistant to antibiotics in Denmark. Nygaard *et al*. (1992) also reported the use of oxytetracycline and oxolinic acid causes the bacteria resistant to other antibiotics. Table 2. Antibiotic analytical results from samples from the area Tuban June-October.

The antibiotic analytical results from samples from the area Tuban (Table 2.) showed that same as Gresik, mud, water and crabs may contains the chloramphenicol. Furthermore Leano *et al*. (1999) reported that *Vibrio* spp. and *Aeromonas* spp. isolated from fish and shrimp were resistant to streptomycin but sensitive to acid oxolinic. Now a lot of bacteria were resistant to more than one antibiotic. *Vibrio harveyi* and *Vibrio* spp. and other species are the most frequent isolated (Tendencia and de la Pena, 2001). The use of drug for cultivation in the USA should get permission from the FDA's Center for Veterinary Medicine. Drug generally used mixed in feed, and it supposed 75% the fish can use it and not mostly discharged into the environment. So the case like in ornamental fish not happened. Ornamental fish are closer to humans will transmit the bacteria that were resistant to more human. Indiscriminate use of antibiotics in Equador, which will cause outbreak of *Vibrio cholerae* in the USA. Which are resistant bacteria can be transmitted from fish to human. Table 3. Antibiotic analytical results from samples from the area Pasuruan June-October. Results of antibiotic analysis from Probolinggo (June-October) see Table 4.

Table 1. Results of antibiotic analysis from Gresik (June-October, 2011)

Sample	Sample quantity	Antibiotic concentration	Antibiotic in feed	Antibiotic
Mud	100 g	0.6 mg/L	1000 mg/kg	Chloramphenicol
Water	100 mL	0.3 mg/L	1000 mg/ kg	Chloramphenicol
Pond organism/crabs	2	1.2 mg/L	1000 mg/kg	Chloramphenicol

Table 2. Results of antibiotic analysis from Tuban (June-October, 2011)

Sample	Sample quantity	Antibiotic concentration	Antibiotic in feed	Antibiotic
Mud	100 g	1.8 mg/L	1000 mg/kg	Chloramphenicol
Water	100 mL	0.8 mg/L	1000 mg/ kg	Chloramphenicol
Pond organism/crabs	2	2.1 mg/L	1000 mg/kg	Chloramphenicol

Table 3. Results of antibiotic analysis from Pasuruan (June-October, 2011)

Sample	Sample quantity	Antibiotic concentration	Antibiotic in feed	Antibiotic
Mud	100 g	0.3 ppb	1000 mg/kg	Chloramphenicol
Water	100 mL	0.1 ppb	1000 mg/ kg	Chloramphenicol
Pond organism/crabs	2	0.6 ppb	1000 mg/kg	Chloramphenicol

Table 4. Results of antibiotic analysis from Probolinggo (June-October, 2011)

Sampel	Sample quantity	Antibiotic concentration	Antibiotic in feed	Antibiotic
Mud	100 g	0.3 ppb	1000 mg/kg	Chloramphenicol
Water	100 mL	0.2 ppb	1000 mg/kg	Chloramphenicol
Pond organism/crabs	2	0.6 ppb	1000 mg/kg	Chloramphenicol

Only 5 kind of drugs are legal in the USA are: oxytetracycline HCl (Terramycin 10), sulfamerazine, and drug combinations containing sulfadimethozine and ormetoprim (Romet-30). Although it found a problem if not used antibiotics in aquaculture National Seafood HACCP Alliance for Training and Education Compendium (USA) "growth" is one reason why farmer use of antibiotics. Antibiotics affect reproduction, growth and prevent disease. Total antibiotic that is used for the production of cattish USA in an estimated between 126,000-252,000 Lb. Antibiotic used for the treatment of enteric septicemia of Catfish (ESC). Although it had been using drugs but still 60% lost, whereas for salmon and trout with the use of antibiotics used 63,000 -104,000 Lb.

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