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RESEARCH ARTICLE

EFFECTIVENESS OF CIPROFLOXACIN AGAINST INFECTION OF AEROMONAS SALMONICIDA IN KOI FISH (*CYPRINUS CARPIO*)

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ABSTRACT

Koi fish (*Cyprinus carpio*) is one of the very potential types of ornamental fish in East Java and has a high economic value. *Aeromonas salmonicida* is a bacterium that includes Quarantine Fish Disease and has spread on the island of Java. Various antibiotics are available on the market for treatment of these bacterial infections, however, only commonly used certain antibiotics. Continuous use of an antibiotic can spur bacterial resistance. This study was conducted to test the effectiveness of ciprofloxacin in the treatment of *A. salmonicida* infection. Ciprofloxacin was chosen because it has the highest inhibitory value of *A. salmonicida* growth of 10 ppm with 11 ppm MBC value. At these doses up to 10 times the value of MIC, ciprofloxacin did not show a toxicity reaction in Koi fish. The research design used Complete Randomize Design with different ciprofloxacin doses, namely treatment A (11 ppm), B (16.5 ppm), C (22 ppm), D (27.5 ppm) and E (33 ppm) repeated 3 times. The results of data analysis with ANOVA showed significant differences between treatments ($p < 0.05$). Analysis continued with least Significant Difference and showed that the treatment of 11 ppm was significantly different from the treatment of 22 ppm, 27.5 ppm and 33 ppm. The treatment of 16.5 ppm was significantly different from the treatment of 22 ppm, 27.5 ppm and 33 ppm, while the treatment of 22 ppm, 27.5 ppm and 33 ppm was not significantly different, the dose of ciprofloxacin was effective and efficient or best for the treatment of *A. salmonicida* in Koi fish is 22 ppm.

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INTRODUCTION

Many pathogenic bacteria are endangered In the cultivation of koi. One of the gram negative bacteria that can cause death is *A. salmonicida*, which occurs in Japan, USA, England, Canada and European countries including France, Norway, Belgium, Austria and Switzerland (Austin and Austin, 1987). Reported (Bullock 1991 and Saroni et al., 1993) in Australia an outbreak of disease caused by *A. salmonicida* bacteria in Gold fish is known as ulcer diseases. In Indonesia *A. salmonicida* is a very important pathogen and included in the list of Quarantine Fish Pests and Diseases in the Decree of the Minister of Marine and Fisheries - No. : KEP. 26 / MEN-KP / 2013 and KEP. 80 and 81 / MEN-KP / 2015 that *Aeromonas salmonicida* is included in the list of group II Quarantine Fish Diseases. The area of spread is in Java, Sumatra, Bali, NTB, Kalimantan, Sulawesi and Maluku. The use of antibacterials with low doses continuously will cause residues in animals / hosts and can result in antibacterial resistance. Thus the use of high doses but have not been able to cure the infection in total must be replaced with new antibacterials (Tollefson et al., 1999; Williams, 2000).

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This study relates to one of the quarantine actions on fish treatment actions caused by infectious diseases, so the curative must be fast, precise and the fish are completely free from harmful diseases. Affirmed (Grant and Laidler, 1993; Hastings and Kay, 1987; Inglis and Richards, 1991; Oppegaard and Sorum, 1994; Tsoumas et al., 1986) that quinolone is used in emergencies. So from that the selection of antibacterial types of Ciprofloxacin is the right choice for treating fish infected with *A. salmonicida*.

Formulation of the problem: To what extent is the antibacterial sensitivity of the type Ciprofloxacin able to inhibit and kill bacteria, what dose is appropriate and effective for controlling *A. salmonicida* infection?

Research purposes: To determine the dosage of ciprofloxacin antibiotics which is appropriate and effective for controlling Koi fish (*Cyprinus carpio*) infected with *A. salmonicida* bacteria.

Benefits of research: The results of this study can be used as one of the proposed materials to remove the type of *A. salmonicida* bacteria from the Quarantine Fish Disease (HPIK)

list of group II types of bacteria on Minister of Agriculture Decree No. KEP. 26 / MEN-KP / 2013 and KEP. 80 and 81 / MEN-KP / 2015.

Hypothesis: It is suspected that there is a difference in the effect of the ciprofloxacin dose on the survival of Koi (*Cyprinus carpio*) infected with *A. salmonicida* bacteria

MATERIALS AND METHODS

Place and Test Time: 3 (three) months starting from January to March 2017. The study was conducted at the Bacteriology Laboratory for Preliminary Tests in Vitro at the Class I Surabaya Fish Quarantine Laboratory on Jl. Raya Ir. H. Juanda Sedati Sidoarjo, is currently undergoing an in Vivo test in the wet laboratory of the Fish Quarantine Center located in Jl. Pagesangan II No. 58 A Surabaya for 3 (three) months starting from January to March 2017.

Research Materials

- ***Aeromonas salmonicida*:** Pure *Aeromonas salmonicida* culture is obtained from the Jakarta Standard Test Center, as a fish quarantine reference laboratory throughout Indonesia.
- ***Koi Fish (Cyprinus carpio)*:** Koi fish (*Cyprinus carpio*) used in research, measuring between 5-7 cm. which is from one parent obtained from Tulungagung, East Java. Koi fish are first adapted for 1 (one) Sunday in a sufficiently aerated reservoir and fed pellets.
- ***Antibiotic*:** The antibiotics used in this study were generic Ciprofloxacin. Antibiotics are obtained from Kimia Farma Pharmacy - Surabaya.
- ***Culture Media and Test Media*:** The media used in bacterial culture are Tryptic Soy Broth (TSB) and sterile aquadest, Tryptic Soy Agar (TSA) and sterile biochemical conventional test media.
- ***Media of Culture*:** The maintenance media used came from filtered well water sources and aerated for 3 (three) days.

Research Equipment

Bacterial examination: autoclave, oven, laminary air flow, 1000x magnification microscope, petridish, micropipette size 0.001-0.01 ml; 0.5-1 ml, dissecting set, aluminum foil test tube, erlenmeyer, measuring cup, electric scale, incubator, object glass, aquarium, filter, refrigerator, glass fiber, Bunsen lamp, ose needle, 70% alcohol bottle, sample bottle .

Sensitivity Test of *A. salmonicida* Against Ciprofloxacin Antibiotics

- The sensitivity test using serial tube dilution method was carried out on the test tube using Ciprofloxacin with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ppm. With 3 (three) replications on the TSB media has been infected with 24-hour-old bacterium Anderson and Conroy (1969).
- Sensitivity test using the disc method was carried out with filter paper spheres soaked in Ciprofloxacin solution with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ppm. With 3 (three) replications. The TSA media which had been sprinkled with pure bacteria 24 hours of

age evenly with bent glass, put the disc from each of these concentrations and incubated at 27°C for 24 hours. Statement that zones formed from spheres / discs on TSA media indicate bacterial sensitivity to antibiotics (Anonymous, TT).

Ciprofloxacin Toxicity Test: To determine the toxicity of antibiotics for koi fish, a test is carried out by maintaining koi in an antibiotic solution. The antibiotic concentration used is 0 (Control), the MIC results up to 10 times. If up to the highest dose there are no dead koi, it is assumed that antibiotics are not harmful to fish and can be used for treatment.

Research Design (Main Test): The design in this study used a Completely Randomized Design (CRD). This design is used because the experimental medium is homogeneous, so that the effect of the research results is only treatment (Sastrosupadi, 1977). That is to find out the effect of antibiotics on the survival of Koi (*Cyprinus carpio*.) Fish which are infected with *A. salmonicida*. Treatment treatment in the main test consisted of 5 treatments, 2 controls with immersion method (long deeping) (Lewbart, 1997), for 3 days and given once a day at the same hour (Anonymous, TT) and observed every 6 hours in the aquarium. Details of treatment are as follows:

Treatment: Koi fish infected with *A. salmonicida*, after clinical symptoms arise, are given antibiotic treatment A 11 ppm. B 16.5 ppm. C 22 ppm. D 27.5 ppm. E 33 ppm for 3 (three) days. The number of replications is 3x for the determination of treatment and replication. The formula can be used as follows (Anonymous, 2002; Juknis, 2002): $(t - 1) (n - 1) > 12$ where: t = number of treatments, n = number of replications

Observation of Survival Rate

To get the survival rate in the study used the following formula;

$$SR = \frac{Nt}{No.} \times 100\% \text{ (Effendi, 1979)}$$

Where :

SR: Survival Rate

Nt: Number of End Individuals.

No: Number of Early Individuals

Data analysis: To find out the difference in average survival rate, an ANOVA (Analysis of Variance) test was carried out, followed by the LSD test (the smallest real difference) or post hoc to find out the treatment that had the best (efficient) and effective dose. Data analysis was carried out using the SPSS program

RESULTS AND DISCUSSION

Postulate Koch Bacteria *A. salmonicida*: The results of identification of Koch's Postulate test showed that bacterial cells were in the form of coccobacilli, gram negative, non motile. The results of the Postulate Koch test for *A. salmonicida* bacteria infected with healthy koi in freshwater, have been reported by Saron (1983) and have been investigated by Bullock (2001).

Table 1. Survival of Koi Fish until the End of Research.

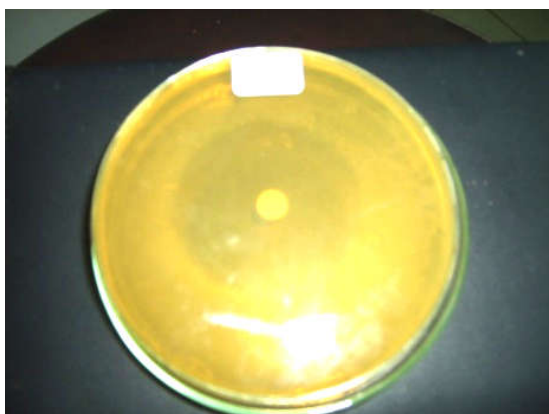
| DOSAGE (ppm) | Repeat | | | Amount | Average | SR (%) |
|--------------|--------|----|----|--------|---------|--------|
| | 1 | 2 | 3 | | | |
| 11 | 7 | 7 | 7 | 21 | 7,00 | 70,0 |
| 16,5 | 8 | 7 | 8 | 23 | 7,33 | 73,3 |
| 22 | 9 | 10 | 10 | 29 | 9,96 | 96,6 |
| 27,5 | 10 | 10 | 9 | 29 | 9,66 | 96,6 |
| 33 | 9 | 10 | 10 | 29 | 9,66 | 96,6 |
| K(-) | 10 | 9 | 10 | 29 | 9,66 | 96,6 |
| K(+) | 1 | 0 | 0 | 1 | 0,33 | 3,3 |

Table. 2 ANOVA Test Results

| SR | Sum of Squares | df | Mean Square | F | Sig. |
|----------------|----------------|----|-------------|--------|------|
| Between Groups | 2026.667 | 4 | 506.667 | 19.000 | .000 |
| Within Groups | 266.667 | 10 | 26.667 | | |
| Total | 2293.333 | 14 | | | |

Table 3. Smallest Significant Different Test Results (Post Hoc Tests)

| Treatment | N | Subset for alpha = 0.05 | |
|---------------|---|-------------------------|----------|
| | | 1 | 2 |
| dose 11 ppm | 3 | 70.0000a | |
| dose 16,5 ppm | 3 | 76.6667a | |
| dose 22 ppm | 3 | | 96.6667b |
| dose 27,5 ppm | 3 | | 96.6667b |
| dose 33 ppm | 3 | | 96.6667b |
| Sig. | | .145 | 1.000 |



The sensitivity of the Disc from Ciprofloxacin concentration

- MIC Sensitivity Test of Tube Dillution Method The invitro preliminary test results showed that the sensitivity level of *A. salmonicida* to ciprofloxacin antibiotics was at least 10 ppm. g ml⁻¹ ...μ 0.125 ≥ This is consistent with the results of the study (Giraud, 2004) in France in 1998-2000 which stated that the sensitivity of ciprofloxacin to *A. salmonicida* in Red fish in Vitro using the Polymerase Cain Reaction (PCR) method, the MIC value reached.
- MIC Sensitivity Test with Disc Method Sensitivity to ciprofloxacin antibiotics formed a diameter of the inhibition zone of ≥ 21 mm Koneman et. Al 1997 in Geraud, 2004. At a concentration of 10 ppm formed a resistance zone of 49 mm which stated that ciprofloxacin had a high sensitivity to bacteria *A. salmonicida* compared to zones formed above 21 (ciprofloxacin 5 μg). These results indicate that ciprofloxacin can inhibit the action of enzymes DNA gyrase bacteria *A. salmonicida*. so that the resistance zone formed is shown in the following figure 10 ppm.

Base on the picture above it can be explained that the bacteria *A. salmonicida* is sensitive to the antibiotic Ciprofloxacin by inhibiting the activity of DNA gyrase as an enzyme which plays a role in preventing the formation of supercoiling in bacterial cells that are carrying out DNA replication.

MBC Sensitivity Test Tube Dilution Method: The MBC test results showed that at concentrations of 6 to 10 ppm, *A. salmonicida* was still grown on TSA media. Starting from the concentration of 11 ppm the bacteria did not experience growth as it meant that at a concentration of 11 ppm Ciprofloxacin was able to kill *A. salmonicida* bacteria and subsequently used as a treatment dose in the in Vivo study, according to Yanong (2003) statement that administration of low and deep doses of antibiotics short time will not kill bacteria, but will increase bacterial resistance to antibiotics.

Ciprofloxacin Toxicity Test: The results of the toxicity test of ciprofloxacin starting from the dose of 10 ppm up to. 100 ppm does not cause death in the test host and has a high tolerance for the host. This is shown that the concentration of 100 ppm does not interfere with the life of koi fish so it is assumed that ciprofloxacin is not toxic so that the use of antibiotics at doses below 100 ppm, ie doses of 11 to 33 ppm in the study in Vivo is within the safe limits of control.

Survival Rate: The observation of the survival rate of Koi fish during the main test was compared between controls and all treatments with the design of dose variations, listed in the following table. Table 1 shows that in the control (+), Survival Rate (SR) 3.3% means that the attack power of the bacteria is quite malignant because it is capable of killing 97.7% within 6 days after infection with a dose of 10 7.sel / ml. There is a tendency for higher doses of ciprofloxacin, the average survival rate of koi fish is higher, starting from the dose of 22 ppm to 33 ppm experiencing SR 96.6%. The ANOVA results showed significantly different between treatments (P <0.05) as in Table 2. This shows that the activity of ciprofloxacin is very influential on the bacteria *A. salmonicida* with a density of 10 7.sel / ml. This result is evidenced by the Smallest Significant Different Test obtained from the interpolation (Post Test Tests) of the Social Science Program Statistic which is made in the form of notations, listed in Table 3 below. From the table above it can be seen that the treatment of 11 ppm and 16.5 ppm is not significantly different. This proves that the use of ciprofloxacin in the range of 11 to 16.5 ppm has not been able to completely kill the bacterium *A. salmonicida* in Koi, but it is very significantly different at doses of 22 ppm, 27.5 ppm and 33 ppm. This proves that the ciprofloxacin dose of 22 ppm, 27.5 and 33 ppm has been able to kill all bacteria in the body of koi fish and live fish media. The dosage treatment of 22 ppm to 33 ppm was not significantly different. This shows that in this dosage range ciprofloxacin has the same ability to control *A. salmonicida* in koi fish so that the ciprofloxacin dose of 22 ppm is the best or effective and efficient dose that can be used as an antibiotic controlling *A. salmonicida* bacteria.

Conclusion and Recommendation

Conclusion

From the results of the study it can be concluded that ciprofloxacin antibiotics can inhibit and kill *A. salmonicida* in Koi fish at a dose of 22 ppm, for 3 days and given once a day.

Suggestion

Given that in the study of ciprofloxacin antibiotics given to Koi ornamental fish, it is necessary to try other types of ornamental fish. Likewise, it can be tried to the non-consumption fish or brood fish. Control in the future should be considered biologically and integrated therapy.

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