

# Resistance Test of *Vibrio sp.* From Milkfish (*Chanos chanos*) in Fishpond Jabon Sidoarjo to Heavy Metals and Antibiotics

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**Keywords:** *Vibrio sp.*, Resistance test, Lead (Pb), Cadmium (Cd), Tetracycline antibiotic, Chloramphenicol antibiotic.

**Abstract:** The milkfish (*Chanos chanos*) obtained from Fishponds Jabon Sidoarjo contained heavy metals such as Pb and Cd. Heavy metals pollution can be controlled by bioremediation. The objective of this research is to determine the resistance of *Vibrio sp.* isolated from milkfish to heavy metals Pb, Cd, at various concentrations and to know the resistance of these bacteria to antibiotics such as tetracycline and chloramphenicol. Resistance tests of *Vibrio sp.* were performed using dilution and pour plate method. The Nutrient agar media used in this study contained heavy metals PbCl<sub>2</sub> and CdCl<sub>2</sub> with concentrations of 5 ppm, 10 ppm, 25 ppm. Heavy metals-resistant *Vibrio sp.* was tested its antibiotic resistance with diffusion method using antibiotic disks such as Tetracycline 30 µg and Chloramphenicol 30 µg. The results showed that *Vibrio sp.* was resistant to heavy metals Pb and Cd at all concentrations. Antibiotic sensitivity test using chloramphenicol 30 µg showed that *Vibrio sp.* was resistant to chloramphenicol at concentration of 5ppm. Pb-resistant *Vibrio sp.* was shown resistant to chloramphenicol at concentration of 10 ppm and 25 ppm. Pb- and Cd-resistant *Vibrio sp.* were shown resistant to Tetracycline 30 µg at all concentrations.

## 1 INTRODUCTION

In 2014, there were approximately 79% rivers with status of slight, moderate, and severe pollution, while in 2015 there was increasing number of polluted rivers. One of polluted rivers in East Java was Porong River in Kabupaten Sidoarjo due to disposal of lapindo mud by Lapindo Brantas Inc. This led to reduction of ecosystem caused by heavy metals contamination such as Lead (Pb) and Cadmium (Cd).

Kholidiyah (2010) stated that concentrations of heavy metals in the water of Porong river and lapindo mud exceeded the standard level, showing Lead (Pb) at concentration of 7,2876 mg/L in lapindo mud and 0,6949 mg/L in the water of Porong river, whereas Cadmium (Cd) contained in lapindo mud were at concentration of 0,3063 mg/L and of 0,0271 mg/L in the water of Porong river. This caused pollution of nearby fishponds.

Both Lead (Pb) and Cadmium (Cd) are heavy metals that can accumulate inside the body of living creatures especially fishes in water contaminated with heavy metals, potentially become toxic. If those fishes are consumed by human, toxic will

accumulate inside tissues of human body and endanger their health (Nuhman, 2003) One of the known contaminated fishes is Bandeng or Milkfish (*Chanos chanos*), a brackish water fish commonly bred in fishponds.

Larasati (2015) in her research stated that milkfish bred in fishpond Jabon Sidoarjo located at 5 km, 10 km, and 15 km away from lapindo mud showed average concentration level of Pb of 1,0985 mg/kg, 1,3408 mg/kg, and 1,0839 mg/kg. Based on those results it could be concluded that milkfish was contaminated with Pb at concentration exceeding standard of heavy metal concentration according to SNI 7387:2009 which is 0,3 mg/kg. Screening test to detect heavy metals contained in milkfish living in fishpond Jabon Sidoarjo (2017) showed the result as much as 4,82 ppm and 6,11 ppm of Pb, while Cd was as much as 2,63 ppm and 3,24 ppm.

One of methods to overcome contamination caused by heavy metals is by bioremediation, a technique using certain microorganisms to eliminate effect of contaminants (toxic substances), in order to reduce contaminating substances (Sitana, dkk., 2008). Microorganisms which can be used in bioremediation are those resistant to heavy metals.

After screening test of bacterial isolation from milkfish (2017) was performed, the result showed that *Vibrio* sp was contained inside the milkfish. *Vibrio* sp, according to Utomo (2016) is indigenous bacteria naturally found in fish.

Ayu (2013) stated that if certain microorganisms contain any substances resistant to heavy metals and antibiotics, they will negatively affect to human's health as treatment failure. Therefore, it is necessary to perform resistance test using antibiotics, a chemical substance produced by certain microorganisms that inhibit growth or kill other microorganisms. (Simangunsong, *et al.*, 2015). Antibiotics often used in medical treatment are tetracycline with wide spectrum for certain indication from bacteria (BPOM, 2015) and chloramphenicol which is a preferred antibiotic for treating infection of gram-positive and gram-negative bacteria (Uddin, *et al.*, 2016).

Based on facts above, It was necessary to perform a study about resistance of *Vibrio* sp isolated from Milkfish (*Chanos chanos*) in fishpond Jabon Sidoarjo to heavy metals such as Lead (Pb) and Cadmium (Cd) and to antibiotics such as Tetracycline and Chloramphenicol.

The aim of this study was to identify the resistance of *Vibrio* sp to Pb and Cd at concentrations of 5 ppm, 10 ppm and 25 ppm, and to identify the resistance of heavy metals-resistant *Vibrio* sp to Tetracycline and Chloramphenicol.

## 2 RESEARCH METHOD

### 2.1 Type of Research

This study used explorative descriptive method.

### 2.2 Place and Time

This study took place in Microbiology Laboratory of study program of Medical Laboratory Technology, Health Polytechnic Surabaya starting from January until June 2017.

### 2.3 Tools and Materials

Tools used in this study were autoclave, incubator, inoculating loop, spirit burner, laboratory tripod, erlenmeyer flask, petri dish, laboratory cottons, analytical balance, glass stirring rod, sterile alcohol swab, pH-indicator papers, graduated cylinder, laboratory funnel, tweezers, rubbers, aluminium foil, volumetric flask, beaker glass, and watch glass.

Materials used in this study were isolates of *Vibrio* sp from milkfish, NA (*Nutrient Agar*) media, PbCl<sub>2</sub>, CdCl<sub>2</sub>, NB (*Nutrient Broth*) media, MH (*Mueller Hinton Agar*) media, *Mc Farland* standards, antibiotic sensitivity disks Tetracycline 30 µg and Chloramphenicol 30 µg.

## 2.4 Procedures

### 2.4.1 Isolation of Bacteria from Milkfish

Milkfish firstly was cleaned from bones and mashed. Serial dilution of fish samples then was performed using saline solution to create dilution of 10<sup>-3</sup>. The diluted samples were inoculated on *Mac Conkey Agar* and incubated on 37 °C for 24 hours. Colonies grown on *Mac Conkey Agar* were picked off and inoculated on *Eosin Methylene Blue Agar* media, incubated on 37 °C for 24 hours. Colonies grown on EMB media were inoculated on Triple Sugar Iron Agar media, incubated on 37 °C for 24 hours. Colonies on TSIA media were further identified using IMViC test (Indol, *Methyl Red*, *Voges Proskauer* and Citrate) and Semi solid.

### 2.4.2 Resistance Test to Heavy Metals

Isolates of *Vibrio* sp were diluted until 10<sup>-5</sup> then poured into NA-PbCl<sub>2</sub> and NA-CdCl<sub>2</sub> media with concentrations used were 5 ppm, 10 ppm and 25 ppm. In addition, NA-PbCl<sub>2</sub> and NA-CdCl<sub>2</sub> media were used as negative controls in the absence of *Vibrio* sp isolates. After 24 hours of incubation, the grown isolates were shown to be resistant to Pb and Cd, and negative controls media. Then, CFU (Colony Forming Unit) was determined by comparing the number of colony in media test and that of negative control media.

### 2.4.3 Resistance test to Antibiotics

Resistance tests of *Vibrio* sp to antibiotics were performed by inoculating heavy metals-resistant bacteria on MH (*Mueller Hinton*) media, putting tetracycline disk and chloramphenicol using sterile tweezers on the surface of media inoculated with bacteria, and incubating those media for 24 hours. Transparent zones were measured and results were interpreted.

### 3 RESULT

#### 3.1 Resistance Test to Heavy Metals

Resistance tests of *Vibrio sp* to heavy metals were performed by determining the viability of Pb- and Cd-resistant *Vibrio sp*. The CFU/ml of *Vibrio sp* isolates treated with Pb (Table 1) showed constant number at all concentrations, while the CFU/ml of *Vibrio sp* isolates treated with Cd showed certain decline at concentration of 5 ppm, 10 ppm and 25 ppm (Table 1).

Isolates of *Vibrio sp* from milkfish were able to grow on Pb- and Cd- containing media with concentration of 5 ppm, 10 ppm, and 25 ppm (Figure 1). According to Zulaika *et.al* (2012), bacteria isolated from heavy metals-contaminated environment were resistant to surrounding heavy metals.

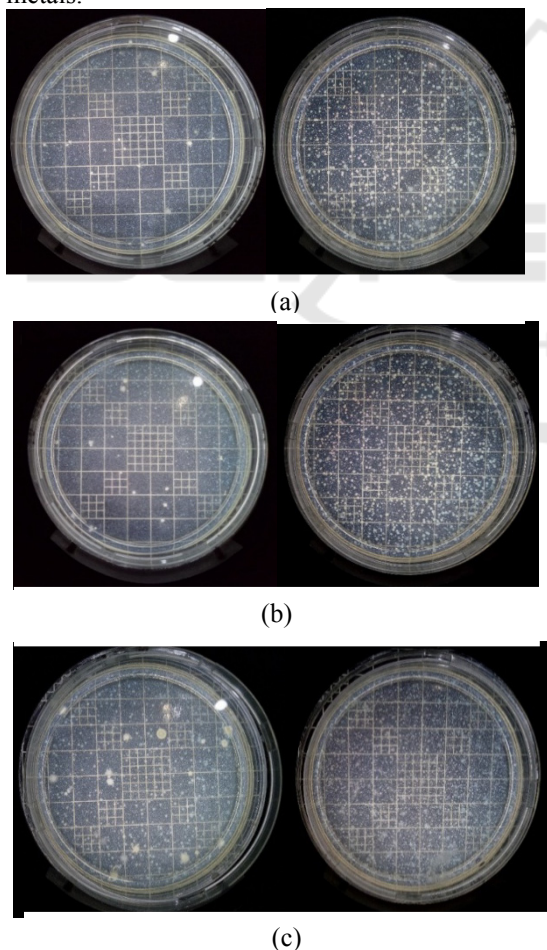


Figure 1: Result of inoculation of *Vibrio sp* to Pb and Cd with concentration of 5 ppm (a), 10 ppm (b), 25 ppm (c).

Table 1: Result of resistance test of *Vibrio sp* to Pb and Cd.

Concentrations of heavy metals	Pb	Cd
	CFU/ml	
5 ppm	13,55 x 10 <sup>6</sup>	30,0 x 10 <sup>6</sup>
10 ppm	13,3 x 10 <sup>6</sup>	16,55 x 10 <sup>6</sup>
25 ppm	13,7 x 10 <sup>6</sup>	12,2 x 10 <sup>6</sup>

#### 3.2 Antibiotic Sensitivity Test

Result of antibiotic sensitivity test shown in Figure 2 and Table 2 showed that Pb-resistant *Vibrio sp* was resistant to Tetracycline 30 µg at all concentrations of heavy metals and was resistant to Chloramphenicol at Pb concentration of 10 ppm and 25 ppm. Meanwhile, Pb-resistant *Vibrio sp* was susceptible to Chloramphenicol 30 µg at Pb concentration of 5 ppm.

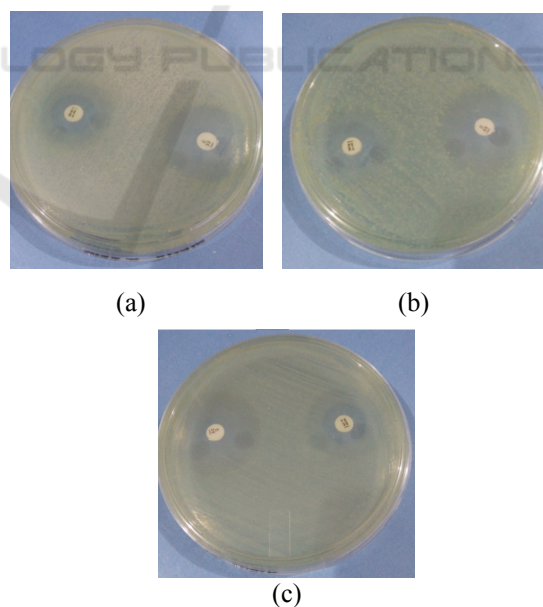


Figure 2: Result of antibiotic sensitivity test of Pb-resistant *Vibrio sp*.

Table 2: Result of antimicrobial resistance test of Pb-resistant *Vibrio* sp.

Pb	Inhibition zone diameter of Tetracycline 30 µg	Inhibition zone diameter of Chloramphenicol 30 µg
5 ppm	17 mm, resistant	29 mm, susceptible
10 ppm	18 mm, resistant	16 mm, resistant
25 ppm	16 mm, resistant	15 mm, resistant

Antibiotic sensitivity test of Cd-resistant *Vibrio* sp to Tetracycline 30 µg and Chloramphenicol 30 µg using Cd at concentration of 5 ppm, 10 ppm, and 25 ppm showed that Cd-resistant *Vibrio* sp was resistant to Tetracycline 30 µg and Chloramphenicol 30 µg at all concentrations of Cd (Table 3).

Table 3: Result of antibiotic sensitivity of Cd-resistant *Vibrio* sp.

Cd	Inhibition zone diameter of Tetracycline 30 µg	Inhibition zone diameter of Chloramphenicol 30 µg
5 ppm	6 mm, resistant	17 mm, susceptible
10 ppm	6 mm, resistant	15 mm, resistant
25 ppm	6 mm, resistant	15 mm, resistant

## 4 DISCUSSIONS

### 4.1 Resistance Test to Heavy Metals

*Vibrio* sp was able to grow in such condition by a mechanism called biotransformation. Biotransformation is a process of enzyme production possessed by microorganisms through a chemical alteration of pollutants in order to modify toxic pollutants. This process will lead to biodegradation process which is the ability of microorganisms to cleave chemical structure of toxic pollutants into noncomplex substances with low toxicity (Perdana, 2012). Biodegradation is classified into two types, namely extracellular and intracellular. Extracellular mechanism is the ability of bacteria to detoxify the effect of heavy metals with the availability of polyphosphate precipitates or by forming nonspecific bond with extracellular polysaccharides or natural polymers in the cell wall whereas intracellular mechanism of heavy metals is unactivated through precipitation by polyphosphate, binding to Metallothionein (MT), and efflux system (Jaroslawiecka and Seget, 2014).

### 4.2 Antibiotic Sensitivity Test

Based on the results above, Pb and Cd-resistant *Vibrio* sp were all resistant to Tetracycline 30 µg, shown by inhibition zone diameters less than 18 mm. According to Byarugaba (2009) resistance can naturally occur because microorganisms are not easily affected by antibiotics. This statement was supported by Johnson *et.al* (2011) stating that mechanisms of antimicrobial are classified into two, namely nongenetic mechanism caused by loss of specific targeted structures and genetic mechanism caused by chromosomal/ extrachromosomal resistance in bacteria.

In addition to Tetracycline, chloramphenicol 30 µg was also used for antibiotic sensitivity test. Based on the results above, it was found that Pb-resistant *Vibrio* sp was resistant to Chloramphenicol at concentration of 10 and 25 ppm and was resistant to Cd at all concentrations, shown by inhibition zone diameters less than 19 mm. In contrast, Pb-resistant *Vibrio* sp was susceptible to Chloramphenicol at concentration of 5 ppm, shown by inhibition zone diameters more than 19 mm. This was caused by the wide-spectrum ability of antibiotics to effectively inhibit growth of gram-negative bacteria by blocking the activity of enzyme peptidyl transferase. This



enzyme plays a role in the process of forming peptide bonds between new amino acids attached to tRNA and the last amino acids which are still developing in the bacteria. As the result, protein synthesis in bacteria will be completely interrupted (Pratiwi, 2008).

Gram-negative bacteria are more resistant to drugs compared to gram-positive bacteria due to efflux system possessed by gram-negative bacteria. This system allows accumulated drugs inside cells to be carried out, enabling drug concentration to be reduced. The antibiotic sensitivity test showed that bacteria can modify themselves to reduce effectivity of certain drugs. As the result, the bacteria can survive living and reproduce themselves to be more endangering (Dwyana and Fahrudin, 2012).

## 5 CONCLUSIONS AND SUGGESTIONS

### 5.1 Conclusions

According to study above, it could be concluded that:

1. *Vibrio sp* was resistant to heavy metals such as Pb and Cd at concentration of 5 ppm, 10 ppm, and 25 ppm, suggesting that the bacteria could survive living in the water regardless the availability of Pb dan Cd.
2. Antibiotic sensitivity test showed that Pb-resistant *Vibrio sp* was resistant to Tetracycline 30 µg at all concentrations but susceptible only to Chloramphenicol 30 µg at concentration of 5 ppm. Antibiotic sensitivity test showed that Cd-resistant *Vibrio sp* was resistant to Tetracycline 30 µg and Chloramphenicol 30 µg at all concentrations.

### 5.2 Suggestions

1. Society should not excessively consume milkfish due to side effect of heavy metals contained in water where it lives and due to bacteria in it which are resistant to heavy metals.
2. We suggest that the next researchers perform a study using Pb and Cd with higher concentration and do resistance test using different strains of bacteria, heavy metals, and antibiotics.

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