J. Mar. Biotechnol. Immunol
Vol. 1 No. 1 (2023) e-ISSN:xxxx-xxxx

Journal of Marine Biotechnology and Immunology

Journal homepage : https://ejournal.immunolmarbiotech.com

Susceptibility of *Vibrio* spp. from Gill of Barramundi (*Lates calcarifer*) Towards Antibiotics

Eny Heriyati*[1], Prita Lintang Larasati[2]

[1]Marine Science Study Program, Kutai Timur Agricultural College Jl. Soekarno Hatta No 02, East Kalimantan, 75387, Indonesia
[2]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro, Jl Prof. Sudharto, Tembalang, Semarang 50275, Indonesia

OPEN ACCESS

Article Info

Received: September 01, 2023
Accepted: September 20, 2023
Published: September 23, 2023
Available online: September 25, 2023

Keywords:

Antibiotics
Sensitivity
Susceptibility
Vibrio spp.

*Corresponding Author email: enystiper70@gmail.com

Abstract

Vibrio spp. bacteria are aquatic bacteria that can be found in brackish water and estuaries, and they have opportunistic pathogenic properties. These bacteria are usually dominant in brackish and estuarine areas such as river mouths, rivers, ponds, and seas with salinities between 20-40 ppt and a temperature of 37°C. The aim of this research is to determine the susceptibility and sensitivity of *Vibrio* spp. bacteria to various types of antibiotics. The *Vibrio* spp. bacteria isolated in this study were obtained from barramundi fish (*Lates calcarifer*). If they are still sensitive, the growth of *Vibrio* spp. bacteria can be inhibited by antibiotic administration. The effectiveness of antibiotics against *Vibrio* spp. bacteria in barramundi fish was tested using eight types of antibiotics, namely Co-Amoxiclav, Gentamicin, Doxycycline, Tetracycline, Azithromycin, Chloramphenicol, Ciprofloxacin, and Ampicillin. Bacteria that are sensitive to specific types of antibiotics have larger inhibition zones. The measurement of inhibition zones was carried out using a caliper by measuring the vertical and horizontal diameters and then dividing by 2 to obtain the average. This average was compared to a standard interpretative zone diameter chart to determine antibiotic sensitivity and resistance status using the Disk Diffusion method. Tetracycline and Ampicillin antibiotics can inhibit or are resistant to *Vibrio* spp. bacteria with a percentage of 0.08%. Gentamicin has greater sensitivity compared to the other tested antibiotics. In this study, the *Vibrio* spp. obtained are still sensitive to certain types of antibiotics and resistant to Ampicillin.

1. Introduction

Indonesia is a maritime country with abundant fisheries and marine resources that must be preserved and conserved. Barramundi is one of the commodities in the aquaculture sector in Indonesia. Specifically, the cultivation of barramundi, especially the white barramundi (*Lates calcarifer*), has been widely developed in Indonesia for commercial purposes (Santika *et al*., 2021). Barramundi grows relatively fast, with a harvest time ranging from 6 to 24 months. It is easy to cultivate and tolerant to high salinity levels (Zaenuddin *et al*., 2019). Barramundi from the aquaculture sector holds high economic value, as evidenced by prices ranging from 75,000 to 80,000 IDR per kilogram at the producer level in Teluk Lampung (Yaqin *et al*., 2018).

However, barramundi aquaculture often faces various challenges, including issues related to feed, water quality, and diseases (Jaya *et al*., 2013). Vibriosis is one of the diseases that frequently infect barramundi, particularly white barramundi. This disease is typically caused by bacteria from the *Vibrio* genus and can lead to mass mortality in white barramundi. Antibiotics and vaccination are common strategies to mitigate the impact of Vibriosis (Zaenuddin *et al*., 2019).

Vibrio spp. bacteria are opportunistic pathogens and aquatic bacteria. They are found in estuarine and brackish water environments, with optimal growth occurring at salinities between 20-40 ppt and a temperature of 37°C, particularly in brackish and seawater. These bacteria can associate with marine and freshwater animals (Ihsan and Retnaningrum, 2017). Antibiotics can be used to inhibit the growth of *Vibrio* spp. bacteria, as they are quite effective against these pathogens. However, over time, bacteria can develop resistance to antibiotics, which is why it is necessary to test their resistance/sensitivity to antibiotics. The aim of this research is to determine which antibiotics can control the growth of *Vibrio* spp. bacteria and to assess the susceptibility of *Vibrio* spp. bacteria from barramundi gills to different types of antibiotics.

2. Material and methods

2.1 Collection of *Vibrio* spp. Isolates

The *Vibrio* spp. isolates used in this study were obtained from the Laboratory of Biology, Faculty of Fisheries and Marine Sciences, Diponegoro University. The *L. calcarifer* fish used in the study were sourced from aquaculture at the Marine Science Technopark, Diponegoro University, Jepara, Faculty of Fisheries and Marine Science, Diponegoro University, Jepara, Indonesia.

2.2 Solid Agar Medium Preparation Method

This research utilized several types of media, one of which was the solid agar medium for *Vibrio* spp. samples from TR1 10^{-6} gills. The preparation of the solid agar medium involved dissolving 1.3 grams of nutrient broth (NB) and 1.5 grams of agar in 100 mL of distilled water in an Erlenmeyer flask, and this process was repeated three times. The media was homogenized using a hot plate stirrer and a magnetic stirrer, then transferred to vials in 5 mL aliquots. Once the media had cooled down, it was covered with cotton and then wrapped in aluminum foil. The vials were sterilized in an autoclave for 15 to 20 minutes. After autoclaving, the media was allowed to solidify while being tilted on supports (making sure the media does not touch the cotton).

2.3 Morphological Identification of *Vibrio* spp. Samples from TR1 10^{-6} Gills

Vibrio spp. samples were isolated from the gills of infected barramundi and cultured on TCBSA medium containing 88 g/L of TCBS agar, 13 g/L of nutrient broth, and 28 g/L of nutrient agar. The isolated *Vibrio* spp. samples were then incubated in an incubator at a temperature of 38°C. The TR1 10^{-6} gill samples, after incubation, were observed by examining the bacteria from a petri dish with the aid of a flashlight. They were identified morphologically by observing the shape and color of the colonies. If the morphology was known, colonies with the same shape and color were counted (note that if there were many colonies, they did not need to be counted, and if there were few colonies, they needed to be counted). These colonies were marked with a marker to avoid recounting.

2.4 Preparation of Liquid and Solid *Vibrio* spp. Media, Isolation, and Purification of TR1 10^{-6} Gill

Vibrio spp. Samples Liquid media were prepared using 1.3 grams of nutrient broth and 2.5 grams of NaCl dissolved in 100 mL of distilled water in an Erlenmeyer flask. The media were homogenized using a hot plate stirrer and a magnetic stirrer, then transferred to 5 mL vials once they had cooled down. The vials were sealed with cotton and covered with aluminum foil. Subsequently, the vials were sterilized in an autoclave for 15 to 20 minutes and allowed to cool.

Solid media were prepared using 1.5 grams of Agar, 2.5 grams of NaCl, and 1.3 grams of Nutrient Broth (NB) dissolved in 100 mL of distilled water in an Erlenmeyer flask. The media were homogenized using a hot plate stirrer and a magnetic stirrer, then poured into petri dishes as needed and allowed to solidify. The solid media were then wrapped and stored in a refrigerator in an inverted position.

2.5 Isolation, Cultivation, and Purification of TR1 10^{-6} Gill

Vibrio spp. Samples The *Vibrio* spp. samples from TR1 110^{-6} gills, which had been morphologically identified and had their colonies counted, were collected using a sterilized needle heated with a Bunsen burner. The needle, containing *Vibrio* spp. scrapings from the petri dish, was then streaked in a zigzag pattern onto solid agar media. The vials were sealed with cotton and labeled accordingly. These vials were incubated in a container box that had been sprayed with alcohol.

The isolated *Vibrio* spp. bacteria from TR1 10^{-6} gills were then incubated at 38°C and morphologically identified. Subsequently, they were purified from solid media to liquid media that had been prepared. *Vibrio* spp. bacteria were collected horizontally using a sterilized loop from the agar media that had grown previously. The vials were sealed with cotton and covered with aluminum foil. These steps were performed using aseptic techniques. The labeled vials were incubated in a container box that had been sprayed with alcohol.

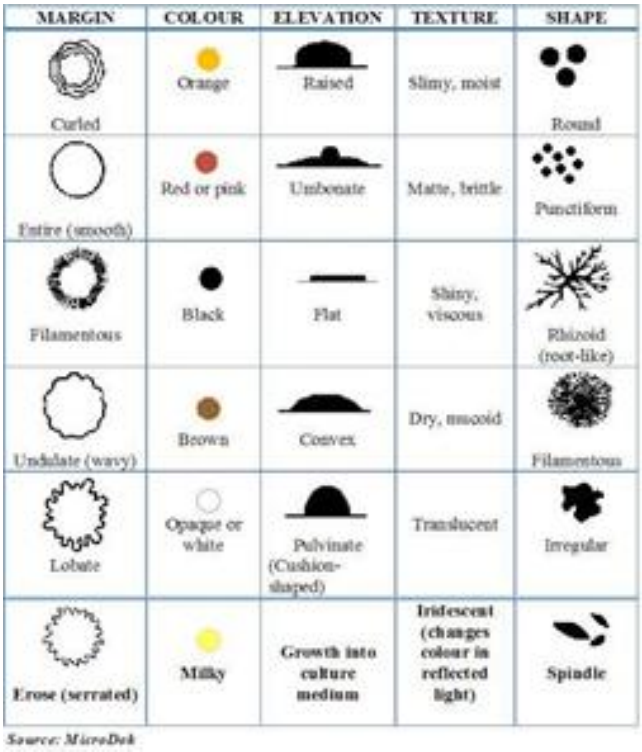

Figure 1. Characterization of Bacteria (Microbiology Class, 2020).

2.6 Testing the Effectiveness of Antibiotic Resistance in *Vibrio* spp.

The effectiveness of antibiotic resistance by *Vibrio* spp. bacteria was assessed by preparing a saline solution for McFarland of *Vibrio* spp. bacteria. This solution was prepared by dissolving 1.7 grams of NaCl in 200 mL of distilled water in an Erlenmeyer flask, which was then homogenized. The homogenized saline solution was poured into 26 vials, each containing 5 mL, and sterilized using an autoclave. McFarland 0.5 standardization was carried out after preparing the McFarland saline solution. This was done by transferring *Vibrio* spp. bacteria from the purification into the vials containing the saline solution until they reached the turbidity level specified in the McFarland 0.5 standard. Standardization was performed for 26 vials.

For this antibiotic resistance test, stock antibiotic solutions were prepared first. The stock antibiotic solutions were prepared by sterilizing vials with an autoclave and then adding 10 mL of 96% ethanol to each vial. The antibiotics used in the antibiotic resistance test for *Vibrio* spp. bacteria were as follows: 500 mg of Ciprofloxacin, 100 mg of Doxycycline, 500 mg of Tetracycline, 250 mg of Chloramphenicol, 500 mg of Ampicillin, 125 mg of Co-Amoxiclav, and 500 mg of Azithromycin. The antibiotics were added to the vials containing 10 mL of 96% ethanol (except for Ampicillin, which was dissolved in 10 mL of distilled water) and homogenized. The vials were then wrapped. After preparing the stock antibiotic solutions, dilution was performed.

Dilution of the antibiotics required small vials containing sterile distilled water, which were sterilized. After sterilizing the distilled water, each antibiotic was diluted with the sterilized distilled water as the solvent. The antibiotic dilution was done with the following calculations: 10 µL of Ampicillin + 990 µL of sterile distilled water, 500 µL of Gentamicin + 500 µL of sterile distilled water, 150 µL of Doxycycline + 850 µL of sterile distilled water, 30 µL of Tetracycline + 970 µL of sterile distilled water, 60 µL of Chloramphenicol + 940 µL of sterile distilled water, 5 µL of Ciprofloxacin + 995 µL of sterile distilled water, 80 µL of Co-Amoxiclav + 920 µL of sterile distilled water.

This antibiotic resistance test also required the addition of antibiotics to paper disks, which were then placed on media that had already been cultured with *Vibrio* spp. bacteria. Thirteen paper disks were needed in one petri dish, with each antibiotic requiring two petri dishes, resulting in 13 paper disks per dish. The antibiotics that had been previously diluted were added in 20 µL aliquots to each paper disk and allowed to dry but not excessively (they should remain slightly moist). *Vibrio* spp. bacteria were planted by transferring 100 µL of bacteria that had reached the McFarland standard into the solid media in the petri dish. The bacteria were then spread evenly with a spreader, and the petri dish was allowed to dry until the bacterial liquid was absorbed into the media. The previously dried paper disks were then placed on the solid media containing *Vibrio* spp. bacteria, according to the antibiotic label, using sterilized forceps. The media with the paper disks was wrapped and incubated at 38°C.

The antibiotics used had different concentrations. Based on the statement by Sarker *et al*. (2014), the concentrations of the antibiotics were as follows: Ampicillin 30 µg/disk, Ciprofloxacin 15 µg/disk, Doxycycline 30 µg/disk, Tetracycline 30 µg/disk, Chloramphenicol 30 µg/disk, Co-Amoxiclav 10 µg/disk, Azithromycin 15 µg/disk, and Gentamicin 10 µg/disk.

2.7 Preparation of Liquid Media and Purification of *Vibrio* spp.

Bacteria from Solid Agar to Liquid Media The preparation of liquid media began with the setup of the required equipment and materials. The weighed ingredients were 1.3 grams of Nutrient Broth and 1.5 grams of NaCl, which were then added to an Erlenmeyer flask along with 100 ml of distilled water. A magnetic stirring bar was placed inside the Erlenmeyer flask, and it was homogenized on a hot plate magnetic stirrer. Once all the ingredients were well homogenized, the Erlenmeyer flask was removed from the hot plate magnetic stirrer and allowed to cool slightly. Then, the liquid media was transferred into vials, with each vial containing 5 ml. The vials filled with liquid media were sealed with cotton and aluminum foil, followed by sterilization using an autoclave.

Vibrio bacteria that had grown in test tubes were streaked with a sterilized needle. The needle was sterilized using a Bunsen burner until it became red-hot. After the needle had cooled down, it was streaked across the bacteria on the solid agar medium. The needle, now containing the bacterial streaks, was then dissolved in the liquid media inside a vial. The vial was sealed with cotton in an aseptic manner, wrapped with plastic wrap, labeled according to the sample, and incubated in a sterile container.

2.8 Preparation of Solid Media

The preparation of solid media began with the setup of the required equipment and materials. The weighed ingredients were 1.3 grams of Nutrient Broth and 1.5 grams Agar, which were then added to an Erlenmeyer flask along with 100 ml of distilled water. A magnetic stirring bar was placed inside the Erlenmeyer flask, and it was homogenized on a hot plate magnetic stirrer. Once the media had foamed, the Erlenmeyer flask was removed from the hot plate magnetic stirrer and covered with cotton and aluminum foil. The media in the Erlenmeyer flask was then sterilized using an autoclave. After sterilization, the media was allowed to cool slightly and then poured aseptically into petri dishes. The petri dishes were sealed with plastic wrap and covered with plastic secured with rubber bands. The dishes were stored in separate sterile containers to avoid contamination.

2.9 Antibiotic Resistance Testing of *Vibrio* spp. Bacteria

2.9.1 Preparation of McFarland 0.5 Standard Solution and Standardization of *Vibrio* spp. Bacteria with McFarland Standard Solution

The preparation of McFarland 0.5 standard solution began with the setup of the required equipment and materials. NaCl weighing 0.85 grams was added to an Erlenmeyer flask along with 100 ml of distilled water. A magnetic stirring bar was placed inside the Erlenmeyer flask, and it was homogenized on a hot plate magnetic stirrer. Once all the ingredients were well homogenized, the Erlenmeyer flask was removed from the hot plate magnetic stirrer and allowed to cool slightly. Then, the McFarland standard solution was transferred into vials, with each vial containing 5 ml. The vials filled with the standard solution were sealed with cotton and aluminum foil, followed by sterilization using an autoclave.

The standardization of *Vibrio* spp. bacteria was done by adding *Vibrio* spp. bacteria from the liquid media purification into vials containing the McFarland standard solution using a micropipette in an aseptic manner until reaching the turbidity level according to McFarland 0.5 standard. Vials that reached McFarland 0.5 standard were sealed using plastic wrap, labeled according to the sample, and stored in a sterile box.

2.9.2 Preparation of Stock and Dilution of Antibiotics

Before starting the preparation of antibiotic stock, 20 ml vial bottles and their caps were sterilized using an autoclave. Seven types of antibiotics, namely Ciprofloxacin 500mg, Doxycycline 100mg, Tetracycline 500mg, Chloramphenicol 250mg, Ampicillin 500mg, Co Amoxiclav 125mg, and Azithromycin 500mg, were ground into fine powder using a mortar and pestle. Each antibiotic was then placed into separate vial bottles and labeled accordingly. Ethanol 96% was added to the vial bottles containing Ciprofloxacin 500mg, Doxycycline 100mg, Tetracycline 500mg, Chloramphenicol 250mg, Co Amoxiclav 125mg, and Azithromycin 500mg, each with 10 ml of ethanol. Ampicillin 500mg was dissolved in 10 ml of distilled water (aquades). The antibiotic solutions were homogenized and then sealed with vial bottle caps and plastic wrap. The antibiotic stock solutions were stored in the refrigerator.

The dilution of antibiotics began by sterilizing distilled water (aquades) in test tubes and 10 ml vial bottles using an autoclave. Each vial bottle was labeled for easy identification of the antibiotics. Then, 10µL of Ampicillin was added to a vial bottle using a micropipette, followed by the addition of 990µL of sterile distilled water. For Genta-100, 500µL was added to a vial bottle using a micropipette, followed by the addition of 500µL of sterile distilled water. For Doxycycline, 150µL was added to a vial bottle using a micropipette, followed by the addition of 850µL of sterile distilled water. Tetracycline received 30µL into a vial bottle using a micropipette, followed by the addition of 970µL of

sterile distilled water. Chloramphenicol received 60µL into a vial bottle using a micropipette, followed by the addition of 940µL of sterile distilled water. Ciprofloxacin received 5µL into a vial bottle using a micropipette, followed by the addition of 995µL of sterile distilled water. Co Amoxiclav received 80µL into a vial bottle using a micropipette, followed by the addition of 920µL of sterile distilled water. The dilution of antibiotics was done aseptically, and the vial bottles were sealed with caps and plastic wrap.

2.9.3 Preparation of Paper Disks and Injection of Antibiotics into Paper Disks

Whatman paper No. 3 was cut using a paper hole punch to obtain paper disks. These paper disks were then placed in a glass beaker and covered with aluminum foil. The glass beaker containing paper disks was sterilized using an autoclave.

The injection of antibiotics into paper disks began with the preparation of sterilized petri dishes using an autoclave. Sterilized paper disks were placed in the petri dishes using sterilized forceps in an aseptic manner. The paper disks were placed without overlapping to avoid sticking together. Antibiotics were injected into the paper disks, with each receiving 20µL of antibiotic solution. The number of paper disks used was adjusted to the number of samples in the petri dish. Petri dishes containing the antibiotic disks were sealed with plastic wrap and allowed to air dry until the paper disks were semi-dry. If the inner top of the dishes had wet paper disks, they could be dried using sterile gauze. Petri dishes containing antibiotic disks were labeled for antibiotic identification.

2.9.4 Testing the Antibiotic Potency via Paper Disk Diffusion

Prepared petri dishes containing solid media were used. *Vibrio* bacteria that had been standardized using the McFarland solution were inoculated onto the solid media with 100µL of bacterial suspension using a micropipette. Each bacterial strain was inoculated onto two solid media plates. Inoculation was done aseptically. Bacteria were then spread evenly across the surface of the solid media using a sterilized spreader. Petri dishes were sealed with plastic wrap, labeled, and placed in an incubator until the bacteria had fully incorporated into the solid media.

The next step was to place antibiotic disks onto the solid media. Four different antibiotic disks were placed in a single petri dish. Labels on paper were used to indicate the antibiotic disks placed in each dish. Antibiotic disks were placed on the solid media using sterilized forceps. The antibiotic disks were spaced apart and not placed too close to the edge of the petri dish. Petri dishes containing solid media with antibiotic disks were sealed with plastic wrap and incubated in an incubator for 24 hours. After the incubation period, zones of inhibition on the media were observed. The zones of inhibition were measured using a vernier caliper at opposite edges of the inhibition zones. The diameter of the inhibition zones was measured perpendicularly. The results of the inhibition zone measurements were recorded, and then, they were processed using Microsoft Excel to determine the sensitivity of bacteria to each type of antibiotic, whether the bacteria were resistant, sensitive, or intermediate, following the CLSI (2011) criteria.

3. Results

The results of the measurement of the inhibition zones and the criteria for the inhibition zones of Gentamicin, Co-Amoxiclav, and Tetracycline HCl can be seen in Table 1.

The results of the measurement of the inhibition zones and the criteria for the inhibition zones of Ciprofloxacin, Ampicillin, and Chloramphenicol can be found in Table 2.

The results of the measurement of the inhibition zones and the criteria for the inhibition zones of Azithromycin and Doxycycline can be found in Table 3.

The percentage effectiveness of antibiotics in inhibiting the growth of *Vibrio* spp. bacteria can be seen in Table 4.

4. Discussion

Based on the observations made, the macroscopic shapes of *Vibrio* spp. bacteria in the TR1 10^{-6} gill sample can be categorized into four macroscopic shapes. The first shape is irregular with a flat elevation. The next shape is irregular with an umbonate elevation. Another macroscopic shape is punctiform with a convex elevation, and there are also rounded shapes with entire margins and convex elevations. This is consistent with the statement by Widyastana *et al*. (2015) that bacterial colonies grown on media can be observed macroscopically based on their shape, size, texture, and color. Furthermore, macroscopic characteristics can also be observed in terms of margin and elevation.

Antibiotics are a group of drugs used to treat and prevent bacterial infections and can inhibit bacterial growth. Each type of antibiotic has different effects on inhibiting or controlling bacterial growth, especially *Vibrio* spp. bacteria. According to Utami (2012), the mechanism of action of antibiotics is to stop the metabolic processes of bacteria. The goal of antibiotics is to inhibit bacterial growth. The antibiotics used in this study are Co-Amoxiclav, Gentamicin, Doxycycline, Tetracycline, Azithromycin, Chloramphenicol, Ciprofloxacin, and Ampicillin. These types of antibiotics have varying effectiveness or impact on inhibiting the growth of *Vibrio* spp. bacteria. Tetracycline and Ampicillin antibiotics can inhibit or are resistant to *Vibrio* spp. bacteria.

The administration of antibiotics to the gills of snapper fish affected by *Vibrio* spp. bacteria leads to resistance to Tetracycline and Ampicillin antibiotics, while Gentamicin antibiotics have a greater sensitivity compared to other antibiotics tested. According to Hermanti *et al*. (2009), bacteria are considered sensitive to certain types of antibiotics if the diameter of the inhibition zone is large. This can be observed from the clear zones formed, indicating sensitivity or resistance to the antibiotics being tested. The inhibition zone can be measured using calipers. The measurement is performed by measuring either the horizontal or vertical diameter, and then the two diameters are added together and divided by 2. This will yield an average or mean. The unit of the inhibition zone is in millimeters. The inhibition zone has its own criteria when different types of antibiotics are applied. According to Surjowardojo *et al*. (2015), the categories for the inhibition zone are divided into four: ≤5 mm diameter is categorized as weak, 6-10 mm diameter is moderate, 11-20 mm is strong, and ≥21 mm is very strong. Sarker *et al*. (2014) also provide a standard interpretative zone diameter chart for determining antibiotic sensitivity and resistance status using the disk diffusion method.

5. Conclusions

The *Vibrio* spp. bacteria isolated from the gills of White Snapper (*Lates calcarifer*) fish exhibit different levels of resistance and sensitivity to eight different types of antibiotics. In this study, *Vibrio* spp. remained sensitive to certain antibiotics but showed resistance to Ampicillin.

Table 1. Measurement Results and Inhibition Zone Criteria for Gentamicin, Co-Amoxiclav, and Tetracycline HCl

Sample	Shape	Isolate code	Gentamycin		Co-Amoxiclav		Tetracyclin HCl	
			Mean	Criteria	Mean	Criteria	Mean	Criteria
TR1^6	IRE	1	16,6	S	13,05	R	8,5	R
	IRE	2	8,5	R	8,5	R	6	R
	IRE	3	12,8	I	12,1	R	6	R
	IRE	4	13,7	I	10,35	R	6,35	S
	IRE	7	23,5	S	10,45	R	15	S
	IRE	8	17,75	S	16,1	I	16,75	R
	IRE	9	15,8	S	11,5	R	6	R
	IRE	11	25,2	S	10,1	R	6,75	R
	IRE	12	14,45	I	13,2	I	8,45	R
	IRE	14	13,75	I	14,2	R	6,35	R
	IRE	15	9,7	R	9,45	R	6	R
	IRE	16	12,7	I	10,2	R	6	R
	IRE	17	32,35	S	15,85	I	7,25	R
	IRE	19	10,8	R	11,85	R	6	R
	IRE	20	12,95	I	13,7	I	6	R
	IRE	21	28,7	S	11,15	I	11,6	I
	IRE	25	16,8	S	15,4	I	12	R
	IRE	26	28,45	S	17,25	S	8	R
	IRE	30	17,25	S	16	R	8,8	R
	IRE	32	13,6	I	13,7	R	6	S
	IRE	33	21,85	S	20,7	I	19,7	R
	IRE	35	14,95	I	13,9	R	6,75	R
	IRE	37	12,1	R	10,95	R	6	R
	IRE	38	11,05	R	11,8	R	6	S
	IRE	40	20,2	S	13,1	I	6	R
	IRE	41	10	R	8,1	R	6	R
	IRE	42	13,4	I	10,35	R	6	R
	IRE	43	8,65	R	9,6	R	6	R
	IRE	44	11,95	R	12,4	R	7	R
	IRE	45	12,6	I	17,5	R	7,15	S
	IRE	46	25,05	S	17,95	I	16,1	R
	IRE	48	14	I	12,75	R	6	R
	IRE	49	17,15	S	16,55	I	10,8	R
	IRE	18	12,15	I	13,6	R	6	R
	IRU	5	14,6	I	11,65	R	17,9	R
	IRU	6	16	S	14,45	I	8,9	S
	IRU	10	17,1	S	17,15	I	7,85	R
	IRU	13	24,2	S	20,4	I	8,3	R
	IRU	50	17,3	S	17,65	I	9,25	R
	PUNC	47	11,95	R	9,65	I	6	R
	RO	22	17,95	S	11,55	R	13,15	I
	RO	23	20,25	S	14,9	I	11,65	R
	RO	24	29,55	S	14,4	I	7,6	I
	RO	27	21,75	S	7	R	6	R
	RO	28	15,6	S	14,45	I	10	S
	RO	31	9,75	R	10,5	R	6	R
	RO	34	19,55	S	20,4	I	7,35	R
	RO	36	15,3	S	14,95	R	10,9	R
	RO	39	27,7	S	16,95	I	16,55	R
	RO	29	27	S	13,95	R	20,35	R

Information ; S : Sensitive ; I : Intermediate ; R : Resistance

IRE = Ireguler, Flat; IRU= Ireguler, Umbonate; PUNC= Punctiform, Convex; RO = Entire Rounded, Convex;

Table 2. Measurement Results and Inhibition Zone Criteria for Ciprofloxacin, Ampicillin, and Chloramphenicol.

Sample	Shape	Isolate code	Ciprofloxacin		Ampicillin		Chloramphenicol	
			Mean	Criteria	Mean	Criteria	Mean	Criteria
TR1^6	IRE	1	15,95	I	9	R	13,05	I
	IRE	2	9,55	R	10,15	R	15,4	I
	IRE	3	13,6	R	10	R	17,55	I
	IRE	4	13	R	9,7	R	14,05	I
	IRE	7	15,1	R	7,9	R	13,45	I
	IRE	8	18,25	I	11,5	R	14,85	I
	IRE	9	10,8	R	10,9	R	17,3	I
	IRE	11	11,15	R	10	R	12,9	I
	IRE	12	16,9	I	11,95	R	21,25	S
	IRE	14	13,65	R	9,55	R	14,6	I
	IRE	15	12,2	R	7,9	R	12,95	I
	IRE	16	12	R	8,65	R	9,35	R
	IRE	17	15,2	R	10,15	R	20,55	S
	IRE	19	12,1	R	9,85	R	14,5	I
	IRE	20	16	I	12,3	R	16,85	I
	IRE	21	17,15	I	9,6	R	23,35	S
	IRE	25	16,3	I	14,5	I	17,75	I
	IRE	26	21,95	S	11,8	R	19,1	S
	IRE	30	14,5	R	12,85	R	18,45	S
	IRE	32	14,55	R	9	R	13,55	I
	IRE	33	18,9	I	12,8	R	18,75	S
	IRE	35	13,55	R	9	R	11,05	R
	IRE	37	14,6	R	9,6	R	15,35	I
	IRE	38	10,2	R	8,1	R	12,15	R
	IRE	40	17,7	I	12,25	R	10,3	R
	IRE	41	11,55	R	8,4	R	12,85	I
	IRE	42	10,75	R	9,3	R	14,3	I
	IRE	43	11	R	6,9	R	10,65	R
	IRE	44	14,2	R	17,8	S	11,3	R
	IRE	45	15	R	12,65	R	22,55	S
	IRE	46	18,25	I	10,35	R	20,25	S
	IRE	48	13,2	R	12,55	R	22,65	S
	IRE	49	15,95	I	10,2	R	14,55	I
	IRE	18	11,8	R	10,7	R	16,5	I
	IRU	5	14,45	R	10,65	R	15,3	I
	IRU	6	16,1	I	9,55	R	15,65	I
	IRU	10	16,55	I	11,2	R	15,05	I
	IRU	13	16	I	10,15	R	18,15	S
	IRU	50	18,75	I	12,35	R	21,9	S
	PUNC	47	16,9	I	6,75	R	13,55	I
	RO	22	14	R	12	R	14,9	I
	RO	23	16,95	I	12	R	18,3	S
	RO	24	17,15	I	11,4	R	15,95	I
	RO	27	11,9	R	9,2	R	15,3	I
	RO	28	15,1	R	12,6	R	23,35	S
	RO	31	13,2	R	10,4	R	18,35	S
	RO	34	16,85	I	8,25	R	16,05	I
	RO	36	13,8	R	11,85	R	19,1	S
	RO	39	19,4	I	8,3	R	11,85	R
	RO	29	14,85	R	11,95	R	18,2	S

Information ; S : Sensitive ; I : Intermediate ; R : Resistance

IRE = Ireguler, Flat; IRU= Ireguler, Umbonate; PUNC= Punctiform, Convex; RO = Entire Rounded, Convex.

Table 3. Measurement Results and Inhibition Zone Criteria for Azithromycin and Doxycycline

Sample	Shape	Isolate code	Azithromycin		Doxycycline	
			Mean	Criteria	Mean	Criteria
TR1^6	IRE	1	9,95	R	9,2	R
	IRE	2	9,55	R	8,9	R
	IRE	3	10,25	R	15,65	S
	IRE	4	9,75	R	9,2	R
	IRE	7	11	R	6,75	R
	IRE	8	12,5	S	12,45	I
	IRE	9	11,9	R	9,05	R
	IRE	11	11,85	R	6	R
	IRE	12	22,25	S	10,2	R
	IRE	14	9,6	R	15,05	S
	IRE	15	11,65	R	6,3	R
	IRE	16	8,1	R	11,05	I
	IRE	17	9,85	R	10,15	R
	IRE	19	7,8	R	9,05	R
	IRE	20	10,55	R	20,9	S
	IRE	21	19,1	S	10,85	I
	IRE	25	10,7	R	12,15	I
	IRE	26	13,7	S	10,3	R
	IRE	30	11,4	R	15,4	S
	IRE	32	8,15	R	9,6	R
	IRE	33	10	R	12,15	I
	IRE	35	7,25	R	9	R
	IRE	37	8,6	R	10,6	I
	IRE	38	9,7	R	9	R
	IRE	40	17,65	S	15	S
	IRE	41	7,7	R	9,4	R
	IRE	42	11,75	R	7,25	R
	IRE	43	8,5	R	6	R
	IRE	44	13,5	S	11,35	I
	IRE	45	24,75	S	13,75	I
	IRE	46	15,85	S	11,35	I
	IRE	48	11,5	R	12,05	I
	IRE	49	9,2	R	10	R
	IRE	18	14,75	S	10,05	R
	IRU	5	11,1	R	13,15	I
	IRU	6	9,35	R	11,5	I
	IRU	10	11,75	R	11,25	I
	IRU	13	14,95	S	13,8	I
	IRU	50	18,2	S	16,1	S
	PUNC	47	16,1	S	6	R
	RO	22	11,8	R	7,8	R
	RO	23	11,7	R	10,5	I
	RO	24	10,3	R	10,15	R
	RO	27	6	R	10,55	I
	RO	28	15,85	S	14,95	S
	RO	31	9,6	R	10,5	I
	RO	34	6,25	R	8,6	R
	RO	36	17,45	S	11,9	I
	RO	39	7,75	R	9,5	R
	RO	29	9,25	R	15,3	S

Information ; S : Sensitive ; I : Intermediate ; R : Resistance

IRE = Ireguler, Flat; IRU= Ireguler, Umbonate; PUNC= Punctiform, Convex; RO = Entire Rounded, Convex.

Table 4. Percentage Effectiveness of Antibiotics in Inhibiting the Growth of *Vibrio* spp. Bacteria.

Types of antibiotics	Shape			
	IRE	IRU	PUNC	RO
Gentamicin	41,2% (S)	80% (S)	100% (R)	90% (S)
Co-Amoxiclav	67,6% (R)	80% (S)	100% (I)	50% (R)
Tetracycline HCl	82,4% (R)	80% (R)	100% (R)	70% (R)
Ciprofloxacin	67,6% (R)	20% (R)	100% (I)	60% (R)
Ampicillin	94,1% (R)	100% (R)	100% (R)	100% (R)
Chloramphenicol	55,9% (I)	60% (I)	100% (I)	50% (S)
Azithromycin	73,5% (R)	60% (R)	100% (S)	80% (R)
Doxycyclin	53,9% (R)	80% (I)	100% (R)	40% (R)

Ethics approval
No need permit to *Lates calcarifer*

Data availability statement
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Credit authorship contribution statement
Eny Heriyati: Conceptualization, methodology, validation, investigation, resources, writing original draft preparation, writing review and editing, visualization, supervision, project administration, funding acquisition. Prita Lintang Larasati: formal analysis, writing original draft preparation, visualization. Both authors have read and agreed to the published version of the manuscript.

Funding
No funding available.

Acknowledgments
I would like to state my special thanks of gratefulness to Diponegoro University.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Referrences

CLSI. 2011. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guidline-Second Edition. *CLSI document M45-A2*. 30(18).

Hermanti, A., R. Nuchsin dan J. Dewi. 2009. Screening Bakteri Penghambat Untuk Bakteri Penyebab Penyakit Pada Budidaya Ikan Kerapu Dari Perairan Banten Dan Lampung. Poklit Biologi Oseanografi, Puslit Oseanografi LIPI, Jakarta Utara. Balai Budidaya Besar Lut DKP, Teluk Hurun Lampung. *Jurnal Makara Sains*. 13(1) : 81-86.

Ihsan, B. dan E. Retnaningrum.2012. Isolasi Dan Identifikasi Bakteri *Vibrio* sp. Pada Kerang Kapah (*Meretrix meretrix*) Di Kabupaten Trenggalek. *Jurnal Harpodon Borneo*. 10(1) : 23-27. Jaya,
B., F. Agustriani dan Isnaini. 2013. Laju Pertumbuhan dan Tingkat Kelangsungan Hidup Benih Kakap Putih (*Lates calcarifer*, Bloch) dengan Pemberian Pakan yang Berbeda. *Maspari Journal: Marine Science Research. 5*(1) : 56-63.

Microbiology Class. 2020. Microorganisms and Their Colonal Characteristics. https://microbiologyclass.com/microorganisms_and their-colonial-characteristics/. [diakses 8 Agustus 2023]

Santika, L., Diniarti, N., dan Lestari, D. P. 2021. Pengaruh Penambahan Ekstrak Kunyit Pada Pakan Buatan Terhadap Pertumbuhan Dan Efisiensi Pemanfaatan Pakan Ikan Kakap Putih (*Lates calcarifer*). *Jurnal Kelautan: Indonesian Journal Of Marine Science And Technology*. 14(1) : 48-57.

Sarker, M. R., K. N. Islam, H. Z. Huri, M. Rahman, H. Imam, B. Hosen, N. Mohammad and Md. Z. I. Sarker .2014. Studies Of The Impact Of Occupational Exposure Of Pharmaceutical Workers On The Development Of Antimicrobial Drug Resistance. *Journal of occupational health*. 56 : 260-270.

Utami, P. 2012. Antibiotik Alami untuk Mengatasi Aneka Penyakit. Jakarta, Agromedia Pustaka. 120 hlm.

Widyastana, I. W. Y., R. Kawuri, dan A. A. G. R. Dalem. 2015. Keberadaan Bakteri Patogen *Vibrio cholerae* Pada Beberapa Hasil Perikanan Yang Dijual Di Pasar Tradisional Kota Denpasar. *Metamorfosa: Journal of Biological Sciences. 2*(1) : 16-22.

Yaqin, A. A., L. Santoso dan S. Saputra. 2018. Budidaya Ikan Kakap Ini Khususnya Ikan Kakap Putih (*Lates calcarifer*) Sudah Banyak Dikembangkan Di Indonesia Yang Bersifat Komersil. *Jurnal Sains Tekonologi Akuakultur*. 2(1) : 12-19.

Zaenuddin, A., Y. L. Nuraini, A. Faries dan S. Wahyuningsih. 2019. Pengendalian Penyakit *Vibrio*sis Pada Ikan Kakap Putih. *Jurnal Peekayasaan Budidaya Air Payau dan Laut*. 14 : 77-83.