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Susceptibility of *Vibrio* spp. from Viscera Organ and Flesh of *Lates calcarifer* Against Antibiotics

Zaenal Arifin[1]*, Amalia Tasyakurnia Rahman[2]

[1]National Research and Innovation Agency (BRIN), Earth and Marimite Organization Research, Fisheries Research Center, CSC-BG LIPI, Jl. Raya Bogor Km 46, Cibinong, Bogor, 16915, INDONESIA.
[2]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro, Jl Prof. Sudharto, Tembalang, Semarang 50275, INDONESIA.

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*Corresponding Author email: zarifin091961@gmail.com

Abstract

Cultivation of *Lates calcarifer* is widely developed in Indonesia due to its high market demand and economic value. The biggest challenge in aquaculture is the diseases caused by pathogenic bacteria. The presence of pathogenic bacteria can disrupt the cultivation process and even lead to mass mortality in fish. *Vibrio* spp. is one of the pathogenic bacteria found in the cultivation of white snapper. The growth and development of *Vibrio* spp. bacteria can be controlled by the use of antibiotics. However, excessive and improper use of antibiotics can trigger the development of antibiotic resistance. Therefore, resistance testing is necessary to determine the level of resistance of *Vibrio* spp. bacteria from the viscera organ and flesh of *L. calcarifer* to 8 types of antibiotics, namely Gentamicin, Co-Amoxiclav, Tetracycline HCl, Ciprofloxacin HCl, Ampicillin, Chloramphenicol, Azithromycin, and Doxycycline. Resistance testing of *Vibrio* spp. bacteria from the viscera organ and flesh of *L. calcarifer* to these 8 types of antibiotics is carried out using the disc diffusion method. *Vibrio* spp. bacteria from the viscera organ of fish have resistance to Ampicillin and Co-Amoxiclav with a resistance percentage of 35.7%, while *Vibrio* spp. bacteria from the flesh of *L. calcarifer* have resistance to Ampicillin and Azithromycin with a resistance percentage of 57.1%. *Vibrio* spp. bacteria from the viscera organ and flesh are sensitive to Gentamicin with a percentage of 100%. *Vibrio* spp. bacteria from the viscera organ and flesh of *L. calcarifer* have different resistance and sensitivity to various types of antibiotics. Overall, the *Vibrio* spp. obtained in this study are still sensitive to antibiotics.

1. Introduction

Cultivation of Seabass (*Lates calcarifer*) is widely practiced in Indonesia. Cultivation can be carried out in floating net cages or in ponds. Several advantages of Seabass serve as the basis for its high cultivation activity. These advantages include the high economic value of Seabass, with prices ranging from Rp. 75,000 to Rp. 80,000 per kilogram (Santika *et al*., 2021). Seabass has a wide market coverage both domestically and internationally, including countries like Germany, Spain, England, and Italy (Asdary *et al*., 2019). Seabass has a broad physiological tolerance range and rapid growth, making the cultivation process easier (Hasibuan *et al*., 2018).

The biggest challenge in aquaculture is diseases caused by pathogenic bacteria. The presence of pathogenic bacteria can disrupt cultivation activities and even lead to mass mortality in fish (Azhar *et al*., 2020). This can be detrimental to the aquaculture sector and pose risks to human health in the vicinity. Factors that can potentially contribute to the development of pathogenic bacteria in fish farming include poor water conditions (Lein *et al*., 2020). Pathogenic bacteria can also originate from contamination of the aquaculture equipment and the use of inappropriate cultivation techniques (Palawe *et al*., 2018).

Vibrio spp. is one type of pathogenic bacteria that can cause diseases in marine organisms (Azhar and Yudiati, 2023). *Vibrio* spp. bacteria are commonly found in shallow tropical waters because they thrive in waters with temperatures up to 37°C (Rahmaningsih *et al*., 2012). The use of antibiotics is one of the measures taken to inhibit the growth rate of *Vibrio* spp. bacteria (Santi *et al*., 2017). Antibiotics are widely used in fish farming to control diseases caused by bacteria (Nurhasnawati *et al*., 2016). However, this has led to cases of antibiotic-resistant bacteria, necessitating

resistance testing to determine the level of bacterial resistance to the antibiotics to be used. This research aims to identify the types of antibiotics that can be used to treat diseases in *L. calcarifer* caused by *Vibrio* spp. bacteria and to determine the resistance of *Vibrio* spp. bacteria isolated from the viscera organs and flesh of *L. calcarifer* affected by the disease to antibiotics.

2. Material and methods

2.1 Collection of *Vibrio* spp. Isolates.

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

2.2 Preparation of Agar Slant Solid Media

The preparation of slant solid media begins by assembling the necessary equipment and materials. The weighed materials are 1.3 grams of Nutrient Broth (Merck) and 1.5 grams of Agar (Merck), which are placed into an Erlenmeyer flask and mixed with 100 ml of distilled water. A magnetic stirring bar is added to the Erlenmeyer flask, and the mixture is homogenized on a hot plate magnetic stirrer. Once the media starts foaming, the Erlenmeyer flask is removed from the hot plate magnetic stirrer and allowed to warm up. When the media is sufficiently warm, it is poured into reaction tubes, filling them to a volume of approximately 5 ml. The reaction tubes are then sealed with cotton and aluminum foil and sterilized using an autoclave. After autoclaving, the tubes containing the media are tilted on a stand on a sterile table surface. Be cautious not to tilt them too much to prevent spillage onto the cotton covering, and allow the media to solidify.

2.3 Characterization and purification of *Vibrio* spp. bacteria

The *Vibrio* spp. bacteria to be characterized originate from samples of the visceral organs and muscles of snapper fish, which have been previously incubated. The bacteria are observed from petri dishes with the assistance of a flashlight, then marked and identified based on their characteristics following the bacterial characterization method by Ejikeugwu (2017). The characteristics of the bacteria are recorded, and colonies with similar characteristics are counted. Once characterized, the bacteria are purified on prepared solid agar media. Purification is carried out using a sterilized needle that has been heated until it becomes red-hot with a Bunsen burner. After it cools down,

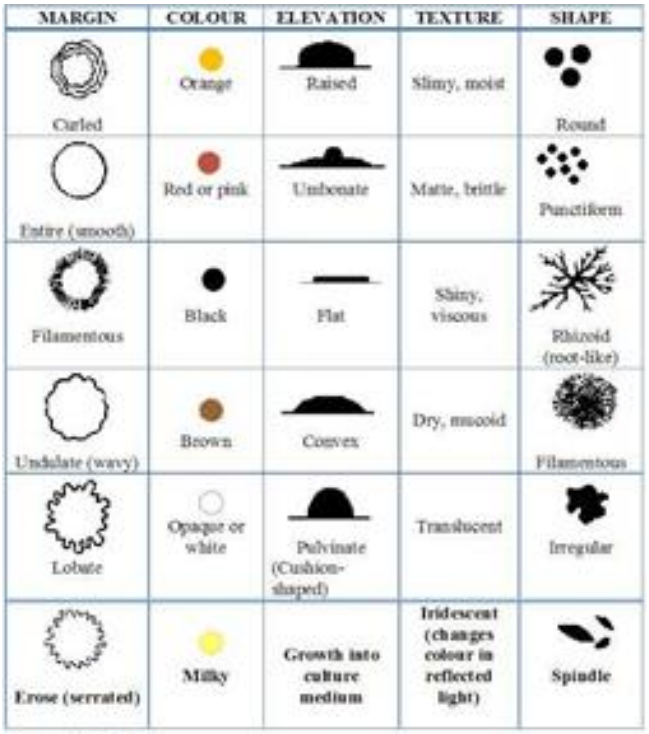

Figure 1. Bacterial Characterization (Microbiology Class, 2020).

the needle is streaked through the bacteria on the petri dish and then streaked onto the solid agar media. Bacteria with different characteristics are placed into separate test tubes and labeled accordingly. The test tubes are sealed with plastic wrap in an aseptic manner and incubated in a sterile container.

2.4 Preparation of Liquid Media and Purification of *Vibrio* spp. Bacteria from Solid Agar to Liquid Media

The preparation of liquid media begins with the preparation of the necessary equipment and materials. Nutrient Broth weighing 1.3 grams and NaCl weighing 1.5 grams are placed into an Erlenmeyer flask, followed by the addition of 100 ml of distilled water. A magnetic stirring bar is placed inside the Erlenmeyer flask, which is then homogenized using a hot plate magnetic stirrer. Once all the ingredients are homogenized, the Erlenmeyer flask is removed from the hot plate magnetic stirrer and left to warm. The liquid media is then transferred into separate vials, each containing 5 ml. The filled vials are sealed with cotton and aluminum foil, followed by sterilization using an autoclave.

Vibrio bacteria grown in the test tubes are scraped with an inoculating needle. The inoculating needle is sterilized using a Bunsen burner until it becomes red hot.

Tabel 1. Interpretive Criteria for Disk Diffusion Susceptibility Testing *Vibrio* spp.

Antibiotik	Zone Diameter (mm) Interpretive Criteria		
	resistance	Intermediate	sensitive
Chloramphenicol (30ppm)	≤ 12	13–17	≥ 18
Gentamicin (10ppm)	≤ 12	13–14	≥ 15
Co-Amoxiclav (10ppm)	≤ 13	14–17	≥ 18
Ciprofloxacin HCL (5ppm)	≤ 15	16–20	≥ 21
Ampicilin (10ppm)	≤ 13	14–16	≥ 17
Tetracyclin (30ppm)	≤ 11	12–14	≥ 15
Azithromycin (15ppm)	≤12	–	≥13
Doxycycline (30ppm)	≤10	11–13	≥14
Erythomycin (15ppm)	≤ 13	14–17	≥ 18

After the needle has cooled down, it is used to scrape the bacteria from the solid agar medium. The inoculating needle, now containing a trace of bacteria, is then dissolved in the liquid media inside the vial. The vial is sealed again with cotton in an aseptic manner, sealed with plastic wrap, labeled according to the sample, and incubated in a sterile container.

2.5 Preparation of Solid Media

The preparation of solid media begins with the preparation of the necessary equipment and materials. The weighed ingredients, Nutrient Broth (1.3 grams) and Nutrient Agar (1.5 grams), are placed in an Erlenmeyer flask, followed by the addition of 100 ml of distilled water (aquades). A magnetic stirring bar is inserted into the Erlenmeyer flask, and the contents are homogenized on a hot plate magnetic stirrer. Once the media has foamed, the Erlenmeyer flask is removed from the hot plate magnetic stirrer and sealed with cotton and aluminum foil. The media in the Erlenmeyer flask is then sterilized using an autoclave.

After sterilization, the media is allowed to cool slightly and is poured aseptically into petri dishes. The dishes are sealed with plastic wrap and covered with plastic secured with rubber bands. The petri dishes are then stored separately in sterile containers to prevent contamination.

2.6 Testing the Resistance of *Vibrio* spp. Bacteria to Antibiotics

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

The preparation of the McFarland 0.5 standard solution begins with the preparation of the necessary equipment and materials. Sodium chloride (NaCl) weighing 0.85 grams is placed into an Erlenmeyer flask and then 100 ml of distilled water (aquades) is added. A magnetic stirring bar is placed inside the Erlenmeyer flask, and the mixture is homogenized using a hot plate magnetic stirrer. Once all the components are homogenized, the Erlenmeyer flask is removed from the hot plate magnetic stirrer and allowed to cool slightly. The McFarland standard solution is then transferred into individual vials, each containing 5 ml of the solution. These vials are sealed with cotton and aluminum foil, and then sterilized using an autoclave.

The standardization of *Vibrio* spp. bacteria is performed by aseptically transferring the bacteria from the liquid media purification into the vials containing the McFarland standard solution, using a micropipette, until the turbidity level matches the McFarland 0.5 standard. Vials that have reached the McFarland 0.5 standard are sealed with plastic wrap, labeled according to the sample, and stored in a sterile container.

2.6.2 The preparation of antibiotic stock solutions.

Before starting the preparation of antibiotic stock, 20 ml vials and their caps are sterilized using autoclave. Seven types of antibiotics, namely Ciprofloxacin 500mg, Doxycycline 100mg, Tetracycline 500mg, Chloramphenicol 250mg, Ampicillin 500mg, Co-Amoxiclav 125mg, and Azithromycin 500mg are ground into a fine powder using a mortar and pestle. They are then transferred into separate vials and labeled accordingly. Ethanol 96% is added to the vials containing Ciprofloxacin 500mg, Doxycycline 100mg, Tetracycline 500mg, Chloramphenicol 250mg, Co-Amoxiclav 125mg, and Azithromycin 500mg, each with 10 ml of ethanol. Aquades (sterile water) is added to the vial containing Ampicillin 500mg, also with 10 ml of aquades. The antibiotic solutions are homogenized, capped, and sealed with plastic wrap. The antibiotic stock is stored in the refrigerator.

The antibiotic dilution process begins with the sterilization of aquades in test tubes and 10 ml vials using autoclave. Each vial is labeled for easy identification of the antibiotics. 10µL of Ampicillin is added to the vial using a micropipette, followed by the addition of 990µL of sterile aquades. 500µL of Genta-100 is added to the vial using a micropipette, followed by the addition of 500µL of sterile aquades. 150µL of Doxycycline is added to the vial using a micropipette, followed by the addition of 850µL of sterile aquades. 30µL of Tetracycline is added to the vial using a micropipette, followed by the addition of 970µL of sterile aquades. 60µL of Chloramphenicol is added to the vial using a micropipette, followed by the addition of 940µL of sterile aquades. 5µL of Ciprofloxacin is added to the vial using a micropipette, followed by the addition of 995µL of sterile aquades. 80µL of Co-Amoxiclav is added to the vial using a micropipette, followed by the addition of 920µL of sterile aquades. The antibiotic dilution process is carried out aseptically, and the vials are capped and sealed with plastic wrap.

2.6.3 Preparation of Paper Disks and Antibiotic Injection into Paper Disks

The Whatman paper No. 3 is cut using a hole punch to create paper disks. These paper disks are then placed into a glass beaker and covered with aluminum foil. The beaker containing the paper disks is sterilized using an autoclave.

The injection of antibiotics into the paper disks begins with the preparation of sterilized petri dishes using an autoclave. Next, the sterilized paper disks are aseptically arranged in the petri dishes using sterile forceps. The paper disks are placed with some spacing between them to avoid sticking together. The injection of antibiotics is performed with aseptic techniques. Each paper disk is injected with 20µL of the respective antibiotic. The number of paper disks used corresponds to the number of samples in the petri dish. The dishes containing the paper disks are sealed with plastic wrap and left to air dry until the paper disks are partially dry. If the top surface of the dishes with paper disks is still wet, it can be dried using sterile gauze. Each dish containing antibiotic disks is labeled to differentiate its antibiotic content.

2.6.4 The testing of antibiotic potency through paper disk diffusion

The process involves the preparation of agar plates. Bacteria that have been standardized using the MacFarland solution are inoculated into the solid media with 100µL each, using a micropipette. Each type of bacteria is inoculated into two solid media plates. The bacterial inoculation is performed aseptically. The bacteria are then evenly spread across the surface of the solid media using a sterilized spreader heated by a Bunsen burner. Petri dishes are then sealed with plastic wrap, labeled, and placed in an incubator until the bacteria have fully integrated with the solid media.

The next step involves placing antibiotic disks onto the solid media. Four different types of antibiotic disks are placed in a single media plate. Paper labels are used to identify the antibiotic disks placed in each dish. Antibiotic disks are positioned on the solid media using sterilized forceps. They should be spaced apart and not too close to the edge of the dish. Petri dishes containing the media and antibiotic disks are then sealed with plastic wrap and incubated in the incubator for 24 hours.

After the incubation period, zones of inhibition will become visible in the media. These zones are measured using

calipers at opposite edges of the inhibition zones, perpendicular to the edge. The diameters of the inhibition zones are measured. The results, including the diameter of the inhibition zones, are recorded. These results are then processed using Microsoft Excel to determine the sensitivity of the bacteria to each type of antibiotic, whether the bacteria are resistant, sensitive, or intermediate, following the criteria for the diameter of the inhibition zone as defined by CLSI (2011) as shown in Tabel 1.

3. Results

The results of the measurement of inhibition zones and the criteria for the Gentamicin inhibition zone can be seen in Table 2. The measurement results and criteria for Tetracycline HCl inhibition zones can be found in Table 3. The measurement results and criteria for Ciprofloxacin HCl inhibition zones can be seen in Table 4. The measurement results and criteria for Ampicillin inhibition zones can be seen in Table 5. The measurement results and criteria for Chloramphenicol inhibition zones can be seen in Table 6. The measurement results and criteria for Azithromycin inhibition zones can be seen in Table 7. The measurement results and criteria for Doxycycline inhibition zones can be seen in Table 8. The inhibition zones formed on the isolates can be seen in Figure 2. The percentage effectiveness of antibiotics against *Vibrio* spp. bacteria can be seen in Table 9.

Table 2. Measurement Results and Criteria for Gentamicin Inhibition Zones

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR3[7]	Round Convex	Q	36,5	S
	Round Convex	R	30	S
	Round Convex	S	30	S
	Round Convex	T	34,45	S
	Round Convex	U	28,7	S
	Round Convex	V	27,25	S
	Round Convex	W	28,95	S
OT TR3[6]	Round Convex	A	27,15	S
	Round Convex	B	28,65	S
	Round Convex	C	31	S
	Round Convex	D	27,3	S
	Round Convex	E	32,8	S
	Round Convex	V	24,95	S
	Round Convex	G	22,85	S
	Round Convex	H	23,05	S
	Round Convex	I	31,1	S
	Round Convex	J	31,3	S
	Round Convex	K	34,8	S
	Round Convex	N	31,75	S
	Round Convex	O	34,85	S
	Round Convex	P	38	S
	Irregular Convex	L	28,9	S
	Irregular Convex	M	31,75	S

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

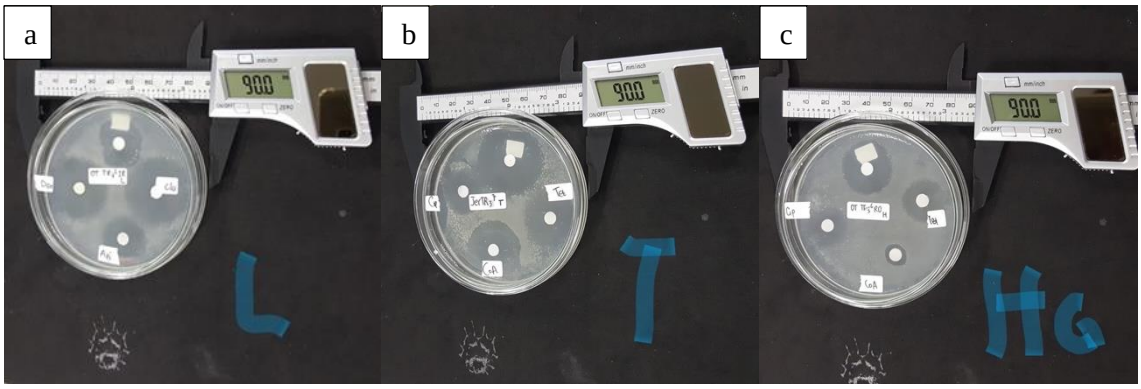

Figure 2. Inhibition zones on a petri dish. Isolate OTTR36 Irregular (a), JerTR37 Round (b) and OTTR36 Round (c).

Table 3. Measurement Results and Criteria for Tetracycline HCl Inhibition Zones

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR3[7]	Round Convex	Q	30	S
	Round Convex	R	26,2	S
	Round Convex	S	30	S
	Round Convex	T	28,2	S
	Round Convex	U	17,8	S
	Round Convex	V	6	R
	Round Convex	W	6	R
OT TR3[6]	Round Convex	A	16,9	S
	Round Convex	B	22,45	S
	Round Convex	C	27,05	S
	Round Convex	D	22,8	S
	Round Convex	E	24,65	S
	Round Convex	V	18,95	S
	Round Convex	G	21,95	S
	Round Convex	H	18,8	S
	Round Convex	I	25,1	S
	Round Convex	J	28,15	S
	Round Convex	K	28,3	S
	Round Convex	N	24,75	S
	Round Convex	O	26,4	S
	Round Convex	P	33,2	S
	Irregular Convex	L	26,75	S
	Irregular Convex	M	27,75	S

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

Table 4. Measurement Results and Criteria for Ciprofloxacin HCl Inhibition Zones.

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR37	Round Convex	Q	28,75	S
	Round Convex	R	19,8	S
	Round Convex	S	30	S
	Round Convex	T	25,1	S
	Round Convex	U	14,35	S
	Round Convex	V	21,25	I
	Round Convex	W	16,55	R
OT TR36	Round Convex	A	14,15	R
	Round Convex	B	14,05	S
	Round Convex	C	19,95	I
	Round Convex	D	14,45	R
	Round Convex	E	20,45	I
	Round Convex	V	12,6	R
	Round Convex	G	16,6	I
	Round Convex	H	16,6	I
	Round Convex	I	17,45	S
	Round Convex	J	12,45	R
	Round Convex	K	32,6	S
	Round Convex	N	27,35	I
	Round Convex	O	28,3	I
	Round Convex	P	32,6	I
	Irregular Convex	L	31,75	S
	Irregular Convex	M	20,5	I

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

Table 5. Measurement Results and Criteria for Ampicillin Inhibition Zones.

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR37	Round Convex	Q	25,3	S
	Round Convex	R	0	R
	Round Convex	S	19,55	S
	Round Convex	T	33,75	S
	Round Convex	U	6	R
	Round Convex	V	6	R
	Round Convex	W	6	R
OT TR36	Round Convex	A	13,8	R
	Round Convex	B	10,85	R
	Round Convex	C	35,75	S
	Round Convex	D	9,35	R
	Round Convex	E	27,35	S
	Round Convex	V	12	R
	Round Convex	G	15,25	I
	Round Convex	H	6	R
	Round Convex	I	30,65	S
	Round Convex	J	33,7	S
	Round Convex	K	34,6	S
	Round Convex	N	30	S
	Round Convex	O	35,5	S
	Round Convex	P	34,95	S
	Irregular Convex	L	22,35	S
	Irregular Convex	M	36,7	S

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

Table 6. Measurement Results and Criteria for Chloramphenicol Inhibition Zones.

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR37	Round Convex	Q	11,7	R
	Round Convex	R	32,15	S
	Round Convex	S	25,5	S
	Round Convex	T	34,4	S
	Round Convex	U	6	R
	Round Convex	V	19,6	S
	Round Convex	W	6	R
OT TR36	Round Convex	A	29,15	S
	Round Convex	B	23,9	S
	Round Convex	C	27,25	S
	Round Convex	D	20,2	S
	Round Convex	E	21	S
	Round Convex	V	23,25	S
	Round Convex	G	25,15	S
	Round Convex	H	16,5	I
	Round Convex	I	20,95	S
	Round Convex	J	30,6	S
	Round Convex	K	33,9	S
	Round Convex	N	30	S
	Round Convex	O	31,6	S
	Round Convex	P	30,35	S
	Irregular Convex	L	29,65	S
	Irregular Convex	M	34,65	S

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

Table 7. Measurement Results and Criteria for Azithromycin Inhibition Zones.

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR37	Round Convex	Q	16,45	S
	Round Convex	R	28,3	S
	Round Convex	S	22,3	S
	Round Convex	T	12,0	R
	Round Convex	U	6	R
	Round Convex	V	6	R
	Round Convex	W	6	R
OT TR36	Round Convex	A	20,3	S
	Round Convex	B	16,1	S
	Round Convex	C	20,6	S
	Round Convex	D	13,65	S
	Round Convex	E	20,1	S
	Round Convex	V	16,45	S
	Round Convex	G	15,9	S
	Round Convex	H	11,95	R
	Round Convex	I	12	R
	Round Convex	J	21,75	S
	Round Convex	K	27,5	S
	Round Convex	N	29,1	S
	Round Convex	O	26	S
	Round Convex	P	28,85	S
	Irregular Convex	L	19,7	S
	Irregular Convex	M	36,1	S

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

Table 8. Measurement Results and Criteria for Doxycycline Inhibition Zones.

Sample	Characteritation	Isolate	Gentamicin	
			Mean (mm)	Criteria
Jer TR37	Round Convex	Q	30,1	S
	Round Convex	R	20,4	S
	Round Convex	S	29,95	S
	Round Convex	T	24,8	S
	Round Convex	U	16,4	S
	Round Convex	V	6	R
	Round Convex	W	6	R
OT TR36	Round Convex	A	31,45	S
	Round Convex	B	22,4	S
	Round Convex	C	26,9	S
	Round Convex	D	18,85	S
	Round Convex	E	29,25	S
	Round Convex	V	25,45	S
	Round Convex	G	23,5	S
	Round Convex	H	19,7	S
	Round Convex	I	24,45	S
	Round Convex	J	32,1	S
	Round Convex	K	33,65	S
	Round Convex	N	29,7	S
	Round Convex	O	37,35	S
	Round Convex	P	37,55	S
	Irregular Convex	L	29,75	S
	Irregular Convex	M	35,5	S

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

Table 9. Percentage Effectiveness of Antibiotics against *Vibrio* spp.

Type of Antibiotics	Bacteria Isolate		
	Jer TR3[7] Round Convex	OT TR3[6] Round Convex	OT TR3[6] Irregular Convex
Gentamicin	100%	100%	100%
Co-Amoxiclav	71,4%	64,3%	100%
Tetracyclin HCl	71,4%	100%	100%
Ciprofloxacin HCl	71,4%	21,4%	50%
Ampicillin	42,9%	57,1%	100%
Chloramphenicol	57,1%	92,9%	100%
Azithromycin	42,9%	85,7%	100%
Doxycycline	71,4%	100%	100%

4. Discussion

Testing the resistance of *Vibrio* spp. bacteria from the viscera organ and flesh of *L. calcarifer* begins with the characterization of bacterial colonies. Characterization is done by observing bacterial colonies on agar plates. Colonies are characterized and grouped based on their margin shape, color, and elevation. Bacterial colonies isolated from the JerTR37 plate have a round shape with a convex elevation and are yellow in color. This is consistent with the morphological characteristics of *Vibrio* spp. colonies as reported by Arisandi *et al*. (2019), which describes round colonies with a yellow color. In contrast, bacterial colonies isolated from the OTTR36 plate have round and irregular shapes with a convex elevation. The differences in the shape of *Vibrio* spp. bacterial colonies in one plate are influenced by abiotic environmental factors such as pH, temperature, oxygen levels, nutrient availability in the growth medium, and temperature (Situmeang *et al*., 2016). The color of *Vibrio* spp. bacterial colonies is influenced by their ability to utilize sucrose. This is supported by the statement from Ilmiah *et al*. (2012), which explains that yellow colonies of *Vibrio* spp. indicate the ability to utilize sucrose, while green colonies indicate the inability to utilize sucrose.

The level of antibiotic resistance is determined by measuring the inhibition zones formed by the reaction of *Vibrio* spp. bacteria from the viscera organ and flesh of *L. calcarifer*. *Vibrio* spp. bacteria from the viscera organ showed resistance to Azithromycin and Ampicillin with percentages above 50%. This is consistent with data from Kusmarwati *et al*. (2017), which reported that *Vibrio* spp. bacteria found in shrimp ponds exhibited resistance to Ampicillin, reaching up to 73%. *Vibrio* spp. bacteria from the white snapper muscle did not show resistance to excessive antibiotics, as indicated by resistance percentages below 50%. The highest resistance percentage was 35.7%, which was observed for Ampicillin and co-Amoxiclav antibiotics. Based on the results obtained, it can be concluded that *Vibrio* spp. bacteria from the viscera organ and flesh of *L. calcarifer* have the highest sensitivity to Gentamicin, with a sensitivity percentage of 100%.

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5. Conclusions

Vibrio spp. bacteria from the viscera organ and flesh of *Lates calcarifer* exhibit varying levels of resistance and sensitivity to different types of antibiotics. In general, the *Vibrio* spp. obtained in this study are still sensitive to antibiotics.

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

Zaenal Arifin: Conceptualization, methodology, validation, investigation, resources, writing original draft preparation, writing review and editing, visualization, supervision, project administration, funding acquisition. Amalia Tasyakurnia Rahman: formal analysis, writing original draft preparation, visualization. Both authors have read and agreed to the published version of the manuscript.

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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

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