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Resistance of *Vibrio* sp bacteria in Vaname shrimp (*Litopenaeus vannamei*) rearing ponds in Jepara to antibiotics amoxicillin and enrofloxacin

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Abstract
This study aims to determine the morphological characteristics of *Vibrio* sp. bacteria, identify the types of *Vibrio* sp. bacteria present in vannamei shrimp ponds in Jepara, and analyze the resistance of *Vibrio* sp. bacteria to the antibiotics amoxicillin and enrofloxacin. The method used in this study is exploratory confirmatory. Water and shrimp gut samples were obtained from vannamei shrimp ponds in Jepara, Central Java. The morphological results of *Vibrio* sp. bacteria showed two colors, green and yellow, circular and irregular shapes, convex and flat elevations, and smooth and undulating edges. *Vibrio* sp. bacteria found in the water and shrimp gut included *Vibrio parahaemolyticus*, *Vibrio anguillarum*, and *Vibrio metschnikovii*. The analysis of amoxicillin and enrofloxacin antibiotics tested in water and shrimp gut samples fell into the resistant category, indicating that *Vibrio parahaemolyticus*, *Vibrio anguillarum*, and *Vibrio metschnikovii* bacteria were resistant to amoxicillin and enrofloxacin antibiotics.

Keywords: Antibiotics, resistance, vaname shrimp, *Vibrio* sp.

Introduction
Vaname shrimp (*Litopenaues vannamei*) is an aquatic commodity that is economically valuable as an export commodity because it has a significant market interest in the world market. Vaname shrimp production continues to increase yearly; in 2013, it was 390,279 tons; in 2014, it reached 411,729 tons; in 2016, it was 488,091 tons (KKP, 2018) [28]. Vaname shrimp has a high market interest, especially in Indonesia, due to losses from tiger shrimp (Penaeus monodon) cultivation caused by virus and disease attacks. According to Sa'adah and Ahmad (2018) [49], vaname shrimp has a high level of productivity because it has a high survival rate, so it can utilize the entire pool of water from the bottom to the surface, allowing it to be cultivated with high stocking density conditions. In addition to high survival rates, vaname shrimp have a cultivation time that can be harvested within 120 days (Prawitasari and Musyaffa, 2022) [41].
Vaname shrimp farming has the potential to generate large profits, so farmers cultivate vaname shrimp with high stocking densities (Febriana *et al*., 2016) [17]. Intensive cultivation technology with high stocking density is done because of the increasing demand for vaname shrimp production from domestic to foreign countries (Yuniarty and Diana, 2021) [66]. High stocking density cultivation is expected to help increase production. Still, suppose the stocking density used exceeds a specific limit. In that case, it will affect the carrying capacity or water quality of the aquaculture pond so that it can no longer support the life of shrimp in a specific biomass (Rahim *et al*., 2021) [44]. Suppose the water quality in shrimp farming has decreased, and the pond's carrying capacity can no longer support the metabolism of cultured shrimp. In that case, it will impact shrimp health, causing diseases that will affect cultured shrimp production. According to Junaidi *et al*., (2020) [25], the disease that most often attacks vaname shrimp is a bacterial disease such as *Vibrio*sis caused by *Vibrio* sp.
Vibrio sp. is a bacterial disease often occurring in shrimp farming (Sarjito *et al*., 2022) [53]. *Vibrio*sis is one type of disease that usually attacks shrimp farming; one of the agents that cause *Vibrio*sis disease is *Vibrio harveyi* bacteria (Widarnani *et al*., 2014) [62].

The bacteria *V. Harveyi* is an opportunistic pathogen often found in shrimp or marine fish farming environments. According to Rahim and Dheni (2023) [45], *V. harveyi* bacteria cause *Vibrio*sis disease and are so dominant that they can cause mass mortality in shrimp farming. Mass mortality of vaname shrimp reaches 80%-100%, caused by *Vibrio* sp. bacteria that attack their hosts when water conditions are not good (Annisa *et al*., 2015) [3]. Bacterial diseases caused by the genus *Vibrio* have become a significant concern for the shrimp farming industry (Sarjito and Sabdono, 2021) [53]. Handling this disease can use chemicals such as antibiotics. Antibiotics are an alternative treatment widely used for disease control, so antibiotics are often considered a god's medicine that is effective in curing many diseases (Lusiastuti, 2021) [29].

Antibiotics are generally used to treat human infectious diseases, eventually developing into industries such as fisheries, livestock, and food. Antibiotics are chemical compounds that can inhibit or kill the growth of disturbing microorganisms that can cause problems (Davies and Dorothy, 2010) [11]. The use of antibiotics for an extended period impacts the environment and poses a global threat to health, such as bacterial resistance to antibiotics (Fauziah, 2016) [16]. Antimicrobial resistance (AMR) or antimicrobial resistance occurs due to excessive use of antibiotics and is not by the dosage limit. According to Chowdhury *et al*. (2022) [8], irrational use of antibiotics is due to a lack of knowledge of the limits of antibiotic use for cultivation activities. Antimicrobial resistance causes the death of 700,000 people/per year due to pathogens caused by antibiotic-resistant diseases. It will continue to increase, with predictions of 10 million people/year by 2050 if antibiotic use is not adequately controlled (Hou *et al*., 2023) [22]. Amoxicillin and Enrofloxacin are antibiotics that are still often used to handle aquaculture diseases. Some studies that use amoxicillin and enrofloxacin as antibacterials on pathogenic bacteria that attack aquaculture such as *Vibrio* sp. and *A. hydrophilla* (Rahayuningsih *et al*., 2023) [43], *Vibrio parahaemolyticus* (Pattipeilohy *et al*., 2023) [36], *A.hydrophilla* (Hermawan *et al*., 2022) [20], *Salmonella thypii*, *Salmonella thypi* (Suwandi and Jefri, 2017) [58], *Vibrio harveyi* (Naina *et al*., 2019), *A.hydrophilla* (Safitri *et al*., 2023) [50], *Vibrio*sis (Sarjito *et al*., 2015) [53], *Pantoea* sp. (Sugiani *et al*., 2018) [57], *Vibrio*sis (Yasin, 2021) [65], *A.hydrophilla* (Mustahal, 2022) [35]. Amoxicillin is an antibiotic in the penicillin group active against gram-negative bacteria (Suwandi and Jefri, 2017) [58]. At the same time, enrofloxacin is a type of antibiotic that is included in the fluoroquinolone group. According to Widiyanti *et al*. (2015) [64], enrofloxacin is a broad-spectrum antibiotic that can effectively treat diseases caused by gram-negative and positive bacterial infections.

Efforts can be made to control the excessive use of antibiotics by following the applicable procedures and dosage limits. In addition to managing the use of antibiotics, it is necessary to observe the dose that can be used, and there is still a reasonable limit on the dose of antibiotics that are still widely used in cultivators (Kapoor *et al*., 2017) [26]. Some studies on antimicrobial resistance include amoxicillin, chloramphenicol, ciprofloxacin, ceftriaxone, trimethoprim (Suwandi and Jefri, 2017) [58], ampicillin, gentamicin, amoxicillin, tetracycline, chloramphenicol (Rahmaniar *et al*., 2019) [47], enrofloxacin, erythromycin, oxytetracycline (Sugiani *et al*., 2018) [57], enrofloxacin, oxytetracyline, tetracyline (Safitri *et al*., 2023) [50]. Farmers still often use the antibiotics used in this study, including amoxicillin and enrofloxacin. The sampling location was obtained from Jepara, Central Java. This location was chosen because Jepara has many widespread vaname shrimp farms. According to the Central Java Provincial Government (2019), Jepara has 1,605 hectares of shrimp ponds spread across five coastal districts. One of the five vaname shrimp farms is at DSTP (Directorate of Science and Techno Park) Jepara. Sampling from DSTP Jepara shrimp ponds was conducted to determine the diversity of *Vibrio* sp. bacteria and antibiotic resistance of amoxicillin and enrofloxacin in rearing ponds in DSTP Jepara.

Methodology

Ethical Approval

In this study, animals were not handled directly. It focused only on the observations of Bacteria. All experimental and rearing procedures involving animals were conducted under the National Accreditation for Animal Welfare as outlined in the Republic of Indonesia's SNI 7311:2009.

Time and Place

This research was conducted in December 2023-February 2024. Water sampling water sampling was conducted in Jepara, Central Java. Laboratory sample research was undertaken in the FPIK Laboratory in Building C and J, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang. Marine Science, Diponegoro University, Semarang.

Research Materials

The tools used in this study include the first ose needle as a tool for moving / taking bacterial culture, Petri dish as a container for culturing bacteria, Erlenmeyer as a container for making agar media, test tubes as a container for single colonies, test tube racks as a place to put test tubes, bunsen which functions as a sterilizing device in bacterial culture, paper discs as a tool to test the microbial activity of antibiotics against bacteria, stirring rods as a tool for stirring agar media materials, micropipettes which function as a tool for taking bacterial samples, laminar air flow as a place to culture bacterial samples, matches as a tool to turn on the fire on the bunsen, spatula as a tool for taking agar media material, aluminum foil which functions as a container for making agar media material, digital scales as a tool for weighing agar media making materials, plastic wrap which functions as a test tube wrapper, incubator as a tool for incubating bacteria, magnetic stitter as a tool for stirring the media, autoclave as a tool for sterilizing tools and materials that will be labeled as a marker for samples, hotplate as a tool for heating the media, drop pipette as a tool for taking bacterial samples in liquid media, vortex as a solution mixing tool and the last is a spectrophotometer as a tool for calculating absorbance values. The test materials used in this study are pond water and vaname shrimp as samples of bacterial isolation and amoxicillin and enrofloxacin as test antibiotics. Supporting materials used in this study are bacterial growth media, namely TCBS and TSA, 70% alcohol as an antiseptic, distilled water and sea water as a solvent for agar media materials, tissue as a cleaning agent, sprites as bunsen fuel, sterile gauze, and cotton wool as test tube cover material.

Research Design

The method used in this research is a confirmatory

exploratory method with descriptive analysis. The confirmatory exploratory method aims to test a theory or hypothesis (Aprilia *et al*., 2016) [4]. Exploratory techniques are creative, flexible, and open, where in conducting research, all sources are considered essential to be used as information (Mudjiyanto, 2018) [32]. Descriptive analysis is an analysis carried out to find out each value of a variable, both one or more, which are independent without making a comparative relationship with other variables (Purnia *et al*., 2020) [42]. That way, research using confirmatory exploratory methods and descriptive analysis is carried out to provide an overview or description of the variables.

Work Procedure

The isolation of bacteria used in this study was *Vibrio* sp. bacteria obtained from vaname shrimp rearing ponds in Jepara. Bacterial isolation was done by testing TPC (Total Plate Count) with serial dilution (Yunita *et al*., 2015) [67]. Isolation of bacteria that have been obtained, then gram staining is done to determine the bacteria from gram positive and gram negative and the shape of the bacterial cells after the gram staining process is continued with biochemical tests. Bacterial characterization is carried out by biochemical testing, which consists of various tests, including oxidase test, indole test, Voges Proskauer test, urease test, motility test, ONPG test, starch hydrolysis test, H2S test, and glucose fermentation test. Cowan and Steel's 1993 [9] Manual guides the identification of *Vibrio* bacteria for identifying Medical Bacteria. The inhibition zone test was carried out to determine the extent to which the isolated *Vibrio* sp. bacteria survived against antibiotics used to treat the disease. The initial stage of the inhibition zone test is to prepare agar media in a sterile Petri dish and disc paper. Furthermore, the test bacteria that have been inoculated are diluted to about 0.1 ml and spread on the surface of the agar media with sterile conditions and sterile tools. Sterile paper discs were then dipped in antibiotics that had been dissolved according to the solution of each antibiotic. The antibiotics used were amoxicillin and enrofloxacin. After the disc paper is dipped into the antibiotic solution, the disc paper is carefully placed on the surface of the inoculated agar until the disc paper sticks well to the agar surface. Next, incubation was carried out in an incubator with a temperature of 37ºC for 1-2 x 24 hours. After the incubation process is complete, the Petri dish is taken, and the inhibition zone that has been formed is observed. The inhibition zone can be seen from the clear zone formed around the disc paper. Suppose the clear zone is visible around the disc paper. In that case, it indicates that the *Vibrio* bacteria are sensitive to the antibiotics used. Still, if there is no clear zone around the disc paper, it suggests that the bacteria are resistant to the antibiotics used. The diameter of the clear zone formed is measured using a push-pull term. Resistance tests are carried out to see how bacteria become susceptible or resistant to antibiotics. Resistance tests are carried out by taking bacterial isolates obtained and tested with the antibiotics used. The antibiotics used are amoxicillin and enrofloxacin. Amoxicillin is used with ten micrograms (Tan *et al*., 2020) [60]. The enrofloxacin antibiotic used is enrofloxacin, with a concentration of 3 micrograms (Grabowski *et al*., 2022) [19]. After being given different concentrations of each antibiotic, bacteria that grow in TCBS agar media will show a reaction to the antibiotics used. The response of bacteria to antibiotics can be seen from the observation of the inhibition zone.

Data Analysis

The data obtained from the research were analyzed descriptively. Descriptive analysis is conducted to find out each variable's value, either one or more, that is independent without making a comparative relationship with other variables (Purnia *et al*., 2020) [42]. The results were obtained in the form of the abundance of *Vibrio* sp. bacteria and common bacteria with the TPC method and identification of *Vibrio* sp. bacteria, which includes morphological observations, microscopic observations, biochemical tests, and antibiotic resistance tests.

Result and Discussions

Bacteria Isolation

Table 1 shows the results of calculations on TCBS and TSA media based on the number of bacteria calculated in rearing water and shrimp intestines using the TPC (Total Plate Count) method.

Table 1: TPC calculation results.

TPC (Total Plate Count)/ CFU/mL				
Sample	**Media**			
	Dilution	**TCBS**	**Dilution**	**TSA**
Rearing Ponds	10^{0} 10^{-1}	5 3 x 10^{1}	10^{-9}	TBUD
			10^{-10}	TBUD
			10^{-11}	2,92 x 10^{13}
			10^{-12}	2,49 x 10^{14}
			10^{-13}	2,43 x 10^{13}
Average		1,75 x 10^{2}		1,01 x 10^{14}
	Dilution	TCBS	Dilution	TSA
Shrimp Intestine	10^{-1}	5 x 10^{1}	10^{-9}	TBUD
	10^{-2}	2 x 10^{2}	10^{-10}	2,43 x 10^{12}
	10^{-3}	1 x 10^{3}	10^{-11}	2,1 x 10^{13}
Average		3,4 x 10^{2}		1,15 x 10^{13}

Table 1. shows that the calculation of the average TPC of *Vibrio* sp. bacteria in rearing water on TCBS media obtained is 1.75 x 102 CFU/mL, while the calculation of the average TPC of *Vibrio* sp. bacteria in the shrimp intestine on TCBS media obtained is 3.4 x 102 CFU/mL. The calculation of the average TPC of common bacteria in the rearing water on TSA media obtained is 1.01 x 1014 CFU/mL, while the calculation of the average TPC of common bacteria in the shrimp intestine on TSA media obtained is 1.15 x 1013 CFU/mL. The results of the average *Vibrio* sp. bacteria in rearing water and shrimp's intestines are still in the reasonable category. Still, the results of the average general bacteria in water and shrimp intestines need to be alerted because they have a high average result. According to Ambat *et al*. (2022) [1], the limit value of

the abundance of *Vibrio* sp. bacteria in water is 104 CFU / ml, and the limit value of general bacteria in water is 106 CFU / ml. According to Anjasmara *et al*. (2018) [2], the limit of *Vibrio* sp. abundance in the shrimp body is 104 CFU / ml. The limit of bacterial abundance in the shrimp body is 106 CFU / ml (Eliyani *et al*., 2022) [13]. The abundance of bacteria that have exceeded the maximum threshold, mainly *Vibrio* sp. bacteria, can infect shrimp; if shrimp have been infected with bacteria, the ability of shrimp to survive will decrease over time and can cause mass death in shrimp (Fatmala *et al*., 2019) [15]. The abundance of *Vibrio* sp. and general bacteria in rearing ponds and shrimp intestines is thought to be caused by several factors, including poor water quality (Farrosyi *et al*., 2022) [14].

Several factors, including water pollution, can cause poor water quality. Water pollution is adding material or energy into the waters that causes changes in water quality to damage the use value of water and water resources (Tejo and Tharsisius, 2022) [61]. Water pollution occurs due to human activities that produce various kinds of waste, including household and industrial waste that is discharged into water bodies without management or because currents carry it wind (Siegfried *et al*., 2017) [55]. When the water source used is a polluted supply water source, it can cause various problems in cultivation, such as strains of bacteria that eventually live and develop in the cultivation carried out. The factor of bacterial strains that already exist and grow in aquaculture activities can occur due to uncontrolled feeding, causing an increase in organic matter that triggers the development of *Vibrio* sp. in ponds (Ambat *et al*., 2022) [1]. This can occur because 15% of the feed given to vaname shrimp farming will dissolve in water, and the remaining 85% is consumed, and some become waste at the bottom of the waters, which can increase organic matter (Situngkir *et al*., 2019) [56]. High organic matter can grow bacteria in general; a high-density cultivation system also causes a diversity of bacterial species in water (Ariadi and Tholibah, 2021) [5]. Given the importance of shrimp health level, the countermeasures against the triggers of the onset of disease in shrimp are essential. According to Kharisma and Abdul (2012) [27], efforts that can be made to overcome the abundance of bacteria in ponds that trigger disease can be made by monitoring water quality, giving the correct dose of feed, giving probiotics, or giving antibiotics in the proper dose.

Vibrio sp. bacteria are pathogenic bacteria that infect and can cause disease in weak shrimp conditions and an uncontrolled environment. According to Idami and Rizki (2020) [23], several species of *Vibrio* sp. which can cause *Vibrio*sis disease infections include *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio anguillarum*, *Vibrio alginolitycus*, *Vibrio splendidus* and *Vibrio harveyi*. Apart from the danger of *Vibrio* sp. bacteria on the survival of shrimp, it will cause the same thing to humans, namely foodborne diseases. Foodborne diseases are human diseases caused by pathogenic bacteria through contaminated food or drink. According to Devi *et al*. (2019) [12], three species of *Vibrio* sp. can cause foodborne diseases in humans: *Vibrio cholera*, *Vibrio parahaemolitycus*, and *Vibrio vulminicus*.

Bacteria Characteristics

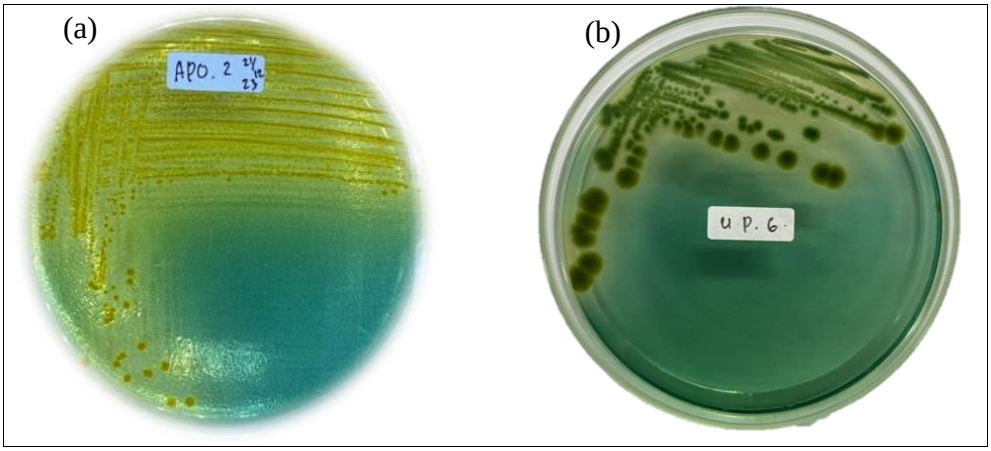
The results of bacterial isolation and Gram staining of rearing water and intestines of vaname shrimp (*Litopenaeus vannamei*) with samples carried out on TCBS media obtained nine pure isolates of bacteria, and the observation results of Gram staining showed that the bacteria are Gram-negative characterized by bacteria that appear in red or pink color after the staining process. The nine characters of bacterial isolates and Gram staining can be seen in Table 2.

Table 2: Bacterial Isolate Character and Gram.

Isolate	Shape	Color	Colony Characteristics		Gram	Shape Gram
A1	Round	Green	Entire	Convex	Negative	Comma
A2	Round	Yellow	Entire	Convex	Negative	Comma
A3	Round	Green	Entire	Convex	Negative	Comma
A4	Round	Green	Entire	Convex	Negative	Comma
UU1	Irregular	Green	Undulate	Convex	Negative	Comma
UU2	Irregular	Green	Irregular	Flat	Negative	Comma
UU3	Round	Yellow	Undulate	Convex	Negative	Comma
UU4	Round	Green	Entire	Convex	Negative	Comma
UU5	Irregular	Green	Irregular	Convex	Negative	Comma

Description: A: Rearing Ponds, UU: Shrimp intestine

The results obtained from the nine isolates consist of water isolates (4 isolates) and shrimp intestines (5 isolates), which have characters including round and irregular shapes, green and yellow colors, smooth, jagged, and irregular edges, and convex and flat elevations. The results of Gram staining showed Gram-negative. Single isolates obtained from enlarged water and shrimp intestines can be seen in Figure 1.



Description: (a) Pure isolate of rearing ponds A2, (b) Pure isolate of shrimp intestine UU4

Fig 1: Pure isolates from rearing ponds and shrimp intestines.

Results of biochemical identification of bacteria with identification referring to Cowan & Steel's (1993) [9] guidebook to see the level of similarity in the results of bacterial types. The results of identifying the nine bacteria can be seen in Table 3.

Table 3: Results of Identification of 9 Bacterial Isolates.

Isolate	Type of bacteria	Cowan & Steel's (1993) [9]
A1	*Vibrio parahaemolyticus*	81,4%
A2	*Vibrio anguillarum*	92,5%
A3	*Vibrio metschnikovii*	81,4%
A4	*Vibrio parahaemolyticus*	85,1%
UU1	*Vibrio parahaemolyticus*	81,4%
UU2	*Vibrio anguillarum*	92,5%
UU3	*Vibrio parahaemolyticus*	81,4%
UU4	*Vibrio parahaemolyticus*	85,1%
UU5	*Vibrio parahaemolyticus*	81,4%

Description: A: Rearing Ponds, UU: Shrimp intestine

The results of biochemical bacterial identification showed that the nine isolates were identified as *Vibrio parahaemolyticus* (A1, A4, UU1, UU3, UU4, UU5), *Vibrio anguillarum* (A2, UU2) and *Vibrio metschnikovii* (A3).

Based on the results of the morphological identification of *Vibrio* sp., it has two colors: green and yellow. The color difference occurs due to different types of *Vibrio* sp. bacteria. According to Ambat *et al*. (2022) [1], green bacterial colonies are caused by the kind of bacteria *Vibrio* sp., which is unable to ferment sucrose, while the yellow bacterial colonies are caused by the type of bacteria *Vibrio* sp., which can ferment sucrose in agar media, namely TCBS. The shape of the bacterial cell *Vibrio* sp. shows two shapes, namely round (circular) and irregular (irregular); this is by research by Ashofa *et al*. (2014) [7] indicates that *Vibrio* sp. has round (circular) and irregular (irregular) cell shapes. Elevation of the bacteria *Vibrio* sp. shows convex and flat elevations; this is by research by Rosmalina *et al*. (2023) [48], which shows that the bacteria *Vibrio* sp. has convex and flat elevations. The edge shape of the bacteria *Vibrio* sp. shows a smooth edge shape (entire) with more dominance and a wavy edge shape (endulate); this is by research by Hidayat (2014) [21] which shows that the bacteria *Vibrio* sp. has a smooth edge shape (entire) and a wavy type shape (endulate).

Vibrio sp. bacteria. can develop when there is already a carrier of bacterial disease or from water media contaminated with bacteria thought to come from leftover feed. According to Feliatra *et al*. (2014) [18], several species of *Vibrio* sp. which are often found to cause disease in shrimp include *Vibrio alginolyticus*, *Vibrio anguilarum*, *Vibrio parahaemolitycus*. One of the shrimp diseases caused by the bacteria *Vibrio* sp. is White Feces Disease (WFD) (Marbun *et al*., 2019) [31]. This disease occurs due to the combination of *Vibrio* sp., yellow like *Vibrio* alginolyticus, with the bacteria *Vibrio* sp. green ones like *Vibrio parahaemolyticus* (Widigdo *et al*., 2021) [63]. *Vibrio* sp. bacteria. With green colonies have greater pathogenicity than yellow colonies, so they are more often the cause of shrimp disease (Ramadhani *et al*., 2022) [46].

Based on the results of microscopic observations of Gram staining carried out using the staining technique of Hans Christian Gram (1884), the bacteria *Vibrio* sp. shows that the gram color of the bacteria turns pink. According to Dahlia *et al*. (2017) [10], the bacteria *Vibrio* sp. observed under a red microscope indicates that the bacteria are Gram-negative. *Vibrio* sp. bacteria. They are classified as harmful bacteria because they have a long and round shape, are included in Gram-negative bacteria characteristics, are facultative anaerobes, or can live with or without oxygen. *Vibrio* sp. bacteria. It is Gram-negative whose single cells have a short, bent or straight rod shape, has a length of 1.4-5.0 and a width of 0.3-1.3, are motile (move), and has a polar flagellum (Mahulauw *et al*., 2022) [30]. *Vibrio* sp. bacteria. It is pink because the crystal violet dye complex dissolves in the alcohol solution, so it turns red from adding safranin. According to Nurhidayati *et al*. (2015) [35], the difference in the color of Gram-positive and Gram-negative occurs due to differences in the cell wall structure. Gram-positive has thick cell walls with peptidoglycan content, while Gram-negative has high lipid content. They are observing Gram bacteria in a microscope with a magnification of 10x-40x, showing that the shape of the bacterial cells is short rod-like comma shape. This is by Hidayat (2014) [21], who stated that the form of the bacteria *Vibrio* sp. is a brief, bent stem (coma). Gram staining is also helpful in knowing the characteristics of *Vibrio* sp. bacteria before further testing is carried out.

Antibiotic Resistance Test

The antibiotic resistance test uses two types of drugs that can be given to aquatic bacteria, including Amoxicillin and Enrofloxacin. The results of every kind of antibiotic have different inhibitory abilities. The doses used for the two antibiotics are 60ppm, 100ppm, 200ppm, 300ppm, 400ppm and control. The results of the antibiotic resistance tests were observed for 24 hours, and the inhibition zone formed by each

antibiotic treatment of *Vibrio* sp. bacteria was examined. The results of the antibiotic resistance test for amoxicillin and enrofloxacin are seen in Table 4.

Table 4: Resistance test results for the antibiotics amoxicillin and enrofloxacin.

***Vibrio* sp.**	**Obstacles Zone (mm)**									
	Amoxicillin (ppm)					**Enrofloksasin (ppm)**				
	60	**100**	**200**	**300**	**400**	**60**	**100**	**200**	**300**	**400**
Vibrio parahaemolyticus	4.50	3.00	0.00	0.50	3.50	1.50	0.50	2.50	1.50	5.00
Vibrio anguillarum	1.00	2.00	3.00	1.00	8.50	1.00	1.00	2.50	2.50	4.50
Vibrio metschnikovii	0.00	1.50	0.00	0.50	0.50	0.00	1.25	5.50	1.50	6.00
Vibrio parahaemolyticus	4.00	14.0	9.00	10.0	7.00	2.50	2.00	3.00	5.00	8.00
Vibrio anguillarum	0.00	0.00	0.50	0.00	1.50	0.00	2.00	0.00	2.00	1.50

The inhibition zone formed's results are different for each dose depending on the sensitivity and susceptibility of the test bacteria. The results of the antibiotic test can be seen in Figure 2.

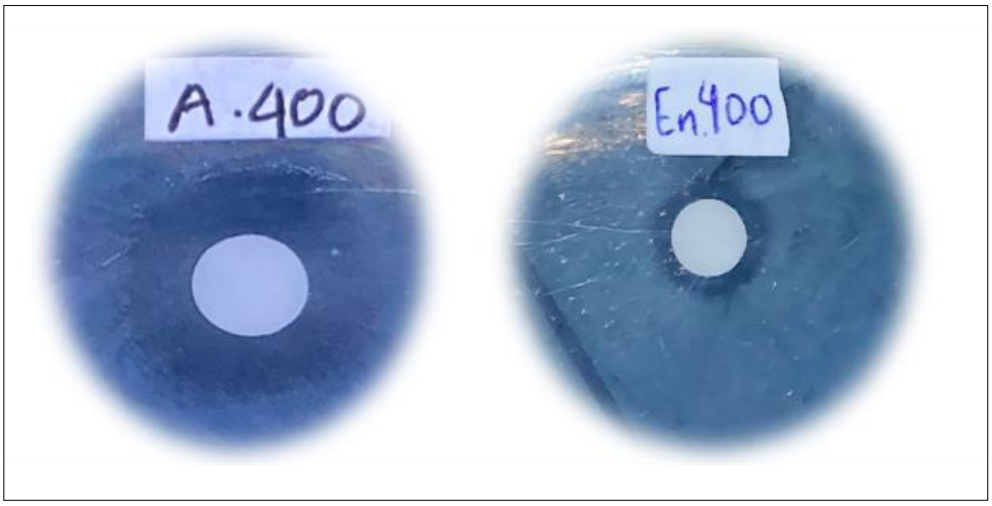


Fig 2: Inhibition zone results in the antibiotic test. Description: (a) Inhibition Zone 8.50 mm Amoxicillin dose 400 ppm, (b) Inhibition Zone 4.50mm Enrofloxacin dose 400 ppm

The antibiotic resistance test uses two types of antibiotics, namely amoxicillin and enrofloxacin. Each type of antibiotic used can inhibit and kill the growth of different bacteria. The solubility of antibiotics in water is significant because treatment for fish farming is often carried out using the soaking method. The antibiotics amoxicillin and enrofloxacin results at the doses given were in the resistant category. The sensitivity test refers to the provisions of the Clinical and Laboratory Standards Institute (CLSI, 2912). The sensitivity value is at an inhibitory zone diameter of > 17 mm, intermediate is 13-16 mm, and resistant is < 12 mm. In *Vibrio parahaemolyticus* bacteria with the antibiotic amoxicillin, the average result of the inhibition zone formed was 2.41 mm. This result shows that this bacteria is resistant, which refers to the standard of the inhibition zone formed (Rahmaniar *et al*., 2019) [47]. The antibiotic enrofloxacin on this bacteria showed that the average result of the inhibition zone formed was 1.98 mm. This result indicates that this bacteria is resistant, which refers to the standard of the inhibition zone formed (Sugiani *et al*., 2018) [57]. In *Vibrio anguillarum* bacteria with the antibiotic amoxicillin, the average result of the inhibition zone formed was 1.75 mm. These results indicate that the bacteria are resistant, referring to the standard of the inhibition zone formed (Syafriana *et al*., 2020) [59]. The antibiotic enrofloxacin on this bacteria showed that the average inhibition zone formed was 1.7 mm. These results indicate that this bacteria is resistant, referring to the standard inhibition zone formed (Safitri *et al*., 2023) [50]. In *Vibrio metcshnikovii* bacteria with the antibiotic amoxicillin, the average result of the inhibition zone formed was 0.5 mm. These results indicate that the bacteria are resistant, which refers to the standard of the inhibition zone formed (Artati *et al*., 2016) [6]. The antibiotic enrofloxacin on this bacteria showed that the average inhibition zone formed was 2.85 mm. These results indicate that this bacteria is resistant, referring to the standard inhibition zone formed (Perkasa *et al*., 2019) [38]. The resistance test results using a reference to standards have shown resistance values for each antibiotic at different doses. However, several antibiotics tested at various doses still formed an inhibition zone/clear zone, as seen in Figure 2. By looking at the results of the inhibition zone/clear zone that is formed, it indicates that several antibiotics at various experimental doses can still fight the *Vibrio* sp. bacteria. Even though the clear zone formed is still at a low value or the inhibitory power is weak. According to Zulkarnain *et al*. (2021) [68], one of the criteria used in determining antibacterial strength is to look at the diameter of the inhibition zone formed; an inhibition zone of 15-20 mm is potent inhibition, 10-14 mm is medium inhibition and 0 -9 is weak resistance. The low level of sensitivity can also be caused by the wall structure of Gram-negative bacteria, which is more complex than the cell structure of Gram-positive bacteria. According to Pelczar *et al*. (2008) [37], the wall structure of Gram-negative bacteria has three layers, namely the outer, middle, and inner layers, while Gram-positive bacteria only have one layer. This could be the reason why the level of antibiotic sensitivity is relatively tiny. According to Jamili *et al*. (2014) [24], the relatively complex structure of the walls of Gram-negative

bacteria makes it difficult for antibiotics to enter cells and find targets to work on. Other factors thought to cause antibiotic resistance can also be genetic or non-genetic. According to Setyaningsih (2004) [54], genetic resistance occurs by conjugation and transudation between the same strains, while non-genetic resistance can occur through excessive administration of antibacterials. Antibiotics for treatment must be done correctly and rationally, considering the dose or indication of disease and if alternative treatments other than antibiotics can also be applied. According to Suwandi and Jefri (2017) [58], improper use of antibiotics causes antibiotics to work optimally no longer. Another impact resulting from using antibiotics too often and over a long period is the emergence of resistance in microorganisms to various antibiotics (Pratiwi, 2017) [40].

Conclusion

Morphology of *Vibrio* sp. bacteria. has two colors, green and yellow. The shape of the bacterial cell *Vibrio* sp. shows two shapes: round (Cilular) and irregular (irregular). Elevation of the bacteria *Vibrio* sp. shows convex and flat elevations. The edge shape of *Vibrio* sp. shows a smooth edge shape (entire) with more dominance and a wavy edge shape (endulate). Identify the type of bacteria *Vibrio* sp. in vaname shrimp rearing ponds in Jepara; various kinds of *Vibrio* sp. bacteria were found. These include *Vibrio parahaemolyticus*, *Vibrio anguillarum*, and *Vibrio metschnikovii*. The antibiotics amoxicillin and enrofloxacin tested in rearing ponds have entered the resistant category, which indicates that the bacteria *Vibrio parahaemolyticus*, *Vibrio anguillarum*, and *Vibrio metschnikovii* are resistant to the antibiotics amoxicillin and enrofloxacin.

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