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Sensitivity of *Vibrio alginolyticus* to Antibiotics Tetracycline, Amoxicyllin, and Ampicillin from Intensive Shrimp Cultivation Media in Kendal Regency

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A B S T R A C T :

Vannamei shrimp farming in Indonesia has been widely practiced using intensive farming systems. In practice, intensive cultivation encounters numerous problems, such as disease outbreaks caused primarily by infections from Vibrio spp. bacteria. Vibrio bacterial infections can lead to shrimp mortality rates of up to 100%. The high usage of antibiotics by farmers to treat vibriosis has resulted in antibiotic resistance. This study aimed to identify and characterize the types of Vibrio bacteria resistant to tetracycline, amoxicillin, and ampicillin antibiotics from intensive ponds in Kendal Regency. This study employed a confirmatory exploratory research method. The colony counts indicated that Vibrio colonies ranged from 5.8×10^4 to 1.82×10^7 CFU/mL, with two isolates classified as TSUD. The number of common bacterial colonies was higher, ranging from 4.2×10^7 to 2.95×10^8 CFU/mL.Five of the eleven successfully isolated colonies exhibited a round morphology with the typical green-yellow color associated with Vibrio colonies, smooth texture, flat edges, and convex and umbonate elevations. Based on the biochemical characterization results, the five isolates were identified as Vibrio alginolyticus. Sensitivity tests showed that four of the five isolates were sensitive to the three antibiotics, while the remaining isolate was resistant to all tested antibiotics. This resistance pattern indicates a potential threat of antibiotic resistance in environmental pathogenic bacteria, which could impact disease control efforts in the aquaculture sector. These findings are expected to inform improved aquatic environmental management and the selection of natural therapies as alternatives to antibiotics for Vibrio alginolyticus infections.

Keywords: antibiotics; intensive farming; sensitivity; shrimp; Vibrio

1. Introduction

Shrimp is a leading fishery commodity widely cultivated in Indonesia because of its delicious taste and high protein content. A 7.32% increase in global demand in 2022 is expected to drive national shrimp production to 1.19 million tonnes (KKP, 2023). Furthermore, shrimp contribute up to 60% of the value of fishery exports (Suhana *et al.,* 2023). Shrimp exports in 2023 reached 220,889.26 tons, valued at USD 1,729,521.19 (KKP Statistics 2024). Given the significant potential of the export market, the government is targeting an increase in shrimp production to 2 million tons by 2024 (Directorate General of Aquaculture Public Relations 2021). To meet market demand, many farmers have implemented intensive cultivation systems.

Intensive shrimp farming has been implemented in various regions, including Kendal Regency, which has significant potential for the development of whiteleg shrimp farming (Awanis *et al*., 2017). The location for shrimp farming development in this area is considered safe and strategic (Sagita *et al*. 2015). This is evidenced by the increase in the intensive land area from 745,000 m² in 2022 to 753,000 m² in 2023 (KKP, 2025). However, intensive systems pose challenges, such as pollution due to high stocking densities and leftover feed, which degrade the water quality (Dauda, 2019; Said *et al*., 2024). These conditions trigger stress and disease in shrimp, particularly *Vibrio* sp. bacterial infections, which can cause mortality in the postlarval and juvenile stages (Kennedy *et al*., 2016; Sanguanrut *et al*., 2018).

To address bacterial infections, cultivators typically use antibiotics such as tetracycline, which are still permitted for use (PERMEN-KP, 2019; Phan *et al*., 2024). However, excessive use of antibiotics can lead to antimicrobial resistance (AMR), cause residue accumulation, and pose risks to human health (Zago *et al*., 2020; Dewi *et al*., 2023; Nguyen *et al*., 2024). The issue of AMR in aquaculture has become a global concern, with deaths due to resistant pathogens reaching 700,000 people per year and potentially increasing to 10 million (Lusiastuti, 2021; Murray *et al*., 2022). Sarjito *et al*. (2016) found resistance to erythromycin, enrofloxacin, and oxytetracycline in Kendal ponds. Resistant bacteria are difficult to treat and control (Sundari, 2022). Therefore, further studies on bacterial sensitivity to antibiotics are required to determine effective treatment methods for vibriosis in shrimp.

This study aimed to examine the sensitivity of *Vibrio* bacteria derived from intensive shrimp farming media in Kendal to several types of antibiotics. The research findings are expected to provide scientific information regarding the resistance levels of *Vibrio* to antibiotics and serve as a basis for implementing judicious and sustainable disease control strategies in intensive shrimp farming systems.

2. Materials and Methods

Research Design

This research is an exploratory-confirmatory research with a quantitative approach, conducted through field and laboratory studies. The exploratory method is a research approach aimed at seeking or exploring new ideas and findings (Stebbins, 2012; Megawati *et al*., 2014). Exploration is conducted to gather data regarding a hypothesis or issue from multiple sources. After conducting the exploration, the researcher validated or confirmed the findings by testing the data obtained and aligning them with the existing hypothesis. The purpose of the confirmatory method is to validate and test the compatibility of theories through identification (Reio & Shuck, 2014; Isaputra *et al.,* 2024). This method is used to determine the development of AMR in *Vibrio* sp. bacteria during the cultivation of vannamei shrimp (*L. vannamei*), which is widespread. Data were collected by exploring samples taken from intensive shrimp ponds in Kendal. The obtained samples underwent antibiotic sensitivity testing, followed by morphological characteristic observation. The collected data were sorted to determine which isolates were identified using the VITEK 2 Compact system.

2.2. Research site

This study was conducted on November 2024 – April 2025. The samples used were culture media sourced from intensive vannamei shrimp ponds in Kendal. All laboratory activities, including isolation, serial dilution, purification, bacterial morphological observation, and bacterial sensitivity testing, were performed in the microbiology laboratory of the Aquaculture Department at Diponegoro University, Semarang.

2.3. Research Materials

The tools used during the research included an inoculating needle, spreader, Petri dishes, test tubes, test tube racks, Erlenmeyer flasks, autoclave, Laminar Air Flow, refrigerator, incubator, calipers, hot plate, stirrer, measuring cylinder, analytical balance, orbital shaker, cotton swabs, vortex, Bunsen, micropipettes, sample bottles, and cool boxes. The materials used in this research are Thiosulphate Citrate Bile Salt Sucrose (TCBS) media, Zobell Marine Agar (ZMA) media, Zobell Marine Broth (ZMB) media, Nutrient Agar (NA) media, Mueller Hinton Agar (MHA) media, 70% alcohol, physiological saline solution (NaCl), paper discs, Phosphate Buffer Saline (PBS) solution, aluminum foil, plastic wrap, and labels.

2.4. Sterilization

Sterilization is an important initial step in microbiological activities to kill microbes on equipment and media to avoid contamination (Junaini *et al*., 2019). Media such as Mueller Hinton Agar (MHA) and Zobell were sterilized using an autoclave at 121°C for 15 min by covering the Erlenmeyer flask with aluminum foil (Koyongian *et al.,* 2020). Heat-resistant glassware, such as Erlenmeyer flasks, test tubes, L-glasses, loop needles, and measuring cylinders, were washed, dried, and wrapped before being placed in the autoclave. According to Ambat *et al*. (2022), test tubes are covered with sterile cotton and wrapped in plastic and paper, whereas Erlenmeyer flasks are covered with aluminum foil. After ensuring the appropriate water content in the autoclave, the equipment was sterilized at 121°C for 15 min, then removed after the temperature dropped to 70°C, and placed in a sterile area. Meanwhile, non-glass and heat-resistant tools were sterilized using 70% alcohol and a Bunsen burner.

2.5. Media Preparation

The media used in this study included TCBS, ZMA, ZMB, NA, and MHA. TCBS media is a selective medium for growing *Vibrio* sp. and is prepared by dissolving TCBS powder in distilled water using a magnetic stirrer and a hotplate at 250–300°C until boiling. The mixture was then poured into Petri dishes in a LAF near a Bunsen burner and allowed to solidify (Ambat *et al*., 2022). The media were ready for use after 24 h to ensure that they were free from contamination.

The preparation of NA, ZMA, ZMB, and MHA media was carried out in a similar manner, but was sterilized using an autoclave. The agar powder was weighed to the nearest 0.001 g, added to seawater, and then dissolved using a hotplate at 220–270°C until homogeneous. The media were then sterilized at 121°C for 15 min (Susanti *et al*., 2023). After cooling, the media were poured into their respective containers: NA into test tubes (tilted until solidified and covered with plastic wrap), ZMB into test tubes with sterile cotton and plastic wrap, and ZMA and MHA into petri dishes and left for 24 h before use (Jannah *et al*., 2017).

2.6. Sampling

The samples used were water samples from intensive vannamei shrimp (*L. vannamei*) ponds in Kendal. Samples were collected from the ponds, with sampling points located at the edge (Sivaraman *et al*., 2021). The samples were obtained from three different ponds and were coded N2.A1 for pond 1, N2.A2 for pond 2, N2.A3 for pond 3, and N2.AI for the inlet pond. The samples were placed in coded sample bottles and stored in a cool box until analysis. The samples were isolated and subjected to sensitivity testing at the Microbiology Laboratory, Building C, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University.

2.7. Serial Dilution

Serial dilutions were performed to reduce the bacterial density in the samples. The serial dilution method used was a modification of that described by Argiantini *et al*. (2021) and Wantania *et al*. (2016). Serial dilutions were performed using a 1:9 ratio, which involved taking 100 µL of sample water, equivalent to 0.1 mL, using a micropipette. This was then dissolved in 900 µL or 0.9 mL of PBS solution and homogenized using a vortex. For the second dilution, 0.1 mL of the sample solution from the first dilution was taken and dissolved in 0.9 mL of PBS solution. The same procedure was used for the subsequent dilutions. Sample dilutions were performed several times to reduce the bacterial density in the initial sample.

2.8. Total Plate Count (TPC) Calculation

Bacterial colony counting was performed using the Total Plate Count (TPC) method to determine the microbial density in a sample by counting colonies growing on agar media (Rizki *et al.,* 2022). TPC was performed on common bacteria on NA media and *Vibrio* bacteria on TCBS. A 50 µL sample was taken using a microtip and spread over the surface of the media using the spread plate technique with an L-glass (Irawati *et al*., 2022). The process was carried out aseptically in a LAF near a Bunsen burner and then incubated for 18–24 h at 35°C. After incubation, the number of colonies was counted using a colony counter, and the results were expressed using the following formula:

$$\text{TPC (CFU/mL)} : \frac{\text{Number of colonies per plate} \times \text{Dilution factor}}{\text{Sample volume}}$$

2.9. Bacterial Purification

Purification is carried out by isolating bacteria from TCBS media to grow and separate the desired bacteria from other types in the sample (Sabbathini *et al*., 2017). The goal was to obtain a pure isolate (single colony) for further testing. The streak plate method was used in this process, which involved scratching the isolated bacterial colonies onto new media using a sterile loop needle in three quadrants (Wulandhani *et al*., 2024). The medium was then wrapped in plastic and incubated for 24 h at 34°C (Anjani *et al*., 2022). The entire process was performed aseptically in a LAF near a Bunsen burner to prevent contamination. After incubation, the morphology of the isolates was observed, including color, shape, elevation, edges, optical appearance, and size.

2.10. Bacterial Identification

Bacterial characterization was performed through biochemical identification using the VITEK 2 Compact, an automated tool with 48 reagent slots for bacterial identification (Pincus, 2014; Wahid, 2020; Sarjito *et al.,* 2021). The working process of this tool includes sterilization, inoculation, incubation, and analysis of results based on biochemical reactions. Identification was performed using an ID-GNB card containing 64 wells with various fermentation, assimilation, decarboxylase, and enzymatic tests (Sibadu *et al*. 2023). A suspension of bacterial isolates with a 0.5 McFarland standard was inoculated onto the card using a vacuum device, sealed, and inserted into the VITEK 2 Compact reader module. Fluorescence was measured every 15 min, and identification results were obtained within 3 h (Stefaniuk *et al*., 2005). Identification is based on color changes due to enzymatic reactions between substrates and chromophore reagents, such as 7-AMC and 4-MU, which produce fluorescence to automatically determine bacterial species (Al-Kraety *et al*., 2020; Sobhy and Shaltout, 2020).

2.11. Antibiotic Susceptibility Test (AST)

The Antibiotic Susceptibility Test (AST) aims to determine the level of bacterial sensitivity to antibiotics using the agar diffusion method on Mueller Hinton Agar (MHA) media (Dwyana and Murniati, 2020; CLSI, 2021). Bacterial isolates were cultured on ZMB media for 24–48 h on a shaker at 1200 rpm until the media became cloudy, indicating bacterial growth (Wantania *et al*., 2016). The culture turbidity was adjusted to the McFarland 0.5 standard to ensure uniform bacterial counts (Rosmania and Fitri, 2020).

Sterilized MHA media were aseptically poured into Petri dishes and allowed to solidify. A cotton swab was dipped into the bacterial suspension and streaked evenly across the medium. Four paper disks were placed on the surface of the media, each treated differently: sterile distilled water (control), antibiotics tetracycline (30 µg), ampicillin (10 µg), and amoxicillin (20 µg) (CLSI 2021; Devads *et al*. 2024). After the antibiotics were absorbed, the Petri dishes were wrapped in plastic wrap and incubated for 24 h at 30°C. The resulting inhibition zones were measured using a vernier caliper (mm) and categorized as resistant (R), moderately sensitive (MS), or sensitive (S) (CLSI, 2021). The inhibition zone was calculated using the following formula:

$$\frac{(\text{Dv} - \text{Ds}) + (\text{Dh} - \text{Ds})}{2}$$

where Dv, Dh, and Ds are the vertical, horizontal, and disc diameters, respectively.

3. RESULTS AND DISCUSSION

3.1. Total Plate Count (TPC)

Bacterial colony counts were performed to determine the number of bacteria in the samples. This count included common bacteria and *Vibrio* sp. obtained from each pond: ponds 1, 2, 3, and the inlet pond. Of the four ponds, the highest TPC of common bacteria was observed in pond 3, whereas the highest TPC of *Vibrio* sp. was observed in pond 1 (Table 1).

Table 1. Results of Total Plate Count (TPC) Calculation

Total Plate Count (TPC) CFU/mL calculation				
Sample Code	**Sample Origin**	**Media**		**Comparison**
		TCBS	**NA**	
N2.A1.	Pond 1	$1.82x10^7$	$5.66x10^7$	1:3,1
N2.A2	Pond 2	TSUD	$4.2x10^7$	can't be compared
N2.A3	Pond 3	$5.8x10^4$	$2.95x10^8$	1:5086
N2.AI	Inlet pond	TSUD	$2.44x10^8$	can't be compared

The results of bacterial density calculations showed that the abundance of *Vibrio* sp. was less than that of general bacteria. Among the four ponds, pond 3 had the highest density of general bacteria, with a bacterial density of $2.95x10^8$ CFU/mL. The highest abundance of *Vibrio* sp. was observed in the first pond, with a density of $1.82x10^7$ CFU/mL. The density of *Vibrio* sp. in pond two and the inlet pond was classified as TSUD. TPC is said to be TSUD if the number of *Vibrio* sp. bacterial colonies is less than the TPC calculation limit of 25-250 colonies so it does not meet the TPC calculation requirements (Aris *et al.,* 2024). The density of general bacteria in all ponds is classified as unsafe and exceeds the threshold for shrimp cultivation. Meanwhile, the density of *Vibrio* sp. bacteria that exceeded the threshold was only in pond 1. The maximum limit for general bacterial density in water is $10^{6,}$ whereas the threshold for *Vibrio* sp. bacterial density is 10^4. which is suitable for shrimp cultivation, namely no more than 10^4 CFU/mL (Kharisman and Manan, 2012; Ariyati *et al*., 2023).

High bacterial counts in water are closely linked to the emergence of diseases and even mass mortality. High bacterial densities cause physiological damage to cultivars, such as gill and other organ damage due to bacterial stress (Wang *et al*., 2020). Furthermore, the pathogenic nature of *Vibrio* sp. bacteria emerges when their abundance exceeds $8.3x10^4$ CFU/mL (Idami and Nasution, 2020). Bacterial density is influenced by cultivar stocking density, nutrient abundance, and the physics and chemistry of pond water. Cultivator stocking density affects environmental conditions and water quality because of the feeding intensity (Esparza Leal *et al*., 2020). Nutrient abundance resulting from excessive feed residue triggers competition for nutrient utilization by bacteria, resulting in an increase in bacterial numbers (Bauer *et al*., 2018; Tinh *et al*., 2023).

3.2. Characterization of Vibrio sp. Bacteria

Bacterial morphology was assessed based on the colony shape, color, elevation, margin, optical appearance, and size. Bacterial purification yielded 11 isolates with the morphology shown in Table 2.

Table 2. Bacterial Colony Morphology from Water Samples in TCBS Media

Isolates Code	**Origin of Pond**	**Colony Morphology**					
		Colour	**Shape**	**Margin**	**Elevation**	**Opacity**	**Size**
N2.A1.B.2.A	1	yellow	round	entire	umbonate	opaque	large
N2.A2.B	2	yellow	round	entire	convex	opaque	large
N2.A2.1	2	green	round	entire	convex	opaque	medium
N2.A3.C.2	3	green	round	entire	flat	trans-lucent	large
N2.A3.I.2.B	3	green	round	entire	flat	trans-lucent	large
N2.A3.I.2.A	3	dark green	round	entire	convex	trans-lucent	large
N2.A3.I.1	3	green	round	entire	convex	trans-lucent	large
N2.A3.I.2	3	yellow	round	entire	convex	opaque	medium
N2.AI.B.2. B.H	Inlet	yellow	round	entire	convex	opaque	medium
N2.AI.B.2. B.K	Inlet	yellow	round	entire	convex	opaque	large
N2.AI.2.B.2	Inlet	greenish yellow	round	entire	convex	trans-lucent	large

From 11 isolates originating from intensive shrimp culture media in four different ponds, five isolates were selected based on their morphology and location of origin. The results for the selected isolates are presented in Table 3.

Table 3. Morphology of the Five Selected Isolates

Isolates Code	Origin of Pond	Colony Morphology					
		Colour	Shape	Margin	Elevation	Opacity	Size
N2.A1.B.2.A	1	yellow	round	entire	umbonate	opaque	large
N2.A2.B	2	yellow	round	entire	convex	opaque	large
N2.A3.I.2.A	3	dark green	round	entire	covex	translucent	large
N2.AI.B.2. B.H	*inlet*	yellow	round	entire	convex	opaque	medium
N2.AI.B.2. B.K	*inlet*	yellow	round	entire	convex	opaque	large

Based on the results in Table 3. five isolates were selected based on differences in morphology performance and source. The morphology was yellow and dark green; round; entire margin; convex and umbonate elevation; opaque and translucent; and large and medium size. The five isolates are shown in Figure 1.

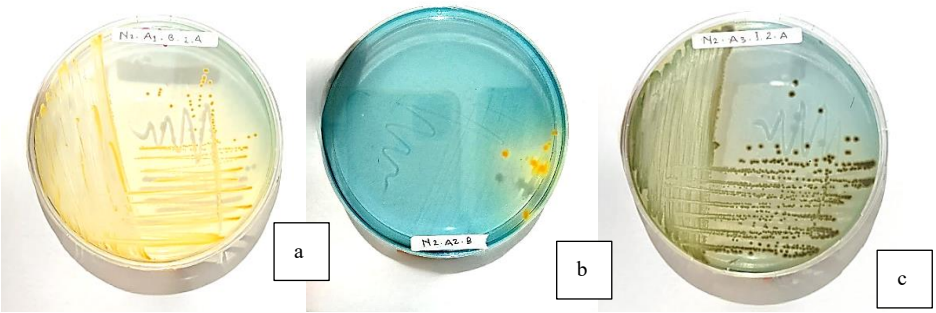

Figure 1. Morphology of isolate from shrimp cultivation media

Remarks: a) Isolate N2.A1.B.2.A; N2.AI.B.2.B.H, and N2.AI.B.2.B.K b) Isolate N2.A2.B, c) Isolate N2.A3.I.2.A

Based on the morphology of the five isolates, they were similar to the general morphology of *Vibrio* sp. bacteria. Several studies have stated that *Vibrio* bacteria are generally characterized by a round shape, entire edges, converging elevations, and a yellow to green color with a diameter of 2-5 mm (Hikmawati *et al*., 2019; Handayani *et al*., 2020). In several studies, it has been stated that yellow *Vibrio* colonies are identical to *V. cholerae, V. alginolyticus,* and *V. harveyi*, while green colonies are identical to *V. parahaemolyticus, V. vulnificus, V. fischery,* and *V. mimicus* (Widigdo *et al*., 2021; Tamrin *et al*., 2024). Green colonies are unable to ferment sucrose on TCBS media, while yellow colonies are able to ferment sucrose and lower the pH (Ihsan and Retnaningrum, 2017; Handayani *et al*., 2020; Ambat, *et al.*, 2022).

3.. Identification of *Vibrio* species Bacteria

Isolates selected based on their morphology were biochemically identified using VITEK 2 Compact. This instrument identifies bacteria by analyzing their biochemical profiles. The identification results based on the bacterial biochemical profiles indicated that all five isolates were *V. alginolyticus* with a high degree of similarity (Table 4).

Table 4. Results of Bacterial Biochemical Tests Using VITEK 2 Compact

Test Parameters	N2.A1. B.2.A.	N2.A3. I.2.A	N2.AI. B.2.B.K	N2.AI. B.2.B.H	N2.A2.B
APPA	+	+	+	+	+
ADO	-	-	-	-	-
Pyr A	+	+	+	-	+
lARL	-	-	-	-	-
dCEL	-	-	-	-	-
BGAL	-	-	-	-	-
H2S	-	-	-	-	-
BNAG	+	+	+	+	+
AGLTp	-	-	-	-	-
dGLU	+	+	+	+	+
GGT	-	-	-	-	-
OFF	-	-	-	-	-
BGLU	-	-	-	-	-
dMAL	+	+	+	+	+
dMAN	+	+	+	+	+
dMNE	+	+	+	+	+
BXYL	-	-	-	-	-

Balap	-	-	-	-	-
ProA	+	+	+	+	+
LIP	-	-	-	-	-
PLE	-	-	-	-	-
Tyr A	+	+	-	-	-
URE	-	-	-	-	-
dSOR	-	+	-	-	-
SAC	+	+	+	+	+
dTAG	-	-	-	-	-
dTRE	+	+	+	+	+
CIT	-	-	-	-	-
MNT	-	-	-	-	-
5KG	-	-	-	-	-
lLATk	+	+	-	-	-
AGLU	-	-	-	-	-
SUCT	-	-	-	-	-
NAGA	-	-	-	-	-
AGAL	-	-	-	-	-
PHOS	-	+	+	+	-
GlyA	-	-	-	-	-
ODC	-	-	-	-	-
LDC	-	-	-	-	-
lHISa	+	-	-	-	-
CMT	+	+	+	+	+
BGUR	-	-	-	-	-
O129R	-	-	-	-	-
GGAA	+	+	+	+	+
lMLTa	+	+	-	-	-
ELLM	-	-	-	-	-
lLATa	+	-	-	-	-
Bacterial Species	*V. alginolyticus (97%)*	*V. alginolyticus (97%)*	*V. alginolyticus (97%)*	*V. alginolyticus (99%)*	*V. alginolyticus (99%)*

Biochemical test results showed that *V. alginolyticus* was positive for acid production from glucose, mannitol, and sucrose; utilization of glucose, mannitol, N-acetyl-glucosamine, gluconate, and malate; and negative for H_2S production, acid production from inositol, sorbitol, ranose, arabinose, lactose, and salicin; urea hydrolysis; arginine dihydrolase; and ß-galactosidase. Parmar *et al*. (2020) stated that *V. alginolyticus* was able to produce acid from the fermentation of sugars such as cellobiose, dextrose, fructose, galactose, maltose, mannose, sucrose, and trehalose. In addition, there were differences in the probability figures caused by differences in the test results of the eight parameters. These parameters included PyrA, TyrA, dSOR, lLATk, phosphatase, lHISa, lMLTa, and lLATa. In the study by Kwok *et al*. (2024), the results of the VITEK 2 Compact test on *V. alginolyticus* bacteria on the parameters PyrA, TyrA, dSOR, phosphatase, and lHISa were positive. The parameters lHISa, lMLTa, and lLATa showed negative results.

These differences are caused by several factors, such as differences in enzyme expression that occur during the isolation process, varying intrastrain biochemical profiles, and technical and system-related factors. Lowe *et al*. (2002) stated that variations in bacterial biochemical profiles cause differences in test results using the VITEK 2 Compact. These variations in biochemical profiles are directly influenced by genetic mutations. The effects of gene mutations are related to metabolism and cause changes in enzyme activities, metabolic end products, and metabolic pathways, thus affecting the production of acids, gases, and other compounds, which means changes in the bacterial biochemical profile (Gots and Benson, 1974; Lopatkin *et al*., 2021; Jiao et *al*., 2024). The presence of gene mutations that affect enzyme activity indicates a deviation from the characteristics of *V. alginolyticus* strain

.3.4 **Antibiotic Susceptibility Test (AST)**

The level of bacterial sensitivity to an antibiotic was determined by the size of the inhibition zone. The results of the antibiotic sensitivity test showed that the majority of the selected isolates were still sensitive to the three antibiotics used (tetracycline, amoxicillin, and ampicillin) (Table 5).

Table 5. Results of the Average Inhibition Zone Calculation

Isolates Code / **Antibiotic**	**Average Inhibition Zone Calculation (mm)**				
	N2.A1. B.2.A	N2.A2.B	N2.A3. I.2.A	N2.AI. B.2.B.K	N2.AI. B.2.B.H
Tetracycline	16.90	16.53	10.65	20.48	16.37
Amoxicillin	21.42	22.5	17.15	20.23	19.7
Ampicillin	21.32	17.08	0	17.58	16.43

Based on Table 5, the isolate that is most sensitive to tetracycline antibiotics is isolate N2.AI.B.2.B.K with a diameter of 20.48 mm, the isolate that is most sensitive to amoxicillin is N2.A2.B with a diameter of 22.5 mm, and the isolate that is most sensitive to ampicillin is N2.A1.B.2.A with a diameter of 21.32 mm. The results of the antibiotic susceptibility test can be seen in Figure 2 Futhermore, the results also indicated that three isolates (N2.A1.B.2.A, N2.A2.B, and N2.AI.B.2.B.H) were sensitive to all the antibiotics tested, whereas isolate N2.AI.B.2.B. K is sensitive to ampicillin antibiotics and isolate N2.A3.I.2.A was resistant to all tested antibiotics. The differences in sensitivity levels are influenced by the action of antibiotics on the target. Tetracycline antibiotics have a mechanism of action that binds to the 30s ribosomal subunit in bacteria and prevents protein synthesis (Schmidt, 2023). Amoxicillin and ampicillin are ß-lactam antibiotics that inhibit bacterial cell wall synthesis (Dutta *et al*., 2021; Natan *et al*., 2024; Zhang *et al*., 2020). Keller *et al*. (2022) stated that *Vibrio* sp. bacteria are more sensitive to ß-lactam antibiotics due to the synthesis of cell wall precursors. However, there were indications of multi-drug resistance (MDR) in isolate N2.A3.I.2.A, which is resistant to the three types of antibiotics.

The occurrence of resistance of bacteria is influenced by several factors, such as modification of antibiotic targets, inactivation of antibiotics by enzyme production, and the presence of efflux pumps. Target modifications, such as mutations in the 16 rRNA gene, can reduce the effectiveness of tetracycline antibiotics on their targets (Urban-Chmiel *et al*., 2022). In addition, efflux pump activity causes low-level resistance. The mechanism of efflux pump activity, especially from the resistance-nodulation-division (RND) group, actively pumps erythromycin out of bacterial cells, thereby reducing its concentration and effectiveness (Yasir *et al*., 2022; Stephen *et al*., 2022). Horizontal gene transfer (HGT) is another cause of the rapid spread of resistance genes. HGT can spread widely in the environment and is carried through water and biofilms (Li and Zhang, 2022; Michaelis and Grohmann, 2023). HTG occurs via three mechanisms: transformation, transduction, and conjugation. The gene transfer process through direct contact between bacterial cells (conjugation) is the most influential factor in the spread of antibiotic resistance genes (Lerminiaux & Cameron, 2019; Liu *et al*., 2024).

4. Conclusion

Based on the research results, the following conclusions can be drawn:

1. The TPC of common bacteria in all ponds exceeded the threshold, whereas the density of *Vibrio* bacteria remained within safe limits, except in pond 1.
2. The five selected isolates were identified as *Vibrio alginolyticus*.
3. Four isolates were sensitive to the test antibiotics (tetracycline, amoxicillin, and ampicillin), whereas one isolate (N2.A3.I.2.A) was resistant to all tested antibiotic

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