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Research Article

Level of Antibiotic Resistance in *Vibrio parahaemolyticus* Bacteria Isolated from Shrimp: Observation of Shrimp Ponds in South Sulawesi Province

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ABSTRACT

The presence of Vibrio parahaemolyticus in shrimp ponds needs to be a concern because it is an opportunistic pathogen. Although V. parahaemolyticus can aid shrimp digestion, an increase in its population can negatively impact shrimp conditions. Currently, there are concerns about antibiotic-resistant V. parahaemolyticus due to the use of antibiotics in aquaculture activities. Antibiotic resistance is not only caused by unwise use of antibiotics but can also occur due to mutations between bacteria in the aquatic environment. Therefore, it is important to determine the presence or absence of antibiotic-resistant V. parahaemolyticus from shrimp farming activities in ponds. Early detection will be crucial for policymakers regarding the use of antibiotics in pond shrimp farming and provide information for shrimp farmers about the effectiveness of the antibiotic use they have taken. This study aims to observe antibiotic resistance in V. parahaemolyticus bacteria obtained from ponds in South Sulawesi Province. Based on observations made in four districts, the results showed that no V. parahaemolyticus was found to be resistant to the three types of antibiotics tested. It can be concluded that shrimp from ponds in South Sulawesi Province are still free from antibiotic-resistant V. parahaemolyticus. Nevertheless, continuous vigilance and monitoring of this problem is still needed to maintain the sustainability of the shrimp farming industry and food safety.

1. INTRODUCTION

As a Muslim, consuming food that is halal and thayyib (good) is an obligation set forth in Islamic teachings. Allah SWT has commanded Muslims to eat what is halal and good, as mentioned in the Qur'an, Surah Al-Baqarah verse 168:

يٰٓاَيُّهَا النَّاسُ كُلُوْا مِمَّا فِى الْاَرْضِ حَلٰلًا طَيِّبًاۖ وَّلَا تَتَّبِعُوْا خُطُوٰتِ الشَّيْطٰنِۗ اِنَّهٗ لَكُمْ عَدُوٌّ مُّبِيْنٌ

"O mankind, eat from whatever is on earth [that is] lawful and good and do not follow the footsteps of Satan. Indeed, he is to you a clear enemy."

Halal food refers to food that is permissible to consume according to Islamic law, while thayyib refers to food that is good, clean, healthy, and beneficial for the body (Saeed, 2023; Zakaria, 2023). Consuming halal and thayyib food is important not only for physical health but also affects spiritual health and the acceptance of one's worship. Therefore, every Muslim should be careful in choosing food, ensuring its source is halal, its processing complies with Islamic law, and it has good nutritional value for health, including shrimp from aquaculture farms (Pye, 2022). By adhering to this command, a Muslim not only maintains their health but also demonstrates obedience to Allah SWT and preserves their purity.

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The consumption of safe shrimp is crucial for health, especially concerning the threat of antibiotic-resistant bacteria. These resistant bacteria can develop due to the excessive use of antibiotics in shrimp farming, potentially contaminating the final product (Ali, 2022; Fenitra, 2024). If humans consume contaminated shrimp, it can lead to infections that are difficult to treat because these bacteria do not respond to standard antibiotic treatments. Therefore, it is important to ensure that the shrimp consumed comes from reliable sources and has undergone rigorous testing to confirm it is free from resistant bacteria. Such measures not only protect consumer health but also help prevent the broader spread of antibiotic resistance (Maulana, 2022; Pradana, 2022).

At present the aquaculture industry has developed quite rapidly, which has contributed almost more than half of the total number of fishery commodities consumed by humans worldwide. Aquaculture with an intensive system or semi-intensive system has been able to produce fishery commodities in large quantities (Jaree 2018; Liu 2016). However, the cultivation sometimes fails due to disease infection, so cultivators use antimicrobials or antibiotics on a wide scale to control the disease. The use of drugs in aquaculture is carried out at the stage of disease control (sub-therapeutic) for a considerable time through application in maintenance water or through feed (Cui 2017; Kamal 2022; Schleder 2020). This condition allows for the emergence of strains of bacteria that are resistant to antibiotics (resistant) and can even lead to horizontal gene transfer from resistant bacteria to other bacteria. Global attention and efforts should be made to prevent or stop the excessive use of antimicrobials in aquaculture and encourage policymakers to establish preventive measures (Ray 2019). Establishing new policies is critical to contain the development and increase of antibiotic-resistant bacteria (Santos and Ramos 2018).

Antibiotic resistance is the ability of bacteria to survive and multiply despite having been given a supposedly effective dose of antibiotics. Antibiotic resistance can occur when bacteria adapt, mutate, or undergo genetic changes so that they are resistant to antibiotics that have previously been used (Nadella 2021; Pham 2018; Suyamud 2021). In this condition, the treatment applied will no longer be effective and can increase the risk of more severe infection or even death. Antibiotic resistance is often caused by excessive or inappropriate antibiotic use, such as interacting too often or too long with antibiotics that are not needed, using the wrong antibiotic, or not following recommended doses (Nadella 2021). Therefore, it is important to adhere to the instructions or instructions for use in applying antibiotics and only use antibiotics wisely (Sharma 2021; Suyamud 2021; Yasin 2022). Aquaculture farmers' understanding of antibiotic resistance is important, but it seems to be lacking for now. Let alone in the field of fisheries in the field of human health, the understanding of nurses and caregivers is still lacking as revealed in a survey related to the field of human health (medical) to see the knowledge of caregivers related to the use of antibiotics and resistance in Indonesia shows the results that the level of knowledge of caregivers related to the occurrence of antibiotic resistance is still low (Herawati et al. 2020).

Antibiotic resistance in shrimp farming in ponds is a problem that may have occurred because antibiotics have often been used in maintaining shrimp health and preventing bacterial infections (Liu 2019; Yasin 2022). The results of research in Vietnam revealed that the use of antibiotics in aquaculture, used for treatment (therapeutic) and prevention of infection (prophylaxis), antibiotics used sometimes from types of antibiotics commonly used in humans and the use of antibiotics in health management aquaculture raises concerns about residues in aquatic products and allows the emergence of bacteria resistant to these antibiotics (Nguyen, Van, and Coloe 2016).

The results of the study in Malaysia showed that from 118 bacterial isolates obtained from six aquaculture cultivation sites, 58 isolates were resistant to antibiotics, types of antibiotics namely rifampicin, ampicillin, and sulfafurazol (Kam et al. 2022). Excessive use of antibiotics in shrimp farming can cause damage to the aquatic environment, as well as endanger the health of humans who consume farmed shrimp. Bacterial resistance to antibiotics can reduce the effectiveness of treatment in humans and animals, thus threatening public safety and health at large (Dai 2020; Saejung 2014; Zhou 2021).

So to overcome the problem of antibiotic resistance in shrimp farming in ponds, a holistic and sustainable approach is needed. And the first step of that meeting is to collect information data on the number and type of bacteria that have been resistant to pond shrimp farming activities (Ekasari 2014; Stalin 2016; Wada 2016). In addition, there needs to be an increase in the understanding of fishery farmers in maintaining shrimp health naturally by providing optimal environmental conditions, such as ensuring the cleanliness of ponds and maintaining water quality. The use of sustainable cultivation methods can help reduce unnecessary antibiotic use and can prevent antibiotic resistance in bacteria (Cui 2017; Kamal 2022).

South Sulawesi Province is one of the largest producers of pond shrimp in Indonesia, so it is important to detect early the emergence of antibiotic-resistant pathogenic bacteria. Observation of resistance bacteria was carried out for

the type of bacteria Vibrio parahaemolyticus. This observation activity was carried out in several districts that are the center of pond shrimp production, including Takalar, Barru, Pinrang, and Jeneponto Regencies.

This study provides a new perspective on the problem of antibiotic resistance in shrimp farming in ponds, especially in South Sulawesi Province, Indonesia. The focus of research on the early detection of the emergence of pathogenic bacteria resistant to antibiotics in several pond shrimp production center districts (Takalar, Barru, Pinrang, and Jeneponto) is an approach that has not been widely carried out before. This is important considering that South Sulawesi is one of the largest shrimp producers in Indonesia, so the results of this study can provide a more comprehensive picture of the situation of antibiotic resistance in one of the main shrimp production areas in the country.

Another novelty lies in the focus of research on Vibrio parahaemolyticus bacteria. Although previous studies have examined antibiotic resistance in different types of bacteria in the aquaculture environment, this study specifically highlighted V. parahaemolyticus which is an important pathogen in shrimp farming. By observing the level of antibiotic resistance in these bacteria, the study could provide new insights into the potential health risks associated with shrimp consumption and its impact on food safety more broadly.

Another innovative aspect is the proposed holistic approach to addressing the problem of antibiotic resistance. The study not only focuses on resistance detection, but also emphasizes the importance of improving pond farmers' understanding of sustainable farming practices and natural shrimp health management. This approach reflects a paradigm shift away from overuse of antibiotics towards more sustainable prevention strategies. By combining technical (resistance detection) and social aspects (farmer education), this study offers a more comprehensive perspective in addressing the problem of antibiotic resistance in the aquaculture sector

2. RESEARCH METHODS

2.1 Materials and Tools

The materials and instruments employed for the experimental procedures consist of:Enrichment media formulated as a 10% peptone solution for the purpose of isolating Vibrio sp bacterial strains. Bacterial identification was performed utilizing the Polymerase Chain Reaction (PCR) methodology.Antimicrobial susceptibility testing (AST) was conducted through the Agar Diffusion Method utilizing Mueller-Hinton Agar (MHA) media, in conjunction with chilled antibiotics. Engineering activities pertaining to antibiotic resistance in Vibrio sp bacteria isolated from pond shrimp were executed from February to June at the Testing Laboratory of the Takalar Brackish Aquaculture Fisheries Center.

2.2 Research Methods

The methodology employed in assessing antibiotic resistance is delineated into three distinct components: The initial phase involves the procurement of Vibrio sp isolates from aquaculture environments, specifically shrimp ponds. A random sampling method was applied to select a total of ten shrimp specimens from a designated pond bed. The subsequent step entails the extraction of bacterial organisms from the hepatopancreas of the shrimp, utilizing sterile cotton swabs as the means of collection. The isolated bacteria are subsequently cultured on a medium consisting of a 10% peptone solution. Following a 24-hour incubation period, the isolated bacteria from the 10% peptone solution are further cultured onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates. The identification of Vibrio parahaemolyticus is conducted on the Vibrio sp isolates harvested from the ponds. The selection of bacteria cultivated on TCBS solid media is performed based on the chromatic characteristics of the colonies. Colonies exhibiting a green pigmentation are subjected to a purification process through two successive culturing steps on TCBS solid media. The genomic characterization of the bacterial isolate is subsequently performed utilizing the Polymerase Chain Reaction (PCR) technique to accurately identify the specific strain of Vibrio parahaemolyticus bacteria in accordance with the SNI 2332.13-2020 standards. The isolates confirmed to possess Vibrio parahaemolyticus characteristics are then recultured in Tryptic Soy Agar (TSA) oblique media within test tubes for the purpose of establishing a collection of isolates.

Inhibitory power evaluation: preparation for testing. The preparation phase involves the formulation of Mueller Hinton Agar media with the incorporation of sodium chloride (NaCl) in concentrations ranging from 1.5% to 2.5%. The bacterial isolates selected for this antimicrobial resistance (AMR) assessment are aged between 18 to 24 hours, with approximately 3 to 5 colonies chosen for each targeted bacterial strain. A bacterial culture is prepared to achieve an optical density equivalent to the McFarland 0.5 standard, corresponding to approximately 1.5 x 10^8 CFU/ml. Additionally, antibiotic discs for tetracycline, oxytetracycline, and enrofloxacin are prepared for testing purposes.

Escherichia coli (ATCC 25922) is utilized as a standard control organism. Sterile cotton swabs are employed for the inoculation process.

Execution of the test: The sterile cotton swab is immersed in a bacterial broth, followed by a streaking technique across the agar surface in a diagonal manner from left to right. This streaking procedure is repeated 2 to 3 times, altering the position of the quarter-circle cup with each application. A similar streaking method is employed for the standard ATCC bacterial strain and the control strains. Antibiotic paper discs are then placed onto the agar surface, ensuring an even distribution to prevent the accumulation of inhibition zones. The setup is allowed to equilibrate at room temperature (between 18°C and 22°C) for a duration of 15 to 30 minutes. Following this, the media is incubated at a controlled temperature of 25°C ± 3°C for a period of 16 to 24 hours, during which the zones of bacterial growth inhibition are subsequently measured in millimeters. The interpretation of the test results is conducted through a comparative analysis of the inhibition zones observed, by the standards delineated in the accompanying Table 1.

Table 1. Standard Interpretation of Test Results (Melvin P. Weinstein. et al. 2020)

Antibiotics	Disc unit	Inhibition zone (diameter, unit mm)		
		Resist (R)	Intermediate (I)	Sensitive (S)
Tetracycline	30 µg	≤ 14	15-18	≥ 19
Oxytetrasiklin	30 µg	≤ 14	15-18	≥ 19
Enrofloksasin	5 µg	≤ 15	16-20	≥ 21

The elucidation of the resistance test outcomes is articulated in the following manner: In instances where the microorganism is designated as sensitive (S), the application of the antibiotic is permissible. Conversely, should the microorganisms be categorized as intermediate (I), the utilization of the antibiotic is not advised. Lastly, if the microorganism is classified as resistant (R), the antibiotic is deemed unsuitable for therapeutic intervention.

2.3 Time and Place

Antibiotic resistance engineering activities in *Vibrio sp* bacteria derived from pond shrimp were carried out from February to June, at the Test Laboratory of the Takalar Brackish Aquaculture Fisheries Center.

2.4 Data Analysis.

The data obtained are processed and analyzed descriptively

3. RESULTS AND DISCUSSION

3.1 Activity Results

Isolates of Vibrio sp bacteria obtained from shrimp in 4 districts, namely Takalar, Barru, Pinrang, and Jeneponto, were further purified and then identified for the type of *Vibrio parahaemolitycus*.

3.1.1 Takalar County

The results of observations on shrimp ponds obtained the following results in Table 2.

Table 2. Bacterial isolate

Isolate Code	Address		Identification Results
	Village	District	
1	Mappakalompo	Galesong	Negative
2	Mappakalompo	Galesong	Negative
3	Mappakalompo	Galesong	Negative
4	Mappakalompo	Galesong	Negative
5	Mappakalompo	Galesong	Negative
6	Mappakalompo	Galesong	Negative
7	Mappakalompo	Galesong	Negative
8	Mappakalompo	Galesong	Negative
9	Mappakalompo	Galesong	Negative
10	Mappakalompo	Galesong	Negative
11	Boddia	Galesong	Negative
12	Boddia	Galesong	*Positive*
13	Boddia	Galesong	Negative
14	Boddia	Galesong	Negative

15	Boddia	Galesong	Negative
16	Boddia	Galesong	Negative
17	Boddia	Galesong	Negative
18	Boddia	Galesong	Negative
19	Boddia	Galesong	Negative

In Table 2 above, it is known that of the 19 isolates of Vibrio sp., there is only one isolate from the type of Vibrio parahaemolyticus, based on identification using the polymerase chain reaction (PCR) method, while other isolates may be from other types.

The number of Vibrio sp. bacterial isolates obtained can be an indication of disease due to bacterial infection. The presence of Vibrio sp. bacteria in the pond will not cause serious problems as long as the shrimp's body condition is in top condition, meaning that Vibrio sp. bacteria will infect shrimp if the condition of the shrimp's immune system decreases. So that Vibrio sp. bacteria include opportunistic bacteria, this is revealed by Saha S (2021) dan Baker-C (2018) that some opportunistic bacteria from Vibrio sp. have infected mangrove crabs and that they can transfer to humans causing diarrhea, nausea, fever, wheezing, or gastroenteritis (Baker- et al. 2018; Saha, Dash, and Sciences 2021).

The results in Table 2 above were then tested with oxytetracycline, tetracycline, and enrofloxacin antibiotics to determine the response of Vibrio parahaemolyticus bacteria, and the results as follows:

Table 3. Antibiotic inhibition zone

Isolate Code	Antibiotic Disc (mm)		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
12	26,6±0,6	27,6±0,8	28,4±0,5

The data in Table 3 above shows the average results of antibiotic inhibition zone measurements tested on *Vibrio parahaemolyticus* grown on Mueller-Hinton Agar media. Based on the results in Table 3 above, then interpreted based on the standards of CLSI Edition 30 of 2021, the results are as follows:

Table 4. Interpretation Test Results

Isolate Code	Antibiotic		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
12	Sensitive	Sensitive	Sensitive

Based on the results in Table 4 above, *Vibrio parahaemolyticus* bacteria are still "*sensitive"* to antibiotics. This means that oxytetracycline, tetracycline or enrofloxacin antibiotics can still kill *Vibrio parahaemolyticus* bacteria at recommended doses. The ability of these antibiotics to inhibit bacterial growth is in line with what was developed by Dang T.H.O., (2022) that in a study to determine the antibacterial resistance of *Vibrio parahaemolyticus* isolated from shrimp ponds located in the eastern coastal region of the Mekong Delta of Vietnam, it has revealed that *Vibrio parahaemolyticus* bacteria isolated from shrimp that showed symptoms of AHPND disease, after being tested with doxycycline and oxytetracycline antibiotics was still included in the sensitive category (Dang, Truong, and Nguyen 2022).

3.1.2 Barru County

The results of observations on shrimp ponds obtained the following results:

Table 5. Bacterial isolate

Isolate Code	Address		Identification Results
	Village	District	
1	Binuang	Balusu	*Positive*
2	Binuang	Balusu	Negative
3	Tanete	Tanete Rilau	*Positive*
4	Tanete	Tanete Rilau	*Positive*
5	Coppo	Barru	*Positive*
6	Coppo	Barru	*Positive*
7	Coppo	Balusu	Negative

Isolate Code	Address		Identification Results
	Village	District	
8	Siawing	Balusu	*Positive*
9	Siawing	Balusu	*Positive*
10	Siawing	Barru	*Positive*
11	Siawing	Barru	*Positive*

In Table 5 above, it is known that after identifying bacterial isolates using the PCR method, there were nine isolates of *Vibrio parahaemolyticus* bacteria from 11 isolates of *Vibrio sp* bacteria collected. *Vibrio parahaemolyticus* bacteria are quite dominant in shrimp originating from Barru Regency ponds. Research conducted by Khimmakthong (2017) revealed that *V. parahaemolyticus* bacteria are the cause of Acute Hepatopancreatic Necrosis Disease (AHPND), or Early Mortality Syndrome (EMS) in white shrimp, which in the process infects important shrimp organs, namely hepatopancreas, and then secretes and separates certain compounds that are toxic to shrimp (Khimmakthong and Sukkarun 2017).

The results in Table 5 above continued with inhibition testing of three types of antibiotics the results are as follows:

Table 6. Antibiotic inhibition zone

Isolate Code	Antibiotic Disc (mm)		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
1	24,4±1,1	25,7±0,0	25,4±1,0
3	25,0±0,1	26,7±0,8	28,5±1,3
4	24,6±0,9	25,3±0,6	27,2±1,2
5	25,6±0,0	27,5±0,0	29,3±1,4
6	23,0±0,2	24,1±0,9	27,1±0,0
8	22,0±0,0	22,9±1,1	22,1±0,0
9	23,0±0,0	23,8±0,0	23,7±0,2
10	24,2±1,7	23,7±0,0	24,7±0,9
11	22,8±0,2	21,9±1,1	22,4±1,4

The data in Table 6 above is the result of measuring the average antibiotic inhibition zone against the growth of *Vibrio parahaemolyticus* bacteria in MHA media. The data is then interpreted, and the results are as follows:

Table 7. Interpretation Test Results

Isolate Code	Antibiotic		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
1	Sensitive	Sensitive	Sensitive
3	Sensitive	Sensitive	Sensitive
4	Sensitive	Sensitive	Sensitive
5	Sensitive	Sensitive	Sensitive
6	Sensitive	Sensitive	Sensitive
8	Sensitive	Sensitive	Sensitive
9	Sensitive	Sensitive	Sensitive
10	Sensitive	Sensitive	Sensitive
11	Sensitive	Sensitive	Sensitive

Based on the results in Table 7, Vibrio *parahaemolyticus* bacteria are still "*sensitive*" to oxytetracycline, tetracycline, and enrofloxacin. These antibiotics are still effective enough to kill *Vibrio parahaemolyticus* bacteria.

3.1.3 Pinrang Regency

The results of observations on shrimp ponds obtained the following results:

Table 8. Bacterial isolate

Isolate Code	Address		Identification Results
	Village	District	
1	Mallonggilonggi	Lanrisang	*Positive*
2	Mallonggilonggi	Lanrisang	*Positive*
3	Mallonggilonggi	Lanrisang	Negative
4	Mallonggilonggi	Lanrisang	Negative
5	Mallonggilonggi	Lanrisang	*Positive*
6	Mallonggilonggi	Lanrisang	Negative
7	Datta	Duampanua	*Positive*
8	Datta	Duampanua	*Positive*
9	Datta	Duampanua	*Positive*
10	Datta	Duampanua	*Positive*
11	Datta	Duampanua	*Positive*
12	Datta	Duampanua	Negative
13	Datta	Duampanua	*Positive*
14	Bababinanga	Duampanua	Negative
15	Paria	Duampanua	Negative
16	Paria	Duampanua	Negative

In Table 8 above, it can be seen that there are quite a lot of isolates of *Vibrio sp.* bacteria obtained from pond shrimp in Pinrang Regency, and after PCR identification, it turns out that the number of Vibrio parahaemolyticus bacteria is quite dominant, namely there are 9 out of 16 isolates of *Vibrio sp*. The dominance of *Vibrio parahaemolyticus* bacteria can be a strong indication that there have been cases of bacterial infection in pond shrimp [9,11,12].

Diseases that are quite worrying attacking shrimp in ponds today one of them is Acute Hepatopancreatic Necrosis Diseases or White Stool Diseases (AHNPD). This disease in shrimp is usually accompanied by an increase in the population of *Vibrio parahaemolyticus*, type bacteria in the shrimp body even if sometimes the bacteria are resistant to antibiotics (Kumar et al. 2021).

Based on the results in Table 8 above, antibiotic inhibitory testing was carried out to isolate positive bacteria identified *as Vibrio parahaemolyticus* and the results were as follows:

Table 9. Antibiotic inhibition zone

Isolate Code	Antibiotic Disc (mm)		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
1	23,5±0,0	24,7±1,2	26,2±0,0
2	25,1±0,6	25,6±0,8	22,9±0,0
5	21,6±0,0	23,2±0,0	24,8±0,0
7	21,1±0,4	21,7±0,0	24,0±0,0
8	20,8±2,0	21,8±0,9	24,0±0,0
9	21,7±1,0	21,5±0,6	22,2±0,4
10	21,5±0,8	21,9±0,1	24,0±0,0
11	20,9±0,0	22,0±0,0	24,0±1,1
13	27,0±0,0	30,5±1,1	34,5±0,8

Table 9 above shows the average diameter of the antibiotic inhibition zone against the growth of *Vibrio parahaemolyticus* bacteria. The inhibitory zone is formed because bacteria cannot live properly in the zone so the area will look clear. Based on the results of the inhibition zone measurement, further interpretation is carried out based on the standards set by CLSI and the test results as follow (Melvin P. Weinstein. et al. 2020)

Table 10. Interpretation Test Results

Isolate Code	Isolate Code		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
1	Sensitive	Sensitive	Sensitive
2	Sensitive	Sensitive	Sensitive
5	Sensitive	Sensitive	Sensitive
7	Sensitive	Sensitive	Sensitive
8	Sensitive	Sensitive	Sensitive
9	Sensitive	Sensitive	Sensitive
10	Sensitive	Sensitive	Sensitive
11	Sensitive	Sensitive	Sensitive
13	Sensitive	Sensitive	Sensitive

The data in Table 10 above show that oxytetracycline, tetracycline, and enrofloxacin antibiotics are still effective enough to inhibit the growth or kill the bacterial isolate *Vibrio parahaemolyticus.* This means that *Vibrio parahaemolyticus* bacteria are still sensitive to these antibiotics. Antibiotics can inhibit bacterial growth and reproduction or can damage bacterial cell structure, and each antibiotic has one or more mechanisms of action for a particular bacterium (Kohanski, Dwyer, and Collins 2010).

3.1.4 Jeneponto County

The results of observations on shrimp ponds obtained the following results in Table 11.

Table 11. Bacterial isolate

Isolate Code	Address		Identification Results
	Village	District	
1	Tujuh	Bangkala	Negative
2	Arungkeke	Arungkeke	*Positive*
3	Pallengo	Bangkala Induk	Negative
4	Pallengo	Bangkala Induk	Negative
5	Pallengo	Bangkala Induk	Negative
6	Manyombeng	Binamu	Negative
7	Manyombeng	Binamu	Negative
8	Manyombeng	Binamu	Negative
9	Manyombeng	Binamu	Negative
10	Manyombeng	Binamu	Negative

The data in Table 11 above shows that there was only one isolate of *Vibrio parahaemolyticus* bacteria that was successfully identified from 10 isolates *of Vibrio sp* bacteria collected. The lack of isolate of *Vibrio parahaemolyticus* bacteria obtained from ponds in Jeneponto Regency because there may be rare cases of disease due to bacterial infection.

The results in Table 11 above were then tested for antibiotic inhibitory power for isolates of positive bacteria identified *as Vibrio parahaemolyticus* and the results were as follows:

Table 12. Antibiotic inhibition zone

Isolate Code	Antibiotic Disc (mm)		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
2	20,4±0,9	21,4±0,1	22,5±1,0

Based on the results in Table 12 above, then interpreted based on the standards of CLSI Edition 30 of 2021, the results are as follows:

Table 13. Interpretation Test Results

Isolate Code	Antibiotic		
	Oxytetrasiklin	Tetracycline	Enrofloxacin
2	Sensitive	Sensitive	Sensitive

Based on the results in Table 13 above, Vibrio *parahaemolyticus* bacteria are still "*sensitive*" to antibiotics. Although the presence of *Vibrio parahaemolyticus* bacteria in shrimp originating from Jeneponto Regency ponds is not too worrying, you still have to be aware of the emergence of resistant bacteria that can come from other types of bacteria. A research report conducted in Nigeria by Justice-Alucho (2021) has found that there are several types of bacteria from seafood samples including *Staphylococcus gallinarum, Vibrio rotiferanus, Vibrio parahaemolyticus, Klebsiella aerogenes and Klebsiella quasipneumoniae,* and based on the results of this study raises concerns because these bacteria are opportunistic and there is a risk to transfer antibiotic-resistant genes (C.H., E.E., and W. 2021).

3.2 Discussion

In this activity, shrimp samples were taken from the pond randomly, not distinguishing healthy shrimp or sick shrimp. The pond shrimp taken has been aged over 30 days (DOC 30), so the weight size ranges from 1-3 gr with a length ranging between 5-6 cm. Bacteria from shrimp are taken in the hepatopancreas organ using a sterile cotton bud, which is then etched on a specific medium for *Vibrio sp.* bacteria, namely Thiosulfate Citrate Bile Salt Sucrose (TCBS) media. In general, *Vibrio parahemolyticus* bacteria will form green colonies on TCBS media (Hikmawati, Susilowati, and Setyaningsih 2019).

Based on the green color of the colony, purification of bacterial isolate up to 2x culture was carried out on TCBS media, which was then identified by the polymerase chain reaction (PCR) method which followed the identification standard *of Vibrio parahaemolyticus bacteria* in the Indonesian National Standard (SNI 2332.13-2020). The Shrimp and *Vibrio sp*. Bacteria can be seen in Figure 1.



(a) (b)

Figure 1. (a) Shrimp from Takalar Regency Pond, (b) *Vibrio sp.* bacteria on TCBS media

In the pond environment, many types of *Vibrio sp.* bacteria live and symbiosis with shrimp. *Vibrio sp.* bacteria are opportunistic, which means that in a small population, the *Vibro sp* bacteria are helpful in shrimp digestion and control zooplankton populations. However, if the population of *Vibrio sp.* is too much, it will be detrimental to shrimp in the pond, especially if the shrimp's immune system decreases, eating will be a serious problem (Baker- et al. 2018; Khimmakthong and Sukkarun 2017; Saha et al. 2021).

At the stage of pure isolation and the identification process by polymerase chain reaction (PCR) method, it will be ascertained from the type of *Vibrio parahaemolyticus*, then antibiotic resistance testing is carried out, namely oxytetracycline, tetracycline, and enrofloxacin. The antibiotic testing process is carried out on Muller Hilton Agar (MHA) media. Antibiotics that can inhibit or kill bacteria will form a clear zone. Next, the inhibitory zone of each antibiotic was measured using a caliper (Melvin P. Weinstein. et al. 2020).

Penicillin is an antibiotic that was originally found to kill bacteria. The ability to kill bacteria is a complex interaction between drug molecules and bacterial cells. The interaction between drug molecules and bacterial cells causes changes in cell biochemistry, causing ultrastructural changes in bacterial cells (Kohanski et al. 2010). The pure isolates of Vibrio can be seen in Figure 2.



Figure 2. (a) Pure isolates *of Vibrio sp* on TCBS , (b) Measurement of antibiotic inhibitory zones on MHA media, (c) Reading of the identification results of Vibrio parahaemolyticus with Polymerase Chain Reaction method (SNI 2332.13-2020)

When the research was carried out, shrimp farming conditions in Takalar Regency were proven to be still relatively safe from *Vibrio sp.* bacterial infection and especially from the type of *Vibrio parahaemolyticus,* although still have to be vigilant to avoid antibiotic resistance in aquaculture activities. Efforts are needed to take steps such as minimizing the use of antibiotics and only using antibiotics when necessary, using antibiotics that are appropriate to the type of infection that occurs, and testing antibiotics for bacteria that cause infection to determine the type of antibiotic that is effective. Rotate antibiotics to prevent the formation of bacterial resistance to one type of antibiotic, and provide appropriate treatment to maintain shrimp health, for example, providing nutritious feed, maintaining water quality, and carrying out good maintenance. By taking appropriate precautions, antibiotic resistance in aquaculture activities can be reduced to create a healthy and sustainable cultivation environment. The existence of continuous monitoring efforts to detect early the emergence of resistant bacteria, including the existence of rules that can control the use of antibiotics is necessary to anticipate the spread of antibiotic-resistant bacteria (Preena et al. 2020).

The presence of *Vibrio parahaemolyticus* bacteria must still be watched out for, especially if it has entered the body of shrimp because it may be a problem for humans who consume it, as happened in Malaysia. The results of research in Malaysia conducted by MY AlOthrub S., (2014) showed that shrimp and shellfish commodities that are often traded in the market contain *Vibrio parahaemolyticus* bacteria as the cause of gastroenteritis in humans, and these bacteria turned out to be resistant to ampicillin.

Based on the results obtained from bacterial isolate in shrimp farming, Barru Regency shows that the level of bacterial infection attack in shrimp is quite high, especially from the type of *Vibrio parahaemolyticus* because of the eleven *Vibrio sp* bacteria isolated, nine of them or 82% including from the type of *Vibrio parahaemolyticus.*

Based on the results obtained from shrimp farming in Barru Regency, there is a need to increase understanding of the use of antibiotics and their impacts, especially for shrimp farmers or farmers in the area. Based on the results of the study revealed that good management of antibiotic use is not only determined by the knowledge and perception of healthcare workers but also by their caregivers or in this case shrimp farmers (Herawati et al. 2020).

The rapid development of aquaculture today requires the use of antibiotics as a control of diseases caused by pathogenic bacteria. Even though there have been rules or regulations issued by the government, there are still resistant bacteria that can be isolated from the aquaculture system by researchers (Gazal et al. 2020).

The results of observations of shrimp farming in Pinrang Regency show that many *Vibrio sp.* bacteria can be isolated. And of the 16 isolates *of Vibrio sp.,* nine of them, or 56% are types *Vibrio parahaemolyticus*. These results can be the basis for suspicion that the rate of bacterial infection is quite high in farmed shrimp in Pinrang Regency. The dominance of *Vibrio parahaemolyticus* bacteria found has become a concern for the emergence of serious problems in shrimp farming in the future, although in research it is still proven to be controlled with the use of antibiotics. However, if from now on efforts are not made to improve the cultivation system, of course, these bacteria have the potential to be resistant, especially *Vibrio parahaemolyticus* bacteria, including gram-negative bacteria that can form biofilms. The ability of bacteria to form biofilms can increase the ability of bacteria resistance to antibiotics (Sanhoury, Khalil, and Sleim 2021).

Shrimp farming can play a role in the spread of global AMR because it is an international trade commodity, whose connectivity is direct to the aquatic environment, and most shrimp production comes from low- or middle-income countries, where the quality and use of antibiotics are poorly regulated (Thornber et al. 2020).

Antibiotic resistance in *Vibrio parahaemolyticus* bacteria occurs through several mechanisms, such as genetic mutations, bacteria can undergo genetic mutations that make them more resistant to antibiotics. Mutations like this

can occur naturally or are exposed to antibiotics, and gene transfer, bacteria can transfer resistance genes through bacterial conjugation, transformation, and transduction. Resistance genes from other bacteria can transfer to *Vibrio parahaemolyticus* and make it more resistant to antibiotic enzyme expression, *Vibrio parahaemolyticus* bacteria can increase the expression of enzymes that break down or inactivate antibiotics before they work. These enzymes are called beta-lactamase, aminoglycosides, and ring-opening enzymes. Because antibiotics cannot be recognized by bacteria, they are no longer effective. Target modification, bacteria can also change the target structure of the antibiotic molecule. For example, *Vibrio parahaemolyticus* can change the proteins on its cell walls that are targeted by antibiotics, so that the antibiotic is no longer effective.

The results of research in Malaysia by Banerjee S., (2012) revealed the presence of multi-drug resistant antibiotics in *Salmonella sp.* and *Vibrio sp.* bacteria in *Litopenaeus vannamei* shrimp ponds so antibiotic resistance will be a worrying problem in the treatment process for shrimp infectious diseases and human diseases in the future [21].

The mechanism of antibiotic resistance in bacteria can involve one or more factors including the increasingly widespread and easy-to-use availability of antibiotics, which can induce resistance in bacteria such as *Vibrio parahaemolyticus*. This then requires the development of new antibiotics or alternative strategies to address bacterial infections that are resistant to antibiotics.

Several ways can be done to overcome antibiotic-resistant bacteria, including implementing appropriate and rational antibiotic use policies, such as limiting the use of antibiotics only to infectious conditions that require it. Choose an antibiotic that suits the type of bacteria and the severity of the infection. The user has the right dosage as well as the appropriate duration of treatment. Making the development of new and effective antibiotics. Develop alternative therapeutic strategies such as combination therapy or immune therapy. Optimize vaccine development to prevent bacterial infections. Maintain environmental cleanliness and sanitation, such as avoiding contact with antibiotic-resistant bacteria, and avoiding unhealthy eating habits. Strengthen the body's immune system with a healthy diet and exercise. Increase public awareness and education about the importance of complying with antibiotic use policies, recognize the symptoms of bacterial infections and how to avoid them, and understand the adverse effects of inappropriate antibiotic use, improve cooperation and coordination between health workers, researchers, governments, and communities to address the problem of antibiotic resistance comprehensively.

The results of observations on shrimp farming in Jeneponto Regency showed that 10 isolates of *Vibrio sp* bacteria could be obtained, and one of them, or 10% was a type of *Vibrio parahaemolyticus*. The bacteria *Vibrio parahaemolyticus after testing with antibiotics oxytetracycline, tetracycline, and enrofloxacin showed that the antibiotic is still sensitive to* Vibrio parahaemolyticus. Although antibiotics are still quite effective in shrimp farming activities in Jeneponto Regency, they still have to be aware of the emergence of resistant bacteria due to the use of antibiotics. As a result of the administration of drugs aimed at inhibiting or suppressing certain bacteria, which in the sub-therapeutic process will accumulate in water and sediment will make ideal conditions for the emergence and selection of resistant bacterial strains or can even stimulate horizontal transfer of resistant genes [1].

The emergence of antibiotic resistance and the spread of resistant bacteria in aquaculture depend on the type of antibiotic used, inappropriate use of antibiotics, the use of antibiotic-contaminated animal feces, cultivation methods that are integrated with other activities, the presence of resistant genes, the presence of antibiotic residues in animals or the environment (Aly and Albutti 2014). The main cause of antibiotic resistance is the use of antibiotics that are suboptimal and not accompanied by sufficient data on their use [23].

The results of research in Indonesia by Yudiati E., (2021) showed that *Vibrio parahaemolyticus* bacteria isolated from several white shrimp ponds (Litopenaeus vannamei) have been resistant to amoxicillin and ampicillin antibiotics (Yudiati, Subagiyo, and Azhar 2021). If antibiotic-resistant bacteria are present in shrimp farms, then there are several possible dangers, among others, reducing shrimp production: Antibiotic-resistant bacteria can cause disease in shrimp in ponds. If not treated properly, this disease can spread and result in the mass death of shrimp. This will certainly reduce shrimp production from the farm, reducing the quality of shrimp: Antibiotic-resistant bacteria can cause infections in shrimp that can affect the quality of shrimp produced. Infected shrimp can be of poor quality and reduce their marketability, increasing antibiotic resistance in humans: If the antibiotic-resistant bacteria spread to humans, it could be that humans are infected and difficult to treat because antibiotics do not work. Then it can also increase antibiotic resistance in humans. Therefore, it is important to anticipate the emergence of antibiotic-resistant bacteria in shrimp ponds by paying attention to the cleanliness of the water in the pond and avoiding the use of antibiotics that are not the right dose and duration of use.

Alternative substitutes for the use of antibiotics that have been resistant to bacteria can usually use extracts from certain plants or by using natural competitors from these bacteria. In research conducted in Vietnam on bacteria

originating from ponds, some of them can inhibit the growth of *Vibrio parahaemolyticus* bacteria, including the types of *Streptomyces sp* and *Bacillus subtilis* (Phuong, Thanh, and Diep 2020; Prates et al. 2022). Some types of seaweed including *Kappaphycus striatum, Hydropuntia edulis, and Eucheuma denticulatum* can inhibit the growth of *Salmonella arizonae* bacteria [22].

4. CONCLUSION

Based on the results of observations of *Vibrio parahaemolyticus* bacteria from pond shrimp in 4 districts in South Sulawesi Province, there have been no bacteria resistant to antibiotics for oxytetracycline, tetracycline, and enrofloxacin types. It can be recommended to continue routine observation at that place and other places to detect early the emergence of antibiotic-resistant bacteria in brackish water aquaculture activities, especially for shrimp commodities.

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