JURNAL ILMU PENDIDIKAN INDONESIA
Website:http://ejournal.uncen.ac.id/index.php/JIPI
Jurnal Terakreditasi Nasional, Keputusan, No 225/E/KPT/2022

Jurnal **Ilmu Pendidikan Indonesia**

Vol 13, No 1, Pages 65 –72 February 2025
P – ISSN 2338-3402, E-ISSN 2623-226X

STUDY ON THE SUSTAINABILITY OF THE USE OF ANTIMICROBIAL (AMU) AND ANTIMICROBIAL RESISTANCE (AMR) IN MARINE FISH FARMING IN THE BAY

Syaripuddin[1)], Bethsy J Pattiasina[2)], Shelly M Pattipeiluhu[3)]

[1)] Ambon Fisheries and Aquaculture Center, Postgraduate Program of Patimura University Ambon, Faculty of Fisheries and Marine Sciences, Indonesia; syaripuddinsima@gmail.com
[2)] Ambon Fisheries and Aquaculture Center, Patimura Ambon University Postgraduate Program, Faculty of Fisheries and Marine Sciences, Indonesia; bpattiasina@yahoo.com
[3)] Faculty of Fisheries and Marine Sciences, Pattimura University, Indonesia; shellymieke@gmail.com

Abstract: *Use of antibiotics and chemicals substances were done to prevent bacterial attacks especially by Vibrio sp, which if used excessively will cause bacterial resistance. Another problem that arises is the risk of damage to the surrounding environment of fish farming and human health, therefore the use of these substances must be effective and efficient. The research aims to determine the antimicrobial inhibition during the handling of cage-cultured fish diseases and to determine the level of resistance to antimicrobial substances (Antimicrobial Resistance/AMR) to obtain recommendations for the sustainability of antimicrobial use. Disk diffusion test method with antibiotics Oxytetracycline, Enrofloxacin, and Tetracycline in paper disc form was used in the inhibition test. Bacterial identification used biochemical tests with bacterial culture media (TCBS) also biochemical tests using the API 20E kit. The results show that the inhibitory power of using the three types of antibiotics is able to provide sensitive and intermediate responses to Vibrio sp bacteria. Sensitivity inhibition in white snapper, trevally, grouper, and humpback grouper with oxytetracycline antibiotics all at 60%, enrofloxacin antibiotics respectively 80%, 100%, 100%, and 80%; and tetracycline antibiotics respectively 60%, 100%, 60%, and 80%. Intermediate inhibition with the same group of fishes with oxytetracycline antibiotics all at 40%, enrofloxacin antibiotic in all fish 0%, except in humpback grouper 20% and tetracycline antibiotics respectively 40%, 0%, 40%, and 20%. Thus, it can be concluded that the use of oxytetracycline, enrofloxacin, and tetracycline antibiotics can still be recommended for the management of diseases in aquaculture. For common bacteria, two types of bacteria Vibrio parahaemolyticus and Vibrio alginolyticus have been found.*
Keywords: Resistance, *Vibrio* spp, antibiotics, fish farming

1. INTRODUCTION

Indonesia's fisheries and marine sector is a high-value sector and has the potential to be developed in improving the economy of the Indonesian people (Luhur *et al*., 2019). This potential shows an increase in the value of gross domestic product from 2015 (5,363,274 tons) to 6,242,846 tons in 2018 (Miar *et al.*, 2020). The export value of fishery products in 2011-2015 showed an increase of up to 2.29% in the categories of live fish, frozen fish, mollusk products and crustaceans (Luhur *et al*, 2019). However, this potential is also challenged in terms of decreasing product quality, causing cases of rejection of fishery product exports in destination countries (Nurilmala *et al*., 2020). One of them is pathogenic bacterial contamination in fishery products starting from the cultivation

or capture process to product processing (Schmidt *et al*., 2018).

Aquaculture production in Indonesia is quite high, both brackish water, freshwater and marine commodities. However, the production process is often hampered by diseases that infect farmed fish in the form of viruses, bacteria, fungi and parasites. Infectious diseases, need to get special treatment so that they can be localized, do not develop and do not endanger the life of cultivated organisms, one of the efforts made is to use anti-microbial materials. Antimicrobials are microbial killers, especially microbes that are detrimental to chemoterpeutics (antimicrobials) are defined as chemical drugs used to eradicate microoranistic infectious diseases such as bacteria, fungi, viruses and protozoa as well as infections by worms (Tjay, *et al*, 2010).

Antimicrobials as medicinal ingredients have an important role in achieving healing, but in their use, caution is needed. The uncontrolled use of antimicrobials as antibiotics can cause other problems, namely the onset of resistance. As a result, it will accelerate the process of transmitting resistant microbes and further worsen the quality of antimicrobial work. Currently, antimicrobials are one of the global problems that need serious attention. Regulations on antimicrobial resistance have been regulated by the OIE in the *Aquatic Animal Health Code* of 2019. This regulation was followed up by the Government of Indonesia by issuing the Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 52/KEPMEN-KP/2014 concerning the classification of fish drugs as antibacterial, namely erythromycin, enrofloxacin, chlortetracycline, oxytetracycline and tetracycline which regulates the use of antimicrobials, especially antibiotics that are allowed in aquaculture in accordance with the use of antimicrobials, especially antibiotics that are allowed in aquaculture if appropriate, it will not be a serious problem if used correctly. Proper use will result in *overuse* and *misuse* which results in antimicrobial resistance in pathogenic bacteria.

Antimicrobial resistance occurs when microorganisms such as parasites (protozoa), fungi, viruses and bacteria undergo changes/mutants so that the drugs used to cure the infection caused are ineffective and unable to eliminate these microorganisms. The causes of antimicrobial resistance are: excessive and improper use of antimicrobials in livestock and fisheries, inadequate hygiene and sanitation, slow development of new antimicrobials (WHO 2021).

Antibiotic resistance in bacteria can lead to complications, longer treatment periods, treatment failures and death from resistant bacterial infections. Various efforts have been made to prevent the attack of Vibrio alginolitycus bacteria, including the use of chemicals and antibiotics. The use of these two materials requires a fairly expensive cost and if used excessively and

continuously, it can cause bacterial resistance. Another problem that arises is the impact on the environment, farmed fish, and humans (Anonymous, 2005), so the use of these ingredients must be effective and efficient, which is based on the concentration of antibiotics used and takes into account the toxicity level of the bacteria. The purpose of this study is to identify the types of bacteria distributed in farmed fish in Floating Net Cages (KJA), find the need for antimicrobial use (*Anti Microbial Use*/AMU) when handling floating net cage fish diseases in Ambon Dalam Bay, and analyze the level of microbial resistance to antimicrobial materials (*Antimicrobial Resistance/AMR*) as a feasibility study for its use in floating net cages in Ambon Dalam Bay.

2. RESEARCH METHODS

Time and Place

This research was carried out at the Microbiology Laboratory of the Ambon Marine Aquaculture Fisheries Center, Fish Disease and Environmental Pest Laboratory from July to December 2023. Fish sampling at a floating net cage fish farming business unit in the Ambon Dalam Bay environment (Figure 1). All samples taken in the form of live fish are followed by testing as soon as possible after arriving at the laboratory, to avoid death and deterioration of the quality of the samples.

Sampling

The method of sampling bacteria was carried out according to the *interagated* method based on SNI 698. Meanwhile, the fish sampling method is carried out *purposively*, which is the selection of samples for certain purposes (FAO, 2004). Sampling was carried out by considering the entry route of marine environmental disease polluting agents, the exposure period and the transport mechanism in the water body. Samples were taken from tiger grouper, white snapper, bubara fish and duck grouper in healthy condition

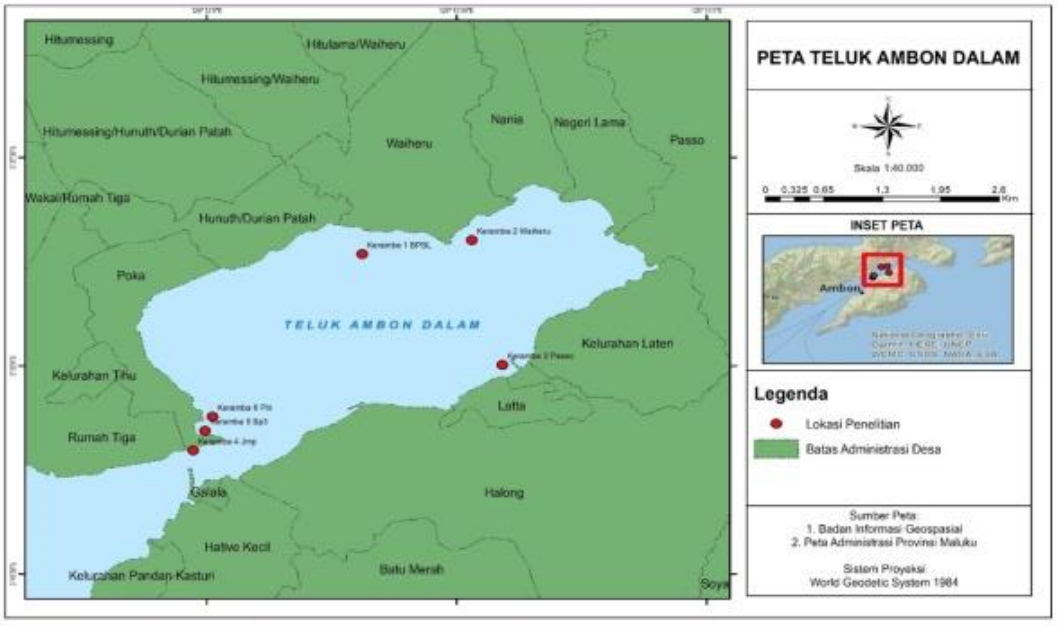

Figure 1. Map of sampling location points in Ambon Dalam Bay

Material

The materials used in this study are: Vibrio algynolitycus *bacterial isolate*, ATCC 17749 *Vibrio algynolitycus Standard Bacteria,* Mueller Hinton Broth Merck, 0.5 Mc Farland Standard, Oxytetracycline Standard, Enrofloxacin (Sigma), Disc antibiotic oxytetracycline, enrofloxacin (oxoid), and Aquadest sterile.

Tool

The tools used in this study are: analytical scales, test tubes, sterile petri dishes, volumetric pipettes, refrigerators, tip pipettes. Hot plate, ose needle, vortex, autoclave, incubator, and bunsen.

Anti Microbial Use (AMU)

To determine the use of antimicrobial (*Anti Microbial Use*) in the cultivation of floating net cage fish, surveillance of the use of antibiotics and fish health is carried out, namely systematic observation activities to obtain data and information about the incidence of fish diseases or health problems and conditions that affect the increase and transmission of diseases in order to direct control and countermeasures effectively and efficiently. The basic concept of surveillance activities includes: data collection, data processing, data analysis and data interpretation, feedback, good dissemination and quick response. Data collection is carried out with a questionnaire that has been provided

Antimicrobial Sensitivity Test *Method*

The Antimicrobial Sensitivity (AST) test uses *the Agar Diffusion method*. This method is performed using antibiotic discs and the level of antibiotic sensitivity is calculated based on the inhibitory zone formed

Test preparation

Preparation for the test was carried out by making Mueller Hinton agar media and Mueller Hinton media liquid. The test material included 11 isolates *of Vibrio alginolitycus* and standard bacteria, the concentration of test bacteria was made 1.5 x 106 cfu/ml for MIC testing and 1.5 x 108 cfu/ml for AMR testing, respectively. Next, a sterile 0.85% NaCl physiological solution and a standard antibiotic concentration of oxytetracycline and endroflosaxin were 512µg/ml, respectively, for MIC testing.

Test Implementation

The implementation of the test begins by dipping sterile ose (*sterile cotton stick*) into broth containing bacteria, then streak the agar surface from left to right diagonally. Streak the surface from left to right by changing/rotating the position of the cup in a quarter circle 2-3 times. The same is done for ATCC standard bacteria. Next, the antibiotic paper disc is placed on the surface of the water so that, with such a distance that it is expected that there will be no accumulation of inhibition zones, it is left for about 15-30 minutes at room temperature (180C -220C). The dish was incubated at 250C-300C for 16-24 hours (CLSI, 2022) for *Enterobacteriaceae*), the bacterial growth inhibition zone was measured in millimeters. The interpretation of test results is carried out by comparing the inhibition zones formed with the Test Result Interpretation Standard.

3. RESULTS AND DISCUSSION

Antimicrobial Use (AMU)

The use of antimicrobials by floating net cage fish farmers in Ambon Dalam Bay is relatively low, from all respondents, only 25% use antimicrobials. The results of monitoring the use of antimicrobials can be seen in Table 1.

Table 1. Survey results of antibiotic use in KJA in Teluk Ambon Dalam

No	Lokasi Survey (KJA)	Penggunaan antibiotic (ya/tidak)	Tujuan (pencegahan/ pengobatan)	Merek Antibiotik	Jumlah Antibiotik (gr/ml)	Pengelolaan Limbah Antibiotik
1	Kelompok Sinar Waiheru	Ya	pencegahan	• C-SAN BOSTER PT SAMBE FARMA • Vitamineral BOSTER PT SAMBE FARMA. • Fish Imonovit BOSTER PT INDOSCO DWIJAYA SAKTI Surabaya.	• 1gr/kg pakan • ml/kg pakan • 5000 ml	Air sisa perendaman di buang kelaut
2	Kelompok Ikan Kerapu	Tidak	-	-	-	-
3	Kelompok Tuna	Tidak	-	-	-	-
4	Kelompok Siloam	Tidak	-	-	-	-
5	Kelompok Arid	Tidak	-	-	-	-
6	Kelompok Baronang	Ya	pengobatan	• Enrofloxacin-12 BOSTER PT Indosco Surabaya. • Enrofloxacin-25 BOSTER PT Indosco Surabaya. • Vitamin B kompleks BOSTER KKP RI No D1707309 PBS. • Oxytetracycline BOSTER *Streptomyces rimosi* • Acriflavine Paul Erhlich, Perusahaan Kimia I.G. FARBEN Jerman	• 15gr/50 L • 15gr/50 L1 • gr/ kg pakan • 1 gr/2-4 kg pakan • 1 gr/50 L	Air sisa perendaman dibuang kelaut

The total number of 24 KJA units in the waters of Ambon Dalam Bay, there are six KJA (25%) surveyed and only two KJA units (33%) use antibiotics. More KJA do not use antibiotics for several reasons such as difficult to obtain, relatively expensive prices and farmers know very well that fish do not need medicines if the right maintenance system is implemented. The two KJAs who use antibiotics get it from the internet and also the Ambon Fisheries and Marine Aquaculture Center where they are fostered. The amount of antibiotics used has been in accordance with the manufacturer's recommendations written on the packaging. Fish antibiotic soaking is carried out before feeding, for 2 hours if done in a seed rearing tub while if done in KJA soaking is only 10-20 minutes to avoid stress.

The problem is that cultivators still dispose of antibiotic waste after soaking into the sea. The management of antibiotic waste in farmed fish is an important topic because its use in fish farming can cause problems of antibiotic resistance and environmental pollution. The use of antibiotics in this study is carried out only if there is a disease attack of both bacteria and parasites infested with floating nets. Some previous research results to prevent pollution, for example, the use of chemical reagents such as calcium hypochlorite can break the betalactan ring with 100% efficiency, which helps reduce antibiotic pollution in water. Likewise, filtration systems and aerators help maintain

water quality by minimizing waste and ensuring that water remains clean and oxygenated. The biofiltration method involves the use of microorganisms to decompose the waste of organisms including antibiotic residues thereby reducing their impact on the aquatic ecosystem. In addition, biodigister technology can process waste into biogas and can be used as an alternative energy source for fiber to produce fertilizer that is useful for agriculture. Last but not least is the cessation of pre-harvest antibiotic administration, cultivators are required to stop administering antibiotics to ensure that antibiotic residues have been cleared from the body of fish so that fishery products are safe for human consumption and the environment. No research has been conducted to study the effect of waste disposal on the surrounding waters or on fish cultivated in Ambon Dalam Bay.

Efforts to prevent fish diseases in KJA that do not use antibiotics are by soaking in fresh water, usually done once in 2 weeks routinely with a soaking duration of 10-15 minutes or depending on the condition of the fish with the aim of avoiding ectoparasites in the fish. For the administration of vitamins to cultivated fish, they usually do it only once a week to increase the appetite of the fish even if the vitamin supply is available.

By understanding the current use of antibiotics, several factors can be known that can accelerate the emergence and spread of resistant antimicrobials (AMR). This includes factors that are difficult to control and factors that can be controlled, factors that are difficult to control such as an increase in the human population and an increase in human movement around the world whereas factors that can be controlled such as efforts to manage antimicrobials through efforts to reduce the use of inappropriate and improper antimicrobials. Controllable AMRs, such as those relating to improper or improper use, should be fully encouraged to be implemented.

Uji Sensitivitas Antibiotik (***Antimicrobial Sensitivity Test*) (AST)**

Sensitivity is a state in which microbes are very sensitive to antibiotics or sensitivity is the sensitivity of an antibiotic to a microbe to be able to show in conditions that are appropriate for its inhibitory effect on microbes, a decrease in antimicrobials that shows small changes that cannot be shown by chemical methods, so that microbiological and biological testing is usually a standard method to overcome doubts about loss of microbial activity (Djide, 2008)

In the bacterial sensitivity test, it is Agar Diffusion, which is by observing the inhibition of the growth of microorganisms by known extracts from the area around the paper *disk* that are not overgrown by microorganisms. This growth inhibition zone shows the sensitivity of bacteria to antibacterial ingredients (Junairiah, 2005). The sensitivity of bacteria to antibiotics depends on the

ability of the antibiotic to penetrate the bacterial cell wall. Antibiotics are more effective against gram-positive bacteria because they have higher cell wall permeability than gram-negative so an antibiotic is said to have a narrow spectrum if it is able to inhibit the growth of gram-positive bacteria, while broad-spectrum antibiotics if the growth of gram-positive and gram-negative bacteria can be inhibited by antibiotics (Sumadio, et al, 1994). Compounds that can inhibit the growth of bacteria are called bacteriostatics and those that can kill bacteria are called bactericides or in other words also called antibiotics, which are bio-sourced materials that at low levels have inhibited the growth of living microorganisms (Paturusi, 2008).

Intermediate is a state where there is a shift from a sensitive state to a resistant state but not completely resistant while resistance is a state where microbes are sensitive or resistant to antibiotics (Djide, 2008). The cause of resistance to microorganisms is the inappropriate use of antibiotics, for example the use of inadequate doses, irregular use, as well as the treatment time is not long enough, so to prevent or slow down the occurrence of resistance, the use of antibiotics needs to be considered (Djide, 2008) The inhibition zone is a place where the growth of the bacteria is inhibited due to antibacterial or antimicrobial. The inhibition zone is the area to inhibit the growth of microorganisms in the agar medium by antibiotics such as Tetracycline, Oxytetracycline, Enrofloxacin are antibiotics that have a wide spectrum so that they can inhibit the growth of bacteria widely (Djide, 2008).

Resistance test is a test that is carried out to determine the sensitivity of bacteria to an antibiotic. Excessive or uncontrolled use of antibiotics causes dangerous side effects, which cause certain bacteria to become resistant to antibiotics. The mechanism of action of this antibiotic is by inhibiting the formation process of DNA supercoil related to the enzyme DNA gyrase which is an enzyme that is important for DNA replication and repair (Shulman *et al,* 1994). Bacterial resistance to these antibiotics can occur due to the presence of gene mutations that encode polypeptide subunit A of the DNA gyrase enzyme (Jawetz *et al*., 2001). The resistance, sensitivity and intermediate tests to be tested are *Vibrio sp* bacteria isolated against three antibiotics, namely Oxytetracycline, Enrofloxacin and Tetracycline, the test results can be seen in the table below:

The results of this study have been adjusted to the standard criteria from the Clinical and Laboratory Standards Institute (CLSI 2022) of the antibiotics enrofloxacin, oxytetracycline and tetracycline. The average amount of the diameter of the resistance force formed and the results can be seen in Table 2.

Table 2. AST test results for Vibrio sp bacteria in fish samples

NO		AST Oxytetracycline (S/I/R)	AST Enrofloxacin (S/I/R)	AST Tetracycline (S/I/R)
1	Sampel	14,75 (S)	30 (S)	15,25 (S)
Ikan Kakap putih		16 (I)	16,75 (I)	15,5 (I)
		16,5 (I)	18 (S)	16,5 (I)
		18,75 (S)	17,75 (S)	19,25 (S)
		18,25 (S)	19,25 (S)	19,5 (S)
2	Sampel	AST Oxitetracyclin (S/I/R)	AST Enrofloxacin (S/I/R)	AST Tetracyclin (S/I/R)
Ikan Bubara		15,75 (S)	21 (S)	19 (S)
		20,5 (S)	21,75 (S)	18,75 (S)
		17 (I)	29,5 (S)	16,5 (S)
		15 (S)	27,5 (S)	26,5 (S)
		17 (I)	21,65 (S)	18,75 (S)
3	Sampel	AST Oxitetracicline (S/I/R)	AST Enrofloxacin (S/I/R)	AST Tetracycline (S/I/R)
Ikan Kerapu macan		16,75 (I)	25,75 (S)	16,75 (S)
		21,5 (S)	21,5 (S)	16,7/ (I)
		20,6 (S)	29 (S)	15,75 (I)
		18,75 (S)	27 (S)	16,5 (S)
		17,5 (I)	25,75 (S)	21,25 (S)
4	Sampel	AST Oxitetracycline (S/I/R)	AST Enrofloxacin (S/I/R)	AST Tetracycline (S/I/R)
Ikan Kerapu bebek		20,75 (S)	20,5 (S)	21 (S)
		19,25 (S)	19,25 (S)	18 (S)
		19,75 (S)	19 (S)	16,75 (I)
		12,5 (I)	20,5 (S)	14,25 (S)
		16 (I)	16,75 (I)	15,5 (S)

From the table above, we can see that the standard of each antibiotic is different for a particular bacterium tested. The test results are marked with the letters "S" and "I" (intermediates) while resistant antibiotics are marked with the letters "R" Sensitive indicates that the antibiotic has an inhibitory power greater than the criteria that should be, the intermediate is in the lowest minimum range until it reaches sensitivity and resistance indicates that the inhibitory force formed is far below the specified *criteria.(National Committee For Clinial Laboratory Standards) NCCLS* . Oxytetracycline 30 µg sensitive at ≥ diameter 21 mm intermediate 16-20 mm resistant ≤ 15 Enrofloxacin 5 µg, sensitive at ≥ inhibitory zone diameter 21 mm, intermediates 16 – 20 mm, resistant ≤15 mm, Tetracycline 30 µg, sensitive at inhibitory zone diameter ≥19 mm, intermediates 15 – 18 mm, resistant ≤ 14 mm. Oxytetracyline antibiotic 30 µg, sensitive to the diameter of the inhibition zone ≥18 mm, intermediates 13 – 17 mm, resistant ≤ 12 mm (Clinical and Laboratory Standarts Institute, 2022). For intermediate criteria, sensitive and resistant on. The antibiotic

enrofloxacin has almost the same spectrum of action as the antibiotic oxite tracycline, which is against bacteria by stopping bacterial multiplication by inhibiting bacterial reproduction and repairing genetic material or DNA, but enrofloxacin has good acting power compared to oxtetracycline because the antibiotic enrofloxacin works effectively in all layers of body tissues. This reason is why this antibiotic is widely used to treat organisms when infected with bacteria (www. Wikipedia. org/), bacteria are categorized as sensitive to certain antibiotics, if with certain concentrations there can be a large diameter of bacterial growth inhibitor. Resistance occurs due to the excessive use of antibiotics in humans, but some of it may be due to the use of antibiotics as growth promoters in animal feed (Johnson *et al*., 2006). These antibiotics are widely chosen because antibiotics can kill gram-positive and gram-negative bacteria (Shoki *et al*., 2010). Since its discovery, *V. Parahaemolyticus* has been found to be the cause of 20–30% of food poisoning cases in Japan and seafood-borne diseases in many Asian countries (Nature, et al., 2002). *Vibrio Parahaemolyticus* is also recognized as the leading cause of gastroenteritis in humans associated with seafood consumption in the United States (Kaysner and DePaola, 2001; Newton et al., 2012). The global prevalence of gastroenteritis cases emphasizes the need to understand the virulence factors involved and their impact on humans.

The AST test results of 20 isolates of *Vibrio sp* bacteria in white snapper using three types of antibiotics, namely oxytetracycline, enrofloxacin and tetracycline, were found to be sensitive in white snapper with antibiotics of 30%, 40% and 30% respectively and (Figure 2)

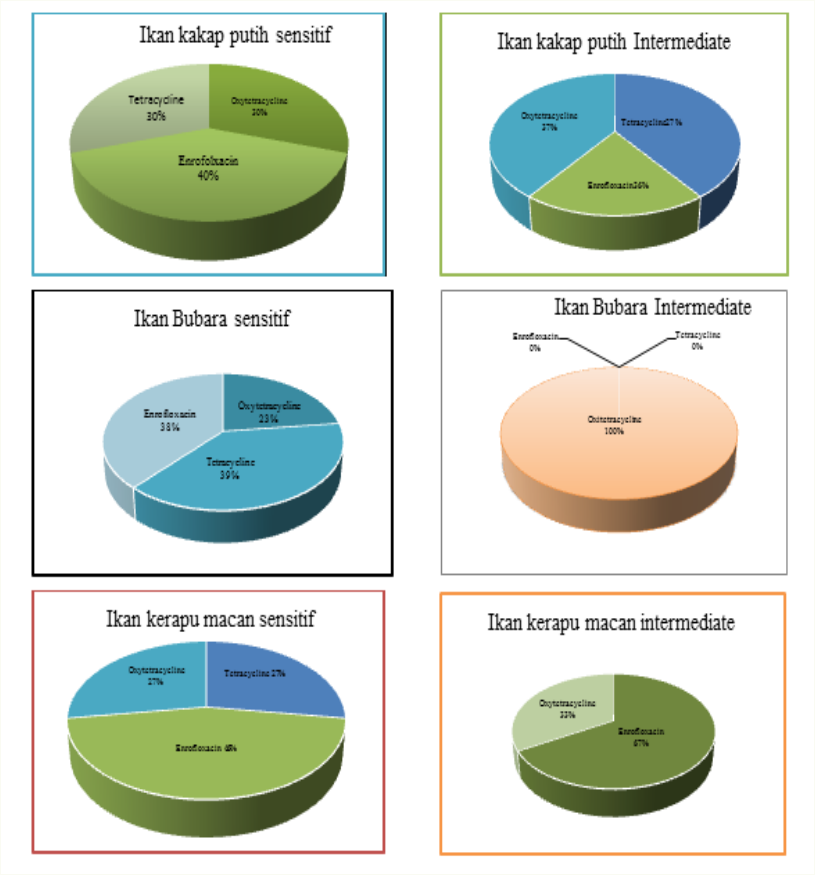

Figure 2. The results of the AST test of Vibrio sp bacteria from fish meat samples are different against three types of antibiotics

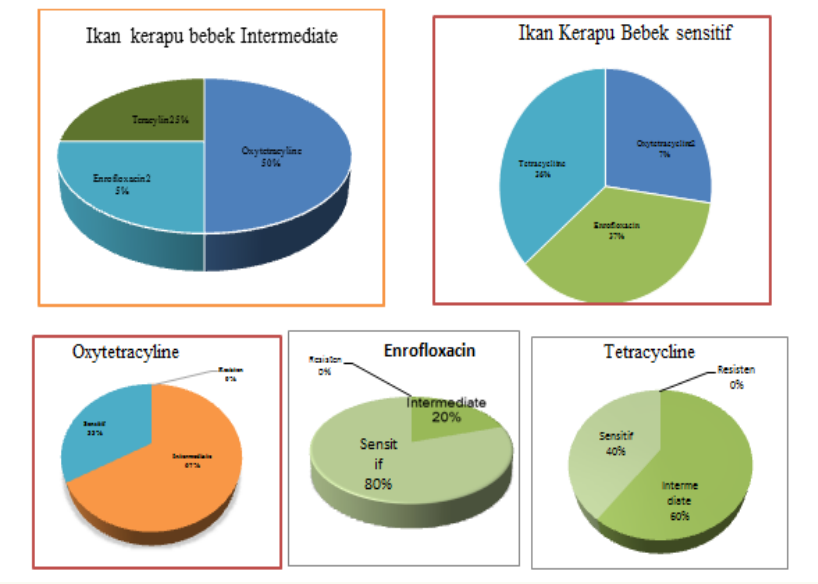

Figure 3. AST Test Results of *Vibrio sp* Bacteria against oxytetracycline, Tetracycline and Enrofloxacin

Inhibition Zone Measurement

Antibacterial inhibition varies, antibacterial is a material or compound that is specifically used for a group of bacteria. Antibacterial can be differentiated based on its mechanism of action, namely antibacterial that inhibits cell wall growth, antibacterial that results in changes in cell membrane permeability or inhibits active transport through cell membranes, antibacterial that inhibits protein synthesis and antibacterial that inhibits cell nucleic acid synthesis. The antibiotic Enrofloxacin does not work effectively on anaerobic bacteria, and will act selectively when an organism is attacked or infected *streptococcus.* Herwigh et al., (1979) said that the action of antibiotics as sidal bacteria and bacteria is highly dependent on the dose or active power of the antibiotic concentration given. AST test results from bacterial isolate *vibrio parahaemyliticus* By using three types of antibiotics, namely oxytetracycline, enrofloxacin and tetracycline, it was obtained as a sensitive TV, intermediate in white snapper using antibiotics there was an inhibitory power of Oxytetracycline, Sensitive 33% while intermediate 67%. The antibiotic enrofloxacin has the bioactive content of the antibiotic enrofloxacin which is included in the fluoroqiunolone group derivative. The mechanism of action of this enrofloxacin antibiotic is difficult to understand This antibiotic can be used to cure diseases caused by gram-positive bacteria or gram-negative bacteria which include a variety of species which include *Pseudomonas aeruginosa*, *Klebsiella, E. coli*, *Enterobacter*, *Campylobacter*, *Shigella*, *Salmonella*, *Aeromonas*, Haemophillus, Proteus, Yersinia, Serratia, Vibrio, Brucella, Chlamydia, Staphylocci mycoplasma, dan *Mycobacterium* (www. Wikipedia. org / enrofloxacin/ 2010). The results showed that oxytetracycline could significantly inhibit antibody levels, while slightly reducing phagocyte activity and lymphocyte count, but had no obvious effect on fish survival rates. Oxytetracycline can inhibit the peak of the fish's humoral response is in Figure 4 below.

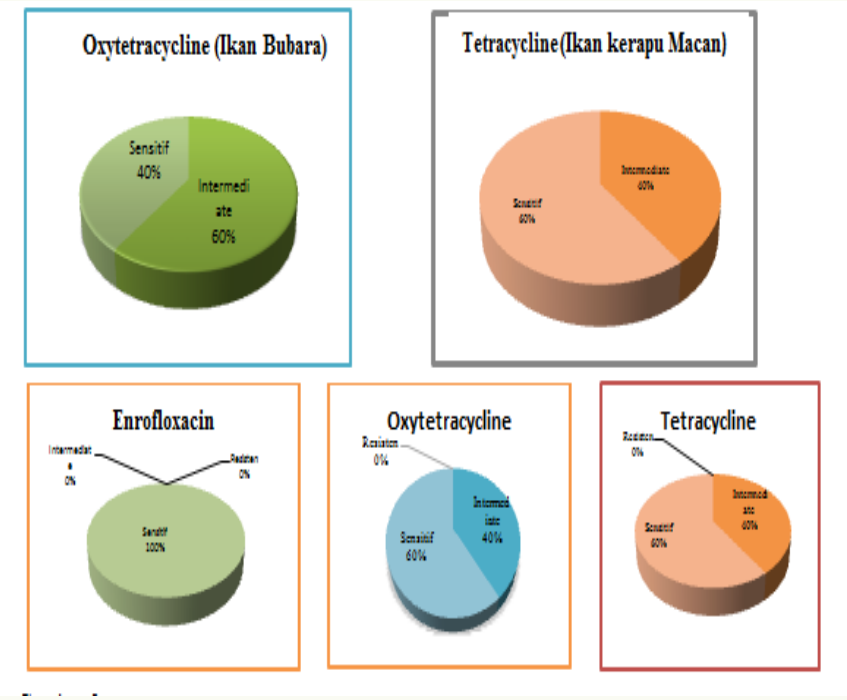

Figure 4. AST Test Results Inhibitory Ability of Oxytetracyline Antibiotic against *Vibrio sp* bacteria in bubara fish

AST test results from *vibrio sp* bacterial isolate in tiger grouper using oxytracycline antibiotics with sensitive inhibition of 60% and Intermediate 40%, Resistance 0%. The results showed that oxytetracycline could significantly inhibit antibody levels, while slightly reducing phagocyte activity and lymphocyte count, but had no obvious effect on fish survival rates. Oxytetracycline can inhibit the peak of the fish's humoral response.

Enrofloxacin antibiotics have bioactive content, namely vitamins B and C and have several uses, namely eradicating bacteria quickly and effectively, curing fish diseases such as bleeding bodies, enlarged stomachs, melting mucus, rotten ulcers, peeling scales, fish often appear on the surface, and decreased appetite, Kamiso (1996), increasing the immunity of fish and shrimp due to bacterial diseases, increasing the appetite of fish and shrimp so as to accelerate growth and increasing fish/shrimp activity caused by bacterial diseases.

Tetracycline is an antibiotic that is primarily bacteriostatic (Agustanty & Andre, 2022). Tetracycline is an antibiotic that has a broad spectrum and can inhibit various gram-positive, gram-negative bacteria, both aerobic and anaerobic, as well as other microorganisms such as Ricketsia, Mycoplasma, Chlamydia, and several mycobacterial species (Permenkes, 2011). The diversity of distribution of tetracycline resistance depends on environmental conditions such as waste, soil and

water. Horizontal gene transfer is the main mechanism that can lead to the high spread of resistance genes in other bacteria in the environment. The existence of high antibiotic resistance can be a special concern because the transfer of resistance can also occur due to environmental factors, ecological factors such as from other livestock, rodents, pets or from cage workers (Anggita et al, 2022). The prevalence and distribution *of V. parahaemolyticus* is known to be influenced by several environmental factors including water temperature, salt and oxygen concentrations, interactions with plankton, the presence of sediments, organic matter in suspensions and marine organisms (Cabrera-Garcia et al., 2004). To find out the results of the use of the three antibiotics in samples of white snapper, bubara, tiger grouper and duck grouper from the Floating Net Cage, see Figure 5.

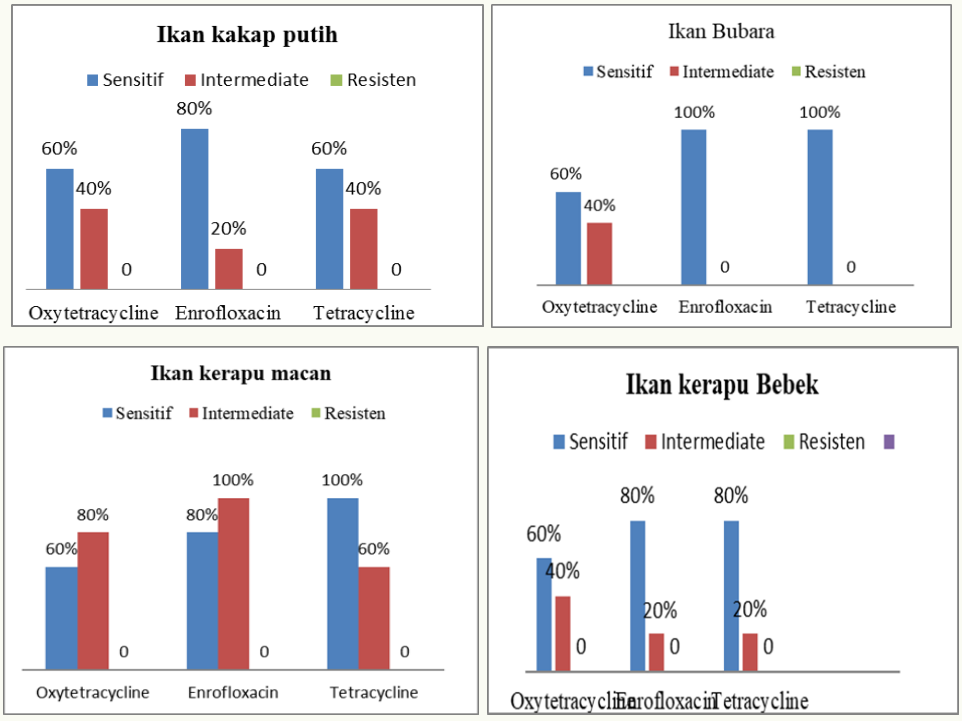

Figure 5. Response of *Vibrio sp* bacteria in white snapper (A), Bubara fish (B), Tiger Grouper (C) and Duck Grouper (D) to antibiotics

Based on the picture above, it can be seen that white snapper with oxytetracyline antibiotics is sensitive by 60%, intermediate 40% while enrofloxacin is sensitive 80% and intermediate 20% then tetracycline is sensitive 60% and intermediate 40% resistant is not found in the use of antibiotics then the fish disperse by using antibiotics oxytetracyline sensitive by 60%, intermediate 66% while enrofloxacin is sensitive 100% and intermediate 40% then tetracycline sensitive 100% and 100% intermediate resistance is not found in the use of antibiotics, it can also be known that

tiger grouper fish with oxytetracyline antibiotics is sensitive by 60%, intermediate by 80%, while enrofloxacin is sensitive by 80%, and intermediate by 100% then tetracycline is sensitive by 100%, and intermediate by 60%, resistance is not found in the use of antibiotics, then it can also be known that duck grouper with oxytetracyline antibiotics is sensitive by 60%, Intermediate 40% while enrofloxacin is 80% sensitive and 20% intermediate then tetracycline is 80% sensitive and intermediate 20% resistant is not found in the use of antibiotics.

4. CONCLUSIONS AND SUGGESTIONS

CONCLUSION

The types of bacteria identified in farmed fish samples in floating net cages in the Inner Bay are *Vibrio alginolyticus* and *Vibrio parahaemolyticus*.

The use of antibiotics oxytetracycline, endrofloxacin and tetracycline can still be recommended for disease management in aquaculture, because the inhibitory use of the three types of antibiotics is able to provide a sensitive and intermediate response to *Vibrio parahaemolyticus* and *Vibrio alginolyticus bacteria*.

SUGGESTION

The science e-module on Heat Transfer based on Papuan local wisdom can be used to improve science learning outcomes in elementary schools.

5. ACKNOWLEDGMENTS

Gratitude is mainly addressed to the Head of the Ambon Fisheries and Aquaculture Center, the Head of the Microbiology Laboratory of the Ambon Marine Aquaculture Fisheries Center, and the Fish and Environmental Pest and Environmental Laboratory who have provided permission and assistance in this research.

BIBLIOGRAPHY

Agustanty, D., & Andre, W. (2022). Tetracycline: Antibiotic with Broad Spectrum and Bacteriostatic Properties. Journal of Microbial Resistance, 15(3), 210-220.

Alam, M., et al. (2002). Vibrio parahaemolyticus and Its Role as a Foodborne Pathogen in Japan and Southeast Asia. International Journal of Food Microbiology, 82(1-2), 137-145.

Anggita, R., et al. (2022). Antibiotic Resistance in Farm Animals and Transfer of Resistance through Environmental and Ecological Factors. Journal of Public Health, 17(2), 215-224.

Anonymous. (2005). The Impact of Antibiotic Use on the Environment and Human Health in Aquaculture Environmental Monitoring Report, 9(3), 35-42.

Cabrera-Garcia, M. et al. (2004). Prevalence and Distribution of Vibrio parahaemolyticus Influenced by Environmental Factors: Temperature, Salinity, Oxygen, and Interaction with Plankton. Journal of Environmental Microbiology, 15(4), 87-98.

Clinical and Laboratory Standards Institute (CLSI). (2022). Performance Standards for Antimicrobial Susceptibility Testing (M02-Ed14). CLSI.

Djide, M. (2008). Antibiotic Sensitivity Testing to Pathogenic Bacteria.

FAO. (2004). Guidelines for the Use of the Codex Alimentarius and Other Standards. Food and Agriculture Organization of the United Nations (FAO).

Herwigh, A., et al. (1979). Antibiotic Action as Bactericidal or Bacteriostatic Depending on Dosage and Concentration. Journal of Antibiotics, 32(2), 83-90.

Jawetz, E., Melnick, J. L., & Adelberg, E. A. (2001). Microbiology: Antibiotic Resistance and Mechanisms. In Medical Microbiology (23rd ed., pp. 428-430). Appleton & Lange.

Johnson, J. R., et al. (2006). Antimicrobial Resistance in Foodborne Pathogens: Implications for Public Health. Clinical Infectious Diseases, 43(5), 758-765.

Junairiah, S. (2005). Antibiotic Sensitivity Testing by the Agar Diffusion Method. Journal of Microbiology, 10(2), 121-126.

Kaysner, C. A., & DePaola, A. (2001). Vibrio parahaemolyticus: A Review of the Pathogen and Its Transmission in Seafood. Food Control, 12(5), 253-260.

Luhur, E.S., Suryawati, S.H., & Kurniawan, T. (2019). The Contribution of the Fisheries Sector in the Development of Rote Ndao Regency: Location Quotient (LQ) and Shift Share (SS) Approaches. Scientific Bulletin "MARINA" Socio-Economics of Marine and Fisheries, 5(1), 11-19.

Miar, M., et al. (2020). Analysis of Factors Affecting the Growth of the Fisheries Industry in Indonesia. Journal of Economics and Development Studies, 21(2), 123-135.

Newton, A. E., et al. (2012). Vibrio parahaemolyticus: A Leading Cause of Seafood-Related Gastroenteritis in the United States. Journal of Food Protection, 75(6), 1147-1154.

Nurilmala, E., et al. (2020). Analysis of Factors Causing Decline in the Quality of Fishery Products and Their Impact on Export Rejection to Destination Countries. Journal of Economic Development, 8(2), 105-113.

Paturusi, S. (2008). Antibiotics and Their Mechanism of Action against Microorganisms. Journal of Microbiology, 12(1), 34-45.

Permenkes (2011). Regulation of the Minister of Health of the Republic of Indonesia No. 1010/Menkes/Per/IX/2011 concerning Guidelines for the Use of Antibiotics. Ministry of Health of the Republic of Indonesia.

Schmidt, V., et al. (2018). Contamination of pathogenic bacteria in fishery products from the cultivation process to processing. Journal of Food Safety, 12(3), 45-56.

Shoki, J., et al. (2010). Antibiotic Use in Animal Feed and the Development of Antimicrobial Resistance. Journal of Antimicrobial Chemotherapy, 65(6), 1183-1189.

Shulman, S. T., et al. (1994). Mechanism of Action of Antibiotics and Interaction with DNA Gyrase Enzymes. Journal of Microbiology and Antibiotic Therapy, 19(3), 123-130.

Sumadio, et al. (1994). Mechanism of Action of Antibiotics Against Gram-Positive and Gram-Negative Bacteria. Indonesian Journal of Microbiology, 8(3), 45-53.

Tjay, T. H., et al. (2010). Pharmacology and Therapeutics: Antimicrobial and Immunotherapy. Jakarta: Aesculapius Media.

Wikipedia contributors. (2010). "Enrofloxacin." Wikipedia, The Free Encyclopedia. Retrieved from https://en.wikipedia.org/wiki/Enrofloxacin.

World Health Organization (WHO). (2021). Antimicrobial resistance: Global report on surveillance 2021. Geneva: World Health Organization.