Asian Journal of Aquatic Sciences, April 2024. Vol 7, Issue (1) 52-60
e-issn: 2716-4608
p-issn: 2655-366X

ISOLATION OF *Escherichia coli* FROM VANAME SHRIMP (*Litopenaeus vannamei*) POND AND THE SENSITIVITY TOWARD ANTIBIOTICS

Resty Fauziah Putri[1], Nursyirwani Nursyirwani[1], Ummi Mardhiah Batubara[1]
[1]Department of Marine Science, Faculty of Fisheries and Marine,
Universitas Riau, Pekanbaru, 28293 Indonesia
*resty.fauziah1775@student.unri.ac.id

ABSTRACT

Vannamei shrimp (*Litopenaeus vannamei*) is a fishery commodity widely cultivated in various parts of Indonesia. The main problem often found in vannamei shrimp production is poor water quality during the rearing period. This study aims to isolate and identify *E.coli* contained in water, sediment, and meat of vannamei shrimp cultivated in shrimp ponds and examines its sensitivity to antibiotics. The research was conducted from March to May 2023 at the Technical Implementation Unit (UPT) Vannamei Shrimp Ponds of the Maritime Affairs and Fisheries Service, Tanjung Punak Village, Rupat Utara District, Bengkalis Regency, Riau. The survey method was used in this study. The Most Probable Number (MPN) method was employed for *E. coli* isolation to calculate bacterial density, and the Kirby-Bauer method was used to test bacterial sensitivity to antibiotics. Based on the identification of the six isolates carried out, it was found that only two isolates were positively identified as *E. coli* bacteria, namely SD C 3.1 and IP B 1.1, which were isolated from sediment and the Wastewater Treatment Plant (WWTP) pond. The diameter of the inhibition zone for sediment isolate RF01 against the antibiotic chloramphenicol was 18.75 mm, indicating intermediate sensitivity. On the other hand, WWTP isolates RF02 exhibited an inhibition zone of 17 mm for chloramphenicol, indicating medium sensitivity as well. However, it showed resistance to penicillin with a diameter of 6.5 mm and resistance to ampicillin with a diameter of 2 mm.

Keywords: Antibiotics, *Escherichia coli*, Sensitivity, Vannamei shrimp

1. INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) is a fishery commodity widely cultivated in various parts of Indonesia, such as in Tanjung Punak Village, Bengkalis Regency, and Riau. This shrimp has the potential to improve the village's economy. This is because vannamei shrimp is easy to cultivate and has promising market opportunities[1]. The price is relatively lower in the market, encouraging people to increase their consumption of vannamei shrimp to fulfill nutritional health needs. There is an excellent opportunity for efforts to increase vannamei shrimp consumption, which will affect market demand and encourage the development of vannamei shrimp farming business activities[2].

The main problem often found in vannamei shrimp production is failure due to poor water quality during the rearing period. This production failure occurred because the vannamei shrimp intended for export did not meet standards. They contain pathogenic bacteria that could harm consumers. These pathogenic bacteria, such as *Salmonella* sp, *Shigella* sp, and *Escherichia coli*, indicate pollution from the coliform group. *E.coli* in the water will be used to measure pollution caused by human activities around the coast. *E.coli* can survive in water relatively long, making it the best indicator of coliform bacteria

Received : 18 January 2024
Accepted : 10 March 2024

species resulting from fecal contamination and pathogens[3].

Research related to bacterial isolation of *E.coli* in shrimp ponds has been conducted. The study's results stated that *E.coli* bacteria in shrimp ponds, especially in sediments, are more abundant than *E.coli* bacteria in water and shrimp. This is because more nutrients or organic compounds accumulate at the bottom of the water, providing a favorable environment for bacterial growth[4].

There are several ways to inhibit the growth of *E.coli* bacteria, one of which is administering antibiotics. One of the most widely used antibiotics in aquaculture is chloramphenicol. Using chloramphenicol offers many advantages, including inhibiting disease development in vannamei shrimp farming, enhancing endurance, and increasing the weight of cultivated shrimp[5]. Research related to the bacterial sensitivity of *E.coli* against antibiotics was conducted by Rahmaniar et al.[6], which stated that *E.coli* bacteria are 100% resistant to the antibiotic amoxicillin and 22.2% to ampicillin. Meanwhile, the antibiotics that are still effective are tetracycline and chloramphenicol.

One method used to calculate the density of *E.coli* bacteria is the Most Probable Number (MPN) method. MPN is a technique for estimating the number of microbes using a liquid medium in test tubes, typically employing 3 or 5 series of tubes for each dilution. The calculations involved in this method are based on a statistical approach.

The Kirby-Bauer method, also known as the disc diffusion method, can be used to determine the sensitivity of bacteria to antibiotics. This method involves measuring the clear zone area formed around the paper disc that contains the antibiotic, which indicates the level of antimicrobial activity. The disc diffusion method offers several advantages, including its speed, relatively low cost, ease of use, and the fact that it does not require special skills[7].

2. RESEARCH METHOD

Time and Place

The research was conducted from March to May 2023 at the Technical Implementation Unit (UPT) Vannamei Shrimp Ponds of the Fisheries Service in Tanjung Punak Village, Rupat Utara District, Bengkalis Regency, Riau. Bacterial analysis of *E.coli* in water, sediment, and shrimp samples at the Laboratory of Marine Microbiology and Marine Chemistry, Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Riau.

Methods

The method used in this study is a survey method, namely by collecting primary data by measuring water quality, collecting samples directly in the field, and analyzing them at the Marine Microbiology and Marine Chemistry Laboratory. Secondary data was obtained from literature studies, journals, books, and articles.

Procedures

This research begins with conducting a literature study on the topic, collecting and processing the data needed, analyzing data, and compiling a research report. The determination of the sampling location was based on a purposive sampling technique, namely by observing the activities that occurred in the UPT Vannamei Shrimp Ponds Fisheries Service, Tanjung Punak Village, Rupat Utara District, Bengkalis Regency, Riau by researchers. Samples taken were from vannamei shrimp, influent (effluent) water, Wastewater Treatment Plant (WWTP), pond water with three repetitions (taken from three ponds), and sediment with three repetitions.

Water Quality Measurement

Water quality must be constantly monitored by measuring an aquatic ecosystem's physical, chemical, and biological parameters. The water quality measured in each pond was temperature,

pH, salinity, dissolved oxygen, and ammonia levels.

Bacterial Isolation

The isolation of bacteria began with diluting the sample in stages, which were then isolated in liquid media using Lactose Broth (LB) media. The *E.coli* MPN method was used to calculate the density of bacteria. There are three stages of testing carried out with this method: the presumptive test, the confirmation test, and the completed test. The number of coliform bacteria is calculated based on the MPN Table using a 3-tube series (Thomas Formula). The analytical procedure using the MPN method is as follows:

Presumptive Test

Before the presumptive test, multilevel dilution was carried out until dilution. A total of 1 mL of the solution was put into a test tube containing 9 mL of Lactose Broth (LB) medium, and the test tube already contained a Durham tube. Replications were carried out three times using the MPN 3-tube series method. All tubes that had been isolated were incubated for 24-48 hours at 37°C.

Confirmed Test

A total of 1 ml of the solution isolated from LB media was poured into a test tube containing Brilliant Green Lactose Bile Broth (BGLBB) media and a Durham tube (3 repetitions were made). All tubes were incubated for 48 hours at 44.5°C.

Completed Test

This test was carried out by streaking the results of the BGLBB media test, which were positive for Fecal coliform, onto the Eosin Methylene Blue Agar (EMBA) medium; all tubes were incubated for 24 hours at 37°C.

Identification of Bacteria

The identification of bacteria can be done in two ways: morphologically and physiologically. Morphological identification included colony shape, colony structure, cell shape, cell size, and Gram staining of bacteria. Gram staining aims to differentiate bacteria into two groups: Gram-positive and Gram-negative.

Physiological observations can be carried out using biochemical tests. A biochemical test is a test used to identify bacteria physiologically. The test parameters used to identify bacteria *E. coli* physiologically are the Indole test, Methyl red, Voges Proskauer, and Citrate (IMViC). The IMViC test is a way to differentiate between fellow microbes belonging to the Enterobacteriaceae group, with the target being bacteria *E.coli* [8].

Antibiotic Sensitivity Test

The sensitivity test of bacteria to antibiotics can be carried out using the *Kirby-Bauer* method, which uses disc diffusion and measures the diameter of the clear zone, which indicates a response to inhibition of bacterial growth by antibiotic compounds. The way to measure the inhibition zone is to measure the outermost zone of the disc paper to the outer limit of the inhibition zone using a caliper. The sensitivity test results are read based on the Clinical and Laboratory Standard Institute Table[9]. The standard diameter of the inhibition zone for *E.coli* bacteria against antibiotics can be seen in Table 1.

Table 1. The Standard Diameter of the Inhibition Zone for *E.coli* against Antibiotics

Antibiotics	Inhibition zone category (mm)		
	resist	Intermediates	Sensitive
Ampicillin	≤ 13	14-16	≥ 17
chloramphenicol	≤ 12	13-17	≥ 18
Penicillin	≤ 13	14-16	≥ 17

The stage in the sensitivity test is that the MHA media is compacted first. Then, one dose of *E. coli* bacteria was dissolved in 0.9% NaCl solution and inoculated on Mueller Hinton Agar (MHA) media using a sterile cotton bud. *E.coli* has spread aseptically on the surface of the MHA media. There was a positive control with three paper discs containing antibiotics (chloramphenicol, penicillin, and ampicillin) with a dose of 10 µg and empty discs given distilled water as a negative control.

3. RESULT AND DISCUSSION

Condition of Vannamei Shrimp Ponds

The UPT Shrimp Pond of the North Rupat Fishery Service is in Tanjung Punak Village. The area is located in geographical coordinates of 2°08'99.0"N 101°70'56.1"E. The shrimp that are cultivated in these ponds are vannamei shrimp. Apart from ponds, there is also a hatchery room, which produces shrimp seeds from spawning to making larvae. Water entering the ponds comes from the sea, channeled through pipes to seawater storage ponds, and then flows to each pond. Water from each pond is directed into the water-holding pond located between the ponds and then flows to the WWTP pool. There are 3 (three) WWTP ponds, and pipes interconnect all three.

Water Quality Parameters

The water quality parameters studied were temperature, salinity, pH, dissolved oxygen, and ammonia levels. Water quality measurement parameter data can be seen in Table 2.

Table 2. Water Quality Parameters for Vaname Shrimp Ponds

Sample	Water Quality Parameters				
	Temperature °C	Salinity (ppt)	pH	DO (mg/L)	Ammonia (mg/L)
Influent	29.4	21.5	7.50	1.3	0.18
Effluent	28.5	19.6	7.17	6.0	0.20
WWTP	29.4	15.0	6.90	6.5	0.28
Pond K-2	27.5	18.7	7.89	7.6	1.12
Pond K-3	28.5	20.6	7.63	7.6	0.96
Pond K-6	28.0	20.6	7.84	7.5	1.26

The optimum temperature for vannamei shrimp growth ranges from 26-32°C[10]. Based on data from Table 3, the temperature range in pond waters ranges from 27.5-29.4°C, indicating that this temperature is optimal for vannamei shrimp growth. The measurement results obtained ranged from 15-21.5 ppt. The optimal salinity range for vannamei shrimp ranges from 15-30 ppt[11], indicating that this salinity is still considered optimal for the growth of vannamei shrimp. The optimal pH values for vannamei shrimp cultivation range from 7.0 to 8.5. The measurement results obtained a pH ranging from 6.90 to 7.89, meaning it is included in the optimal category for vannamei shrimp growth.

The optimum dissolved oxygen quality standard for shrimp growth is 4-9 mg/l and is suitable for survival. The results of dissolved oxygen measurements in ponds ranged from 1.3-7.6 mg/L. The dissolved oxygen obtained is high in pond water and low in inlet water. Ammonia levels in ponds ranged from 0.18-1.26 mg/L; the highest was in pond 6. This could be due to several factors, such as the number of shrimps, the amount of feed, and the age of the shrimp. This is supported by the opinion of Suhendar et al.[12], stating that ammonia levels contained in the waters are the accumulation of organic matter resulting from the metabolism of shrimp or fish in the form of solid waste (feces) and dissolved (ammonia), which is excreted through the anus, kidneys, and gill tissue.

Bacterial Isolation *E.coli*

Bacterial isolation *of E.coli* starts with the presumptive, confirmed, and completed tests. For the results of the prediction test and confirmation test, refer to the MPN 3 tube series table (Thomas formula). The results obtained in this prediction test can be seen in Table 3.

Table 3. Presumptive Test Results

Test sample	10^{-1}			10^{-2}			10^{-3}			Draw	Index (MPN/100mL)
	I	II	III	I	II	III	I	II	III		
Influent	-	+	-	-	+	-	-	+	+	1-1-2	15
Effluent	+	+	+	+	+	+	+	+	+	3-3-3	≥1898
Pool water	+	+	-	+	+	+	+	-	+	2-3-2	38
WWTP	-	-	+	-	-	-	+	-	-	1-0-1	7
Sediment	+	+	+	+	+	+	+	+	+	3-3-3	≥1898
Shrimp	+	+	+	+	+	+	+	+	+	3-3-3	≥1898

Note : + = positive result; - = negative result

For the abundance of coliform bacteria based on the presumptive test, the highest was found in the discharge water, sediment, and vannamei shrimp, which was ≥1898 MPN/100 mL. Coliform bacteria consist of fecal and non-fecal bacteria. *E.coli* bacteria are included in the fecal coliform group. The high abundance of coliform bacteria has not been a determinant of the presence of *E.coli* bacteria. Suppose only the high coliform count is known. In that case, it is most likely caused by the environment, which indicates pollution, which can indirectly become agents for other pathogens[13].

In the confirmed test, positive results from the presumptive test were then inoculated on BGLBB media, which was incubated at 44.5°C for 48 hours. The results obtained in this confirmed test can be seen in Table 4.

Table 4. Confirmed Test Results

Test Sample	10^{-1}			10^{-2}			10^{-3}			Draw	Index (MPN/100mL)
	I	II	III	I	II	III	I	II	III		
Influent	-	-	-	-	-	-	-	-	-	0-0-0	0
Effluent	+	+	+	+	+	+	+	+	+	3-3-3	≥1898
Pool water	+	+	+	+	+	+	+	+	+	3-3-3	≥1898
WWTP	-	+	+	+	+	+	-	+	+	2-3-2	38
Sediment	+	+	+	+	+	+	+	+	+	3-3-3	≥1898
Shrimp	+	+	+	+	+	+	+	+	+	3-3-3	≥1898

Note : + = positive result; - = negative result

Based on the results of tests, the highest abundance of fecal coliform bacteria was found in 4 samples, namely discharge water, pond water, sediment, and vannamei shrimp, which was ≥1898 MPN/100 ml. In the WWTP, there were few fecal coliform bacteria, namely 38 MPN/100 ml, and in the incoming water, there were no fecal coliform bacteria. In WWTP, a few fecal coliform bacteria can occur because bacteria do not accumulate much in the water but accumulate a lot in WWTP sediments. This is because more nutrients or organic compounds reach the bottom of the seas[4]. According to Government Regulation Number 22 of 2021 regarding the quality standard for fecal coliform bacteria in class 2 waters, namely 1000 MPN/100 mL. If the abundance of bacteria exceeds the quality standards, then the pond waters are

indicated to be polluted by fecal coliform bacteria.

Furthermore, a completed test was carried out to determine the presence of *E.coli* bacteria in the sample. Of the six samples that have been tested, three samples indicate the presence of *E. coli* bacteria, namely sediment samples (SD C 3, SD B 3, SD A 1), shrimp (UD C 2), and WWTP (IP A 2, IP B 1). This was obtained from the results of the bacteria injection using EMBA media, namely that the streaks had a metallic green color. The results of inoculation of *E.coli* bacteria on the EMBA media showed a color change on the surface of the media to a sparkling rainbow color, also called a violet color, indicating the presence of *E.coli* [14].

Morphological and Physiological Characteristics of Bacteria

Based on the morphological characteristics of the bacteria obtained, it is known that the shape of the bacterial colonies obtained from the six isolates was irregular and round. Then, the bacterial colonies' elevation was convex, raised, and flat. The edges of the bacteria are wavy and smooth. Bacterial colonies obtained were metallic green in all isolates. Colonies of *E.coli* bacteria on EMBA media are metallic green. This is due to the ability of bacteria to ferment lactose and methylene blue, while bacteria belonging to the species *Enterobacter aerogenes* will be pink to colorless[15].

In Gram staining of bacteria, the shape of the cell and the nature of the Gram of bacteria. This Gram-negative nature is seen from the color of the bacterial cells on a 1000x magnification microscope. *E.coli* bacteria are rod-shaped bacteria that are Gram-negative, facultatively anaerobic, do not form spores, and are natural flora in the intestines of mammals[16]. Of the six isolates tested, it was found that the bacterial cells were rod-shaped and Gram-negative.

A biochemical test was carried out to see the physiology of bacteria. Because each bacterium has different biochemical properties, the identification process will be more accessible. The indole test was seen from the formation of a red ring at the top of the media after being dropped by Kovac's reagent. The test results showed four indole-positive isolates and two indole-negative isolates. The motile test can be seen from the results of the media being stabbed with bacteria using a straight loop needle, but there is growth of bacteria that do not match the puncture or spread. Of the six isolates that have been tested, it was found that all isolates were motile. In the TSIA media, sugar tests were carried out on glucose, sucrose, and lactose. Of the six isolates tested, the slant and butt turned yellow, and the TSIA media lifted. The yellow media indicates that the bacteria can ferment sucrose and lactose-type sugars. Meanwhile, the raised medium indicates that the bacteria produce gas.

In the citrate test, four samples were positive for citrate, and two isolates were negative for citrate. Citrate-positive means that bacteria can utilize citrate as a carbon source, while citrate-negative bacteria cannot utilize citrate as a carbon source. In the catalase test performed on six isolates, it was found that they were given a solution of 3%, producing bubbles. These bubbles indicate that the bacteria can produce the catalase enzyme. In the methyl red test, six isolates of *E.coli* showed a positive result because a red color change in the medium was indicated after dropping the methyl red solution. Voges proskauer's test was performed on 6 isolates *E.coli* in the Naphthol and KOH solution drops, one isolate was positive because the media turned red, and five isolates were negative because the media did not change color.

Based on Holt et al.[17], *E.coli* bacteria have biochemical properties: positive indole, motile, positive for producing catalase enzymes, negative for citrate, positive for methyl red, and negative for Voges Proskauer. Based on the identification of the six isolates carried out, it was found that only two isolates were positively identified as *E.coli* bacteria,

namely SD C 3.1 and IP B 1.1, which were isolated from sediments and WWTP. Then, we proceeded to the sensitivity test for the chloramphenicol, ampicillin, and penicillin isolates from sediment and WWTP.

Bacterial Sensitivity to Antibiotics

This sensitivity test was conducted to determine the sensitivity of the *E.coli* bacteria found in vannamei shrimp ponds to antibiotics. The sensitivity test results of each bacterial isolate of *E.coli* can be seen in Figure 1.

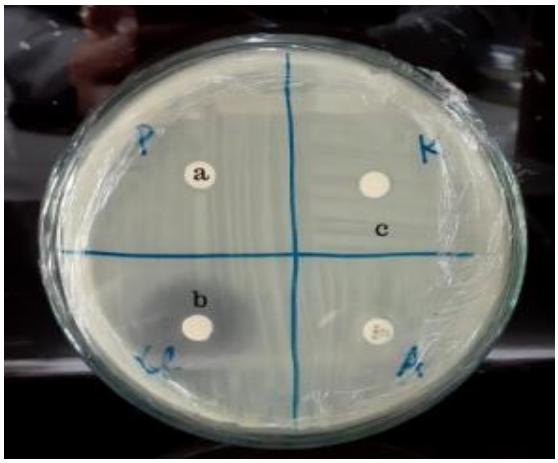

Figure 1. Sensitivity Test Results of *E.coli* Bacteria on MHA media; (a) Antibiotic Disc (b) Clear Zone Area; (c) *E.coli* Bacteria

Based on the results of the tests conducted, it was found that the inhibition zone formed on the source of intermediate Sediment RF01 isolates against the antibiotic chloramphenicol had a diameter of 18.75 mm. At the same time, the inhibition zone for the source of intermediate IPAL RF02 isolates to the antibiotic chloramphenicol was 17 mm in diameter, resistant to penicillin antibiotics with a diameter of 6.5 mm, and resistant to the antibiotic ampicillin with a diameter of 2 mm.

Based on the test results, it can be seen that the effective antibiotic to inhibit the growth rate of *E.coli* bacteria is by producing a clear zone or large inhibition zone, namely the antibiotic chloramphenicol type. Chloramphenicol is a broad-spectrum antibiotic effective against several anaerobic bacteria and germs[18]. Chloramphenicol can inhibit the development of the disease and can increase the resistance and weight of cultured shrimp. The price is relatively low, making vannamei shrimp cultivators use these antibiotics. Besides being used as an antibiotic for shrimp farming, chloramphenicol is a disinfectant for rinsing ponds during production to avoid disease[19].

Differences in the bacterial strains obtained can cause differences in the effectiveness of antibiotics in inhibiting bacterial growth. Several factors influence the resulting difference in the inhibition zone. Factors that affect the size of the inhibition zone are the organism's sensitivity, pH, type of microbe, anti-microbial agent used, culture medium, incubation conditions, and agar diffusion rate. Factors that affect the speed of agar diffusion are the concentration of microorganisms, media composition, incubation temperature, and incubation time[20].

4. CONCLUSION

Based on the identification of the six isolates carried out, it was found that only two isolates were positively identified as *E.coli* bacteria, namely SD C 3.1 and IP B 1.1, which were isolated from sediments and WWTP ponds. The results of the sensitivity test of *E.coli* bacteria to 3 types of antibiotics were carried out on two isolated sources that indicated positive *E.coli*, namely the source of sediment isolates RF01 and WWTP RF02. The inhibition zone on the source of intermediate Sediment RF01 isolates against the antibiotic chloramphenicol was 18.75 mm in diameter. The inhibition zone for the source of intermediate IPAL RF02 isolates to the antibiotic chloramphenicol was 17 mm in diameter, resistant to penicillin antibiotics with a diameter of 6.5 mm, and resistant to the antibiotic ampicillin with a diameter of 2 mm.

REFERENCES

1. Arsad, S., Afandy, A., Purwadhi, A.P., Maya V, B., Saputra, D.K., Buwono, N.R. Studi Kegiatan Budidaya Pembesaran Udang Vaname (*Litopenaeus vannamei*) dengan Penerapan Sistem Pemeliharaan Berbeda. *Jurnal Ilmiah Perikanan dan Kelautan*, 2017; 9: 1–5.
2. Sa'adah, W., Milah, K. Permintaan Udang Vannamei (*Litopenaeus vannamei*) di Kelompok Pembudidaya Udang At-Taqwa Paciran Lamongan. *Jurnal Pemikiran Masyarakat Ilmiah Berwawasan Agribisnis*, 2019; 5(2): 243–251.
3. Khairunnida, G.R., Rusmini, H., Maharyun, E. Isolasi dan Identifikasi Bakteri *Escherichia coli* Penyebab Waterborne Disease pada Air Minum Kemasan dan Isi Ulang. *Jurnal Ilmiah Kesehatan Sandi Husada*, 2020; 9: 634–639.
4. Wahyuni, Y., Jamilah, I., Suryanto, D. Isolasi Bakteri Patogen Oportunistik dari Tambak Udang Sumatera Utara. *Jurnal Agrohita*, 2017; 1: 71–75.
5. Alghifari, D., Kuswandi, B., Pratoko, D.K. Pengembangan Sensor Kloramfenikol Berbasis Imobilisasi Bovine Serum Albumin (BSA) pada Selulosa Asetat dengan Metode Spektroflorometri. *Jurnal Pustaka Kesehatan*, 2017; 5(1): 40–45.
6. Rahmaniar, R.P., Widhowati, D., Hidayah, N. Sensitivitas Antimikroba terhadap Bakteri *Escherichia coli* yang Diisolasi dari Udang di Pasar Keputran Surabaya. *Jurnal Kajian Veteriner*, 2019; 7(2): 93–100.
7. Intan, K., Diani, A., Suci, A., Nurul, R., Barat, J. Aktivitas Antibakteri Kayu Manis (*Cinnamomum burmanii*) terhadap Pertumbuhan *Staphylococcus aureus* Karlina. *Jurnal Kesehatan Perintis Journal*, 2021; 8(2), 121–127.
8. Sapitri, A., Afrinasari, I. Identifikasi *Escherichia coli* pada Cincau yang Dijual di Pasar Baru Stabat. *Journal of Pharmaceutical and Sciences*, 2019; 2(2): 18–23.
9. [CLSI] Clinical and Laboratory Standard Institute. *Performance Standards for Antimicrobial Susceptibility Testing. Ed*. CLSI supplement M100. Wayne, PA, 2020.
10. Sahrijanna, A., Septiningsih, E. Variasi Waktu Kualitas Air pada Tambak Budidaya Udang dengan Teknologi Integrated Multitrophic Aquaculture (IMTA) di Mamuju Sulawesi Barat. *Jurnal Ilmu Alam dan Lingkungan,* 2017; 8(16): 52–57.
11. Nurhasanah., Junaidi, M., Azhar, F. Tingkat Kelangsungan Hidup dan Pertumbuhan Udang Vaname (*Litopanaeus vannamei*) pada Salinitas 0 ppt dengan Metode Aklimatisasi Bertingkat Menggunakan Kalsium $CaCO_3$. *Jurnal Perikanan,* 2021; 11(2): 166–177.
12. Suhendar, D.T., Zaidy, A.B., Sachoemar, S.I. Profil Oksigen Terlarut, Total Padatan Tersuspensi, Amonia, Nitrat, Fosfat dan Suhu pada Tambak Intensif Udang Vanamei. *Jurnal Akuatek*, 2020; 1(1): 1–11.
13. Jamilatun, M., Aminah, A. Isolasi dan Identifikasi *Escherichia coli* pada Air Wudhu di Masjid yang Berada di Kota Tangerang. *Jurnal Medikes*, 2016; 3(1): 81–90.
14. Susilo., Setyaningsih, M., Mulyawati, D. Strain *Escherichia coli* dari Usus Ayam: Karakterisasi Profil Resistensi Antibiotika Ciprofloxacin dan Erythromycin. *Jurnal Pembelajaran dan Biologi Nukleus,* 2022; 8(1): 103–113.
15. Trisno, K., Suarjana, I.G.K. Isolasi dan Indentifikasi Bakteri *Escherichia coli* dari Udara pada Rumah Potong Unggas Swasta di Kota Denpasar. *Indonesia Medicus Veterinus,* 2019; 8(5): 685–694.
16. Rahayu, W.P., Nurjanah, N., Komalasari, E. *Escherichia coli: Patogenitas, Analisis dan Kajian Resiko*. IPB Press. Kota Bogor, 2018.
17. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, W., Stanley, T. *Bergey's Manual of Determinative Bacteriology*. 9th edition. Baltimore: Williams and Wilkins, 1994.

18. Dian, R., Fatimawali, F., Budiarso, F. Uji Resistensi Bakteri *Escherichia coli* yang Diisolasi dari Plak Gigi terhadap Merkuri dan Antibiotik Kloramfenikol. *Jurnal E-Biomedik (EBM)*, 2015; 3(1): 59–63.
19. Virgianti, S.E., Moelyaningrum, A.D., Ningrum, P.T. Kandungan Residu Kloramfenikol pada Udang Putih (*Litopenaeus vannamei*). *Buletin Kesehatan Lingkungan Masyarakat*, 2022; 41(4), 149–155.
20. Yusriyani, Y., Asfi, D., Yusliati, R.K. Uji Daya Hambat Ekstrak Etanol Daun Miana Merah (*Coleus benth*) terhadap *Staphylococcus Aureus*. *Jurnal Kesehatan Yamasi Makassar*, 2023; 7(1): 10–16.