Exploration of the Antibacterial Potential from Rice Eel Skin Mucus (*Monopterus albus*) Against Bacteria *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus*

Ratih Novita Praja[1*], Aditya Yudhana[1], Didik Handijatno[1], Wardatul Qoryah Al-Madinah[2], Jonathan Mark Hamonangan[3], Alivia Khairina Insani[1], Nindya Pradnya Prameswari[1], Dhenatra Rifqy Prasetyo[1], Shifa Salsabilla Praja[1]

[1]Veterinary Medicine Study Program, Department of Health, Medicine, and Life Sciences, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Wijaya Kusuma Street 113, Banyuwangi, East Java, Indonesia.
[2]Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo Street, Surabaya, East Java, Indonesia.
[3]School of Biodiversity, One Health, and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom.

*email: ratihnovitapraja@fkh.unair.ac.id

ABSTRACT

The skin mucus of the rice eel (*Monopterus albus*) contains various antibacterial compounds and has the potential as a synthetic antibiotic. This research was conducted to explore the potential antibacterial power of the rice eel skin mucus against some pathogenic bacteria in freshwater fish. The bacteria were isolated from five samples of rice eel cultivation ponds belonging to Mr. Sabwan and then challenged with the mucus of the eel's skin through diffusion tests using paper discs. The rice eel skin mucus tested its antibacterial activity against three species of freshwater bacteria, *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus*, by testing sensitivity and inhibitory zones. The inhibitory zones of each bacterium were measured using Vernier caliper which refers to the standardization of the inhibitory zone: < 4 mm no activity, 5-9 mm weak, 10- 14 mm medium, and > 15 mm strong. Test results showed that the eels' skin mucus could inhibit the growth of bacteria *Aeromonas hydrophila, Escherichia coli, and Staphylococcus aureus*.

Keywords: Antibacterial, Infectious disease, *Monopterus albus*, Skin mucus.

INTRODUCTION

Cases of AMR (antimicrobial resistance) have become a global concern, and the spread of bacteria resistant to various antibiotics has created severe health problems (Van Duin & Paterson, 2016). According to the National Institute of

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

Allergy and Infectious Disease in Kurniawan (2017), the cause of antibiotic resistance is the genetic mutation of microbes, so they become more resistant to antibiotics. Increased antibiotic resistance to antibiotics commonly used in aquaculture has motivated researchers to look for new antibacterial agents (Patil et al., 2015). The last ten years, many researchers have discovered the natural antibacterial agent of skin mucus from many fish species against several pathogenic microbes (Reverter et al., 2018).

In this study, researchers wanted to analyze the antibacterial potential of the skin mucus of rice eel (*Monopterus albus*) using the disc diffusion method. The challenge that often attacks fish farming businesses in Indonesia is a bacterial infection that is pathogenic in water (Directorate of Production and Cultivation Business, 2017). *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus* are bacterial diseases that generally attack fish. *Aeromonas hydrophila* is a pathogenic bacterium that causes Motile Aeromonas Septicemia (MAS) disease in fish (Saputra et al., 2018). According to Hanson et al. (2012), fish infected with *Aeromonas hydrophila* are characterized by redness of the fins, bleeding on the body surface, exophthalmia, swollen abdomen, erosion of the fins, skin and ulcers. *Escherichia coli* is a bacterium commonly found in aquaculture, especially in freshwater fish. *Escherichia coli* infection can cause death characterized by septicemia (Razak et al., 2019). *Staphylococcus aureus* is not a normal flora in fish. *Staphylococcus aureus* infection in fish is characterized by septicemia, endocarditis, renal carbuncle, epidural abscess, kidney, spleen and liver disorders (Li & Hu, 2012).

Fish skin mucus contains innate immune components such as lectins, pentraxin, lysozyme, proteolytic enzymes, alkaline phosphatase, C-reactive protein, complement, AMPs (antimicrobial peptides), and immunoglobulins that have antibacterial potential (Hedmon, 2018; Praja et al., 2021). According to research conducted by Setiawan (2016), rice eel skin mucus contains bioactive such as glycoproteins, lectins, hemagglutinins, and hemolysins, which are released through Mucus glands from eel skin that act as antibacterial, in addition to research conducted by Hilles (2018), said that rice field eel skin mucus has antibacterial properties and has shown a significant bacteriostatic effect. Rice eel skin mucus contains antimicrobial peptides, which is helpful as an alternative therapy in aquaculture because of its potential as an antibacterial (Dash et al., 2018).

Banyuwangi is one of the eel-producing districts in Indonesia. Eel production in Banyuwangi District has increased by 140.82%, from 4.9 tons in 2014 to 11.8 tons in 2017 (Banyuwangi District Fisheries and Food Service, 2018). The high demand for fish consumption has seen the emergence of many fish farming businesses, one of which is the cultivation of rice eel. Rice eels are freshwater fish belonging to the Synbranchidae family under the order Synbranchiformes (Hilles et al., 2019).

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

MATERIALS AND METHODS

Materials

The study was conducted from May to August 2023. Five rice eel skin mucus were collected from rice eel cultivation in the Licin Subdistrict, Banyuwangi District, East Java, Indonesia. The materials used for collecting rice eel skin mucus are 70% alcohol and ice cubes. Materials needed for bacterial isolation are Nutrient Broth (NB) (Merck®), Eosin Methylene Blue Agar (EMBA) (Merck®), Tryptic Soy Agar (TSA) (Merck®), Mannitol Salt Agar (MSA) (HIMEDIA®), Methyl Red and Voges Proskauer (MR-VP) (Merck®), Buffer Peptone Water (BPW) (HIMEDIA®), D-Mannitol (Merck®), Sulfide Indole Motility (SIM) (Merck®), Simmons Citrate Agar (SCA) (Merck®), methyl red indicator, liquid paraffin, oxidase strip, 0.5 ml rabbit plasma, 40% potassium hydroxide (KOH) solution, 5% alpha naphthol, 95% ethanol, Kovach reagent (Merck®), hydrogen peroxide 3% (H_2O_2), sterile aquadest and water samples from aquaculture ponds of rice eel. For staining, malachite green, crystal violet, Lugol, alcohol acetone, safranin, oil immersion are used. The materials needed for the diffusion test are Mueller Hinton Agar (MHA) (Merck®), McFarland 0.5, rice eel skin mucus, sterile aquadest, chloramphenicol antibiotic discs, blank disc, 0.9% physiological NaCl, and bacterial suspension of *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus*.

This study is a laboratory experimental study to determine the effect of antibacterial activity of rice eel skin mucus against *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus* bacteria through a test of bacterial activity using Kirby-Bauer disc diffusion method using paper discs containing eel skin mucus.

Antibacterial activity tests can be done using inhibition test. The method used in this research is Kirby-Bauer agar diffusion method using disc diffusion with selective media, namely Mueller Hinton Agar (Oktovia, 2017). The diffusion test is the most frequently used inhibitory test because its implementation is easy, inexpensive, and its measurement is not difficult (Al Rosyad, 2012). MHA media is the standard medium used for antibacterial susceptibility testing. According to Himedia (2018), the use of MHA was chosen by the Clinical Laboratory Standards Institute (CLSI) because this medium supports the growth of most pathogenic bacteria.

The data obtained were analyzed descriptively. The results of the antibacterial sensitivity test of rice eel skin mucus are presented in tables and figures (Kusriningrum, 2011). This study uses a data collection technique that measures the zone of inhibition by making direct observations on the object under study by looking at the diameter of the inhibition zone formed and then measured using a Vernier caliper. The inhibition zone of each treatment was measured using a Vernier caliper which refers to the standardized inhibition zone: < 4 mm no activity, 5 – 9 mm weak, 10 – 14 mm medium, and > 15 mm strong (Nonutu *et al*., 2021). The larger the inhibition zone formed, the more effective the material tested against bacteria (Widyaningsih, 2019).

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

RESULTS AND DISCUSSION

The results of this study were obtained from the results of the isolation and identification of bacteria *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus*. The isolation and identification of *Aeromonas hydrophila* were classified as a Gram-negative bacterium, and the results of biochemical tests showed positive for motility, catalase, fermentative oxidase, and oxidase. *Escherichia coli* is classified as Gram negative with biochemical results tests showing positive for motility and Methyl Red, negative for Voges Proskauer and Simon Citrate Agar. Meanwhile, *Staphylococcus aureus* is classified as Gram positive with biochemical test results showing positive for catalase, mannitol fermentation, coagulation, and Voges Proskauer.

The results of the antibacterial power test of rice eel skin mucus from cultured pond water against the three bacteria were obtained with the Kirby-Bauer agar diffusion test using disc diffusion. The positive control in this study of rice eel skin mucus has a concentration of 100%, and the antibiotic chloramphenicol. Based on the results of the research conducted, samples of rice eel skin mucus that were challenged against *Aeromonas hydrophila*, *Escherichia coli*, and *Staphylococcus aureus* bacteria had antibacterial potential.

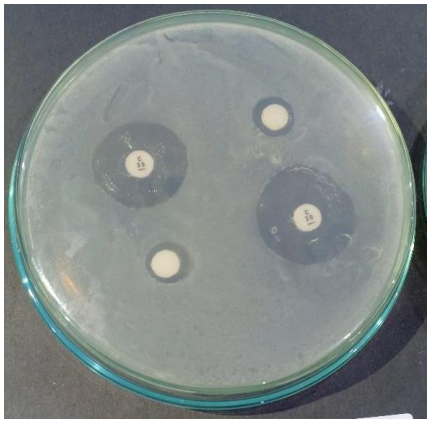

Figure 1. Inhibition zone of antibacterial activity test result

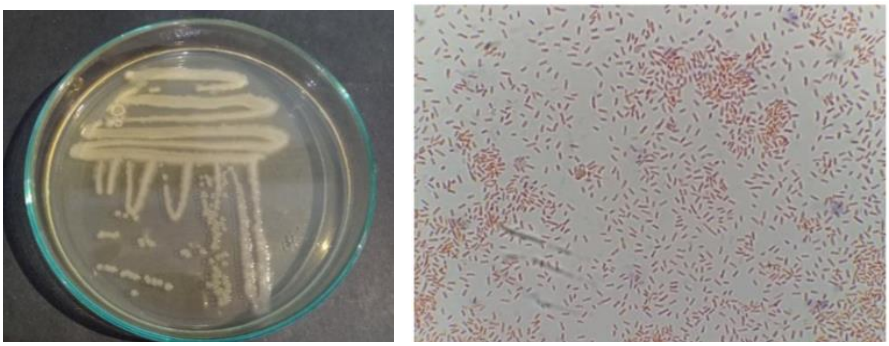

Figure 2. *Aeromonas hydrophila* yellowish cream on TSA media (left) and results of Gram staining of *Aeromonas hydrophila* 1000x magnification (right)

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

Table 1. Biochemical test results for *Aeromonas hydrophila*

No	Substrate Test	Test Results		
		Positive	Negative	Final Result
1	Gram Staining		-	Gram-negative
2	Motility	+		Motile
3	Catalase	+		Catalase enzyme is present
4	Fermentative Oxidase	+		Oxidizes and ferments bacteria toward glucose
5	Oxidase	+		Oxidase enzyme is present

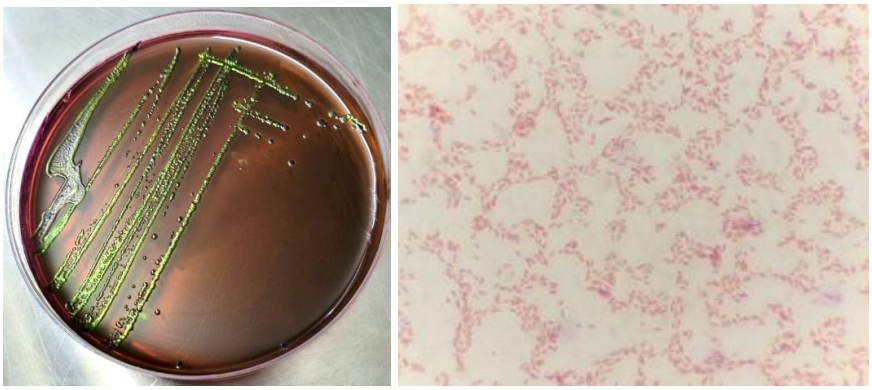

Figure 3. *Escherichia coli* is metallic green in EMBA media (left) and *Escherichia coli* Gram Stain Results 1000x magnification (right).

Table 2. Biochemical test results for *Escherichia coli*

No	Substrate Test	Test Results		
		Positive	Negative	Final Result
1	Gram Staining		-	Gram-negative
2	Motility	+		Motile
3	MR (Methyl Red)	+		Ferments glucose to acid
4	VP (Voges-Proskauer)		-	Does not produce acetonin
5	SCA (Simmons Citrate Agar)		-	Maintains green color

Table 3. Biochemical test results for *Staphylococcus aureus*

No	Substrate Test	Test Results		
		Positive	Negative	Final Result
1	Gram Staining	+		Gram-positive
2	Catalase	+		Catalase enzyme is present
3	Mannitol Fermentation	+		Acid is present
4	Coagulation	+		Coagulase enzyme is present
5	VP (Voges-Proskauer)	+		Produces acetoin

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

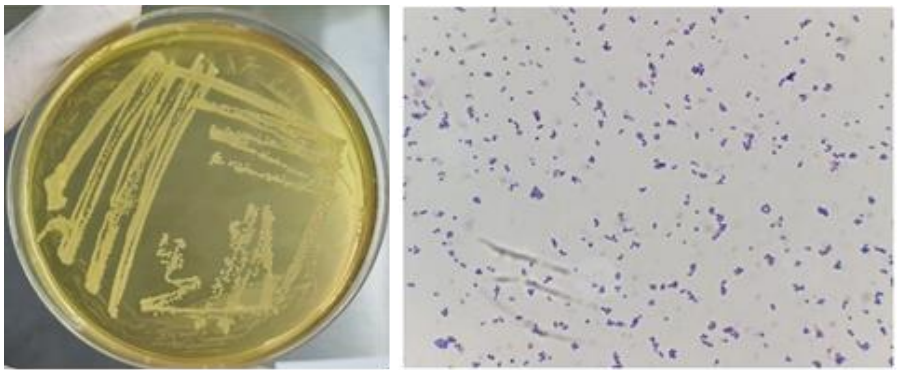

Figure 4. *Staphylococcus aureus* is golden yellow on MSA media (left) and *Staphylococcus aureus* Gram Stain Results. 1000x magnification (right)

Table 4. Diffusion test results of rice eel skin mucus against *Aeromonas hydrophila, Escherichia coli*, and *Staphylococcus aureus*

No	Tests	Inhibition zone					
		A. Hydrophila (mm)		*E. coli* (mm)		*S. aureus* (mm)	
		Mucus	Control (+)	Mucus	Control (+)	Mucus	Control (+)
1	I	10.78	29.50	10.38	25.65	10.20	21.33
2	II	10.50	28.70	9.83	25.80	9.90	23.35
3	III	10.98	28.18	9.35	21.15	11.28	24.80
4	IV	9.05	25.60	9.70	23.63	11.45	26.90
5	V	11.28	38.33	9.05	25.40	11.05	23.38
Total Average		**10.52**	**30.06**	**9.66**	**24.33**	**10.78**	**23.95**

The antibacterial activity results indicate that eel skin mucus can inhibit the growth of *Aeromonas hydrophila* bacteria with an average inhibition zone diameter of 10.52 mm, *Escherichia coli* bacteria with 9.66 mm, and *Staphylococcus aureus* bacteria with 10.78 mm. Furthermore, according to the table, Chloramphenicol antibiotic as a positive control shows inhibition of *Aeromonas hydrophila* bacteria with an average inhibition zone diameter of 30.06 mm, *Escherichia coli* bacteria with 24.33 mm, and *Staphylococcus aureus* bacteria with 23.95 mm.

The *Aeromonas hydrophila* bacteria obtained are in accordance with the statement of Akmal et al. (2020) that *Aeromonas hydrophila* bacteria is one of the most common bacteria infecting freshwater fish. According to Tamala et al. (2021), *Aeromonas hydrophila* bacteria have been isolated and identified in freshwater fish cultures, including eels. In addition, Hanson et al. (2012) stated that redness of the fins, bleeding on the body surface, exophthalmia, swollen abdomen, erosion of the fins, and skin and ulcers on rice eels were caused by infection with *Aeromonas hydrophila* bacteria.

Escherichia coli bacteria that have been isolated and identified are in accordance with Razak et al.'s (2019) finding that

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

Escherichia coli is a Gram-negative bacterium often found in aquaculture as an indicator of water, soil, food and beverage contamination. *Escherichia coli* infection in rice field eels can cause death in fish characterized by septicemia.

The *Staphylococcus aureus* bacteria obtained are in accordance with Hammad et al. (2012) that *Staphylococcus aureus* bacterial infection in eels indicates contamination of water hygiene or secondary infection in eels. In addition, Li and Hu (2012) stated that eel organ disorders such as kidney, spleen and liver were caused by infection with *Staphylococcus aureus* bacteria.

The average inhibition zone obtained shows that rice eel skin mucus can inhibit the growth of *Aeromonas hydrophila* bacteria with an average inhibition zone diameter of 10.52 mm, *Escherichia coli* bacteria at 9.66 mm and *Staphylococcus aureus* bacteria at 10.78 mm. Based on the table, the results are that the antibiotic chloramphenicol as a positive control can inhibit the growth of *Aeromonas hydrophila* bacteria with an average inhibition zone diameter of 30.06 mm, *Escherichia coli* bacteria 24.33 mm and *Staphylococcus aureus* bacteria 23.95 mm. The emergence of an inhibitory zone is due to the antibacterial activity of fish skin mucus containing innate immune such as lectins, pentraxin, lysozyme, proteolytic enzymes, alkaline phosphatase, C-reactive protein, complement, antimicrobial peptides (AMP) and immunoglobulins which have potential antibacterial power (Hedmon, 2018).

CONCLUSION

The conclusions obtained from the results of the study were that the eel skin mucus tested against *Aeromonas hydrophila* bacteria had a medium antibacterial activity with an inhibition zone diameter of 10.52 mm, *Escherichia coli* had a weak antibacterial activity with an inhibitory zone diameter of 9.66 mm, and *Staphylococcus aureus* had a medium antibacterial activity with an inhibition zone diameter of 10.78 mm.

ETHICS APPROVAL

This research does not involve the use of experimental animals; therefore, it does not require ethical testing.

ACKNOWLEDGMENT

Thanks go to those who funded the research and we acknowledge of institutions and individuals who have helped the research or scientific writing processes.

REFERENCES

Akmal, M., Rahimi, M. A., Hafeez, U. R. M., Hussain, A., & Choi, T. J. (2020). Isolation, Characterization, and Application of a Bacteriophage Infecting the Fish Pathogen *Aeromonas hydrophila*. *Pathogens*, *9*(3):215.

Al Rosyad, F. A. (2012). *Uji Aktivitas Antibakteri Ekstrak Etanol Pare (Momordica charantia L.) terhadap Pertumbuhan Escherichia coli secara in vitro*. Skripsi. Universitas Jember.

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

Dinas Perikanan dan Pangan Kabupaten Banyuwangi. (2018). *Profil Perikanan Budidaya Dinas Perikanan dan Pangan Kabupaten Banyuwangi*. p. 16.

Direktorat Produksi dan Usaha Budidaya. (2017). Buku Panduan Pengelolaan Paket Bantuan Pemerintah. Budidaya Ikan Lele Sistem Bioflok. pp. 1-7.

Hammad, A. M., Watanabe, W., Fujii, T., & Shimamoto, T. (2012). Occurrence and Characteristics of Methicillin Resistant and Susceptible *Staphylococcus aureus* and Methicillin Resistant Coagulase-negative Staphylococci from Japanese Retail Ready to Eat Raw Fish. *International Journal of Food Microbiology*, *156*: 286-89.

Hanson, L. A., Liles, M. R., Hossain, M. J., Griffin, M. J., & Hemstreet, W. G. (2012). Motile Aeromonas Septicemia. *FHS*, *129*: 1-11.

Hilles, A. R., Mahmood, S., Kaderi, M. A., & Hashim, R. (2019). Evaluation of The Antimicrobial Properties of Eel skin mucus from *Monopterus albus* Against Selected Oral Pathogens and Identification of the Anti-Oral Bioactive Compounds Using Lc Qtof Ms. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(1): 140.

Himedia. (2018). *Technical Data: Mueller Hinton Agar*. Himedia Laboratories. Mumbai, India.

Kurniawan, N. S. (2017). Pengaruh Pemberian Edukasi Door to Door Penggunaan Antibiotik pada Infeksi Saluran Pernapasan Akut terhadap Pengetahuan Ibu-Ibu di Desa Wanogara Kulon Kecamatan Rembang Kabupaten Purbalingga. [Doctoral Dissertation]. Universitas Muhammadiyah Purwokerto. Kusriningrum, R. S. 2011. Perancangan Percobaan. Surabaya. Penerbit Dani Abadi, pp. 31 – 39.

Li, Y. J., &Hu, B. (2012). Establishment of Multi Site Infection Model in Zebrafish Larvae for Studying *Staphylococcus aureus* Infectious Disease. *JGG, 39*: 521-34.

Maryland Hedmon, O. (2018). Fish Mucus: A Neglected Reservoir for Antimicrobial Peptides. *AJPRD*, *6*(4): 6-11.

Nonutu, S. E., Pangemanan, D. H., & Mintjelungan, C. N. (2021). Uji Daya Hambat Ekstrak Ikan Nike *(Awous melanocephalus)* terhadap Pertumbuhan Bakteri *Fusobacterium nucleatum*. *eGiGi*. *9*(2): 238-242.

Patil, R. N., Kadam, J. S., Ingole, J. R., Sathe, T. V., &. Jadhav, A. D. (2015). Antibacterial Activity of Fish Mucus from *Clarias batrachus (Linn.)* Against Selected Microbes. *Biolife*, 3(4): 788-91.

Praja, R. N., Yudhana, A., Haditanojo, W., & Oktaviana, V. (2021). Short Communication: Antimicrobial properties in cloacal fluid of olive Ridley sea turtle (*Lepidochelys olivacea*). *Biodiversitas*, *22*(9): 3671-76.

Razak, L. A. A., Musa, N., Jabar, A., & Musa, N. (2019). Therapeutic Potentials of Excoecaria Agallocha Against Gram Positive and Gram-Negative Fish Bacterial Pathogens. *JIDHealth*, *2* (2): 87-94.

Saputra, I., & Indaryanto, F. R. (2018). Identifikasi Bakteri *Aeromonas hydrophila* pada Komoditas Ikan yang di Lalulintaskan menuju Pulau Sumatera melalui Pelabuhan

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index

Penyeberangan Merak. *Jurnal Perikanan dan Kelautan*, *2089*:3469.

Tamala, A. R., Ramadhani, F. R., Hasanati, J. N., Ryandra, R., Annisa, R. T., Putri, I. R., & Fifendy, M. (2021). Persepsi Peternak Ikan Air Tawar terhadap Parasit pada Budidaya Perikanan. *Prosiding Seminar Nasional Biologi*, *1*(1): 510-25.

Van Duin D., & Paterson, D. L. (2016). Multidrug Resistant Bacteria in the Community: Trends and Lessons Learned. *Infectious Disease Clinics*, *30*(2): 377-90.

Widyaningsih, W., Supriharyono, & Widyorini, N. (2016). Analisis Total Bakteri Coliform di Perairan Muara Kali Wiso Jepara. *MAQUARES*, *5*(3):157-64.

Received : 25-03-2024, Accepted : 15-05-2024, Published online : 17-09-2024
Available at https://e-journal.unair.ac.id/MKH/index