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Identification of Pathogenic Bacteria from Nile Tilapia *Oreochromis niloticus* in Freshwater of Sigi Regency, Central Sulawesi

Identifikasi Bakteri Patogen pada Ikan Nila *Oreochromis niloticus* di Lokasi Budidaya Ikan Air Tawar Kabupaten Sigi, Provinsi Sulawesi Tengah

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ABSTRACT

This research aims to identify the types of bacteria that infect tilapia (*Oreocromis niloticus*) cultivated in Sigi Regency and analyze air quality parameters (temperature and pH). This research was carried out from May to July 2023. Bacteria was isolated and identified at the Fish Quarantine Station Testing Laboratory, Quality Control and Safety of Fishery Products in Palu City. The target organ examined is the kidney. Bacteria were grown on TSA media and then isolated. On bacteria, Gram staining, motility, aerobic, catalase, oxidase, O/F, glucose, and Rimlershots media tests were carried out for *Aeromonas hydrophila*. Based on the test results, bacterial identification was done using the Manual to Identify Medical Bacteria. Of the 50 samples identified, 25 types of pathogenic bacteria were found that attack tilapia. In Sigi Biromaru District, there were seven types of bacteria *Bordetella* sp, *Pseudomonas alcaligenes*, *Pasteurella* sp, *Moraxella* sp, *Shigella* sp, *Enterobacter* sp, *Achromobacter* sp. In Dolo District, there were ten types of bacteria, namely *Flavobacterium* sp, *Achromobacter* sp., *P.haemlytica*, *Alcaligenes* sp, *A.salmonicida*, *Actinobacillus* sp, *Plesiomonas shigelloides*, *A.sobria*, *Citrobacter* sp, and *Chromobacterium* sp. In the Dolo Barat District, there are eight types of bacteria: *P.shigelloides*, *Veillonella* sp, *Acinetobacter* sp, *Pasteurella* sp, *Plesiomonas* sp, *Bacillus* sp, *Enterococcus* sp, and *A.caviae*. In Sigi Kota District, there are eight types of bacteria, namely *Plesiomonas* sp, *P.haemlytica*, *P.alcaligenes*, *Klebsiella* sp, *Legionella* sp, *A.caviae*, *Eikenella corrodens*, and *Moraxella* sp. In Marawola District, there are seven types of bacteria, namely *P.haemlytica*, *E.corrodens*, *Klebsiella* sp, *Acinetobacter* sp, *Pasteurella* sp, *P.shigelloides*, and *Moraxella* sp.

Keywords: Pathogenic bacteria, Prevalence, Aquaculture, *Oreochromis niloticus*.

ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi jenis-jenis bakteri yang menginfeksi ikan nila *(Oreocromis niloticus)* yang dibudidayakan di Kabupaten Sigi, serta menganalisa parameter kualitas air (suhu dan pH). Penelitian ini dilakukan pada bulan Mei hingga Juli 2023. Isolasi dan identifikasi bakteri dilakukan di Laboratorium Penguji Stasiun Karantina Ikan, Pengendalian Mutu dan Keamanan Hasil Perikanan Kota Palu. Organ target yang diperiksa adalah ginjal. Bakteri ditumbuhkan pada media TSA kemudian di isolasi. Pada bakteri dilakukan uji pewarnaan Gram, motility, aerobis, katalase, oksidase, O/F, glukosa dan media *Rimler shots* untuk *Aeromonas hydrophila.* Berdasarkan hasil uji tersebut, dilakukan identifikasi bakteri menggunakan buku *Manual for the Identification of Medical Bacteria*. Dari 50 sampel yang di identifikasi, didapat 25 jenis bakteri patogen yang menyerang ikan nila, pada Kecamatan Sigi Biromaru terdapat tujuh jenis bakteri *Bordetella sp*, *Pseudomonas alcaligenes*, *Pasteurella* sp, *Moraxella* sp, *Shigella* sp, *Enterobacter* sp, dan *Achromobacter* sp. Kecamatan Dolo terdapat sepuluh jenis bakteri, yaitu *Flavobacterium* sp, *Achromobacter* sp, *P.haemlytica*, *Alcaligenes* sp, *A.salmonicida*, *Actinobacillus* sp, *Plesiomonas shigelloides*, *A.sobria*, *Citrobacter* sp, dan *Chromobacterium* sp. Kecamatan Dolo Barat terdapat delapan jenis bakteri, yaitu *P.shigelloides*, *Veillonella* sp, *Acinetobacter* sp, *Pasteurella* sp, *Plesiomonas* sp, *Bacillus* sp, *Enterococcus* sp, dan *A.caviae.* Kecamatan Sigi Kota delapan jenis bakteri, yaitu *Plesiomonas* sp, *P.haemlytica*, *P.alcaligenes*, *Klebsiella* sp, *Legionella* sp, *A.caviae*, *Eikenella corrodens*, dan *Moraxella* sp. Kecamatan Marawola terdapat tujuh jenis bakteri, yaitu *P.haemlytica*, *E. corrodens*, *Klebsiella* sp, *Acinetobacter* sp, *Pasteurella* sp, *P. shigelloides*, dan *Moraxella* sp.

Kata Kunci: Bakteri patogen, Prevalensi, Budidaya, *Oreochromis niloticus*

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INTRODUCTION

Tilapia (*Oreocrhomis niloticus*) is one of the most widely cultivated fish because of its ability to adapt to a high environment. Tilapia farming is greatly affected by fish diseases that may occur due to environmental changes. Cultivation of *O. niloticus* faces several problems associated with the emergence of pathogenic bacteria and causes high monetary losses. The disease can be caused by several pathogens, such as fungi, parasites, viruses, and bacteria (Fikri, 2023). Tilapia originated from fresh waters in Africa. Subsequently, tilapia expanded and was cultivated in various countries, including Taiwan, Thailand, Vietnam, Bangladesh, and Indonesia. In the Asian region, the distribution area of tilapia was initially centered in several countries, such as the Philippines and China (Riauwaty & Syawal, 2016).

Freshwater fish farming is one of the businesses pursued by fish farmers in Tahuna, Sangihe Islands Regency. Tilapia is a fish that is widely cultivated due to its high tolerance to the environment. In addition, tilapia has a high economic value. The price of fresh tilapia in the traditional Tahuna market has reached IDR. 50,000.00/kg Along with the development of aquaculture, there are detrimental problems, such as pests and diseases, that result in the economic income of the community. Diseases in fish arise due to unbalanced interactions between host, environment, and pathogen. The bacterium is one of the disease-causing pathogens that attack fish (Azhari et al. in Manurung & Susantie, 2017).

Tilapia aquaculture activities cannot be separated from obstacles such as fish mortality due to disease outbreaks and water quality changes due to toxic waste entry. The spread of disease in cultured fish can occur due to the interaction between fish, the environment, and disease agents. Changes in water quality can cause disturbances in fish, such as fish becoming stressed so that fish become weak, and the condition of the fish will cause fish to be susceptible to pathogenic microorganisms (Lukistyowati et al. in Riauwaty & Syawal, 2016).

Aquaculture significantly affects the mechanism of disease outbreaks; because of environmental manipulation, the balance between the host and disease-causing bacteria will be disturbed and unstable; besides that, the fish will be easily stressed, so bacteria easily infect the fish (host) and transmit to other fish. Based on this, it is necessary to identify pathogenic bacteria in tilapia in aquaculture ponds to determine whether the pond waters are full of pathogenic bacteria so that prevention and treatment can be carried out (Lubis et al., 2015).

Diseases found in fish are one of the problems in aquaculture. The percentage of fish diseases caused by bacteria reached 33.9%. Protozoa caused 20.7%, while viruses, worms, fungi, and crustaceans caused others. (Manurung & Susantie, 2017). Tilapia is also often attacked by bacteria, such as *Aeromonas hydrophila*, caused by *Motile Aeromonas Septicemia* (MAS). Bacteria are also infectious diseases that often cause death in large numbers and in a reasonably short time. Identifying bacteria in freshwater fish is very important to know the type of pathogenic bacteria so that prevention efforts can be made as early as possible against the attack of these bacteria.

MATERIALS AND METHOD

Time and place of research

This research was conducted at the Laboratory of Fish Quarantine Testers, Quality Control and Safety of Fishery Products in Palu City, and the implementation time was from May to July 2023. The sample used was tilapia. Sampling was conducted in five sub-districts of Sigi Regency, namely Sigi Kota sub-district, Sigi Biromaru sub-district, Dolo sub-district, Dolo Barat sub-district and Marawola sub-district.

Data collection

The research was conducted using the descriptive method and purposive sampling technique. Water sampling as supporting data was performed simultaneously with tilapia sampling.

Fish sampling

Tilapia sampling was conducted randomly, and water parameters were measured in the culture ponds. As many as ten fish were in each sample location per sub-district, so the total sample was fifty (50) fish. After that, the samples were brought to the Laboratory of Fish Quarantine, Quality Control and Safety of Fishery Products of Palu City for identification, and the target organ of the test was the kidney. The tilapia samples were taken to the BKIPM laboratory in Palu for analysis.

Water quality measurement

Water quality parameters were collected along with fish samples. Water quality parameters measured include temperature, dissolved oxygen, and pH acidity.

Data analysis

The identified bacteria were recorded for the type found, and the prevalence was calculated (Angreni et al., 2018). The prevalence formula is as follows:

$$\text{Prevalence} = \frac{\text{Number of fish infected with bacteria}}{\text{Number of fish examined}} \text{ x } 100\%$$

RESULT AND DISCUSSION

Types of pathogenic bacteria in Sigi Regency

Based on the results of 50 samples identified, 25 types of pathogenic bacteria attack tilapia; in Sigi Biromaru District, there are seven types of bacteria: *Bordetella sp*, *Pseudomonas alcaligenes*, *Pasteurella* sp, *Moraxella* sp, *Shigella* sp, *Enterobacter* sp, *Achromobacter* sp. In the Dolo District, there are ten types of bacteria, namely *Flavobacterium* sp, *Achromobacter* sp, *P.haemlytica*, *Alcaligenes* sp, *A.salmonicida*, *Actinobacillus* sp, *Plesiomonas shigelloides*, *A.sobria*, *Citrobacter* sp, and *Chromobacterium* sp. In Dolo Barat District, there are eight types of bacteria, namely *P.shigelloides*, *Veillonella* sp, *Acinetobacter* sp, *Pasteurella* sp, *Plesiomonas* sp, *Bacillus* sp, *Enterococcus* sp, and *Aeromonas caviae*, in Kecamatan Sigi Kota there were eight types of bacteria namely *Plesiomonas sp*, *Pasteurella haemlytica*, *Pseudomonas alcaligenes*, *Klebsiella sp*, *Legionella* sp, *A.caviae*, *Eikenella corrodens*, and *Moraxella* sp. In Kecamatan Marawola, there were seven types of bacteria: *P.haemlytica*, *E.corrodens*, *Klebsiella* sp, *Acinetobacter* sp, *Pasteurella* sp, *P. shigelloides*, and *Moraxella sp.*

The bacteria identified are thought to be caused by factors from the environment (water quality) and from within the fish itself. Water quality, which is the habitat of farmed fish, is an essential factor for the health of farmed fish. In addition to a decrease in aquaculture, water quality will cause fish to become stressed; density is also a factor that can stress fish due to limited oxygen. This follows Lubis et al. (2015), who state that the success of a tilapia farming business is closely related to the rearing system and the environment. Fish farming, especially tilapia, often experiences mortality caused by bacteria. Some types of bacteria that commonly attack tilapia are *Aeromonas* sp and *Edwardsiella tarda* in Java, *Streptococcus* sp in Sumatra, and *Mycobacterium* sp in Sulawesi.

Diseases caused by bacteria show symptoms such as loss of appetite, wounds on the body surface, bleeding in the gills, enlarged abdomen filled with fluid, loose scales, and loose tail fin. If surgery is performed, swelling and damage will be seen in the fish liver, kidney, and spleen (Arfiandi, 2020).

Identification of bacteria based on biochemical tests

Based on the results of the biochemical test above, it can be seen that the bacteria that infect tilapia obtained seven types of pathogenic bacteria, namely *A.hydrophila*, *Corynebacterium* sp, *Enterobacteria* sp, *Listeria* sp, *Pseudomonas* sp, *Plesiomonas* sp, and *Kurtiha* sp. From the identification results, *A.hydrophila* bacteria were detected in 4 sub-districts, namely *Corynebacterium* sp bacteria, which were detected in 3 sub-districts: Tamako, Tabukan Utara, and Tahuna. *Enterobacteria* sp bacteria were detected in 3 sub-districts: Tabukan Tengah, Manganitu, and Tahuna. *Listeria* sp was only detected in the Tamako sub-district. *Pseudomonas* sp bacteria were only detected in the Tahuna sub-district. *Plesiomonas* sp bacteria were only detected in the Manganitu sub-district, and *Kurtiha* sp. bacteria were only indicated in the North Tabukan sub-district (Figure 1).

The presence of disease in the aquatic environment is one of the constraints in developing the aquaculture subsector. The cause of the disease can be physical and chemical factors of the environment, feed and metabolism, and stress as part of the psychological reaction of fish. The attack of the disease can disrupt aquaculture productivity and even cause failure to cause losses. In addition, sudden increases or decreases in temperature can cause stress in fish. High fish density will cause fish to be easily stressed, making them more susceptible to disease, and climate change is a contributing factor to the onset of disease (Sulistiyono & Mutiara, 2023).

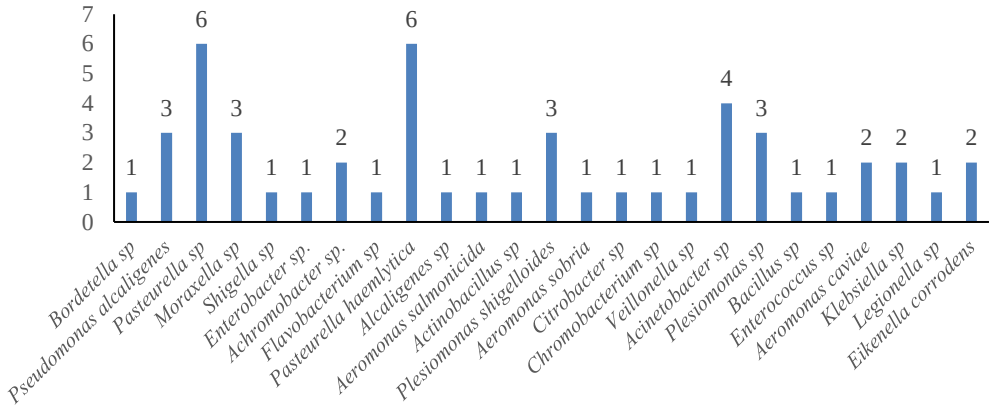

Figure 1. Percentage chart of pathogenic bacteria types that attack tilapia fish

Description of bacteria found in tilapia fish

Aeromonas hydrophila

Aeromonas hydrophila is the most common bacteria in fish samples, 36.6%. Transmission of *A.hydrophila* can take place through contaminated equipment and fish infected with *A.hydrophila*. Its movements become slower, weaker, and more accessible to catch. *A. hydropila* is a pathogenic bacterium that causes *Motile Aeromonas Septicemia* (MAS) or Hemorrhage Septicemia. This bacterium is more detrimental to fish health than other bacteria (Manurung & Susantie, 2017). *Aeromonas hydrophila* is Gram-negative, with a beige colony color, flat colony edges, convex elevations, rod-shaped, motile, fermentative oxidase, catalase positive, and indole positive (Cowan, 1974). This bacterium generally lives in fresh water. *Aeromonas* sp. can appear at any time under deplorable environmental conditions. *Aeromonas* sp bacteria can be transmitted through water, body contact, and contact with contaminated equipment. This follows Lubis et al.'s (2015) statement that *A.hydrophila* bacteria are Gram-negative, oxidase and catalase-positive, motile, and fermentative. These bacteria can live at a temperature of 22-28 ℃.

Corynebacterium sp

Corynebacterium sp is the second most commonly found bacteria at 20%. Fikri (2023) revealed that fish infected with Enterobacter sp. showed pale internal organs, such as the liver and kidneys, and caused anemia, intestinal congestion, anal ulceration with mucus, and bleeding in the infected external organs. Wahjuningrum et al. (2009) added that the character of the genus Enterobacter. is aerobic and facultative anaerobic, producing gas and ornithine decarboxylase. Some strains produce yellowish pigments. *Enterobacter* sp with IP ≥ 2.00 was isolated from gifted tilapia from diseased and healthy fish gills. *E. ictaluri* is one of the bacteria that attack arowana fish. The symptoms are minor wounds on the skin and flesh of the Arowana accompanied by bleeding. The wounds will ulcerate and ooze pus. Further attacks can cause injury to the liver and gizzard (Manurung & Susantie, 2017).

Listeria sp

Listeria sp bacteria are facultative bacteria and are included in Gram-positive. Based on the presumptive test, *Listeria* sp bacteria showed positive oxidase, Gram-positive, and positive catalase results. Based on morphological observations from the results of Gram staining in the microscope, *Listeria* sp bacteria have the characteristics of purple cells, short rod-shaped, and cells that form short chains.

Pseudomonas sp.

Pseudomonas sp is found in fish kidneys, including Gram-negative bacteria, and is aerobic, short rod-shaped, catalase positive, oxidase positive, and can oxidize glucose/ other carbohydrates (Cowan, 1974). This bacterium belongs to the Pseudomonadaceae family and causes illness in fish. *Pseudomonas* bacteria are opportunistic pathogens that attack freshwater fish and are classified into the group of fin-damaging bacteria (bacterial fin rot).

***Plesiomonas* sp**

Plesiomonas sp is a facultative anaerobic bacterium, Gram-negative, rod-shaped, oxidase-positive, non-spore, motile and has lophotrichous and peritrichous flagella, grows at an optimum temperature of 30°C and has a range of 29-41°C. *Plesiomonas* sp is an opportunistic pathogenic bacterium that can cause petechial hemorrhage in fish intestines (Raudia et al., 2021). Under normal conditions, this bacterium can be found in the gastrointestinal tract of freshwater fish (Arfiandi, 2020). Bacteria *P.shigelloides*, usually found in the oral cavity or skin of fish, has characteristics that include being gram-negative and rod-shaped. These bacteria are motile and fermentative, can produce catalase enzymes and indole, and can decarboxylase amino acids lysine and ornithine. Acid production from the sugar fermentation can be obtained from glucose, inositol, and trehalose. According to Sulistiyono & Mutiara (2023), the pathogenicity of *P. shigelloides* can cause diarrhoea in humans as this bacterium produces enterotoxins. The geographical distribution of this organism is limited to tropical and subtropical regions.

***Kurtiha* sp.**

Kurtiha sp is usually found in the environment, as well as in animal feces. Kurthia sp. is not pathogenic and is a normal flora in salmon waters *Scomberomus* sp (Manurung & Susantie, 2017). Bacterial isolation is performed on the kidney. Macroscopically, the kidneys of teleostei fish look dark brown or black, divided into anterior and posterior kidneys. The anterior part functions as a lomhomyeloid organ, while the posterior part functions as a lomhomyeloid organ. Its posterior functions as an excretory organ.

Water quality

The study's water quality measurement results can be seen in (Table 1).

Table 1. Water quality parameters

Location	Temperature (ºC)	Oxygen (mg/L)	pH
Mpanau	28.5	2	6
Kaleke	25	3	7
Kota Pulu	29	2	7
Sidera	31	5	6
Sunju	30	3	6

Based on the measurement results of several water quality parameters in the five sub-districts, the temperature is in the range of 25 - 28℃, where the lowest value is 25℃ in the Tamako sub-district and the highest value is 29o C in the Tahuna sub-district. According to Kordi (2010), The suitable temperature for fish rearing in aquaculture activities is 23-32℃. However, fish in the tropics can live well in the temperature range of 30-35℃ (Ashari et al., 2014). Based on the quality standards of water quality according to PP No. 82 of 2001, the temperature in the research location is still average according to natural conditions.

Field measurements in the five sub-districts showed that the pH value ranged from 6-7, and following the quality standard limit of PP No. 82 of 2001, this value is suitable for aquaculture. Aquatic organisms, especially fish, can grow well with a neutral pH. pH values that are too low and too high can kill fish. The ideal pH in aquaculture fisheries is 5-9 (Lubis et al., 2015).

CONCLUSION

Based on the test results, bacterial identification was carried out using the Manual for the Identification of Medical Bacteria. Of the 50 samples identified, 25 types of pathogenic bacteria were found to attack tilapia namely *Bordetella* sp, *Pseudomonas alcaligenes*, *Pasteurella* sp, *Moraxella* sp, *Shigella* sp, *Enterobacter* sp, *Achromobacter* sp, *Flavobacterium* sp, *P. haemlytica*, *A.salmonicida*, *Actinobacillus* sp, *Plesiomonas shigelloides*, *A. sobria*, *Citrobacter* sp, *Chromobacterium* sp, *Veillonella* sp, *Bacillus* sp, *Acinetobacter* sp, *A. caviae*, *Enterococcus* sp, *Klebsiella* sp, *Legionella* sp, *Eikenella corrodens*, and *Moraxella* sp,

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