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IDENTIFICATION AND PREVALENCE OF *Edwardsiella tarda* BACTERIA INFECTING TILAPIA (*Oreochromis niloticus*) AT CV SERAMBI IKAN CENTER, LAMBARO

Identifikasi dan Prevalensi Bakteri *Edwardsiella tarda* yang Menginfeksi Ikan Nila (*Oreochromis niloticus*) di CV Serambi Ikan Center, Lambaro

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ABSTRACT

Edwardsiella tarda is a gram-negative pathogen that can cause serious disease in fish, including mass mortality. Tilapia fish infected with *E. tarda* bacteria show clinical symptoms such as sore skin, swelling and decreased appetite. Bacterial identification was carried out at the SKIPM Laboratory, Aceh Besar, to confirm the presence of *E. tarda* using biochemical tests. The target organs taken for the isolation process are the liver, kidneys and spleen. This research uses a descriptive survey method with sampling using a purposive sampling technique. The results of the research show the morphological characteristics of the bacterial colony which, when viewed from above, is round, flat, intact and whitish in color. The observation table data from the identification results showed that two fish samples with sample codes N5 and N7 were infected with *Edwardsiella tarda* bacteria in the same target organ, namely the liver. Bacteriological examination through biochemical tests, *Edwardsiella tarda* bacteria showed negative results in the 3% KOH test, positive in the indole test, and negative in the oxidase test. The prevalence rate of bacterial attacks obtained from the 10 fish samples used was 20% of the total samples.

Keywords: *Edwardsiella tarda*, Identification, Prevalence

ABSTRAK

Edwardsiella tarda merupakan pathogen gram negatif yang dapat menyebabkan penyakit serius pada ikan, termasuk kematian massal. Ikan nila yang yang terserang bakteri *E. tarda* menunjukkan gejala klinis seperti luka pada kulit, pembengkakan, dan penurunan nafsu makan. Identifikasi bakteri dilakukan di Laboratorium SKIPM, Aceh Besar, untuk memastikan keberadaan *E. tarda* dengan uji biokimia, organ target yang diambil untuk proses isolasi yaitu hati, ginjal, dan limpa. Penelitian menggunakan metode survey deskriptif dengan pengambilan sampel menggunakan teknik purposive sampling. Hasil penelitian menunjukkan karakteristik

morfologi bentuk koloni bakteri yang jika dilihat dari atas berbentuk bulat, dataran yang timbul, berbentuk utuh dan berwarna keputih-putihan. Data tabel pengamatan hasil identifikasi ditemukan dua sampel ikan dengan kode sampel N5 dan N7 terinfeksi bakteri *Edwardsiella tarda* pada organ target yang sama yaitu Hati. Pemeriksaan bakteriologis melalui uji biokomia, bakteri *Edwardsiella tarda* menunjukkan hasil negative pada uji KOH 3%, positif pada uji indol, dan negative pada uji oksidase. Tingkat prevalensi serangan bakteri yang didapat dari 10 sampel ikan yang digunakan sebesar 20 % dari jumlah sampel.

Kata Kunci: *Edwardsiella tarda*, Identifikasi, Prevalensi

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a type of freshwater fish with high economic value and is widely cultivated in Indonesia. However, tilapia cultivation often experiences health problems such as bacterial infections caused by stress due to high population density, inadequate water quality, and poor environmental conditions. When fish are stressed, their immune systems can be weakened, increasing the risk of infection (Pramytha et al., 2014).

Diseases caused by bacteria are characterized by clinical symptoms such as skin lesions, organ swelling, and decreased appetite, which can lead to death. Edwardsiellosis is an example of a disease caused by the bacterium *Edwardsiella tarda*. This disease causes damage to vital organs such as the liver and kidneys, and disrupts the fish's metabolic function. This disease, which attacks the fish's immune system and has a detrimental impact on mortality, can have significant economic losses for farmers (Yunin et al., 2019).

In this case, researchers observed a disease in tilapia (*Oreochromis niloticus*) caused by the bacterium *Edwardsiella tarda* collected from CV Serambi Ikan Center, Lambaro. This study aims to identify bacteria that attack tilapia and also to determine the prevalence level of *Edwardsiella tarda* bacteria that attack tilapia (*Oreochromis niloticus*).

RESEARCH METHODS

Time and Place

The research was conducted from March to June 2024 for approximately 20 days at the Fish Quarantine Station Laboratory, Quality Control, and Fishery Product Safety, Aceh Besar.

Equipment and Materials

The equipment used in the research included surgical instruments, an autoclave, a Bunsen burner, a Pteri dish, a hot plate stirrer, an incubator, loop needles, a laminar flow hood, a magnetic stirrer, a sprayer, an Elenmeyer flask, a surgical tray, an oven, test tubes, droppers, cotton swabs, a refrigerator, an analytical balance, a test tube rack, rubber bands, aluminum foil, label paper, masks, gloves, spatulas, and syringes.

The materials used were 10 tilapia test samples, distilled water, 70% alcohol, Tryptone Soy Agar (TSA) medium, Triple Sugar Iron Agar (TSIA) medium, Oxidative/Fermentative (O/F) medium, Citrate medium, Motility Indole Ornithine (MIO) medium, Methyl Red Voges Proskauer (MRVP) medium, phenol red broth (base), sugar medium (arabinose, glucose, inositol, mannitol, rhamnose, sorbitol, sucrose, trehalose), stripe oxidase, 3% KOH, 3% H_2O_2, paraffin, Kovac's, α-napthol, methyl red, bromothymole blue, phenylalanine, nutrient agar, bacterogolical peptone, ferrixamonium citrate, urea, and myo-inosit.

Research Procedure

This study used a conventional, simple method for bacterial examination. The process begins with observing clinical symptoms, sterilizing equipment, preparing media, preparing samples/necropsies, isolating bacteria, and identifying bacteria using basic and biochemical

tests. This will then calculate the prevalence of bacterial infestation in tilapia (*Oreochromis niloticus*) samples.

Equipment Sterilization and Media Preparation

The equipment used, namely test tubes and petri dishes, is wet sterilized using an autoclave. The test tubes and petri dishes are boiled, washed with disinfectant, and then dried. Once dry, the test tubes are covered with rolled cotton, wrapped in paper, and placed in an autoclave at 121°C for 15 minutes at 1 atm of pressure. After the autoclave, the equipment is dried in an oven at 40°C.

The media for TSA, O/F, TSIA, MIO, MRVP, and Citrate are prepared by dissolving the media ingredients in distilled water in an Erlenmeyer flask at the appropriate volume. The mouth of the Erlenmeyer flask is then covered with aluminum foil and secured with a rubber band. The solution is then sterilized along with the other equipment. Sterilization is performed using an autoclave at 121°C for 15 minutes to eliminate all microorganisms present on the equipment and materials. The sugar medium is mixed with phenol red broth. After sterilization, the medium is cooled to room temperature and then transferred to a Petri dish or test tube.

Sample Preparation

Before the necropsy, the culture medium for the test samples is prepared by writing a sample code on each medium. The necropsy process begins from the lower body, working towards the anus and then towards the head. The target organs are observed: the liver, kidneys, and spleen.

Bacterial Isolation/Inoculation

The target organ is punctured with a loop needle and then implanted into TSA medium, spread through a scratch using the streak plate method. The culture medium is then incubated at 27°C for 24 hours. Bacterial colonies growing in the culture medium will be selected based on basic characteristics (color, shape, type, and size). Colonies that match the characteristics of the target bacteria will be purified on fresh TSA medium for further identification.

Bacterial Identification

Bacterial colonies growing in the purification medium will be observed for morphological structure, including shape, color, and colony margins. Basic tests (3% KOH gram, oxidase, catalase) and biochemical tests will then be performed. The 3% KOH gram test is performed by dropping a drop of 3% KOH solution onto a cover glass. The bacteria are then collected using a loop and homogenized. Changes in the bacteria are observed by mucus formation. Gram-negative bacteria will form mucus when mixed with 3% KOH solution, while gram-positive bacteria will not. The oxidase test is performed by streaking the colony on an oxidase strip, then observing the oxidation reaction on the streak. Observations are made by observing the color change on the strip. A blue color change on the oxidase strip indicates positive bacteria, while no color change indicates negative bacteria. The catalase test is performed by dropping 2-3 drops of 3% H_2O_2 solution onto a cover glass. The isolate is then homogenized in the solution and observed for changes. The bacterial reaction will foam if the colony is catalase positive, and not foam if it is catalase negative. The O/F test inoculates bacteria on the O/F medium by pricking the bacteria on the medium. Separate the Oxidase and Fermentative media, one tube will be filled with 2-3 drops of sterile liquid paraffin on the surface of the O/F medium and incubated. If the Oxidative and Fermentative media turn yellow then the bacteria are Oxidative. Motility and indole tests are carried out by pricking an ose needle on the MIO medium, motile bacteria will be marked by bacterial growth that spreads not only on the puncture line. For the indole test, the medium is dripped with 10 drops of

Kovac's reagent, indole-positive bacteria will form a red ring while indole-negative bacteria do not form a red ring. The ornothin test is carried out by observing the color change of the media, ornithine is positive if the media is white or yellow and ornithine is negative if the media is gray, purple and blue. In the TSIA test, bacterial isolates are inserted into the base of the medium and then streaked upwards in the slant area. The medium is then incubated aseptically at 27°C for 24 hours. Changes that occur indicate an acidic reaction that turns the medium yellow, if there is no color change, the reaction is alkaline. Observations are made on the butt and slant sections. Gas formation is indicated by the rise of the base of the medium, the medium is broken up, or there are cavities in the medium. Meanwhile, the formation of H2S is indicated by a black color change in the media. The citrate test inoculates bacteria by inserting the base of the medium and streaking upwards in the slant media. A positive reaction will turn the media blue and will produce an alkaline reaction. While a negative reaction occurs if there is no color change in the media. The MR-VP test is carried out by dividing 2 MR and VP test tubes. The MR test method is carried out by dripping 3 drops of methyl red into the bacterial isolate inoculation tube. Meanwhile, the VP test is carried out by adding half of the α-napthol reagent to the medium and homogenizing it until it turns milky brown and feels warm. Then add 40% KOH again to homogenize the VP medium again. Observation of changes is seen in the color changes that occur. Positive MR results will change the color to red and negative MR will remain yellow (no color change). Positive VP results will change color to red after 10-15 minutes, while negative VP results do not change color. The bacterial inoculation sugar test is carried out on a sugar medium by dipping. The sugar medium consists of arabinose, glucose, inositol, mannitol, rhamnose, sorbitol, sucrose, and trehalose. The medium will be incubated for 24 hours at a temperature of 25°C. Observation of color changes will indicate a positive result if the media turns yellow in each sugar medium, while a negative result does not show a color change.

Bacterial Infestation Prevalence

Prevalence calculations are conducted to determine the number of fish in a population infected with a disease. The prevalence of identified bacterial microorganisms is recorded by recording the type, number, and location of the affected organs in the fish, and the prevalence is calculated using the formula (Haryadi, 2006).

$$\text{Prevalence} = \frac{\text{Number of diseased fish}}{\text{Number of fish examined}} x\ 100\%$$

Test Data Analysis

Descriptive research provides research data that will be presented in the form of figures and tables. Data were collected using a descriptive survey method, with data collection using conventional methods and purposive sampling. Observations were made through direct observation of the objects being studied, ensuring that the data or events reported are based on reality.

RESULT

Identification of *Edwardsiella tarda* Bacteria

Based on research conducted at the Bacterial Testing Laboratory of the Fish Quarantine Station for Quality Control and Safety of Fishery Products, Aceh Besar, the results are presented in the following table.

Table 1. Biochemical Test Results

Test	Sample Code		Information
	N5a	N7a	
Shape	C, R, E	C, R, E	Single colony
Color	White	White	Milky white
KOH 3%	-	-	Negative, slimy
Motility	+	+	Motile
Catalase	+	+	Positive, bubbly
Oxidase	-	-	Negative, colorless
Glucose	+	+	Positive, acid-forming
O/F Fermentative	F	F	Fermentative properties
Ornithine	+	+	Positive
Indole	+	+	Positive
TSIA	k/m	k/m	Positive, yellow/red
Citrate	-	-	Negative
MR	+	+	Positive
VP	-	-	Negative
Sucrose	-	-	Negative
Rhamnose	-	-	Negative
Arabinose	-	-	Negative
Sorbitol	-	-	Negative
Inositol	-	-	Negative
Lactose	-	-	Negative
Mannitol	-	-	Negative
Trehalose	-	-	Negative
H_2S	+	+	Positive, blackish
Gas	G	G	Forms gas
EIM Test	+	+	Positive, blackish green
Inspection results	+	+	Positive, *Edwardsiella tarda*

Description: C (circular): round; R (raised): raised flat; E (entire): intact

Clinical Symptoms and Organ Examination

(a) Sample code N5 (b) Sample code N7 (c) Fish are not infected

Figure 1. Examination of Tilapia Fish Organs (*Oreochromis niloticus*)

The results of clinical symptoms and organ examinations on fish samples were based on direct observation of behavioral indicators (reflex responses and appetite), as well as clinical symptoms such as changes in color, eyes, and skin and fin condition.

Infestation Prevalence

The prevalence of infestations in tilapia identified through the Laboratory of the Fish Quarantine Station, Quality Control and Fishery Products, Aceh Besar, is shown in Table 2 below.

Table 2. Prevalence (%) of *Edwardsiella tarda* Bacteria Infecting Tilapia

No	Cultivators	Number of Samples	Number of Infected	Prevalence (%)
1.	CV. Serambi Ikan Center	10	2	20

Prevalence is calculated from the number of fish samples examined and the number of infected fish from examination of each target organ.

DISCUSSION

The results of bacterial testing with biochemical tests can be seen that the identification of tilapia (*Oreochromis niloticus*) tested contained *Edwardsiella tarda* bacteria in sample codes N5a and N7a in the target organ of the liver. The results of bacterial identification can be seen in table 4.3 adjusted to the results of the characteristics of *Edwardsiella tarda* based on SNI 7663: 2011. In the process of isolating the organs taken for observation of *Edwardsiella tarda* bacteria are the liver, kidney, and spleen. The target organ will be pierced with a sterile ose needle and then inoculated into TSA media in a petri dish by scratching. TSA is a general (non-selective) medium used as a growth medium, aiming to observe the morphology of bacterial colonies, develop pure cultures, and biochemical test growth. Bacterial colonies to be purified are taken from colonies with characteristics that match the target bacterial colonies. From the morphological examination of the shape of the *Edwardsiella tarda* bacterial colony is a single white colony of 1 mm. pure culture that grows on pure TSA media, will be continued with basic tests and biochemical tests to determine the characteristics and biochemical properties of bacteria. Based on the results of the gram test, the bacterial isolate from the tilapia fish sample has gram-negative properties which are characterized by the formation of mucus in the bacteria when given 3% KOH reagent. The presence of this mucus is based on the nature of the gram-negative bacterial cell wall which has many layers of thin lipid cell walls, the cell wall will be damaged and the bacterial cell will burst, releasing bacterial DNA. This was stated by Anggraini et al. (2016) gram-negative bacteria have a thin peptidoglycan component that makes it easier for bacterial cells to burst. The results of the oxidase test showed that the isolate from the tilapia fish sample was negative, meaning the bacteria did not have the cytochrome oxidase enzyme which can be seen by the change in color of the oxidase strip paper that was scratched with the bacteria. According to Purnamawati (2016), the oxidase enzyme functions to accelerate the combination of O_2, with a substrate which at the same time also reduces O_2, so that H_2O can be formed. From the results of the catalase examination carried out, the isolate in the catalase test was positive, characterized by the formation of air bubbles when mixed with 3% H_2O_2 with the bacterial isolate. According to Mac Faddin (1980), hydrogen peroxide is formed as an oxidative end product of aerobic sugar breakdown. H_2O_2, if allowed to accumulate, is toxic to bacteria and can cause death. Catalase utilizes H_2O_2 to oxidize other substrates (phenol, formic acid, formaldehyde, and alcohol). The sugar test conducted for biochemical tests uses 9 types of sugars, namely arabinose, glucose, inositol, lactose, mannitol, rhamnose, sucrose, sorbitol, and trehalose. Based on Table 1, the TSIA test results of isolates from tilapia samples showed an acidic reaction in the butt and an alkaline reaction in the slant. This indicates that the bacteria are able to ferment glucose in the butt, but cannot ferment lactose and sucrose in the slant. According to Anggraini et al. (2016), bacteria have the ability to degrade and ferment carbohydrates accompanied by acid production. Positive sugar test results indicate that the bacteria can degrade that type of sugar and produce an acidic reaction, while negative results indicate that the bacteria are unable to degrade that type of sugar and produce an alkaline reaction. The O/F test on all isolates in tilapia samples showed fermentative properties. This is because the media that is not covered and covered with paraffin changes color from green to yellow, so the bacteria are able to utilize carbohydrates under anaerobic

conditions through the fermentation process. According to Anggraini et al. (2016), some bacteria cannot grow without oxygen and some bacteria continue to grow in the presence of oxygen. The motility test showed that the isolates were motile. This is because the bacteria growing in the test medium spread from the puncture line. This indicates the presence of flagella, which function to move. According to Anggraini et al. (2016), flagella are one of the main structures outside the bacterial cell that cause movement (motility) in bacterial cells. In the indole test, the isolate showed a positive reaction, indicating the production of indole from tryptophan. Indole production can occur because the bacteria are able to produce the enzyme tryptophanase, which degrades the amino acid macromolecule tryptophan into pyruvic acid, ammonia, and indole. The results of the Ornithine test showed a positive result, indicating the bacteria are able to break down ornithine by decarboxylase. According to Barow & Feltham (1993), bacteria generally have the ability to break down various proteins and utilize them for cell synthesis as well as an energy source. Decarboxylase is an enzyme that plays a role in the separation of carboxyl groups to produce CO_2. The Citrate test on the tilapia sample isolate showed a negative result. According to Sudarsono (2008), citrate is produced in the condensation process of active acetyl with oxalacetic acid. Citrate acts on the enzyme citrase, which produces oxalacetic acid with acetic acid. This product is then enzymatically converted into pyruvic acid and carbon dioxide. If the microorganism is able to utilize citrate, the acid is removed from the culture medium, causing an increase in pH and changing the medium color from green to blue. The MRVP test on tilapia samples for all isolates isolated on MR media was positive. This indicates that the bacterial isolates are capable of oxidizing glucose by producing high concentrations of acid as the end result. The VP test on isolates from tilapia samples showed negative results. According to Anggraini et al. (2016), the VP test identifies microorganisms that ferment carbohydrates into 2,3-butanediol as the main ingredient, resulting in the accumulation of this material on the surface of the growth medium. For more accurate test results, the *Edwardsiella tarda* Media (EIM) test was performed, which showed positive results with colonies growing on green media with a blackish center.

In the calculation of the prevalence of *Edwardsiella tarda* bacteria that infect tilapia, there were 2 samples infected in the same target organ, namely the liver. The calculation shows a prevalence rate of 20% of the total number of samples. This number indicates that *Edwardsiella tarda* bacteria is a significant threat to fish health. *Edwardsiella tarda* bacteria cause serious diseases, especially when the fish's immune system is weakened. From the observation of clinical symptoms of fish infected with *Edwardsiella tarda* bacteria, symptoms such as decreased appetite, unstable swimming, white spots and sores around the mouth, lesions that cause bleeding around the operculum, peeling scales, damaged tail feathers, and loss of color pigment in the fish. This is in accordance with the statement that the external condition of the fish's body causes small wounds, loss of color, and if the wound is scratched, it will smell of H_2S (Yunin et al., 2019). The results of observations of symptoms in target organs show the most significant changes in the liver, swelling and discoloration. The liver plays a role in the body's metabolic processes, acting as a detoxification tool and phagocytizing foreign objects that enter the organ. If infected, the fish's liver will show signs of inflammation and tissue damage, including hepatomegaly (an enlarged liver). This can disrupt the fish's metabolic and digestive functions, and lead to further disruption of the body's metabolic processes if the liver is exposed to infection (Pramytha et al., 2014). *Edwardsiella tarda* bacteria can enter the body through wounds in the skin or through the digestive system and can spread to various organs, including the liver. Symptoms of *Edwardsiella tarda* infection in the liver can include tissue inflammation, liver cell damage, decreased liver function, yellowish or greenish discoloration, and liver swelling. On the skin, this infection can progress to the dermis and muscles, causing blisters and loss of color pigment (Ali et al., 2021).

CONCLUSION

Based on the results of research conducted at the Fish Quarantine Station for Quality Control and Safety of Fishery Products, it can be concluded that the results of the examination of three target organs in tilapia (*Oreochromis niloticus*) samples detected the presence of *Edwardsiella tarda* bacteria in fish experiencing symptoms of bacterial infection. The examination was carried out by observing clinical symptoms in fish, morphology and physiology of colonies growing on TSA media and biochemical testing with characteristic results adjusted to the SNI 7663: 2011 guideline. *Edwardsiella tarda* bacteria are opportunistic pathogens that can cause serious infections, especially when the fish's immune system is weakened. The target organ that detects the presence of *Edwardsiella tarda* bacteria is the liver. The prevalence rate of *Edwardsiella tarda* bacterial attacks is 20% of the 10 samples used, this can also be a significant threat to fish health. Good fish health management, including monitoring water quality and preventing infection is very important to reduce the risk of *Edwardsiella tarda* bacterial attacks.

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