



IDENTIFICATION and PREVALENCE OF *Aeromonas hydrophila* in *Cyprinus carpio* at CV. SERAMBI IKAN CENTER

IDENTIFIKASI DAN PREVALENSI BAKTERI *Aeromonas hydrophilla* PADA IKAN MAS *Cyprinus carpio* DI CV. SERAMBI IKAN CENTER

Received: March 10, 2026, Revised: May 05, 2026, Accepted: August 23, 2026
DOI: 10.35308/ja.v10i2.14947

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Abstract

This research aims to identify and determine the prevalence of *Aeromonas hydrophila* bacteria in goldfish (*Cyprinus carpio*) cultivated in CV. Serambi Ikan Center, Lambaro Street, Aceh Besar. *Aeromonas hydrophila* is a pathogenic bacteria that can cause disease in fish and has the potential to cause economic losses in aquaculture. This research is based on the importance of controlling fish diseases to support the success of aquaculture. The method used is goldfish sampling, namely the descriptive survey method, followed by the isolation and identification of bacteria using culture techniques and biochemical tests. The results of the study showed that *Aeromonas hydrophila* was identified in a number of goldfish samples with a prevalence of 20%. This finding indicates that there is a potential risk of bacterial infection in goldfish farming at the research site.

Keywords: Abundance, Plankton, Bah Bolon River, Diversity

1. Introduction

Common carp (*Cyprinus carpio*) is a freshwater fish of high economic value that is widely cultured because of its good adaptability to the environment, ease of spawning, omnivorous feeding habits, and relatively fast growth (Supriatna, 2013). Afifah et al. (2014) stated that common carp is also one of the food fish favored by Indonesians, so the demand for this commodity continues to rise along with increasing production each year. The good adaptability of common carp also enables this commodity to survive changing environmental conditions, including fairly extreme temperature fluctuations (Mustofa et al., 2018).

Abstrak

Penelitian ini bertujuan untuk mengidentifikasi dan mengetahui prevalensi bakteri *Aeromonas hydrophila* pada ikan mas (*Cyprinus carpio*) yang dibudidayakan di CV. Serambi Ikan Center, Jalan Lambaro, Aceh Besar. *Aeromonas hydrophila* merupakan bakteri patogen yang dapat menyebabkan penyakit pada ikan dan berpotensi menimbulkan kerugian ekonomi dalam budidaya perikanan. Penelitian ini dilatarbelakangi oleh pentingnya pengendalian penyakit ikan untuk menunjang keberhasilan budidaya. Metode yang digunakan adalah pengambilan sampel ikan mas yaitu metode survey deskriptif, dilanjutkan dengan isolasi dan identifikasi bakteri menggunakan teknik kultur dan uji biokimia. Hasil penelitian menunjukkan bahwa *Aeromonas hydrophila* teridentifikasi pada sejumlah sampel ikan mas dengan prevalensi sebesar 20%. Temuan ini mengindikasikan adanya potensi risiko infeksi bakteri tersebut dalam budidaya ikan mas di lokasi penelitian.

Kata Kunci : Kelimpahan, Plankton, Bah Bolon, Keanekaragaman

In aquaculture activities, disease remains one of the main constraints that can reduce productivity and cause economic losses. Disease outbreaks in fish can be caused by infectious agents such as viruses, bacteria, fungi, protozoa, and parasites (Aryani, 2004). Among these agents, bacterial diseases are a problem frequently encountered in fish farming, one of which is caused by *Aeromonas hydrophila* (Haryani et al., 2012).

Aeromonas hydrophila is an aquatic bacterium that can be found in both fresh and marine waters and is opportunistic in stressed fish. This bacterium is known as the cause of *hemorrhagic septicemia*, or red spot disease, which is characterized by hemorrhages on the fish body and tissue damage (Yogananth et al., 2009). Safratilo (2017) reported that infection with this bacterium can cause high mortality within a short time, namely approximately 80–100%

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within 1–2 weeks. Common clinical signs include red spots on the body, lesions on the abdomen, chest, fins, and tail, and reduced mucus production on the body surface of the fish (Afrianto et al., 2015).

However, data on the prevalence of *Aeromonas hydrophila* in common carp in Aceh Besar are still limited. Information on the presence and infection rate of this bacterium is important to support disease prevention and control efforts in common carp farming systems. In addition, the liver can be used as one of the observation indicators because it plays an important role in metabolism, detoxification, and phagocytosis of foreign substances entering the fish body (Pramytha et al., 2014). Therefore, this study aimed to identify and calculate the prevalence of *Aeromonas hydrophila* in common carp (*Cyprinus carpio*) at CV. Serambi Ikan Center, Aceh Besar.

2. Materials and Methods

2.1. Time and Location

This study was conducted from March to June 2024 at the Laboratory of the Fish Quarantine, Quality Control, and Fishery Product Safety Center, Aceh Besar.

2.2. Sampling Location

The fish samples used in this study were collected at CV. Serambi Ikan Center, Lambaro Street, Aceh Besar. The collected samples were then taken to the Laboratory of the Fish Quarantine, Quality Control, and Fishery Product Safety Center, Aceh Besar.

2.3. Sampling Method

Fish samples were taken from culture ponds at CV. Serambi Ikan Center, Aceh Besar. The study site was selected purposively based on the availability of common carp culture and ease of access. From the selected site, the fish were collected randomly to minimize bias.

The study sample consisted of 10 common carp weighing approximately 250 g each. This sample size was determined based on operational considerations and as a preliminary study to obtain initial data on the presence of *Aeromonas hydrophila* in common carp. The fish were caught using a scoop net and then placed in an aquarium filled with clean water equipped with an aerator to maintain the condition of the fish during transportation to the laboratory. Upon arrival at the laboratory, the samples were observed and further analyzed to detect the presence of *A. hydrophila*.

2.4. Equipment Preparation

The first step in equipment preparation was to sterilize all the equipment to be used, such as Petri dishes, Erlenmeyer flasks, and test tubes. First, all the equipment was boiled in a pot until the water boiled to clean it. After that, the equipment was washed thoroughly, dried, and wrapped in sterile paper. Next, the equipment was sterilized in an autoclave at 121°C for 15-20 minutes. After the sterilization process was completed, the equipment was placed in an oven at 60°C to keep it clean until ready

for use (SNI 7303-1-2015, Buku panduan penelitian SKIPM).

2.5. Preparation of Materials

a. Preparation of TSA

The first step in preparing the TSA medium was to weigh 40 grams of TSA, which was then placed into an Erlenmeyer flask, and 1000 ml of distilled water was added. Next, the mixture was sterilized in an autoclave at 121°C for 15-20 minutes for homogenization. After sterilization was complete, the medium was left to reach room temperature. Once cooled, the TSA medium was poured into Petri dishes and left until it solidified into agar. After the medium solidified, the Petri dishes were inverted so that the residual vapor could disappear and the medium was ready for use.

b. Preparation of TSIA

A total of 19.5 grams of TSIA medium was weighed and placed into an Erlenmeyer flask, then 300 ml of distilled water was added and the flask was covered with aluminum foil. The mixture was then homogenized in an autoclave at 121°C for 15-20 minutes. After that, the Erlenmeyer flask was removed from the autoclave and allowed to cool to room temperature. Once it reached room temperature, the liquid was poured into sterile test tubes up to one-quarter of the tube volume in a slanted position so that a slant agar medium would form.

c. Preparation of MIO

A total of 9.3 grams of MIO medium was weighed and placed into an Erlenmeyer flask, then 300 ml of distilled water was added and the flask was covered with aluminum foil. The mixture was then homogenized in an autoclave at 121°C for 15-20 minutes. After that, the Erlenmeyer flask was removed from the autoclave and allowed to cool to room temperature. Once it reached room temperature, the liquid was poured into sterile test tubes up to one-quarter of the tube volume. Once it had become agar, the MIO medium was ready for use.

d. Preparation of O/F

A total of 2.345 grams of the medium was weighed and placed into an Erlenmeyer flask, then 250 ml of distilled water was added and the flask was covered with aluminum foil. The mixture was then homogenized in an autoclave at 121°C for 15-20 minutes. After that, the Erlenmeyer flask was removed from the autoclave, 5 grams of glucose was added and stirred slowly until dissolved, and the O/F medium was left to reach room temperature. Once it reached room temperature, the liquid was poured into sterile test tubes up to one-quarter of the tube volume. After the medium turned into agar, the O/F medium was ready for use.

e. Preparation of RS Medium

A total of 21.24 grams of RS Medium was weighed and placed into an Erlenmeyer flask, then 495 ml of distilled water and a magnetic stirrer were added. The Erlenmeyer flask was covered with aluminum foil and homogenized on a hotplate until boiling; after boiling, the flask was removed from the hotplate, and once it reached room temperature, the medium was poured into sterile Petri dishes.

2.6 Bacterial Isolation

The preparation process began with sterilizing the work or dissection table and the dissecting instruments with 70% alcohol until everything was sterile. Next, the fish was euthanized by piercing the middle of the head with an instrument. After the fish died, necropsy was performed using sterile dissecting instruments. A sterile inoculation loop was then inserted into the target organ and streaked onto TSA medium. The streaked medium was incubated at 25°C to 28°C for 18 to 24 hours (SNI 7303-1-2015, buku panduan penelitian SKIPM).

2.7 Colony Purification

Before colony purification, the work area inside the laminar air flow cabinet was first cleaned with 70% alcohol to maintain cleanliness and prevent contamination. Next, TSA medium was prepared inside the laminar air flow cabinet, and the inoculation loop was sterilized with the flame of a Bunsen burner. Colonies that had been incubated for 24 hours were taken from the incubator, and a single colony was selected on the TSA medium. This single colony was then streaked onto fresh TSA medium and reincubated for 24 hours at 25°C to 28°C (SNI 7303-1-2015, Buku panduan penelitian SKIPM).

2.8 Observed Parameters

a. Clinical Signs

Clinical signs in this study were observed based on the indicators shown in the following Table:

Table 1.
Clinical Signs

No	Clinical Sign Indicator	Clinical Sign Observation
1.	Faded or darkened body color	The body color of the fish becomes darker or faded
2.	Protruding eyes	The eyes of the fish appear to protrude from their sockets
3.	Hemorrhage	Hemorrhage occurs on the gills, fins, or other body parts
4.	Scale discoloration	Scales detach or change color
5.	Pale or damaged internal organs	Pale liver, blackish kidney, blackish intestine, and tissue damage in other internal organs
6.	Body lesions	Wounds, sores, or ulcers appear on the body surface, with detached scales

b. Bacterial Identification

1. Gram Test (3% KOH)

The Gram test with 3% KOH solution aims to determine the nature of the tested bacteria, whether Gram-positive or Gram-negative. The test

procedure was performed by mixing the purified bacterial isolate on the surface of a cover glass using an inoculation loop, then adding 3% KOH solution. The mixture was observed for slime formation. If the mixture does not produce a slimy reaction, the bacterium is Gram-positive. Conversely, if the solution becomes slimy, the bacterium is Gram-negative (SNI 7303-1-2015).

2. Oxidase Test

The oxidase test serves to determine the presence or absence of the oxidase enzyme in bacteria. The oxidase test procedure began by dropping Kovac's reagent onto oxidase paper. Next, an incubated bacterial colony was taken with an inoculation loop and smeared onto the oxidase paper treated with Kovac's reagent. Observation was made by noting the color change of the oxidase paper. If the oxidase paper turns blue, the test result is declared positive, indicating that the bacterium produces the oxidase enzyme. Conversely, if no color change occurs, the test result is considered negative, meaning that the bacterium does not produce the oxidase enzyme (SNI 7303-1-2015).

3. H₂O₂ Catalase Test

This test aims to determine the presence of the catalase enzyme in bacteria. The catalase enzyme functions to break down hydrogen peroxide (H₂O₂) into water and oxygen. If air bubbles form in the solution, the test result is declared positive, meaning that the bacterium produces the catalase enzyme. Conversely, if no air bubbles form, the test result is considered negative. The test procedure began by preparing a cover glass and then dropping H₂O₂ solution onto its surface. Next, the incubated bacterial isolate was taken using a sterile inoculation loop and mixed with the H₂O₂ solution. The reaction was observed to determine the catalase test result (SNI 7303-1-2015).

4. MIO Test

The test procedure began by taking a bacterial isolate with a sterile inoculation loop, which was then inoculated into a tube containing MIO medium by stabbing the loop vertically into the medium. After that, the medium was incubated at 28°C for 24 hours. After incubation, the medium was observed for color changes and growth to determine the motility, indole, and ornithine test results. To determine the indole test result, after the MIO medium had been incubated for 24 hours, 2 drops of Kovac's reagent were added to the MIO medium. If bacterial growth spreads from the stab line in the test medium, the motility test result is positive. If a red ring layer appears on the surface of the medium, the indole result is positive; conversely, if no red ring layer forms on the surface of the medium, the indole result is negative. A positive ornithine test result is indicated by a change in the color of the medium to yellow at the bottom of the medium (SNI 7303-1-2015, buku panduan penelitian SKIPM).

5. OF Test

Two tubes containing O/F (Oxidation-Fermentation) medium were prepared, one with paraffin and one without paraffin. The bacteria were then inoculated into the medium by stabbing a sterile inoculation loop carrying the bacterial isolate straight into the O/F medium tube without paraffin. In the other O/F test tube, after the bacteria were stabbed in, 3 to 4 drops of liquid paraffin were added to the tube. Both tubes were then incubated at 25°C to 28°C for 18- 24 hours. After the incubation period was completed, the color change in the medium was observed. A positive fermentative OF test result is indicated by a change in the color of the medium to yellow in the paraffin-sealed tube (anaerobic), whereas an oxidative result is indicated by a yellow color change in the non-paraffin tube (aerobic). If both tubes turn yellow, the bacterium is oxidative-fermentative (SNI 7303-1-2015, Buku panduan penelitian SKIPM).

6. Triple Sugar Iron Agar (TSIA) Test

Prepare a test tube containing TSIA medium, then take a bacterial colony with a sterile loop. Stab the loop into the base of the medium and streak the slanted part of the medium perpendicularly to a depth of ¾ of the base or butt. Streak the loop over the slanted surface in a zigzag pattern. Next, incubate the medium at 35-37°C for 18-24 hours to observe the test results. Observe the color change of the medium in the slant and the butt, gas formation (cracking and lifting of the medium), and the formation of a black precipitate (indicating H₂S production) (SNI 7303-1-2015, buku panduan penelitian SKIPM).

7. RS Medium Test

The RS.Medium test began by taking a bacterial isolate with a sterile inoculation loop and streaking it onto the surface of the RS.Medium. The medium was then incubated for 18-24 hours at 37°C, and the colonies that grew after incubation were observed. If the bacteria streaked onto the medium turn yellow without black in the center or at the edge of the colony, the medium is positive for *Aeromonas hydrophila* (SNI 7303-1-2015, buku panduan penelitian SKIPM).

2.9 Infection Prevalence

Infection prevalence is a term used to describe the percentage of fish affected by a particular disease compared with the total population of fish examined at a given time. Prevalence indicates how many fish in the population are infected with the disease, expressed as a percentage (Kementerian Kelautan dan Perikanan RI, 2021).

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

The categories of prevalence levels according to (Syukran *et al*, 2018) are presented in the following Table of Prevalence Categories:

Table 2.
Prevalence Categories

No	Prevalence	Category	Description
1	100-99%	Always	Very severe infection
2	98-90%	Almost always	Severe infection
3	89-70%	Usually	Moderate infection
4	69-50%	Very often	Very frequent infection
5	49-30%	Commonly	Common infection
6	29-10%	Often	Frequent infection
7	9-1%	Occasionally	Occasional infection

3.2 Data Analysis

The test data in this study were presented systematically using tables and figures to facilitate understanding of the results. Tables were used to present quantitative data, such as prevalence percentages, as well as the results of biochemical tests and bacterial characteristics. Figures in the form of photographs were used to clarify the visual depiction of the clinical condition of the fish and the results of the identification of *Aeromonas hydrophila* (Cahyani, 2019; Weto, 2022).

The presence of bacteria was tested using the conventional bacterial examination method referring to the Indonesian National Standard (SNI) 7303.1:2015. Data such as bacterial species and the dominant bacteria infecting common carp (*Cyprinus carpio*) were analyzed descriptively and discussed with reference to relevant literature. This method included bacterial isolation from fish tissues, observation of colony morphology, and a series of biochemical tests to confirm bacterial identification in accordance with standardized procedures.

3. Results and Discussion

3.1. Clinical Signs

Based on observations referring to the clinical sign indicators in Table 1, the observation results are presented in Table 3.

Table 3.

No	Fish	Clinical Signs	Figure
1	Fish 2	Tail scales peeled off	
2	Fish 6	Reddish lesion on the operculum and protruding eyes	
3	Fish 7	Black spots on the back of the fish	
4	Fish 10	lesion on the ventral surface of the fish	

Before the fish were dissected, observation of clinical signs for approximately two days showed that the fish suspected of being infected with *Aeromonas hydrophila* exhibited several typical signs, namely reddish lesions on the operculum, the appearance of black spots, a change in

skin color to pale, and lesions on the ventral surface of the fish. In addition, the scales on the tail also peeled off. These signs are consistent with the findings of Haryani *et al* (2012), who stated that *Aeromonas hydrophila* is a pathogenic bacterium that causes aeromonas disease in fish.

3.2 Identification of *Aeromonas hydrophila*

The results of the identification of *Aeromonas hydrophila* through biochemical tests in accordance with the Indonesian National Standard (SNI) are presented in detail in Table 4. These biochemical tests comprise a series of examinations used to confirm the presence and characteristics of *Aeromonas hydrophila* based on the applicable SNI protocol, thereby providing an accurate picture of the bacterial identification in the tested samples.

Table 4.
Biochemical Examination Results

Tabel 4.2 Hasil Pemeriksaan Biokimia

Parameter Pemeriksaan	Kode Sampel Pemeriksaan																													
	Sampel 1			Sampel 2			Sampel 3			Sampel 4			Sampel 5			Sampel 6			Sampel 7			Sampel 8			Sampel 9			Sampel 10		
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fermentative	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H ₂ S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ornithine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malonate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose (Oxid)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ketone (Oxid)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Keterangan: Angka 1 sampai 30 digunakan untuk kode organ target secara berurutan dengan abjad H(Hati), G(Ginjal), K(Kulit), M(Merah), K(Kuning), O(Oksidatif), F(Fermentatif), H(Hisus).

Based on the results in Table 4, the target parts or samples showing characteristics consistent with the pathogenic bacterium *Aeromonas hydrophila* can be identified. The main characteristics of this bacterium include positive motility, positive oxidase, positive glucose fermentation, fermentative properties, positive indole, positive sucrose and lactose fermentation, and no production of H₂S or gas.

Based on the main characteristics of *Aeromonas hydrophila* analyzed through biochemical tests, of the 10 common carp samples examined in three target organs, namely the liver, kidney, and skin, two samples showed indications of *Aeromonas hydrophila* infection. The samples indicated as infected were the sample with code 16 from the liver and the sample with code 17 from the kidney.

1. 3% KOH Test

Based on the results of the 3% KOH test, all isolates showed a slimy reaction indicating Gram-negative properties, except for the skin sample of fish 1, the kidney sample of fish 3, and the kidney sample of fish 10. In the kidney samples of fish 1 and 2, the bacteria did not grow, so they could not be evaluated further. These results are consistent with the characteristics of *Aeromonas hydrophila*, which is generally classified as a Gram-negative bacterium. According to Anggraini *et al.* (2016), a slimy reaction in the KOH test indicates that the bacterium

is Gram-negative because its cell wall is thinner and easily broken down by the KOH solution, producing slime when stirred. This finding strengthens the presumption that the obtained isolates have characteristics consistent with *A. hydrophila*. In the kidney samples of fish 1 and 2, the bacteria did not grow. The Gram test results are shown in Figure 1.

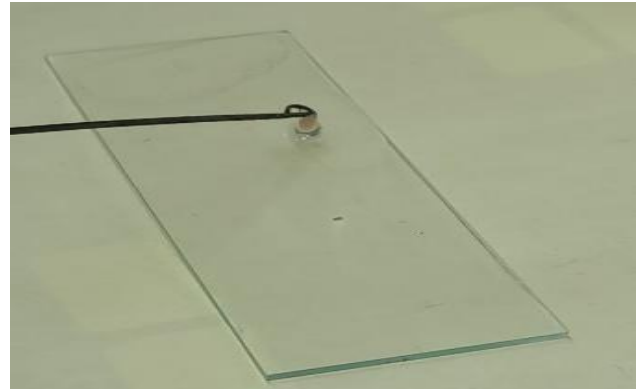


Figure 1. 3% KOH Test

2. Catalase Test

The catalase test is used to detect the presence of the catalase enzyme in bacterial isolates. A positive reaction is indicated by the formation of air bubbles due to the breakdown of 3% H₂O₂ into water and oxygen, whereas a negative reaction shows no bubble formation (Dwidjoseputo, 2005; Sulistiyono and Mutiara, 2022). The results showed that the isolates from fish 6 from the liver (code 16) and kidney (code 17) gave a negative reaction, whereas the isolates from fish 3, 4, 5, 7, 8, 9, and 10 showed a positive reaction. In the kidney samples of fish 1 and 2, there was no bacterial growth, so observation could not be performed. The catalase test results are shown in Figure 2.

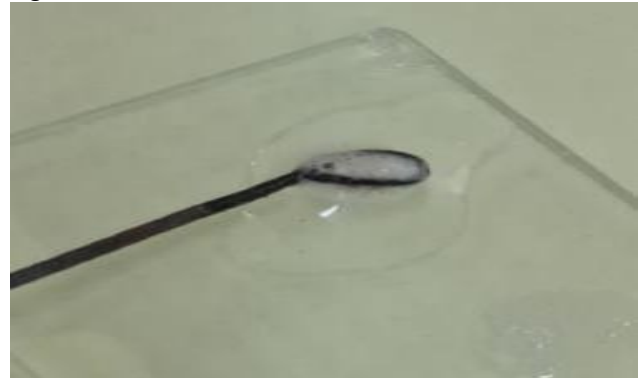


Figure 2. Catalase Test Results

3. Oxidase Test

The oxidase test showed that most isolates were oxidase-positive, indicated by the oxidase paper turning blue (Sulistiyono and Mutiara, 2022). Negative results were obtained in the skin of fish 7 and the kidney of fish 8, whereas no bacterial growth was found in the kidneys of fish 1 and 2, so the test could not be performed. The oxidase test results are shown in Figure 3.



Figure 3. Oxidase Test

4. MIO Test (Motility, Indole, Ornithine)

The MIO (*Motility Indole Ornithine*) test aims to determine the motility, indole production, and ornithine decarboxylation ability of bacteria (Sulistiyanto and Trimulyono, 2019). Motility indicates the ability of bacteria to move, characterized by the spread of growth around the inoculation line. The indole test is used to determine the ability of bacteria to produce indole, with a positive result indicated by the formation of a red ring after the addition of Kovac's reagent to the surface of the medium (Ester and Mukarlina, 2022). The ornithine test is used to detect the ability of bacteria to utilize ornithine, with a positive result indicated by a change in the color of the medium to yellow at the bottom of the medium (Wahyunio, 2011).

The results showed that all bacterial isolates gave positive results in the motility test, indicated by the spread of growth from the inoculation line. Positive indole results were found in the liver and skin of fish 1, the skin of fish 2, the kidney of fish 3, the liver, kidney, and skin of fish 4, the liver and kidney of fish 5, the liver of fish 6, the liver, kidney, and skin of fish 7, the kidney of fish 8, the liver and kidney of fish 9, and the liver of fish 10. In contrast, negative indole results were obtained in the liver of fish 2, the liver and skin of fish 3, the skin of fish 5, the kidney and skin of fish 6, the liver and skin of fish 8, the skin of fish 9, and the kidney and skin of fish 10. In some samples, such as the kidneys of fish 1 and fish 2, the bacteria did not grow, so the test could not be performed. All isolates showed negative results in the ornithine test, indicating that the bacteria lack the ability to decarboxylate ornithine. The MIO test results are shown in Figure 4.

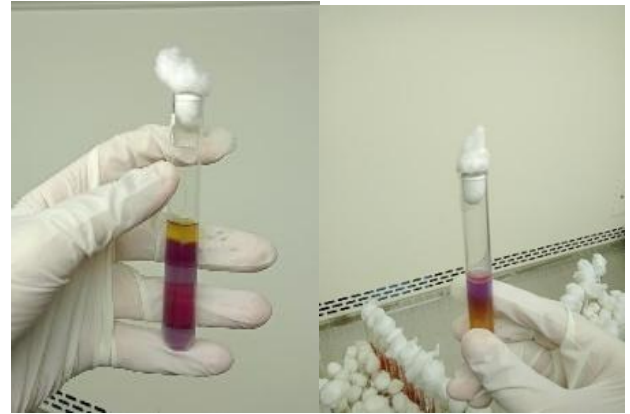


Figure 4. MIO Test

5. OF Test (Oxidative/Fermentative)

The O/F test is a biochemical test that aims to determine the ability to oxidize and ferment glucose in the presence or absence of oxygen, because bacteria have different oxygen requirements (Sulistiyono and Mutiara, 2022). Anaerobic respiration is used by microorganisms to live without oxygen, whereas aerobic organisms only use anaerobic respiration and will die in the presence of oxygen (Pardamean *et al.*, 2021).

The O/F test results were determined by observing the color change in the medium. If the O/F medium without paraffin changes color to yellow and its surface becomes purplish blue, the result is oxidative positive (+); if the medium does not change color, the result is oxidative negative (-). Meanwhile, if the O/F medium with paraffin also changes color to yellow, the result is fermentative positive (+), and if the medium does not change color, the result is fermentative negative (-).

The O/F test showed a fermentative (F) reaction in the skin of fish 1, the kidney of fish 3, the liver, kidney, and skin of fish 4, the liver of fish 6, the kidney of fish 7, the liver and kidney of fish 9, and the liver of fish 10. Meanwhile, an oxidative (O) reaction was found in the liver of fish 1, the liver and skin of fish 2, the liver and skin of fish 3, the liver, kidney, and skin of fish 5, the kidney and skin of fish 6, the liver and skin of fish 7, the liver, kidney, and skin of fish 8, the skin of fish 9, and the kidney and skin of fish 10. The OF test results are shown in Figure 5.



Figure 5. OF Test

6. TSIA Test

The TSIA test aims to differentiate bacterial species based on their ability to break down glucose, lactose, and sucrose; in addition, the TSIA test serves to determine whether or not the bacterium produces H_2S gas (Holt *et al.*, 2000). If the medium turns black or there is black color in the medium, the result is +Glucose, + H_2S . If there are bubbles or air spaces in the agar medium, the result is +Gas. If the medium is colored or has black color, the result is -Lactose and -sucrose; if the TSIA medium turns yellow or reddish pink, the result is +lactose and +sucrose (Wahyuni *et al.*, 2018).

The TSIA test results for each fish sample showed different reaction variations. The TSIA test showed that H_2S production was found in the samples of fish 7 (skin) and fish 10 (skin). Gas production was observed in the samples of fish 1 (skin), fish 3 (kidney), fish 4 (liver), fish 5 (kidney and liver), fish 7 (kidney), fish 8 (liver, kidney, and skin), and fish 9 (liver). The M/M reaction (acid in the slant and butt) was found in the samples of fish 2 (liver), fish 3 (liver and skin), fish 5 (liver), fish 6 (skin), fish 8 (skin), fish 9 (kidney and skin), and fish 10 (liver, kidney, and skin). The K/M reaction (alkaline slant, acid butt) occurred in the samples of fish 1 (skin), fish 4 (kidney and skin), fish 5 (kidney), and fish 8 (liver). Meanwhile, the M/K reaction (acid slant, alkaline butt) was found in the samples of fish 1 (liver), fish 2 (skin), and fish 8 (kidney). The K/K reaction (alkaline slant and butt) was observed in the samples of fish 3 (kidney), fish 4 (liver), fish 5 (skin), fish 6 (liver and kidney), fish 7 (liver, kidney, and skin), and fish 9 (liver). Meanwhile, in some organs, such as the kidneys of fish 1 and fish 2, no bacterial growth was found. The TSIA test results are shown in Figure 6.



Figure 6. TSIA Test

7. Rimler-Shotts Test (RS.Medium)

Rimler-Shotts medium is a selective medium for the growth of *Aeromonas hydrophila*, in which the growing bacteria show a characteristic change in the color of the medium to yellow. A positive indication of the presence of *Aeromonas hydrophila* is marked by the appearance of a yellow color on the medium (Hamny *et al.*, 2019).

The results of the Rimler-Shotts (RS) test on fish sample 6, with target organ code numbers 16 (liver) and 17 (kidney), showed a yellow color, indicating that the samples were positive for *Aeromonas hydrophila*. The yellow color on RS medium is a characteristic indicator of the presence of *Aeromonas hydrophila*. The RS.Medium test results are shown in Figure 7.

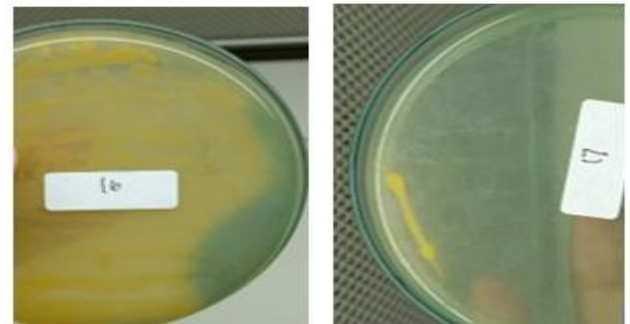


Figure 7. RS.Medium Test Results

3.3 Infection Prevalence

Of the 10 fish samples examined in three target organs, it was found that in fish number 6, *Aeromonas hydrophila* infected two target organs, namely target organ code 16 (liver) and target organ code 17 (kidney). The prevalence of *Aeromonas hydrophila* infecting common carp is shown in the Table of Prevalence (%) of *Aeromonas hydrophila* Infecting Common Carp

Table 5.
Infection Prevalence

No	Farmer	Number of samples	Number of infected fish	Prevalence (%)	Category
1	CV. Serambi Ikan Center	10	1	10%	Frequent infection

The prevalence of *A. hydrophila* in this study was 10%, obtained from 1 infected fish out of the 10 samples examined, and was therefore categorized as a frequent infection. This result is lower than the 93% incidence of *Aeromonas* sp. infection reported in freshwater fish in Sleman and Bantul Regencies. This difference in prevalence was likely influenced by differences in culture environmental conditions, fish health status, stocking density, and the design and sample size of the studies. This finding is also in line with a study on tiger grouper, which showed an infection prevalence of 50–60% at different culture sites.

4. Conclusion

Based on the study on the identification and prevalence of *Aeromonas hydrophila* in common carp (*Cyprinus carpio*) at CV. Serambi Ikan Center, 2 out of 10 fish samples (20%) were found to be infected with *A. hydrophila* based on biochemical tests (Gram-negative, oxidase-positive, motile, indole-positive, fermentative, and positive on Rimler-Shotts medium), supported by clinical signs in the form of erythema on the operculum, hyperpigmentation, abdominal ulceration, and desquamation of tail scales. This 20% prevalence indicates that *A. hydrophila* infection is a significant risk in common carp farming at the study site, although it is lower than that reported in a study on goldfish in Aceh (50%). Therefore, disease prevention and control efforts such as improving water quality, managing fish stress, and routine health monitoring should be prioritized to reduce negative impacts on fish health and maintain the sustainability of common carp farming.

Acknowledgments

The authors would like to thank Dr. Suri Purnama Febri, S.Kel, M.Si, as the First Supervisor, and Andika Putriningtias, S.Kel, M.Si, as the Second Supervisor, for their extensive guidance in the preparation of this journal article, and M. Ramli, A.Md, as the Field Supervisor, for the assistance and effort provided during the research. The authors also thank all parties who have helped, from the preparation of this article until its publication.

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