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Identification and characterization of bacteria from faeces and rivers in East Bekasi using biochemical test

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ABSTRAK

Identifikasi dan karakterisasi bakteri adalah dua deteksi utama dalam menentukan tingkat spesies patogenisitas suatu bakteri. Penelitian ini bertujuan mengisolasi bakteri dari feses orang normal, feses cair, dan beberapa yang berasal dari air sungai yang masih digunakan oleh masyarakat setempat untuk mandi, berenang, dan memancing, hasilnya dikonsumsi. Air yang terkontaminasi dengan mikroorganisme seperti bakteri paling sering disebabkan oleh kontaminasi dari polusi, limbah industri, bahkan feses hewan dan manusia. Media yang digunakan adalah media Salmonella Shigella Agar (SSA) diferensial selektif. Koloni yang tumbuh kemudian dikarakterisasi dengan menguji aktivitas biokimia mereka. Hasil uji biokimia dibandingkan dengan Bergey's Manual of Determinative Bacteriology. Dari tujuh sampel di setiap lokasi, Salmonella *sp. diidentifikasi dari Sungai jalan Kiai Haji Abu Bakar, dan* E. coli *diidentifikasi dari Sungai Bumi Palapa. Kedua spesies ini dikategorikan sebagai patogen manusia. Sampel lainnya mengandung bakteri oportunistik, termasuk* Enterobacter *sp. dan* Serratia *sp. Penelitian ini menyimpulkan bahwa dua dari tujuh lokasi sampel berisiko tinggi terhadap kesehatan manusia. Identifikasi ini dapat membantu menentukan patogen yang menyebabkan berbagai penyakit dengan dampak besar pada masyarakat.*

Kata kunci: *Biokimia; identifikasi; karakterisasi bakteri; sungai*

ABSTRACT

Identification and characterization of bacteria are the two main detection in determining the species level of pathogenicity of a bacterium. This study aims to isolate bacteria from normal person faeces, liquid faeces, and some sourced from river water which is still used by local communities for bathing, swimming and fishing activities, the results of which are consumed. Water contaminated with microorganisms such as bacteria is most caused by contamination from pollution, industrial waste, even animal and human faeces. The media used was selective differential Salmonella Shigella Agar (SSA) media. Colonies that grown were then characterized by testing their biochemical activity. The results of the biochemical tests were compared with Bergey's Manual of Determinative Bacteriology. From seven samples for each location, Salmonella *sp. was identified from Kiai Haji Abu Bakar street River, and E. coli was identified from Bumi Palapa river. Both species were categorized as human pathogens. The rest of samples were contained the opportunistic bacteria, including* Enterobacter *sp. and* Serratia *sp. This study concluded that two from seven samples location were high risk for human health. This identification*

can help to determine the pathogens that cause various diseases with a large impact on society.

Keywords: *Bacterial characterization; biochemical; identification; river*

INTRODUCTION

Salmonella and *Shigella* genera are Gram-negative bacteria that can affect human health. *Salmonella* and *Shigella* bacteria can contaminate the air. The disease that arises from *Salmonella* in humans causes typhoid fever. Meanwhile, *Shigella* can cause symptoms of diarrhoea, fever and abdominal pain (Sulaeman, 2015). *Salmonella* and *Shigella* that generally found in faeces, so rivers containing faeces can be contaminated by *Salmonella* and *Shigella* bacteria.

Faeces are the final product of a person after passing through final metabolism in the body. When a person carries out activities, such as eating food, which is then digested by the body, faeces will form as remains that are excreted. Recently, many Indonesian people still use rivers as a source for daily life, including washing clothes, bathing, cooking water, and defecation which is also take place in rivers, so that it often becomes contaminated, and makes the water unhealthy for consumption.

Salmonella and *Shigella* bacteria can contaminate the drinking water. One of the differential isolation media for *Salmonella* and *Shigella* is Salmonella-Shigella Agara (SSA) media. SSA media is a very common medium for isolating *Salmonella* sp. SSA is a daily-routine isolation medium for isolating *Salmonella* sp. and *Shigella* sp. from faeces, urine and food samples (Arlita et al., 2014). As a confirmation, identification then carried out using a biochemical to determine *Salmonella* and *Shigella* bacteria. The biochemical tests used are TSIA (Triple Sugar Iron Agar), SIM (Sulphite Indole Motility), MR (Methyl Red), VP (Voges Proskaver) and sugar tests consisting of glucose, lactose, sucrose, maltose and mannitol.

Poor hygiene and sanitation problems can cause diseases such as typhoid fever and diarrhea. *Salmonella* can also cause salmonellosis. The salmonellosis prevalence rate in Indonesia in 2019 was 5.82 per 100,000 population with a mortality rate of 2.42. The prevalence of salmonellosis in North Sulawesi in 2018 was 2.9%, then the prevalence of salmonellosis in North Minahasa Regency in 2018 was 2.7%. Meanwhile, *Shigella* causes shigellosis. The prevalence rate in Indonesia for shigellosis cases was 11.7% in the West Java area (North Sulawesi Provincial Health Service, 2021). Moreover, study in 2023 showed that Bekasi river contained *E. coli* with gene that responsible to cephalosporins resistant (Gusti et al., 2024). This fact indicates the survey of the presence of *Salmonella* and *Shigella* becomes important, in particular in Urban area, such as Bekasi city, West Java Province, Indonesia. Based on this, the problems that will be discussed in the manuscript are formulated.

METHODOLOGY

We use purposive sampling methods in collecting our samples throughout the study area.

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Studies sites and species sampling

Six water samples were obtained on March 27, 2024 which were taken from various places. The water intake locations are located in the Kalimalang River, Perum Bumi Bekasi Baru, Rawalumbu, Bekasi River, and Palapa River which are the main hydrological arteries in this study area. This river flows through varied landscapes, from domestic waste to estuaries or hillsides, creating heterogeneous ecological conditions. Located on geographic coordinates, this location is accessed via main road access and footpaths that provide easy access to the riverbank.

TABLE 1. Sampling locations and coordinates

Sample number	Location	Coordinate
1	New Bumi Bekasi River	-6.284054234486066, 106.99836925697083
2	Kalimalang River	-6.2574881584092905, 107.00829860080516
3	Rawalumbu River	-6.260525771194329, 107.00041036074764
4	Kalimalang River (Kiai Haji Abu Bakar Street)	-6.2718769079612064, 107.05232479117112
5	Bekasi River	-6.243555658932897, 107.01374180836362
8	Bumi Palapa Rive	-6.2553529872827065, 106.98902387976021
4	Kalimalang River (Kiai Haji Abu Bakar Street)	-6.2718769079612064, 107.05232479117112

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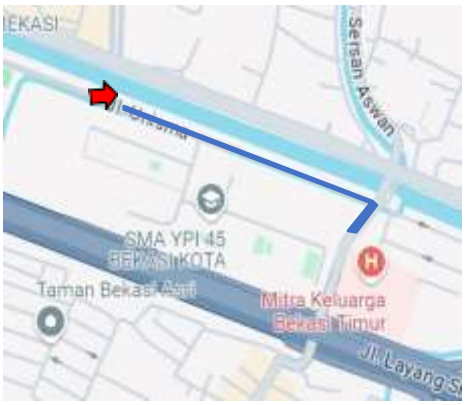

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FIGURE 1. Map of sampling location (1) New Bumi Bekasi river, (2) Kalimalang River, (3) Rawalumbu River, (4) Kiai Haji Abu Bakar Street River, (5) Bekasi River, (8) Bumi Palapa River

Research design

1. Faeces samples

Two faeces samples were obtained from normal person and gastroenteritis symptom person in 26, March 2024. Fecal samples from normal individuals were taken using the sterile containers provided and stored fresh at the right temperature for bacteriological testing. Sample collection is carried out by ensuring sterile procedures and avoiding contamination from external sources. This normal stool sample will be a control to compare the bacterial composition with stool samples from sick individuals.

TABLE 2. Number and source of samples

Samples Number	Source
6	Normal person
7	Gastroenteritis symptom person

Stool samples from sick individuals are taken as a representation of conditions that allow pathogenic bacterial infections in the digestive tract. Individuals who provide stool samples experience symptoms of gastrointestinal illness that include diarrhea.

Research procedures

River water and faeces samples were collected from predetermined locations using sterile sample bottles (Fig. 1). Sampling is carried out by ensuring sterilization of tools and avoiding cross contamination. Then the SSA media solution is heated to dissolve all components, then poured into a sterile petri dish and cooled to solidify. Then the river water sample was diluted 10x, 100x, and 1000x using 9ml of NacL in a test tube, then inoculated the sample with each dilution, and the river water sample was inoculated onto the surface of the SSA media using a sterile technique such as a spread plate. And for faeces samples, normal individual faeces are weighed using an analytical balance as much as 1 gram and then diluted with 0.9ml NacL and then diluted 10x, 100x, 1000x. In sick individuals, weighing is not necessary because samples taken from sick individuals are generally in liquid form. Therefore, measuring sample weight is not relevant in the

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context of taking stool samples from individuals who are experiencing digestive disorders or gastrointestinal diseases. Then inoculate the sample into each dilution, and the river water sample is inoculated onto the surface of the SSA media using a sterile technique such as a spread plate. After the sample on the SSA medium is ready, the sample is incubated at a temperature suitable for the growth of pathogenic bacteria (usually around 37°C) for 24-48 hours. After the samples are incubated, a biochemical test is carried out to identify the presence of pathogenic bacteria in the river water and faeces samples

1. Biochemical Test

Biochemical testing is a test used to determine previously unknown bacterial species. Each bacteria have different biochemical properties so this biochemical test stage is very helpful in the identification process. After the sample is inoculated on differential or selective media, then the germ colonies are inoculated on biochemical test media. There are 12 types of tests that are often used in biochemical tests, although there are many more media that can be used.

2. SIM Test

Sulfide indole motility (SIM) is a differential medium. This medium is used to test the ability of organisms to determine hydrogen sulfide (H2S) production, indole formation, and motility. The ability of bacteria to convert the amino acid tryptophan into indole. Bacteria have the enzyme tryptophanase to produce indole, indole (+) comes from the automeric group (Anurogo et al., 2024).

The steps used in carrying out the SIM test are, Inoculate the culture on the SIM media by inserting it with a needle into the media 3/4 of the way deep. Then incubation was carried out at a temperature of 37° C for 24 h. After incubation for 24 hours, interpret the results obtained.

3. TSIA Test

The TSIA test aims to identify bacteria that come from the Enterobacteriaceae class. This test is also usually used to differentiate between gram-negative ones that are capable of catabolizing glucose, lactose, sucrose, and those that are capable of liberating H2S or not. The working principle of this test is to detect bacteria that can ferment lactose, sucrose and glucose. Detect bacteria that produce gas and H2S.

The steps used in using the TSIA test are inoculating the sample on TSIA media by means of puncture inoculation then continuing by spreading it straight upright on slanted agar then incubating the media at a temperature of 37° C for 24 h. Interpretation of positive results on the TSIA test is:

- Carbohydrate positive: the medium turns yellow
- Positive gas: the media contains air bubbles

Positive H2S: there is black sediment at the bottom of the tube

4. Methyl Red Test

The methyl red test is a test that aims to confirm whether the microorganisms that have been found have the ability to produce and maintain the final product. Where the final product in the form of acid is obtained through the glucose oxidation process. Where positive results are indicated by a change in the top of the media to red after 3-5 drops of 1% methyl red reagent are added (Puspadewi et al., 2017).

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The steps used in carrying out the Methyl Red test are the tube is heated until it is red hot then allowed to cool. After that, one tube of isolate from the bacteria is taken and then attached to the wall of the MR-VP Broth Base media tube, then incubated for 24 hours at a temperature of 37° C. After incubation, drop 5 drops of Methyl Red reagent through the tube wall. The interpretation of positive results on the TSIA test is:

a. Positive: red color on the media after dropping the methyl red reagent
b. Negative: yellow color on the media after dropping the methy red reagent

5. Voges Proskauer Test

The Voges Proskauer (VP) test is useful for understand the condensation reaction between diacethyl. Positive results show a color change from yellow to red and the result is negative characterized by no color change red on the tube (Hayati et al., 2019).

The steps used in carrying out the Voges Proskauer test are, heat the tube until it is red hot, then leave it to cool, then one bacterial isolate is taken and placed on the wall of the tube on the MR-VP Broth Base medium, then incubated for 24 hours at a temperature of 37 degrees Celsius, after carrying out Incubate, add 0.6 ml of alpha napthol and continue with the addition of 6.2 ml of 40% KOH reagent then homogenize. The interpretation of the results of the VP test is:

- Positive: red color on the media after adding VP reagent
- Negative: there is no color change in the media after the VP reagent is dropped

6. Carbohydrate Fermentation Test

This test is helpful in the identification of bacteria that can ferment carbohydrate or those cannot ferment the carbohydrate. This test is based on the principle of acid or gas production. The type of media used for this test glucose, sucrose, lactose, mannitol, and maltose (Hemraj et al., 2013).

The steps taken in the Carbohydrate Fermentation Test are, 1 bacterial isolate is inoculated on each sugar medium (glucose, lactose, sucrose, mannitol, maltose, sucrose) then incubated at 37° C for 24 hours. After incubation, interpret the results obtained. Interpretation of the results of the Carbohydrate Fermentation Test:

- Positive : Color change, indicating acid production. If there is a Durham tube (a small tube inside the test tube), gas bubbles indicate gas production.
- Negative : No color change, indicating the absence of acid fermentation. If there are no gas bubbles in the Durham tube, this means there is no gas production.

Data analysis techniques

This study was conducted using descriptive method with bacterial species identification as a result for each river.

RESULTS AND DISCUSSION

Samples that have been grown on SSA media and show the growth of black, pink, and colorless colonies are continued with the carbohydrate fermentation test. The results of the carbohydrate fermentation test can be seen in Table 4 and Fig. 2.

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TABLE 3. Isolation Result, Gram Staining and Embedding on SSA Based on Bergeys Manual Determinative Bacteriology Book (Bergey & Holt, 1994)

Sample	Colony	Gram staining	Bacterial shape	Identification
Normal faeces	Circle	Negative	Cocobacil	*Enterobacter* sp.
Liquid faeces	Circle	Negative	Cocobacil	*Serratia* sp.
Kiai Haji Abu Bakar street river	Circle	Negative	Cocobacil	*Salmonella* sp.
Rawalumbu river	Circle	Negative	Cocobacil	*Serratia* sp.
Kalimalang river	Circle	Negative	Cocobacil	*Serratia* sp.
Bumi palapa river	Circle	Negative	Cocobacil	*E. coli*
Bekasi river	Circle	Negative	Cocobacil	*Enterobacter* sp.

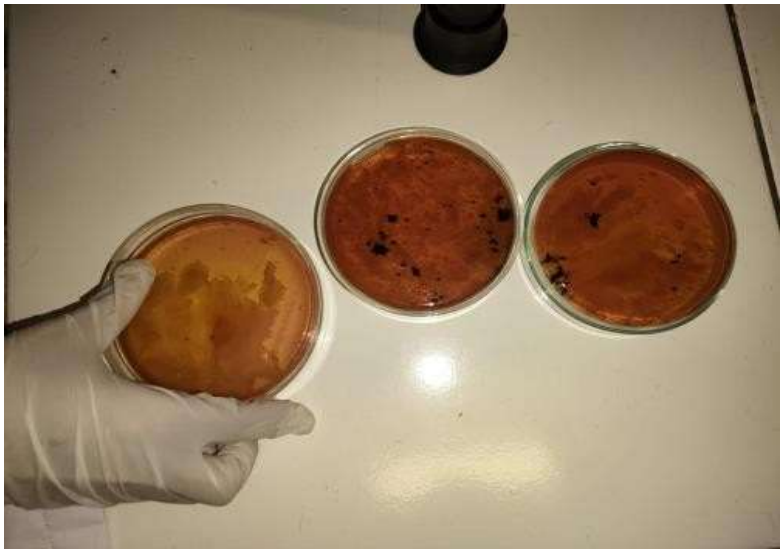

FIGURE 2. Bacteria grown in SSA medium while samples isolation

TABLE 4. Carbohydrate Fermentation Result

Sample	Lactose	Glucose	Mannitol	Sucrose	Maltose
Normal faeces	Positive	Positive	Positive	Positive	Positive
Liquid faeces	Positive	Negative	Positive	Negative	Positive
Kiai Haji Abu Bakar street river	Negative	Negative	Positive	Negative	Negative
Rawalumbu river	Positive	Positive	Positive	Negative	Negative
Kalimalang river	Positive	Positive	Positive	Negative	Positive
Bumi palapa river	Positive	Positive	Negative	Positive	Positive
Bekasi river	Positive	Positive	Positive	Positive	Positive

Samples that have been grown on carbohydrate fermentation test media and show colony growth characterized by the presence of gas and turbidity are continued with biochemical tests. Biochemical test results can be seen in the following table (Table 5)(Fig. 3 & 4).

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TABLE 5. Biochemical Test Result

Sample	SIM	TSIA	Methyl Red	Voges Proskauer
Normal Faeces	H_2S Negative Indole Negative Motility Positif	Slant Yellow Butt Yellow Gas Positive H_2S Negative	Positive	Positive
Liquid Faeces	H_2S Negative Indole Negative Motility Positive	Slant Yellow Butt Yellow Gas Negative H_2S Negative	Positive	Negative
Kiai Haji Abu Bakar street river	H_2S Positive Indole Negative Motility Positive	Slant Red Butt Red Gas Negative H_2S Positive	Positive	Negative
Rawalumbu River	H_2S Negative Indole Negative Motility Positive	Slant Yellow Butt Yellow Gas Positive H_2S Negative	Positive	Negative
Kalimalang River	H_2S Negative Indole Negative Motility Positive	Slant Yellow Butt Yellow Gas Positive H_2S Negative	Positive	Negative
Bumi Palapa River	H_2S Negative Indole Positive Motillty Positive	Slant Yellow Butt Yellow Gas Positive H_2S Negative	Positive	Negative
Bekasi River	H_2S Negative Indole Negative Motility Positive	Slant Yellow Butt Yelllow Gas Positive H_2S Negative	Positive	Positive

FIGURE 3. Sugar fermentation results

FIGURE 4. SIM, TSIA, MR, and VP results

River is an important water source, in particular in the big city. River contaminated by bacteria can cause disease such as many symptoms related to foodborne and

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waterborne disease. Several bacterial species are harmfull or can not be tolerated in the river, such as *Salmonella* and *E. coli.* The result of this study showed that from seven rivers in Bekasi, two of them contained *Salmonella* sp. and *E. coli,* identified from Kiai Haji Abu Bakar street River and Bumi Palapa River, respectively. Bacteria from Kiai Haji Abu Bakar street River showed Gram-negative (grown in Salmonella-Shigella agar), lactose fermentation negative (table 2), indole test negative, motility positive, and H2S production positive (table 3). This species identification as described in Bergeys Determinative Bacterial Identification.

Bacteria based on their cell wall components are divided into 2 groups, namely Gram- positive bacteria and Gram-negative bacteria. To differentiate between the two groups of bacteria you can use Gram staining. In Gram staining, a primary dye (crystal violet), mordant (iodine/lugol), alcohol and then a counter dye (safranin) are used. The complex red colored Gram-negative bacteria dissolve when given a bleaching solution and then take on a second red color. Gram-positive bacteria are purple in color because when staining the violet-iodine crystals are retained even though they are given a whitening solution (alcohol). Gram-positive bacteria have a thicker peptidoglycan composition compared to Gram-negative bacteria and contain teichoic acid, whereas in Gram- negative bacteria the cell wall consists of lipopolysaccharides and does not contain teichoic acid (Sylvia & Demas, 2018).

Salmonella media, Shigella Agar (SSA) is a selective medium for *Salmonella* sp bacteria so that the growth of non-Salmonella bacteria can be inhibited. Used for the isolation of *Salmonella* and many *Shigella* species (i.e. non-lactose-fermenting enteric bacteria) from lactose-fermenting enteric (coliforms). *Shigella* isolation is no longer recommended because Hektoen agar and XLD are more effective, but it is still used to isolate *Salmonella* species. The principle of the SSA medium is that it is an undefined, differential, and selective medium with bile salts and brilliant green dye acting as selective agents against gram positives and many Gram negatives. Lactose is a fermentable carbohydrate and sodium thiosulfate provides a source of reducible sulfur. Neutral red is a pH indicator and iron citrate react with HS to form a black precipitate, thus indicating sulfur reduction. Lactose fermenters will produce reddish colonies because the neutral red color changes from colorless to red at low pH. *Salmonella* and *Proteus* species usually reduce sulfur which is characterized by colonies with black centers (Bentum et al., 2025).

The Methyl Red test aims to identify the ability of bacteria to produce stable acidic end products through mixed acid fermentation of glucose. The principle of the MR-VP test is a combination medium used for testing methylene red and Voges Proskauer. This is a simple solution containing peptone, glucose, and phosphate buffer. Peptone and glucose provide fermentable proteins and carbohydrates and potassium phosphate resists pH changes in the medium. The Proskauer Voges test is designed for organisms capable of fermenting glucose but rapidly converting the acid products to acetoin and 2,3 butanediol. Adding VP reagent to the medium after incubation will oxidize acetoin (if present) to diacetyl, which then reacts with the guanidine core of the peptone to produce a red color. Therefore, positive VP produces a red color, there is no color change (or development of copper color) after adding the negative reagent. The copper color is the

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result of interactions between different reagents with the actual red color in positive results, using positive and negative controls as a comparison is usually recommended (Ali et al., 2022).

The Voges Proskauer test has a principle the reaction of acetoin from bacteria with KoH. Acetoin oxidizing diacetyl is a reaction catalyzed by alpha-naphthol. The diacetyl formed will react with compounds containing guanidine such as arginine contributed by Repton in the media to form a red product as a positive result of the VP test (Patil & Pradhan, 2014). Bacteria that react positively in the VP test are *Klebsiella, Serratia marcescens*, *Enterobacter*, *Vibrio*, while negative VP are *Escherichia coli*, *Shigella*, *Salmonell*a bacteria (Krieg et al., 2010).

The TSIA (Triple Sugar-iron Agar) test is used to differentiate between different groups within the *Enterobacteriaceae* and all of them are Gram negative bacillus bacteria which can ferment glucose and accompanied by acid formation to differentiate *Enterobacteriaceae* from other Gram-negative intestinal bacilli. TSIA slant agar contains lactose and sucrose with a concentration of 1% and glucose has a concentration of 0.1% to detect the use of these substrates only. The red renol acid-base indicator is added to the medium to identify carbohydrate fermentation which is characterized by a change in the color of the medium from red orange to yellow due to the formation of acid. The agar slants are inoculated using a puncture-scratch method (Cappuccino & Welsh, 2018).

The SIM (Sulfide Indole Motility) test is a differential bacterial growth medium that is useful for identifying 3 different characteristics of organism. S is sulfur reduction and H2S is sulfide production, I is indole and M is motility. The principle of the SIM test is that the test organism is inoculated into a semi-solid medium, namely SIM agar medium and tested for the production of hydrogen sulfide, indole and bacterial motility. Bacteria that produce hydrogen sulfide will produce H2S which then reacts with iron salts, iron sulfate and iron ammonium citrate from the SIM, producing a black precipitate. Bacteria that produce indole will break down the tryptophan in SIM agar with the tryptophanase enzyme and produce indole. Indole production will be detected with Kovac/Ehrlich reagent containing 4 (P)-dimethylamino-benzaldehyde. If it reacts with indole, it will show the red compound rosindole dye. Non-motile bacteria will grow in the area around the puncture while motile bacteria will grow spread throughout the tube (Saimin et al., 2020).

The sugar fermentation test or what is also called the carbohydrate test is used to see the ability of bacteria to ferment carbohydrates into organic acids, gas and alcohol. The medium used is a liquid medium with the addition of carbohydrates such as glucose, lactose, mannose, maltose and sucrose at a content of 1% and added with bromotymol blue or phenol red as a pH indicator. Color changes will occur in the media if the bacteria being tested could ferment a carbohydrate or are accompanied by the presence of gas produced (Asril et al., 2023; Penna et al., 2002).

CONCLUSIONS

Biochemical test result and comparing it with Bergey's determinative, the results identified a pathogen suspected to be *Salmonell*a sp. in samples from Kiai Haji Abu Bakar street river. Further study is needed to confirm the molecular identification of this isolate.

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AUTHOR CONTRIBUTIONS

NAI, MI.: project conception; NAI, MI, LPE, SDA.: methodology; NAI, MI.: data analyses; NAI.: original manuscript draft; NAI, MI.: manuscript review and editing.

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CONFLICTS OF INTEREST STATEMENT

There are no conflicts to declare.

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