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Identification of *Escherichia coli* and *Salmonella* sp. bacteria on dishwashing sponges based on length of use

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Abstract
Background: Sponges are tools used to clean cutlery. Dishwashing sponges are the main source of bacterial cross-contamination because they can transmit foodborne pathogens. Sponges that are used for a long time will have more potential to grow various microorganisms, such as bacteria from the *Enterobacteriaceae* family, including *Escherichia coli* and *Salmonella* sp. *Objectives*: The purpose of this study was to identify *Escherichia coli*, *Salmonella* sp..and the proper duration of use on sponges to avoid bacterial contamination. *Materials and Methods*: The type of research is descriptive, with a Quota sampling technique with a sample size of 6 dishwashing sponges at 6 points in the house (2 samples at one time), which have been determined on three indicators, namely 3,5,7 weeks of use. Observation and testing data collection methods. *Results*: The results showed negative *Esherichia coli* bacteria in all sponge samples with a duration of use of 3, 5, and 7 weeks, while positive *Salmonella* sp. bacteria in 1 sponge sample with a duration of use of 7 weeks. *Conclusions*: There were *Salmonella* sp. bacteria in 1 sample, namely a sponge used for 7 weeks and no *Escherichia coli* bacteria were found in all samples, all samples of dishwashing sponges with a usage period of 3, 5, and 7 weeks tested positive for bacterial contamination, so the minimum usage period for the sponge is 2 weeks, to avoid bacterial contamination from the dishwashing sponge.

Keywords
Sponge; bacteria; *Escherichia coli*; *Salmonella* sp.; Contamination

1. Introduction

Eating utensils are one of the factors that play a role in the transmission of disease. Eating utensils can be an intermediary medium for bacteria to move to food, this is one of the factors in the occurrence of foodborne diseases (Hanum and Annisa in Siregar et al., 2023).

Sponges are tools used to clean cutlery. Dishwashing sponges are a major source of contamination because they can cross-contaminate foodborne pathogens and microorganisms that are spoiling

food residues (Osaili et al., 2020). There is concern that sponges can spread pathogenic bacteria to kitchen surfaces and hands, posing a threat to consumers rather than a means of reducing cross-contamination of food or the mouth. The use of sponges and other cleaning equipment and tools varies from country to country. Sponges are commonly used for cleaning kitchens in most of the 10 European countries surveyed (Møretrø et al., 2021).

The habit of leaving dishwashing sponges in a sponge container filled with water for a long time, not washed and dried after use is not right because bacteria from food residue left behind from the sponge can grow in the water (Gusti et al, 2022). The water that comes into contact with the foam used for washing causes the sponge to be filled with bacteria. Using a sponge in this condition to wash dishes will cause the plate to become a nest for bacteria. Sponges that are used for a long time will have more potential to grow various types of microorganisms (Andini et al, 2021). Cases of poisoning can be caused by eating utensils contaminated with bacteria. Poor hygiene of eating utensils plays an important role in the growth and spread of germs (Noviarini, 2019).

One type of bacteria that is often found in the results of bacterial identification on eating utensils is *E. coli* and *Salmonella* sp.. Research Agustin et al (2019) with 10 dishwashing sponges at 10 food vendors at Margahayu Market, East Bekasi, bacteria of the species *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, and Proteus sp. were found.*

Based on research Andini et al (2021) stated that sponge samples with use of more than 4 weeks showed positive results for *E. coli* bacterial contamination so it was concluded that the safe time span for using sponges to be replaced is 4 weeks. Based on the research Anita et al (2021) stated that with a total of 5 samples of used dishwashing sponges soaked for 3 days, all of them contained *Salmonella* sp. bacteria.

Based on research Siregar et al (2023) stated that samples with more than 4 weeks showed positive results for *E. coli* so that from the study it was concluded that the safe time for using sponges to be replaced was 4 weeks of use. Based on the study Gusti et al (2022) it can be concluded that kitchen sponges that are washed and dried after use have a lower germ content compared to kitchen sponges that are not washed and dried after use. The third day of use showed results that exceeded the specified threshold, namely more than 100 colonies.

Enterobacteriaceae is a family of gram-negative, facultative anaerobic rod-shaped bacteria that are the cause of most food-borne illnesses. This family includes many harmless bacteria that make up the flora of the body and the environment but also includes pathogens such as *Salmonella, E. coli, Yersinia pestis, Klebsiella, Shigella, Proteus, Enterobacter, Serratia and Citrobacter*. The type genus is Escherichia (AraSains, 2021).

Escherichia coli or *E. coli* is a bacteria found in the environment, food, human intestines, and

animals. Types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, through contact with animals or humans. In addition, *Salmonella* sp. is also one of the main causes of acute diarrheal disease even though preventive measures have been implemented. The clinical picture of salmonellosis varies from common gastroenteritis to enteric fever which is a life-threatening disease (Popa and Popa, 2021).

Based on the description above, this study aims to identify *E. coli* and *Salmonella* sp. bacteria on dishwashing sponges based on the duration of use. The data obtained in this study can be used for additional information and education to the public regarding the importance of maintaining the cleanliness of food utensils and the duration of use of dishwashing sponges.

2. Materials and Methods

2.1. Type and Design of Research

The type of research in this study is descriptive research. The design used is a qualitative design. This research was conducted in Karangbale Village RT 04/RW 04, Larangan District, Brebes Regency in April-June 2024.

2.2. Population and Sample

The population in this study was the dishwashing sponge that will be placed at the house point of Karangbale Village RT 04/RW 04. The sample in this study was obtained using Quota sampling, 6 samples of dishwashing sponges at 6 house points (2 samples at one time/indicator) which have been determined on three indicators, namely the use of 3,5,7 weeks.

2.3. Method of collecting data

The method used in this research is observation. The data in this study are primary data obtained directly from the results of tests carried out in duplicate, identification of bacteria on household dishwashing sponges based on the duration of use in Karangbale Village RT 04/RW 04. Data on the identification of *E. coli* and *Salmonella* sp. bacteria were obtained by testing methods.

2.4. Research Procedures

The tools used are petri dishes, tubes, round loops, needle loops, spirits, Erlenmeyer flasks, autoclaves, incubators, pipettes, microscopes, glass objects, measuring cups, scales, stirring rods, cotton covers. The materials used are MCA media (Mac Conkey Agar), TSIA media, SIM, Simmon citrate and Ehrlich reaction reagents, aquadest, 0.5% crystal violet, lugol, 96% alcohol, 0.5% fuchsin water, immersion oil, sponge samples.

1. Preparation of used sponges

   Each sponge is placed in 6 households that have been determined for a certain period of use, each 2 sponges in one usage period (3, 5, 7 weeks). After reaching the usage limit, samples of the remaining sponges are taken and taken to the Laboratory for identification.

2. Culture and identification

   Sterilization Sterilization of tools and materials using an autoclave at a temperature of 121℃ for 15 minutes. Media creation *MacConkey agar (MCA)* Weigh 6 grams of MacConkey media powder, and dissolve with 120 ml of distilled water. Put into an Erlenmeyer flask. Heat until boiling to dissolve the media (indicated by no media granules still sticking to the Erlenmeyer). After being heated, the pH of the media was measured at 7.4 ± 0.2 at a temperature of 250℃. If the pH is not suitable and is too alkaline, then 0.1 N HCl solution is added. If it is too acidic, 0.1 N NaOH is added, and the aim is for the bacteria to grow. The Erlenmeyer flask is closed with cotton. Sterilize in an autoclave at 121℃ for 15 minutes. Wait for the temperature to warm up (45-50℃). Homogenize the media. The media is poured into the plate at a temperature of 25℃ or room temperature. Each pouring is done while fixing the Erlenmeyer flask mouth aseptically. The media is poured into a 15-25 ml plate with a thickness of 1.5 cm. The goal is for the bacteria to grow to get enough nutrition and to facilitate the observation process. After the media solidifies the pink MCA media, the media is stored in an inverted position so that the remaining water vapor on the plate lid does not fall onto the surface of the media and cause contamination. The media is put into the refrigerator or can be used immediately. Isolation of positive results of *E. coli* colonies on Mac conkey agar media, dry colonies are round donut-shaped, dark pink surrounded by a cloudy zone (area of dark pink bile salt deposits), 2 mm in size, flat edges, convex elevation, soft concentration, and lactose fermentation. While *Salmonella* sp. colonies on MCA media are colorless, clear, medium, convex, smooth, round, flat and non-lactose fermentation.

   Biochemistry: TSIA (Triple Sugar Iron Agar). The TSIA media used was weighed, which was 3.9 g in 60 ml of distilled water. The media was poured into an Erlenmeyer flask, homogenized, and then heated until dissolved. Poured into a test tube and sterilized again in an autoclave for 15 minutes. After sterilization, the media was removed from the autoclave and left in a half-tilted position until solidified. SIM (Sulfode Indole Motility) The media used was weighed as much as 1.8 g in 60 ml of distilled water. The media was poured into an Erlenmeyer flask, homogenized and then heated until dissolved. Poured into a test tube and sterilized again in an autoclave for 15 minutes. After sterilization, the media was removed from the autoclave and left in an upright position.

   SC (Simon Citrate) Weighed 1.35 g of media in 60 ml. The media was poured into an Erlenmeyer flask, homogenized, then heated until dissolved. The media was poured into a test tube and

sterilized again in an autoclave for 15 minutes. After sterilization, the media was removed from the autoclave and left in a very tilted position until solidified.

a. Planting in the media using the streak plate technique (scratch plate) T scratches

Mark the outside bottom of the petri dish by dividing the dish into 3 sections, forming a T shape. Heat the ose until it glows over the Bunsen burner, then distance it from the Bunsen burner and let it cool. Use a cold loop to take a pure bacterial culture (take 1 loop) for each sponge. Scratching on the surface of the medium to start from one end on part 1 with a zig-zag streak. The ose that is touched on the surface of the medium should not be pressed too deeply. Reheat the ose over the Bunsen burner and let it cool. Turn the cup to face part 2 and make zig-zag strokes by connecting from the tail of stroke 1. Do the same on part 3. Incubate the petri dish containing the microbes in an inverted position at room temperature 37℃ in an incubator for 24 hours and observe the growth of the bacterial colonies.

b. Gram staining

Sterilize the object glass over a Bunsen burner, add 1 drop of distilled water to the object glass using a sterile ose. Take 1 loop of bacterial colony on the media, then place it on the object glass in a circle. Fixation over the Bunsen flame. Flood the preparation with 0.5% Gentian Violet, leave for 1 minute, and rinse with running water. Pour Lugol and let it sit for 1 minute, then throw away the paint. Dilute with 96% alcohol until no dye is dissolved, then rinse with water. Pour 0.5% fuchsin water for 1 minute, then wash with running water and dry it. Observe with a microscope with 1000x magnification, namely a 100x objective lens with added immersion oil and a 10x ocular lens. Positive results of the morphology of long rod-shaped bacteria, red in color and gram-negative, are *Salmonella* sp. bacteria, while coccobacillus-shaped bacteria (short rods), red in color and gram-negative, are *E. coli* bacteria [20].

c. Biochemical test

TSIA (Triple Sugar Iron Agar): Bacteria are taken on selective media with a sterile needle loop and inserted into the vertical part of the medium then lifted and scratched in a zigzag manner on the surface of the slanted part of the medium and incubated for 24 hours at 37℃. Observed acid slant, acid butt which is marked by a change in the color of the media, cracks in the media or the media will be lifted to the end of the tube and H_2S gas production will be seen if the media turns black.

SIM (Sulfode Indole Motility): To determine the nature of bacteria in producing H_2S, indole and the movement of bacteria (motility). How it works, bacteria are taken on selective media with a sterile needle loop then pricked perpendicularly on the media and incubated at 37℃ for 24 hours. H_2S production is marked by black media, indole production can be seen after being dripped with 0.5 ml of erlich reagent on the tube wall so that a line is visible separating the media from the reagent. If indole is positive, a red ring forms on the surface of the media,

motility can be seen if there is a blurring of the media at the puncture site of the loop.

SC (Simon Citrate): Bacteria are taken from selective media with a sterile loop and then rubbed in a zigzag pattern on the surface of the media from the base to the other end. The media is incubated at 37℃ for 24 hours. Positive results are indicated by a change in color on the media from green to blue, which means that the bacteria are able to utilize citrate as a carbon source.

2.5. Data Analysis

The data analysis used in this study is qualitative descriptive analysis. Data analysis in this study will be presented in the form of tables and figures.

3. Results and Discussion

3.1. Isolation results on Mac Conkey Agar media

Sponge samples 3, 5 and 7 weeks on mediaMac Conkey incubated for 24 hours at 37℃ can be seen in table and figure 1.

Table 1. Results of bacterial colony identification on Mac Conkey media

Sample code	Identification results		Information
	E. coli	*Salmonella* sp.	
A1	+	+	There was growth of *E. coli* and *Salmonella* sp. colonies.
A2	-	+	Only *Salmonella* sp. colonies grew.
B1	+	+	There was growth of *E. coli* and *Salmonella* sp. colonies.
B2	+	+	There was growth of *E. coli* and *Salmonella* sp. colonies.
C1	+	+	There was growth of E. coli and *Salmonella* sp. colonies.
C2	-	+	Only *Salmonella* sp. colonies grew.

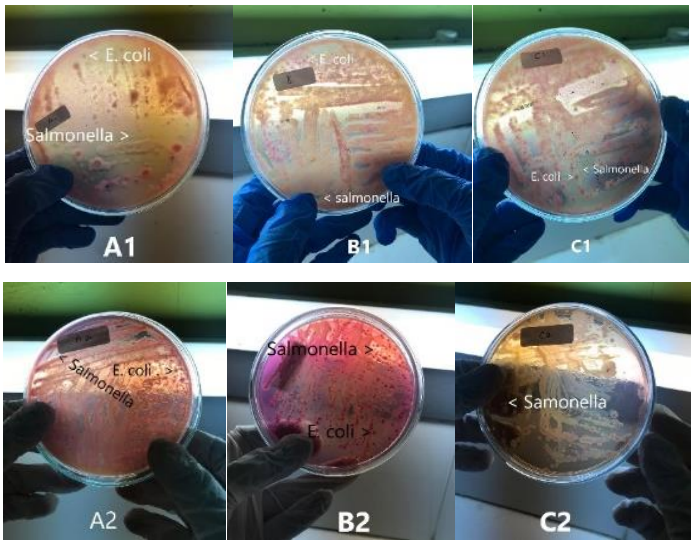

Figure 1. bacterial colonies on MacConkey agar media

Based on the image and table above, colonies suspected of being *E. coli* grew in 4 samples with codes A1, B1, B2, C1, the colonies appeared dry, round, convex, medium, dark pink surrounded by dark pink, smooth with even edges, *Escherichia coli* bacteria that can ferment lactose produce dark pink colonies surrounded by a cloudy zone (area of dark pink bile salt deposits).

The results are by the statementAbu-sini et al (2023);Susanti and M. Agung (2022), that the characteristics of *E. coli* colonies growing on MacConkey media are dry, round, donut-shaped colonies, dark pink surrounded by a cloudy zone (area of dark pink bile salt deposits), 2 mm in size, flat edges, convex elevation, soft concentration, and lactose fermentation. Meanwhile, according to research references Sinaga et al (2021) growth of *Salmonella* sp. bacteria on Macconkey agar media, colonies are colorless, clear, medium, convex, smooth, round, flat, and do not ferment lactose.

Colonies suspected of Salmonella sp. grew in all samples A1, A2, B1, B2, CI, C2 in a round, colorless, convex, smooth shape with flat edges; the media turned yellow because it did not ferment lactose. This is by the reference statement Sinaga et al (2021). Based on these results, all samples suspected of being positive for *E. coli* and *Salmonella* sp. will be continued with Gram staining.

3.2. Results of gram staining observations

Colonies taken from MacConkey media were stained with Gram to determine the morphological characteristics of bacteria. The results of the observations are as seen in the table and figure 2.

Table 2. Results of gram staining observations

Sample code	Observation result				Information
	Form		Color		
	E. coli	*Salmonella*	*E. coli*	*Salmonella*	
A1	cocobacilli	basil	red	red	Gram negative
A2	-	cocobacilli	Red	red	Gram negative
B1	cocobacilli	basil	Red	red	Gram negative
B2	Basil	basil	Red	red	Gram negative
C1	cocobacilli	basil	Red	red	Gram negative
C2	-	basil	Red	red	Gram negative

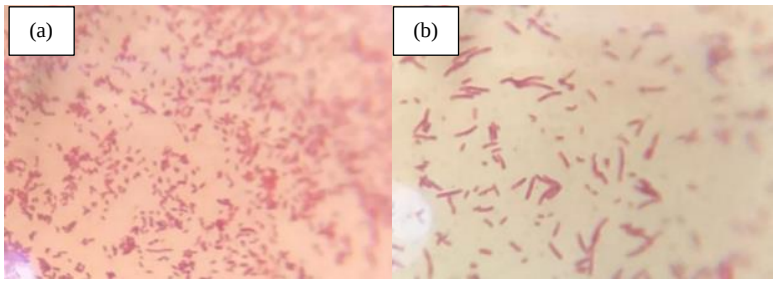

Figure 2. (a) short rods/cocobacilli (E. coli) and (b) long rods/bacilli (Salmonella sp.)

Based on the results of gram staining observations under a microscope with a magnification of 100x, it was found that all bacteria were gram-negative bacteria, because the bacteria appeared red. Furthermore, from a total of 4 samples suspected of being *E. coli*, only 3 samples (A1, B1, C1) were short rod-shaped (cocobacilli), these samples were declared positive for *E. coli* species bacteria. While 1 other sample (B2) was declared negative, because it was long rod-shaped (bacilli), this did not match the morphological characteristics of *E. coli*.

According to Lisdewi et al (2023) The morphological characteristics of *E. coli* bacteria are short rod-shaped/cocobacilli, and red in color. The red color is produced because the cell walls of gram-negative bacteria absorb the second dye, namely safranin, while according toPrincess (2016) in gram-negative *Salmonella* sp. bacteria, the morphology is long rod-shaped/bacillus, red in color.

Furthermore, from a total of 6 samples suspected of *Salmonella* sp., only 5 samples (A1, B1, B2, C1, C2) in the form of long rods (bacilli) were declared positive, this is in accordance with the reference. While 1 sample (A2) in the form of short rods (cocobacilli) was declared negative, because it did not match the morphological characteristics of *Salmonella* based on the reference. Samples suspected of being positive for *E. coli* and *Salmonella* bacteria will be continued to biochemical tests, while those that are negative will not be continued.

3.3. Biochemical test results

Biochemical tests are advanced tests to identify isolated bacterial cultures through their physiological properties. By means of colonies from Mac conkey media inoculated into several specific biochemical media, such as TSIA, SIM, Simmon citrate and incubated at 37℃, Ehrlich reagent is added to the SIM media to determine whether or not there is indole production. The results are as seen in table and figure 3.

Table 3: Results of biochemical test inoculation

Sample code	Biochemical test						Information
	TSIA			SIM		SC	
	Slope/base	H_2S	Gas	Indo	Mot		
A1c	A/A	+	+	-	+	+	Not E.coli
A1b	A/A	+	+	+	-	+	Not *Salmonella* sp.
B1c	A/A	+	+	+	-	+	Not E.coli
B1b	K/K	-	-	+	+	+	Not *Salmonella* sp.
B2b	A/A	-	+	-	+	+	Not *Salmonella* sp.
C1c	A/A	+	-	+	+	+	Not E.coli
C1b	K/A	+	+	-	+	+	*Salmonella*sp.
C2b	K/K	-	-	-	+	+	Not *Salmonella* sp.

*Information : c : cocobacilli (*E. coli*); b : basil (*Salmonella sp.*)

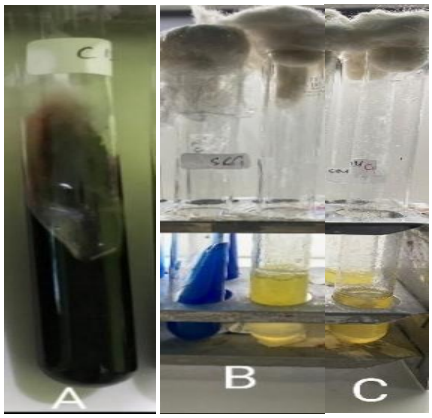

Figure 3: (a) TSIA K/A H_2S gas, (b) citrate positive and indole negative (motile), (c) indole positive (motile)

According to the reference Khakim and Rini (2018); Harsono et al (2015); Abu-sini et al (2023), in the TSIA test, *Escherichia coli* bacteria are able to ferment acidic compounds such as glucose and lactose/sucrose, indicated by the slope and base of the yellow media that are acidic (A/A), producing gas in the form of cavities/cracks in the media, and negative H_2S. These bacteria can produce indole, indicated by the presence of a red ring on the surface of the media. Have movement/motility, because *E. coli* has a flagellum as a means of locomotion. Negative citrate, meaning that these bacteria do not use citrate as a carbon source (indicated by the media remaining green).

Based on the results of the biochemical test, the samples suspected of being *Escherichia coli*, namely samples A1c, B1c, and C1c, were declared negative, this was due to a discrepancy between the test results and the existing references. Sample A1c was declared negative because the TSIA test produced H_2S in the form of black sulfide in the media, this did not match the research reference because *E. coli* bacteria do not produce H_2S.

In the negative indole SIM test, this is not in accordance with the reference because *E. coli* positive indole is marked by a red ring on the surface of the media, meaning that the bacteria can form indole from tryptophan acid as an energy source. In the positive citrate Simmon test, it is marked by a change from green to blue, this is not in accordance with the reference because *E. coli* bacteria are citrate negative, meaning that these bacteria do not use citrate as a carbon source (marked by the media remaining green).

Sample B1c was declared negative because the TSIA test produced H_2S in the form of black sulfide in the media, this is not in accordance with the reference because *E. coli* bacteria do not produce H_2S, this is not in accordance with the research reference because *E. coli* bacteria do not produce H_2S. In the SIM test there was no movement/motility, this is not in accordance with the reference because in *E. coli* bacteria there is movement/motility indicated by a cloudy color like fog along the puncture mark of the loop. In the positive citrate Simmon test, it is indicated by a change from green to blue, this is not in accordance with the reference because *E. coli* bacteria are citrate negative, meaning that these bacteria do not use citrate as a carbon source (indicated by the media remaining green).

Sample C1c was declared negative because the TSIA test produced H_2S in the form of black sulfide in the media, did not produce gas, this is not in accordance with the reference because *E. coli* bacteria do not produce H_2S. In the Simmon citrate test, all suspected *E. coli* samples (A1c, B1c, C1c) showed positive citrate results marked by changing from green to blue, this is not in accordance with the research reference above because *E. coli* bacteria are citrate negative, meaning that these bacteria do not use citrate as a carbon source (marked by the media remaining green).

According to Khakim and Rini (2018) *Salmonella* sp. bacteria in the TSIA, SIM and Simmon citrate tests, in the TSIA test these bacteria are able to ferment glucose, indicated by the media slope changing to red indicating alkaline properties (K), the base of the yellow media indicating that the glucose compound is acidic (A), produces H_2S which is black in color, and is able to produce gas so that the media forms bubbles like empty cavities/cracks. Nocan produce indole (the surface of the media is yellow), meaning that the bacteria cannot form indole from tryptophan acid as an energy source. There is movement/motility, because *Salmonella* sp. has a flagellum

as a means of locomotion, marked by a cloudy color like fog along the puncture marks of the loop. Using citrate as a carbon source (the media changes from green to blue).

Furthermore, from a total of 6 samples suspected of *Salmonella* sp., only 1 sample, namely sample C1b, was declared positive because it was in accordance with the reference. While the other samples, namely A1b, B1b, B2b, C2b, were declared negative because they did not match the biochemical properties of *Salmonella* sp. based on the reference.

Sample C1b was declared positive, the results as shown in Figure IV-3 (a, b). Because in the TSIA test, this bacteria is able to ferment glucose (K/A), indicated by the media slope changing to red indicating alkaline properties (K), the base of the yellow media indicating that the glucose compound is acidic (A). Produces H_2S in the form of black sulfide in the media, and produces gas indicated by cracks/cavities at the base of the media. The SIM test showed that the results did not produce indole (the surface of the media was yellow), meaning that the bacteria could not form indole from tryptophan acid as an energy source. The presence of movement/motility is indicated by a cloudy color like fog along the puncture marks of the loop. Using citrate as a carbon source (the media changes from green to blue due to increased pH). This is in accordance with existing references.

Sample A1b was declared negative because in the TSIA test it was able to ferment acidic compounds such as glucose and lactose/sucrose, indicated by the slope and base of the yellow media which are acidic (A/A), this is not in accordance with the reference because *Salmonella* bacteria are only able to ferment glucose, indicated by the media slope turning red indicating alkaline properties (K), the base of the yellow media indicates that the glucose compound is acidic (A). In the SIM test it can produce indole/positive indole (the presence of a red ring on the surface of the media), meaning that bacteria can form indole from tryptophan acid as an energy source, this is not in accordance with the reference because *Salmonella* bacteria cannot produce indole (the surface of the media is yellow), meaning that bacteria cannot form indole from tryptophan acid as an energy source.

Sample B1b was declared negative because in the TSIA test, it did not ferment sugar as indicated by the red slope and base (buut) which are alkaline (K/K), and did not produce H_2S and gas. This is not in accordance with the reference because *Salmonella* bacteria are able to ferment glucose, indicated by the media slope turning red indicating alkaline properties (K), the yellow media base indicating that the glucose compound is acidic (A), producing H_2S in the form of black sulfide in the media and producing gas in the form of cracks/cavities in the media. The SIM test showed that the results can produce indole (the presence of a red ring on the surface of the media, and no movement/motility. This is not in accordance with the reference because *Salmonella* bacteria

cannot produce indole (the surface of the media is yellow), meaning that bacteria cannot form indole from tryptophan acid as an energy source and the presence of movement/motility is indicated by a cloudy color like fog along the puncture marks of the ose.

Sample B2b was declared negative because in the TSIA test it was able to ferment acidic compounds such as glucose and lactose/sucrose, indicated by the slope and base of the yellow media which were acidic (A/A), and did not produce H_2S. This is not in accordance with the reference because *Salmonella* bacteria are only able to ferment glucose, indicated by the media slope changing to red indicating alkaline properties (K), the base of the yellow media indicating that glucose compounds are acidic (A). This is not in accordance with the reference because *Salmonella* bacteria produce H_2S in the form of black sulfide in the media.

Sample C2b was declared negative because in the TSIA test, it did not ferment sugar as indicated by the red slope and base (buut) which are alkaline (K/K), and did not produce H_2S and gas. This is not in accordance with the reference because *Salmonella* bacteria are able to ferment glucose, indicated by the media slope turning red indicating alkaline properties (K), the yellow media base indicating that the glucose compound is acidic (A), producing H_2S in the form of black sulfide in the media and producing gas in the form of cracks/cavities in the media.

Other research related to biochemical tests to identify *Salmonella* sp. contamination was conducted by Geletu et al (2022) with stool samples, bulk milk, hand swabs, and floor swabs. Biochemical reactions TSIA K/A slant base (red), acid base (yellow), H_2S and gas production, citrate utilization as carbon source, indole negative. Based on the research results, it is known that all sponge samples were positive for bacterial contamination, both in sponges with a usage period of 3, 5, and 7 weeks. However, none of the samples were positive for E. coli contamination, and there was only 1 sample that was positive for *Salmonella* sp. contamination, namely sample C1b, which is a sponge with 7 weeks of use.

Salmonella infection generally does not cause complications. However, in some people such as pregnant women, toddlers, the elderly, and people with weak immune systems, it can cause complications. Complications that can occur include: dehydration, *Salmonella* infection can cause diarrhea even in severe cases. So that it can lead to dehydration, especially if the lost body fluids are not immediately replaced. Signs of dehydration can include decreased urine frequency, dry mouth and tongue, sunken eyes and reduced tear production. Bacteremia, *Salmonella* infection entering the bloodstream can infect tissues throughout the body. Including tissue around the brain and spinal cord (meningitis), the lining of the heart or heart valves (endocarditis), bones or bone marrow (osteomyelitis), blood vessel lining. Reactive arthritis, people with *Salmonella* infection are at higher risk of developing reactive arthritis or Reiter's

syndrome, which is arthritis caused by infection. Reactive arthritis usually causes eye irritation, pain when urinating, and causes pain in the joints (Fadli, 2019).

Based on other research, bacteria from the *Enterobacteriaceae* family that have been identified as being able to grow on dishwashing sponges are *Proteus* sp., *Enterobacter aerogenes, Pseudomonas*, and other bacteria such as *Staphylococcus aureus, Acinotebacter, Chryseobacterium, Enhydrobacter* (Moretro *et al.*, 2022).

4. Conclusions

In the study on 'The Relationship between C-Reactive Protein Levels and HbA1c Levels in Type II Diabetes Mellitus Patients,' the Chi-Square test results showed a p-value of 0.536. Because the Sig value > 0.05, this indicates that there is no significant relationship between C-Reactive Protein (CRP) levels and HbA1c levels in Type II Diabetes Mellitus patients.

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