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(RESEARCH ARTICLE)

Detection of Pathogenic bacteria (*Escherichia coli* and *Salmonella* sp.) in Banjar Mackerel (*Rastrelliger kanagurta*) in West Jakarta Traditional Markets

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Abstract

Banjar mackerel (*Rastrelliger kanagurta*) is a marine fish commodity widely consumed by the people of West Jakarta due to its high nutritional content and affordable price. However, fish sold in traditional markets are susceptible to contamination by pathogenic bacteria such as *Escherichia coli* and *Salmonella* sp. due to inadequate hygiene standards. This study aims to detect the presence of pathogenic bacteria *Escherichia coli* and *Salmonella* sp. in Banjar mackerel sold in traditional markets in West Jakarta. The method used includes random fish sampling at 5 locations in the traditional market with 5 repetitions at each location. Isolation and identification of bacteria using selective media, as well as biochemical tests to confirm the presence of *Escherichia coli* and *Salmonella* sp. The results of this study provide a clear picture that the safety and suitability of mackerel consumption in West Jakarta Traditional Markets are in accordance with SNI 2729:2021 concerning the safety and consumption of fresh fish. This research is important to support consumer health protection and maintain the quality of fishery products circulating in the community.

Keywords: Mackerel; *Escherichia coli*; *Salmonella* sp.; Food safety

1. Introduction

Banjar mackerel (*Rastrelliger kanagurta*) is one of Indonesia's marine fish commodities that is widely consumed by the public, especially the people of West Jakarta due to its high nutritional content such as protein, vitamins and minerals, omega-3, easy availability, and affordable price. Mackerel contains nutrients including energy of 103–824 kcal, protein 20.80%, fat 7.56%, calcium 0.76 ppm, and minerals 1.42–1.49% [1]. In addition, mackerel contains essential amino acids, namely lysine 12.65% and methionine 1.49% as well as non-essential amino acids, namely glutamic acid 11.20% and cysteine 0.20% [2]. Its abundant availability and relatively affordable price make Banjar mackerel (*Rastrelliger kanagurta*) popular, especially in traditional markets, as a source of animal food.

Traditional markets often have less adequate hygiene standards than modern markets. Fish sold in traditional markets is highly susceptible to microbial contamination, particularly the pathogenic bacteria *Escherichia coli* and *Salmonella* sp., due to poor handling, sanitation, and storage conditions, which often do not meet hygiene standards. These bacteria can survive in mackerel and can only be destroyed by cooking at specific temperatures. Based on research conducted by Yulianto et al., 2024, it was shown that mackerel which Fish sold in traditional markets often test positive for *E. coli* contamination in quantities exceeding the threshold set by the Indonesian National Standard, indicating the need for

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monitoring and early detection of the presence of pathogenic bacteria in consumed fish. Furthermore, detecting *Salmonella* sp. bacteria is also crucial to ensure food safety [3].

Pathogenic bacteria are bacteria that can cause disease in their hosts by altering tissue through genetic changes [4]. In addition to causing disease, pathogenic bacteria can also reduce and affect the quality and degrade fishery products. Some bacteria that frequently contaminate fishery products include *E. coli* and *Salmonella*.

The presence of pathogenic bacteria in food can harm consumer health. *E. coli* is a Gram-negative bacterium that is part of the normal flora of the human digestive tract. If its presence exceeds normal limits, this bacterium will become pathogenic and endanger human health. Pathogens in food are a major cause of food poisoning. *E. coli* can cause disease symptoms after invading the host and adapting to survive in the human body. It then attacks the immune system and ultimately causes disease [5]. *Salmonella* can cause diarrhea, salmonellosis, typhus fever, and other infectious diseases. The disease that occurs when humans are infected with *Salmonella* bacteria is typhus fever in humans which causes high fever with vomiting [6].

Fishery products, especially mackerel, consumed by the public must be safe and healthy, and free from contamination by pathogenic bacteria. This is a matter of concern to address the widespread circulation of low quality fishery products containing pathogenic bacteria that can cause poisoning [6]. Therefore, the purpose of this study was to determine the contamination of pathogenic bacteria (*Eschericia coli* and *Salmonella* sp.) in Banjar mackerel (*Rastrelliger kanagurta*) sold in traditional markets in Jakarta, particularly in West Jakarta.

2. Materials and Methods

The research was conducted from August to November 2025 at the Central Laboratory of Fishery Product Production, Inspection, and Certification (PPISHP) of the Jakarta Fisheries and Marine Affairs and Fisheries Department. The tools used included microbiological equipment such as glassware, Petri dishes, micropipettes, stomachers, incubators, autoclaves, and microscopes. The materials used included samples of Banjar mackerel and microbiological media and reagents for testing *Escherichia coli* and *Salmonella* sp.

Banjar mackerel samples were randomly collected from traditional markets in West Jakarta at five locations, with five replicates at each location. Fresh fish were stored in an ice box and transported to the laboratory for analysis. *E. coli* testing was performed using the 5-point method. SNI 2332.1:2015 [7] includes the stages of the presumptive test using Lauryl Tryptose Broth (LTB), the booster test using EC Broth, and the complementary test on Eosin Methylene Blue Agar (EMBA) media. Suspected isolates are then confirmed through the IMViC biochemical test. *Salmonella* testing is carried out using the SNI ISO 6579-1:2017 [8] method including the pre-enrichment stage using Buffered Peptone Water media. (BPW), selective enrichment using RVS and MKTTn, and isolation on selective media XLD and BSA. Identification was continued with biochemical tests and morphological observations. The data obtained will be analyzed descriptively and displayed in the form of tables and figures.

3. Results and Discussion

3.1. *Escherichia coli* Bacteria Identification Test

The results of observations from the identification test of *Escherichia coli* bacteria in mackerel in the traditional market of West Jakarta using the SNI 2332.1:2015 method are as follows.

Table 1 Results of *Eschericia coli* Bacteria Identification Test

Code	**Coliform**	***Eschericia coli***			**Conclusion**
	LTB	EC	LEMB	Biochemistry	
Control +	+	+	+	+	Positive 2 APM/g
Point Sampling 1	+++	---	---	---	Negative 1,8 APM/g
Point Sampling 2	++++	----	----	----	Negative 1,8 APM/g
Point Sampling 3	+++++	-----	-----	-----	Negative 1,8 APM/g

Point Sampling 4	+	-	-	-	Negative 1,8 APM/g
Point Sampling 5	++	--	--	--	Negative 1,8 APM/g

3.2. *Salmonella* sp. Bacteria Identification Test

The observation results from the identification test of *Salmonella* sp. bacteria in frozen salmon meat using SNI ISO 6579-1:2017 are as follows.

Table 2 Results of *Salmonella* sp. Identification Test

Code	**Pre-Enrichment**	**Enrichment**	***Salmonella* sp.**		**Conclusion**
			Selektive Agar	Biochemistry	
Control +	+	+	+	+	Positive
Point Sampling 1	+	+	+	-	Negative
Point Sampling 2	+	+	-	-	Negative
Point Sampling 3	+	+	+	-	Negative
Point Sampling 4	+	+	+	-	Negative
Point Sampling 5	++	++	++	--	Negative

Based on Table 1. The results of the *Escherichia coli* bacteria test show that the presence of *E. coli* in the Banjar mackerel samples from the West Jakarta Traditional Market, it still meets the standards set by the Indonesian National Standard (SNI 2332.1:2015) regarding the determination of coliform and E. coli in fishery products, namely below the maximum threshold of.

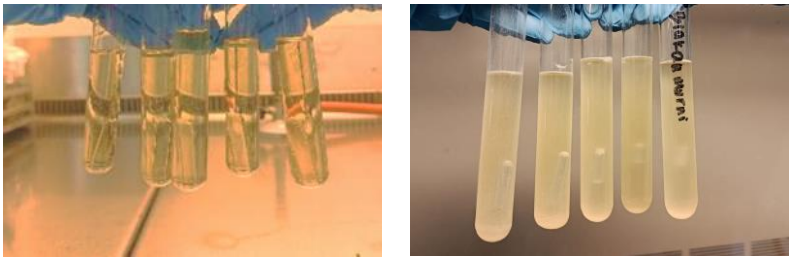
At the E. coli predictor test stage on LTB media, varying results were obtained for each sampling points. Positive results were obtained in the 10-1 dilution tube at sampling points 1(1), 1(2), 1(4), 2(1), 2(4), 2(5), 3(1), 4(1), 5(4), and 5(5).

(a) (b)

Figure 1 Positive Results of the *E. coli* Predictor Test in LTB Media on Test Samples (a) and Positive Control (b)

One of the main signs of a positive result is a change in turbidity and gas formation in the LTB medium, as shown in Figure 1. This turbidity indicates that bacteria have multiplied in the addition to turbidity, the formation of gas bubbles in the Durham tube is also an important indicator. This gas is produced as a byproduct of bacterial metabolism and is evidence that E. coli or other coliform bacteria are present in the test sample [9].

A positive result in the previous predictive test was followed by a booster test on EC Broth media. The result obtained in this test was negative because it showed no color change to cloudiness and no the presence of gas bubbles, as shown in Figure 2(a). If the result is negative, the next test, namely the complementary test and biochemical test, is not continued. This is in contrast to the positive control, which shows a color change to turbidity and gas production, as shown in Figure 2(b). In this test, the positive control proves that the media and test method function properly in detecting E. coli.



(a) (b)

Figure 2 Negative Results of the *E. coli* Booster Test in LTB Media in Test Sample (a) and Positive Results in Positive Control (b)

In the E. coli complement test, a positive tube in EC broth was inoculated into L-EMBA media to confirm the presence of the bacteria. The test results showed one positive petri dish at a $10ÿ^{1}$ dilution with a black colony in the center and a metallic green sheen typical of E. coli as shown in Figure 3. This color appears due to lactose fermentation which produces acid and precipitates eosin and methylene blue dyes. This characteristic is a strong indicator that the growing colony is E. coli [10].

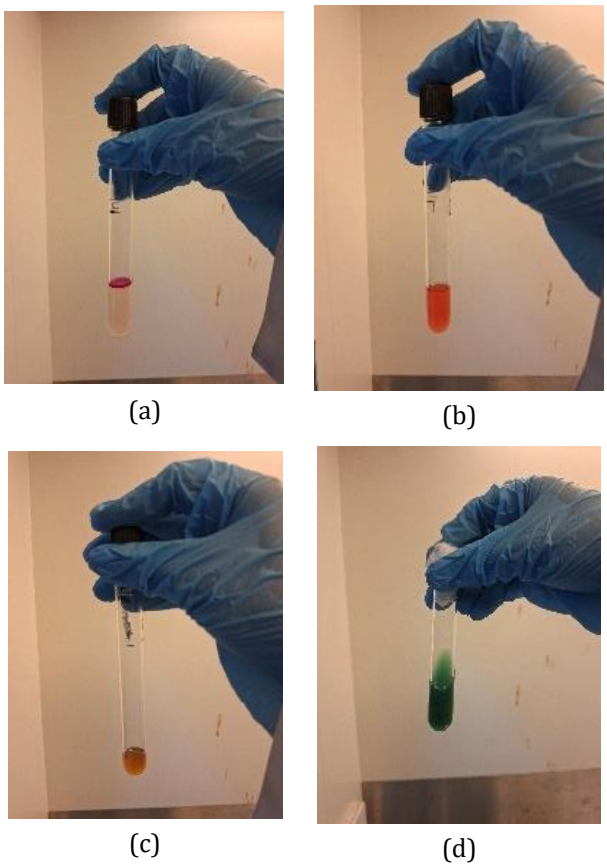
Figure 3 Results of Complementary Test on Positive Control in EMBA Media

E. coli colonies detected on EMBA media were then purified on PCA slant media at test tube. This purification process aims to obtain a single isolate ready for further testing. The isolate was then confirmed through a series of IMViC biochemical tests, including Indole and Methyl Red tests. (MR), Voges-Proskauer (VP), and Citrate, with the test results shown in Table 3.

Table 3 Interpretation of Escherichia coli Biochemical Test Results in Positive Controls

Criteria	Biotipe 1	Biotipe 2
Gas in the LTB cylinder	(+)	(+)
Indole	(+)	(-)
Methyl Red (MR)	(+)	(+)
Voges-Proskauer (VP)	(-)	(-)
Citrate	(-)	(-)

Based on the results of the biochemical tests presented in Table 3, the isolate showed positive results in the test. Indole, Methyl Red (MR), and gas formation in Lauryl Tryptose Broth (LTB) tubes. Negative results were obtained in the Voges-Proskauer (VP) and Citrate tests. This pattern of results is consistent with the typical characteristics of E. coli which is able to ferment glucose and produce acid and gas, but cannot utilize citrate as a carbon source. Positive tests for Indole and MR strengthen the indication that the isolate belongs to the fecal coliform bacteria group. These biochemical results confirmed that the tested isolate was E. coli [11].



(a) (b)

(c) (d)

Figure 4 Results of Eschericia coli Biochemical Testing ; Indole Test (a); Methyl Red Test (b); VP Test (c); and Citrate(d)

Based on the observations, the sample showed a positive reaction in the indole test as shown in Figure 4(a) with the formation of a red ring on the top layer of the medium. This indicates that Escherichia coli produces the enzyme tryptophanase, which can break down tryptophan into indole, which then reacts with the aldehyde in Kovac's reagent to form a characteristic red color [122].

A positive result in the Methyl Red test is indicated by a change in the color of the media to red, such as Figure 4(b), while a negative result will produce a yellow color [13]. Based on the test results, the sample showed positive results with the formation of a red color, this indicates that E. coli is capable of oxidizing glucose to acid at high concentrations. The resulting acid reacts with methyl red, causing a color change to red due to a decrease in pH [14].

A positive reaction in the VP test is indicated by the formation of a pink to ruby red color. as in Figure 4 (c), while negative results do not show a color change.

Based on the test results, E. coli showed a negative result because there was no color change in the media. This indicates that the bacteria did not produce acetylmethylcarbinol (acetoin) during the glucose fermentation process [12].

A positive citrate test result is indicated by a change in the color of the media to blue as shown in Figure 4. (d), while a negative result is indicated by a persistent green color [15]. Based on the observation results, E. coli showed a negative result, where the media remained green, indicating that this bacterium cannot utilize citrate as its primary carbon source [12]. These results are consistent with the general characteristics of E. coli , which relies more on glucose and lactose fermentation as energy sources.

The presence of E. coli in a sample is often used as an indicator of cleanliness and sanitation levels in product processing. E. coli's natural habitat is in the large intestines of animals and humans, and in general, this bacteria cannot survive long outside the digestive tract. Therefore, the presence of E. coli in water, the environment, or food usually indicates contamination from feces. This makes E. coli an important indicator in assessing the effectiveness of sanitation implementation in food production processes. Detection of this bacteria in fishery products This indicates the need for

stricter hygiene controls to prevent microbiological contamination [11]. This study indicates that the mackerel tested is still suitable for consumption in terms of microbiological contamination, especially E. coli. The presence of E. coli This low amount may be caused by mild environmental contamination during the handling process.

Observation of the morphology of E. coli colonies in positive controls was carried out macroscopically. Generally circular in shape with a convex surface, flat edges, and smooth texture, grows optimally at a temperature of 37°C for 24 hours, producing homogeneous colonies that are easy to observe. The characteristic metallic green color of Eosin Methylene Blue Agar (EMBA) media indicates lactose fermentation by E. coli, which is an indicator of media selectivity and identification reliability [17].

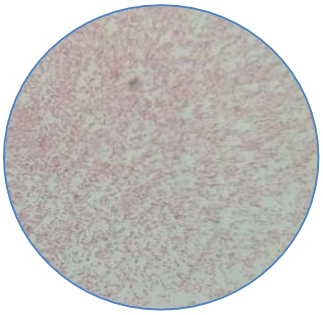


Figure 5 Microscopic form of E. coli bacteria at 40X Magnification

Cell observations were carried out microscopically. E. coli cells are short rod-shaped (bacilli) with a length of 2.0–6.0 µm and a width of 0.4–0.7 µm, are Gram-negative and appear pink after staining. Gram staining due to thin cell walls with a lipopolysaccharide outer membrane.

Based on Table 2. The results of testing for Salmonella sp. bacteria on all mackerel samples showed negative results, which means that no typical colony growth was detected on the media. XLD and BSA selectively, as well as positive results in further biochemical tests. These results indicate that there is no Salmonella sp. contamination in the Banjar mackerel sold at the West Jakarta Traditional Market.

The initial stage of Salmonella testing is pre-enrichment. According to Sandel et al., (2003); Gracias & McKillip (2004), the pre-enrichment stage helps reduce the death rate of Salmonella bacterial cells. sp. due to heating, cooling, drying and osmotic pressure during the sample handling process.

Figure 6 Enrichment Test

The test results at this stage are observed through the turbidity of the media and the distinctive odor after 18 hours of incubation. at a temperature of 34-38°C as in Figure 15, indicates positive bacterial growth before proceeding to the selective enrichment stage. The pre-enrichment stage aims to increase the number of Salmonella bacteria cells that may be present in the sample and repair any bacterial cells that may have been damaged by production processes such as cooling or heating. The enrichment stage aims to increase the number of Salmonella bacteria and inhibit the growth of other bacteria [8].The test results are observed in Figure 7 (a) on RVS media and 7 (b) on MKTTn media after 24 ± 3

hours of incubation at a temperature of 41.5 °C, with positive indications in the form of turbidity or bacterial growth which were then confirmed at the isolation stage.

The selection stage in selective media in Salmonella testing involves isolating bacteria from enrichment results using XLD (Xylose Lysine Deoxycholate) and BSA (Bismuth Sulphite Agar) differential agar to distinguish Salmonella colonies from other bacteria. In positive Salmonella colonies cultured on XLD media, if a sample contains Salmonella sp. bacteria, the colonies that grow on XLD media will be pink with or without shiny dots or almost all black colonies will appear as in Figure 7. XLD media has the ability to inhibit the growth of Gram positive bacteria because it contains sodium deoxycholate, and contains thiosulfate as an H2S indicator that is visible in colonies growing in XLD media. If a sample contains *Salmonella* sp. bacteria cultured on BSA media, it shows a positive result for *Salmonella* through the formation of metallic black or blackish-gray colonies with a shiny metallic sheen as in Figure 8, caused by the reaction of bismuth sulfite with hydrogen sulfide (H2S) produced by the bacteria. The media around the colonies often turns dark brown or black.

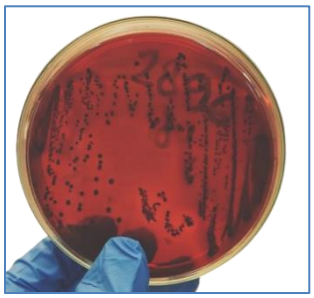


Figure 7 Positive Results on Selective Media in XLD Media

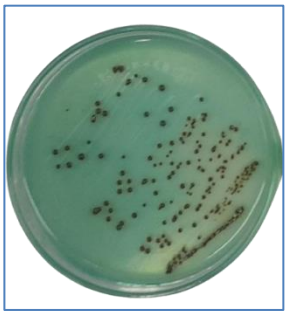


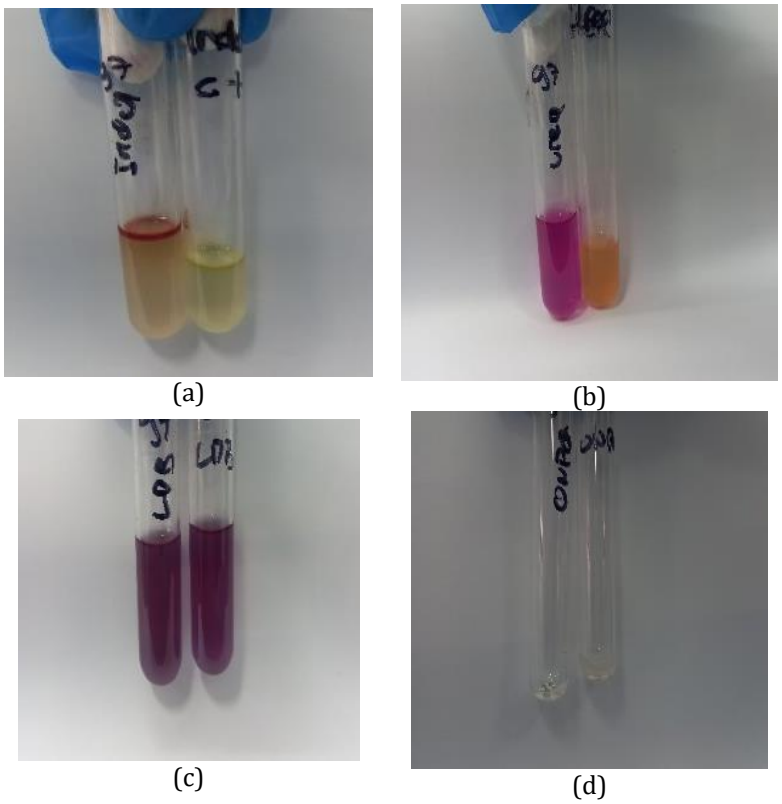
Figure 8 Positive Results on Selective Media in BSA Media

Suspected *Salmonella* on XLD and BSA media, purified with TSIA media in tubes reaction and confirmed using biochemical tests consisting of Indole Test, Urea Test, LDC Test and ONPG Test.

The indole test is used to assess the ability of bacteria to hydrolyze tryptophan into indole. After reacting with Kovac's reagent , a red ring forms on the top layer of the medium, indicating a positive result. Salmonella sp. generally produces a negative indole result, indicated by the absence of a red ring and color change. The yellow ring formed on the top layer of the medium in Figure 9(a) indicates a negative result [8].

The urea broth test uses a medium containing urea and a phenol red indicator to detect the activity of the urease enzyme, which breaks down urea into ammonia and increases the pH. If the reaction is positive, urea will release ammonia, which changes the phenol red color to pink and then to a deep cherry red, which occurs and is often visible after 2 to 4 hours [8]. Salmonella sp. are generally urease-negative, so the color of the medium remains pale yellow or unchanged after incubation, as shown in Figure 9 (b).

The LDC test is used to assess the ability of lysine to decarboxylate to base, using Lysine Decarboxylase Broth medium or through interpretation on Lysine Iron Agar (LIA). The majority of Salmonella sp. showed positive LDC, which was indicated by the formation of a purple color after incubation. as in Figure 9 (c) [8].



(a) (b) (c) (d)

Figure 9 Results of Salmonella sp. Biochemical Testing ; Indole Test (a); Urea Test (b); LDC Test (c); and ONPG Test(d)

The ONPG (o-nitrophenyl-ÿ-D-galactopyranoside) test is used to detect the enzyme ÿ-galactosidase. which breaks down ONPG into yellow *o-nitrophenol* . Salmonella sp. generally produces ONPG is negative, meaning there is no color change to yellow as in Figure 9 (d), which indicates the absence of significant ÿ-galactosidase activity [15].

The morphological test of Salmonella sp. includes two main aspects, namely macroscopic observation of colonies on solid media and microscopic observation of bacterial cells after staining using a microscope which is used as the basis for identification after further biochemical tests. In macroscopic morphology tests, Salmonella colonies grown on Xylose Lysine Desoxycholate (XLD) Agar media are generally described as round with flat colony edges, convex surfaces, and black or transparent pink with a black center because the bacteria are able to reduce thiosulfate to sulfide compounds that react to produce blackening of the colony and the surrounding zone [8]. Meanwhile, Salmonella colonies grown on Bismuth Sulphite Agar (BSA) media appear as brown, dark gray to black colonies with a metallic sheen. surrounded by a media zone that is initially brown then turns black over incubation time due to the production of hydrogen sulfide (HÿS) which reacts with bismuth and metal ions in the media.

In microscopic morphological tests, Salmonella sp. has a straight rod shape (bacilli) with a size of around 2–4 µm long and 0.3–0.8 µm wide, is Gram negative so that after Gram staining it appears pink to red because it does not retain crystal violet . Gram staining shows that the cells are arranged singly or scattered, do not form long chains, no endospores are visible in the cells, and in several studies it is stated that they have peritrichous flagella, which cover the entire surface of the cell.

There are several factors that cause *Salmonella* sp. bacteria not to be detected. sp. easily grows in fishery products when sanitation is poor and there is cross-contact between raw fish and contaminated surfaces or water [25]. The negative results in this study indicate that fish handling in the market was quite hygienic, for example by cooling with ice in Styrofoam. Salmonella sp. generally lives in the digestive tract of land animals, especially birds and mammals, not in marine fish. Based on its physiological characteristics, Salmonella requires a neutral–slightly alkaline pH (6–8) and an optimum temperature of around 37°C [17]. Mackerel itself lives in saltier seas, and low temperatures can inhibit the growth and shorten the lifespan of Salmonella sp. Based on research by Christanti and Azhar (2019) [18] the characteristics of the research location taken are that fish sold in traditional markets in West Jakarta mostly come from

large suppliers who apply cold chains during shipping. According to the Ministry of Maritime Affairs and Fisheries (Ministerial Regulation of the Ministry of Maritime Affairs and Fisheries No. 9 of 2024 concerning *Good Fish Distribution Practices* – (CDIB), Cold Chain is a series of activities for handling, storing, and distributing fishery products carried out in low (controlled) temperature conditions to maintain food quality and safety from the point of capture to the end consumer. Cold Chain Suppresses the activity of enzymes and spoilage microorganisms such as Pseudomonas, E. coli, and Salmonella sp. at low temperatures, the rate of bacterial metabolism slows down so that the growth of pathogenic microbes is inhibited [18]. These low temperature conditions are effective in suppressing the growth of pathogenic bacteria such as *Salmonella* sp.

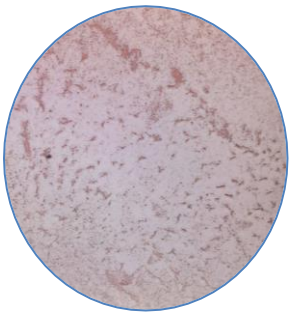


Figure 10 Microscopic form of *Salmonella* sp bacteria at 40X Magnification

4. Conclusion

Based on the detection test of pathogenic bacteria (*Escherichia coli* and *Salmonella* sp.) in fish the results of the study on Banjar mackerel (*Rastrelliger kanagurta*) in the West Jakarta Traditional Market have been carried out, the conclusion obtained from the *E. coli* test using the SNI 2332.1:2015 method obtained results <1.8 APM/g which indicates that the number of *E. coli* bacteria is still within safe limits and the *Salmonella* sp. test using the SNI ISO 6579-1:2017 method showed negative results. The results of this study provide a clear picture that the safety and suitability of mackerel consumption in the West Jakarta Traditional Market is in accordance with SNI 2729:2021 concerning the safety and consumption of fresh fish.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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