Original Research Report

TOTAL PLATE COUNT ANALYSIS AND FOOD-CONTAMINATING BACTERIAL IDENTIFICATION OF SMOKED *TATIHU* (*Thunnus albacares*) SOLD IN SEVERAL TRADITIONAL MARKETS IN AMBON, INDONESIA

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ABSTRACT

The large marine area and abundant fish resources of Maluku Province, Indonesia, are in contrast to the poor hygiene of its traditional markets, which can cause microbial contamination and taint processed products, such as smoked *tatihu* (yellowfin tuna). In Ambon city, Maluku, Indonesia, no research had been conducted concerning total plate count analysis and food-contaminating bacterial identification that could guarantee the microbiological safety of smoked *tatihu*. Therefore, this study aimed to assess the microbiological quality of smoked *tatihu* according to Indonesian National Standards (INS 2725:2013) and to identify any presence of food-contaminating bacteria. This research was a quantitative descriptive study with a true experimental laboratory approach. The samples used were smoked *tatihu* collected from three traditional markets in Ambon, Indonesia. The spread plate method was used in the isolation process, while the total plate count analysis was performed to estimate the quantity of colonies on each petri dish. Bacterial identification was carried out macroscopically and microscopically. The microscopic identification involved Gram staining to determine the shape and color of the bacteria. Additionally, the bioMérieux VITEK 2 Compact system was utilized for biochemical identification to ascertain the species of bacteria present. The results revealed that the colony counts in smoked *tatihu* from the Mardika market and Hative Kecil market were 1.1 x 10^4 CFU/g and 8.2 x 10^6 CFU/g, respectively. However, smoked *tatihu* from the Batu Meja market had an excessive number of colonies that were difficult to quantify. The contaminating bacteria were identified as *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Rothia kristinae*, and *Staphylococcus pseudintermedius*. In conclusion, smoked *tatihu* fish from the Mardika market are considered safe for consumption as the microbiological parameters do not exceed the Indonesian National Standards, whereas those obtained from the Hative Kecil and Batu Meja markets are unsafe for consumption due to the excessive presence of food-contaminating bacteria.

Keywords: Food contamination; Gram-positive bacteria; INS; traditional market; human and health

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Highlights:
1. This research provides important information regarding the food safety of smoked *tatihu* (yellowfin tuna) sold in several traditional markets in Ambon, Indonesia.
2. We find that smoked tatihu fish samples examined at 2 of 3 traditional markets in Ambon city are unsafe for consumption according to the Indonesian National Standard due to bacterial contamination.
3. The findings indicate that smoked fish can be contaminated by Gram-positive bacteria, such as *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Rothia kristinae*, and *Staphylococcus pseudintermedius*.

INTRODUCTION

The Maluku Province in eastern Indonesia has a vast water area that offers great potential for fish production. One of the abundant fish resources is the yellowfin tuna (*Thunnus albacares*), commonly referred to as *tatihu* fish in the local community (Sigmarlatu 2023). The abundance of fish in Maluku has emerged as a potential contributor to the diversity of processed fish products. Ambon, the capital of Maluku Province, is known as the "City of Fish." Ambon earns the title due to its production of fish products that possess a distinctive taste,

distinguishing them from processed fish products in other regions, particularly smoked fish (Pormes et al. 2022). The production of smoked fish involves a smoking process that lasts 4–5 hours. The chemical compounds in the smoke are introduced into the fish and dissolve on the surface, resulting in the fish product being infused with distinctive flavors, colors, and aromas (Tamtama & Dwi Pratiwi 2023). The traditional method of producing smoked fish has been passed down through generations. This has made smoked fish widely consumed by the people of Maluku and popular as a souvenir for tourists. However, the quality of processed fish products in the region is prone to rapid degradation. The deterioration in quality can be attributed to poor hygiene during the production and sale of fish products, especially smoked fish. Additionally, smoked fish are often distributed to traditional markets in Ambon, where cleanliness may not be guaranteed. Inadequate hygiene and sanitation in traditional markets are a major risk factor that can increase bacterial contamination through both direct and indirect means, including the air. Excessive bacterial contamination in food can result in cases of foodborne disease (Tuhumury et al. 2022).

According to Pitri et al. (2020), the Indonesian Food and Drug Authority (*Badan Pengawas Obat dan Makanan*) reported 27 cases of food poisoning in 2017 across several regions. Commonly, the early symptoms of food poisoning include foodborne disease symptoms, such as diarrhea. It is important not to underestimate this symptom, as prolonged diarrhea may lead to death (Putri & Kurnia 2018). The data provided by the World Health Organization in 2010 revealed an estimated 582 disease outbreak cases. From the total number of cases, 22 were categorized as foodborne disease outbreaks (Trigunarso 2020).

The quality and safety requirements for smoked fish are specified in the Indonesian National Standards (INS 2725:2013). The standards require smoked fish products to be free from microbial contamination (Jeujanan 2022). However, prior research conducted by Fitria & Oktaviani (2024) has identified the presence of *Escherichia coli* in smoked skipjack tuna sold at the central market of Batang, Indonesia. Out of four samples obtained, three were confirmed to be contaminated with *E. coli*. In addition, Haryati (2020) conducted microbiological testing of smoked yellowtail fusilier from the Youtefa market in Jayapura, Indonesia. The study identified the presence of *Staphylococcus* and *Salmonella* bacterial groups. Subsequently, another study conducted by Tuhumury et al. (2022) provided additional evidence regarding the inadequate quality of fish products sold in traditional markets. The study also revealed that smoked skipjack tuna sold at the Mardika market in Ambon, Indonesia, contained *Salmonella* sp. The microbiological content of food products can be tested using the total plate count method in accordance with the specific criteria established by the Indonesian National Standards (Maulana et al. 2022).

Prior research by Tuhumury et al. (2022) that investigated bacterial contamination in smoked fish sold in Ambon, Indonesia, has focused solely on one type of processed fish product and a specific traditional market, namely smoked skipjack tuna from the Mardika market. There had been no research undertaken to analyze the microbiological quality of smoked *tatihu* available in various markets in Ambon City. Hence, the objectives of this study were to conduct total plate count analysis and identify food-contaminating bacteria in smoked *tatihu* (*Thunnus albacares*) sold in several markets in Ambon City, in accordance with the Indonesian National Standards.

MATERIALS AND METHODS

This study used a quantitative descriptive research design with a true experimental laboratory approach. The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Universitas Pattimura, and the Office for Health Laboratory and Medical Device Calibration of Maluku Province, Ambon, Indonesia, from June to July 2024. The samples consisted of smoked yellowfin tuna (*Thunnus albacares*), locally known as *tatihu*, which were purchased from several traditional markets in Ambon, Indonesia. After conducting a survey, it was found that smoked *tatihu* were available in only three traditional markets: Mardika, Batu Meja, and Hative Kecil traditional markets. As there were several vendors selling smoked *tatihu* in each market, this study employed a purposive random sampling technique to choose the vendors from whom the samples should be purchased (Suen et al. 2014). The sample criteria included smoked *tatihu* sold in the three traditional markets for a sale duration beyond one day.

The equipment used in this study included an autoclave, blender (Philips), magnetic stirrer (Cimarec), analytical balance (PioneerTM), 100 mL beaker glass (Duran), 100 mL measuring cup (Iwaki), pen, laptop, camera, tweezers, HVS papers, 1,000 mL Erlenmeyer flask (Iwaki), 500 mL Erlenmeyer flask (Iwaki), 250 mL Erlenmeyer flask (Iwaki), disposable petri dishes (OneMed), test tubes (Iwaki), micropipette (Socorex), spreader (Iwaki), microscope, vortex mixer (VM-300 Gemmy), inoculating needle, aluminum foil (Klinpak), plastic wrap (Klinpak), sterile cotton (OneMed), Bunsen burner, glass slides (Sailbrand), and VITEK 2 Compact (bioMérieux). Additionally,

smoked *tatihu*, 0.85% sodium chloride (NaCl) solution (MJB Pharma), plate count agar media (Merck), Gram staining reagents, sterile distilled water (Waterone), 95% alcohol (OneMed), 70% alcohol (OneMed), spirit, and immersion oil (Merck).

The microbiological testing process started by subjecting HVS papers to autoclave sterilization at 121°C for 15 minutes. The sample preparation was carried out by dividing the samples into parts and homogenizing them using a blender. Subsequently, 22.5 g of plate count agar media were weighed and dissolved in 1,000 mL of sterile distilled water. The mixture was then heated on a hotplate and stirred using a magnetic stirrer. The dilution process began with preparing 9 mL of 0.85% NaCl solution in several test tubes to produce dilutions ranging from 10^{-1} to 10^{-5}. In order to achieve a 10^{-1} dilution, 1 g of the samples was added to a test tube containing 0.85% NaCl. For the preparation of a 10^{-2} dilution, 1 mL of the suspension obtained from the 10^{-1} dilution was transferred to a tube specifically meant for the 10^{-2} dilution. The suspension was mixed using a vortex before taking the 1 mL aliquot. The same procedure was implemented to yield the dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. The diluted isolates were then inoculated onto the plate count agar media using the spread plate technique. The suspension was mixed using a vortex, and a volume of 0.1 mL was spread onto the media using the spread plate technique. The plates were subsequently incubated at room temperature for 48 hours (Tuhumury et al. 2022).

The total plate count analysis involved counting the colonies present on the petri dish. The purpose of colony counting was to determine the level of microbial contamination, and the counting was conducted only for petri dishes with colony counts ranging from 30 to 300 (Jamilatun 2022, Yunita et al. 2022a). The total plate count of smoked *tatihu* was determined by the formula of multiplying the number of bacterial colonies by 1 per dilution factor. The purification process was carried out to obtain a pure bacterial colony. A four-quadrant zigzag method was employed to undertake this process on a petri dish (Jamilatun 2022, Yunita et al. 2022a, 2022b). One colony obtained from the purification process was collected using an inoculation needle and underwent inoculation in five tilted tubes using a zigzag pattern. The purpose of this process was for the microscopic and biochemical identification using the bioMérieux VITEK 2 Compact system (Rosmania & Yanti 2020).

In addition to the microscopic observation, the bacterial identification was also performed macroscopically. The purpose of the macroscopic identification was to observe the morphology of the colonies resulting from the purification process and categorize them according to their size, shape, margin, elevation, and color (Fauziah 2023). The microscopic identification was performed using Gram staining to determine the shape and color of the bacteria under the microscope. The biochemical identification of bacterial species in smoked *tatihu* was carried out using the bioMérieux VITEK 2 Compact system (Nimer et al. 2016, Astuty et al. 2023).

RESULTS

This study revealed varying results in the total plate count of smoked *tatihu* samples purchased from three markets. An analysis of samples from the Hative Kecil market showed a colony count of 8.2 x 106 CFU/g at a 10-5 dilution. Samples from the Mardika market had colony counts of 1.1 x 104 CFU/g at a 10-2 dilution, 8.1 x 104 CFU/g at a 10-3 dilution, 5.1 x 105 CFU/g at a 10-4 dilution, and 5.1 x 106 CFU/g at a 10-5 dilution. However, smoked *tatihu* samples from the Batu Meja market exhibited colonies that were too many to count (TMTC) at a dilution ranging from 10-1 to 10-5. The results of the bacterial total plate count in this study are presented in Table 1.

Table 1. Results of the total plate count analysis of smoked *tatihu*.

Dilution	Traditional markets						INS
	Hative Kecil	Indication	Mardika	Indication	Batu Meja	Indication	
10^{-1}	TMTC	Unsafe	TMTC	Unsafe	TMTC	Unsafe	
10^{-2}	TMTC	Unsafe	$1.1 \times 10^{4} \pm 5.5$	Safe	TMTC	Unsafe	
10^{-3}	TMTC	Unsafe	$8.1 \times 10^{4} \pm 3.5$	Safe	TMTC	Unsafe	5×10^{5}
10^{-4}	TMTC	Unsafe	$5.1 \times 10^{5} \pm 8$	Unsafe	TMTC	Unsafe	
10^{-5}	$8.2 \times 10^{6} \pm 3.5$	Unsafe	$5.1 \times 10^{6} \pm 11$	Unsafe	TMTC	Unsafe	

Legends: TMTC=too many to count; INS=Indonesian National Standards.

After the bacterial purification was performed, the different macroscopically bacterial colonies were identified according to their distinct characteristics, including size, shape, margin, elevation, and color. The results of the bacterial purification are presented in Figure 1.

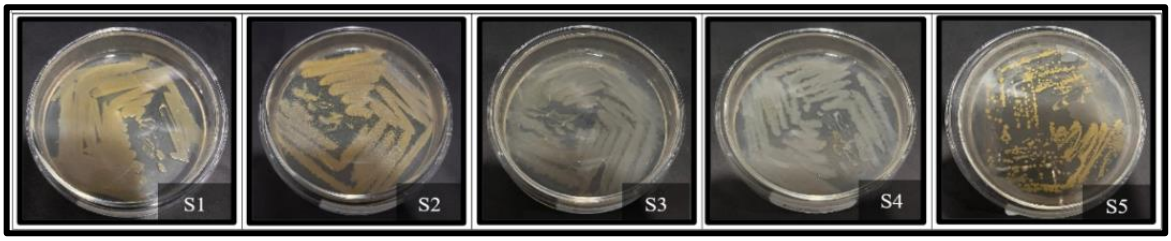

Figure 1. Purification results of five selected bacterial isolates (S1, S2, S3, S4, and S5) after 48 hours of incubation. Each circled spot represents a single colony of the purified isolates.

The bacterial colonies resulting from purification were recultured and subsequently examined macroscopically. The results of the observations are detailed in Table 2. Isolate S1 was small, irregularly shaped, yellow, with an undulate margin and a crateriform elevation. Isolate S2 was small, irregularly shaped, milky white, with a curled margin and a flat elevation. Isolate S3 was medium-sized, irregularly shaped, with a cloudy yellow color, an undulate margin, and a raised elevation. Isolate S4 was medium-sized, irregularly shaped, milky white, with an entire margin and a raised elevation. Lastly, isolate S5 was large, filamentous, cloudy yellow, with filiform edges and a flat elevation.

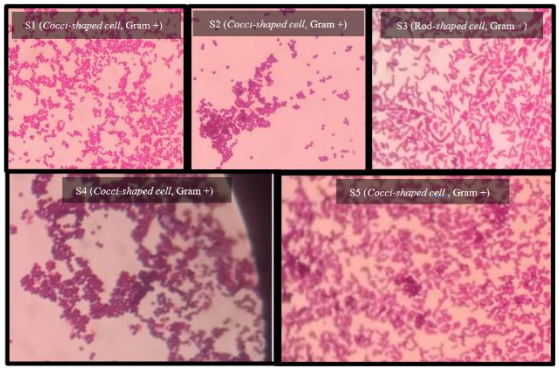

Figure 2. Results of the bacterial Gram staining.
Description: S1=*Staphylococcus gallinarum*; S2=*Staphylococcus sciuri*; S3=*Rothia kristinae*; S4=*Staphylococcus pseudintermedius*; S5=*Staphylococcus gallinarum*.

Table 2. Macroscopic characterization of selected bacterial isolates from smoked *tatihu* samples.

Isolates	Morphology characteristics of the colonies				
	Size	Shape	Color	Margin	Elevation
S1	Small	Irregular	Yellow	Undulate	Crateriform
S2	Small	Irregular	White	Curled	Flat
S3	Medium	Irregular	Yellow	Undulate	Raised
S4	Medium	Irregular	White	Entire	Raised
S5	Large	Filamentous	Yellow	Filiform	Flat

Legends: S1=*Staphylococcus gallinarum*; S2=*Staphylococcus sciuri*; S3=*Rothia kristinae*; S4=*Staphylococcus pseudintermedius*; S5=*Staphylococcus gallinarum*.

Bacterial contamination in smoked *tatihu* was assessed by microscopic characterization of cell shape and Gram staining. The microscopic characterization results can be seen in Figure 2. Isolate S1 had a Gram-positive coccus shape, was arranged in clusters, and exhibited a purple coloration. Isolate S2 was also a Gram-positive coccus, characterized by its clustered arrangement and purple coloration. Isolate S3 was a Gram-positive rod, which appeared elongated, paired, and purple in color. Isolate S4 was a Gram-positive coccus with a clustered arrangement and a purple coloration. Similarly, isolate S5 was a Gram-positive coccus, arranged in a cluster, and appeared purple. These observations indicated that all five bacteria were Gram-positive.

Table 3. Results of bacterial identification using the VITEK 2 Compact system.

Isolates	Bacterial species	Significance	Habitat
S1	*Staphylococcus gallinarum*	99%	Poultry and human saliva
S2	*Staphylococcus sciuri*	91%	Mice
S3	*Rothia kristinae*	87%	Human oral cavity
S4	*Staphylococcus pseudintermedius*	99%	Dogs and cats
S5	*Staphylococcus gallinarum*	99%	Poultry and human saliva

The rapid identification of the five selected bacteria was performed using the VITEK 2 Compact system. The results confirmed that the VITEK 2 system had an excellent level of accuracy in directly and quickly identifying the examined samples. It was determined that the five bacterial species were *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Rothia kristinae*, and *Staphylococcus pseudintermedius*. Comparable identification results were obtained for the five bacterial species, i.e., 99% for *S. gallinarum* (isolate S1), 91% for *S. sciuri* (isolate S2), 87% for *R. kristinae* (isolate S3), 99% for *S. pseudintermedius* (isolate S4), and 99% for *S. gallinarum* (isolate S5).

DISCUSSION

Total plate count analysis of processed fish products

Total plate count is a method used to test the suitability and safety of food products. The number of microorganisms in food products sold in Indonesia is expected to comply with the Indonesian National Standards. In this study, the total plate count method was employed to count the colonies on petri dishes after 48 hours of incubation, specifically for colony counts ranging from 30 to 300. The research findings indicated that the total plate count for the three smoked *tatihu* samples did not meet the Indonesian National Standards of 5 x 105 CFU/g. These results might be attributed to various factors, such as inadequate sanitary facilities and equipment as well as the sellers' insufficient hygiene practices. A study conducted by Dewi et al. (2023) analyzed the bacterial total plate count of smoked skipjack tuna in the traditional markets of Krembangan District, Surabaya, Indonesia. The results showed colony counts ranging from 5.9 x 10^4 to 22.19 x 10^5 CFU/g. The study also revealed that the high microbial counts in smoked fish were likely due to a variety of factors, including poor sanitation and hygiene around the vendors. According to Akerina (2018), processed fish products, such as smoked fish, that are not properly packaged and sold in traditional markets are vulnerable to bacterial contamination. Additionally, selling and displaying unwrapped products in close proximity to main roads can lead to cross-contamination from vehicle exhaust and dust. In addition to its function as a protective barrier for processed products, packaging also helps maintain the product's quality and enhances the product's attractiveness in traditional markets (Nanlohy et al. 2022). Failure to fulfil these criteria may increase the risk of bacterial contamination in food products.

The Hative Kecil market is among the several markets in Ambon, Indonesia, that sell smoked *tatihu*. However, its location is far from the city center, which led people to prefer the Mardika market for shopping, despite the less hygienic conditions of the market. Although the Hative Kecil market was relatively quiet, smoked *tatihu* sold there remained susceptible to bacterial contamination. This was mostly due to its close proximity to main roads and industrial facilities, which posed an indirect risk of contamination through the air. Additionally, the little activities in the Hative Kecil Market might result in extended durations of smoked *tatihu* storage, potentially affecting the quality of the product (Tuhumury et al. 2022).

The Batu Meja market in Ambon, Indonesia, also sells smoked *tatihu*. The traditional market is located in the city center, along the roadside. Such location increases the potential for bacterial contamination in the food products available for sale. Humidity and the surrounding environment, especially during the rainy season, might affect the quality of smoked *tatihu* at the time of purchase for this research. These factors could potentially promote bacterial growth in the food. Despite performing serial dilutions, the yield from the colony growth remained too many to count, making it impossible to determine the bacterial colony count in smoked *tatihu* from the Batu Meja market. The results were in line with research conducted by Katiandagho et al. (2017), who found that environmental conditions and humidity in a sales area can affect the shelf life and quality of a product. On the contrary, lower moisture content in processed food products can maintain their quality and extend their shelf life.

Analysis of smoked *tatihu* samples for bacterial identification

The bacterial identification was carried out to determine the species of bacteria present in smoked *tatihu* samples according to their macroscopic,

microscopic, and biochemical characteristics. The identification process began with the purification of five bacterial isolates, which were selected based on their distinct macroscopic characteristics. The microscopic identification was then performed using Gram staining, followed by biochemical identification using the bioMérieux VITEK 2 Compact automated system at the Office for Health Laboratory and Medical Device Calibration of Maluku Province, Ambon, Indonesia (Nimer et al. 2016, Astuty et al. 2023). The Gram staining results indicated that all five bacterial isolates exhibited Gram-positive characteristics. The bacterial species identified using the bioMérieux VITEK 2 Compact system were *S. gallinarum*, *S. sciuri*, *S. pseudintermedius*, and *R. kristinae*.

The *S. gallinarum* (isolates S1 and S5) is widely distributed in nature and has predominantly been reported in poultry, as well as isolated from the saliva of healthy humans. This bacterium can cause bacteremia in patients with chronic hepatitis B virus infection, presenting with symptoms such as mild fever, upper abdominal pain, and nausea (Shi et al. 2015). According to Sugiarti & Nursanty (2020), *S. sciuri* (isolate S2) is a commensal bacterium found in animals, particularly rats. *S. pseudintermedius* (isolate S4) is a Gram-positive bacterium that inhabits the mucous membranes of dogs and cats. As previously mentioned, traditional markets are often associated with unsanitary and unhygienic conditions. These shortfalls let animals, such as rats and cats, to inhabit the sales areas and induce food contamination (Fungwithaya et al. 2022).

Bacteria from the genus *Staphylococcus* sp. are generally cocci-shaped, with a diameter of 0.5 to 1 µm. The bacteria are Gram-positive and exhibit a purple color under a Gram stain. *Staphylococcus* sp. is parasitic to both humans and animals due to their endotoxin content. *Staphylococcus* sp. contamination in processed fish products is suspected to result from interactions between sellers and buyers, as well as a lack of attention to sanitation and hygiene by producers and sellers along the entire supply chain, from post-harvest fish cleaning to processing and marketing. Smoked fish vendors often sell their products without packaging, thus exposing them to the open air. This practice increases the risk of food contamination by *Staphylococcus* sp. (Haryati 2020).

This study identified another Gram-positive bacterium, namely *R. kristinae* (isolate S3). The genus *Rothia* was first introduced in 1967 by George and Brown. It is naturally found in the human oral cavity. There have been few published case reports of human infections caused by *Rothia*, which include infective endocarditis, pneumonia, meningitis, and bacteremia. Because *Rothia* is part of the normal human flora, its presence in blood cultures is often considered a contamination rather than an invasive infection. There is currently no research supporting the contamination of food and beverages by *R. kristinae* (Odeberg et al. 2023). Therefore, it was suspected that contamination in smoked *tatihu* in this study might be due to the indirect transfer of saliva droplets from either the seller or buyer into the air, leading to bacterial spread through the air.

Strength and limitations

This study provides essential information to the public regarding the food safety of smoked *tatihu* sold in several traditional markets in Ambon, Indonesia. However, this research has limitations, as the findings revealed that bacteria contaminating the smoked *tatihu* samples were not Gram-negative coliform bacteria. This was possible due to the transmission of contamination from the seller to the smoked *tatihu* samples. Thus, future research is recommended to use a different method from this study, such as the most probable number (MPN) method, to analyze bacterial contamination.

CONCLUSION

Smoked *tatihu* collected from the Hative Kecil and Batu Meja markets in Ambon, Indonesia, exceeds the microbiological parameters set by the Indonesian National Standards. Therefore, the findings suggest that smoked *tatihu* sold in both markets is not safe for consumption. On the other hand, smoked *tatihu* purchased from the Mardika market comply with the parameters established by the Indonesian National Standards, ensuring its safety for consumption. The bacterial identification of smoked *tatihu* exhibits the presence of different Gram-positive bacteria, including *S. gallinarum*, *S. sciuri*, *S. pseudintermedius*, and *R. kristinae*.

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Conflict of interest

None.

Ethical consideration

This study received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, under reference No. 101/FK-KOM.ETIK/VIII/2024 on 07/06/2024.

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Author contribution

ERH contributed to the data analysis and interpretation in this study. MY contributed to the conception and critical revision of the article for important intellectual content. S contributed to the drafting of the article. All authors gave their approval to the final article.

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