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Pathogenic Microbial Analysis and Antibiotic Susceptibility Testing of Smoked Skipjack (*Katsuwonus pelamis*) Sold in Traditional Market of Ambon

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ABSTRACT

This study aimed to analyze the presence of pathogenic microbes in smoked *Katsuwonus pelamis* sold in traditional markets of Ambon and to examine their antibiotic resistance patterns. A total of 12 smoked fish samples were collected from three major markets and were analyzed using bacterial isolation on TCBS selective media. Subsequently, antibiotic resistance testing was conducted using the disk diffusion method. The results revealed that *Vibrio parahaemolyticus* and *Vibrio cholerae* were the predominant pathogenic bacteria found in the smoked fish samples. The highest levels of contamination were detected in samples from Mardika and Galala markets, whereas Batu Meja Market exhibited lower contamination levels or was free from contamination. Antibiotic resistance testing indicated that bacterial isolates exhibited high resistance to β-lactam antibiotics such as ampicillin and cephalothin, suggesting the presence of bacterial defense mechanisms against these antibiotics. In contrast, nalidixic acid and gentamicin demonstrated higher efficacy in inhibiting the growth of pathogenic bacteria. The primary factors contributing to microbial contamination were poor hygiene in processing and storage methods. Therefore, strict monitoring of hygiene standards for smoked fish and improved regulations on antibiotic use in the fisheries industry are necessary. This study provides critical insights into food safety and highlights the need for better quality control measures to prevent the spread of antibiotic-resistant bacteria in seafood products.

INTRODUCTION

Smoked fish is a widely consumed fishery product in Indonesia, particularly in coastal regions such as Ambon. The smoking process is traditionally used to extend the shelf life of fish by inhibiting microbial growth and enhancing its flavor. One of the most smoked fish species is *Katsuwonus pelamis* (skipjack tuna), which has high economic value and plays a significant role in local diets **(Tapotubun *et al.* 2024)**. However,

unhygienic smoking processes and inadequate storage techniques can lead to pathogenic microbial contamination, posing potential health risks to consumers **(Huss *et al*., 2000)**.

Several studies have shown that smoked fish is susceptible to microbial contamination, particularly by pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Vibrio* spp., and *Staphylococcus aureus*, which can cause gastrointestinal disorders and serious infections **(Rakha *et al*., 2022)**. Moreover, the presence of antibiotic-resistant pathogenic bacteria has become an increasing concern in food safety and public health. Antibiotic resistance in pathogenic microbes may arise due to antibiotic exposure in aquatic environments or through the food chain **(Cabello *et al*., 2016)**. Therefore, analyzing pathogenic microbial contamination and antibiotic resistance in smoked fish is crucial for assessing the safety of this product.

Previous studies have identified microbial contamination in various fishery products **(Abigaba *et al.*, 2021; Iriobe & Awoyale, 2021)**; however, specific research on smoked *Katsuwonus pelamis* sold in traditional markets of Ambon remains limited. Most research has focused on the chemical composition and sensory quality of smoked fish, while studies on the presence of pathogenic microbes and their antibiotic resistance patterns are still scarce **(Ubaka *et al*., 2019)**. Given the increasing consumption of smoked fish in Ambon, a deeper understanding of its potential microbiological hazards is necessary.

The emergence of antibiotic-resistant bacteria in seafood poses a significant threat to public health, as the consumption of contaminated fish can contribute to the spread of antimicrobial resistance (AMR). The persistence of resistant *Vibrio* species in smoked fish may be linked to the overuse of antibiotics in aquaculture and poor hygiene during processing and storage **(Dutta *et al.*, 2021)**. According to the World Health Organization (WHO), AMR is a growing global concern that requires urgent action, particularly in food production systems. Understanding the prevalence of resistant bacteria in smoked fish is crucial for assessing potential health risks and guiding regulatory measures to ensure food safety.

This study aimed to analyze the presence of pathogenic microbes in smoked *Katsuwonus pelamis* sold in traditional markets of Ambon and to assess their antibiotic resistance patterns. Several pathogenic bacteria commonly associated with fishery products include *Vibrio* spp., *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli*. However, this study specifically focuses on *Vibrio* spp. due to its high prevalence in fishery products, particularly smoked fish, and its potential health risks.

Compared to previous studies, this research adopted a more comprehensive approach to evaluating the microbiological safety of smoked fish by identifying specific pathogenic bacteria and analyzing their antibiotic resistance patterns. Furthermore, the findings provide updated insights into the food safety status of smoked fish in traditional markets, serving as a scientific basis for policy development on quality control and food safety regulations in Ambon.

The findings of this study are expected to benefit various stakeholders, including consumers, smoked fish traders, and local authorities, in efforts to improve hygiene standards and food safety. Furthermore, information on antibiotic resistance patterns in pathogenic bacteria could aid healthcare professionals in managing infections linked to fishery products.

Thus, this study not only contributes to the field of food microbiology but also has broader implications for public health and food safety risk management. With the growing concern over global antibiotic resistance, this research may serve as a reference for understanding the role of fishery products in the spread of resistant bacteria in the environment.

MATERIALS AND METHODS

Data sampling

This study was conducted at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. Smoked fish (Skipjack tuna – *Katsuwonus pelamis*) samples were collected from three roadside market locations: Mardika Market, Batu Meja Market, and Galala Market. A total of 12 smoked fish samples were obtained from different vendors to ensure a broader representation of the products available in the market.

Isolation and identification of pathogenic bacteria

The isolation of pathogenic bacteria was performed using Thiosulfate Citrate Bile Salts Sucrose (TCBS) selective media (OXOID). Bacterial identification followed the standard procedures outlined in the *Bacteriological Analytical Manual* (BAM) Chapter 9 **(Kaysner *et al*., 2004)**, which is widely recognized for microbiological food analysis.

Antibiotic susceptibility testing

The antibiotic susceptibility of pathogenic bacteria was assessed using the Disk Diffusion Method (Kirby-Bauer). A total of 12 antibiotics from OXOID were tested: Azithromycin (15µg), Gentamycin (10µg), Cephalothin (30µg), Neomycin (30µg), Chloramphenicol (30µg), Ampicillin (10µg), Nalidixic Acid (30µg), Amoxicillin (25µg), Erythromycin (15µg), Streptomycin (10µg), Tetracycline (30µg), and Kanamycin (30µg).

This test aimed to determine the resistance levels of bacteria to various antibiotics commonly used in the treatment of pathogenic microbial infections. The research methodology was designed to provide a comprehensive understanding of the potential contamination of smoked fish with pathogenic microbes and their resistance patterns to the tested antibiotics.

RESULTS AND DISCUSSION

1. Bacterial isolated from smoked fish

A total of 12 smoked fish samples were collected from three roadside market locations: Mardika Market, Batu Meja Market, and Galala. Each sample was obtained from different vendors to ensure a broader representation in the microbiological analysis. Isolation using Thiosulfate Citrate Bile Salts Sucrose (TCBS) selective media revealed that 7 pathogenic bacterial isolates were successfully obtained from the tested samples. These isolates were distributed as follows:

- Mardika Market: M2.1 and M2.2
- Galala Market: G3.1, G3.2, G3.3, G2.1, and G2.2
- Batu Meja Market: No Vibrio contamination detected

Based on colony morphology characteristics observed on TCBS selective media, the identified isolates belonged to two *Vibrio* species. The isolates from Mardika Market (M2.1 and M2.2) and some from Galala Market (G3.1, G3.2, and G3.3) were identified as *Vibrio parahaemolyticus* (Fig. 1a). Meanwhile, the other isolates from Galala Market (G2.1 and G2.2) were identified as *Vibrio cholerae* (Fig. 1b). In contrast, no *Vibrio* contamination was detected in the samples from Batu Meja Market (BM, TL, and P) (Fig. 1c).

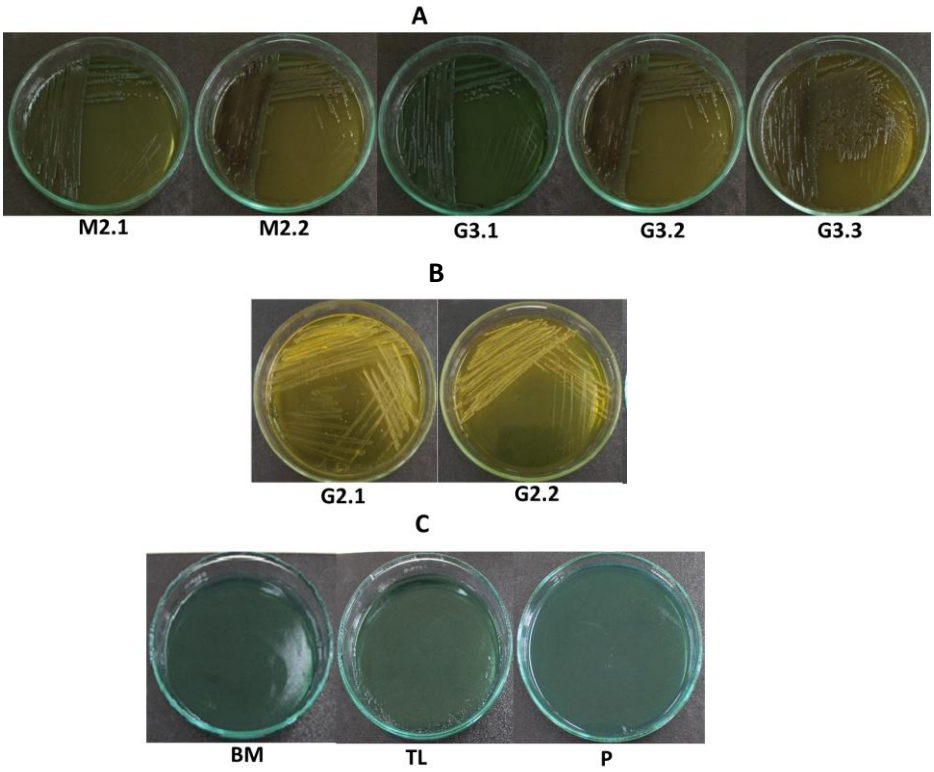


Fig. 1. Bacterial isolates from smoked fish are grown on TCBS media: **(A)** *Vibrio parahaemolyticus* isolate; **(B)** *Vibrio cholerae* isolate; **(C)** TCBS medium without *Vibrio* growth

TCBS (Thiosulfate Citrate Bile Salts Sucrose) agar is specifically designed for the selective isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus* from clinical and non-clinical specimens. Most *Vibrio* species can grow on TCBS agar, except for *Vibrio hollisae* **(Aryal, 2022)**.

The absence of *Vibrio* in smoked fish samples from Batu Meja Market could be attributed to several factors. One primary possibility is the difference in storage and handling conditions in this location. According to **Oliver (2015)**, *Vibrio* bacteria are highly sensitive to temperature and salinity changes and have low tolerance to more hygienic storage conditions. If smoked fish in Batu Meja Market were stored at lower temperatures or had reduced moisture content due to extended drying, *Vibrio* growth could have been inhibited **(Huq *et al*., 2012)**.

Additionally, the salt content and pH levels of smoked fish may influence *Vibrio* presence. **Sampaio *et al.* (2022)** reported that *Vibrio* species thrives in moderate salinity environments, and optimal fish processing methods—such as higher salt concentrations or prolonged smoking—can suppress bacterial growth. Another contributing factor could be the quality of raw fish, where fresher or higher-quality fish reduce the likelihood of initial contamination from aquatic environments **(DePaola *et al*., 1994)**.

Therefore, differences in storage practices, processing methods, and raw material quality are suspected to be the primary reasons for the absence of *Vibrio* in Batu Meja Market samples. Visualization of the TCBS agar isolation results is presented in Fig. (1).

2. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed on the seven pathogenic bacterial isolates obtained from smoked fish sold at Mardika Market and Galala Market, Ambon City. This test aimed to determine the resistance and sensitivity patterns of the isolates against 12 antibiotics. Based on the inhibition zone measurements, the isolates exhibited varying responses to the tested antibiotics, indicating differences in susceptibility among the bacterial species.

In general, some isolates demonstrated complete resistance to certain antibiotics, particularly Cephalothin and Ampicillin, where no inhibition zones were observed for all tested isolates. This suggests that the pathogenic bacteria found in smoked fish possess resistance mechanisms against these β-lactam antibiotics. Resistance to Ampicillin and Cephalothin has been widely reported in pathogenic bacteria such as *Vibrio parahaemolyticus* and *Vibrio cholerae*, which produce β-lactamase enzymes capable of hydrolyzing β-lactam antibiotics **(Silvester *et al*., 2019)**.

Among the tested antibiotics, Nalidixic Acid, Tetracycline, and Gentamycin exhibited the highest effectiveness against most isolates. Nalidixic Acid produced the largest inhibition zones in almost all isolates, particularly in those from Mardika Market, with diameters reaching 24–25mm (Table 1). As a first-generation quinolone, Nalidixic Acid inhibits bacterial DNA gyrase, preventing DNA replication and leading to bacterial cell death **(Oh & Edlund, 2003; Azargun *et al*., 2020)**.

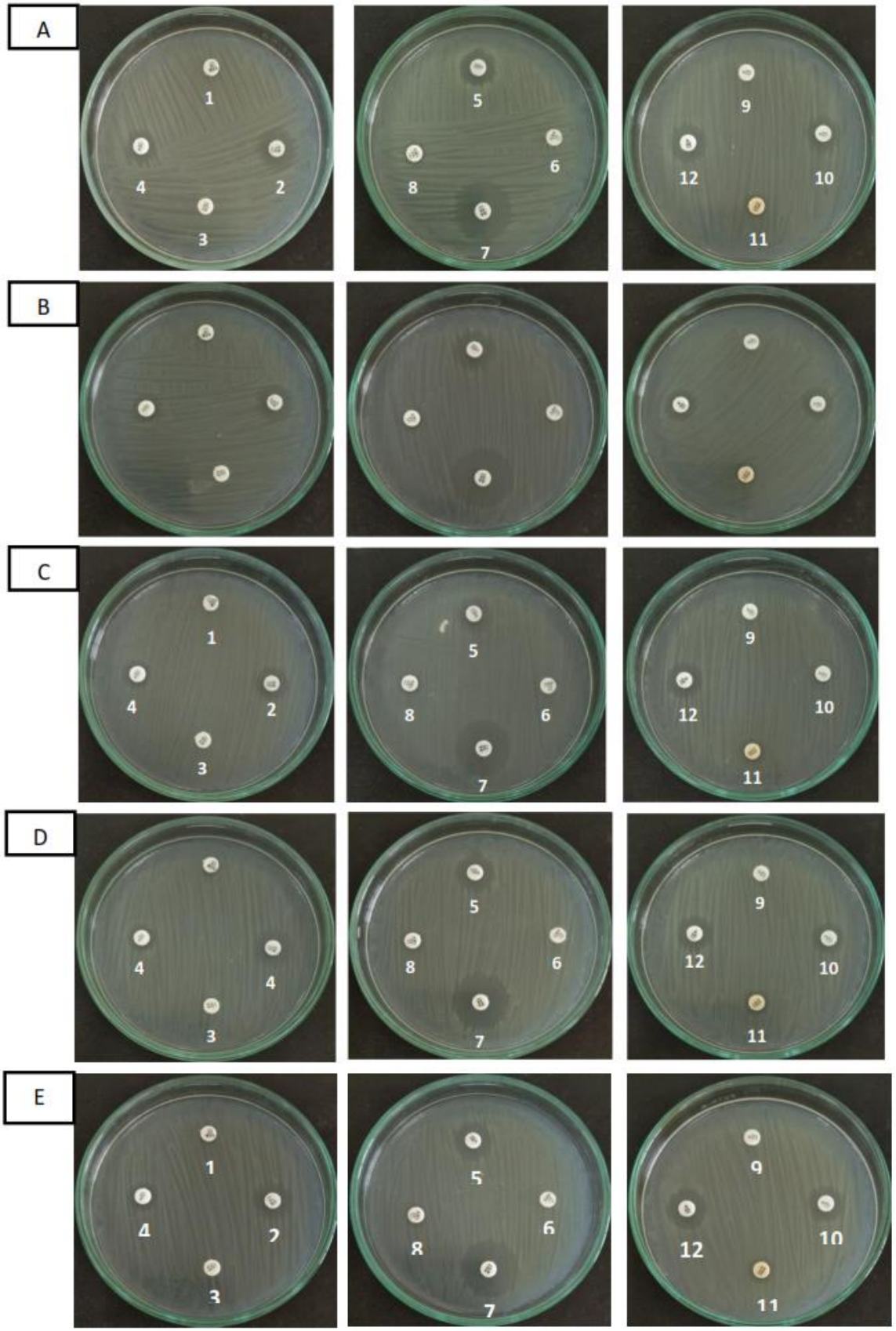
Tetracycline and Gentamycin also showed strong antibacterial activity, especially against isolates from Galala Market. Tetracycline had the largest inhibition zones in isolates G2.1 and G2.2, with diameters of 27 and 23mm, respectively. This antibiotic functions by inhibiting protein synthesis through binding to the bacterial 30S ribosomal subunit. Meanwhile, Gentamycin was effective against almost all isolates, with inhibition zones ranging from 14 to 20mm. As an aminoglycoside antibiotic, Gentamycin causes mRNA misreading, which disrupts essential bacterial protein synthesis and inhibits bacterial growth **(Gauba & Rahman, 2023)**.

Conversely, some antibiotics exhibited variable effectiveness depending on the tested isolates. For instance, Azithromycin was ineffective against isolates from Mardika Market and some isolates from Galala Market but demonstrated effectiveness against isolates G2.1 and G2.2, with inhibition zones of 16 and 12mm, respectively. This suggests that Azithromycin resistance may be strain-specific within *Vibrio* spp., potentially due to efflux pump mechanisms or target site modifications **(Tian *et al*., 2023; Zhang *et al*., 2024; Wang *et al*., 2025)**.

Table 1. Results of sensitivity tests of several antibiotics to smoked fish contaminant pathogens

No.	Types of antibiotics	Jamming zone diameter (mm)						
		Isolate M2.1	Isolate M2.2	Isolate G3.1	Isolate G3.2	Isolate G3.3	Isolate G2.1	Isolate G2.2
1.	Azithromycin	0	0	0	0	0	16	12
2.	Gentamycin	16	14	14	16	16	20	18
3.	Cephalothin	0	0	0	0	0	0	0
4.	Neomycin	13	14	12	14	12	15	12
5.	Chloramphenicol	15	15	10	16	15	19	15
6.	Ampicillin	0	0	0	0	0	0	0
7.	Nalidixic acid	24	25	22	24	24	11	10
8.	Amoxicillin	8	7	9	9	0	10	8
9.	Erythromycin	0	0	0	0	0	23	15
10.	Streptomycin	14	14	8	13	15	18	10
11.	Tetracycline	11	9	10	10	10	27	23
12.	Kanamycin	15	14	15	14	16	23	13

These findings indicate that *Vibrio* isolates from smoked fish exhibit a high level of resistance to several commonly used antibiotics for bacterial infections in humans. Factors such as excessive antibiotic use in aquatic environments, particularly in aquaculture, may contribute to the development of antibiotic resistance in pathogenic bacteria in coastal ecosystems **(Cabello *et al*., 2013)**. Therefore, this study highlights the importance of monitoring antibiotic usage and ensuring the quality control of smoked fish sold in traditional markets to prevent the spread of drug-resistant pathogenic bacteria.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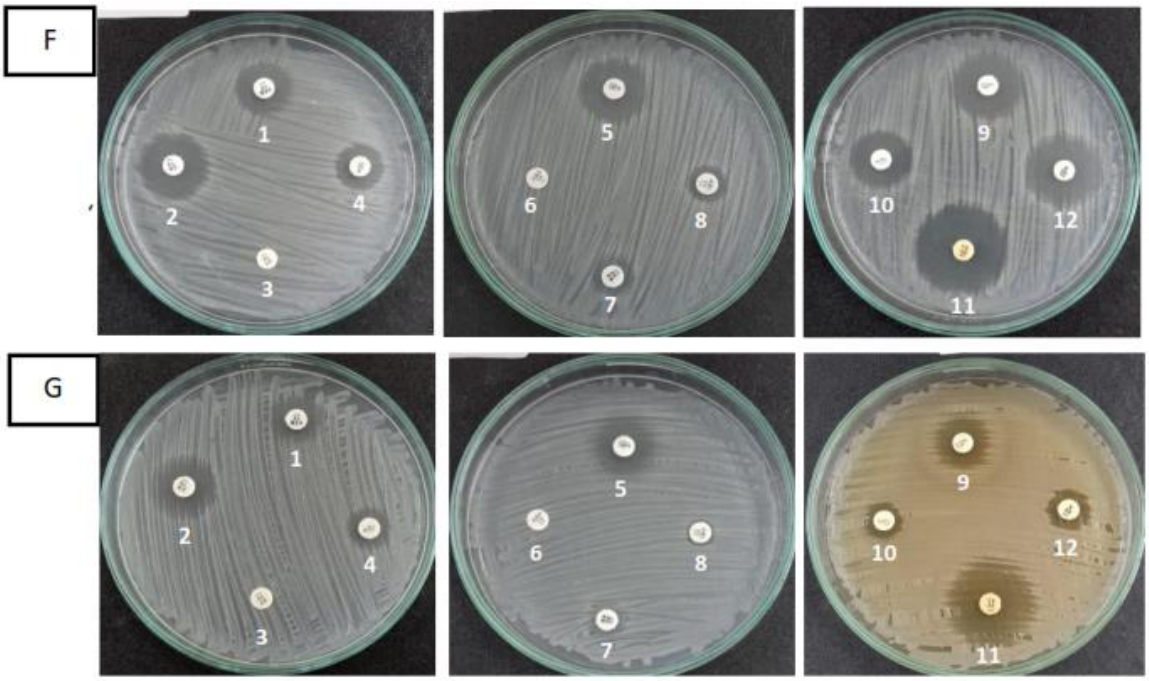

Fig. 2. Test the antibiotic sensitivity of smoked fish on several samples. **(A)** M2.1; **(B)** M2.2; **(C)** G3.1; **(D)** G3.2; **(E)** G3.3; **(F)** G2.1; **(G)** G2.2; (1) Azithromycin, (2) Gentamycin; (3) Cephalothin; (4) Neomycin; (5) Chloramphenicol; (6) Ampicillin, (7) Nalidixic acid, (8) Amoxicillin; (9) Erythromycin; (10) Streptomycin; (11) Tetracycline; (12) Kanamycin

Thus, this research provides valuable insights into the resistance and sensitivity patterns of *Vibrio* spp. to commonly used antibiotics. These findings serve as a foundation for food safety management, particularly in handling and processing smoked fish, and offer crucial information for health authorities in developing strategies to control bacterial infections that pose public health risks.

The antibiotic susceptibility test results, as presented in Table (1), can be further confirmed through the observation of inhibition zones on agar media. Fig. (2) illustrates the inhibition zone patterns formed around antibiotic discs, reflecting the effectiveness of each antibiotic against the tested bacterial isolates.

3. General discussion

This study highlights the presence of pathogenic microbes in smoked *Katsuwonus pelamis* sold in traditional markets in Ambon and their antibiotic resistance patterns. These findings reinforce evidence that inadequate processing and storage of fishery products can increase the risk of pathogenic bacterial contamination. Previous studies have demonstrated that *Vibrio parahaemolyticus* and *Vibrio cholerae* are the predominant microorganisms frequently found in seafood products, particularly those that have not undergone an adequate sterilization **(Oliver, 2015)**. Therefore, monitoring the processing methods of smoked fish is crucial to reducing health risks for consumers.

One of the contributing factors to high bacterial contamination is the suboptimal storage conditions in traditional markets. According to **DePaola *et al*. (2003)**, *Vibrio* bacteria can proliferate rapidly under warm temperatures and appropriate salinity levels. The results of this study indicate that smoked fish sold in Mardika Market and Galala Market exhibited higher contamination levels than those in Batu Meja Market, likely due to differences in storage methods and environmental hygiene. This aligns with the findings of **Huss *et al*. (2000)**, elucidating that unhygienic storage practices are a major factor in the spread of pathogenic microbes in seafood products.

Antibiotic resistance testing in this study revealed that bacterial isolates from smoked fish exhibited high resistance to several antibiotics, particularly β-lactams such as Ampicillin and Cephalothin. This phenomenon has been widely reported in previous studies, where *Vibrio* bacteria possess the ability to produce β-lactamase enzymes, which hydrolyze β-lactam antibiotics and reduce their efficacy **(Silvester *et al*., 2019)**. Such antibiotic resistance poses an increasing public health threat, especially if resistant pathogenic bacteria enter the human food chain.

Conversely, antibiotics such as Nalidixic Acid and Gentamicin demonstrated higher effectiveness against *Vibrio* isolates. This is attributed to their mechanisms of action, which inhibit bacterial DNA replication or protein synthesis, thereby suppressing the growth of pathogenic bacteria **(Azargun *et al*., 2020)**. However, the varying resistance patterns among isolates suggest that bacterial defense mechanisms may differ depending on environmental factors and prior antibiotic exposure.

From a food safety perspective, the findings of this study emphasize the importance of quality control for smoked fish sold in traditional markets. Improving hygiene standards, such as implementing lower storage temperatures and optimizing the smoking process, could help reduce bacterial contamination risks **(Huq *et al*., 2012)**. Additionally, educating fish vendors on the importance of proper sanitation practices in processing and storing smoked fish could be an effective measure in preventing the spread of pathogenic bacteria that pose a health risk to consumers.

Furthermore, the findings on antibiotic resistance highlight the need for stricter regulations on antibiotic use in the fisheries sector. The excessive use of antibiotics in aquaculture has been linked to the increased resistance of bacteria to various antibiotics, which can subsequently spread through the food chain **(Cabello *et al*., 2016)**. Therefore, controlling antibiotic use and developing alternative strategies, such as utilizing natural antimicrobial compounds, could serve as more sustainable approaches to addressing antibiotic resistance issues in aquatic environments.

Moreover, this study provides insights into how traditional fish processing practices contribute to the dynamics of pathogenic microbes in coastal environments. With the growing demand for smoked fish in various regions, regular microbiological monitoring is crucial to ensuring the safety of seafood products for public consumption **(Rakha *et al*., 2022)**. Additionally, the findings of this study could serve as a foundation for public

health policies in developing mitigation strategies against microbial contamination risks in fishery products.

In conclusion, this study underscores that the presence of pathogenic microbes in smoked fish sold in traditional markets in Ambon and their antibiotic resistance patterns are critical issues requiring further attention. A collaborative effort between the government, academia, and the fisheries industry is necessary to enhance food safety standards and mitigate the negative impacts of antibiotic resistance in aquatic environments. With a more integrated approach, the safety of fishery products can be better ensured, while also reducing the risk of spreading harmful pathogenic bacteria to the broader community.

CONCLUSION

This study reveals that smoked skipjack tuna (*Katsuwonus pelamis*) sold in traditional markets in Ambon is highly susceptible to contamination by pathogenic microbes, particularly *Vibrio parahaemolyticus* and *Vibrio cholerae*. Antibiotic resistance tests indicate that bacterial isolates exhibit high resistance to several antibiotics, especially β-lactams, which could pose significant public health risks. The primary factors contributing to this contamination are inadequate hygiene practices in storage and processing methods. Therefore, improving hygiene standards and implementing strict monitoring of smoked fish products are essential to reduce the risk of bacterial contamination. Additionally, controlling antibiotic use in the fisheries sector is crucial to preventing the escalation of bacterial resistance to drugs. Thus, this study underscores the importance of collaboration among government authorities, academia, and industry stakeholders in enhancing seafood safety and mitigating the negative impacts of antibiotic resistance in aquatic environments.

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