Study of Chloramphenicol Antibiotic Residues in Processed Food Products of Honey, Shrimp, and Fish in Sulawesi and Maluku

Ma'rifah Ebtasari[a,1,*], Alifah Nur Aini [a,2]

[a]Indonesian Food and Drug Authority Regional Office in Palu, Indonesian Food and Drug Authority, Jl. Undata No. 3, Palu, Central Sulawesi, 94111, Indonesia
[1] marifah.ebtasari@pom.go.id*;[2] alifah.nuraini@pom.go.id
* corresponding author

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ABSTRACT

Antibiotic residues in food products can cause significant public health problems. Using antibiotics in livestock farming can potentially cause antibiotic residues in animal-based foods. We conducted this study to determine the results of chloramphenicol residue testing in processed honey, shrimp, and fish food products in the Sulawesi and Maluku regions. This study can determine the distribution of test results based on business type. This study is expected to produce recommendations to stakeholders to minimize the use of chloramphenicol, which is not covered by the provisions. The research samples came from processed honey, shrimp, and fish food products distributed in the Central Sulawesi, Ambon, Sofifi, Mamuju, Morotai, and Tanimbar regions in 2021-2022. Testing was carried out at the laboratory of the Indonesian Food and Drug Authority Regional Office in Palu according to the analysis method of PPPOMN 071/PA/17 concerning the Determination of Chloramphenicol Residue Levels in Shrimp by ELISA and MA PPPOMN 11/PA/09 concerning the Determination of Chloramphenicol Residue Levels in Honey by ELISA. Based on the test results, processed honey, shrimp, and fish food products were found to contain chloramphenicol residues. Of the total 92 samples tested, there were 15 honey products, four processed shrimp food products, and seven processed fish food products that did not meet the requirements (TMS). Chloramphenicol residue levels ranged from 0.01 to 0.09 ppb. The number of TMS samples in honey and fish food products with PIRT distribution permits was more significant in percentage when compared to products with MD/ML distribution permits. The rate of TMS in honey and processed fish food products with PIRT distribution permits was 53% and 21%, respectively. Further research on the source of chloramphenicol residues and the origin of honey, shrimp, and fish-producing areas needs to be conducted by relevant institutions or academics. In addition, it is necessary to conduct socialization and education among the public regarding the use of chloramphenicol antibiotics according to the provisions for both the treatment of diseases and livestock practices to minimize the presence of chloramphenicol residues in processed food products.

Residu antibiotik dalam produk makanan dapat menimbulkan masalah kesehatan masyarakat yang signifikan. Praktek penggunaan antibiotik pada budidaya ternak berpotensi menimbulkan residu antibiotik pada pangan berbahan dasar dari hewan. Penelitian ini dilakukan untuk mengetahui hasil pengujian residu kloramfenikol pada produk pangan olahan madu, udang, dan ikan di wilayah Sulawesi dan Maluku. Selain itu, melalui penelitian ini dapat diketahui sebaran hasil uji berdasarkan jenis izin edar. Pada akhirnya diharapkan menghasilkan suatu rekomendasi kepada stakeholder terkait untuk meminimalisir penggunaan kloramfenikol yang tidak sesuai ketentuan. Sampel penelitian berasal dari produk pangan olahan madu, udang, dan ikan yang terdistribusi di wilayah Sulawesi Tengah, Ambon, Sofifi, Mamuju, Morotai, dan Tanimbar tahun 2021-2022. Pengujian dilakukan di laboratorium Balai POM Palu sesuai metode analisis PPPOMN 071/PA/17 tentang Penetapan Kadar Residu Kloramfenikol dalam Udang secara ELISA dan MA PPPOMN 11/PA/09 tentang Penetapan Kadar Residu Kloramfenikol dalam Madu secara ELISA. Berdasarkan hasil pengujian ditemukan produk pangan olahan madu, udang, dan ikan yang mengandung residu kloramfenikol. Dari total 92 sampel yang diuji, terdapat 15 produk madu, 4 produk pangan olahan udang, dan 7 produk pangan olahan ikan yang tidak memenuhi syarat. Kadar residu kloramfenikol berkisar dari 0,01-0,09 ppb. Jumlah sampel

TMS pada produk pangan madu dan ikan dengan izin edar PIRT lebih besar persentasenya jika dibandingkan dengan produk dengan izin edar MD/ML. Presentase TMS produk madu dan pangan olahan ikan dengan izin edar PIRT secara berturut -turut 53% dan 21 %. Riset lebih lanjut mengenai sumber residu kloramfenikol dan asal wilayah penghasil madu, udang, dan ikan perlu dilakukan oleh lembaga atau akademisi terkait. Selain itu, perlu dilakukan sosialisasi dan edukasi kepada masyarakat mengenai penggunaan antibiotik kloramfenikol sesuai ketentuan baik untuk pengobatan penyakit ataupun praktik peternakan untuk meminimalisir adanya residu kloramfenikol pada produk pangan olahan.

Keywords: *antibiotic residue, chloramphenicol, honey, processed fish, processed shrimp*
Kata Kunci: residu antibiotik**,** kloramfenikol, madu, olahan ikan, olahan udang.

1. Introduction

Food safety has become an essential issue in the era of globalization and international trade; consumers are increasingly aware of the quality and safety of products to be consumed. One aspect that is important to note is the presence of antibiotic residues. In 2014-2023, there were 77 cases of rejection of fresh fishery food exports from the US Food and Drug Administration (US FDA) due to residues of chloramphenicol, nitrofurans, and veterinary drugs. (BPOM, 2023)

Antibiotic residues in food products pose a public health problem, as they can trigger antibiotic resistance and adversely affect consumer health. (Menkem et al., 2019). According to the Food and Agriculture Organization (FAO) (2021), 700,000 people die from infectious diseases every year due to antibiotic-resistant microbes. (BPOM, 2023).

Exposure to antibiotic residues can occur from consuming seafood that contains residues or while processing products that contain residues. (Aly and Albutti, 2014). According to FAO and the World Veterinary Association, residues of chloramphenicol and its metabolites are hazardous if found in food. (Wang et al., 2021). One of the effects that can be caused by residual chloramphenicol in food is spinal cord toxicity. (Nisha, 2008)

According to Adi Saputra and Arfi (2021), consuming shrimp containing chloramphenicol residues in excess for a long time causes spinal cord depression. This can disrupt the red blood cell production, causing aplastic or hypoplastic anemia, thrombocytopenia, and granulocytopenia. In addition, several studies have shown a direct correlation between antibiotic resistance in humans and animals and the use of antibiotics in pet food. (Aly and Albutti, 2014).

Chloramphenicol is an antibiotic used in livestock. Apart from therapeutic purposes, it is also used as a growth-inducing substance. Usually, it is added to artificial feed. (Wibowo et al., 2010). Antibiotics are commonly used in beekeeping to treat bacterial infections that attack honey bee larvae.

The Indonesian government has realized the potential dangers of chloramphenicol contaminating food, so relevant ministries and agencies have made several regulations. The Indonesian Ministry of Health regulates the chloramphenicol contamination limit in Permenkes Number 33 of 2012 (Kemenkes, 2012) . In addition, the Indonesian Food and Drug Authority (BPOM) regulates the use of chloramphenicol in PerBPOM Number 7 of 2018 regarding Prohibited Raw Materials in Processed Food. (BPOM, 2018). The National Standardization Agency also regulates the maximum limits of microbial contaminants and residues in food ingredients of animal origin in SNI 01-6366 of 2000. (BSN, 2000).

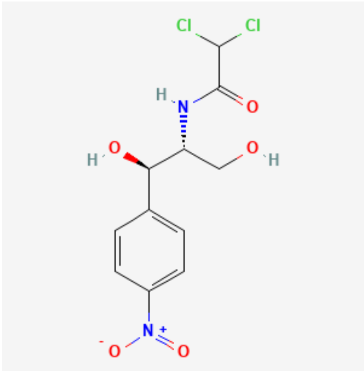

Figure 1. Chemical structure of chloramphenicol antibiotic
(source: *National Center for Biotechnology Information* (2023)).

To ensure that food products are safe, BPOM supervises products both before they are distributed and after they are on the market. Products in circulation were randomly sampled for laboratory testing according to requirements. The test results on honey, shrimp, and fish processed food products at the Indonesian Food and Drug Authority Regional Office in Palu in 2021-2022 showed that there were samples that did not meet the requirements (TMS) for the chloramphenicol residue test. The Indonesian Food and Drug Authority Regional Office in Palu conducted chloramphenicol residue testing of honey, shrimp, and fish processed food products from the Central Sulawesi, Ambon, Sofifi, Mamuju, Morotai, and Tanimbar regions. The comprehensive coverage of the sampling area provides results that are pretty representative of chloramphenicol residue tests on food products in circulation.

Previous studies have analyzed chloramphenicol residue levels in honey, shrimp, and carp products. The methods used were Enzyme-Linked Immunosorbent Assay (ELISA) and High-Performance Liquid Chromatography (HPLC) (Wibowo et al., 2010). Other research on different regions has been carried out, such as the Analysis of Chloramphenicol Antibiotic Residue Levels in *Vannamei* Shrimp (*Litopenaeus Vannamei*) in Bangkalan Regency with the ELISA Method by Sari and Hafiludin (2023). Research on chloramphenicol residue levels in processed food products in the Sulawesi and Maluku regions has never been done. Thus, it is necessary to conduct this research to find out how big the risk of processed food made from honey, shrimp, and fish containing chloramphenicol residues is.

This study will analyze the test results of chloramphenicol residue content in processed food products of honey, shrimp, and fish in Sulawesi and Maluku regions, analyze the type of business in samples that do not meet the requirements (TMS), and the distribution of TMS samples based on the type of business.

2. Methodology

This research is a retrospective quantitative analysis. All research samples came from processed food products of honey, shrimp, and fish tested by the food laboratory of the Indonesian Food and Drug Authority Regional Office in Palu in 2021-2022. Honey samples were in the form of honey products in bottles or cassettes. Shrimp-based samples are in the form of cikua products and shrimp meatballs. The fish samples are shredded products, sardines in cans, fish balls, tempura, and fish pempek. In 2021, the samples tested came from processed food products distributed in the Central Sulawesi region. In 2022, the samples came from processed food products distributed in the Central Sulawesi, Ambon, Sofifi, Mamuju, Morotai, and Tanimbar regions. Sampling is carried out randomly at distribution facilities scattered in the area. The random sampling method can provide an overview of samples circulating in the community.

The test was carried out using the PPPOMN 071/PA/17 analytical method on determining Chloramphenicol Residue Levels in Shrimp by ELISA and the PPPOMN 11/PA/09 analytical method on determining Chloramphenicol Residue Levels in Honey by ELISA. In the ELISA analysis, a positive control is used to ensure the validity of the test results. In addition, a standard series is used to ensure the validity of the results.

The data obtained were then categorized based on the conclusion of the test results, namely samples that meet the requirements (MS) and samples that do not (TMS). The samples were grouped based on the type of business to see the profile of TMS samples. The first group is nationally produced TMS products with Domestic Food (MD) and Foreign Food (ML) distribution permits. The second group of locally produced TMS products had a Home Industry Food (PIRT) distribution permit. The two data groups were analyzed descriptively to determine the trend and distribution of the data.

3. Results and Discussion

Based on the test results of the Indonesian Food and Drug Authority Regional Office in Palu, chloramphenicol residues were still found in several samples. Details of the test results of chloramphenicol residue levels are listed in Table 1.

Table 1. Chloramphenicol Residue Testing Results of Indonesian Food and Drug Authority Regional Office in Palu in 2021-2022

Types of processed food products	**Total Samples Tested**	**Product Qualified (MS)**		**Unqualified Product (TMS)**	
		Total	**MS percentage (%)**	**Total**	**TMS Percentage (%)**
2021					
Honey	13	0	0,00	13	100,00
Shrimp	5	1	20,00	4	80,00
Fish	0	0	0,00	0	0,00
Total	**18**	**1**	**5,56**	**17**	**94,44**
2022					
Honey	26	24	92,31	2	7,69
Shrimp	8	8	100,00	0	0,00
Fish	40	33	82,50	7	17,50
Total	**74**	**65**	**87,84**	**9**	**12,16**

The results of chloramphenicol residue testing in honey experienced a decrease in TMS samples from 100.00% (2021) to 7.69% (2022). Likewise, the results of chloramphenicol residue testing in shrimp experienced a decrease in TMS samples from 80.00% (2021) to 0.00% (2022). Despite the decrease in TMS samples, the threat of chloramphenicol residues in these processed food products still exists. This is in line with the results of research by Sari and Hafiludin (2023), where chloramphenicol residues were still found in *Vannamaei* shrimp. In addition, Luo et al. (2021) Also, chloramphenicol residues in shellfish, fish, and shrimp were found based on research conducted in South China waters. This indicates that chloramphenicol residues in food products derived from aquaculture are still a real threat. Using antibiotics in animal farming can result in antibiotic residues in fresh food from fish (PSAI), which are eventually carried over into processed food products. (BPOM, 2023).

Tables 2, 3, and 4 list the detailed test results for chloramphenicol residue levels in honey, shrimp, and fish products.

Table 2. Testing Results of Chloramphenicol Residue Levels in Honey Based on Business Type

Tested Products	Business Type	Chloramphenicol residual level (ppb)	Test Results
Honey 1	PIRT	0,02	TMS
Honey 2		0,02	TMS
Honey 3		0,02	TMS
Honey 4		0,01	TMS
Honey 5		0,01	TMS
Honey 6		0,01	TMS
Honey 7		0,02	TMS
Honey 13		0,02	TMS
Honey 20		0,08	TMS
Honey 24		0,05	TMS
Honey 8	MD/ML	0,04	TMS
Honey 9		0,05	TMS
Honey 10		0,05	TMS
Honey 11		0,04	TMS
Honey 12		0,06	TMS

Table 3. Testing Results of Chloramphenicol Residue Levels in Shrimp by Business Type

Tested Products	Business Type	Chloramphenicol residual level (ppb)	Test Results
Shrimp 1	MD/ML	0,02	TMS
Shrimp 3		0,02	TMS
Shrimp 4		0,03	TMS
Shrimp 5		0,02	TMS

Table 4. Chloramphenicol Residue Test Results in Fish by Business Type

Tested Products	Business Type	Chloramphenicol residual level (ppb)	Test Results
Fish 22	PIRT	0,01	TMS
Fish 23		0,01	TMS
Fish 38		0,01	TMS
Fish 13	MD/ML	0,05	TMS
Fish 14		0,09	TMS
Fish 17		0,01	TMS
Fish 35		0,01	TMS

ppb = *part per billion* (µg/Kg)
TMS = Does not meet the requirements for chloramphenicol residue based on Permenkes No. 33 of 2012
PIRT = Home Industry Food
MD= Domestic Food (domestically produced food products)
ML= Outside Food (imported food products produced abroad)

Honey residue levels detected ranged from 0.01 to 0.08 ppb, processed shrimp food products ranged from 0.02 to 0.03 ppb, and processed fish food products ranged from 0.01 to 0.09 ppb. Based on the results of research conducted by Sari and Hafiludin (2023), chloramphenicol antibiotic residue levels in *Vannamei* shrimp in Bangkalan Regency ranged from 0.006 to 0.027 ppb. The results of research conducted by Adi Saputra and Arfi (2021) showed that the residual levels of chloramphenicol antibiotics in Windu shrimp from the Kutaradja Ocean Fishing Port (PPS) were 2.4634 ppb—likewise, the results of research conducted by Virgianti et al. (2022). The residual levels of chloramphenicol antibiotics in white shrimp ranged from 0.12 to 0.14 ppb. From the results of these studies, it appears that chloramphenicol residue levels in fresh shrimp are higher than in processed shrimp food products. The processing of food products may be able to reduce chloramphenicol residue levels. The process of washing repeatedly or heating at certain temperatures can be a factor in significantly reducing chloramphenicol residue levels. Research conducted by

Gowtham et al. (2020) showed that food processing reduces antibiotic residue levels that vary in food products.

Research on the use of antibiotics in beekeeping, which is at risk of causing antibiotic residues in honey, has been carried out. The results of a study entitled Detection of Antibiotic Residues in Blossom Honeys from Different Regions in Turkey by LC-MS/MS Method showed that of the 80 honey samples tested, various types of antibiotic residues were still found in them. (Demirhan and Demirhan 2022). Several other international reports also reported antibiotic residues in honey samples. Antibiotics can cause antibiotic residues in honey to treat bee diseases caused by bacteria. Antibiotic residues mostly come from the environment and improper beekeeping practices. (Al-Waili et al., 2012). Honey products do not undergo many processes during production, so the levels are not significantly different after harvesting or packaging.

Apart from the composition of the main ingredient, residual chloramphenicol can come from other raw materials used. For this reason, the composition label listed on the product packaging was observed. Other raw materials suspected of containing chloramphenicol residues are milk and eggs. Of the 11 samples of shrimp/fish-based processed food products with TMS test results, four samples included egg white, either egg white powder, premix flour, or *batter* flour containing eggs. Chloramphenicol residues can be found in chicken eggs due to the use of antibiotics as growth promoters for chicken animals. Chicken farmers intentionally add chloramphenicol to these animals' artificial feeds and drinks.

Furthermore, this study analyzed the type of business with chloramphenicol residue content. Business types for processed food were divided into two major groups, namely national-scale and local-scale business types. The national scale includes products with BPOM MD and BPOM ML distribution permit numbers. The local-scale category includes products with PIRT distribution permits. The distribution of TMS products based on the type of distribution permit is listed in Figure 2.

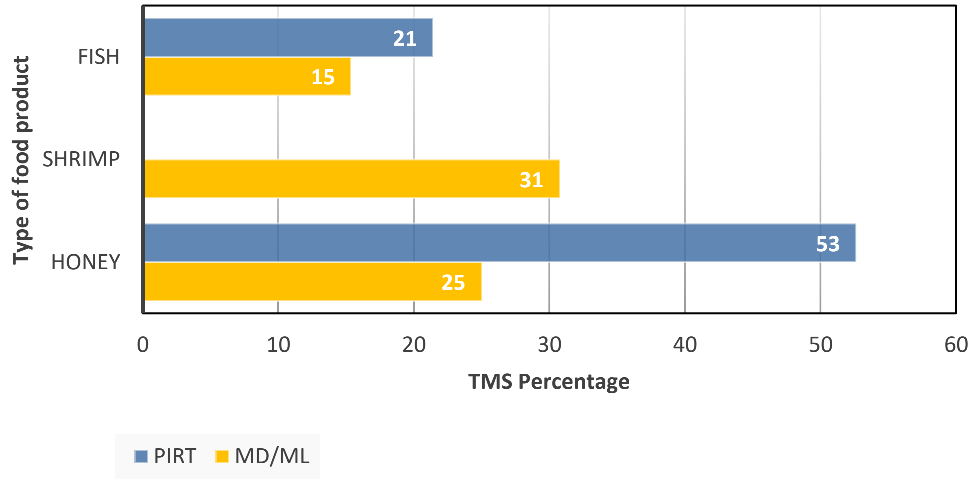

Figure 2. Percentage of TMS Samples by Circulation Permit Category
PIRT = Home Industry Food
MD= Domestic Food (domestically produced food products)
ML= Outside Food (imported food products produced abroad)

Samples of TMS honey with a PIRT distribution permit amounted to 21%, which is higher than the MD/ML distribution permit (15%). Most of the honey with PIRT distribution permits were sourced from areas around Sulawesi and Maluku. Improper beekeeping practices can lead to chloramphenicol residues in honey. According to Al Waili et al. (2012), antibiotic residues consumed with food and honey can cause resistance in bacterial populations. This global public health issue continues to be a challenging problem. (Al-Waili et al., 2012). It is challenging for the government to investigate local honey farming practices in Sulawesi and Maluku.

The shrimp-based food products tested by the Indonesian Food and Drug Authority Regional Office in Palu are products with the MD/ML distribution permit number, so they cannot be compared with local products. Both types of products still have the same risk of chloramphenicol residue. Products with an MD/ML distribution permit are not guaranteed to be free from the risk of chloramphenicol residues. This concerns relevant stakeholders, who must be more prudent in using chloramphenicol. Likewise, for PIRT products, local raw materials should be more selective so that the products are safe for public consumption.

Samples of TMS-processed fish products with PIRT distribution permits were also more significant than those with MD/ML distribution permits. The raw material source area can be one of the factors causing samples to contain chloramphenicol residues. Of the 7 TMS samples, three were sampled from Central Sulawesi, two in Ambon, and two in Mamuju. Central Sulawesi and Mamuju samples were local products with PIRT distribution permit numbers. PIRT-scale businesses usually use fish raw materials from the waters of the area. Based on the description of the composition label, it appears that the fish used is marine fish, not farmed fish. This needs to be a concern, especially for the governments of Central Sulawesi and West Sulawesi (Mamuju), to be more aware of chloramphenicol contamination in their regional marine waters.

The use of antibiotics in the treatment of fish or farmed animals has been commonly practiced. This is regulated by the Ministry of Marine Affairs and Fisheries Number 1 of 2019, which regulates the types of antimicrobials permitted in fish farming. However, the use should be under the supervision of a veterinarian. Inappropriate antibiotic use practices can trigger antibiotic residues in food products made from aquaculture. (BPOM, 2023).

The Indonesian Food and Drug Authority (BPOM), which grants distribution permits for processed food products, has set product criteria that must be met. These criteria are regulated in BPOM Regulation Number 27 of 2017 Regarding the Registration of Processed Food. The first is safety criteria, including physical, chemical, and biological safety. Processed food that will be circulated must be free from these three contaminants. In addition, food additives (BTP) and auxiliary materials must be used according to applicable requirements. The second criterion is quality requirements according to predetermined requirements. Third, nutritional criteria must be met; the product must be ensured to contain nutritional value by the requirements before obtaining a distribution permit number.

In addition to these three criteria, the registered processed food must also meet the labelling requirements. Production facilities must also meet the requirements of suitable processed food production methods (CPPOB). Implementing some of these regulations is expected to ensure that MD/ML and PIRT products are of the same quality. To improve the competitiveness of PIRT products, household food industries, before producing processed food, must have a certificate of processed food production for household industries by the provisions of laws and regulations. Basic knowledge about food safety is expected to be a provision for producers to produce quality products.

All products that had been tested were products that obtained MD/ML or PIRT numbers, indicating that the producers followed good CPPOB. However, honey, shrimp, and fish products were still found to contain chloramphenicol residues. The presence of this substance cannot be confirmed as coming from environmental contamination or deliberately added by honey, shrimp, and fish suppliers. Further research on the source of chloramphenicol residues in honey, shrimp, and fish must be conducted by relevant institutions or academics to minimise chloramphenicol residues in food products.

Related institutions or academics need to conduct further research on the source of chloramphenicol residues in honey, shrimp, and fish. In addition, it is necessary to socialize and educate the public regarding using chloramphenicol according to the provisions for animals and humans to reduce chloramphenicol residues in food products spread across Sulawesi and Maluku. In addition, The Indonesian Food and Drug Authority (BPOM) can consider making a policy of listing the source of raw materials on product labels so that it is easier to trace if chloramphenicol residue results exceed the predetermined threshold.

4. Conclusion

The results of testing the residual chloramphenicol content of the laboratory of the Indonesian Food and Drug Authority Regional Office in Palu in 2021-2022 from a total of 92 tests showed that there were 15 samples of honey products, four samples of processed shrimp food products, and seven samples of processed fish food products that did not meet the requirements (TMS). Chloramphenicol residue levels in the samples ranged from 0.01 to 0.09 ppb. Honey and processed fish products with PIRT distribution permits showed a higher percentage of TMS than those with MD/ML distribution permits. Raw materials for honey, shrimp, and fish from Sulawesi and Maluku must be studied further to determine the primary source of chloramphenicol residues. This is important as a basis for policy-making in maintaining the quality of processed honey, shrimp, and fish products.

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