Chloramphenicol Residues and Bacterial Contamination in Farmed African Catfish (*Clarias gariepinus*) from Banyuwangi Traditional Markets: A Risk Assessment

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Abstract

This study aimed to determine chloramphenicol residues and bacterial contamination (Aerobic Plate Count-APC, *Escherichia coli* count, *Salmonella* sp., and *Vibrio cholerae*) in farmed African catfish (*Clarias gariepinus*) that are marketed in Banyuwangi, Indonesia. A total of ninety samples of *C. gariepinus* were collected from several markets in Banyuwangi, namely, Kertosari, Blambangan, and Banyuwangi Kota. Using standard procedures, APC, *E. coli* count, *Salmonella* sp., *V. cholerae*, and chloramphenicol residues were determined. 36.37% of samples from Kertosari markets; 23.33% of samples from Banyuwangi Kota markets; and 16.67% of samples from Blambangan markets contained chloramphenicol residues, but less than 0.3 ppb. Only 40% of samples from Kertosari markets, 36.67% of samples from Banyuwangi Kota markets, and 26.67% of samples from Blambangan markets were contaminated by *E. coli*, but were less than 3 $MPNg^{-1.}$ The highest APC was from Banyuwangi Kota, followed by Kertosari and Blambangan. No samples were contaminated by *Salmonella* sp. and *V. cholerae* from any of the markets, and there was no significant difference between all markets in APC, *E. coli* count, or chloramphenicol residues. All farmed catfish marketed in Banyuwangi were safe to eat because no samples exceeded the maximum chloramphenicol residue and bacterial contaminant standards. Further studies are needed to detect other antibiotic residues used in aquaculture, including tetracycline, sulphonamide, enrofloxacin, and nitrofuran.

Keywords: bacterial contamination, Banyuwangi traditional markets, chloramphenicol residues, *Clarias gariepinus*

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INTRODUCTION

Increasing the demand for African catfish (*Clarias gariepinus*) for consumption every year encourages the intensive system. During 2011–2019, the production of farmed catfish in Indonesia increased to 190.78% (Ministry of Maritime Affairs and Fisheries, 2020). One of the high-risk factors in this system was fish mortality caused by infectious diseases. Infectious diseases are often associated with stress conditions because of their high density, ammonia concentration, and low immunity defenses (Assefa and Abunna, 2018). Therefore, the utilization of antibiotics, especially chloramphenicol (CAP), as a drug of choice to prevent or treat bacterial disease in farmed catfish (Chuah *et al*., 2016).

Currently, chloramphenicol has been banned because it can cause serious problems, including residues in fish meat and antibiotic resistance in humans (Lulijwa *et al*., 2020). Furthermore, chloramphenicol residues in humans can cause digestive disease, anemia, allergy, bone disruption (Prajwal *et al*., 2017), optic neuropathy, and brain abscess in varying degrees of severity and clinical presentations (Jayalakshmi *et al*., 2017). Not only residues of antibiotics in fish meat but also bacterial contamination caused the disease in humans and decreased the quality of fish meat (Roca-saavedra *et al*., 2016). Fish meat has promiscuous connective tissue, so the amino acid content is easier for bacteria's metabolism and production of ammonia, biogenic amines, organic acids, ketones, and sulfur components (Novoslavskij *et*

al., 2016). Several foodborne pathogens associated with fish meat, including *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae, V. parahemolyticus, Salmonella* sp., *Listeria monocytogenes, Clostridium botulinum,* and *Shigella* spp. (Gauthier, 2015), cause diarrhea, digestive disruption, food intoxication, and human mortality because of their toxicants (Antunes *et al*., 2019; Jääskeläinen *et al*., 2019; Sheng and Wang, 2021).

Previous studies on the detection of chloramphenicol residues in fish meat have been reported in tilapia (*Oreochromis niloticus*) fillets in Brazil (Bortolotte *et al*., 2021); sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) in Turkey (Doğan *et al*., 2020); and seafood products in Shenzen, South China (Luo *et al*., 2021). Several studies reported bacterial contamination in fish meat, including *Bangasius hypophanmus* (Basa), *Mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel), and *Sparus aurata* (Denise) (Ia and Ta, 2017); seafood products marketed in Yucatan Peninsula, Mexico (Jorge *et al*., 2016); salmon fillets (Møretrø *et al*., 2016); packaged yellowfish tuna (*Thunnus albacares*) and salmon (*Salmo salar*) (Jääskeläinen *et al*., 2019); catfish (*Clarias* sp.) and tilapia (*Tilapia mossambica*) (Budiati *et al*., 2015).

In Banyuwangi, not many studies were done on this subject, and there is currently no report of antimicrobial residues and microbial contamination in African catfish raised in Banyuwangi. Therefore, this study aimed to determine chloramphenicol residues and bacterial contamination of marketed African catfish from traditional markets in Banyuwangi City. This result could be used to improve the hygiene and safety of fishery products concerning human health.

MATERIALS AND METHODS

Ethical Approval

This study did not require animal ethics approval. All procedures were carried out based on standard operating procedures (SOP).

Study Period and Location

This study was conducted from April to November 2018 in the Instrumental Laboratory, Faculty of Health, Medicine and Life Sciences, Universitas Airlangga, Banyuwangi, Indonesia. Fish samples were collected from several fish markets in Banyuwangi City.

Sample Collection

A total of ninety samples of live African catfish were collected randomly from April to November 2018 from three traditional markets in Banyuwangi City, consisting of Kertosari, Blambangan, and Banyuwangi markets, respectively. Samples were placed in a sterile plastic bag and kept in a coolbox at approximately 4°C during transportation to the laboratory.

Determination of Chloramphenicol Residues

A total of 150 g of fillet was homogenized and accurately weighted into the 4 centrifuge tubes to approximately 10 g. Aquadest (4 mL) was added to each tube, vortexed (Thermo, USA) for 3 min, and kept at 27°C for 10 min. Ethyl acetate (12 mL) was added to the tube, vortexed (Thermo, USA) for 5 min, and then centrifuged (Thermo, USA) at 4000 rpm for 3 minutes until separated into three layers. An 8.4 mL aliquot of ethyl acetate supernatant (upper layer) was dried at 50°C under a weak steam of N_2. The residue was suspended in a 2.8 mL n-hexane and chloroform (1:1) mixture. Then 1.4 mL of 2% ethanol was added, vortexed (Thermo, USA) for 3 minutes, and centrifuged (Thermo, USA) at 4000 rpm for 10 min. One hundred µL of the ethanolic (upper) layer was used and analyzed by high-performance liquid chromatography (HPLC). The HPLC system was equipped with a quaternary pump on a silent phase C18 (150 mm × 4.6 mm inside diameter (i.d.), particle size 5 µm). The mobile phase consisted of a mixture of methanol and water (40:60, v/v) with a flow rate of 1.0 mL/min. The injection volume was 5, 10, or 20 µL and the column temperature was set at 30°C. CAP was detected by its UV absorbance at a wavelength of 277 nm and quantified by comparison with the standard under the

Indonesian Ministry of Maritime Affairs and Fisheries Regulation 39/PERMEN-KP/2019.

Determination of Aerobic Plate Count

Twenty-five grams of catfish meat were mixed with 225 mL of 0.1% peptone water (PW, Sigma Aldrich, St. Louis, USA) and homogenized using a stomacher (Interscience, Singapore) for 2 min. The dilution series was made from 10^{-1} to 10^{-6} by pipetting 1 mL of aliquot and mixing it with 9 mL of 0.1% PW. 100 µL of aliquot was spread on Tryptic Soy Agar (TSA, Oxoid, Hampshire, UK) and incubated at 37°C for 24–48 hours. The total aerobic count was expressed as $CFUmL^{-1}$ and quantified by comparison with the standard ISO 7388-2009.

Determination of *E. coli*

Twenty-five grams of samples were accurately weighed, transferred into a sterile glass bottle, and then added to a 225 mL sterile Butterfield's Phosphate Buffer (BPB, Merck, Darmstadt, Germany), subsequently homogenized by blenders for 2 min. The dilution was prepared by mixing a 1 mL aliquot with 9 mL BPB until 10^{-3}. A total of 1 mL diluent was pipetted and transferred to the three tubes containing 9 mL of Lauryl Sulfate Tryptose Broth (LSTB, HiMedia, Mumbai, India) and then incubated at 35°C for 24–48 hours. Approximately 10 µL of LSTB-positive tubes (turbid tubes with gas) were inoculated into *E. coli* broth (ECB, HiMedia, Mumbai, India) and incubated at 45.5°C for 24–48 hours. The positive reaction was identified by a turbid tube filled with gas. The confirmation test was performed by pipetting 1 mL mixture from the positive ECB tube and spreading it onto Levine Edosin-Methylene Blue Agar (L-EMBA, HiMedia, Mumbai, India) and incubated at 35°C for 24 hours. Positive L-EMBA (metallic green colony with black center) was inoculated on Plate Count Agar (HiMedia, Mumbai, India) for morphology and biochemistry testing (Indole, MR-VP, Citrate/IMViC) for *E. coli* confirmation. The *E. coli* counts were expressed as MPN g^{-1} and quantified by comparison with the standard ISO 7388-2009.

Determination of *Salmonella* sp.

A total of 25 g of each sample was added to sterile 225 mL Lactose Broth (LB, Sigma Aldrich, St. Loius, USA) and homogenized for 2 min, then transferred to a sterile bottle and incubated at 35°C for 24 hours. A total of 1 mL solution was inoculated into 10 mL Tetra Thionate Broth (TTB, HiMedia, Mumbai, India), 0.1–10 mL Rappaport Vassiliadis (RV, HiMedia, Mumbai, India), and 1–10 mL Selenite Cystine Broth (SCB, HiMedia, Mumbai, India) and incubated at 35°C for 24 hours respectively. Isolates from each medium were spread onto Hectoen Enteric (HE, Oxoid, Hampshire, UK), Xylose Lysine Deoxycholate (XLD, HiMedia, Mumbai, India), and Bismuth Sulfite Agar (BSA, HiMedia, Mumbai, India), and incubated at 35°C for 24 hours. The *Salmonella* sp. colony in HE was green with black center, in XLD it was pink and in BSA it was brown. Biochemistry tests (Indole, MR-VP, Citrate/IMViC, triple sugar iron agar (TSIA), lysine iron agar (LIA), and sugar media) were used to confirm the *Salmonella* sp. colony.

Determination of *V. cholerae*

Twenty-five grams of samples were mixed with 225 mL of alkaline peptone water (APW, HiMedia, Mumbai India) in an Erlenmeyer flask, mixed well, and incubated at 35–37°C for 6–8 hours. One mL of solution was pipetted and spread to thiosulfate-citrate bile salt sucrose (TCBS, Merck, Darmstadt, Germany) and then incubated at 37°C for 18–24 hours. The *V. cholerae* colony in TCBS was characterized as a smooth, yellow circle with a turbid center of 2–3 mm diameter. To verify the presence of the *V. cholerae* colony, confirmation tests involving Gram staining, Triple Sugar Iron Agar (TSIA), and Lysine Iron Agar (LIA) were necessary.

Statistical Analysis

The statistical analysis of chloramphenicol residues, APC, and *E. coli* count was determined using One Way ANOVA and Duncan Multiple Range Test (DMRT) (SPSS software for Windows version 18, Chicago, USA) to verify mean differences ($p < 0.05$) among the various markets in Banyuwangi. All data are reported as

the mean value ± standard deviation (SD). While the *Salmonella* sp. and *V. cholerae* were analyzed descriptively with a table.

RESULTS AND DISCUSSION

The detected and measured levels of APC, *E. coli*, *Salmonella* sp., *V. cholerae*, and chloramphenicol residues in the flesh of farmed African catfish from 3 traditional markets in Banyuwangi are presented in Table 1. A total of 23 (25.56%) samples were detected to have chloramphenicol residues ranging from 0.07 ± 0.01 ppb. A total sample from Kertosari market contained chloramphenicol residues (36.67%) more than those from Banyuwangi Kota (23.33%) and Blambangan market (16.67%). However, there were no samples that exceeded the standard and no significant difference ($p > 0.05$) among chloramphenicol residues between all markets.

Table 1. Occurrence and levels of APC, *E. coli* count, *Salmonella* sp., *V. cholerae*, and chloramphenicol residues in farmed African catfish (*C. gariepinus*) from different Banyuwangi markets

Parameter	Markets	Total sample	Number of positive samples (%)	Mean ± SD[1]	Number of samples over standard (%)[3]
Chloramphenicol residues (ppb)	Kertosari	30	11 (36.37)	0.06 ± 0.01[a]	0 (0)
	Blambangan	30	5 (16.67)	0.06 ± 0.01[a]	0 (0)
	Banyuwangi Kota	30	7 (23.33)	0.09 ± 0.01[a]	0 (0)
APC ($\times 10^5$ CFU mL^{-1})	Kertosari	30	30 (100)	5.67 ± 0.39[a]	30 (100)
	Blambangan	30	30 (100)	4.75 ± 0.38[a]	10 (33.33)
	Banyuwangi Kota	30	30 (100)	6.40 ± 0.43[a]	30 (100)
E. coli counts (MPN g^{-1})	Kertosari	30	12 (40)	1.08 ± 0.67[a]	0 (0)
	Blambangan	30	8 (26.67)	0.57 ± 0.07[a]	0 (0)
	Banyuwangi Kota	30	11 (36.67)	0.49 ± 0.06[a]	0 (0)
Salmonella sp.	Kertosari	30	0 (0)	N/A[2]	0 (0)
	Blambangan	30	0 (0)	N/A	0 (0)
	Banyuwangi Kota	30	0 (0)	N/A	0 (0)
V. cholerae	Kertosari	30	0 (0)	N/A	0 (0)
	Blambangan	30	0 (0)	N/A	0 (0)
	Banyuwangi Kota	30	0 (0)	N/A	0 (0)

[1]The values with the same superscript in the same parameters means no significantly different ($p > 0.05$)
[2]N/A = not available
[3]Based on the standards in ISO 7388-2009 and Indonesian Ministry of Maritime Affairs and Fisheries Regulation 39/PERMEN-KP/2019; APC less than 5×10^5 CFUmL^{-1}, *E. coli* count less than 3 MPNg^{-1}, *Salmonella* sp. negative, *V. cholerae* negative, and chloramphenicol residues less than 0.3 ppb.

In this study, the mean APC values ranged from 4.75 to 6.4 × 10^5 CFU mL^{-1}. All samples from the Kertosari and Banyuwangi Kota markets were above the standard of APC, and only 10 samples (33.33%) from Blambangan market were above the standard of APC. There was no significant difference ($p > 0.05$) among APCs in the 3 markets.

From the 90 samples obtained from 3 traditional markets in Banyuwangi, 12 (40%), 8 (26.67%), and 11 (36.67%) samples were contaminated by *E.coli*, particularly in Kertosari, Blambangan, and Banyuwangi Kota markets, with a range of 0.49 to 1.08 MPN g^{-1}. No sample has exceeded the standard and no significantly different ($p > 0.05$) in terms of *E. coli* count between the 3 markets. The results of the *Salmonella* sp. and *V. cholerae* tests showed that all samples of African catfish taken from the Kertosari, Blambangan, and Banyuwangi Kota traditional markets were not contaminated by the pathogen i.e. *Salmonella* sp. and *V. cholerae.*

Antibiotic residues (Hassan *et al*., 2021) and bacterial contamination (Sheng and Wang, 2021) in fish flesh could have negative effects on consumer's health. The present results showed

that chloramphenicol residues were detected in catfish muscles in all locations, however, the residues were less than the standard set by the Indonesian Ministry of Maritime Affairs and Fisheries Regulation 39/PERMEN-KP/2019 (< 0.3 ppb), indicating that the catfish sold in the markets are safe to consume. Similar food safety-related studies were conducted outside Indonesia such as those reported by Duncan *et al*. (2022), who demonstrated that *C. gariepinus* cultivated in Ghana contained chloramphenicol residues (4.154 µg/L). Chloramphenicol residues were also detected in Nile tilapia muscle from the Rosetta branch of the river Nile, Egypt (Eissa *et al*., 2020), and in other seafood products such as crustaceans, and mollusks in Shanghai, China (Ni *et al*., 2021).

Recently, the use of antibiotics, especially chloramphenicol, in Indonesia's aquaculture has been tightly controlled. Based on the Indonesian Fisheries and Maritime Ministry Regulations number 01/PERMEN-KP/2019, only several types of antibiotics are permitted to be used in aquaculture, for instance, amphenicol (thiamphenicol, chloramphenicol, and fluorphenicol); nitrofuran (nitrofurantoin, nifurpirinol, furazolidone, nifurtoinol, and furaltadon); nitroimidazole (dimetridazole, metronidazole, fluconazole, and tinidazole); and macrolide (virgiamycin, tilosina, and spiramycin). The restriction of antibiotics used in aquaculture because of their residues could harms human health, for example, antibiotic resistance in bacteria which could cause devastating effects to human health and the environment. Prajwal *et al.* (2017) showed that antibiotic residues could lead to drug hypersensitivity, carcinogenic effects, disruption of normal intestinal flora, mutagenic effects, and teratogenic effects.

Arsène *et al*. (2022) reported the impact of antibiotic residues could be seen in multiple biological aspects such as organ toxification (disruption of liver, kidney, and hematopoietic tissue), microbial community in the host (disruption of normal intestinal flora), immunopathology (drug hypersensitivity), and neurological dysfunction (Alzheimer's disease). It is important to note that chloramphenicol residues have been closely associated with deadly blood dyscrasia, aplastic anemia, bone disruption, hepatic problems, reproductive abnormalities, and myelotoxicity (Kyuchukova, 2020; Falaye *et al*., 2024).

Aerobic plate count (APC) of microbes in catfish flesh ($4.75–6.4 \times 10^5$ CFU mL^{-1}) obtained from Kertosari and Banyuwangi Kota traditional markets were found above the ISO 7388:2009 standard. This indicated poor personal hygiene of fish handlers and poor sanitation of the environment were responsible for the bacterial contamination. Fish can become contaminated throughout the handling, processing, and transportation phases. The raw materials, workers, and processing equipment could all be connected to this contamination. Furthermore, contamination of seafood can occur during processing and storage. Contamination may be caused by food-borne pathogens which naturally exist in waters (Novoslavskij *et al*., 2016; Fikri *et al*., 2022). The contaminated aquaculture products can be harmful to human health through disease transmission or intoxication (Ali *et al*., 2020; Wiradana *et al*., 2023). The presence of several bacteria in fish, including human pathogenic bacteria, has been connected to direct contact with a contaminated water environment as well as the consumption of bacteria from sediments or contaminated feed (Kartikasari *et al*., 2019; Wardhana *et al*., 2021; Praja *et al*., 2024). As a result, bacteria found in fish reflect the health and safety of aquatic habitats (Gufe *et al*., 2019). Similarly, Wong *et al*., (2015) and Kim *et al*., (2017) demonstrated the total viable counts (TVCs) of bacteria in raw ready-to-eat seafood were significantly higher than standard (5 log CFU mL^{-1}) from Taiwan and Korea, respectively.

Our results showed that *E. coli* was detected in catfish muscles in all fish markets (34%), however, the bacterial count is lower than the tolerance level based on the ISO 7388:2009 standard (less than 3 MPN g^{-1}). Studies showed that fish products are prone to *E. coli* contamination. *E. coli* is a commensal bacterium that is commonly found in the intestinal of numerous healthy animal species, including humans. Nevertheless, certain strains have the

potential to induce certain illnesses (Abebe *et al*., 2020). Furthermore, Li *et al*., (2021) reported the presence of *E. coli* bacteria was utilized to predict fecal contamination levels more accurately than total coliform counts. It is caused by *E. coli* can express the enzyme activity of B-D-glucuronidase, a sensitive and specific marker for detecting fecal contamination. Ava *et al*. (2020) reported that 75.6% of farmed and 75 % of marketed fish in Dinajpur, Bangladesh, were contaminated by *E.coli*. Other studies by Yohans *et al*., (2022) found that 20% of raw and 13.8% of cooked fish (Nile tilapia, *Labeo barbus*, and African catfish) were contaminated by *E.coli* in Northwest Ethiopia. In Nairobi, Kenya, 35.29% of tilapia (*Oreochromis niloticus*) were found contaminated with *E. coli* (Mumbo *et al*., 2023).

Most *E. coli* is harmless, but the virulent strains can cause important diseases worldwide. The pathogenic strains produce a toxin called verotoxin. This toxin has a distinct effect on Vero cells (Onmaz *et al*., 2020). Based on their antigenic properties, *E. coli* produces two forms of verotoxin/Shiga toxin (Stxs): Shiga toxin type 1 (Stx1) and Shiga toxin type 2 (Stx2). Purified Stx2 is 1,000 times more toxic than Stx1 to human renal endothelial cells (Terajima *et al*., 2017). The pathogenicity of *E. coli* depends on several virulence factors, such as adhesins, iron uptake factors, protectins, toxins, invasins (Sora *et al*., 2021), metabolism, and secretion systems (Hu *et al*., 2022). The diseases that are associated with *E. coli* in humans are watery diarrhea, hemorrhagic colitis (HC), and/or hemolytic uremic syndrome (HUS) (Mohamed and Habib, 2023; Wibawati *et al*., 2024). Infection is mainly via oral route, i.e., the ingestion of food contamination from poor handling and poor sanitation (Rahman *et al*., 2022; Tahir *et al*., 2024).

Based on the present findings, *Salmonella* sp. and *V. cholerae* were not detected in all *C. gariepinus* obtained from the markets. Previous study also reported the similar result in shellfish (shrimps, clams, and cephalopods) from landing centers and retail markets of Mumbai India (Prabhakar *et al*., 2020) and prawns from wet markets in Besut district and Kuala Terengganu, Malaysia (Sudin *et al*., 2018). It indicated that there was no secondary contamination from anthropogenic pollution in the cultivation process. The accessibility of clean water for washing fresh fish and ice manufactured from potable water also contributes significantly to the reduction of *Salmonella* sp. and *V. cholerae* contamination of fresh seafood. *Salmonella* sp. and *V. cholerae* in freshwater fish have typically been linked to fecal contamination of the water where the fish was taken (Ali *et al*., 2020). Other studies indicate that seafood contamination with two or more microorganisms might significantly raise the risk factors in consumers (Dumen *et al*., 2020).

Salmonellosis, caused by *Salmonella* sp. is a significant pathogen in the animal production industry. Not only in humans, *Salmonella* sp. causes animal diseases and is spread by trade in animals and non-heated animal food products, including fishery products (Ehuwa *et al*., 2021). *Salmonella* sp. produces adhesins, invasins, fimbriae, hemagglutinins, exotoxins, and endotoxins (Jajere, 2019). These compounds could disrupt human bodies and subsequently lead to diarrhea, abdominal pain, nausea, gastroenteritis, and mild fever. Prostation, vomiting, headache, anorexia, and enteric fever may also occur (Wang *et al*., 2020). Good sanitation is the best way to prevent *Salmonella* sp. contamination in fishery products (O'Bryan *et al*., 2022).

While *V. cholerae* is an opportunistic bacterium that can survive in freshwater and marine environments. Humans are infected by these pathogens through the ingestion of undercooked or mishandled seafood (Parlapani, 2021). The clinical symptoms of cholera are septicemia, cholecystitis, wound infections, cellulitis, necrotizing fasciitis, meningitis, and endophthalmitis (Afreen and Ucak, 2021). *V. cholera* produces Cholera toxin (CT), accessory cholera enterotoxin (ACE), zonula occludens toxin (ZOT), heat-stable enterotoxin (ST), cholix toxin (Chx), hemagglutinin protease (HAP), and *V. cholerae* cytolysin (VCC) which are highly toxic to humans (Ramamurthy *et al*., 2020). These bacteria are not only important in the seafood

industry, but they can also contaminate water (Fida *et al*., 2023), fruits and vegetables, milk, cakes, and meat (Chowdhury *et al*., 2022).

CONCLUSION

All farmed catfish marketed in Banyuwangi were safe to eat as no samples were found to exceed the limits of chloramphenicol and bacterial contaminants following the standards. Studies are needed though to further investigate other antibiotics (such as tetracycline, sulphonamide, enrofloxacin, and nitrofuran) used and their residues in aquaculture products to ensure food safety among the local markets around Banyuwangi, Indonesia.

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AUTHORS' CONTRIBUTIONS

MFU: Supervision, Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft, writing-review and editing. HKJ: Validation, Investigation and Formal analysis. DSB: Investigation and Formal analysis. MAP: Formal analysis, Investigation, Writing-original draft, writing-review and editing. JYL: Supervision, Formal analysis, Writing-original draft, writing-review and editing. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review on Major Food-Borne Zoonotic Bacterial Pathogens. *Journal of Tropical Medicine*, 2020.

Afreen, M., & Ucak, I. (2021). Food-borne pathogens in seafood. *Eurasian Journal of Agricultural Research*, 5(1), 44–58.

Ali, A., Parisi, A., Conversano, M. C., Iannacci, A., D'Emilio, F., Mercurio, V., & Normanno, G. (2020). Food-borne bacteria associated with seafoods: A brief review. *Journal of Food Quality and Hazards Control*, *7*(1), 4–10.

Antunes, P., Novais, C., & Peixe, L. (2019). Food-to-humans bacterial transmission. *Microbial Transmission*, 161–193.

Arsène, M. M. J., Davares, A. K. L., Viktorovna, P. I., Andreevna, S. L., Sarra, S., Khelifi, I., & Sergueïevna, D. M. (2022). The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. *Veterinary World*, 15(3), 662–671.

Assefa, A., & Abunna, F. (2018). Maintenance of Fish Health in Aquaculture: Review of Epidemiological Approaches for Prevention and Control of Infectious Disease of Fish. *Veterinary Medicine International*, 2018, 1–10.

Ava, A., Faridullah, M., Lithi, U., & Roy, V. (2020). Incidence of Salmonella and Escherichia coli in fish farms and markets in Dinajpur, Bangladesh. *Bangladesh Journal of Scientific and Industrial Research*, 55(1), 65–72.

Bortolotte, A. R., Daniel, D., & Reyes, F. G. R. (2021). Occurrence of antimicrobial residues in tilapia (*Oreochromis niloticus*) fillets produced in Brazil and available at the retail market. *Food Research International*, 140(3).

Budiati, T., Rusul, G., Wan-abdullah, W. N., Ahmad, R., & Arip, Y. M. (2015). Microbiological Quality of Catfish (*Clarias Gariepinus*) and Tilapia (*Tilapia Mossambica*) Obtained from Wet Markets and Ponds in Malaysia. *Journal of Aquaculture Research and Development*, 6(1), 1–5.

Chowdhury, F., Ross, A. G., Islam, M. T., McMillan, N. A. J., & Qadri, F. (2022). Diagnosis, Management, and Future Control

of Cholera. *Clinical Microbiology Reviews*, 35(3).

Chuah, L. O., Effarizah, M. E., Goni, A. M., & Rusul, G. (2016). Antibiotic Application and Emergence of Multiple Antibiotic Resistance (MAR) in Global Catfish Aquaculture. *Current Environmental Health Reports*, 3(2), 118–127.

Doğan, Y. N., Pamuk, Ş., & Gürler, Z. (2020). Chloramphenicol and sulfonamide residues in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) fish from aquaculture farm. *Environmental Science and Pollution Research*, 27(33), 41248–41252.

Dumen, E., Ekici, G., Ergin, S., & Bayrakal, G. M. (2020). Presence of Foodborne Pathogens in Seafood and Risk Ranking for Pathogens. *Foodborne Pathogens and Disease*, 17(9), 541–546.

Duncan, A. E., Adoko, C., Osei-Marfo, M., Barnie, S., Sakyi, A. G., & Adjei, J. (2022). Analysis and Risk Assessment of Pharmaceutical Residues in Fish from Three (3) Water Bodies in Ghana. *Journal of Water and Health*, 00(3), 1–10.

Ehuwa, O., Jaiswal, A. K., & Jaiswal, S. (2021). Salmonella, food safety and food handling practices. *Foods*, 10(5), 1–16.

Eissa, F., Ghanem, K., & Al-Sisi, M. (2020). Occurrence and human health risks of pesticides and antibiotics in Nile tilapia along the Rosetta Nile branch, Egypt. *Toxicology Reports*, 7(3), 1640–1646.

Falaye, A. E., Abah, A., & Sule, S. O. (2024). Effect of Gum Arabic (*Acacia senegal*) on Growth Performance, Carcass Quality and Health of *Clarias gariepinus* Juveniles. *Jurnal Medik Veteriner*, 7(1), 163–176.

Fida, M., Li, P., Wang, Y., Alam, S. M. K., & Nsabimana, A. (2023). Water Contamination and Human Health Risks in Pakistan: A Review. *Exposure and Health*, 15(3), 619–639.

Fikri, F., Wardhana, D. K., Purnomo, A., Khairani, S., Chhetri, S., & Purnama, M. T. E. (2022). Aerolysin gene characterization and antimicrobial resistance profile of *Aeromonas hydrophila* isolated from milkfish (*Chanos chanos*) in Gresik, Indonesia. *Veterinary World*, 15(7), 1759–1764.

Gauthier, D. T. (2015). Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. *Veterinary Journal*, 203(1), 27–35.

Gufe, C., Canaan Hodobo, T., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, P. V., & Machakwa, J. (2019). Antimicrobial Profiling of Bacteria Isolated from Fish Sold at Informal Market in Mufakose, Zimbabwe. *International Journal of Microbiology*, 2019.

Hassan, M. M., El Zowalaty, M. E., Lundkvist, Å., Järhult, J. D., Khan Nayem, M. R., Tanzin, A. Z., Badsha, M. R., Khan, S. A., & Ashour, H. M. (2021). Residual antimicrobial agents in food originating from animals. *Trends in Food Science and Technology*, 111(1), 141–150.

Hu, J., Afayibo, D. J. A., Zhang, B., Zhu, H., Yao, L., Guo, W., Wang, X., Wang, Z., Wang, D., Peng, H., Tian, M., Qi, J., & Wang, S. (2022). Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic Escherichia coli (APEC). *Frontiers in Microbiology*, 13(12), 1–13.

Ia, S., & Ta, H. (2017). Enterobacteriaceae in Some Imported Fish. *Journal of Dairy and Veterinary Sciences*, 3(5), 4–9.

Jääskeläinen, E., Jakobsen, L. M. A., Hultman, J., Eggers, N., Bertram, H. C., & Björkroth, J. (2019). Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage. *International Journal of Food Microbiology*, 293(12), 44–52.

Jajere, S. M. (2019). A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and adaptation and antimicrobial resistance including multidrug resistance. *Veterinary World*, 12(4), 504–521.

Jayalakshmi, K., Paramasivam, M., Sasikala, M., & Sumithra, A. (2017). Review on antibiotic residues in animal products and its impact on environments and human health. ~ *1446 ~ Journal of Entomology and Zoology Studies*, 5(3), 1446–1451.

Jorge, R. J., Redondo, P. N., & Monroy, C. (2016). Bacterial load comparison of marine fish collected and commercially obtained for human consumption in western region of Yucatan Peninsula, Mexico. *International Journal of Aquatic Science*, 7(1), 6–12.

Kartikasari, A. M., Hamid, I. S., Purnama, M. T. E., Damayanti, R., Fikri, F., & Praja, R. N. (2019). Isolation and identification of *Escherichia coli* as bacterial contamination in broiler chicken meat in poultry slaughterhouse lamongan district. *Jurnal Medik Veteriner*, 2(1), 66–71.

Kim, H. W., Hong, Y. J., Jo, J. I., Ha, S. D., Kim, S. H., Lee, H. J., & Rhee, M. S. (2017). Raw ready-to-eat seafood safety: microbiological quality of the various seafood species available in fishery, hyper and online markets. *Letters in Applied Microbiology*, 64(1), 27–34.

Kyuchukova, R. (2020). Antibiotic residues and human health hazard – review. *Bulgarian Journal of Agricultural Science*, 26(3), 664–668.

Li, E., Saleem, F., Edge, T. A., & Schellhorn, H. E. (2021). Biological indicators for fecal pollution detection and source tracking: A review. *Processes*, 9(11).

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture*, 12(2), 640–663.

Luo, L., Lu, S., Huang, C., Wang, F., Ren, Y., Cao, H., Lin, Q., Tan, Z., & Wen, X. (2021). A survey of chloramphenicol residues in aquatic products of Shenzhen, South China. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 38(6), 914–921.

Mohamed, M. Y. I., & Habib, I. (2023). Pathogenic *E. coli* in the Food Chain across the Arab Countries: A Descriptive Review. *Foods*, 12(20), 1–18.

Møretrø, T., Moen, B., Heir, E., Hansen, A., & Langsrud, S. (2016). Contamination of salmon fillets and processing plants with spoilage bacteria. *International Journal of Food Microbiology*, 237, 98–108.

Mumbo, M. T., Nyaboga, E. N., Kinyua, J. K., Muge, E. K., Mathenge, S. G. K., Rotich, H., Muriira, G., Njiraini, B., & Njiru, J. M. (2023). Antimicrobial resistance profiles of *Salmonella* spp. and *Escherichia coli* isolated from fresh nile tilapia (*Oreochromis niloticus*) fish marketed for human consumption. *BMC Microbiology*, 23(1), 1–18.

Ni, L., Chen, D., Fu, H., Xie, Q., Lu, Y., Wang, X., Zhao, Y., & Chen, L. (2021). Residual levels of antimicrobial agents and heavy metals in 41 species of commonly consumed aquatic products in Shanghai, China, and cumulative exposure risk to children and teenagers. *Food Control*, 129, 108225.

Novoslavskij, A., Terentjeva, M., Eizenberga, I., Valciņa, O., Bartkevičs, V., & Bērziņš, A. (2016). Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology*, 66(1), 1–15.

O'Bryan, C. A., Ricke, S. C., & Marcy, J. A. (2022). Public health impact of Salmonella spp. on raw poultry: Current concepts and future prospects in the United States. *Food Control*, 132(6), 108539.

Onmaz, N. E., Yildirim, Y., Karadal, F., Hizlisoy, H., Al, S., Gungor, C., Disli, H. B., Barel, M., Dishan, A., Akai Tegin, R. A., & Simsek, E. (2020). *Escherichia coli* O157 in fish: Prevalence, antimicrobial resistance, biofilm formation capacity, and molecular characterization. *Lwt*, 133(May), 109940.

Parlapani, F. F. (2021). Microbial diversity of seafood. *Current Opinion in Food Science*, 37, 45–51.

Prabhakar, P., Lekshmi, M., Ammini, P., Nayak, B. B., & Kumar, S. (2020). Salmonella contamination of seafood in landing centers and retail markets of Mumbai, India. *Journal of AOAC International*, 103(5), 1361–1365.

Praja, R. N., Yudhana, A., Haditanojo, W., Handijatno, D., Purnama, M. T. E., Maslamama, S. T., Insani, A. K., Prasetyo, D. R., Prameswari, N. P., & Praja, S. S. (2024). Comparison of Bacterial Contamination in Sea Turtle Natural and Semi-natural Hatching Nest at Boom Beach Banyuwangi. *The Indian Veterinary Journal*, 101(4), 7–11.

Prajwal, S., Vasudevan, V. N., Sathu, T., Irshad, A., & Nayankumar, S. R. (2017). Antibiotic residues in food animals : Causes and health effects. *The Pharma Innovation Journal*, 6(12), 1–4.

Rahman, M., Alam, M. U., Luies, S. K., Kamal, A., Ferdous, S., Lin, A., Sharior, F., Khan, R., Rahman, Z., Parvez, S. M., Amin, N., Hasan, R., Tadesse, B. T., Taneja, N., Islam, M. A., & Ercumen, A. (2022). Contamination of fresh produce with antibiotic-resistant bacteria and associated risks to human health: A scoping review. *International Journal of Environmental Research and Public Health*, 19(1), 1–15.

Ramamurthy, T., Nandy, R. K., Mukhopadhyay, A. K., Dutta, S., Mutreja, A., Okamoto, K., Miyoshi, S. I., Nair, G. B., & Ghosh, A. (2020). Virulence Regulation and Innate Host Response in the Pathogenicity of Vibrio cholerae. *Frontiers in Cellular and Infection Microbiology*, 10(9), 1–22.

Roca-saavedra, P., Mendez-vilabrille, V., Miranda, J. M., Lamas, A., Nebot, C., Cardelle-cobas, A., Franco, C. M., & Cepeda, A. (2016). Food Additives and Contaminants : Effects on Human Gut Microbiota — A Review. *Preprints*, 2016, 2016120119.

Sheng, L., & Wang, L. (2021). The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 738–786.

Sora, V. M., Meroni, G., Martino, P. A., Soggiu, A., Bonizzi, L., & Zecconi, A. (2021). Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. *Pathogens*, 10(11).

Sudin, N. S., Yew, J., & Tang, H. (2018). Detection, Isolation and Antibiotic Testing of Vibrio cholerae in seafood. *Journal Of Agrobiotechnology*, 9(1), 50–57.

Tahir, N. D. M., Matori, M. F., & Gan, H. M. (2024). First Report of *Aeromonas schubertii* Infection in Striped Snakehead *Channa striata* (Bloch, 1793) Fingerlings in Malaysia. *Jurnal Medik Veteriner*, 7(1), 33–40.

Terajima, J., Izumiya, H., Hara-Kudo, Y., & Ohnishi, M. (2017). Shiga Toxin (Verotoxin)-producing *Escherichia coli* and Foodborne Disease: A Review. *Food Safety*, 5(2), 35–53.

Wang, M., Qazi, I. H., Wang, L., Zhou, G., & Han, H. (2020). Salmonella virulence and immune escape. *Microorganisms*, 8(3), 1–25.

Wardhana, D. K., Haskito, A. E. P., Purnama, M. T. E., Safitri, D. A., & Annisa, S. (2021). Detection of microbial contamination in chicken meat from local markets in Surabaya, East Java, Indonesia. *Veterinary World*, 14(12), 3138–3143.

Wibawati, P. A., Chrismonica, Y., Susanti, R. B., Himawan, M. P., Sintya Devy, A. H., Nirmalasari, J. A., & Abdramanov, A. (2024). Microbial Contamination in Chicken Meat in Traditional Markets in Banyuwangi. *Jurnal Medik Veteriner*, 7(1), 41–47.

Wiradana, P. A., Sudyadnyana Sandhika, I., Widhiantara, I. G., Rizqy, A. N., Soegianto, A., & Yulianto, B. (2023). Contaminants and Human Health Risks Associated with Exposure to Microplastic Ingestion of Green Mussels (*Perna viridis*) Collected from The Kedonganan Fish Market, Bali. *Jurnal Medik Veteriner*, 6(2), 197–208.

Wong, H. C., Jiang, H. Y., Lin, H. Y., & Wang, Y. T. (2015). Microbiological quality of Seafood marketed in Taiwan. *Journal of Food Protection*, 78(11), 1973–1979.

Yohans, H., Mitiku, B. A., & Tassew, H. (2022). Levels of *Escherichia coli* as Bio-Indicator of Contamination of Fish Food and

Antibiotic Resistance Pattern Along the Value Chain in Northwest Ethiopia. *Veterinary Medicine: Research and Reports*, 13, 299–311.

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