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Bacteriological analysis of selected fishes sold in wet markets in Tuguegarao city, Cagayan, Philippines

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ABSTRACT

Fish and fish products are familiar sources of foodborne outbreaks and recalls. Pathogenic and spoilage bacteria can enter any production, processing, or distribution stage, threatening consumer safety. Fish locally sold at Tuguegarao City, Cagayan, Philippines wet market, namely *Oreochromis niloticus* (tilapia), *Chanos chanos* (bangus), *Siluriformes* (hito), and *Decapterus macarellus* (galunggong), were collected and sent to the Department of Agriculture Cagayan Valley Integrated Laboratory (DA-CVIAL) Regional Feed Chemical Analysis Laboratory (RFCAL) for bacteriological analysis. Total bacterial count, total coliform/*E. coli* count, *S. aureus* count, and presence of Salmonella spp. were conducted for analysis. Two tilapia and one hito sample exceeded the threshold for safety standards on the total bacterial count. Aside from these, all fish species have unacceptable levels of *E. coli* except for galunggong and tilapia (not tested for *E. coli*). Analysis of *Salmonella* yielded concerning results. Only two hito samples and three tilapia samples tested negative for the microorganism. This suggests the presence of Salmonella in a significant portion of the fish across all species. *S. aureus* contamination was not detected in any of the fish samples. By the FDA standards (Circular No. 2022-012), no species passed the microbiological tests. High levels of fish contamination may be caused by various factors, including environmental temperature, which can allow certain organisms to thrive, poor personal hygiene of the fish handler, and contaminated water sources that may contain fecal matter.

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INTRODUCTION

Republic Act No. 10611, also referred to as "The Food Safety Act of 2013," was enacted on August 23, 2013, to enhance the food safety regulatory framework in the Philippines (Republic of the Philippines, 2013). It also seeks to improve market access for local foods and food products. This is a joint project of the Department of Agriculture-Department of Health (DA-DOH) Administrative Order that took effect on March 23, 2015, in coordination with the Department of Interior and Local Government (DILG). Despite food safety regulations and guidelines, a significant gap exists between the regulatory frameworks and the actual standards and confidence shown by industry stakeholders and consumers. Competing jurisdictions in particular areas lead to service overextension and redundancy, while ineffective control frameworks make ambiguous enforcement mandates worse. Risk analysis and management implementation are inadequate, and issues associated with the devolution of responsibilities to Local Government Units (LGUs) aggravate systemic inefficiencies.

This study is part of a program that aims to conduct a scientific assessment or risk analysis on fruits and vegetables, meat and meat products, fish and fish products, and street foods sold in Tuguegarao City. One of the components of this study is to conduct a bacterial assessment, which shall determine the presence and contamination rates of pathogenic bacteria. This study's scope is limited to fish sold in Tuguegarao City, Cagayan, Philippines.

Fish and fish products are frequently implicated in yearly foodborne outbreaks and product recalls. Microorganisms play a critical role in determining the safety of these products, as both pathogenic and spoilage microorganisms can be introduced at any stage of the production and supply chain (Sheng and Wang, 2021a). Studies (Giddings *et al.*, 2015a; Shafik and El-Dosoky, 2017) have determined that fish contamination may arise from environmental conditions experienced during transport to landing centers and wholesale markets, in addition to poor handling techniques that can introduce pathogens. Unsanitary, humid conditions and improper storage, display, and packing facilities increase microbial contamination from multiple sources.

Likewise, studies have indicated that elevated contamination levels in fish markets may be attributed to factors such as ambient temperature, which can promote microbial proliferation, and the conduct of fish handlers, notably poor personal hygiene (Alikunhi *et al.*, 2017; Brauge *et al.*, 2024; Sheng and Wang, 2021b). Moreover, contamination may originate from the aquatic environment, where fish might consume water contaminated with fecal matter, resulting in enteric bacteria and other harmful pathogens (Cabral, 2010; Terentjeva *et al.*, 2015a).

In Tuguegarao City, fish and fish products come from across the region, like Cagayan, Isabela, Nueva Vizcaya, and other areas of Luzon, like Dagupan. There are numerous types of fish sold in the city. This includes marine fishes like blue marlin, maya-maya, yellowfin, and tuna; cultured fishes include *tilapia, bangus,* and *malaga;* and imported fishes like salmon. Fish sold in the city are delivered to the city directly by the source, whether marine or culture, to the biggest dealer located at the Riverside Centro 10, or they are fetched by individual dealers directly from the site of the source. In this case, the latter takes responsibility for handling and transporting fish and fish products from the source to Tuguegarao City. The said dealers now distribute the fish and fish products to their retailers and vendors. With these practices, this study will assess the bacterial status and possible sources of contamination of fish sold in the wet market of Tuguegarao City, Cagayan, Philippines.

MATERIALS AND METHODS

Sample handling and collection

The fish samples (*tilapia, bangus, hito, galunggong)* were collected from the fish depot in Tuguegarao City, Cagayan, Philippines. Each sample was placed in a sterile plastic bag, properly labeled, and transported to the DA-CVIAL RFCAL for bacteriological analysis.

Fish sample preparation (Serial dilution)

Twenty-five grams of fish were aseptically introduced into a sterile stomacher bag containing 225 mL of Butterfield's phosphate-buffered diluent to maintain a stable pH. The mixture was homogenized for 2 minutes utilizing a stomacher under sterile circumstances. A 10 mL portion of the homogenate was transferred to a sterile container containing 90 mL of the same diluent to create a 10^{-2} dilution, followed by thorough mixing with a vortex mixer. Serial dilutions were conducted up to 10^{-5} for microbiological analysis and accurate bacterial quantification.

Microbiological analyses (Enumeration and detection)

The total bacterial count (AOAC International #010404), total coliform/*Escherichia coli* count (AOAC International #110402), *Staphylococcus aureus* count (AOAC International #081001), and detection of *Salmonella* spp. were determined using CompactDry™.

Enumeration of total bacterial count

From the prepared serial dilutions, 1 ml of each dilution was dispensed in duplicate on total bacterial count CompactDry™ plates. The inoculated total bacterial count CompactDry™ plates were incubated at 35±2°C for 48±3 hours (AOAC International, 2019). After incubation, colonies that emerged in the total bacterial count CompactDry™ plates were counted and interpreted using the interpretation guide provided by Nissui Pharmaceutical Co., LTD. CompactDry™. The total bacterial count was expressed in CFU (colony-forming units) per gram of fish sample.

Enumeration and detection of pathogenic bacteria

Total coliforms/Escherichia coli count

From the prepared serial dilutions, 1 ml of each dilution (10^{-1} to 10^{-4}) was dispensed in duplicate on *Total Coliforms/Escherichia coli* CompactDry™ plates. The inoculated *Total Coliforms/Escherichia coli* CompactDry™ plates were incubated at 35±2°C for 24±2 hours (AOAC International, 2019). After incubation, colonies that emerged in the *Total Coliforms/Escherichia coli* CompactDry™ plates were counted and interpreted using the interpretation guide provided by Nissui Pharmaceutical Co., LTD. CompactDry™. The *Total Coliforms/Escherichia coli* counts were expressed in CFU (colony-forming units) per gram of fish sample.

Staphylococcus aureus count

From the prepared serial dilutions, 1 ml of each dilution was dispensed in duplicate on *Staphylococcus aureus* CompactDry™ plates. The inoculated *Staphylococcus aureus* CompactDry™ plates were incubated at 35±2°C for 24±2 hours (AOAC International, 2019). After incubation, colonies that emerged in the *Staphylococcus aureus* CompactDry™ plates were counted and interpreted using the interpretation guide provided by Nissui Pharmaceutical Co., LTD. CompactDry™. The *Staphylococcus aureus* count was expressed in CFU (colony-forming units) per gram of fish sample.

Detection of Salmonella spp.

Twenty-five grams of collected fish were aseptically transferred to a sterile stomacher bag and mixed with 225 ml of Buffered Peptone Water. The mixture was homogenized for 2 minutes using a stomacher under aseptic conditions. The resulting mixture was incubated at 36±1°C for 22±2 hours. After incubation, 0.1 ml of pre-enriched media was transferred in duplicate onto *Salmonella* CompactDry™ plates, adding 1.0 ml of sterile distilled water. The inoculated *Salmonella* CompactDry™ plates were incubated at 42±1°C for 22±2 hours (Nissui Pharmaceutical Co., LTD., 2018). After incubation, colonies that emerged in the *Salmonella* CompactDry™ plates were observed and interpreted using the interpretation guide provided by Nissui Pharmaceutical Co., LTD. CompactDry™. *Salmonella* was identified as present or absent per 25 grams of fish sample.

Data analysis

Descriptive statistics such as mean, standard deviation, and range were used to summarize bacterial counts. Frequencies and corresponding

percentages were used to summarize the proportions of samples that did not meet the acceptable threshold. A normality test was done to determine if the bacteriological counts meet the assumptions for one-way ANOVA. Since the assumptions were violated, the Kruskal-Wallis test was used to determine if there was a difference in the bacteriological counts across species. A post hoc analysis of Dunn's test was used to determine the pairwise comparison of the mean ranks of the overall bacteriological counts.

RESULTS AND DISCUSSION

Table 1 reveals that one (1) *hito* and two (2) *tilapia* samples have unacceptable levels of total bacterial count. Additionally, all fish species yielded above-threshold levels of *E. coli,* except for *tilapia,* which was not tested. Among the bacterial parameters done, only *S. aureus* met the bacterial threshold criteria for all fish species. However, all fish tested positive for *Salmonella* spp. with 100% contamination of *bangus* and *galunggong* samples.

Table 1. Batch samples bacteriological summary, CFU/g (n=20)

Parameters	Number of samples	Acceptable levels	Mean actual levels	Number of samples with unacceptable levels (%)	*p*-value
Total bacterial count					0.230
Chanos chanos (Bangus)	5	5 x10^3	1.60 x10^5	0	
Decapterus macarellus (Galunggong)	5	5 x10^3	1.60 x10^5	0	
Siluriformes (Hito)	5	5 x10^3	2.12 x10^6	1 (20%)	
Oreochromis niloticus (Tilapia)	5	5 x10^3	2.36 x10^6	2 (40%)	
Total coliforms/*E. coli* count					0.099
Chanos chanos (Bangus)	5	11	4.16 x10^2	5 (100%)	
Decapterus macarellus (Galunggong)	5	11	2.48 x10^2	3 (60%)	
Siluriformes (Hito)	5	11	1.32 x10^2	5 (100%)	
Oreochromis niloticus (Tilapia)	5	11	-	-	
S. aureus count					-
Chanos chanos (Bangus)	5	1 x10^3	<1 x10^3	0	
Decapterus macarellus (Galunggong)	5	1 x10^3	<1 x10^3	0	
Siluriformes (Hito)	5	1 x10^3	<1 x10^3	0	
Oreochromis niloticus (Tilapia)	5	1 x10^3	<1 x10^3	0	
Detection of Salmonella spp.					-
Chanos chanos (Bangus)	5	Absence	-	5 (100%)	
Decapterus macarellus (Galunggong)	5	Absence	-	5 (100%)	
Siluriformes (Hito)	5	Absence	-	3 (60%)	
Oreochromis niloticus (Tilapia)	5	Absence	-	2 (40%)	

NOTE: Tilapia samples do not have *E. coli* data
Statistical test used: Chi-square test

Table 2 shows the descriptive statistics for the overall bacteriological count and the *E. coli* and *S. aureus* counts on raw fish samples collected from different fish species. Overall, there was a difference in the median bacteriological counts across all species, with *tilapia* having the highest median counts (5.0 x10^5). The analysis also revealed no statistical difference in all species' median *E. coli* counts. However, all species had mean and median *E. coli* counts exceeding the acceptable limits.

On further analysis of the total bacterial counts, the counts of *tilapia* were higher than those collected from *bangus*, *galunggong* and *hito*. No significant differences in the overall counts of all other combinations of fish species were recorded (Table 3).

Bacterial status of raw fish samples

The bacteriological summary of all the fish samples is presented in Table 1. The analysis revealed that the total bacterial counts in *bangus* and *galunggong* samples were within the thresholds set by the FDA (Circular No. 2022-012). However, two *tilapia* samples contained overall bacteriological count levels above safety standards, while one *hito* sample exceeded the threshold.

Table 2. Bacteriological Count, CFU/g (n=20)

Parameters	Mean	SD	Median	Min	Max	*p*-value
Total bacterial count						0.024
Chanos chanos (Bangus)	1.60 x10^5	5.48 x10^4	2.00 x10^5	1.00 x10^5	2.00 x10^5	
Decapterus macarellus (Galunggong)	1.60 x10^5	5.48 x10^4	2.00 x10^5	1.00 x10^5	2.00 x10^5	
Siluriformes (Hito)	2.12 x10^6	4.41 x10^6	2.00 x10^5	1.00 x10^5	1.00 x10^7	
Oreochromis niloticus (Tilapia)	2.36 x10^6	4.27 x10^6	5.00 x10^5	3.00 x10^5	1.00 x10^7	
Total coliforms/*E. coli* count						0.263
Chanos chanos (Bangus)	4.16 x10^2	3.68 x10^2	2.60 x10^2	2.00 x10^1	9.00 x10^2	
Decapterus macarellus (Galunggong)	2.48 x10^2	4.77 x10^2	4.00 x10^2	1.00 x10^1	1.10 x10^3	
Siluriformes (Hito)	1.32 x10^2	9.47 x10^2	1.00 x10^2	7.00 x10^1	3.00 x10^2	
Oreochromis niloticus (Tilapia)	-	-	-	-	-	
S. aureus count						-
Chanos chanos (Bangus)	<1 x10^3	-	<1 x10^3	<1 x10^3	<1 x10^3	
Decapterus macarellus (Galunggong)	<1 x10^3	-	<1 x10^3	<1 x10^3	<1 x10^3	
Siluriformes (Hito)	<1 x10^3	-	<1 x10^3	<1 x10^3	<1 x10^3	
Oreochromis niloticus (Tilapia)	<1 x10^3	-	<1 x10^3	<1 x10^3	<1 x10^3	
Statistical test used: Kruskal-Wallis Test						

Table 3. Pairwise comparison (n=20)

Total bacterial count	Mean rank difference	p-value
Bangus vs Galunggong	0	>0.999
Bangus vs Hito	-0.504	0.614
Bangus vs Tilapia	-2.634	0.008
Galunggong vs Hito	-0.504	0.615
Galunggong vs Tilapia	-2.634	0.008
Hito vs Tilapia	-2.130	0.033
Statistical teDunn'sd: Dunn's Pairwise comparison test		

Tilapia samples were not tested for *E. coli*. All other fish species had samples with unacceptable levels, with *galunggong* having the only samples within the acceptable threshold. Analysis of *Salmonella* yielded concerning results. Only two *hito* samples and three *tilapia* samples tested negative for the microorganism. This suggests the presence of *Salmonella* in a significant portion of the fish across all species. *S. aureus* contamination was not detected in any of the fish samples. According to FDA standards, no species has passed the bacteriological tests.

Surpassing the maximum allowable thresholds for microbiological contamination signifies an intolerably elevated risk to human health, possible food spoiling, and the unsuitability of the product for human consumption (Karanth *et al.*, 2023). A related study (Giddings *et al.*, 2015b) evaluating the microbial quality of fish against the standards established by the U.S. Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) revealed that microbial levels in freshwater fish exceeded these standards. The finding indicates a significant risk to public health.

The findings observed in this study (Table 2) mirror the review article (Gauthier, 2015), which concluded that the reservoirs of bacterial infections associated with human diseases have been discovered in fish. These bacterial infections include pathogenic *Escherichia coli, Salmonella* spp., and *Staphylococcus aureus*. However, the study's findings do not support the previous research regarding *S. aureus,* a non-indigenous bacteria pathogen frequently present in fish of human origin (Fernandes, 2009). *S. aureus* contamination was not detected in any of the fish samples; this result indicates the good personal hygienic practices of fish handlers since *S. aureus* is a good indicator of contamination from human handling (Tan *et al.*, 2014).

The analysis revealed that the total bacterial counts in two *tilapia* samples contained total bacteriological count levels above safety standards, while one *hito* sample exceeded the threshold. Also, *E. coli* counts on all species counts exceeded the acceptable limits. Several studies (Budiati *et al.*, 2013; Novotny *et al.*, 2004; Terentjeva *et al.*, 2015b) frequently reported the presence of *E. coli*

in fresh fish. The Enterobacteriaceae family is a significant indicator of sanitary and environmental contamination in production environments and fisheries products.

Increased levels of Enterobacteriaceae in the gills, intestines, and especially on the fish's skin indicate possible external contamination. A significant Enterobacteriaceae count and an elevated total bacterial count may indicate poor sanitary conditions and potential hazards to environmental and consumer health (Mladenović *et al.*, 2021; Terentjeva *et al.*, 2015b).

A review study emphasizes the widespread occurrence of *Salmonella* in many fish species, with multiple authors concluding that fish and shellfish frequently serve as passive carriers of *Salmonella* (Bibi *et al.*, 2015). These organisms generally exhibit no clinical manifestations of illness yet can excrete *Salmonella* spp. asymptomatically. Fish contamination with *Salmonella* is thought to stem primarily from terrestrial sources, rendering fish possible vectors for the infection. *Salmonella* spp. can infiltrate aquatic ecosystems via contaminated water, frequently tainted by human, wildlife, or domestic animal activities (Popa and Popa, 2021). Prior observations from Asia and Africa have indicated a significant incidence of *Salmonella* in fish, typically attributed to poor sanitary conditions in water sources and subsequent contamination during marketing and handling processes (Marchello *et al.*, 2021).

Moreover, *Salmonella* spp. represent a considerable percentage of hospitalizations resulting from foodborne infections and have been associated with the most extensive fish-related outbreak connected to ingesting contaminated raw tuna (Popa and Popa, 2021). *Salmonella* has been recognized as the predominant bacterial agent responsible for fish-related foodborne outbreaks (Sheng and Wang, 2021a).

The microbiological quality of tilapia revealed that all tissue samples, excluding muscle tissues, were infected with fecal coliforms. Escherichia coli were identified as the predominant contaminant, frequently found at higher concentrations. E. coli, coliforms, and bacteria such as Staphylococcus spp. and rare enterococci frequently represent hazardous conditions during fish processing (Han *et al.*, 2017; Roy *et al.*, 2024).

The quantity of bacteria in fish species typically varies based on environmental and biological factors. Some fish species are intrinsically more vulnerable to contamination due to species variations, eating behaviors, age, size, harvesting season, habitat traits, and geographical location (Alikunhi *et al.*, 2017; Beyari *et al.*, 2021). A significant association exists between environmental factors and bacterial contamination levels. Likewise, several research studies have determined that elevated contamination levels in the gills, intestinal tract, and particularly fish skin are frequently associated with exposure to external environmental pollutants (Dissasa *et al.*, 2022; Elgendy *et al.*, 2023; Svobodová, 1993). Differences in bacterial counts in fish can be ascribed to multiple factors, such as the microbiological quality of water, fish species, feeding behaviors, water temperature, catch size, processing temperature, and storage conditions (Cabral, 2010; Karanth *et al.*, 2023; Sheng and Wang, 2021b, 2021c). In fish products, *E. coli*, *Salmonella*, and *Listeria monocytogenes* also indicate sewage pollution and poor sanitary practices during transportation, distribution, storage, and marketing (Abdelaziz Hassan *et al.*, 2016; Manyi-Loh and Lues, 2025; Ndraha *et al.*, 2024).

Implications for public health

The presence of high bacterial counts on raw fish sold at the local wet market can lead to foodborne illness. Pathogenic bacteria like *Salmonella* spp., *E. coli,* and other coliforms can lead to gastrointestinal problems. This could be life-threatening for individuals like children, older people, or those with diminished immune systems. An increase in disease burden would have economic implications, resulting in higher healthcare costs, primarily because of medication,

hospitalization, and loss of productivity. Additionally, an increase in antibiotic resistance, which poses a greater challenge to public health, is more likely. Notably, bacterial contamination of raw fish poses environmental concerns, especially in terms of improper waste disposal, which can spread pathogens in the environment.

CONCLUSION

According to FDA standards (Circular No. 2022-012), no species has passed the microbiological tests. High levels of fish contamination may be caused by various factors, including environmental temperature, which can allow certain organisms to thrive; poor personal hygiene of the fish handler; and contaminated water sources that may contain fecal matter.

RECOMMENDATION

Improving hygiene is a mitigation measure in the wet market. Better handling practices, from catching to selling, help reduce contamination. To improve hygiene practices, train vendors and educate consumers on proper handling of raw fish. Of course, this would not be possible without the strict regulations and enforcement of safety standards of regulatory bodies.

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