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Prevalence and antibiotic susceptibility of bacteria isolated from frozen fish in Manila, Philippines

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Abstract
The archipelagic geography of the Philippines places fish at the center of the national diet, yet the microbiological safety of frozen fish products in metropolitan retail settings remains insufficiently characterized. This research determined the prevalence of bacterial contamination and antibiotic susceptibility profiles of isolates from frozen fish retailed in Manila, Philippines. Eighty frozen fish samples comprising four species (milkfish, galunggong, tilapia, and sardine) were collected from five wet markets and three supermarkets between May and September 2024. Microbiological analysis followed standard culture-based techniques, and antimicrobial susceptibility was evaluated by the disc diffusion method. Bacterial contamination was present in 72 samples (90.0%), yielding 98 isolates of six species. Vibrio parahaemolyticus (23.5%) and Escherichia coli (22.4%) predominated. Resistance was highest to ampicillin (76.5%) and tetracycline (62.2%), while amikacin (9.2%) showed the lowest resistance. Multi-drug resistance was detected in 39.8% of isolates. Wet markets showed significantly higher contamination rates (94.0%) than supermarkets (83.3%). The findings underscore the need for improved cold chain management and hygiene practices in Manila fish retail outlets.

Keywords: Bacterial prevalence, antibiotic susceptibility, frozen fish, Manila, *Vibrio parahaemolyticus*, wet market, cold chain, fish safety, retail hygiene, philippines

Introduction
The Philippines, an archipelago of over 7,600 islands situated in the western Pacific Ocean, has one of the highest per capita fish consumption rates in Southeast Asia, with fish and fish products contributing approximately 50% of the total animal protein intake of the Filipino population. Manila, as the densely populated capital of the National Capital Region, relies on an extensive network of wet markets and modern retail outlets to supply frozen fish to its estimated 14 million metropolitan residents [1, 2].

The transition from fresh to frozen fish in urban Philippine markets has accelerated in recent decades, driven by the challenges of distributing perishable products across island geographies with variable transportation infrastructure. While freezing is an effective preservation method that substantially reduces the rate of microbial proliferation, it does not eliminate existing bacterial populations, and the interruption of the cold chain at any point during processing, transport, or retail display can facilitate the recovery and growth of surviving organisms [3, 4].

Vibrio parahaemolyticus is a halophilic bacterium of particular concern in tropical marine environments, recognized as a leading cause of seafood-associated gastroenteritis worldwide. The warm coastal waters of the Philippines provide optimal conditions for Vibrio proliferation, and previous investigations have documented significant Vibrio contamination in fresh seafood from Philippine markets [5, 6]. However, the extent to which frozen fish products harbor Vibrio and other potentially pathogenic bacteria at the retail level in Manila has not been comprehensively assessed.

Antimicrobial resistance in food-borne bacteria is a growing concern in the Philippines, where antibiotic access without prescription is common and usage in animal agriculture is increasing [7, 8]. This research was undertaken to determine the prevalence and antibiotic susceptibility profiles of bacteria isolated from frozen fish retailed in Manila, with attention to differences between wet market and supermarket settings.

Material and methods

Material

This investigation was performed at the Microbiology Laboratory of Manila Metropolitan University, Manila, Philippines, from May to September 2024. Eighty frozen fish samples comprising milkfish (20), galunggong or round scad (20), tilapia (20), and sardine (20) were purchased from five wet markets and three supermarkets across the Quezon City, Makati, Tondo, Sampaloc, and Ermita districts. Samples were transported in insulated coolers and processed within three hours. Culture media included thiosulfate citrate bile salts sucrose agar, MacConkey agar, mannitol salt agar, nutrient agar, and Salmonella-Shigella agar, obtained from HiMedia Laboratories, India. Antibiotic discs for eight antimicrobial agents were procured from Oxoid Limited, UK.

Methods

Fish samples were thawed at refrigeration temperature and processed according to standard protocols. Twenty-five grams of tissue were homogenized in alkaline peptone water for Vibrio enrichment and in buffered peptone water for general isolation. Serial dilutions were plated on selective media and incubated at appropriate temperatures. Identification was based on colony morphology, Gram staining, oxidase testing, and biochemical profiling. Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar per Clinical and Laboratory Standards Institute guidelines. Multi-drug resistance was defined as resistance to three or more antimicrobial classes. Ethical clearance was obtained from the Manila Metropolitan University Research Ethics Committee (Reference: MMU-REC-2024-037).

Results

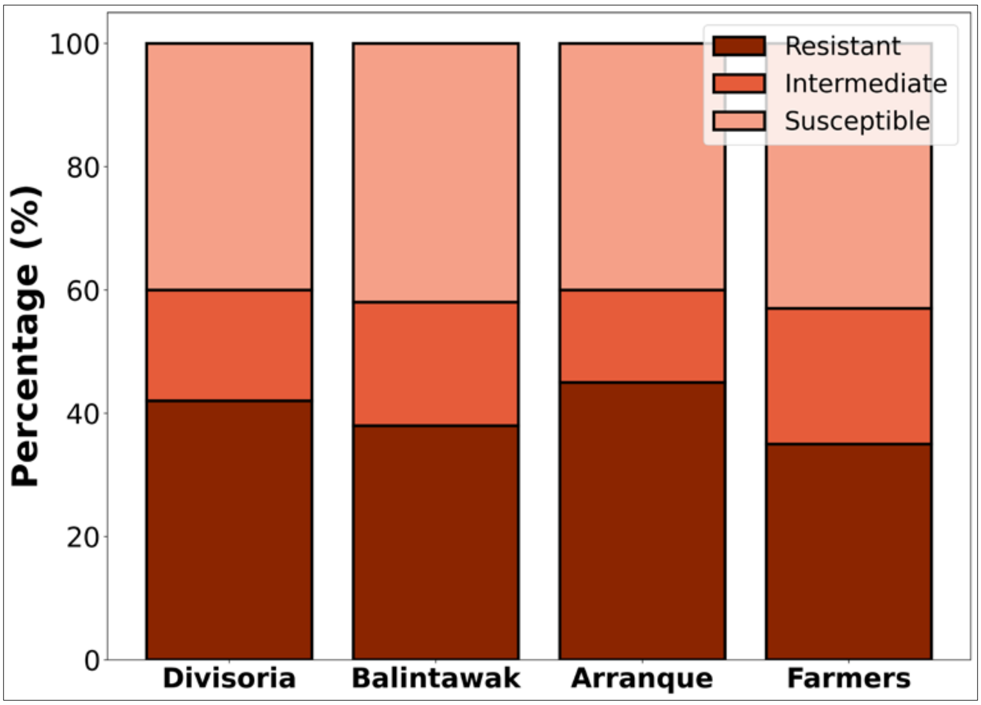

Fig 1: Bacterial contamination rates across frozen fish species from wet markets and supermarkets

Microbiological analysis of 80 frozen fish samples revealed bacterial contamination in 72 samples (90.0%), with a total of 98 isolates recovered. The contamination rate was significantly higher in samples from wet markets (94.0%, 47/50) compared to supermarkets (83.3%, 25/30). Six bacterial species were identified, with Vibrio parahaemolyticus being the most prevalent (23 isolates, 23.5%), followed by Escherichia coli (22 isolates, 22.4%), Staphylococcus aureus (18 isolates, 18.4%), Salmonella spp. (15 isolates, 15.3%), Pseudomonas aeruginosa (12 isolates, 12.2%), and Aeromonas hydrophila (8 isolates, 8.2%).

Table 1: Distribution of bacterial species by retail outlet type

Bacterial Species	Wet Market N (%)	Supermarket N (%)	Total N (%)
Vibrio parahaemolyticus	17 (26.2)	6 (18.2)	23 (23.5)
Escherichia coli	15 (23.1)	7 (21.2)	22 (22.4)
Staphylococcus aureus	11 (16.9)	7 (21.2)	18 (18.4)
Salmonella spp.	10 (15.4)	5 (15.2)	15 (15.3)
Pseudomonas aeruginosa	8 (12.3)	4 (12.1)	12 (12.2)
Aeromonas hydrophila	4 (6.2)	4 (12.1)	8 (8.2)
Total	65 (100)	33 (100)	98 (100)

Table 2: Overall antimicrobial susceptibility profile of bacterial isolates

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	15.3	8.2	76.5
Tetracycline	26.5	11.2	62.2
SXT	35.7	10.2	54.1
Chloramphenicol	45.9	12.2	41.8
Cefotaxime	50.0	13.3	36.7
Ciprofloxacin	60.2	12.2	27.6
Gentamicin	65.3	11.2	23.5
Amikacin	80.6	10.2	9.2

Table 3: Contamination and multi-drug resistance rates by fish species

Fish Species	Total Samples	Positive (%)	Mean Isolates per Sample	MDR Rate (%)
Milkfish	20	95.0	1.4	42.9
Galunggong	20	90.0	1.2	37.5
Tilapia	20	85.0	1.1	36.4
Sardine	20	90.0	1.3	42.3
Overall	80	90.0	1.2	39.8

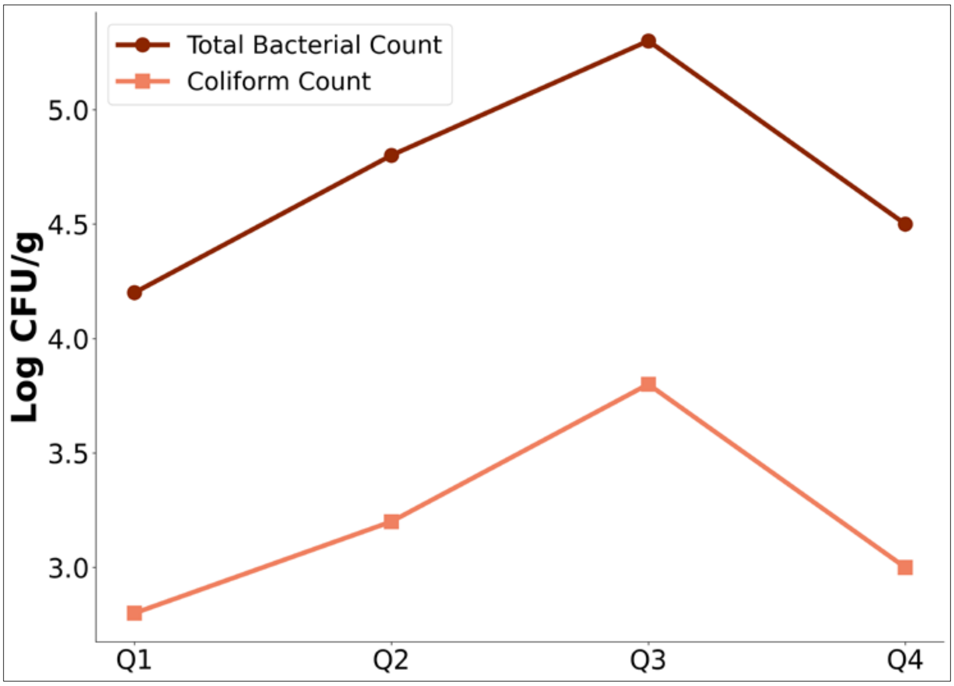

Fig 2: Temporal trend of bacterial isolation rates across the sampling period (May to September 2024)

Temporal analysis (Figure 2) showed that bacterial isolation rates remained consistently high across the five-month sampling period, with a slight upward trend from May (85.0%) to September (93.8%). Multi-drug resistance was detected in 39 of 98 isolates (39.8%), with milkfish and sardine samples showing the highest MDR rates at 42.9% and 42.3%, respectively. V. parahaemolyticus exhibited a multi-drug resistance rate of 34.8%, while E. coli showed the highest rate at 50.0%.

Discussion

This research provides a summary of bacterial contamination and antibiotic resistance in frozen fish from Manila retail outlets, revealing a 90.0% contamination rate and a 39.8% multi-drug resistance prevalence. The predominance of V. parahaemolyticus (23.5%) is in agreement with the expected bacteriological profile of fish products from tropical marine environments, where warm water temperatures and estuarine conditions favor Vibrio colonization [9, 10]. This finding also agrees with earlier Philippine investigations that documented Vibrio as a leading contaminant in fresh seafood [5, 6, 11].

The higher contamination rates in wet markets (94.0%) compared to supermarkets (83.3%) is in agreement with the known differences in infrastructure, temperature control, and handling practices between these two retail formats. However, the observation that supermarkets still showed 83.3% contamination partially disagrees with the expectation of substantially better microbiological quality in modern retail settings, suggesting that contamination may occur upstream in the supply chain before products reach the retail stage [12, 13].

The broader implications of these findings are relevant to Philippine public health planning. The combination of high bacterial prevalence with substantial multi-drug resistance suggests that frozen fish products could serve as vehicles for transmitting resistant organisms to consumers, potentially contributing to the clinical resistance burden. These findings also suggest that current food safety regulations and enforcement mechanisms may be insufficient to ensure the microbiological safety of frozen fish products in Manila [14, 15]. Targeted interventions should address both the retail environment, through improved cold chain compliance and hygiene practices, and the upstream supply chain, through enhanced processing standards and monitoring of antibiotic use in Philippine aquaculture [16].

Data availability statement

All data generated during this research are included in this manuscript. Additional datasets and raw laboratory records are available from the corresponding author upon reasonable request.

Author contributions

MAR conceptualized and designed the research, performed data analysis, and wrote the manuscript. JDS conducted sample collection and microbiological analyses. RLC supervised laboratory work and critically reviewed the manuscript. All authors read and approved the final version.

Conclusion

This research aimed to determine the prevalence and antibiotic susceptibility of bacteria from frozen fish in Manila. The key findings reveal that 90.0% of samples were contaminated, with V. parahaemolyticus and E. coli predominating. High resistance to ampicillin and tetracycline was documented, and multi-drug resistance affected 39.8% of isolates. The practical implication is that both wet markets and supermarkets in Manila require improved cold chain compliance and hygiene enforcement to reduce the burden of antibiotic-resistant bacteria in frozen fish. Future investigations should employ molecular approaches to characterize virulence factors and resistance gene profiles of V. parahaemolyticus and other fish-associated pathogens in the Philippine context.

Acknowledgements

The Manila Metropolitan University provided all laboratory facilities for this investigation. MAR designed and led the project. JDS and RLC contributed to experimental work and manuscript preparation. This research was supported by the MMU Faculty Research Grant (No. MMU-FRG-2024-015).

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