

Retailer-Level Heterogeneity in *Aeromonas* Loads among Nile Tilapia at Thai Wholesale Markets

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Abstract

Aeromonas spp. are opportunistic pathogens commonly found in aquatic environments and are capable of causing gastrointestinal and systemic infections in humans. Nile tilapia (*Oreochromis niloticus*), a widely consumed freshwater fish in Thailand, may serve as a reservoir for these bacteria. Although contamination in aquaculture and retail markets has been reported, data on retailer-level variability in wholesale markets remain scarce. This study investigated the prevalence, bacterial load, and species composition of *Aeromonas* spp. in fresh Nile tilapia sold by individual retailers at two wholesale markets in Pathum Thani Province, Thailand. A total of 32 fish were collected from 19 vendors at Talaad Thai Market (n = 27) and Talaad Rangsit Market (n = 5) between 29 May and 1 June 2024. Intestinal tissue samples were analyzed for *Aeromonas* spp. loads according to ISO 6887-1 guidelines using selective media. Results were reported in log CFU/g and analyzed with non-parametric statistical tests; subsequently, isolates were identified to the species level using biochemical tests. *Aeromonas* spp. were detected in 100% of samples. Median bacterial loads did not differ significantly between markets (5.86 log CFU/g, IQR 5.13–7.18; $p = 1.00$), yet characterized by significant heterogeneity among vendors ($p = 0.010$). Among 42 isolates, *A. hydrophila* was most common (26.2%), followed by *A. caviae* (9.5%), *A. eucrenophila* (7.1%), *A. sobria* (4.8%), and *A. popoffi* (2.4%), respectively; half of the isolates were identified only to the genus level. The high prevalence and presence of potentially pathogenic species suggest that enhanced surveillance and monitoring programs for *Aeromonas* in Thai fish markets should be considered to further ensure food safety. Vendor-level hygiene interventions, molecular species identification, and antimicrobial resistance profiling are recommended to strengthen food safety in aquaculture distribution.

Keywords: *Aeromonas* spp, Nile tilapia, food safety, wholesale market

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most economically important freshwater fish species worldwide, particularly in tropical and subtropical regions. Its rapid growth rate, high feed conversion efficiency, and adaptability to diverse aquaculture systems have contributed to its increasing global production and consumption (El-Sayed, 2020; FAO, 2020). In Southeast Asia, including Thailand, tilapia serves as an affordable and accessible protein source for millions of consumers. The fish is frequently sold fresh in wholesale and retail markets, often in semi-open environments with varying hygiene practices.

Foodborne bacterial contamination in aquatic products remains a major public health concern. Among potential contaminants, *Aeromonas* spp. have emerged as important opportunistic pathogens in both aquatic animals and humans. These Gram-negative bacteria are ubiquitous in aquatic environments and are capable of producing a wide range of virulence factors, including enterotoxins, hemolysins, and cytotoxins, which have been associated with gastrointestinal diseases, wound infections, and, in some cases, septicemia (Igbinosa et al., 2012; Janda & Abbott, 2010; Sakurai et al., 2023). While *Aeromonas* spp. are recognized as part of the natural microflora of freshwater fish, their occurrence at high loads—especially of pathogenic species—poses a potential health risk if fish are undercooked or mishandled. According to International Commission on Microbiological Specifications for Foods (ICMSF) (2018) guidelines, aerobic plate counts exceeding 10^7 CFU/g in raw fish signify incipient spoilage and potential public health risks. Furthermore, *Aeromonas* species are increasingly recognized as reservoirs for antimicrobial resistance genes (ARGs), complicating clinical treatment if transmitted to humans through mishandled aquatic products (Sakurai et al., 2023; Ritter et al., 2022).

Previous studies have documented *Aeromonas* contamination in farmed fish, processing facilities, and retail outlets (Praveen et al., 2016; Sheng & Wang, 2021). However, few investigations have focused specifically on retailer-level variability within wholesale markets—key distribution hubs where fish from multiple sources are aggregated and handled by different vendors under diverse hygienic conditions. This knowledge gap is important, as contamination at the retailer level may not only reflect upstream sources such as aquaculture farms but also be exacerbated by on-site handling, water quality, and equipment sanitation practices.

In Thailand, large-scale wholesale markets such as Talaad Thai serve as national and regional distribution centers for fish and other agricultural commodities, while smaller local markets like Talaad Rangsit provide direct access to community consumers. Differences in market scale, supply chain complexity, and vendor practices could influence microbial contamination levels, yet systematic data comparing such settings are scarce. Furthermore, national microbiological standards currently do not include *Aeromonas* spp., leaving a regulatory gap in food safety monitoring.

Therefore, this study aimed to (i) determine the prevalence and bacterial load of *Aeromonas* spp. in fresh Nile tilapia sold by individual retailers at two wholesale markets in Pathum Thani Province, Thailand, and (ii) characterize the species distribution of isolates. We hypothesized that *Aeromonas* contamination would be ubiquitous among retailers but would vary significantly in bacterial load and species composition between markets and individual vendors. The findings are expected to provide evidence for improving hygiene management and informing future regulatory policies in Thailand's aquaculture distribution chain.

2. Materials and Methods

2.1 Study Design and Sampling Strategy

A cross-sectional study was conducted between 29 May and 1 June 2024 to investigate *Aeromonas* spp. contamination in fresh Nile tilapia sold by retailers at two major wholesale markets in Pathum Thani Province, Thailand: Talaad Thai Market (national-scale hub) and Talaad Rangsit Market (local community market). These sites were selected to represent different scales of distribution and vendor practices.

Sample size was determined pragmatically based on market accessibility and budget constraints, with the aim of capturing at least one sample per vendor. This approach allowed for vendor-level variability assessment while minimizing repeated sampling bias. In total, 32 live tilapias were purchased from 19 independent retailers—14 vendors at Talaad Thai Market (27 fish), an additional five samples were strategically included from Talaad Rangsit Market to enhance the overall sample size for statistical robustness and to provide a broader representation of the wholesale distribution chain. While each fish was treated as an individual sampling unit, multiple specimens were obtained from selected vendors to assess intra-vendor consistency.

2.2 Sample Collection and Transportation

In both markets, tilapias were kept alive in open-water containers until purchase. Once selected, vendors slaughtered and eviscerated and gutted the fish as part of routine practice. To minimize cross-contamination, each fish was handled with single-use gloves and transferred directly into a sterile polyethylene ziplock bag, which was sealed immediately and placed in an insulated cooler with ice packs to maintain 0–4 °C; gloves and tools were changed between vendors. Transport time from market to laboratory did not exceed two hours, in accordance with ISO 6887 (ISO, 2017), and cooler temperature was monitored with a digital probe and recorded at dispatch and on arrival.

2.3 Microbiological Analysis

2.3.1 Sample Preparation

Approximately 25 g of intestinal tissue from each fish was aseptically excised and homogenized in 225 mL of alkaline peptone water using a sterile stomacher bag and laboratory blender to prepare the initial suspension, in accordance with ISO 6887-1 and ISO 6887-3. These standards describe the preparation of initial suspensions and decimal dilutions for the microbiological examination of fishery products.

Separately, the remaining suspension was incubated at 37°C for 24 hours as a qualitative enrichment step to facilitate the subsequent isolation and biochemical identification of *Aeromonas* isolates.

2.3.2 Enumeration of *Aeromonas* spp.

Quantitative analysis was performed immediately using the initial suspension. The initial homogenate was considered a 10^{-1} dilution. Serial tenfold dilutions (10^{-2} to 10^{-4}) were prepared in sterile phosphate-buffered saline. *Aeromonas*-Selective-Agar (Bile Salts Irgasan Brilliant Green (BSIBG) Agar) plates (Neyts et al., 2000) were inoculated with 100 µL aliquots from each dilution and spread evenly using a sterile L-shaped spreader. Plates were incubated at 37 °C for 24–48 h. Presumptive *Aeromonas* colonies were counted and expressed as colony-forming units per milliliter (CFU/g) of homogenate.

2.3.3 Biochemical Identification of Isolates

Presumptive *Aeromonas* colonies—recognized by characteristic morphology and pigment on BSIBG agar—were subcultured onto tryptic soy agar to obtain pure cultures. Species-level identification was determined using biochemical tests including oxidase, triple sugar iron agar (TSI), and motility tests; production of indole, ornithine, and arginine decarboxylase; citrate utilization; urea test; methyl red and Voges-Proskauer (MR-VP) tests; growth in different concentrations of NaCl (0–8%); and carbohydrate fermentation (Abbott et al., 2003; Janda & Abbott, 2010; Ritter et al., 2022) (Table 1). Isolates that could not be fully classified were recorded as *Aeromonas* spp.

Table 1 Biochemical characteristics used for *Aeromonas* species identification.

Biochemical Test	<i>A. schubertii</i>	<i>A. media</i>	<i>A. sobria</i>	<i>A. hydrophila</i>	<i>A. eucrenophila</i>	<i>A. caviae</i>	<i>A. popoffi</i>
Gram stain	-	-	-	-	-	-	-
Oxidase	+	+	+	+	+	+	+
TSI	A/A	K/A	A/A	A/A	K/A	A/A	A/A
Motility	+	-	+	+	+	+	+
Indole	-	v	+	+	-	+	-
Ornithine decarboxylase	-	-	-	-	-	-	-
Arginine decarboxylase	+	+	+	+	+	+	+
Citrate	v	v	+	+	-	+	+
Urea	-	-	-	-	-	-	-
Methyl red indicator	+	+	-	+	-	+	+
Voges-Proskauer	v	-	-	+	-	-	-
0% NaCl	+	+	+	+	+	+	+
1% NaCl	+	+	+	+	+	+	+
3% NaCl	+	+	+	+	+	+	+
6% NaCl	+	+	+	+	+	+	+
8% NaCl	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	+
Mannitol	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Sucrose	-	+	+	+	-	+	v
Galactose	+	+	+	+	+	+	+

Note: Adapted from Abbott et al. (2003), Janda and Abbott (2010), and Ritter et al. (2022).

Symbol definitions: “+” = positive result; “-” = negative result; “A/A” = acid/acid (yellow slant/yellow butt in TSI test);

“A/K” = acid/alkaline (yellow slant/red butt); “v” = variable result; blank cells = not reported or inconsistent results.

2.4 Data Analysis

CFU counts, treating each individual fish as the unit of analysis, were log₁₀-transformed prior to statistical testing to normalize variance. Market-level comparisons were performed using the Mann–Whitney U test, while retailer-level differences were assessed with the Kruskal–Wallis H test to evaluate variability across individual retailers.

Results are presented as median values with interquartile range (IQR). All analyses were conducted in Jamovi software (version 2.6.26) (Jamovi Project, 2025), with statistical significance set at $p < 0.05$.

3. Results and Discussion

3.1 Prevalence of *Aeromonas* spp. in fresh Nile Tilapia

To confirm bacterial viability and demonstrate successful culture conditions, four levels of serial dilution (10^{-1} to 10^{-4}) were performed on representative intestinal homogenates. Serial dilutions from 10^{-1} to 10^{-3} (30–300 CFU/plate) yielded countable colonies, with the 10^{-2} dilution providing the most optimal separation for enumeration (Figure 1). This confirmed culturability and ensured valid quantification of bacterial loads.

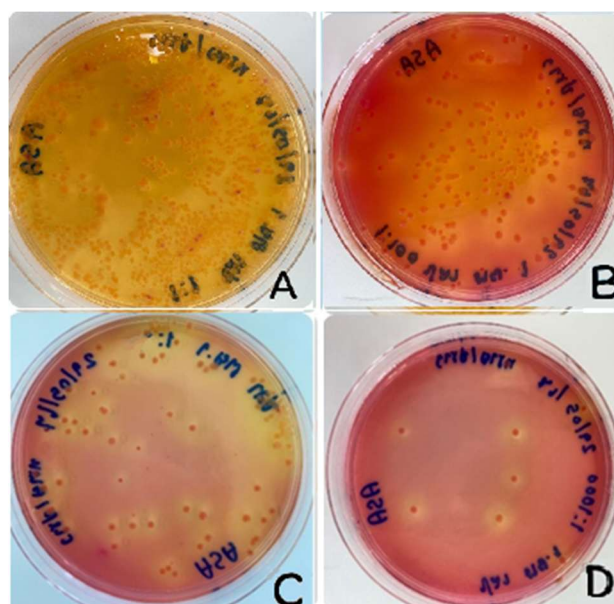


Figure 1 Colony morphology of *Aeromonas* spp. on Aeromonas-Selective-Agar at various dilution levels. Samples were plated from the initial suspension and further serially diluted as follows: (A) 10^{-1} , (B) 10^{-2} , (C) 10^{-3} , and (D) 10^{-4}

All 32 Nile tilapia samples yielded presumptive *Aeromonas* colonies on selective agar, characterized by circular, smooth, and convex morphology, appearing yellowish-green with a distinct dark-green or opaque center (Figure 1), corresponding to a 100% detection rate across both wholesale markets. This universal prevalence indicates that *Aeromonas* contamination is widespread in retail-level fish sold at both the national-scale Talaad Thai Market and the community-scale Talaad Rangsit Market. This underscores that *Aeromonas* spp. are pervasive in the retail fish supply chain, reflecting both environmental ubiquity and the vulnerability of live-holding systems to cross-contamination.

3.2 Bacterial Load and Retailer-Level Variability

Quantitative analysis using non-parametric methods was employed to address the unequal sample sizes ($n=27$ vs. $n=5$). Both markets exhibited identical median bacterial loads of 5.86 log CFU/g (IQR = 5.13–7.32 for Talaad Thai and 5.13–6.03 for Talaad Rangsit); the inter-market difference was not statistically significant ($U = 67.5$, $p = 1.00$) (Figure 2). However, the contamination range was broader in Talaad Thai Market (range: 7.06×10^3 to 8.12×10^7 CFU/g; compared to Talaad Rangsit Market (range: 1.12×10^4 to 1.59×10^6 CFU/g, respectively). Furthermore, significant heterogeneity was observed at the retailer level across all 19 vendors (Kruskal–Wallis $H = 29.12$, $p = 0.010$). These findings highlight that contamination levels are predominantly influenced by individual vendor handling practices rather than the market location itself.

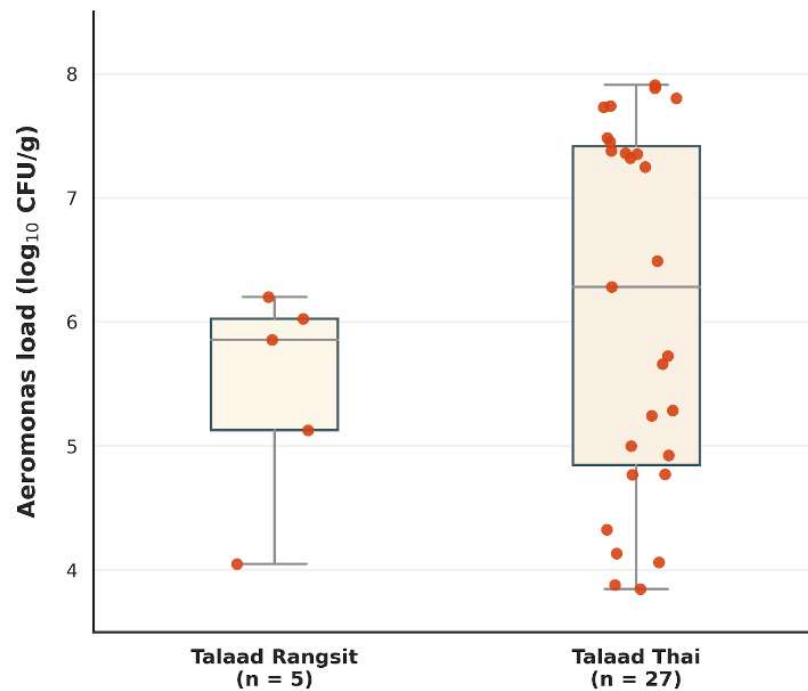


Figure 2 Quantitative distribution of *Aeromonas* spp. loads ($\log_{10}$ CFU/g) in Nile tilapia from Talaad Rangsit Market (n=5) and Talaad Thai Market (n=27). The box plot represents the median and interquartile range (IQR), while the overlaid jittered points illustrate the individual retailer-level heterogeneity

The broad contamination range and higher magnitude of bacterial loads observed in Talaad Thai Market compared with Talaad Rangsit Market may be linked to differences in market scale, throughput, and infrastructure. Talaad Thai Market, as a national-level hub, handles larger fish volumes from multiple provinces, potentially prolonging holding times and increasing opportunities for bacterial proliferation before retail sale. While the median bacterial loads were comparable between the two markets (Figure 2), the greater variation in contamination levels within Talaad Thai Market also suggests inconsistent hygiene practices among vendors, possibly due to variability in stall infrastructure, water replacement frequency, and utensil sanitation.

Bacterial loads exceeded 10^7 CFU/g in several samples, highlighting significant public health concern. Importantly, while overall median loads were similar, the intensity of contamination and the degree of variability differed substantially at the retailer level. Such differences suggest that vendor-level hygiene practices play a crucial role in determining contamination intensity. These observations align with prior studies emphasizing that microbial safety of fish at retail is shaped by both environmental management and individual handling practices (Lamy et al., 2009; Sheng & Wang, 2021).

3.3 Species Distribution of *Aeromonas* Isolates

From the 32 fish samples, 42 presumptive *Aeromonas* isolates were recovered and subjected to biochemical identification. As summarized in Table 2, *A. hydrophila* was the most prevalent species overall (26.2%), followed by *A. caviae* (9.5%), *A. eucrenophila* (7.1%), *A. sobria* (4.8%), and *A. popoffi* (2.4%). Notably, 50% of isolates could not be identified to the species level and were classified as *Aeromonas* spp.

Table 2 Species-level distribution of *Aeromonas* isolates obtained from Nile tilapia at Talaad Rangsit and Talaad Thai markets.

Species	Talaad Rangsit (n)	Talaad Rangsit (%)	Talaad Thai (n)	Talaad Thai (%)	Total (n)	Total (%)
<i>A. hydrophila</i>	3	37.50%	8	23.53%	11	26.19%
<i>A. eucrenophila</i>	1	12.50%	2	5.88%	3	7.14%
<i>A. caviae</i>	1	12.50%	3	8.82%	4	9.52%
<i>A. sobria</i>	2	25.00%	0	0.00%	2	4.76%
<i>A. popoffi</i>	0	0.00%	1	2.94%	1	2.38%
<i>Aeromonas</i> spp.	1	12.50%	20	58.82%	21	50.00%

Note: Values are presented as number and percentage of isolates per market and in total.

Species composition differed between markets. Talaad Thai accounted for 34 (81%) of the total 42 isolates, with a higher proportion of unclassified *Aeromonas* spp., while Talaad Rangsit, despite its smaller sample size, exhibited a more balanced species distribution. The predominance of *A. hydrophila* in both markets, particularly in higher CFU samples, highlights the potential pathogenic significance given its association with multiple virulence factors.

Among isolates, *A. hydrophila*—a species associated with hemolysins, cytotoxins, and multiple virulence factors (Janda & Abbott, 2010; Sakurai et al., 2023)—emerged as the dominant species, reinforcing concerns about the circulation of virulent strains in the retail fish supply chain. While *Aeromonas* spp. are often considered opportunistic pathogens, the predominance of *A. hydrophila* strengthens the argument for its recognition as a foodborne risk organism with broader implications for consumer safety.

3.4 Regulatory and Public Health Implications

Taken together, the high bacterial burden and broad dispersion observed, alongside significant between-retailer heterogeneity, suggest a potential need for targeted hygiene controls at both market and vendor levels. Findings highlight the importance of considering *Aeromonas* testing in future microbiological monitoring for fish products, aligning with ICMSF recommendations (ICMSF, 2018). Vendor-focused interventions such as mandatory hygiene training, standardized water replacement protocols, and the sanitization of storage containers, are critical for reducing microbial loads. Frequent water exchange limits the accumulation of organic matter and fish exudates that serve as substrates for bacterial proliferation, while biofilm removal from surfaces prevents persistent re-contamination of live fish (Sheng & Wang, 2021; Lamy et al., 2009). Routine microbial monitoring at wholesale markets should be prioritized to identify high-risk vendors and enable timely corrective actions.

3.5 Limitations and Future Research

This study was limited by reliance on phenotypic identification methods and market-level sampling, which restricts fine-scale inference. Future research should incorporate molecular identification and antimicrobial resistance profiling and adopt a farm-to-market sampling framework to strengthen traceability, improve public health risk assessment, and inform evidence-based aquaculture food safety policy.

4. Conclusion

Aeromonas spp. contamination was ubiquitous in retail tilapia from wholesale markets in Pathum Thani, with *A. hydrophila* predominance and substantial between-retailer variability. The widespread presence of *Aeromonas* spp. and the identification of potentially pathogenic species suggest that enhanced surveillance and monitoring programs in Thai fish markets should be considered to further ensure food safety. Embedding targeted hygiene interventions and tracking along the farm-to-market continuum will better align retail practices with food-safety management and public health protection.

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