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Prevalence of *Salmonella* and *Edwardsiella* spp. in Nile Tilapia (*Oreochromis niloticus*) sold in some retail fish markets in Tehran, Iran

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Abstract

Salmonella spp. and *Edwardsiella spp.* are major zoonotic pathogens associated with seafood, responsible for foodborne illnesses and significant public health risks. This study investigated their prevalence in Nile tilapia (*Oreochromis niloticus*) sold in retail fish markets in Tehran, Iran. A total of 108 samples were collected in autumn 2024, including 68 fresh whole fish, 24 imported frozen fillets from the main market, and 16 fresh samples from retail outlets. Two skin swabs were taken from each fish and analyzed according to ISO 6579-1:2017 standards, using selective enrichment, bacteriological plating, and biochemical confirmation. Initial screening suggested Salmonella in 48 samples (44.4%) and Edwardsiella in 8 (7.4%). Confirmatory testing identified *Salmonella spp.* in 8 samples (7.4%): 4 from frozen fillets and 4 from fresh retail fish. *Edwardsiella spp.* was confirmed in 4 samples (3.7%), all originating from frozen fillets. Statistical analysis showed a significant difference in Edwardsiella contamination between fresh and frozen samples ($p = 0.0147$). Salmonella contamination also differed significantly between the main market and other retail sources ($p = 0.0026$). These findings suggest contamination may be linked to poor packaging and non-specialized handling in retail settings. As tilapia is increasingly consumed raw or undercooked, routine microbial monitoring is necessary to protect food safety.

Introduction

Tilapia is a freshwater fish native to Africa, belonging to the family Cichlidae. Among the tilapia species, the Nile tilapia (*Oreochromis niloticus*) is the most commercially important, as it is widely distributed in rivers, lakes, and reservoirs and tolerates a diverse range of water conditions (1, 2). Globally, tilapia is the second most widely farmed fish, cultivated in more than 90 countries, with China, Egypt, and the Philippines as the leading producers (1, 3). In Iran, tilapia was introduced in 1999 for research purposes, and commercial farming remains restricted to specific regions due to environmental concerns (4). Its rapid growth, disease resistance, and environmental adaptability support its widespread use in aquaculture, making tilapia a valuable and affordable source of protein (1).

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In recent decades, aquatic foods have become a significant component of the global diet. According to the Food and Agriculture Organization (FAO), global per capita consumption rose from approximately 9–10 kg in 1960–1961 to more than 20 kg between 2014 and 2022 (5, 6). This increase reflects wider recognition of the health benefits of fish, which provide essential fatty acids, vitamins, and minerals (7). In Iran, per capita seafood consumption is about 13 kg, up from just over 9 kg in previous years, although it remains below the global average (8). In 2015, Iran was identified as a country with a marked rise in tilapia imports (9).

Seafood can become contaminated with zoonotic bacteria at multiple points in the supply chain, including processing, storage, and distribution, creating risks for consumers, vendors, and food handlers (10). Among seafood-associated pathogens, *Salmonella* spp. and *Edwardsiella* spp. raise particular concern because of their pathogenicity and potential to cause severe disease in humans. This study, therefore, aimed to determine the prevalence of these two zoonotic bacteria in Nile tilapia (*Oreochromis niloticus*) obtained from retail fish markets in Tehran, Iran.

Edwardsiella species occur naturally in aquatic environments and fish farms, and they infect multiple hosts, including fish and humans, primarily through contaminated water or food (11, 12). In humans, infections range from gastroenteritis to severe systemic disease (13). Likewise, *Salmonella* spp., one of the most common foodborne pathogens worldwide, can contaminate a wide variety of foods, including seafood such as fish and shrimp (14). With global seafood consumption on the rise, particularly in raw or minimally processed forms, fish-associated salmonellosis has become a growing public health concern (15). An estimated 7% of human salmonellosis cases are linked to aquatic products (16). For example, in 2022, a *Salmonella* outbreak linked to fish consumption was reported across four U.S. states, resulting in 39 illnesses and 15 hospitalizations, primarily among individuals who had consumed raw fish or sushi. According to the Centres for Disease Control and Prevention (CDC), approximately 1.2 million cases of salmonellosis occur each year in the United States, with an estimated 450 related deaths (17, 18).

Although many studies worldwide report the presence of zoonotic bacteria in fish, data on the prevalence of *Edwardsiella* and *Salmonella* in Nile tilapia are limited, particularly in Iran. Most studies have examined other fish species or aquaculture settings, leaving uncertainty about the public health risks associated with tilapia sold in retail markets. This study, therefore, aims to determine the prevalence of *Edwardsiella* spp. and *Salmonella* spp. in Nile tilapia (*Oreochromis niloticus*) obtained from retail fish markets in Tehran, Iran.

Materials and Methods

Sampling

A total of 108 Nile tilapia (*Oreochromis niloticus*) samples were collected in autumn 2024 from fish markets in Tehran, Iran, including the main wholesale market and several local retail outlets. The samples included 68 fresh whole tilapia, 24 imported frozen fillets, and 16 fresh specimens obtained from retail markets. Immediately after collection, all samples were placed on ice and transported rapidly to the microbiology laboratory under cold chain conditions (4 ± 1 °C) for conducting the experiments.

For each sample, two sterile disposable swabs (Katan Sadid®, Iran) were collected from the external surface, including the pectoral and dorsal fins. The swabs were pre-moistened with sterile distilled water to improve bacterial recovery. All sampling procedures were conducted beside a Bunsen burner flame to maintain aseptic conditions and reduce the risk of external contamination. For frozen fillets, the samples were thawed under refrigerated conditions (4 ± 1 °C) before swabbing with the same method. In total, 216 swab samples were obtained (two swabs per fish).

Each swab was immediately placed in a sterile screw-cap tube containing 10 mL of Buffered

Peptone Water (BPW; Ibresco, Iran). All samples were maintained on ice during transport, and processing began within 2 hours of arrival at the laboratory to preserve microbial viability.

Isolation and identification of Salmonella spp. and Edwardsiella spp.

The isolation and identification of *Salmonella spp.* and *Edwardsiella spp.* were carried out according to the ISO 6579-1:2017 standard method, with modifications for fish samples, in four steps:

Non-selective pre-enrichment

Each swab sample was inoculated into 10 mL of Buffered Peptone Water (BPW; Ibresco, Iran) and incubated at 37 °C for 24 h to allow recovery of stressed or sub-lethally injured bacterial cells.

Selective enrichment

- For *Salmonella spp.*, 0.1 mL of the BPW culture was transferred into 10 mL of Rappaport–Vassiliadis (RV) broth (Ibresco, Iran) and incubated at 41.5 °C for 24 h.
- For *Edwardsiella spp.*, 1 mL of the BPW culture was transferred into 10 mL of Tryptic Soy Broth (TSB; Ibresco, Iran) and incubated at 35 °C for 48 h.

Selective plating on solid media

Following enrichment, a loopful of each culture was streaked onto selective agar media:

- For *Salmonella spp.*: Xylose Lysine Deoxycholate (XLD) agar and Eosin Methylene Blue (EMB) agar (Himedia, India)were used.
- For *Edwardsiella spp.*: XLD agar, Hektoen Enteric (HE) agar and MacConkey agar (Himedia, India) were used.

All plates were incubated at 37 °C for 24–48 h. Colony morphology and pigmentation were observed to identify presumptive isolates:

- *Edwardsiella spp*. typically presented as small, smooth, and greyish colonies.
- *Salmonella spp.* typically formed pink colonies with black centers on XLD agar.

Biochemical identification

Presumptive colonies were subjected to standard biochemical tests for confirmation, including:

- Citrate utilization test (Simmon's Citrate Agar; Himedia, India)
- Hydrogen sulfide (H_2 S) production on Triple Sugar Iron (TSI) agar; Himedia, India
- Methyl Red (MR) test
- Voges–Proskauer (VP) test
- Urease test
- Indole production test
- Motility test using Motility Indole Ornithine (MIO) medium; (Himedia, India)

All biochemical assays were performed according to the ISO 6579-1:2017 protocol for Salmonella and standard references for Edwardsiella identification.

Quality Control

Blank negative controls were included in each batch of microbiological analyses as part of quality assurance. Sterile Buffered Peptone Water (BPW) tubes without samples were processed in parallel with test samples to monitor potential media contamination, cross-contamination, or procedural errors.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20. Descriptive statistics, including frequencies and percentages, were calculated to summarize the prevalence of bacterial contamination. Due to the limited number of positive cases, Fisher's exact test was used to compare contamination rates between sample groups. A p-value < 0.05 was considered statistically significant.

Results

A total of 108 Nile tilapia (*Oreochromis niloticus*) samples were collected from markets in Tehran, Iran.

Prevalence of Salmonella spp.

Of the 108 samples, 48 (44.4%) were initially suspected of contamination with *Salmonella spp.* based on selective culture media. However, confirmatory biochemical testing showed that only 8 samples (7.4%) were positive, while 100 (92.6%) were negative.

Prevalence of Edwardsiella spp.

Among the 108 samples, 8 (7.4%) were suspected of contamination with *Edwardsiella spp*. based on colony morphology. Confirmatory biochemical tests identified 4 positive samples (3.7%), whereas 104 (96.3%) were negative.

Distribution by Market Type and Fish Condition

- Fresh tilapia samples: None of the 68 fresh tilapia collected from the central market were contaminated with *Salmonella* or *Edwardsiella.*
- Frozen tilapia fillets: Of the 24 frozen fillet samples, 4 (16.7%) were contaminated, all testing positive for both *Salmonella* and *Edwardsiella*.
- Retail market samples: Among the 16 fresh tilapia obtained from retail markets, 4 (25.0%) were positive for *Salmonella*, while no *Edwardsiella* was detected.

Descriptive analysis was performed using SPSS software (version 20). Due to the limited number of positive samples, Fisher's exact test was applied to compare contamination rates between groups. Salmonella contamination differed significantly between fresh and frozen samples ($p = 0.0026$) and between central and retail markets ($p = 0.0016$). Edwardsiella contamination was also significantly higher in frozen samples compared with fresh ones ($p = 0.0147$). A p-value < 0.05 was considered statistically significant.

Table 1. Biochemical Test for *Edwardsiella* and *Salmonella*

Biochemical Test	*Salmonella*	*Edwardsiella*
Citrate utilization	+	-
Hydrogen sulfide	+	-
Methyl red	+	+
Voges-Proskauer	-	-
Lysine decarboxylase	+	-
Urease	-	-
Indole	-	+
Motility	+	+

Table 2. Prevalence of *Salmonella* in Tilapia Fish Samples from Tehran Markets

Category	Number of Samples	Percentage
Total samples collected	108	100
Suspected contaminated samples	48	44.4
Confirmed positive for *Salmonella*	8	7.4
Negative samples	60	55.6

Table 3. *Edwardsiella* Infection Status in Fish Samples

Category	Number of Samples	Percentage
Total samples collected	108	100
Suspected contaminated samples	8	7.4
Confirmed positive for *Edwardsiella*	4	3.7
Negative samples	104	96.3

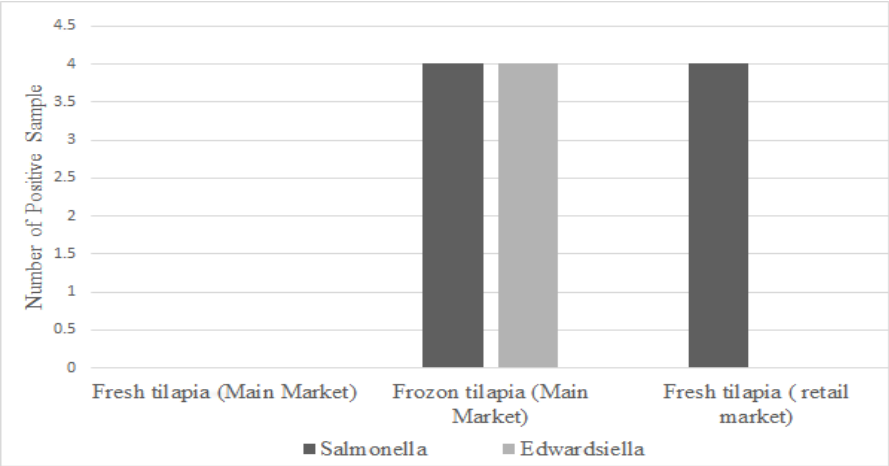

Fig. 1. Detection of *Salmonella* and *Edwardsiella* in tilapia samples

Discussion

This study was conducted from late September to early November, when fresh tilapia was available in the markets. No significant differences were observed in the prevalence of Salmonella or Edwardsiella across the collection time points, despite variations in ambient and water temperatures during this period. Under these conditions, temperature changes did not measurably affect bacterial contamination levels. Prior work suggests that higher water temperatures can promote bacterial proliferation in aquaculture systems and may drive seasonal differences in prevalence. Future studies should use larger sample sizes across multiple seasons to clarify the influence of temperature on contamination patterns. Overall, these findings highlight the importance of strict hygiene, effective cold-chain management, and regular microbiological monitoring, particularly for frozen seafood products, to ensure food safety in both wholesale and retail markets.

In this study, Salmonella and Edwardsiella were detected in 7.4% and 3.7% of tilapia samples, respectively. Contamination occurred exclusively in frozen fillets from the central wholesale market, whereas fresh samples were largely free of these bacteria. Several epidemiological mechanisms may account for these differences.

Frozen fillets often undergo bulk packaging and prolonged storage, conditions that can support bacterial survival and growth, particularly when freezing and thawing are suboptimal. Introduction of bacteria during earlier processing or importation also cannot be ruled out. In contrast, fresh fish typically have rapid turnover, shorter storage, and controlled handling, which limit opportunities for bacterial growth.

Differences between wholesale and retail markets may reflect hygiene standards, staff training, and infrastructure. The absence of Edwardsiella in fresh samples suggests that this bacterium is less tolerant of short-term storage or is introduced primarily via frozen fillets. Previous studies report similar patterns. In Dhaka, Bangladesh, local fish showed higher bacterial contamination than supermarket fish, including *Vibrio cholerae* (62%), Salmonella (24%), and *Escherichia coli* (92%) (19). A study of rainbow trout farms along the Haraz River identified isolates such as *Yersinia* (37%), *Aeromonas* (33%), and Edwardsiella (11%), whereas in Tehran tilapia, the prevalence of Edwardsiella was 3.7% and confined to frozen fillets (20). Likewise, only 3 of 80 randomly collected rainbow trout samples from aquaculture ponds in Chaharmahal and Bakhtiari Province tested positive for Salmonella (21). In Qalyubia, Egypt, contamination was higher in North African

catfish (9.8%) than in tilapia (6%), with Streptococcus, Salmonella, and *E. coli* the most frequently identified bacteria (22). Another study from Sokoto, Nigeria, reported nine bacterial species in fresh tilapia from the central market, including six Gram-positive and three Gram-negative species; *Bacillus pumilus* was most common (19.35%), whereas Salmonella was least frequent (3.2%) (23).

Taken together, these findings suggest that storage conditions, packaging methods, and market management significantly impact the prevalence of zoonotic bacteria. The results align with previous reports and suggest that imported frozen fillets are more likely to harbor bacterial contamination, whereas fresh fish with rapid turnover and proper handling are less susceptible. Overall, these findings indicate the need for strict hygiene, effective cold-chain management, and regular microbiological monitoring, particularly for frozen seafood products, to ensure food safety in both wholesale and retail markets.

Conclusion

This study detected Salmonella and Edwardsiella in 7.4% and 3.7% of tilapia samples, respectively. Positive *Salmonella* cases were detected in fresh tilapia from retail markets and in imported frozen fillets from the main market, whereas Edwardsiella contamination was limited to the frozen fillets. Likely sources of contamination include inadequate packaging of frozen fillets and non-specialized handling at retail outlets. As tilapia consumption grows, valued for its rapid growth, high-quality meat, and affordability, routine monitoring of microbial contamination remains essential, particularly given the increasing consumption of raw or undercooked seafood.

In addition, the following recommendations are suggested to mitigate the risk of bacterial contamination:

- Conduct additional studies to assess the presence of Edwardsiella and Salmonella in other fish species sold in seafood markets.
- Utilize alternative diagnostic methods in conjunction with traditional culture-based assays to enhance pathogen detection.
- Measure contamination on market equipment and surfaces, and compare infection rates in fish sold at markets with those in fish supplied directly from aquaculture farms.
- Evaluate aquaculture water quality for Edwardsiella and Salmonella.
- Implement stricter hygiene practices, packaging protocols, and cold-chain management to reduce the risk of contamination in frozen fish products.

Acknowledgments

Not applicable.

Conflict of Interest

The authors declare no conflicts of interest.

Ethical Approval

Not applicable.

Artificial Intelligence Statement

The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

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