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Microbiological Risk Assessment of Coliforms and *Escherichia coli* Contamination in the Fish Supply Chain: Implications for Food Safety in Local Markets and Restaurants, Omdurman Locality, Khartoum State, Sudan

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ABSTRACT

Fish is a vital source of protein and essential nutrients, but its high perishability makes it particularly vulnerable to microbial contamination during handling, storage, and processing. Coliform bacteria and *Escherichia coli* (*E. coli*) are important indicators of hygiene status and potential fecal contamination along the supply chain. This study aimed to evaluate the levels of coliform and *E. coli* contamination across critical points of the fish supply chain in local markets and restaurants in Sudan, as well as to identify associated risk factors. A total of 90 samples were collected from five sampling points: fish skin swabs, table surfaces, employee hands, pre-cooking fish, and post-cooking fish. Results revealed that employee hands harbored the highest coliform count (81.04 CFU), followed by table surfaces (23.22 CFU) and pre-cooking samples (12.12 CFU), while post-cooking samples had the lowest (5.70 CFU), demonstrating that heat treatment significantly reduced bacterial loads. Mean *E. coli* counts varied significantly across the supply chain, with the highest contamination observed in fish market samples (3.8×10^2 CFU), followed by pre-cooking samples in restaurant refrigerators (1.2×10^2 CFU), and the lowest after cooking (0.6×10^2 CFU) ($p \leq 0.05$). Risk factor analysis indicated strong associations between *E. coli* contamination and poor hygiene practices, including inadequate personal cleanliness, unclean surfaces, and improper handling during transportation and preparation. A qualitative risk assessment classified the fish market pathway as high risk, the restaurant pathway as low risk, and the overall supply chain risk as medium. These findings highlight that human contact, particularly through employee hands, is the most critical source of contamination, while cooking remains effective in bacterial reduction. The study underscores the urgent need for implementing strict hygiene standards, improved handling practices, and better environmental sanitation in both markets and restaurants to minimize microbial hazards, safeguard food safety, and protect public health.

Introduction

Fish is a valuable source of protein and essential nutrients, but it is also highly perishable and can easily become contaminated with harmful bacteria such as *Escherichia coli* (*E. coli*), especially when hygiene practices are poor. Contamination can occur during handling, transportation, storage, or preparation (FAO, 2020).

E. coli is often used as an indicator of fecal contamination and poor sanitation. Its presence in food, particularly fish, can pose serious health risks to consumers. If proper cleanliness is not maintained throughout the fish supply chain, the risk of foodborne illness increases (WHO, 2015).

Contamination in the fish supply chain can occur at multiple stages — from the fish market to restaurants — due to unsafe handling by workers, unclean equipment or surfaces, improper refrigeration, and inadequate cooking (Bhandare *et al.,* 2007). Human contact, especially through unwashed hands, has been identified as a major contributor to bacterial contamination. If food safety measures are not strictly followed, consumers may be exposed to serious health risks, including foodborne illnesses caused by pathogenic strains of *E. coli* (Newell *et al.,* 2010).

In developing countries, challenges such as limited access to clean water, lack of sanitation infrastructure, and poor awareness of food hygiene among workers further increase the risk of contamination. Therefore, evaluating the level of microbial contamination in fish and identifying critical control points along the supply chain is essential for protecting public health and ensuring food safety.

This study was conducted to assess the levels of coliform and *E. coli* contamination in fish at different stages of the supply chain — from markets to restaurants — and to evaluate the associated risk factors. The findings will help highlight the main sources of contamination and inform recommendations to improve hygiene practices and reduce health risks to consumers. This study was conducted to assess the levels of *E. coli* and coliform bacteria in fish from markets and restaurants, and to identify the major sources of contamination. The findings aim to support better hygiene practices and improve food safety.

Materials and Methods

Study Area and Design

A cross-sectional study was conducted between April and December 2021 in Omdurman locality, Khartoum State, Sudan. The investigation targeted two main components of the fish supply chain: the fish market and restaurants. Structured checklists and field observations were used to assess hygiene practices and potential contamination sources.

Sample Collection

A total of 90 fish-related samples were collected from Almorada area, covering three key points in the fish supply chain:

Fish Market (Pre-processing Stage)

Swab samples were taken from fish skin, display tables, and workers' hands (10 samples per source, totaling 30 samples).

Restaurants (Pre-cooking Stage)

Samples were collected from raw fish stored in refrigerators prior to cooking (30 samples).

Restaurants (Post-cooking Stage)

Swabs were collected from cooked fish ready for serving (30 samples).

All samples were taken using sterile cotton swabs, immediately placed in sterile, labeled containers, stored in a cold box, and transported to the Laboratory of Bahri University for microbiological analysis.

Microbiological Analysis and *E. coli* Detection:

Each sample underwent serial dilution using buffered peptone water. One milliliter of each dilution was inoculated onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Colonies with metallic green sheen, indicative of *Escherichia coli*, were counted and recorded.

E. coli identification was confirmed through standard biochemical tests following International Organization for Standardization (ISO) (2005) guidelines and methods described by Barrow and Feltham (2004).

These included:

- Growth on EMB selective agar
- Indole production
- Methyl Red test
- Voges-Proskauer test
- Kligler Iron Agar reaction.

Statistical Analysis

Data were analyzed using SPSS software. Descriptive statistics were calculated, and Analysis of Variance (ANOVA) was used to assess differences in bacterial loads across sample sources. Chi-square tests evaluated the association between identified risk factors and contamination levels. A significance level of $p \leq 0.05$ was used.

Qualitative Risk Assessment of *E. coli*:

Hazard Identification

Following FAO (2011) guidelines, *E. coli* was identified as the biological hazard under investigation. The presence of the organism was confirmed through laboratory culture and identification methods.

Release Assessment

Based on OIE (2014) methodology, the release assessment evaluated the likelihood of *E. coli* introduction during fish handling, processing, and storage in markets and restaurants.

Exposure Assessment

This component estimated the probability of consumer exposure to *E. coli* through two main pathways: contamination during storage or processing and consumption of inadequately cooked fish. Data from field observations and checklists were used to support this evaluation.

Qualitative Risk Estimation

Risk levels were estimated using a tabular scoring framework adopted from Defra (UK), integrating likelihood and impact scores to determine overall risk. The framework ranked risk as:

- Very Low (VL)
- Low (L)
- Medium (M)
- High (H)

Each step in the fish supply chain was assessed based on identified risk factors, mapped geographically and functionally through a structured tabular format.

Overall Risk Estimation

The final risk level was calculated as the product of the estimated likelihood and consequence of exposure (Risk = Likelihood × Impact), following the qualitative scheme developed by FAO (2011) and OIE (2014). Visual aids and flowcharts (Figures 1–4) were used to illustrate the pathways and outcomes of the risk assessment.

Results and Discussion

Coliform Count Along the Fish Supply Chain

The coliform count across different points in the fish supply chain is presented in Table 2. The highest mean coliform count was recorded from employee hand swabs (mean = 81.04 CFU, SE = 34.79), with a maximum value of 290.00 CFU, indicating a critical point of contamination.

Statistical analysis revealed a highly significant difference ($p = 0.000$) between this group and the other sample sources, including skin swabs, pre-cooking, and post-cooking samples. A significant difference was also found between employee hand samples and table surface swabs ($p = 0.002$).

Table surfaces showed the second highest contamination (mean = 23.22 CFU, max = 110.00), though the differences compared to skin and pre-cooking samples were not statistically significant.

Fish skin swabs (mean = 10.10 CFU) and pre-cooking samples (mean = 12.12 CFU) presented similar levels of contamination ($p = 0.890$). However, the difference between skin swabs and employee hands remained highly significant ($p = 0.000$), confirming the impact of direct human handling on bacterial load.

The post-cooking samples showed the lowest contamination levels (mean = 5.70 CFU), demonstrating effective microbial reduction through heat treatment. While not significantly different from skin, table, or pre-cooking samples ($p > 0.05$), the post-cooking bacterial load was significantly lower than that of employee hand samples ($p = 0.000$).

E. coli Count Across the Fish Chain

The mean *E. coli* count across all samples was 405 CFU ($4.05\times10^2 \pm 7.3$), with a range from 50 CFU to 3,000 CFU. Table 3 shows a breakdown of contamination levels by stage:

Fish market: $3.8\times10^2 \pm 1.6$ CFU
Restaurants (before cooking): $1.2\times10^2 \pm 0.4$ CFU
Restaurants (after cooking): $0.6\times10^2 \pm 0.02$ CFU.

Statistical analysis showed highly significant differences between the three stages ($p = 0.00$), with contamination levels decreasing progressively from market to restaurant and post-cooking.

Risk Factors and Hygiene Practices

Observational data revealed that only 60% of fish were stored in refrigerators in the fish market. Just 25% of fish vendors practiced proper waste disposal.

In restaurants, 55% of workers processed fish in a clean environment, and 40% adhered to proper personal hygiene. These findings are summarized in Table 4.

Chi-square analysis indicated a significant association between *E. coli* levels and six identified risk factors ($p \leq 0.05$), as detailed in Table 3.

Qualitative Risk Assessment of *E. coli* Contamination

E. coli contamination was qualitatively categorized as follows:

Very Low Risk: 0–<10 CFU
Low Risk: 10–<100 CFU
Medium Risk: 100–<300 CFU
High Risk: ≥300 CFU

Based on risk pathway analysis:

Fish market pathway: Medium (likelihood) × High (impact) = High Risk
Restaurant (before/after cooking): Medium × Low = Low Risk
Combined Market & Restaurant pathway: High × Low = Medium Risk

Overall, the final risk estimation for *E. coli* contamination in the fish supply chain was Medium, indicating that a risky event is likely to occur more than once within the next three years, as per the DEFRA (UK) risk scoring framework.

The results of this study highlight significant microbial contamination across the fish supply chain in Omdurman, Sudan, with human handling emerging as the most critical point of contamination. The highest *Escherichia coli* (*E. coli*) load was recorded on employee hands (mean = 81.04 CFU), a statistically significant difference ($p < 0.001$) compared to other sources, including fish skin, table surfaces, and pre- and post-cooking samples. This confirms that direct human contact is a major route for microbial transmission in food environments, consistent with the findings of Mensah *et al.,* (2015), who reported that inadequate personal hygiene among fish handlers in Ghana was a major contributor to bacterial contamination.

Table surfaces also demonstrated considerable microbial load (mean = 23.22 CFU), suggesting the potential role of environmental reservoirs and poor sanitation practices. When not regularly disinfected, food preparation surfaces can support biofilm formation and persistent microbial survival (Ryu *et al.,* 2020). This indicates the importance of implementing routine and effective cleaning protocols, particularly in high-contact areas.

Moderate contamination levels were observed in fish skin swabs and pre-cooking samples (means = 10.10 and 12.12 CFU, respectively), reflecting the initial microbial burden from environmental exposure, polluted water sources, or cross-contamination during handling and storage. Since *E. coli* is not a natural inhabitant of fish, its presence indicates fecal contamination from water or human contact. Abakari *et al.,* (2018) reported similar findings in freshwater fish in Ghana, linking *E. coli* presence to contaminated water and unhygienic storage containers.

Table.1 Qualitative risk assessment by Defra- UK.

Likelihood	Description
VL Very low	Rare (the risky event may occur in exceptional circumstances).
L Low	Possible (the risky event may occur in the next three years).
M Medium	Likely (the risky event is likely to occur more than once in the next three years).
H High	Almost certain (the risky event is likely to occur this year or in frequent intervals).

Table.2 Coliform counts across various sources in the fish supply chain.

Source	N	Mean (CFU)	Std. Error	Min	Max	Significance
Skin swab	10	10.10	1.96	3.00	20.00	$p = 0.000$
Table surface	10	23.22	10.23	0.70	110.0	$p = 0.002$
Employee hand	10	81.04	34.79	2.80	290.0	—
Pre-cooking	30	12.12	4.03	0.70	120.0	$p = 0.890$
Post-cooking	30	5.70	1.67	2.30	43.00	$p = 0.000$
Total	90	18.65	4.76	0.70	290.0	—

Table.3 Mean E. coli counts at different stages of the fish chain.

Stage	*E. coli* Mean Count (CFU)	Significance
Fish market	3.8×10^2	$p = 0.000$
Restaurant (before cooking)	1.2×10^2	$p = 0.000$
Restaurant (after cooking)	0.6×10^2	$p = 0.000$

Figure.1 Fish after cooking in Restaurants (Exposure assessment)

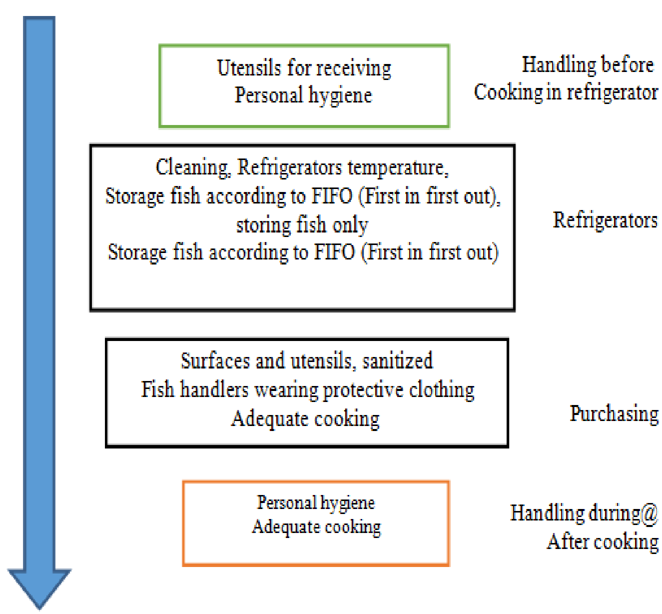

Table.4 Risk Pathways analysis

Fish Market	Fish in Restaurant before Cooking (refrigerators)	Fish in Restaurant after cooking
Poor handling Unhygienic practices Poor Personal Hygiene, Poor waste disposal. E.coli count= (381cfu) Risk Qualifier 50-75% Risk Scoring= high	Improper Clearing, not accurate Temperature with in fish safe range Fish is not stored using the FIFO (first in first out) and not stored fish only E.coli count= (120 cfu) Risk Qualifier 50-75% Risk Scoring= Medium	Improper waste disposal . E.coli count =(60cfu) Risk Qualifier> 50% Risk Scoring=.Low

Table.5 Frequency of risk factors, their associations and the estimated risk.

Refrigerators	Waste disposal	Hygienic practices	Cleaning	Personal hygiene	Storage
All measures in refrigerator was to be = 60% Significance = 0.00 Estimated risk= medium	Proper waste disposal =25% Significance -0.002 Estimated risk= low	Proper hygienic practices = 40% Significance= 0.001 Estimated risk= low	Cleaning practices applied =55% Significance =0.001 Estimated risk= medium	Proper personal hygiene =40% Significance =0.000 Estimated risk=Low	Proper Storage =56 % Significance =0.002 Estimated risk=medium

Thermal processing significantly reduced microbial counts in post-cooking samples (mean = 5.70 CFU), indicating the effectiveness of cooking in eliminating vegetative pathogens. This is in line with the work of Gkogka *et al.,* (2011), who demonstrated that cooking at internal temperatures above 70°C reliably destroys common foodborne bacteria. However, the presence of *E. coli* in some post-cooked samples—reaching up to 43 CFU—raises concerns about either inadequate cooking or post-process contamination, as previously noted by Rahman *et al.,* (2022). Statistical analysis revealed a significant association between *E. coli* levels and six identified hygiene risk factors, namely waste disposal, personal hygiene, surface sanitation, refrigeration conditions, storage, and overall cleanliness ($p \leq 0.05$). This supports the World Health Organization's multi-barrier approach to food safety, which emphasizes the importance of interventions at multiple stages of the food chain (WHO, 2019).

Qualitative risk profiling, based on the FAO/WHO Codex framework, categorized the fish market environment as "high risk." Contributing factors included poor sanitation of storage areas, improper waste disposal, and inadequate refrigeration—a finding echoed in the work of Azanaw *et al.,* (2020), who linked cold chain failures with elevated microbial proliferation in fish products. In contrast, the post-cooking environment exhibited the lowest contamination risk due to effective thermal inactivation of bacteria.

The cumulative risk assessment categorized the overall consumer risk of *E. coli* exposure through fish consumption in Omdurman as "medium." According to Defra's (2015) risk model, this suggests that the likelihood of a foodborne illness event occurring more than once within three years remains substantial under current conditions.

Similar studies from Sudan support these findings. For example, Iman *et al.,* (2022) reported a medium risk of *E. coli* contamination in marine fish from Port Sudan, which aligns with the present study. Meanwhile, Almuatasem *et al.,* (2020) observed a higher contamination level in broiler chickens in Khartoum, indicating variations in risk depending on the food commodity and supply chain management.

To mitigate microbial risks, especially in low- and middle-income countries, integrated strategies are required. These include strengthening hygiene education among fish handlers, enforcing sanitation policies, improving infrastructure such as cold chain systems, and promoting regular inspections and certification of food establishments (Grace, 2015). Without such interventions, the burden of foodborne diseases in urban fish markets will remain a persistent public health concern.

Author Contributions

Mona Gabralla Hamad: Investigation, formal analysis, writing—original draft. Adil M. A. Salman: Validation, methodology, writing—reviewing. Abdalla A. O. Abdelrahim:—Formal analysis, writing—review and editing. Elayis A. Abubaker: Investigation, writing—reviewing. Hayfa Mohammed Ismail: Resources, investigation writing—reviewing. M. I. M. Fangama: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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