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HYGIENIC HABITS AND ITS RELATION TO MICROBIAL LOAD ON SMOKED HERRINGS (*CLUPEA HARENGUS*) IN SABON GARI MARKET, KADUNA STATE, NIGERIA

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ABSTRACT

Smoked fish particularly *Clupea harengus* (herring) is an important and relatively cheap animal protein component in the diet of Nigerians making its consumption and trade a common nutritional and commercial practice. However, the safety of these products is increasingly under scrutiny due to microbial contamination risks associated with poor hygienic practices. This study evaluates the relationship between hygienic habits of vendors and microbial load on smoked herrings sold in Sabon Gari Market, Kaduna State between late March and early May, 2023. A total of 60 C. harengus samples were obtained and analysed using Standard microbiological procedures (serial dilution, pour plate method, and biochemical identification tests). Three different species of microorganisms comprising Escherichia coli, Salmonella spp and Staphylococcus aureus were isolated and Total Viable Count determined (TVC). Descriptive statistics revealed alarmingly high microbial loads, with E. coli (28 CFU/g) and S. aureus (11.57 CFU/g) levels exceeding acceptable thresholds. Although correlation analyses showed a negative association between hygiene scores and microbial levels, ANOVA results indicated no statistically significant differences ($p < 0.05$) in microbial load across hygiene categories. The findings underscore the need for enhanced hygiene training, market infrastructure improvement, and stricter food safety enforcement to protect public health.

Keywords: Smoked fish, *Clupea harengus*, Hygiene practices, Microbial contamination, Food safety

INTRODUCTION

Fish production and consumption are crucial for global food security and nutrition, especially in developing countries. In 2022, global fisheries and aquaculture production reached a record high of 223.2 million tonnes, with aquaculture surpassing capture fisheries for the first time, accounting for over half of this total (FAO, 2024). Fish contributes essential nutrients, with an average global consumption of approximately 20.7 kg per person (FAO, 2025). However, sustainability remains a significant concern, as only 62.3% of marine stocks are fished within biologically sustainable levels (FAO, 2024).

In Africa, Nigeria is a key player in the fish industry, producing around 1.07 million metric tons in recent years, making it one of the continent's top fish producers (FAO, 2020). Despite this, Nigeria faces a substantial supply-demand gap, with fish consumption rate of about 11.3 kg per person annually, significantly below the global average (Barange, 2018). The growing demand for fish, coupled with reliance on imports to meet this demand, underscores the challenges and opportunities within Nigeria's fisheries and aquaculture sector.

The consumption of unhygienically prepared or processed food particularly fresh and smoked fish, poses serious health risks due to microbial contamination even with its swelling demand due to its importance as an alternative and relatively cheaper source of essential animal proteins and omega-3 fatty acids (AIMS Press, 2024). During fish harvesting, processing, or storage, a variety of microorganisms such as bacteria, viruses, molds and parasites, can contaminate the fish and cause foodborne diseases (Chintagari et al., 2017).

Among the pathogens that can infiltrate the seafood supply chain, Escherichia coli, Staphylococcus aureus, and Salmonella are particularly concerning due to their prevalence and the severity of the diseases they can cause (CDC, 2023).

Escherichia coli are often linked to faecal contamination and indicate poor hygiene practices in fish handling and processing (Gonzalez et al., 2003). Pathogenic strains, such as enterohemorrhagic E. coli (EHEC), can result in severe gastrointestinal illnesses characterized by abdominal cramps, diarrhoea, and, in some cases, haemolytic uremic syndrome, which may lead to kidney failure (CDC, 2023). The presence of E. coli in fish raises significant public health concerns, especially for vulnerable populations.

Staphylococcus aureus, commonly found on human skin and in nasal passages, can contaminate fish products through improper handling (Kala, 2006). This bacterium produces enterotoxins that cause food poisoning, with symptoms including rapid onset of nausea, vomiting, and abdominal cramps within hours of ingestion (le Loir et al., 2003). The stability of these toxins at high temperatures poses a risk, as cooking may not eliminate the threat once the toxin has been produced (le Loir et al., 2003).

Salmonella is another major pathogen that poses significant public health risks through contaminated fish and seafood. Infections can cause gastroenteritis, with symptoms such as diarrhoea, fever, and abdominal pain, and can lead to more severe conditions, including septicaemia (Heinitz et al., 2000). This bacterium can enter the seafood supply through contaminated water, poor handling practices, or cross-contamination during processing (CABI Digital Library, 2023). In a study conducted by Ibrahim et al. (2025) on the influence of collection time on nutrients and microbial loads of frozen marine fish species sold in Bichi Local Government Area, Kano State, a significantly higher microbial load was observed in *Clupea harengus* compared to Trachurus trachurus and Scomber scombrus. However, the values did not exceed the recommended limit of 10^6 to 10^7 cfu/g of bacterial load, which was attributed to effective preservation methods and handling practices by fish vendors in cold storage.

The role of smoked fish in Nigeria's food culture and economy cannot be overemphasized as it serves both as a source of protein and of livelihood for many households (Adeyeye, 2016; AIMS Press, 2024). Among the various fish species consumed locally, *Clupea harengus* (Atlantic herring)

is particularly valued due to its affordability, availability, and palatable smoked flavour (Odeyemi et al., 2025). In open markets such as Sabon Gari in Kaduna State, smoked herring is a common commodity, often prepared and sold under varying hygienic conditions.

The nutritional benefits of fish are well documented, offering essential fatty acids, proteins, and micronutrients (FAO, 2016). However, smoked fish is vulnerable to microbial contamination during processing, handling, storage, and retail. Pathogens such as Escherichia coli, Salmonella spp., and Staphylococcus aureus can proliferate if hygienic standards are not adequately maintained, posing serious health risks to consumers (ICMSF, 2002; Akinro et al., 2012). Smoked fish contamination is exacerbated by Nigeria's informal market settings where limited infrastructure, absence of cold chains, and lack of regulatory oversight prevail (Olalekan et al., 2018). Vendors often smoke and display fish in open environments where exposure to dust, flies, and human contact increases microbial risks. Consequently, understanding the relationship between vendor hygiene and microbial contamination is essential for food safety policy and consumer protection.

Although studies have evaluated microbial contamination in fish products, few have directly linked vendor hygienic practices to microbial loads in smoked fish, especially within the context of local markets like Sabon Gari. This study bridges this gap by examining the hygienic behaviours of smoked fish vendors and correlating them with microbiological profiles of *Clupea harengus* samples. The objectives of this study are to: assess the hygienic practices of smoked herring vendors in Sabon Gari Market; determine the microbial load of smoked *Clupea harengus* samples; evaluate the relationship between vendor hygiene scores and microbial contamination levels. By exploring these objectives, the findings should provide insights into the current state of food safety among vendors and potentially inform interventions to enhance hygiene standards and contribute to improve public health through evidence-based recommendations aimed at improving food safety standards in Nigerian fish markets.

MATERIALS AND METHODS

Study Area

The study was conducted in Sabon Gari Market (11º13'N and 07º52'E), one of the busiest markets in Kaduna State, Nigeria during the onset of the raining season, late March to Early May with environmental temperature between 17 – 22°C and relative humidity of 65 – 75% The market is known for its diverse array of food items, including smoked fish products. It serves as a key distribution point for both local and regional trade in frozen *Clupea harengus*.

Study Design

This study employed a cross-sectional descriptive design, combining observational hygiene assessments with microbiological analysis of smoked herring samples. The study also utilized a structured questionnaire to gather data on vendor hygiene practices.

Population and Sample Size

The study population consisted of smoked herring vendors in Sabon Gari Market. A purposive sampling technique was used to select 60 vendors actively selling smoked herring. From each vendor, one smoked fish sample was collected, resulting in a total of 60 samples for microbial testing.

Data Collection Methods

A structured questionnaire and hygiene checklist were administered to vendors to assess hygienic practices. Key variables included: Frequency of hand washing, Use of protective clothing (gloves, aprons), Display and storage methods, cleaning routines of utensils and surfaces, Knowledge and awareness of food safety. Observations were also made to validate self-reported hygiene practices.

Sample Collection and Microbial Analysis

A total of 60 smoked herring samples were collected aseptically from different vendors and transported to the laboratory in sterile containers under cold conditions (4°C). Samples were analyzed for: Total Plate Count (TPC), Coliform Count, Presence of specific pathogens (e.g., Salmonella, Staphylococcus aureus). Standard microbiological procedures were used, including serial dilution, pour plate method, and biochemical identification tests, following APHA (2015) guidelines.

Serial Dilution Method

Preparation of the Stock Solution

25 grams of the smoked herring sample was homogenized and a known volume of sterile buffer (saline or phosphate-buffered saline) was added and mixed thoroughly to ensure even distribution of microorganisms. A known volume (1 mL) of the homogenized sample was transferred into a sterile diluent (9 mL of sterile saline) and thoroughly mixed to perform a 1:10 dilution. This created the first dilution (10^-1). From the first dilution, 1 mL was taken and transferred to another 9 mL of sterile diluent to create the second dilution (10^-2). The process was repeated to create further dilutions up to 10^-6 or 10^-7, depending on the expected microbial load (American Society for Microbiology, 2016).

Pour Plate Method (Preparation of Agar)

A suitable growth medium (Nutrient Agar, MacConkey Agar) for target organisms was prepared by melting the agar and allowing it to cool to approximately 45-50°C. The sterility of the agar is ensured by autoclaving it before use. For each dilution, a specific volume (1 mL) of the diluted sample was taken and placed in a sterile Petri dish. The molten agar was poured into the Petri dish containing the sample. Immediately, the dish was swirled gently to mix the sample with the agar, ensuring even distribution of microorganisms. The agar was allowed to solidify at room temperature for about 15-30 minutes. Once solidified, the plates are incubated at the appropriate temperature (37°C) for 24-48 hours based on the target microorganisms (Endo et al., 2003).

Colonies Counting Method

After the incubation period, the plates are examined for colony growth. The number of colonies formed on each plate is counted. Plates with 30-300 colonies are considered countable for accurate result. The concentration of microorganisms in the original sample was then determined using the formula (Jamali et al., 2013):

$$\text{Concentration (CFU/g)} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}$$

Methodology of Biochemical Identification Tests

Isolate Selection

After performing the pour plate method, individual colonies suspected of being pathogens are selected from the agar plates based on their morphological characteristics. These colonies

are then transferred to sterile broth media to create pure cultures for testing.

Preparation of Pure Cultures
The selected colonies are Incubated in appropriate broth media (Nutrient Broth) at the optimal temperature for 18-24 hours to achieve a sufficient growth density for testing.

Biochemical Tests
A series of biochemical tests were performed based on the expected pathogens as described by Feng et al. (2020). Common tests include:

Indole Test
Used to detect the ability of bacteria to convert tryptophan to indole. Add Kovac's reagent to the culture broth. A red ring indicates a positive result (e.g., E. coli).

Methyl Red Test
Assesses the ability to perform mixed acid fermentation. Add methyl red indicator to the culture. A red colour indicates a positive result.

Voges-Proskauer Test
Detects the production of acetone from glucose fermentation. Add alpha-naphthol and potassium hydroxide. A red colour indicates a positive result.

Citrate Utilization Test
Determines the ability of bacteria to use citrate as the sole carbon source. Inoculate Simmons citrate agar; growth and a colour change to blue indicate a positive result (e.g., Salmonella).

Catalase Test
Tests for the presence of the enzyme catalase, which breaks down hydrogen peroxide. Add a few drops of hydrogen peroxide to the culture. Bubbling indicates a positive result (e.g., Staphylococcus aureus).

Coagulase Test
Specifically used to identify Staphylococcus aureus. Mix the culture with plasma; clot formation indicates a positive result. Results obtained from these tests were compared with standard biochemical profiles to identify the isolates accurately.

Data Analysis
Descriptive statistics (frequencies, percentages, means) were used to summarize vendor hygiene practices and microbial counts. Pearson correlation analysis was used to determine the relationship between hygiene scores and microbial load. ANOVA was also used to compare microbial loads across categories of hygiene compliance (e.g., good, moderate, poor). Statistical significance was set at $p < 0.05$ using SPSS Version 24.

Ethical Considerations
Verbal informed consent was obtained from all vendors prior to participation. The study ensured confidentiality and used anonymized data for analysis. Ethical approval with reference number ABUTHZ/HREC/X43/2022 was obtained from ABU Teaching Hospital Ethical and Research Committee.

RESULTS AND DISCUSSION

The microbial load of smoked *Clupea harengus* was analyzed across 60 samples obtained from vendors in the Sabon Gari market. The descriptive statistics indicated considerable levels of microbial contamination (Table 1).

Table 1: Mean Microbial Contamination Levels on Smoked *Clupea Harengus* in Sabon gari Market

Microorganism	Mean (CFU/g)	Std Dev	Min	Max	F-statistic	p-value
Total Viable Count (TVC)	2,197	685	1,000	3,500	0.043	0.958
Escherichia coli (E. coli)	28	14	5	65	0.195	0.661
Salmonella spp.	3.08	2.41	0	10	0.130	0.720
Staphylococcus aureus	11.57	4.88	2	22	0.404	0.528

Mean values for Total Viable Count (TVC), *E. coli*, *Salmonella spp.*, and *Staphylococcus aureus* were 2,197 CFU/g, 28 CFU/g, 3.08 CFU/g, and 11.57 CFU/g respectively. These values exceed the International Commission on Microbiological Specifications for Foods (ICMSF) standards for ready-to-eat fish products in many cases (ICMSF, 2002). Particularly, *E. coli* and *Staphylococcus aureus* levels were concerning, indicating faecal and handling-related contamination, respectively. This aligns with the view of Karaboz and Dinçer (2002) who affirms that high levels of *Staphylococcus aureus* in food indicates poor hygienic conditions in product preparation, attributable to personnel. According to Kala (2006), the indicators frequently used for determining sanitation conditions are the Coliform bacteria and high levels of these total coliform load indicate lack of hygiene and post-processing contamination (Gonzalez *et al*., 2003).

The self-acclaimed hygiene practices among Smoked *Clupea harengus* Sellers in Sabon gari market is shown in Table 2 with most of them frequently using gloves or washing their hands before and after engaging customers. Also, several vendors consented to covering the fish and carrying out daily checks to ascertain the state and quality of the smoked fish.

Table 2: Self-Reported Hygiene Habits of Smoked *Clupea harengus* Sellers

Hygienic Habit	Most Common Response	Frequency	% Frequency
Hand washing	Always	28	47
Gloves Usage	Always	38	63
Fish Covering	Yes	30	50
Food Safety Training	Yes	27	45
Temperature Knowledge	Yes	38	63
Quality Checks	Daily	18–19	30 – 31.7

While many vendors reported positive hygiene habits (Table 2), microbial counts suggest these practices may not be consistently applied or effective. It reveals no significant difference ($p< 0.05$) across hygiene levels. This could be due to homogeneity in microbial exposure due to shared market conditions and Inaccuracy in self-reported hygiene behaviour since direct or indirect faecal contamination of food increases microbial load. Centre for food safety (CFS) in 2014 reported that Substantial number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage borne out of direct or indirect faecal contamination (CFS, 2014).

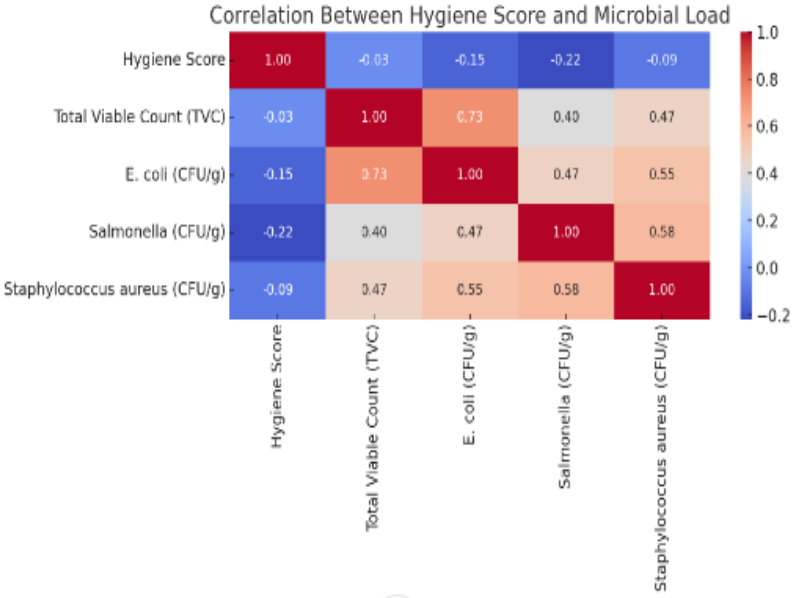

Figure 1: Relationship between overall hygiene score and microbial indicators in smoked *Clupea harengus*

The heat map above shows that Negative correlations were observed between hygiene score and all microbial counts, indicating that better hygiene practices are associated with lower microbial loads.

The strongest inverse relationships were: Hygiene Score vs. *E. coli* showing moderately negative correlation. Hygiene Score vs. *Salmonella* and *Staphylococcus aureus* showed weak to moderate negative correlations. Hygiene Score vs. TVC (Total Viable Count) showed weaker but still negative correlation. These patterns support the hypothesis that improved vendor hygiene reduces contamination in smoked fish. This agrees with Gonzalez *et al*. (2003) who reported that lack of hygiene is directly proportional to high levels of total coliform load. Ibrahim *et al*. (2025) also made clear that proper processing method and handling slows the growth of microbes.

Vendor hygienic behaviour was assessed using self-reported practices (Table 2). These hygiene practices were reportedly moderate among vendors. 63% always used gloves while touching, manipulating and bagging or packaging fish for buyers; 47% washed their hands constantly after making contact with the fish; and 50% covered their fish during display. Despite these claims, high microbial levels observed in this study suggest gaps between reported and actual hygiene behaviour, or the effectiveness of the measures employed were limited. This aligns with the view of Gautam and Curtis (2021) that inadequate food hygiene practices of mothers in the rural hill setting of Nepal is the precursor to frequent exposure of young children to highly contaminated food, water, and milk. Also, the shared market environment and the homogeneity of exposure of these fish product could have aided direct and indirect faecal contamination as reported by CFS (2014).

Hygiene practices were quantitatively scored and correlated with microbial counts (Figure 1). A weak to moderate negative correlation was observed between overall hygiene scores and microbial counts. Higher hygiene scores were associated with lower counts of *E. coli*, *Salmonella spp.*, and *S. aureus*. The strongest negative correlation was with *E. coli*, indicating that better hygienic practices directly reduce faecal contamination risks. These results are consistent with Akinro *et al*. (2012) and FAO (2025) findings, which highlight hygiene as a critical control point in reducing microbial load in smoked fish. In light of the above result also, Mori *et al*. (2020) reported that the frequency and method of cleaning for refrigerators correlates with microbial load count on food stored in them and therefore are important for prevention of food poisoning in consumer households.

The correlation analysis indicated a negative association between hygiene scores and microbial loads, especially for *E. coli*, supporting the hypothesis that improved hygiene reduces contamination (Mueller-Hauser *et al*., 2021). However, one-way ANOVA comparing microbial levels across hygiene categories (Low, Medium, High) revealed no significant differences ($p< 0.05$), suggesting that environmental conditions or systemic market issues may play a larger role in contamination than individual practices. These findings resonate with previous research by Okonta and Ekelemu (2005), who emphasized environmental and infrastructural factors as major contributors to fish spoilage in Nigerian markets. The data highlights the need for integrated food safety interventions beyond individual hygiene improvements.

CONCLUSION

This study concludes that while vendor hygiene practices in Sabon Gari Market are moderately maintained, microbial contamination in smoked *Clupea harengus* remains critically high. The lack of statistically significant differences in

microbial levels across hygiene categories indicates that market-wide interventions are necessary.

RECOMMENDATIONS

Longitudinal studies should be conducted to observe actual hygiene practices and their direct microbial outcomes. Public health inspectors should conduct routine checks to ensure adherence to safety protocols. Awareness campaigns should inform consumers about the importance of visible hygiene in vendor selection.

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