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Bacteriological Quality of Frozen Chicken in Ede, Osun-State, Nigeria

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Abstract

Background: Ensuring the microbiological safety of frozen chicken is crucial for public health, given its extensive consumption and the risk of harbouring harmful bacteria. The microbiological safety of frozen chicken is a significant public health concern due to its widespread consumption and potential to harbour pathogenic bacteria. **Objective**: This study investigated the bacteriological quality of frozen chicken sold in three markets in Ede, Osun State, Nigeria: Elerin, Rector, and Owode markets. **Methodology**: Samples were collected from three shops in each market and repeated three times to ensure accuracy. Bacteriological analysis included total coliform count, total viable count, and detection of specific pathogens such as *Escherichia coli*, *Salmonella spp.*, *Pseudomonas spp.*, and *Klebsiella spp.* **Results**: Results revealed significant bacterial contamination across all markets, with Elerin Market showing the highest contamination levels. Total coliform counts ranged from 1.07×10^4 to 2.30×10^4 cfu/ml, while total viable counts ranged from 1.89×10^5 to 4.18×10^5 cfu/ml. Elerin Market detected *E. coli* and *Pseudomonas spp.* in 88.89% and 100% of samples, respectively, indicating widespread contamination. **Conclusion/Recommendation:** Rector's market exhibited lower contamination levels, suggesting better microbial control, while Owode's market showed moderate but variable contamination. These findings highlight the urgent need to improve hygiene practices and implement stringent safety measures in these markets to ensure the microbial quality of frozen chicken and protect public health.

Keywords: Food toxicology, bacteria, microbial loads, food safety and contamination

Introduction

The poultry industry has evolved into one of the most advanced and modernized sectors within agriculture globally. Through advancements in genetics, nutrition, health, and management techniques, the industry has transformed into a highly efficient and organized system for producing animal proteins of high biological value at a low cost (Bailone et al., 2016). Chicken, in particular, is widely consumed worldwide due to its excellent protein quality, low fat and cholesterol content compared to other meats, and rich nutritional profile, providing high-quality animal proteins, vitamins, and minerals. The rising demand for chicken has resulted in a steady annual growth rate of 6%, with global broiler meat production increasing from 73.1 million tonnes in 2008 to 83.1 million tonnes in 2012 (Chiugo et al., 2022). However, chicken carcasses serve as ideal environments for the proliferation of various food-borne microorganisms, notably *Salmonella*, *Escherichia coli*, *Campylobacter*, and

Staphylococcus aureus. These pathogens are significant contributors to food-borne outbreaks. Consumers expect chicken meat to be fresh, properly chilled, tender, and free from pathogenic microorganisms. Yet, commercial interests often prioritize extended shelf life and storage without signs of spoilage or quality deterioration. Consequently, frozen chicken carcasses and cuts are preferred by manufacturers and retailers over those stored chilled (Atanassova, 2018). Freezing is an effective method for preserving chicken meat quality for extended periods (9–12 months) at temperatures below -18°C. Despite this, drawbacks exist, such as the potential for psychrotrophic bacteria to proliferate even under freezing conditions, leading to undesirable changes in sensory attributes (Atanassova, 2020). Aerobic plate counts (APC) serve as indicators of food quality, reflecting hygienic practices during processing and aiding in assessing food item shelf life (Aberle et al., 2021). Coliform bacteria are also reliable indicators of faecal contamination and improper handling and storage of meat products (Paulsen et al., 2019).

Avian strains of Escherichia coli share many virulence genes with human extraintestinal strains. They can cause various diseases upon consumption of contaminated foods, indicating poor sanitation and potential faecal contamination (Synge, 2021). Salmonella, the most prevalent food-borne pathogen worldwide, can cause human food poisoning in poultry meat (Capita et al., 2021; Muth, 2020). Staphylococcus aureus, which ranks third among the most important food-borne pathogens globally, secretes various staphylococcal enterotoxins associated with staphylococcal food poisoning, posing serious health risks to consumers (Normanno, 2021; María et al., 2019).Given the potential health impacts posed by the bacteriological quality of frozen chicken meat cuts, this study seeks to investigate the bacteriological quality of frozen chicken sold in Ede, Osun State.

Materials and methods

Study area

The study area is Ede, Osun State, located in southwestern Nigeria. Ede is a town approximately 180 kilometres northeast of Lagos and 50 kilometres south of the state capital, Osogbo. It is positioned at coordinates 7°44′N latitude and 4°26′E longitude, providing a tropical climate that influences both its agricultural and economic activities. Ede has a rich historical background dating back several centuries and is known for its contributions to the spread of Islam in southwestern Nigeria. The Timi of Ede is the traditional ruler, and the town's historical and cultural heritage is deeply tied to Yoruba traditions and customs. Ede is predominantly inhabited by the Yoruba ethnic group, which is the most populous ethnic group in southwestern Nigeria, with a population of over 150,000 people. The town is divided into two local government areas, Ede North and Ede South, contributing to the socio-economic dynamics of the region. The economy of Ede is primarily agrarian, with a significant portion of the population engaged in farming and related activities, as well as a growing commercial sector. Key markets include Elerin Market, Rector's Market, and Owode Market.

Sample collection

Three frozen chicken neck and skin samples were collected from each shop in three different markets: Elerin Market, Rector's Market, and Owode Market (Plate 1). The samples were transported in sterile, insulated containers to maintain their cold temperature and prevent microbial growth. Each sample was labelled with the market name and collection date for easy tracking. This sampling process was repeated three times for each shop in each market.

Plate 1: The pictures were taken during the sample collection.

Laboratory analysis:
Microbial contamination testing.
Sample preparation

Ten grams of chicken neck, skin, and flesh were measured and placed into a mortar. Sterile water (90 mL) was added, and the mixture was homogenised using a piston mortar. A 1-millilitre aliquot of each homogenate was used to prepare a ten-fold dilution up to 10^{-6}.

Total plate count (TPC)

For the Total Plate Count (TPC), Nutrient Agar was prepared according to the manufacturer's instructions. Specifically, 28 grams of the agar powder was dissolved in one litre of distilled water and then sterilised by autoclaving at 121°C for 15 minutes. Using the pour plate method, 15 millilitres of the prepared Nutrient Agar was poured into a sterilised Petri dish containing 1 millilitre of a diluted sample (up to five-fold). The mixture was allowed to solidify and was then incubated at a temperature range of 35–37°C for 24–48 hours. After the incubation period, the colonies were counted using a colony counter.

Total Cell Count / 1ml = N× Dilution factor

Where N is the average number of bacteria counted.

Total coliform count

For the total coliform count, MacConkey Agar was prepared according to the manufacturer's procedure by dissolving 54 grams of the agar in one litreof distilled water and then sterilisingas described earlier. To confirm the presence of coliforms, colonies from MacConkey agar were

transferred to Brilliant Green Bile Broth (BGB) tubes and incubated for an additional 24-48 hours. The formation of gas in these tubes confirmed the presence of coliforms.

Enumeration of coliform bacteria

A 1 ml aliquot of the sample dilution was added to a petri dish using a sterile micropipette. Then, 15 ml of pre-prepared medium at a temperature of 44-47°C was added to each petri dish. Plates were inoculated for both the 3rd and 4th dilutions. The medium was added to the petri dish containing the inoculum within 10 minutes. The inoculum was carefully mixed into the medium and solidified in a horizontal position. Plates were incubated according to the type of isolate that was suspected in the sample. After incubation, colonies were observed and counted on each plate. Colonies were enumerated utilizing a digital colony counter Further re-streaking on selective media was performed to obtain pure colonies of organisms for further processing. Pure colonies of bacteria under study were confirmed using biochemical tests on the selective media.

E. coli detection

To detect Escherichia coli, samples with visible growth on MacConkey Agar were subjected to the IMViC (Indole, Methyl Red, Voges-Proskauer, Citrate) test;these samples were further incubated at 44°C for an additional 24 hours. The presence of greenish metallic colonies on Eosin Methylene Blue (EMB) Agar confirmed the presence of E. coli.

Salmonella spp. detection

To detect Salmonella spp., Salmonella-Shigella (SS) Agar was prepared by dissolving 54.5 grams of the agar in one litreof distilled water, followed by sterilization. Samples were streaked onto SS Agar plates and incubated at 35-37°C for 24-48 hours. The absence of black-centred colonies indicated no growth of Salmonella spp.

Total viable cell count

For the total viable cell count, 10 grams of each chicken sample was blended with 100 millilitres of sterile distilled water. Serial dilutions were prepared from this mixture. One millilitreof each dilution was spread onto Nutrient Agar plates, which were incubated at 35-37°C for 24-48 hours. After the incubation period, the colonies were counted. The viable cell counts were calculated using the formula:

$$\text{Colony Count} = {}^{N}/_{V} \times D$$

Where N is the number of colonies, V is the volume of sample plated (in ml), and D is the dilution factor.

Pseudomonas spp. detection

Samples were streaked onto Pseudomonas spp. to detect Pseudomonas Agar plates. These plates were incubated at 35-37°C for 24-48 hours. The presence of visible colonies on the plates indicated the growth of Pseudomonas *spp.*

Statistical analysis

Data from microbial counts were analyzed using descriptive statistics to compare contamination levels across the three markets. The Most Probable Number (MPN) method was used to estimate the density of coliforms and E. coli per 100 ml of the sample.

Quality control

All media and reagents were prepared according to the manufacturer's instructions. Sterility controls were included in each batch of media and reagents to ensure no contamination. To ensure the accuracy and reliability of the results, each set of tests included both positive and negative controls.

Results

Bacteriological analysis was performed on three samples, and the result of the analysis is detailed in Tables 1 to 3.Table 1 presents the mean coliform counts (cfu/ml) for three market locations—Elerin, Rector, and Owode—each with three sampled shops. In Elerin Market, Shop 1 recorded a mean count of 2.06×10^4 cfu/ml;Shop 2 had 2.07×10^4 cfu/ml and Shop 3 showed the highest count at 2.30×10^4 cfu/ml, indicating consistently high coliform contamination. Rector Market displayed lower contamination levels, with Shop

1 recording 1.07×10^4 cfu/ml, Shop 2 at 1.26×10^4 cfu/ml and Shop 3 showing the highest variability at 1.31×10^4. Owode Market exhibited moderate contamination levels, with Shop 1 recording 1.3×10^4 cfu/ml, Shop 2 the highest at 1.59×10^4 cfu/ml and Shop 3 at 1.31×10^4 cfu/ml. Elerin Market demonstrated the highest and most consistent contamination, Rector Market had lower counts with variability, and Owode Market showed moderate contamination with noticeable inconsistencies. Table 2 presents the three market locations' total microbial counts (cfu/ml). In Elerin Market, Shop 1 had the highest microbial load with 4.18×10^5 cfu/ml, followed by Shop 2 at 3.86×10^5 cfu/ml and Shop 3 at3.84×10^5 cfu/ml;this indicates a consistently high level of microbial contamination in Elerin Market. Rector Market had lower microbial counts, with Shop 1 at 1.89×10^5 cfu/ml, Shop 2 at 2.50×10^5 cfu/ml, and Shop 3 at 2.51×10^5 cfu/ml, reflecting better control measures. In Owode Market, Shop 1 recorded 2.63×10^5 cfu/ml, Shop 2 had the highest at 2.83×10^5 cfu/ml, and Shop 3 showed 2.78×10^5 cfu/ml, highlighting moderate levels with variability.

Table 1: Total coliform counts (cfu/mL) across the frozen chicken samples (N=3)

Location	**Elerin market**	**Rector market**	**Owode market**
Shops	Total coliform count in cfu/mL ($x10^4$) Mean/SD	Total coliform count (cfu/mL) ($x10^4$) Mean/SD	Total coliform count cfu/mL ($x10^4$) Mean/ SD
1	2.06 ± 0.07	1.07 ± 0.24	1.3 ± 0.57
2	2.07 ± 0.15	1.26 ±0.27	1.59 ± 0.63
3	2.30 ± 0.35	1.31 ± 0.53	1.31 ± 0.91

Table 2: Total viable count across the frozen chicken samples (N=3)

Location	**Elerin market**	**Rector market**	**Owode market**
Shops	Total viable count in cfu/mL ($x10^5$) Mean	Total viable count in cfu/mL ($x10^5$) Mean	Total viable count in CFU/ml (($x10^5$) Mean
1	4.18±0.46	1.89 ± 0.80	2.63 ± 1.22
2	3.86 ±0.29	2.50 ± 0.58	2.83 ±1.48
3	3.84 ± 0.59	2.51 ± 1.36	2.78 ±1.90

Table 3 shows bacterial isolate prevalence in frozen chicken samples from the three markets, each contributing nine samples. In Elerin Market, E. coli, *Salmonella spp.*, and *Klebsiella spp.* were found in 88.89% of samples, while *Pseudomonas spp.* occurred in 100%. Rector Market had lower contamination, with E. coli and *Salmonella spp.* detected in 66.67%, *Klebsiella spp.* in 55.56%, and *Pseudomonas spp.* in 44.44%. Owode Market recorded E. coli and *Klebsiella spp.* in 88.89%, *Salmonella spp.* in 77.78%, and *Pseudomonas spp.* in 100%. These findings highlight significant contamination, with Elerin and Owode Markets showing particularly high prevalence rates.

Market-specific observations

Elerin market: The Elerin market exhibited the highest levels of bacterial contamination across all parameters. High levels of *Salmonella spp.* and E. coli suggest that contaminated water might have been used for washing dressed chickens. The spoilage bacteria detected were likely responsible for the off-putting smell observed in some samples, emphasizing the need for better hygiene practices.

Rector market: The Rector market had the lowest coliform counts, averaging approximately 1.21×10^4 cfu/ml. The narrower range of microbial counts indicates improved control measures compared to the other markets. However, coliforms suggest that further improvements in sanitation and infrastructure are required to eliminate contamination risks.

Owode market: Owode market's average coliform count was 1.04×104 cfu/ml, with high prevalence rates of bacterial isolates: E. coli and *Klebsiella spp.* in 88.89%, *Salmonella spp.* in 77.78%, and *Pseudomonas spp.* in 100%. These results indicate moderate but inconsistent contamination levels, indicating the need for stricter hygiene protocols to ensure consistent microbial safety.

Table 3: Bacterial isolates across the frozen chicken samples (N = 3)

Isolates	**Elerin market (N=9)**		**Rector's market (N=9)**		**Owode market (N=9)**	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
E. coli	8	88.89	6	66.67	8	88.89
Salmonella *spp.*	8	88.89	6	66.67	7	88.89
Pseudomonas *pp.*	9	100.00	4	44.44	8	100.00
Klebsiella spp	8	88.89	5	55.56	7	88.89

Discussion

The current study evaluated the bacteriological quality of frozen chicken sold in Ede, Osun State, Nigeria, by examining microbial contamination levels across three markets: Elerin Market, Rector's Market, and Owode Market. The results revealed significant variations in microbial load and bacterial types among the markets, reflecting disparities in hygiene and food safety practices. The total plate count, a critical measure of overall microbial load, was highest in Elerin Market, indicating substantial microbial contamination. This result aligns with previous studies (Ledo et al., 2020; Lerma-Fierro et al., 2020), which reported elevated microbial loads in markets with poor hygiene and inadequate sanitation protocols. In contrast, Owode Market recorded the lowest microbial counts, suggesting better hygiene practices than Elerin and Rector's Markets.

Coliform bacteria, especially *Escherichia coli* (*E. coli*), were prominent in Elerin and Owode Markets samples, serving as indicators of faecal contamination. This finding is consistent with research by Desiree et al. (2021), which highlighted the prevalence of coliforms in markets with substandard sanitary practices. Notably, *E. coli* was absent in Rector's Market, pointing to comparatively better sanitation and food handling practices in this location. Microbial contamination of chicken carcasses is often associated with evisceration, during which faecal material from the cloaca contaminates the meat (Umaraw, 2017). Additionally, frozen chicken in Ede is frequently subjected to multiple freeze-thaw cycles during extended transportation from ports to cold storage facilities, creating conditions conducive to microbial proliferation. The findings from this study align with earlier research (Mansour, 2019; Stanley, 2018) that identified similar microbial contaminants in frozen poultry products.

Salmonella spp., a major food-borne pathogen, was detected in four out of 12 samples; this aligns with findings by Gargano et al. (2021) and Mridha et al. (2020), underscoring the need for stringent food safety measures to mitigate the risk of food-borne illnesses associated with poultry products. The presence of *Pseudomonas spp.* in the samples signals potential spoilage risks. This bacterium thrives in temperature-abused conditions, emphasizing the importance of maintaining cold chain integrity during storage and transportation. Similar studies

(Rahman, 2020) have highlighted the role of adequate temperature control in minimizing microbial growth and preserving food quality.

Comparative analysis of microbial and coliform counts across the three markets revealed distinct patterns reflecting the varying hygiene and market management levels. Elerin Market's elevated microbial loads and spoilage indicators underscore the urgent need for enhanced sanitation measures and stricter regulatory oversight. On the other hand, Owode Market's relatively lower microbial and coliform counts suggest implementing more effective hygiene practices, potentially influenced by local regulations or community-driven awareness initiatives. These findings highlight the importance of tailored interventions, such as improved sanitation, cold chain management, and public health education, to ensure the microbiological safety of poultry products in Ede and similar settings.

Conclusion and recommendations:

The study assessed the bacteriological quality of frozen chicken samples from Elerin Market, Rector's Market, and Owode Market in Ede, Osun State, Nigeria. The findings revealed significant variations in microbial contamination levels across the markets. Elerin Market showed the highest levels of microbial contamination, with elevated total plate and coliform counts, and *E. coli* and *Pseudomonas spp.*, indicating poor hygiene and sanitation practices. Rector's market displayed moderate contamination levels, characterized by notable coliform counts but no detection of *E. coli* or *Pseudomonas spp*. Owode Market had the lowest microbial contamination, minimal coliform counts, no *E. coli* or *Pseudomonas spp.*, and the fewest total microbial counts. These results highlight the urgent need for enhanced hygiene practices and effective microbial control measures, particularly in markets with higher contamination levels. The following measures are recommended to mitigate the microbial contamination observed in the studied markets:

- *Enhanced sanitation protocols:* Elerin Market, in particular, should adopt stricter sanitation protocols. Regular cleaning and disinfection of stalls and equipment used in food handling should be enforced. The provision of clean water for washing food items is essential.
- *Regular monitoring and inspection:* Establish routine microbial testing in all markets to monitor contamination trends. Regulatory bodies should conduct periodic inspections and enforce compliance with food safety standards.
- *Public health education:* Organize training sessions for market vendors on proper food handling and storage techniques. Run awareness campaigns for consumers to encourage the purchase of hygienic and safe food products.
- *Improved cold chain management:* Ensure frozen chicken is stored and transported under consistent cold-chain conditions to prevent microbial proliferation.
- *Policy enforcement:* Implement and enforce strict food safety regulations in all markets. Penalties should be imposed for non-compliance to deter poor hygiene practices.

Professional implications of the study: The outcomes of this study hold substantial relevance for public health practitioners, food safety authorities, and market management officials.. By identifying the levels of microbial contamination in frozen chicken sold in Ede, Osun State, Nigeria, this research highlights critical areas that require targeted interventions to improve food safety and reduce the risks of food-borne illnesses. The professional implications include the following:

- *Enhanced public health strategies:* The study underscores the need for comprehensive public health strategies to combat food-borne diseases. Professionals in the public health sector can use the findings to design and implement effective hygiene education programs targeting market vendors and consumers.

- *Policy formulation and regulatory enforcement:* Regulatory bodies can leverage these findings to establish stricter food safety policies and enforce compliance in local markets. The study serves as evidence to advocate for the mandatory implementation of sanitation standards in poultry handling and sales.
- *Training and capacity building:* The results highlight gaps in hygiene practices among market vendors, necessitating capacity-building programs focused on proper handling, storage, and transportation of poultry products. Food safety professionals can organize workshops to improve vendor knowledge and skills.
- *Improvement of market infrastructure:* Market management authorities can prioritize upgrading market infrastructure, including access to clean water, proper waste disposal systems, and cold storage facilities, to minimize microbial contamination risks.
- *Support for Research and Innovation:* The study highlights the importance of ongoing research in developing innovative solutions, such as rapid microbial detection methods and improved cold-chain technologies, to enhance food safety practices.
- *Risk communication and public awareness:* Public health professionals can use these findings to improve communication strategies, emphasizing the risks of consuming contaminated frozen chicken and promoting safer purchasing decisions among the general population.
- *Interdisciplinary collaboration:* The study demonstrates the necessity for collaboration among microbiologists, public health experts, food scientists, and policymakers to address microbial contamination issues comprehensively.

Limitations of the study: While the study provides valuable insights into the bacteriological quality of frozen chicken sold in Ede, Osun State, several limitations should be considered:

- *Limited sample size:* The study focused on a specific number of samples from only three markets, which may not fully represent the overall microbial contamination levels across the entire region.
- *Geographical Scope:* The research was confined to only three markets within Ede, limiting the findings' generalizability to other areas.
- *Seasonal variation:* The study did not account for potential seasonal variations in microbial contamination levels, which could influence results due to differences in temperature, humidity, and market activity throughout the year.
- *Lack of detailed source tracking:* The study did not trace the specific sources of contamination, such as farm-level practices, transportation conditions, or market handling procedures, which could provide more actionable insights.
- *Limited range of microbial testing:* Only a selected range of bacterial species was tested, limiting the comprehensiveness of the assessment.
- *Challenges in ensuring consistency in sampling:* Variability in sampling methods and handling during the collection process may introduce biases, potentially affecting the accuracy and reliability of results.
- *Cold chain and storage factors:* The study did not evaluate the cold chain conditions or storage facilities at different points in the supply chain, which are critical factors influencing microbial growth.
- *Reliance on cultural methods:* The study primarily relied on traditional microbiological methods, which, while reliable, may not detect all bacteria present or provide the same speed and specificity as molecular techniques.
- *Informed consent* was secured from all participants involved in this study, adhering to ethical standards. Participants were briefed on the study's purpose, procedures, and their rights, including voluntary participation and

the ability to withdraw at any time. Confidentiality and anonymity were upheld, and potential risks and benefits were clearly explained. Signed consent forms documented their agreement, ensuring transparency and ethical integrity throughout the research process.

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