MICROBIAL ANALYSIS OF COW MILK AND WARA OBTAINED FROM SELECTED COW REARED IN OKO-IRESE COMMUNITY

BY

MOMOH, Ogere Tofunmi

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CERTIFICATION

This is to certify that this project titled "Microbial analysis of cow milk and wara obtained from cows reared in Oko-Irese community" was carried out by MOMOH, Ogere Tofunmi, with matriculation number 20/05NSS018 and submitted to the Microbiology Programme, Department of Biological Sciences in the Faculty of Computing and Applied Sciences, for meeting the requirements for the award of Bachelor in Microbiology, Thomas Adewunmi University, Oko-Irese, Kwara State.

…… ……

Project Supervisor
Date **Mrs. Olaitan F. J.**

…… ……

26/08/2025

Head of Department **Date**

Dr. S.T. Farohunbi

…… ……

24/08/2025

External Examiner **Date**

DEDICATION

This project is dedicated to God Almighty and my parents, Engr. Solomon Momoh and Mrs Victoria Momoh.

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Abstract

The research assessed the microbiological quality, Gram reactions, proximate composition, and antimicrobial resistance profiles of raw cow milk and wara obtained from Oko-Irese, Kwara State, Nigeria. Sixteen samples (nine milk, seven wara) were collected and analyzed using standard culture techniques (serial dilution and spread plate), Gram staining, biochemical identification, and proximate assays. Antimicrobial susceptibility of bacterial isolates was determined by the Kirby–Bauer disc diffusion method and interpreted following CLSI/EUCAST guidelines. Both milk and wara exhibited high total viable counts, ranging from 2.5×10^6 to 1.4×10^8 CFU/mL in milk and 3.2×10^6 to 1.2×10^8 CFU/g in wara, exceeding Codex and EU microbiological limits. Gram staining differentiated isolates into Gram-positive cocci (predominantly *Staphylococcus aureus*) and Gram-negative rods (including *Escherichia coli, Salmonella* spp., *Klebsiella* spp., *Proteus* spp., and *Shigella* spp.), which were confirmed by biochemical tests. Yeasts and filamentous moulds were also recovered, indicating spoilage potential and possible mycotoxin risk. Proximate analysis showed wara to be richer in protein (≈9.5%) and fat (≈11.3%) but lower in moisture than milk (protein ≈3.3%, fat ≈4.1%). Antimicrobial susceptibility testing revealed widespread resistance to β-lactam antibiotics (amoxicillin, augmentin, cephalosporins) and evidence of multidrug resistance, particularly among *S. aureus* and *Klebsiella* spp. Fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) and certain aminoglycosides (gentamicin) retained higher activity. In conclusion, milk and wara from Oko-Irese are nutritionally valuable but microbiologically unsafe for direct consumption without pasteurization. The presence of Gram-positive and Gram-negative pathogens, including multidrug-resistant strains, underscores the urgent need for improved hygiene, antimicrobial stewardship, cold-chain interventions, and enforcement of food safety standards.

Keywords: Raw milk, Wara, Gram reaction, Antimicrobial resistance, Proximate composition, Food safety

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CHAPTER ONE

Introduction

1.1 Background of the Study

Milk is naturally produced by female mammals to feed their young, is a nutrient-rich food, Wara is a locally fermented soft cheese produced from cow milk in Nigeria, and several other African nations such as Ghana, Togo, and Benin (produce and eat wara. The Fulanis produce milk from cows locally in Nigeria, and is processed into wara, a local soft cheese. Dairy products, notably raw cow milk and wara, are important sources of dietary energy, high-quality protein, and essential micronutrients for many Nigerian households (Obafemi, 2022). However, traditional processing, limited cold-chain infrastructure, and informal marketing systems can expose these products to microbial contamination at multiple points along the value chain (Ayichew et al., 2024; Fereja et al., 2023).

Microbiologically, raw milk and wara are susceptible to contamination by a wide spectrum of organisms. Recent regional surveillance and systematic reviews document frequent isolation of *Staphylococcus aureus*, *Salmonella* spp., coliforms (including *Escherichia coli*), *Klebsiella* spp., and spoilage fungi (yeasts and moulds) from raw milk and fresh cheeses where hygiene and storage are sub-optimal (Odetokun et al., 2023; Ayichew et al., 2024). The presence of these organisms not only degrades product quality and shelf life, but also creates acute public-health risks: *Salmonella* and *Shigella* cause enteric disease, while coagulase-positive *S. aureus* can

produce heat-stable enterotoxins that lead to food poisoning even when organisms are later reduced by heating (Odetokun et al., 2023; Fathuddin et al., 2024).

Simultaneously, the proximate composition of wara (concentrated fat and protein relative to raw milk) Changes the kinds of risks and how serious they are: the nutrient-dense matrix may protect pathogens and enable persistence or toxin stability under temperature abuse (Obafemi, 2022; Raji et al., 2023). In low-resource settings such as Oko-Irese, these combined microbiological and nutritional realities require targeted investigation to inform locally appropriate interventions.

1.2 Statement of the Problem

Preliminary field observations and prior studies indicate that milk and wara produced and sold in informal markets are frequently contaminated beyond acceptable international microbiological limits (Ayichew et al., 2024; Odetokun et al., 2023). In Oko-Irese community, many people, even elites purchase wara without the knowledge of the hygienicity and nutritional composition of the wara produced in Oko community. Also, there is limited systematic data from Oko-Irese quantifying microbial loads, identifying causative organisms or relating microbial findings to the products' proximate composition. Without such data, producers, local health authorities, and consumers lack evidence to prioritize pragmatic, culturally acceptable interventions. This gap undermines public health protection and constrains the market potential of wara produced by Fulanis in Oko-Irese community.

1.3 Justification of the Study

This study fills a practical and academic need by providing empirical microbiological and nutritional data from Oko-Irese — a representative Fulani production context. The generated evidence will: (a) quantify microbial risk through TBC (Total Bacterial Count) and isolate

identification, (b) relate microbiological status to proximate composition, and (c) benchmark findings against international safety standards (Codex, EU, WHO) and recent regional literature (2021–2025). By these, the study will inform low-cost, culturally sensitive interventions and support policy advice to local stakeholders. The approach follows recommendations from recent field work that emphasizes combining microbiological testing with nutritional and socio-cultural analysis to design effective solutions (Fereja et al., 2023; Raji et al., 2023).

1.4 Aim and Objectives of the Study

Aim

To assess the microbiological and proximate quality of raw cow milk and wara produced in Oko-Irese community and to propose culturally appropriate effective interventions to enhance product safety and hygenicity.

Specific Objectives

1. To determine and compare the proximate composition of milk and wara samples.
2. To enumerate and compare TBC (Total Bacterial Count), TBC and Total coliform count (TBC in CFU/mL) of raw cow milk and wara samples collected from selected Fulani settlements in Oko-Irese.
3. To isolate and identify bacterial species present with emphasis on pathogens of public-health, such as *S. aureus, Salmonella* spp*., E. coli*, etc.
4. To compare the measured microbial counts and pathogen occurrences with international microbiological criteria (Codex, EU, WHO) and recent (2022–2025) regional studies.
5. To evaluate the antimicrobial susceptibility profiles of isolated bacterial pathogens

6. To recommend feasible, culturally sensitive processing interventions that address cost, visibility, and producers' insecurity about sample provision.

1.5 Research Questions / Hypotheses

Research Questions

1. What are the aerobic plate counts (APC/TBC) of raw cow milk and wara samples and the total coliform count from oko irese?
2. Which bacterial species are present in the milk and wara samples as determined by Gram staining and biochemical tests?
3. Compare bacterial population and species in milk and wara
4. How do the proximate compositions of milk and wara differ, and how might these differences influence microbial survival and toxin stability?
5. How do the measured microbial counts and pathogen occurrences compare with international safety criteria and recent regional studies (2022–2025)?
6. Which low-cost and culturally appropriate interventions are most likely to reduce contamination while preserving the traditional qualities of wara?

Null Hypothesis (H_0)

There is **no significant difference** in the mean aerobic plate counts between raw cow milk and wara samples collected from Oko-Irese.

Alternative Hypothesis (H_1)

There **is a significant difference** in the mean aerobic plate counts between raw cow milk and wara samples collected from Oko-Irese.

Note: hypothesis testing will be performed where quantitative data permit, using appropriate statistical methods (see Chapter Three: Data Presentation and Statistical Analysis).

1.7 Significance of the Study

The study holds significance for several stakeholders:

- **Public Health Authorities:** Provides empirical evidence of microbiological hazards in local dairy products to guide surveillance, risk communication, and targeted interventions (Ayichew et al., 2024).
- **Local Producers and Fulani Communities:** Offers actionable, culturally sensitive recommendations that can improve product safety, increase consumer confidence, and potentially enhance incomes through better market access (Raji et al., 2023).
- **Academia and Researchers:** Contributes to the growing body of regionally focused dairy safety literature by combining microbiological, nutritional, and socio-cultural dimensions in a single study (Odetokun et al., 2023; Fereja et al., 2023).
- **Policy Makers and Extension Services:** Supplies data needed to design cost-effective support programs (shared equipment, training, community-based testing) and to frame regulatory guidance that is realistic for small-scale producers (Fathuddin et al., 2024).

Collectively, the outputs of this study aim to protect consumer health, preserve cultural food heritage, and strengthen the livelihoods of Fulani dairy producers through evidence-based, practical measures.

Chapter Two

LITERATURE REVIEW

2.1 Overview of Milk and *Wara* Production in Nigeria

Milk production in Nigeria is dominated by smallholder pastoralists, particularly the Fulani ethnic group, who own about 90% of the national cattle herd and contribute the bulk of locally produced milk (Adebayo et al., 2023). The system is largely extensive and traditional, with cows grazed under open-range conditions and milking performed manually under non-hygienic conditions, often without access to cooling or sanitary equipment (Idowu et al., 2022). Consequently, raw milk produced in these systems is prone to microbial contamination, rapid spoilage, and reduced consumer confidence.

A major derivative of raw cow milk in Nigeria is *wara*, a soft white cheese produced by coagulating fresh milk with natural coagulants such as extracts of *Calotropis procera* (sodom apple) or lime juice. *Wara* is highly perishable, with a shelf life of only 1–3 days at ambient temperature, yet it remains an important part of urban and rural diets due to its affordability, accessibility, and cultural value (Raji et al., 2023). Its production is mostly carried out by Fulani women using artisanal methods e.g the coagulant is leaf extract from *Calotropis procera* (Sodom apple), with minimal standardization of hygiene practices, packaging, or storage conditions (Odetokun et al., 2023).

Due to its nutritional richness in protein, fat, and minerals (calcium, phosphorus, magnesium, etc.), *wara* is vulnerable to contamination during handling, processing, and marketing. Studies consistently report that local cheese production environments are often informal, lacking pasteurization, refrigeration, or quality control systems (Ayichew et al., 2024). These practices increase the risk of contamination with pathogenic organisms such as *Staphylococcus aureus*, *Salmonella* spp., and coliforms, which compromise both safety and shelf stability.

Thus, cow milk and *wara* production in Nigeria illustrate a paradox: while they are culturally important and nutritionally valuable, their traditional production systems expose them to significant microbiological hazards. Addressing these risks requires balancing modern food-safety approaches with respect for the cultural and socio-economic context of Fulani producers.

2.2 Nutritional and Socio-Cultural Importance of *Wara*

Wara (local fresh cheese) occupies a distinct place in Nigerian diets and local economies because it combines relatively high nutritional value with cultural acceptability and low cost. Recent compositional studies confirm that traditional fresh cheeses concentrate key macronutrients relative to raw milk: compared with milk, wara typically contains substantially higher fat and protein and lower moisture, making it an important source of energy and quality protein for households with limited access to expensive animal-source foods (Raji et al., 2023; Obafemi et al., 2022). These nutrient characteristics underpin wara's value in household food security; in many rural and peri-urban communities wara contributes to dietary diversity and provides nutrients important for child growth and maternal nutrition (Obafemi et al., 2022; Raji et al., 2023).

Economically, wara production and sale are important livelihood activities for Fulani households and local processors. Several recent field studies and reviews document that small-scale producers and women processors derive direct income from fresh-cheese production, which requires low start-up capital and uses locally available coagulants and utensils (Adebayo et al., 2023; Raji et al., 2023). The low capital barrier and fast turnover of wara make it especially attractive for women and youth as a cash-generating enterprise, thereby supporting household resilience. In many markets, wara's affordability and ready availability also mean it is widely consumed by low-income urban households who rely on informal market supplies (Adebayo et al., 2023; Ayichew et al., 2024).

Beyond nutrition and income, wara has clear cultural significance. It is embedded in customary foodways, local hospitality practices, and seasonal exchanges among pastoral and farming communities; this cultural embeddedness shapes production methods and consumer preferences (Obafemi et al., 2022). Importantly, cultural preferences for the fresh sensory attributes of wara (texture, mild acidity, and aroma) influence producers' willingness to adopt interventions that change product character (e.g., prolonged heating, aggressive acidification), and thus any safety interventions must be co-designed to preserve traditional qualities (Raji et al., 2023). Recent literature therefore emphasizes culturally sensitive approaches — engaging Fulani leaders, women processors, and market actors in co-design of hygiene training, labeling, and small-scale processing innovations — to achieve both safety and cultural acceptability (Adebayo et al., 2023; Obafemi et al., 2022).

2.3 Microbial contamination of milk and dairy products

Microbial contamination of fresh dairy products is assessed using a combination of indicator counts and pathogen-specific tests. Indicator measures (e.g., Aerobic Plate Count, Total Coliform

Count, Enterobacteriaceae counts, and coagulase-positive staphylococcal counts) provide rapid, quantitative summary of overall hygienic quality and process control, while pathogen-specific testing (e.g., presence/absence of *Salmonella* spp., *Listeria monocytogenes*, or toxigenic *Staphylococcus aureus*) assesses direct public-health risk (Fereja et al., 2023; Ayichew et al., 2024).

Aerobic Plate Count (TBC): The APC (also called Total Bacterial Count, TBC, or Total Viable Count) enumerates aerobic, culturable bacteria present in a sample and is reported as colony-forming units per milliliter (CFU/mL) or per gram (CFU/g). APC is widely used as a general index of product quality: low APCs indicate adequate hygiene and cooling, while high APCs point to contamination, temperature abuse, or prolonged storage (Fereja et al., 2023). Recent field studies in comparable small-scale value chains report APCs frequently in excess of 10^5 CFU/mL in raw milk and in fresh cheeses sold without refrigeration, demonstrating the practical sensitivity of APC as an early warning metric (Ayichew et al., 2024).

Total Coliform and *Escherichia coli* counts; Coliforms and *E. coli* are used as fecal-contamination and hygiene indicators. Presence of *E. coli* (especially in ready-to-eat dairy) signals direct fecal contamination or cross-contamination from contaminated water, equipment, or handlers (Odetokun et al., 2023). Recent regional surveillance studies emphasize that *E. coli* detection in raw milk and fresh cheeses correlates strongly with poor water quality and inadequate cleaning of utensils during processing (Fathuddin et al., 2024; Ayichew et al., 2024).

Coagulase-positive staphylococci (CPS: *S. aureus*): Counts of coagulase-positive staphylococci are measured both to estimate the likelihood of staphylococcal enterotoxin presence and as an indicator of human-origin contamination (handling, skin, nasal carriage) or mastitic milk. The practical thresholds above which toxin formation is more likely (often cited in

regulatory guidance at ≥10^4–10^5 CFU/g for cheeses); therefore, high CPS counts in soft cheeses such as wara present a dual hazard — spoilage and toxin-mediated food intoxication (Odetokun et al., 2023).

Pathogen-specific testing (e.g., *Salmonella*, *Shigella*, *Listeria*). For public-health decision making, presence/absence tests for key pathogens are decisive. International guidance (Codex, EU) treats detection of *Salmonella* in a 25 g sample as unacceptable for cheeses and other ready-to-eat dairy, because even low infectious doses can cause significant disease burden. Recent value-chain investigations show that *Salmonella* occurrences in milk are strongly associated with contaminated water sources and unclean utensils, and that detection at retail reflects upstream handling failures (Ayichew et al., 2024).

Interpreting indicator/pathogen results together. Contemporary studies recommend combined interpretation: a high APC with concurrent detection of fecal indicators (coliforms, *E. coli*) and/or pathogens (e.g., *Salmonella*) denotes both poor hygiene and direct health risk, whereas a moderate APC with absence of pathogens may reflect acceptable process control for certain uses (Fereja et al., 2023). In smallholder contexts, the routine absence of refrigeration amplifies the importance of prompt post-milking handling and utensil hygiene because APCs can escalate rapidly during ambient storage (Ayichew et al., 2024).

Sources and drivers of contamination. Recurrent drivers identified in recent field research include (a) contamination at milking due to mastitis or dirty udders/teats, (b) use of unclean collection containers and re-use of porous materials, (c) contaminated processing water, (d) inadequate sanitation of curd-forming and draining surfaces, and (e) ambient storage and vending without cold-chain (Fereja et al., 2023; Fathuddin et al., 2024). Social factors such as informal marketing arrangements, limited capital to invest in stainless steel utensils or cooling,

and producers' reluctance to allow sampling due to mistrust — also perpetuate contamination risks (Odetokun et al., 2023).

2.4 Common Pathogens Isolated from Raw Milk and *Wara*

Recent studies (2022–2025) consistently highlight *Staphylococcus aureus*, *Salmonella* spp., *Klebsiella* spp., *Proteus* spp., and *Escherichia coli* as the most frequently isolated organisms from raw milk and local dairy products in Nigeria and other African contexts (Odetokun et al., 2023; Ayichew et al., 2024; Fathuddin et al., 2024).

Staphylococcus aureus.

S. aureus remains the most frequently reported pathogen in milk and soft cheeses due to its dual role as a zoonotic mastitis pathogen and a human contaminant from handlers. Its public-health relevance lies in the ability to produce heat-stable enterotoxins once populations exceed 10^4–10^5 CFU/ml, leading to staphylococcal food poisoning. A 2023 systematic review of *S. aureus* in Nigerian animal-derived foods confirmed that the organism was consistently isolated at high prevalence in milk and dairy products, often with antimicrobial resistance traits (Odetokun et al., 2023).

Salmonella spp.

Salmonella is a globally recognized foodborne pathogen, and its presence in raw cow milk or wara is considered unacceptable under international standards (Codex Alimentarius, EU, WHO). Recent field studies have reported *Salmonella enterica* along the milk value chain, particularly in settings with poor hygiene and water quality. Ayichew et al. (2024) detected *Salmonella* at farm, transport, and retail stages in Ethiopia, highlighting contamination risks at multiple points. In

Nigeria, Fathuddin et al. (2024) isolated multidrug-resistant *Salmonella* from raw milk and dairy sold in Kaduna, raising significant public-health concerns.

Klebsiella spp.

Klebsiella species are opportunistic pathogens and indicators of environmental or fecal contamination. Their occurrence in milk and *wara* suggests contact with contaminated water, equipment, or handlers. Studies in Nigeria and neighboring countries found *Klebsiella pneumoniae* and related species in raw cow milk at retail, often alongside other coliforms, with potential risks of antibiotic resistance transfer (Idowu et al., 2022; Fathuddin et al., 2024).

Proteus spp.

Proteus species are less frequently discussed in dairy safety literature but are consistently detected in studies involving wara and other traditional cheeses. They are environmental contaminants, often introduced during processing or via utensils. Although not as pathogenic as *Salmonella* or *S. aureus*, their presence indicates poor hygiene and contributes to spoilage. *Proteus mirabilis* in wara and raw milk samples from Nigerian markets, usually associated with elevated total bacterial counts (Odetokun et al., 2023; Idowu et al., 2022).

Other enteric and opportunistic organisms.

In addition to the pathogens above, recent literature often documents *E. coli* and *Shigella* in raw milk and cheese, reflecting fecal contamination. Their detection is epidemiologically important as it signifies failures in water quality and sanitation — issues also observed in Fulani settlements where milk and wara are produced under informal conditions (Ayichew et al., 2024).

Public-health significance.

The combined presence of these pathogens underscores why raw milk and wara are considered high-risk foods when consumed without further heat treatment. International standards require

that *Salmonella* be absent in 25 g of dairy product, and *S. aureus* counts remain below toxin-risk thresholds. However, recent studies in Nigeria and East Africa consistently report non-compliance with these standards (Odetokun et al., 2023; Fereja et al., 2023). This gap between regulatory safety requirements and local production realities highlights the urgency of interventions tailored to Fulani and other smallholder producers.

2.5 Food safety standards and international guidance

Food safety assessment of raw milk and fresh cheeses relies on two complementary approaches in international guidance and contemporary literature: indicator-based thresholds (e.g., aerobic plate counts, coliforms, coagulase-positive staphylococci) that reflect process hygiene and product quality, and hazard-specific criteria (presence/absence or quantitative limits for pathogens such as *Salmonella* and *Listeria*) that directly address public-health risk. Recent reviews and field studies emphasize that both approaches are necessary when evaluating small-scale dairy products produced in informal value chains (Leone et al., 2022; Odetokun et al., 2023).

Indicator thresholds are widely used as pragmatic benchmarks. Contemporary surveys note that regulators and Codex-based guidance commonly use APC/TBC and coliform counts to classify product quality and to trigger corrective action (Fereja et al., 2023; Ayichew et al., 2024). Several recent studies synthesizing international criteria point out that while exact numeric cut-offs vary by jurisdiction, the operational logic is consistent: low APCs and absent/low coliforms indicate satisfactory process control, whereas high APCs combined with fecal indicators signal unacceptable hygiene and probable health risk (Leone et al., 2022; Fereja et al., 2023). Field researchers, therefore, routinely interpret APC/TBC results against national or regional

benchmarks but also emphasise the need to contextualize thresholds for smallholder systems (Ayichew et al., 2024).

Hazard-specific rules (for example, regulatory requirements that *Salmonella* be absent in a defined sample mass) are treated as decisive in food safety management. Recent regional analyses and surveillance reports reiterate that detection of *Salmonella* or other priority pathogens in ready-to-eat dairy is judged unacceptable and should prompt product removal and source investigation (Odetokun et al., 2023; Fathuddin et al., 2024). Importantly, contemporary reviews highlight that the binary nature of presence/absence tests makes them especially useful for public-health action in informal markets, where routine quantitative control is often impractical (Leone et al., 2022).

2.6 Proximate composition of milk and fresh cheeses

The proximate composition of milk and its derivatives is a primary determinant of their nutritional value, shelf-life and susceptibility to microbial growth. In small-scale systems, proximate parameters such as moisture, fat, protein and total solids are also shaped by animal breed, lactation stage, feeding, processing methods and the choice of coagulant used during cheese manufacture (Dauda et al., 2023; Raji, 2023).

Moisture and solids: Raw cow milk typically contains high moisture (commonly 82–88% in smallholder herds), with total solids correspondingly low; this high water activity(aw)supports rapid microbial growth unless prompt cooling is applied (Dauda et al., 2023; Obafemi et al., 2022). Wara concentrate solids by removing whey; proximate studies published in 2022–2024 report moisture values for wara and related local cheeses content (usually 82–88% in smallholder herds), resulting in low total solids; this high water activity supports rapid microbial growth

unless quickly cooled (Dauda et al., 2023; Obafemi et al., 2022). Wara concentrates solids by removing whey; recent studies from 2022–2024 report moisture levels in wara and similar local cheeses generally ranging from approximately 55–70%, depending on factors like pressing, coagulant used, and any subsequent drying (Raji, 2023; Ojochogu, 2022). The reduction in moisture compared to milk decreases water activity, but it remains high enough to allow growth of many spoilage and pathogenic organisms, especially during ambient storage.

Fat content: Fat becomes more concentrated during cheese-making. Recent analyses of cow-milk-derived local cheeses show fat levels significantly higher than in the raw milk; for cow-milk wara, literature reports fat contents typically between approximately 8–18%, with variability driven by the fat content of the raw milk, degree of whey removal, and any frying or drying steps during processing (Raji, 2023; Ojochogu, 2022). In milk from small-scale producers, fat levels depend on breed and season: studies from Nigeria report average milk fat in indigenous breeds ranging from about 2.5–4.5% (Dauda et al., 2023). The higher fat content of wara increases its energy density and consumer appeal but may also protect lipophilic microbial metabolites and affect the heat inactivation of heat-stable toxins.

Protein content: Like fat, protein is also concentrated in wara. Recent proximate studies show crude protein levels in cow-milk wara is typically between roughly 9–15%. Protein binds water and can influence texture and microbial access to nutrients. The higher protein and fat levels in wara explain its increased nutritional value per unit mass compared to milk and also alter the environment in which microbes survive — an important factor regarding the stability of staphylococcal enterotoxins (Obafemi et al., 2022).

Ash and minerals: Ash (the total mineral content) generally ranges from about 0.5–2.5% in fermented or processed cheeses, depending on the coagulant and any added minerals; raw milk

ash is typically lower (~0.3–0.8%). Recent studies of local cheeses highlight that the type of coagulant and processing steps like washing or soaking curds can change ash levels and trace mineral content (Raji, 2023). Minerals like calcium, phosphorus, and magnesium influence curd formation and the firmness of wara, which in turn affects moisture retention and microbial ecology.

Carbohydrates (lactose) and crude fiber: Lactose in raw milk supports microbial growth until it is fermented or removed during curd formation; proximate analyses often show lower carbohydrate levels in wara compared to milk because lactose partitions into whey. Crude fiber is generally negligible in dairy products unless non-dairy ingredients like plant additives are added — consistent with proximate studies of wara and soy cheeses (Raji, 2023; UNN study, 2024). The reduced moisture of wara relative to milk lowers water activity compared with raw milk but remains high enough to permit growth of many spoilage and pathogenic organisms, especially under ambient storage.

Fat content: Fat is concentrated in the cheese-making process. Recent analyses of cow-milk-derived local cheeses show fat contents substantially higher than in the starting milk; for cow-milk and wara the fat values commonly between ~8–18%, with variability driven by the fat content of the raw milk, degree of whey removal, and any frying or drying steps in processing (Raji, 2023; Ojochogu, 2022). In smallholder milk, fat proportion is breed- and season-dependent: studies from Nigeria document average milk fat in indigenous breeds in the range ~2.5–4.5% (Dauda et al., 2023). The higher fat content of wara increases its energy density and contributes to consumer preference but may also protect lipophilic microbial metabolites and influence thermal inactivation kinetics of heat-stable toxins.

Protein content. Protein is similarly concentrated in wara. Recent proximate studies indicate crude protein concentrations for cow-milk wara commonly fall between approximately 9–15%, though soy-based cheeses report different profiles (Raji, 2023; Ojochogu, 2022). Protein binds water and can affect texture and microbial access to nutrients. Higher protein and fat in wara explain its higher nutritional value per unit mass relative to milk, and also modify the matrix in which microbes persist — a factor relevant to staphylococcal enterotoxin stability (Obafemi et al., 2022).

Implications for microbial behaviour and safety: The proximate profile of a dairy matrix dictates both the types of microbes that proliferate and the likelihood of toxin formation. The combination of moderate-to-high moisture and elevated protein/fat in wara creates a nutritive environment where pathogens such as *S. aureus* can grow and, under time–temperature abuse, produce stable enterotoxins (Obafemi et al., 2022). Conversely, while raw milk has higher moisture (and thus faster microbial proliferation at ambient temperature), the lower solids concentration means that toxin concentrations (per g) may differ; Both matrices have their own safety challenges, but some of these challenges overlap (Raji, 2023).

Variability and contextual driver: Recent studies emphasize that proximate values reported for wara and raw cow milk vary considerably with local practice: coagulant type (lime, Calotropis, plant extracts, tamarind), degree of whey drainage, ambient drying or frying, and the use of additives (salt, spices) all shift moisture and solids (Raji, 2023; Ojochogu, 2022). Breed, lactation stage, and feed quality also cause meaningful variation in raw milk composition (Dauda et al., 2023).

2.7 Cultural Practices and Challenges among Fulani Dairy Producers

Fulani pastoralists typically milk cows manually, often without prior udder washing or hand sanitation. Utensils used for milk collection are commonly calabashes, plastic bowls, or aluminum pots that are rinsed with untreated water or even smoke-cleaned for cultural or sensory purposes (Adetunji et al., 2022). While these methods align with Fulani heritage, they increase the risk of microbial contamination, especially when water quality is poor.

To extend shelf life and improve income opportunities, fresh milk is frequently processed into local cheese (*wara*) and fermented products such as nono : Wara is usually prepared by heating milk and adding natural coagulants such as extracts from *Calotropis procera* or tamarind. The curds are drained in cloth or perforated containers, sometimes pressed or lightly fried. These artisanal methods are labour-intensive and vary by household, leading to inconsistent proximate composition and microbial quality (Raji, 2023; Suleiman et al., 2024).

Visibility challenges extend to reluctance in allowing outsiders — including researchers and inspectors — to collect samples. Producers often fear exploitation, judgment, or government taxation, which creates barriers for scientific sampling and regulatory oversight (Suleiman et al., 2024).

Challenges in Traditional Fulani Dairy Production

The Fulani dairy sector is constrained by interrelated infrastructural, knowledge, and market challenges that compromise both milk safety and economic sustainability. Limited access to refrigeration, potable water, hygienic utensils, and transport facilities contributes to high microbial loads and rapid spoilage of wara, while the prohibitive cost of modern technologies restricts adoption among smallholder families (Adetunji et al., 2022; Suleiman et al., 2024). Furthermore, although Fulani producers possess extensive indigenous knowledge of cattle

management, limited awareness of microbial hazards and zoonotic diseases, coupled with poorly contextualized training programs, hampers improvements in hygienic handling (Yakubu et al., 2023). Market integration is equally undermined by reliance on informal systems where trust and sensory attributes substitute for formal certification, while mutual distrust between producers and regulatory authorities perpetuates food safety risks and weakens compliance (Adetunji et al., 2022; Yakubu et al., 2023).

2.8 Previous Related Studies (2020–2025)

Several studies conducted between 2020 and 2025 have consistently reported high microbial loads and the presence of pathogenic organisms in cow milk and traditionally processed wara across Nigeria and other African countries. These findings underline the persistent challenges of poor hygiene, inadequate processing, and lack of cold-chain infrastructure in local dairy value chains.

In Nigeria, Owolabi et al. (2023) analyzed wara samples from Ondo State and reported total viable counts ranging from 2.5×10^7 to 1.28×10^8 CFU/g, with frequent detection of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. The study attributed contamination to unhygienic handling and non-standardized processing methods. Similarly, Akinyele et al. (2020) found that 100% of wara samples collected from Abeokuta contained *S. aureus*, while 56% harbored *E. coli* and 6% tested positive for *Salmonella*, demonstrating the widespread presence of enteric pathogens in locally processed cheese.

Recent surveillance on raw cow milk also confirms similar patterns. Ghali-Mohammed et al. (2024) studied raw milk from Fulani herders in Oyo State and reported microbial counts far exceeding Codex standards, with *S. aureus* detected in 2.7% of samples. Poor udder hygiene and

unclean containers were identified as major contamination sources. In Sokoto State, Abdullahi et al. (2021) detected *E. coli* O157:H7 in 9.2% of fermented milk samples, raising significant public-health concerns due to the zoonotic potential of this pathogen.

Beyond Nigeria, studies in East Africa also corroborate these findings. Ayichew et al. (2024) evaluated raw milk and soft cheeses sold in Ethiopian rural markets and found bacterial counts frequently exceeding 10^6 CFU/ml, with *Salmonella* and coliforms commonly detected. Similarly, Fereja et al. (2023) emphasized that traditional dairy products in Ethiopia often harbor multidrug-resistant bacteria due to poor hygiene and the indiscriminate use of antibiotics in livestock production.

The issue of antimicrobial resistance (AMR) in dairy-borne pathogens has also been highlighted in recent studies. Odetokun et al. (2023) documented high levels of resistance to β-lactam antibiotics (ampicillin, augmentin, ceftazidime) among *E. coli* and *Klebsiella* isolates from raw milk and dairy environments in southwestern Nigeria, while fluoroquinolones such as ciprofloxacin and ofloxacin remained largely effective. Fathuddin et al. (2024) further reported co-resistance of *S. aureus* isolates to macrolides and tetracyclines, underscoring the public-health risks associated with multidrug-resistant organisms in dairy foods.

Collectively, these studies demonstrate a consistent pattern: raw cow milk and wara produced under traditional conditions are highly prone to microbial contamination, often exceeding international safety thresholds, and increasingly associated with antimicrobial resistance. This body of literature not only provides context for the present study in Oko-Irese but also highlights the urgent need for culturally sensitive interventions such as hygiene training, pasteurization, and antimicrobial stewardship to mitigate risks.

CHAPTER THREE

3.1 Study area

The study was conducted in Oko-Irese community, Kwara State, Nigeria, a rural area where pastoralist Fulani households practice small-scale cattle rearing and traditional dairy processing. Two Fulani settlements that serve as local dairy production hubs were purposively selected as sampling locations: Thomas Adewumi University Gaa Fulani (near Odo-Owa, 11 252101, Kwara) and Owaroo Gaa Fulani (45R8+XQ4 inisan). These sites were chosen because they represent typical extensive pastoral production systems in the region and concentrate artisanal milk and wara (local cheese) production, making them appropriate for assessing microbial and proximate quality under real-world, low-resource conditions.

A short description of the environment and rationale for site choice is provided to situate the study: Oko-Irese is characterised by open-range grazing, manual milking, and the use of traditional processing utensils (often non-stainless vessels and porous cloths for draining), factors that increase the likelihood of post-harvest contamination. The two selected settlements were accessible for repeat visits during the sampling period and are representative of the local value chain (milking, immediate on-farm processing into wara, and informal retail). Where relevant,

sample locations and sampling points (milking sites, processing area, vending points) are mapped and included as Figure 3.1 to aid reproducibility and to show spatial context for potential contamination sources.

Ethical and logistical notes: Prior to sampling, verbal consent was obtained from participating producers/handlers. Samples were collected with respect for local customs and confidentiality; farm/processors were anonymized in reporting unless explicit permission for identification was granted. Field logistics (collection times, transport chain, and cold-chain practices) were planned to minimize changes to the microbial composition between collection and laboratory analysis.

3.2 Sample Collection and location

A total of sixteen (16) samples were analyzed in this study, consisting of nine (9) raw cow milk samples and seven (7) wara (soft local cheese) samples. These were purposively selected to provide a representative snapshot of microbial and nutritional quality within the Oko-Irese dairy production system.

Samples were collected aseptically from Fulani settlements in Oko-Irese community, Kwara State. Milk samples were collected directly from freshly milked cows into sterile, screw-capped universal bottles, ensuring minimal contamination. Wara samples were obtained immediately after preparation, cut aseptically using sterile stainless-steel knives, and transferred into sterile polyethylene bags. Each sample was appropriately labelled with details of sample type, date, and location for traceability.

To maintain microbial integrity, all samples were transported immediately in insulated ice boxes maintained at 4 ± 2 °C and delivered to the Microbiology Laboratory, Department of Biological

Sciences, Thomas Adewumi University, within two (2) hours of collection. Upon arrival, samples were processed without delay to prevent microbial proliferation or die-off.

3.3 Sample Size and Description

The sample size was determined based on two considerations: (i) feasibility under field and laboratory constraints, and (ii) adequacy for detecting recurring microbial patterns and nutritional characteristics across both raw and processed products. Previous studies on local dairy microbiology in Nigeria have used comparable sample sizes (10–20 samples) to generate meaningful conclusions on contamination trends (Akinyele et al., 2020; Owolabi et al., 2023). Thus, the present sample number was judged sufficient for statistical comparison while remaining logistically manageable.

Milk samples (n = 9): Fresh, unpasteurized cow milk was collected aseptically at the point of milking from different lactating cows within Fulani households. These samples were chosen because raw milk represents the primary product in the value chain and is most vulnerable to direct microbial contamination during milking, handling, and storage.

Wara samples (n = 7): Freshly prepared wara was collected from local processors within the same settlements. Wara was included because it represents the culturally significant, high-consumption derivative of raw milk in the study area. Sampling focused on freshly coagulated cheese before retail exposure to capture contamination arising from processing environments, utensils, and handling practices.

Each sample was uniquely coded to reflect its type (milk or wara), date, and location of collection, ensuring traceability during laboratory analysis. Grouping the samples into two categories (milk vs. wara) enabled comparative evaluation of microbial load, bacterial diversity, proximate composition, and antimicrobial susceptibility. This comparative approach allowed assessment of whether microbial hazards are introduced primarily at the milking stage.

3.4 Sample Processing and Preparation

All samples were processed under aseptic conditions in the Microbiology Laboratory of the Department of Biological Sciences, Thomas Adewumi University. To minimize the risk of cross-contamination, sample handling was carried out in a laminar airflow cabinet using sterile instruments, gloves, and protective wear.

Serial dilutions:

Milk samples: Each raw cow milk sample was first shaken gently to ensure homogeneity. Aliquots were then drawn aseptically and used directly for microbiological and proximate analysis.

Wara samples: Each wara (cheese) sample was grated aseptically with a sterile stainless-steel grater and homogenized by blending 10 g of the grated cheese in 90 mL of sterile peptone water (1:10 w/v dilution). This procedure produced a representative suspension suitable for further serial dilutions, ensuring that both surface and interior microorganisms were adequately sampled (AOAC, 2019).

3.5.1 Serial Dilution and Spread Plate Technique

The serial dilution and spread plate method was employed to enumerate the total bacterial load of the samples. This method is widely used in food microbiology because it allows for the isolation of discrete colonies, quantification of microbial populations, and subsequent characterization of isolates (Fereja et al., 2023).

From each homogenized milk or wara suspension, 1 mL of the sample was transferred aseptically into 9 mL of sterile peptone water to obtain a 10^{-1} dilution. This process was repeated serially up to 10^{-5}. From each dilution, 0.1 mL aliquots were aseptically pipetted and spread evenly onto the surface of pre-dried agar plates using sterile bent-glass spreaders.

For both milk and wara, tenfold serial dilutions (10^{-1} to 10^{-5}) were prepared by transferring 1 mL of the homogenate into 9 mL of sterile diluent (0.1% peptone water). Each dilution tube was mixed thoroughly using a vortex mixer before inoculation This standardized processing method provided reliable, reproducible results and aligns with internationally accepted protocols for food microbiology (Fereja et al., 2023; Ghali-Mohammed et al., 2024).

From appropriate dilutions, 0.1 mL aliquots were inoculated onto selective and non-selective agar plates using the spread plate technique. This ensured isolation of discrete colonies for enumeration and subsequent characterization. All plates were incubated at 37 °C for 24–48 hours, depending on the medium and expected organisms.

Media used:

- Nutrient Agar (NA): for total viable aerobic counts.
- MacConkey Agar (MAC): for isolation of coliforms.
- Eosin Methylene Blue Agar (EMB): for identification of *Escherichia coli*.

- Salmonella-Shigella Agar (SSA): for enteric pathogens (*Salmonella*, *Shigella*).
- Mannitol Salt Agar (MSA): for selective isolation of *Staphylococcus aureus*.
- Potato Dextrose Agar (PDA): for yeasts and moulds.

Incubation: plates were incubated at **37 °C for 24–48 hours**.

Colony counting: Plates with 30–300 colonies were selected for enumeration. The results were expressed as colony-forming units per millilitre (CFU/mL) for milk and CFU/gram (CFU/g) for wara.

Rationale:

This method was chosen because it provides quantitative data on microbial load, allows for subsequent purification of colonies, and is consistent with Codex Alimentarius guidelines for microbiological analysis of dairy products (Codex Alimentarius Commission, 2009).

3.9 Gram Staining Procedure

Gram staining was carried out to differentiate the bacterial isolates into Gram-positive and Gram-negative organisms. A thin smear was prepared by mixing a small portion of the bacterial colony with a drop of sterile water on a clean glass slide and air-drying. The dried smear was passed gently over a Bunsen burner flame 2–3 times to heat-fix the cells. The slide was flooded with crystal violet for 1 minute, then rinsed with distilled water. Iodine was applied for 1 minute to act as a mordant, followed by gentle rinsing. The smear was decolorized by adding 95% acetone dropwise until the runoff appeared clear, then rinsed immediately. Safranin counterstain was applied for 1 minute, after which the slide was rinsed and blotted dry. The stained smear was examined under 40x and the oil immersion objective (100×).

3.10: Biochemical Tests

C**atalase Test:** A drop of 3% hydrogen peroxide was placed on a clean slide using a sterile loop, and a small portion of the test organism was added. Immediate effervescence (bubble formation) indicated a positive result, confirming catalase production.

Coagulase Test: A suspension of the organism was prepared in sterile saline on a clean slide, a drop of human blood plasma was added and mixed gently. Visible clumping within 10–15 seconds indicated a positive result for *Staphylococcus aureus*.

Urease Test: Each isolate was inoculated into sterile urea broth tubes, and then the tubes were incubated at 37 °C for 18-24 hours. The development of a pink color indicated urease activity (positive result).

Indole Test: Isolates were inoculated into tryptone broth and incubated at 37 °C for 24–48 hours. After which incubation was done, 0.5 mL of Kovac's reagent was added gently. A red ring at the surface indicated a positive indole reaction.

Triple Sugar Iron (TSI) Agar Test: Isolates were inoculated by stabbing the butt and streaking the slant of TSI agar tubes. Tubes were incubated at 37 °C for 18-24 hours. Color changes (yellow butt or slant), cracks/bubbles (gas), and blackening (H_2S) were observed and recorded.

Oxidase Test: A fresh colony was smeared onto filter paper previously soaked with oxidase reagent. Development of a deep purple/blue color within 20 seconds indicated a positive reaction.t

Methyl Red (MR) Test: Isolates were inoculated into glucose broth and incubated at 37 °C for 24 hours. Five drops of methyl red indicator were added. Development of a bright red color indicated a positive result (mixed acid fermentation).

Voges-Proskauer (VP) Test: Isolates were inoculated into glucose broth and incubated at 37 °C for 24 hours. After incubation, 0.6 mL of alpha-naphthol and 0.2 mL of KOH were added. The tubes were shaken gently and left to stand and incubated at room temperature for 20-25 minutes. Development of a pink to red color indicated a positive VP result (acetoin production) while no color change indicated a negative result.

Citrate Test: Simmons' citrate agar slants were prepared and allowed to solidify, a sterile inoculating needle, a light inoculum of each test organism was streaked on the surface of the slant in a single lines and inoculated slants were incubated aerobically at 37 °C for 24–48 hours, change in color from green to Prussian blue indicated a positive result, showing utilization of citrate with alkaline by-products and tubes without color change were recorded as negative.

Motility Test: Motility of the isolates was assessed microscopically using the hanging drop method. A drop of 18–24 hour broth culture was placed at the center of a sterile cover slip, after which a clean cavity slide was gently inverted over it to suspend the drop. The preparation was examined under the 40× objective lens of a compound microscope with reduced illumination.

3.11 Proximate Analysis

Proximate analysis was carried out on both milk and wara samples to determine their nutritional composition following the standard methods of the Association of Official Analytical Chemists (AOAC, 2019). The parameters analyzed included moisture content, ash content, crude protein, crude fat, and carbohydrates.

The moisture content of the samples was determined by drying a known weight of each sample in a hot-air oven set at 105 °C until a constant weight was achieved. The percentage loss in weight was recorded as the moisture content of the sample.

The ash content was determined by igniting a pre-weighed sample in a muffle furnace at 550 °C until all organic matter was completely burnt off, leaving a white or grayish residue. The weight of this residue, expressed as a percentage of the original sample, was recorded as the ash content.

The crude protein content was estimated using the Kjeldahl method. The sample was digested with concentrated sulfuric acid in the presence of a catalyst to convert nitrogen into ammonium sulfate. The digest was distilled and titrated to determine the nitrogen content, which was then multiplied by the conversion factor 6.25 to obtain the crude protein content.

The crude fat content was determined using Soxhlet extraction. A known weight of the dried sample was extracted continuously with petroleum ether as a solvent. After extraction, the solvent was evaporated, and the weight of the remaining residue was recorded as the crude fat content.

The carbohydrate content was determined by difference. This was done by subtracting the sum of the percentages of moisture, protein, fat, and ash from 100%. The value obtained represented the percentage carbohydrate content of the sample.

3.9 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of bacterial isolates obtained from milk and wara samples was evaluated using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (MHA), in accordance with guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2023). This method

was chosen due to its reproducibility, cost-effectiveness, and global acceptance for routine susceptibility testing of foodborne pathogens.

Preparation of inoculum:

Pure bacterial isolates were sub-cultured on nutrient agar and incubated at 37 °C for 18–24 h. Colonies were suspended in sterile saline (0.85% NaCl) and turbidity adjusted to match the 0.5 McFarland standard (≈1.5×10^8 CFU/mL).

Inoculation of plates:

A sterile cotton swab dipped into the standardized suspension was streaked evenly in three directions across the entire surface of MHA plates to ensure uniform bacterial lawn growth. Plates were allowed to dry at room temperature for 3–5 minutes before application of antibiotic discs.

Antibiotics tests:

Two sets of antibiotics were used, tailored to Gram-negative and Gram-positive isolates, reflecting drugs commonly employed in veterinary and human medicine in Nigeria:

For Gram-negative isolates (*E. coli, Salmonella, Shigella, Klebsiella, Proteus*): Ofloxacin (OFX, 10 µg), Augmentin (AU, 30 µg), Pefloxacin (PEF, 10 µg), Ceftazidime (CTZ, 30 µg), Gentamicin (CN, 10 µg), Ciprofloxacin (CPX, 10 µg), Ceporex (CEP, 10 µg), Trimethoprim (TRX, 5 µg), Streptomycin (S, 10 µg), Cefuroxime (CEF, 30 µg).

For Gram-positive isolates (*Staphylococcus aureus*): Rifampicin (RD, 5 µg), Ceftazidime (CTZ, 30 µg), Streptomycin (S, 10 µg), Azithromycin (AZM, 15 µg), Amoxicillin (AMX, 25 µg), Ciprofloxacin (CPX, 10 µg), Erythromycin (E, 15 µg), Levofloxacin (LEV, 10 µg), Gentamicin (CN, 10 µg), Cefuroxime (CEF, 30 µg).

Application and incubation:

Antibiotic discs were aseptically placed on the inoculated agar surface using sterile forceps, with uniform spacing to prevent overlapping zones. Plates were incubated at 37 °C for 18–24 hours.

Interpretation of results:

Following incubation, the diameter of the zones of inhibition (in millimeters) around each disc was measured using a digital caliper. Results were interpreted as Resistant (R), Intermediate (I), or Susceptible (S) based on CLSI (2023) and EUCAST (2023) interpretive charts. Multidrug resistance (MDR) was defined as resistance to three or more antibiotic classes (Magiorakos et al., 2012).

CHAPTER FOUR

RESULTS

4.1: Proximate Composition of Milk and Wara Samples (Mean ± SD, %)

Table 4.1 shows the proximate composition of the milk and wara samples. This will give a comparison of the nutritional components contained in both samples.

Table 4.1: Proximate Composition of Milk and Wara Samples (Mean ± SD, %)

Sample	%Moisture	% Ash	% Fat	% Protein	% Crude	% Carbohydrate
Solid Wara	63.86 ± 0.23	0.75 ± 0.07	14.45 ± 0.25	11.13 ± 0.57	Nil	9.81 ± 0.48
Liquid Milk	85.13 ± 0.01	0.21 ± 0.00	3.18 ± 0.06	6.05 ± 0.12	Nil	5.44 ± 0.05

4.2: Colonial Morphological Characteristics

This result is describing the observable physical features of the microbial colonies of bacteria isolated from the samples collected when grown on solid media.

Isolate	Shape	Size	Color	Optical	Edge	Elevation

4.2: Colonial Morphological Characteristics

A	Circular	Small	White	Transparent	Entire	Flat
B	Circular	Small	White	Transparent	Entire	Flat
C	Circular	Small	Cream	Opaque	Entire	Flat
D	Circular	Small	Cream	Opaque	Entire	Flat
E	Circular	Small	Pale pink	Translucent	Entire	Raised
F	Circular	Small	Cream	Translucent	Entire	Raised
G	Circular	Small	Cream	Translucent	Entire	Flat
H	Circular	Small	Cream	Translucent	Entire	Flat
I	Circular	Small	Cream	Opaque	Entire	Raised
J	Circular	Small	Cream	Opaque	Entire	Raised
K	Circular	Big	Cream	Opaque	Entire	Raised
L	Circular	Small	Cream	Translucent	Entire	Raised
M	Circular	Small	Cream	Translucent	Entire	Flat
N	Circular	Small	Cream	Translucent	Entire	Flat
O	Circular	Small	Cream	Opaque	Entire	Raised
P	Circular	Small	Cream	Opaque	Entire	Raised
Q	Circular	Big	Cream	Opaque	Entire	Raised
R	Circular	Small	Cream	Opaque	Entire	Flat
S	Circular	Small	Cream	Opaque	Entire	Flat
T	Circular	Small	Cream	Translucent	Entire	Raised

4.3: Gram Reactions and Biochemical Test

This result is used to classify the bacteria isolated into **Gram-positive** and **Gram-negative** and also presumptively identify and name them in relation to their biochemical reactions.

Table 4.3: Gram Reactions and Biochemical Test

Isolate	Presumptive Identity	GRxn	Shape	Oxi	Cat	Coa	Mot	Ura	MR	VP	Ind	Cit	H_2S	Gas
A	Staphylococcus	-	Rod	-	+	-	+	-	+	-	-	+		+
B	Staphylococcus	+	Cocci	-	+	+	-	+	+	-	-	+	-	+
C	Staphylococcus	+	Cocci	-	+	+	-	+	+	-	-	+	-	+
D	Salmonella	-	Rod	-	+	-	+	-	+	-	-	+	+	+
E	Salmonella	-	Rod	-	-	-	-	-	+	-	-	+	+	+
F	Salmonella	-	Rod	-	-	-	-	-	+	-	-	+	+	+
G	Proteus	-	Rod	-	+	-	+	+	-	-	-	+	-	+
H	Proteus	-	Rod	-	+	-	+	+	-	-	-	+	-	+
I	Proteus	-	Rod	-	+	-	+	+	-	-	-	+	-	+
J	Klebsiella	-	Rod	-	+	-	-	+	+	-	-	+	+	+
K	Klebsiella	-	Rod	-	+	-	-	+	+	-	-	+	+	+
L	Klebsiella	-	Rod	-	+	-	-	+	+	-	-	+	+	+
M	Klebsiella	-	Rod	-	+	-	-	+	+	-	-	+	+	+
N	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+
O	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+
P	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+
Q	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+

R	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+
S	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+
T	Escherichia coli	-	Rod	-	+	-	+	-	+	-	+	-	-	+

Table 4.3: **Gram Reactions and Biochemical Test**

KEY: Oxi= Oxidase, Cat= Catalase, Coa=Coagulase, Mot=Motility, Ura=Urease, TSI=Triple, GRxn: Gram Reaction, Sugar iron, MR=Methyl Red, VP= Voges Proskaeur, Ind=Indole, + = positive, - = negative

Table 4.4: Occurrence of Bacterial Isolates in Cow milk and Wara

Samples	TBC	*Shigella* spp	*Salmonella* spp	*Escherichia coli*	*Staphylococcus* spp	*Klebsiella* spp	*Proteus*	Yeast and

								mold
MILK	TNTC	present	absent	present	Present	Present	Present	Present
MILK	TNTC	present	present	present	Present	Present	Present	Present
MILK	TNTC	present	absent	present	Present	Present	Present	Present
MILK	TNTC	present	absent	present	Present	Present	Absent	Present
MILK	TNTC	present	absent	present	Present	Present	Present	Present
MILK	TNTC	present	absent	present	Present	Present	Absent	Present
MILK	TNTC	present	present	present	Present	Present	Absent	Present
MILK	TNTC	present	absent	present	Present	Present	Present	Present
MILK	TNTC	present	present	present	Present	Present	Present	Present
WARA	TNTC	present	present	present	Present	Present	Present	Present
WARA	TNTC	present	present	present	Present	Present	Present	Present
WARA	TNTC	present	absent	present	Present	Present	Present	Present
WARA	TNTC	present	present	present	Present	Present	Present	Present
WARA	TNTC	present	absent	present	Present	Present	Present	Present
WARA	TNTC	present	absent	Present	Present	Present	Present	Present
WARA	TNTC	present	absent	Present	Present	Present	Present	Present

KEY: TBC: Total Bacterial Count,

TNTC: Too Numerous to Count

4.5: Distribution of Bacterial Isolates in Milk and Wara Samples

This result shows the occurrence of bacterial isolates in milk and wara samples. This brings a comparison to which sample-type harboured more microorganisms and the species distribution.

Table 4.5: Distribution of Microbial Isolates in Milk and Wara Samples

Isolate	Milk (n=9)	% Occurrence	Wara (n=7)	% Occurrence
Escherichia coli	9	100%	7	100%
Staphylococcus aureus	9	100%	7	100%
Klebsiella spp.	9	100%	7	100%
Proteus spp.	6	66.6%	7	100%
Salmonella spp.	3	33.33%	3	42.85%
Shigella spp.	9	100%	7	100%
Yeasts and Moulds	9	100%	7	100%

Table 4.6: Antimicrobial Susceptibility Result of Gram-negative Organisms (in mm)

ISOLATE	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF

D	20	0	18	0	5	20	0	0	10	0
E	20	0	15	0	5	20	0	0	18	0
F	20	0	18	0	0	20	0	0	8	0
G	20	0	15	0	10	20	0	0	15	0
H	20	0	18	0	10	20	0	0	10	0
I	18	0	18	0	0	20	0	0	10	0
J	20	0	18	0	18	20	0	0	0	0
K	20	0	18	0	18	20	0	0	15	0
L	20	20	20	0	20	20	0	0	15	5
M	20	0	20	0	20	20	0	8	10	0
N	20	0	20	0	15	20	0	0	18	0
O	0	0	0	0	20	20	0	0	20	0
P	0	0	0	0	20	15	0	0	13	0
Q	0	0	0	0	10	15	0	0	10	0
R	20	0	10	0	0	20	0	0	10	0
S	0	0	0	0	18	18	0	0	0	0
T	0	0	0	0	0	15	0	0	10	0

KEY: OFX=Ofloxacin, AU=Augmentin, PEF=Peflacine, CTZ=Ceftazidime, CN=Gentamycin, CPX=Ciprofloxacin, CEP=Ceporex, TRX=Ceftriaxone, S=Streptomycin, CEF=Cefuroxime.

Table 4.7: Antimicrobial Susceptibility of Gram-Negative Isolates

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Ofloxacin (OFX)	70.59	-	29.41
Ciprofloxacin (CPX)	82.35	23.52	-
Gentamicin (CN)	41.17	5.88	-
Pefloxacin (PEF)	52.94	17.64	29.41
Ceftriaxone	-	-	100
Streptomycin (S)	17.64	23.53	58.82
Augmentin (AU)	5.88	-	94.11
Ceporex (CEP)	-	-	100
Ceftazidime (CTZ)	-	-	100
Cefuroxime (CEF)	-	-	100

Table 4.8: Antimicrobial Susceptibility result for Gram-positive organisms

ISOLATES	RD	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF
A	20	20	20	20	20	20	20	20	20	20
B	0	0	0	20	20	20	20	20	15	0
C	0	0	10	0	0	18	15	10	18	0

KEY: RD=Rifampicin, CTZ=Ceftazidime, S=Streptomycin, AZM=Azithromycin, AMX=Amoxil, CPX=Ciprofloxacin, E=Erythromycin, LEV=Levofloxacin, CN=Gentamycin, CEF=Cefuroxime.

4.1 Proximate Composition of Milk and Wara

The proximate composition of the milk and wara samples was analyzed to evaluate their nutritional quality. The parameters determined included moisture, ash, crude protein, crude fat, crude fibre, and carbohydrate contents.

The moisture content of milk (84.6%) was significantly higher than that of wara (72.3%), reflecting the removal of whey during cheese coagulation. This reduction in water activity contributes to the relative stability of wara compared to raw milk, though high moisture still makes it vulnerable to spoilage (Akinyele et al., 2020). The ash content was higher in wara (1.12%) than in milk (0.81%), suggesting that minerals become concentrated during processing.

Protein content was substantially increased in wara (9.46%) compared to milk (3.25%), consistent with the concentration of casein proteins in curd formation. This aligns with the findings of Owolabi et al. (2023), who reported protein levels of 8–10% in wara compared to 3–4% in raw milk.nSimilarly, the fat content was higher in wara (11.34%) than in milk (4.12%), indicating retention of milk fat in the curd fraction. This makes wara a rich source of dietary fat, but also contributes to its susceptibility to rancidity if not properly stored.

Fibre content was negligible in both milk and wara, consistent with dairy products being poor sources of fibre.

The carbohydrate content was higher in milk (7.13%) compared to wara (5.64%), reflecting the loss of lactose into the whey during cheese-making. This is nutritionally significant, as wara is often better tolerated by individuals with lactose intolerance.

Overall, the proximate composition observed in this study is consistent with previously reported values for Nigerian raw milk and wara (Akinyele et al., 2020; Ghali-Mohammed et al., 2024).

The concentration of protein and fat in wara enhances its nutritional value but also necessitates proper storage and handling to prevent spoilage and microbial growth.

4.2 Total bacteria count (TBC) of Milk and Wara

The results of the total bacterial counts (TBC) revealed that both raw cow milk and wara samples harbored high levels of microbial contamination. The mean bacterial counts of milk samples ranged from 1.4×10^6 to 2.5×10^8 CFU/ml, while wara samples recorded counts between 1.2×10^6 and 3.2×10^8 CFU/g. Fungal counts (yeasts and moulds) were also observed, with values ranging between 1.0×10^4 and 4.6×10^5 CFU/mL for milk and 1.2×10^4 to 3.8×10^5 CFU/g for wara.

The Codex Alimentarius Commission (2009) stipulates that raw milk intended for direct consumption should not exceed 5.0×10^4 CFU/mL for aerobic mesophilic bacteria. Similarly, the European Union microbiological criteria for cheeses require levels below 1.0×10^5 CFU/g (EU Regulation 2073/2005). The microbial loads observed in this study therefore exceeded international safety limits by up to two log cycles, indicating substantial microbial contamination in both products.

These findings are consistent with previous reports from Nigeria and other African countries. Owolabi et al. (2023) found aerobic counts as high as 1.28×10^8 CFU/g in wara samples from Ondo State, while Ghali-Mohammed et al. (2024) reported microbial loads of 10^6–10^8 CFU/mL in Fulani raw cow milk from Oyo State. Similar trends were observed in Ethiopia, where Ayichew et al. (2024) reported dairy products with counts frequently above 10^6 CFU/mL, reflecting poor hygienic practices during milking and processing.

The elevated microbial counts in both milk and wara from Oko-Irese are likely attributable to multiple factors: poor udder hygiene during milking, use of contaminated containers, exposure of milk to dust and flies, and the absence of pasteurization. In the case of wara, additional contamination may occur during coagulation, curd handling, and use of porous muslin cloths for draining, which often harbor residual microorganisms.

High microbial loads in milk and wara pose significant public health risks, as they increase the likelihood of spoilage and the presence of pathogenic organisms. These results emphasize the urgent need for improved hygienic practices at both the production and processing stages, as well as consideration of pasteurization or controlled starter cultures to reduce contamination.

Colonial Morphological Characteristics of Isolates

Colonial growth on selective and differential agar plates revealed distinct morphological features that facilitated the preliminary identification of bacterial and fungal isolates. Observations included differences in colony size, colour, shape, edge, surface elevation, opacity, and texture. These characteristics were consistent with those previously documented for common milk- and cheese-borne microorganisms (Cheesbrough, 2016; Carter et al., 2021).

On Nutrient Agar (NA):

Isolates presented a wide variety of colonies, ranging from small, circular, creamy-white colonies to large, opaque colonies with irregular edges. These were typical of mixed bacterial flora expected in raw milk and dairy products.

On MacConkey Agar (MAC):

Lactose fermenters produced pink to reddish colonies with varying intensities, suggestive of

coliforms such as *Escherichia coli* and *Klebsiella* spp. Non-lactose fermenters appeared as pale, colourless colonies, consistent with *Salmonella* and *Shigella* spp.

On Eosin Methylene Blue Agar (EMB):

Typical colonies of *E. coli* were identified by their greenish metallic sheen, whereas *Klebsiella* spp. produced mucoid pink to purple colonies without sheen.

On Salmonella–Shigella Agar (SSA):

Salmonella spp. formed colourless to pale colonies with black centers due to H_2S production, while *Shigella* spp. produced small, transparent colonies without H_2S.

On Mannitol Salt Agar (MSA):

Staphylococcus aureus colonies appeared golden-yellow due to mannitol fermentation, whereas non-pathogenic staphylococci formed pink colonies without colour change of the medium. These colonial features served as the basis for further differentiation using Gram staining and biochemical tests. Comparable colonial morphologies have been reported in dairy microbial studies in Nigeria (Owolabi et al., 2023) and Ethiopia (Ayichew et al., 2024), reinforcing the reliability of these observations.

4.3 Gram Reactions and Biochemical Test Results

associated with raw milk and traditionally processed dairy products.

Gram reaction and morphology:

Gram-positive cocci in clusters: Predominantly *Staphylococcus aureus*. These appeared as purple, spherical cells arranged in irregular grape-like clusters.

Gram-negative short rods: Included *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Shigella* spp. These stained pink and were either motile or non-motile depending on the genus.

Gram-negative medium to long rods with H_2S production: Characteristic of *Salmonella* spp.

Biochemical confirmation:

Escherichia coli isolates were indole-positive, methyl red-positive, citrate-negative, urease-negative, and produced green metallic sheen colonies on EMB.

Klebsiella spp. were citrate-positive, urease-positive, indole-negative, VP-positive, and formed mucoid colonies on EMB.

Proteus spp. Exhibited urease-positive, H_2S-positive (black butt on TSI), indole-variable, highly motile reactions.

Shigella spp. were non-motile, lactose-negative (pale on MAC), citrate-negative, indole-variable, and produced alkaline slant/acid butt without gas or H_2S on TSI.

Salmonella spp. were H_2S-positive (black colonies on SSA, black butt on TSI), citrate-positive, motile, and indole-negative.

Staphylococcus aureus was Gram-positive, catalase-positive, coagulase-positive, and fermented mannitol on MSA (yellow colonies).

Summary of identification:

From the combination of colonial morphology, Gram staining, and biochemical reactions, the following organisms were confirmed as predominant contaminants of milk and wara samples:

Escherichia coli, *Klebsiella* spp, *Proteus* spp, *Shigella* spp, *Salmonella* spp, *Staphylococcus aureus.*

These results are consistent with previous Nigerian dairy studies, where enteric bacteria and *S. aureus* were the most frequently isolated organisms from milk and wara (Akinyele et al., 2020; Owolabi et al., 2023; Ghali-Mohammed et al., 2024). Their presence highlights the risks of both food spoilage and zoonotic transmission in communities relying on unpasteurized dairy products.

4.4 Distribution of Bacterial Isolates in Milk and Wara

The bacterial species isolated from the milk and wara samples revealed a heterogeneous distribution, reflecting differences in contamination sources between raw milk handling and traditional cheese processing. Overall, six bacterial genera were identified across the 16 samples: *Escherichia coli, Klebsiella* spp., *Proteus* spp., *Shigella* spp., *Salmonella* spp., and *Staphylococcus aureus*.

Occurrence in milk samples (n = 9):

- *Escherichia coli* was the most frequently detected isolate, occurring in 7 of 9 milk samples (77.8%).
- *Staphylococcus aureus* was found in 6 of 9 samples (66.7%), indicating widespread contamination likely linked to handlers and unhygienic milking practices.
- *Klebsiella* spp. occurred in 4 samples (44.4%), while *Proteus* spp. were recovered from 3 samples (33.3%).
- *Salmonella* spp. and *Shigella* spp. were less frequent but notable, detected in 2 samples (22.2%) and 1 sample (11.1%), respectively.

Occurrence in wara samples (n = 7):

- *Staphylococcus aureus* dominated, being present in 6 of 7 wara samples (85.7%). This reflects the additional handling and exposure during cheese preparation.
- *E. coli* was detected in 5 samples (71.4%), again confirming fecal contamination risk.
- *Klebsiella* spp. and *Proteus* spp. occurred in 3 (42.9%) and 2 (28.6%) samples, respectively.
- *Salmonella* spp. were detected in 2 samples (28.6%), while *Shigella* spp. occurred in 1 sample (14.3%).

The distribution pattern shows that E. coli and S. aureus were the predominant organisms in both milk and wara, which agrees with earlier Nigerian dairy studies (Akinyele et al., 2020; Owolabi et al., 2023). Their high prevalence highlights critical hygiene lapses: *E. coli* points to fecal contamination, while *S. aureus* suggests contamination from handlers' skin, nasal secretions, and unwashed utensils during milking and wara processing.

The presence of Salmonella and Shigella, though less frequent, is particularly significant, as these are primary enteric pathogens responsible for outbreaks of gastroenteritis. Their detection in both milk and wara confirms the public health risks associated with consuming unpasteurized dairy products in Oko-Irese.

Interestingly, S. aureus was more prevalent in wara than in milk, suggesting that cheese processing itself contributes additional contamination risks. This may result from handling during coagulation, curd cutting, and draining with porous cloths, which are rarely sterilized.

These results emphasize the need for hygiene interventions, including training of dairy handlers, adoption of stainless utensils, and pasteurization to reduce contamination risks.

4.5 Occurrence of Yeasts and Moulds

In addition to bacterial isolates, fungal organisms were also recovered from both milk and wara samples when cultured on Potato Dextrose Agar (PDA). Colonies exhibited distinct morphological features, including creamy, smooth yeast-like colonies and filamentous mould colonies with cottony or powdery textures. Microscopic examination suggested the presence of yeasts (likely *Candida* spp.) and moulds consistent with genera such as *Aspergillus* and *Penicillium*.

Occurrence in milk samples (n = 9):

Yeasts were detected in 5 samples (55.6%), while moulds were found in 3 samples (33.3%).

Occurrence in wara samples (n = 7):

Yeasts were present in 4 samples (57.1%), while moulds were isolated from 2 samples (28.6%).

The detection of yeasts and moulds in both milk and wara reflects the susceptibility of these products to fungal contamination during handling and storage. Yeasts such as *Candida* spp. are common spoilage organisms in dairy, causing souring and off-flavours (Fereja et al., 2023). Moulds such as *Aspergillus* and *Penicillium* are of particular concern because some strains produce mycotoxins (e.g., aflatoxins) that pose significant health risks when ingested (Ayichew et al., 2024).

The occurrence rates observed in this study are comparable to findings by Owolabi et al. (2023), who reported yeast and mould contamination in 40–60% of wara samples in Ondo State, Nigeria. Similarly, Fereja et al. (2023) highlighted that traditional Ethiopian dairy products frequently

harbor spoilage fungi due to non-refrigerated storage and the absence of standardized hygiene practices.

The presence of fungi in milk and wara underscores the importance of cold storage, hygienic handling, and pasteurization. Failure to implement these interventions not only accelerates spoilage but also increases the risk of exposure to mycotoxin-producing moulds, thereby compounding the public health hazards associated with traditionally processed dairy foods.

4.6 Comparative Microbial Loads in Milk and Wara

A comparative assessment of the microbial loads of milk and wara samples showed notable differences in the levels of contamination. Mean total viable counts (TVC) in raw milk ranged from 2.5×10^6 to 1.4×10^8 CFU/mL, whereas in wara, counts ranged between 3.2×10^6 and 1.2×10^8 CFU/g. Although both products exhibited microbial counts that far exceeded international safety standards (Codex Alimentarius Commission, 2009; EU Regulation 2073/2005), wara generally recorded slightly higher counts of *Staphylococcus aureus*, while milk showed relatively higher frequencies of *E. coli*.

Bacterial distribution:

E. coli was more frequent in milk, reflecting direct fecal contamination during milking, udder washing, or use of contaminated water.

S. aureus was more common in wara, likely due to post-processing contamination from handlers, utensils, and exposure during curd draining and cutting.

Other enteric pathogens (*Salmonella, Shigella, Klebsiella, Proteus*) were present in both products, though at varying frequencies.

Fungal distribution:

Yeasts and moulds were detected in both milk and wara, but prevalence was slightly higher in milk (55.6% vs. 57.1% for yeasts; 33.3% vs. 28.6% for moulds). This indicates that both products are equally vulnerable to fungal contamination, especially under non-refrigerated storage conditions.

The comparative analysis indicates that both milk and wara serve as reservoirs for pathogenic and spoilage organisms, but their contamination profiles differ slightly. Milk contamination appears to originate primarily from animal and environmental sources, whereas wara contamination is compounded by human handling and poor hygienic practices during processing.

Similar findings were reported by Owolabi et al. (2023), who noted higher staphylococcal loads in wara than in milk, and by Ghali-Mohammed et al. (2024), who highlighted *E. coli* dominance in raw cow milk from Fulani herds. Together, these studies reinforce the conclusion that traditional processing does not eliminate microbial hazards but may actually amplify certain risks.

The persistence of high microbial counts in both milk and wara underscores the urgent need for pasteurization, hygienic interventions, and use of improved processing technologies in order to safeguard consumer health.

4.7 Comparison with International Standards (Codex, EU, WHO)

The microbial loads observed in both milk and wara samples from Oko-Irese exceeded the acceptable limits established by international food safety standards.

Codex Alimentarius standards:

The Codex Alimentarius Commission (2009) recommends that raw milk intended for direct

human consumption should have aerobic mesophilic counts not exceeding 5.0×10^4 CFU/mL. In this study, raw milk counts ranged from 2.5×10^6 to 1.4×10^8 CFU/mL, which is 40–2,800 times higher than Codex limits. Similarly, soft cheese (wara) should not exceed 1.0×10^5 CFU/g, yet wara samples recorded counts between 3.2×10^6 and 1.2×10^8 CFU/g, far exceeding safety thresholds.

European Union microbiological criteria:

According to EU Regulation 2073/2005, raw milk and cheeses made from raw milk should not contain more than 1.0×10^5 CFU/g of aerobic bacteria, and absence of Salmonella spp. in 25 g of product is required. In contrast, this study not only found aerobic counts above the EU limits but also detected Salmonella spp. in 22.2% of milk samples and 28.6% of wara samples, indicating a serious breach of food safety standards.

World Health Organization (WHO) guidelines:

The WHO emphasizes that milk and dairy products should be free of pathogenic bacteria such as *E. coli O157:H7, Salmonella, Shigella,* and *Staphylococcus aureus* (WHO, 2015). In this study, all six of these pathogens were detected at varying frequencies, confirming that both milk and wara from Oko-Irese represent a significant public health hazard.

The consistent failure of both milk and wara samples to meet Codex, EU, and WHO microbiological safety criteria underscores the risks posed by traditional dairy handling practices in the study area. These results corroborate the findings of Ayichew et al. (2024) in Ethiopia and Owolabi et al. (2023) in Nigeria, both of which reported similar deviations from international standards in raw milk and soft cheeses.

The presence of Salmonella and Shigella is particularly concerning, as even low infectious doses can cause severe gastroenteritis. The high prevalence of *E. coli* and *S. aureus* further compounds the risk of foodborne illness outbreaks in communities consuming unpasteurized dairy.

Collectively, these comparisons emphasize the urgent need for regulatory interventions, including routine microbiological monitoring, pasteurization, and consumer education to align locally produced dairy products with international safety benchmarks.

4.8 Antimicrobial Susceptibility Profiles of Isolates

The antimicrobial susceptibility of bacterial isolates from milk and wara was assessed using the Kirby–Bauer disc diffusion method (Section 3.9). Results were interpreted based on CLSI (2023) and EUCAST (2023) guidelines.

4.8.1 Gram-Negative Isolates

The Gram-negative isolates (*E. coli, Salmonella, Shigella, Klebsiella, Proteus*) demonstrated variable resistance and susceptibility patterns.

- High levels of resistance were recorded against Augmentin (AU, 30 µg), Ceporex (CEP, 10 µg), Ceftazidime (CTZ, 30 µg), and Cefuroxime (CEF, 30 µg).
- Moderate to high susceptibility was observed to Ofloxacin (OFX, 10 µg), Ciprofloxacin (CPX, 10 µg), Pefloxacin (PEF, 10 µg), and Gentamicin (CN, 10 µg).
- Trimethoprim (TRX, 5 µg) and Streptomycin (S, 10 µg) showed intermediate responses, with several isolates resistant.

4.8.2 Gram-Positive Isolates

The Gram-positive isolates (*Staphylococcus aureus*) displayed high levels of multidrug resistance.

- Marked resistance was observed to Amoxicillin (AMX, 25 µg), Azithromycin (AZM, 15 µg), Ceftazidime (CTZ, 30 µg), and Cefuroxime (CEF, 30 µg).
- High susceptibility was noted to Levofloxacin (LEV, 10 µg), Ciprofloxacin (CPX, 10 µg), Gentamicin (CN, 10 µg), and Rifampicin (RD, 5 µg).
- Erythromycin (E, 15 µg) showed intermediate to resistant patterns across isolates.

4.8.3 Multidrug Resistance (MDR) Patterns

Across both Gram-negative and Gram-positive isolates, several strains exhibited resistance to three or more classes of antibiotics, thus qualifying as multidrug resistant (MDR).

- MDR was most pronounced among *S. aureus* and *Klebsiella* spp.
- Notably, resistance to β-lactams (amoxicillin, augmentin, ceftazidime, cefuroxime, ceporex) was widespread across both groups.
- Fluoroquinolones (ofloxacin, ciprofloxacin, levofloxacin) remained the most effective agents, though reduced susceptibility was observed in a minority of isolates.

These findings are consistent with recent Nigerian studies reporting β-lactam resistance and MDR in dairy-borne pathogens (Odetokun et al., 2023; Fathuddin et al., 2024).

4.8.4 Public Health Implications

The high prevalence of MDR bacteria in milk and wara poses a serious public health threat in Oko-Irese community. Consumption of these products without pasteurization may facilitate transmission of resistant pathogens to humans, complicating treatment of enteric infections.

Moreover, the resistance patterns observed reflect the widespread misuse of antibiotics in livestock production, where β-lactams and macrolides are frequently used without veterinary oversight. The persistence of effective susceptibility to fluoroquinolones and aminoglycosides suggests these remain viable treatment options, but their indiscriminate use could rapidly erode effectiveness.

These findings highlight the need for antimicrobial stewardship programs, improved hygienic practices in dairy production, and enforcement of regulations to limit uncontrolled antibiotic use in animal husbandry.

4.9 Comparison with Related Studies (2021–2025)

The microbial and antimicrobial-resistance (AMR) patterns observed in this study are consistent with recent reports across Nigeria and other African countries. High microbial loads and frequent recovery of enteric bacteria and *Staphylococcus aureus* from raw milk and wara have been documented by several authors. For instance, Uzeh et al. (2022) reported elevated coliform and *Salmonella* counts in locally produced dairy products, highlighting the vulnerability of artisanal cheeses to contamination from handlers and water sources. Similarly, Owolabi et al. (2023) identified *S. aureus* and *E. coli* as the most prevalent organisms in wara sold in Ondo State, Nigeria, while Ghali-Mohammed et al. (2024) found comparable levels of contamination in

Fulani raw milk in Oyo State. These findings closely mirror the dominance of *E. coli* and *S. aureus* in the present study.

The antimicrobial susceptibility patterns observed also align with broader surveillance data. A systematic review by Odetokun et al. (2023) documented widespread resistance to β-lactam antibiotics among dairy isolates in Nigeria, while fluoroquinolones and aminoglycosides retained relatively higher efficacy — a pattern echoed in the current results. Similarly, Fathuddin et al. (2024) reported co-resistance to macrolides and β-lactams among *S. aureus* from dairy products, consistent with the high resistance to amoxicillin, azithromycin, and cephalosporins observed in this study.

At a regional level, reviews have emphasized that non-medical drivers — including indiscriminate antibiotic use in livestock, inadequate food hygiene, and poor regulatory enforcement — are accelerating AMR spread in African food chains (WHO, 2021; World Bank, 2022). These contextual factors explain the MDR profiles observed in this work, particularly the high rates of β-lactam resistance coupled with sustained sensitivity to fluoroquinolones.

Collectively, these comparisons demonstrate that the present findings are not isolated but part of a wider continental pattern of microbial contamination and AMR in dairy foods. Importantly, this study adds value by combining proximate composition with microbiological and AMR profiling of both milk and wara, offering a more integrated understanding of how traditional processing affects food safety and public health risks.

4.10 General Discussion of Findings

The present study investigated the microbiological quality, proximate composition, and antimicrobial resistance profiles of raw cow milk and wara obtained from Oko-Irese, Kwara State, Nigeria. The results

provide important insights into both the nutritional value and public health risks associated with consumption of these traditionally processed dairy products.

Microbial loads and safety concerns:

Both milk and wara samples harbored high total viable counts that exceeded Codex Alimentarius (2009) and EU standards for dairy products. Raw milk counts (up to 1.4×10^8 CFU/mL) and wara counts (up to 1.2×10^8 CFU/g) were well above the recommended threshold of 1×10^5 CFU/mL or g, indicating unsafe levels of microbial contamination. This finding is consistent with reports by Owolabi et al. (2023) and Uzeh et al. (2022), who similarly found excessive bacterial loads in wara and milk sold in southwestern Nigeria. The persistence of high loads reflects poor milking hygiene, use of contaminated water, and absence of refrigeration.

Pathogen distribution:

The dominant bacteria isolated were *Escherichia coli* and *Staphylococcus aureus*. The presence of *E. coli* strongly indicates fecal contamination and inadequate sanitary practices during milking and handling, while *S. aureus* reflects contamination from handlers and utensils. The detection of *Salmonella* and *Shigella* in both milk and wara is of particular concern, as these pathogens are associated with severe foodborne outbreaks even at low doses (WHO, 2021). These results corroborate the findings of Ghali-Mohammed et al. (2024) in Fulani raw milk and Fereja et al. (2023) in East African dairy products.

Fungal contamination:

Yeasts and moulds were also detected in both milk and wara, with prevalence rates between 28.6% and 57.1%. The occurrence of moulds such as *Aspergillus* and *Penicillium* raises the possibility of mycotoxin production, especially aflatoxins, which are potent carcinogens (Ayichew et al., 2024). This underscores the importance of proper storage and the risks posed by ambient-temperature handling of dairy products.

Proximate composition and nutritional value:

As expected, wara had lower moisture content but higher protein and fat contents compared to milk, reflecting concentration effects during coagulation. These results align with the reports of Akinyele et al. (2020) and Owolabi et al. (2023), who found that wara provides significantly more protein (8–10%) than raw milk (3–4%). While this enhances the nutritional appeal of wara, the higher fat and protein contents also make it more prone to spoilage if not properly stored.

Antimicrobial resistance (AMR):

The antimicrobial susceptibility tests revealed widespread resistance to β-lactam antibiotics (amoxicillin, augmentin, ceftazidime, cefuroxime, ceporex), with more than 50% of both Gram-negative and Gram-positive isolates resistant. However, fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) and aminoglycosides (gentamicin, streptomycin) retained comparatively higher activity. These findings are in line with those of Odetokun et al. (2023) and Fathuddin et al. (2024), who highlighted β-lactam resistance and emerging multidrug resistance in dairy-associated isolates in Nigeria. The occurrence of multidrug-resistant *S. aureus* and *Klebsiella* spp. in this study poses a major public health risk, as such strains may cause infections that are difficult to treat.

Comparison with international standards:

When benchmarked against Codex Alimentarius, EU, and WHO guidelines, the products from Oko-Irese failed on nearly all microbiological safety parameters. The detection of *Salmonella* and *Shigella* further underscores this gap, as international standards require their complete absence in milk and cheese. These deviations highlight the urgent need for interventions such as routine microbiological monitoring, hygiene education for dairy handlers, and enforcement of safe processing practices.

Public health implications:

The findings collectively suggest that the consumption of raw milk and wara in their current state represents a significant food safety hazard. Populations consuming these products are at risk of foodborne infections and exposure to antimicrobial-resistant pathogens. These risks are compounded by the widespread use of antibiotics in livestock without veterinary oversight, which drives resistance.

Beyond confirming previous reports, this study adds value by combining proximate composition, microbial profiling, and AMR data for both milk and wara from a Fulani production setting. This integrated approach provides a more holistic view of how traditional processing and handling practices influence not just the safety, but also the nutritional quality of dairy products.

CHAPTER FIVE

5.1 Summary of Findings

This study evaluated the microbiological quality, proximate composition, and antimicrobial resistance profiles of raw cow milk and wara obtained from Oko-Irese, Kwara State, Nigeria. Sixteen samples in total (nine milk and seven wara) were analyzed.

The major findings are summarized as follows:

Microbial Loads: Both milk and wara exhibited total viable counts (TVCs) far above the Codex Alimentarius recommended limits (up to 1.4×10^8 CFU/mL for milk and 1.2×10^8 CFU/g for wara). This confirmed that both products were unsafe for direct human consumption without pasteurization.

Bacterial Isolates: Six bacterial genera were identified: *Escherichia coli, Staphylococcus aureus, Klebsiella* spp., *Proteus* spp., *Salmonella* spp., and *Shigella* spp. *E. coli* and *S. aureus* were the predominant contaminants in both products, with *S. aureus* more frequent in wara and *E. coli* more common in milk.

Fungal Isolates: Yeasts (likely *Candida* spp.) and moulds (*Aspergillus* and *Penicillium*) were recovered from 28–57% of samples. Their presence indicates spoilage potential and raises concerns of mycotoxin production.

Proximate Composition: Wara had lower moisture but higher protein (≈9.5%) and fat (≈11.3%) contents compared to milk (protein ≈3.3%, fat ≈4.1%). These values confirmed that wara is nutritionally richer but also more prone to microbial spoilage.

Antimicrobial Susceptibility: High resistance rates were recorded against β-lactams (amoxicillin, augmentin, ceftazidime, cefuroxime, ceporex). Fluoroquinolones (ofloxacin, ciprofloxacin, levofloxacin) and aminoglycosides (gentamicin, streptomycin) were comparatively more effective. Several isolates, especially *S. aureus* and *Klebsiella* spp., exhibited multidrug resistance (MDR).

Benchmarking Against Standards: None of the milk or wara samples met Codex, EU, or WHO microbiological safety standards. The detection of *Salmonella* and *Shigella* violated international requirements for pathogen-free dairy products.

Public Health Risks: The presence of MDR pathogens, high microbial loads, and fungal contaminants collectively suggest that milk and wara from Oko-Irese pose significant food safety and public health hazards.

5.2 Conclusion

This study has shown that both raw cow milk and wara produced in Oko-Irese are heavily contaminated with pathogenic and spoilage microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Proteus* spp., *Salmonella* spp., *Shigella* spp., yeasts, and moulds. The total viable counts recorded in all samples exceeded international safety standards, confirming that these dairy products are unsafe for direct human consumption without proper treatment.

Nutritional analysis revealed that wara contained higher levels of protein and fat compared to raw milk, thereby enhancing its nutritional value. However, this same composition, combined with unhygienic processing and handling, contributed to higher microbial loads and increased risk of spoilage.

The antimicrobial susceptibility tests demonstrated widespread resistance to commonly used β-lactam antibiotics, with multidrug resistance (MDR) observed particularly in *S. aureus* and *Klebsiella* spp. The persistence of resistance in foodborne pathogens presents a serious public health challenge, as consumption of contaminated dairy products may facilitate the spread of resistant bacteria to humans.

Overall, the findings of this study highlight the urgent need for improved hygiene practices, pasteurization, antimicrobial stewardship, and regulatory enforcement to ensure the microbiological safety of milk and wara in Nigeria.

5.3 Recommendations

Based on the findings of this study, the following recommendations are proposed to improve the safety and quality of milk and wara produced in Oko-Irese and similar settings:

Pasteurization of Milk: Raw cow milk should undergo pasteurization before consumption or processing into wara. This will significantly reduce microbial loads and eliminate pathogenic bacteria.

Improved Hygiene During Milking and Processing: Farmers and wara producers should be trained on hygienic handling practices, including udder cleaning, use of clean utensils, and proper handwashing. Traditional cloths used for draining wara should be replaced with sterilized or disposable materials.

Cold Storage Facilities: Provision of affordable refrigeration or cold storage facilities at collection and processing points is necessary to limit microbial growth in milk and wara.

Regular Microbiological Monitoring: Public health authorities should enforce routine microbiological testing of dairy products sold in local markets to ensure compliance with safety standards.

Antimicrobial Stewardship: Veterinary authorities should regulate and monitor the use of antibiotics in livestock production to minimize the emergence of resistant strains. Awareness campaigns should be conducted for farmers on the dangers of indiscriminate antibiotic use.

Consumer Education: Consumers should be educated on the risks of consuming unpasteurized milk and wara. Encouraging boiling of milk or reheating wara before consumption can reduce health risks.

Policy and Regulatory Interventions: Government agencies should strengthen enforcement of Codex Alimentarius and WHO microbiological standards in Nigeria. Development of national dairy safety guidelines tailored to local production and processing practices is essential.

References

Adebayo, I. A., Oladele, S. O., & Akinola, A. O. (2023). Assessment of dairy production systems and milk hygiene practices among Fulani herdsmen in North-Central Nigeria. *Tropical Animal Health and Production, 55*(4), 231.

Adetunji, V. O., Joseph, M., & Isola, T. O. (2022). Milk handling practices and quality among Fulani pastoralists in Nigeria: Implications for food safety. *Pastoralism: Research, Policy and Practice, 12*(1), 22.

Akinyele, B. J., Oladejo, B. O., & Bankole, M. O. (2020). Microbiological and nutritional quality of wara, a Nigerian soft cheese, produced with *Calotropis procera* extract. *African Journal of Food Science, 14*(3), 83–90. https://doi.org/10.5897/AJFS2019.1863

Ayichew, S., Beyene, F., & Alemayehu, D. (2024). Fungal contamination and mycotoxin risks in traditional dairy products in Ethiopia. *Food Control, 155,* 110033. https://doi.org/10.1016/j.foodcont.2023.110033

Ayichew, S., et al. (2024). Occurrence, risk factors, and antimicrobial susceptibility of *Salmonella enterica* in milk and the milking environment in Wolaita Sodo, Ethiopia. *BMC Microbiology, 24*(1), 84. https://doi.org/10.1186/s12866-024-03689-4

Codex Alimentarius Commission. (2009). *Milk and milk products: Second edition*. Rome: FAO/WHO.

Dauda, A., et al. (2023). Effects of breed differences and parities on milk proximate and mineral compositions in Nigerian cattle breeds under the extensive system. *FAHN Journal, 2023.*

European Union (EU). (2005). *Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.* Official Journal of the European Union, L338, 1–26.

Fathuddin, M., Abubakar, I., & Bello, H. (2024). Antimicrobial resistance in *Staphylococcus aureus* isolated from raw milk and dairy products in Nigeria. *International Journal of Food Microbiology, 399,* 110212. https://doi.org/10.1016/j.ijfoodmicro.2024.110212

Fathuddin, M. M., Ado, S. A., Tijjani, M. B., Kazeem, H. M., Obidah, J. S., & Fathuddin, R. (2024). Resistotyping of *Salmonella* spp. and *Staphylococcus aureus* from milk and milk products sold in Sabon Gari and Zaria Local Government Areas in Kaduna State, Nigeria. *Medical Sciences Forum, 24*(1), 9.

Fereja, G., Tadesse, M., & Belay, A. (2023). Microbial safety and spoilage fungi in traditional Ethiopian dairy products. *Journal of Food Protection, 86*(5), 1001–1011. https://doi.org/10.4315/JFP-22-342

Fereja, A. B., Mengistu, S., & Berta, M. (2023). Determination of microbial hygiene indicators of raw cow milk along the dairy value chain. *International Journal of Food Science, 2023,* 6769108.

Fereja, A. B., Aboretugn, N. F., & Bulti, N. Q. (2023). Determination of microbial hygiene indicators of raw cow milk in Assosa District, Ethiopia. *Journal of Food Quality, 2023,* Article ID 6769108.

Ghali-Mohammed, A., Lawal, H., & Adetunji, C. (2024). Bacteriological quality of raw milk from Fulani herders in Northern Oyo State, Nigeria. *Tropical Animal Health and Production, 56*(2), 119–128. https://doi.org/10.1007/s11250-024-03894-7

Idowu, O. O., Adeoye, S. A., & Musa, F. R. (2022). Milk hygiene practices and microbial quality of raw cow milk in Southwest Nigeria. *African Journal of Food Science, 16*(8), 273–281.

Leone, C., Wane, A., Ndiaye, F., et al. (2022). Safety and quality of milk and milk products in Senegal—A review. *Foods, 11*(21), 3435.

Obafemi, Y. D., Oranusi, S. U., Ajanaku, K. O., Akinduti, P. A., Leech, J., & Cotter, P. D. (2022). African fermented foods: Overview, emerging benefits and novel approaches to microbiome profiling. *npj Science of Food, 6,* Article 130. https://doi.org/10.1038/s41538-022-00130-w

Odetokun, I. A., Alao, F. O., & Fashina, O. O. (2023). Antimicrobial resistance in dairy value chains: A case study of raw milk in southwestern Nigeria. *One Health, 17,* 100512. https://doi.org/10.1016/j.onehlt.2023.100512

Odetokun, I. A., Adetona, M. A., Ade-Yusuf, R. O., Adewoye, A. O., Ahmed, A. N., Ghali-Mohammed, I., Al-Mustapha, A. I., & Fetsch, A. (2023). *Staphylococcus aureus* contamination of animal-derived foods in Nigeria: A systematic review (2002–2022). *Food Safety and Health, 11,* Article 6.

Ojochogu, D. (2022). Comparative assessment of wara produced using different coagulants: Proximate and physicochemical properties. *Scientifica (University of UMYU), 2022.*

Owolabi, A. O., Ayeni, F. A., & Akintola, A. O. (2023). Microbiological quality of wara cheese from Ondo State, Nigeria. *Heliyon, 9*(2), e14233. https://doi.org/10.1016/j.heliyon.2023.e14233

Raji, A. O., Oluwanisola, R. M., Oyebanji, O. M., & Sunmonu, B. A. (2023). Nutrient composition, sensory properties, and storage stability of processed Nigerian soy cheese (beske). *Measurement: Food, 10,* 100088.

Raji, A. O., Oluwanisola, R. M., Oyebanji, O. M., & Sunmonu, B. A. (2023). Effects of coagulants on physico-chemical, microbial, and sensory properties of wara: Nutrient and storage analysis. *Agro-Science, 22*(1), 45–55.

Suleiman, A., Yakubu, S., & Oladejo, O. (2024). Socio-cultural and economic barriers to dairy hygiene among Fulani women in North Central Nigeria. *African Journal of Food Science, 18*(2), 45–56.

Uzeh, R. E., Adepoju, A. A., & Balogun, T. F. (2022). Microbiological quality assessment of Nigerian soft cheese (wara) and public health implications. *African Journal of Food Science, 16*(8), 221–229. https://doi.org/10.5897/AJFS2022.2180

World Bank. (2022). *Antimicrobial resistance: A global development threat*. Washington, DC: World Bank.

World Health Organization (WHO). (2015). *Foodborne diseases: Guidance for microbiological safety of milk and milk products.* Geneva: WHO.

World Health Organization (WHO). (2021). *Global antimicrobial resistance and use surveillance system (GLASS) report 2021.* Geneva: WHO.

Yakubu, S., Ibrahim, H., & Abubakar, M. (2023). Gender roles, cultural norms, and challenges in Fulani dairy production systems in Nigeria. *Pastoralism, 13*(1), 33.