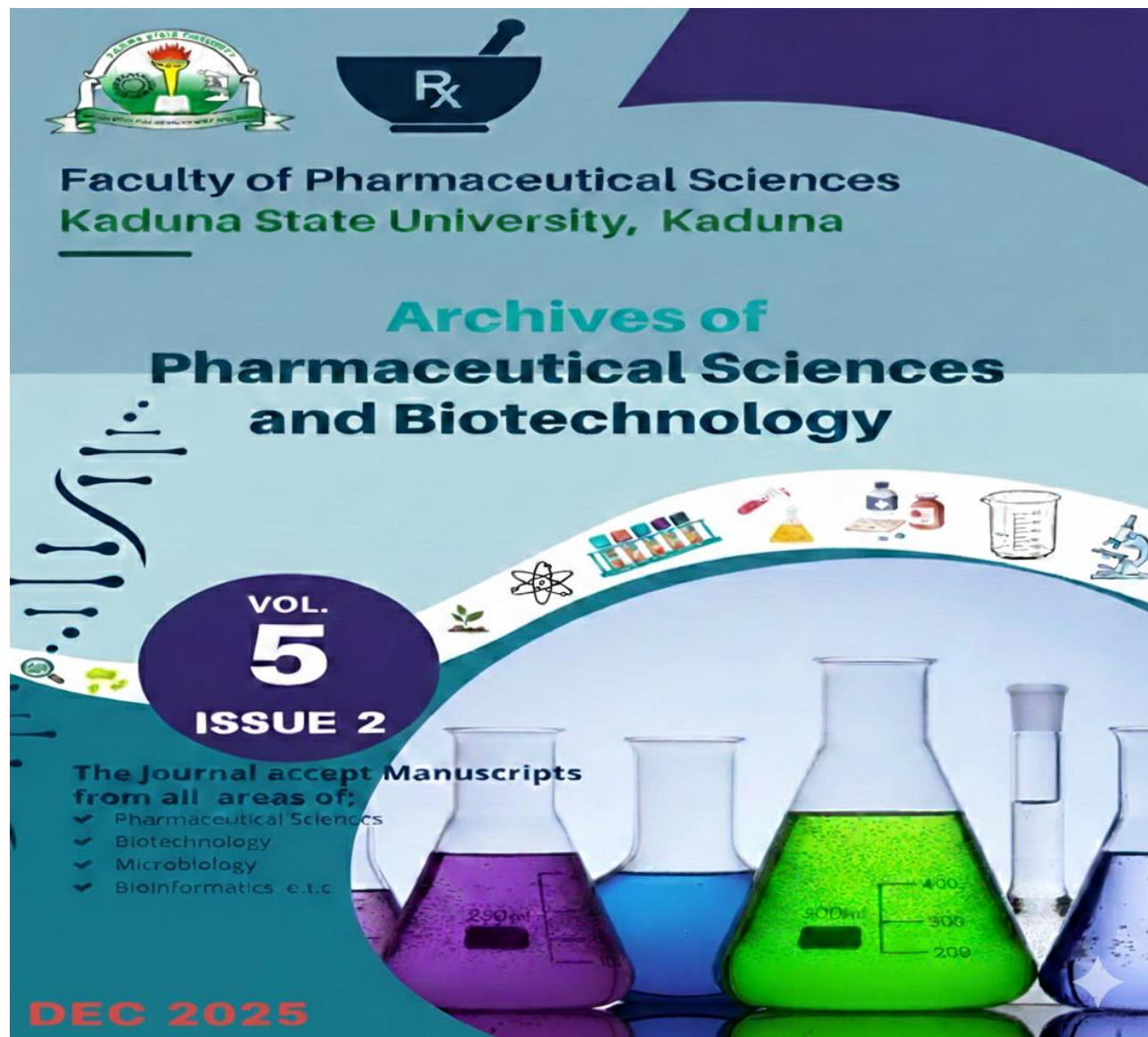




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BACTERIOLOGICAL QUALITY AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF RAW, FERMENTED, AND PASTEURIZED MILK IN KADUNA METROPOLIS, NIGERIA

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ABSTRACT

Background: Milk is a nutrient-rich, microbiologically permissive medium whose contamination across production, processing, and distribution poses significant public health risks, particularly in resource-limited settings.

Objective: To evaluate the microbiological quality of raw cow milk, traditionally fermented milk (nono), and laboratory-pasteurized milk from Kaduna Metropolis, Nigeria, and characterize the antimicrobial susceptibility profiles of isolated pathogens.

Methods: Twelve milk samples (n = 4 per group) were analysed for total plate count (TPC) and coliform count by serial dilution and spread-plate method. *Staphylococcus* spp., *Escherichia coli*, and *Pseudomonas aeruginosa* were selectively isolated and identified by Gram staining and biochemical assays. Antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion against ceftriaxone, cefoxitin, and amoxicillin–clavulanic acid, with categorical interpretation applied only to organisms with defined CLSI breakpoints.

Results: Mean TPC values were 1.47×10^7 , 6.15×10^6 , and 1.53×10^6 CFU/mL for raw, fermented, and pasteurized milk respectively — all exceeding the 1×10^5 CFU/mL regulatory threshold. All three pathogens were recovered from 100% of raw milk samples. Fermented milk yielded *Staphylococcus* spp. and *P. aeruginosa* in 100% and *E. coli* in 75% of samples. Pasteurized milk showed markedly reduced rates (*Staphylococcus* spp. and *E. coli*, 25% each; *P. aeruginosa*, 0%). *Staphylococcus* spp. were susceptible to amoxicillin–clavulanic acid (77.8%) but largely resistant to ceftriaxone (66.7%). Among *E. coli* isolates, susceptibility was highest for ceftriaxone (75.0%) and cefoxitin (62.5%); two isolates showed complete absence of inhibition against all three agents, suggesting broad β -lactam non-susceptibility.

Conclusion: Microbial loads exceeded regulatory limits across all milk types. Pasteurization reduced but did not totally eliminate pathogen burden, consistent with thermotolerant organism survival. These preliminary findings highlight the need for improved milk hygiene practices and antimicrobial stewardship, though larger studies are required to substantiate these observations.

Keywords: milk microbiological quality; Kirby–Bauer disc diffusion; antimicrobial resistance profiling; food-borne pathogen isolation; Kaduna dairy safety

1. INTRODUCTION

Milk is a highly nutritious biological fluid secreted by the mammary glands of mammals, constituting an important dietary source of protein, calcium, vitamins, and essential fatty acids across all age groups (Singhal *et al.*, 2017). As a consequence of its rich biochemical composition (approximately 88% water, 3.5% protein, 4% fat, 4% lactose, and 0.5% minerals) milk is equally a highly permissive growth medium for a broad spectrum of microorganisms (Quigley *et al.*, 2013; Martin *et al.*, 2018). In Nigeria and across sub-Saharan Africa, three principal milk categories are widely consumed: raw milk, traditionally fermented milk (locally known as *nono*), and commercially pasteurized milk. Raw milk that has not undergone any heat treatment and may harbour important food-borne pathogens (Chiarlone *et al.*, 2025). Fermented milk is produced through spontaneous lactic acid fermentation of raw cow milk and, while fermentation lowers pH and can inhibit spoilage organisms, it does not reliably eliminate all pathogenic microorganisms (Marco *et al.*, 2021; Oyedokun *et al.*, 2023). Pasteurization is an established process for inactivating pathogenic bacteria while

preserving nutritional quality of milk (Schafers *et al.*, 2024).

Milk-borne pathogens are responsible for a range of human diseases, from gastrointestinal disturbances (diarrhea, vomiting) to life-threatening conditions such as tuberculosis, brucellosis, and listeriosis (Almashhadany *et al.*, 2022). In developing countries, inadequate refrigeration infrastructure, informal vending practices, and limited regulatory oversight exacerbate the risk of contaminated milk reaching consumers (WHO, 2015).

A growing concern in milk microbiology is the emergence of antimicrobial resistance (AMR). Food animals are significant reservoirs of resistant bacteria, and antibiotic-resistant strains may be transmitted to humans through consumption of contaminated dairy products (Xu *et al.*, 2022). Understanding the resistance profiles of milk-borne pathogens is therefore essential for guiding empiric therapy and public health policy.

This study was therefore designed to: (i) determine the total bacterial and coliform counts in raw, fermented, and pasteurized milk from Kaduna Metropolis; (ii) isolate and characterize key pathogenic bacteria; and (iii)

determine the antimicrobial susceptibility profiles of the isolates against a panel of clinically relevant antibiotics.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The study was conducted in Kaduna Metropolis, which encompasses four Local Government Areas (Kaduna North, Kaduna South, Igabi, and Chikun) in north-central Nigeria, with a combined population exceeding 1.7 million (National Population Commission of Nigeria). A total of 12 milk samples were collected for analysis: four raw cow milk samples and four traditionally fermented milk (nono) samples were obtained from vendors within Kaduna Metropolis, while four pasteurized milk samples were prepared in the laboratory from the raw milks obtained above using the low-temperature long-time (LTLT) pasteurization method (63 °C for 30 min), followed by rapid cooling to 4 °C, as described in standard dairy processing protocols (Walstra *et al.*, 2006). All samples were transported in sterile, pre-labelled screw-cap bottles to the Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Kaduna

State University, and processed within two hours of collection.

2.2 Sample Preparation and Serial Dilution

All media were prepared according to manufacturers' instructions and sterilised at 121°C for 15 min prior to use. Each milk sample was subjected to five-fold serial dilution (10^{-1} to 10^{-5}) in sterile peptone water (0.1%). For TPC and coliform count determinations, 0.1 mL of the 10^{-5} dilution was plated by the spread-plate method onto nutrient agar and MacConkey agar, respectively. For selective isolation of target pathogens, the 10^{-3} dilution was used for EMB agar (*E. coli*) and the 10^{-5} dilution for MSA (*Staphylococcus* spp.) and cetrimide agar (*P. aeruginosa*). All plates were incubated at 37°C for 24 h in an inverted position.

2.3 Enumeration of Total Bacterial and Coliform Counts

Total plate count (TPC) was determined by enumerating visible colonies on nutrient agar after 24 h of incubation at 37°C. Coliform count (CC) was determined by enumerating lactose-fermenting colonies on MacConkey agar. Colony counts were converted to

CFU/mL using the formula: $\text{CFU/mL} = \text{colony count} \times \text{dilution factor} \times 10$ (to account for the 0.1 mL inoculum volume). Samples yielding confluent growth were recorded as "too numerous to count" (TNTC). The regulatory acceptable limit applied was 1×10^5 CFU/mL (National Advisory Committee on Microbiological Criteria for Foods, 2006).

2.4 Selective Isolation and Identification of Pathogens

Target pathogens were isolated on three differential/selective media: mannitol salt agar (MSA, 7.5% NaCl; yellow colonies indicating *Staphylococcus* spp), eosin methylene blue (EMB) agar (blue-green metallic sheen for *E. coli*), and cetrimide agar (blue-green pigmented colonies for *P. aeruginosa*). All plates were incubated at 37°C for 24 h. Presumptive positive colonies were sub-cultured on nutrient agar to obtain pure isolates.

Pure isolates were identified by Gram staining and a standardised panel of biochemical tests: catalase test (3% H_2O_2), coagulase test (citrated human plasma, slide method), oxidase test (1% tetramethyl-p-phenylenediamine dihydrochloride), indole

production (Kovac's reagent after 72 h in tryptophan broth), and citrate utilisation (Simmons' citrate agar). All procedures followed the methods described by Cheesbrough (2005).

2.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023). Bacterial suspensions were standardized to 0.5 McFarland turbidity and uniformly inoculated onto Mueller–Hinton agar plates. Antibiotic discs used were ceftriaxone (30 µg), cefoxitin (30 µg), and amoxicillin–clavulanic acid (30 µg). Plates were incubated aerobically at 35°C for 18–24 h.

Zone diameters were interpreted using organism-specific CLSI breakpoints. Cefoxitin was used as a marker for methicillin resistance in *Staphylococcus* spp. Ceftriaxone and amoxicillin–clavulanic acid breakpoints were applied to *Escherichia coli* isolates only, as CLSI defines interpretive criteria for these agents against *Enterobacteriaceae* but not against *Staphylococcus* spp. For *Pseudomonas*

aeruginosa, no CLSI-defined disc diffusion breakpoints exist for any of the three

antibiotics tested and this is noted as a limitation of this study.

Table 1: Antibiotic Disc Diffusion Breakpoints (CLSI, 2023)

Antibiotic	Abbrev.	Disc Target	Organism	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Ceftriaxone	CRO	30 µg	<i>E. coli</i>	≥ 23	20–22	≤ 19
Cefoxitin	CFX	30 µg	<i>Staphylococcus</i> spp.	≥ 22	—	≤ 21
Amoxicillin–Clavulanic Acid	AMC	30 µg	<i>E. coli</i>	≥ 18	14–17	≤ 13

Abbrev. = Abbreviation; mm = millimetres; µg = micrograms; — = no intermediate category defined.

2.6. Ethical Considerations

Ethical approval was not required for this study, as it involved the analysis of commercially available food samples and did not include human participants, human biological materials, or animal experimentation. Verbal consent was obtained from vendors prior to sample collection.

3. RESULTS

3.1 Total Plate Count and Coliform Count

All milk samples exceeded the permissible microbial threshold of 1×10^5 CFU/mL (Table 2). Mean TPC values were highest in raw milk (1.47×10^7 CFU/mL), followed by fermented milk (6.15×10^6 CFU/mL) and pasteurized milk (1.53×10^6 CFU/mL). Two raw milk samples (A and D) yielded counts too numerous to count (TNTC) on nutrient agar. Mean coliform counts followed the same trend: raw milk (7.5×10^6 CFU/mL), fermented milk (2.375×10^6 CFU/mL), and pasteurized milk (1.5×10^5 CFU/mL). One pasteurized milk sample (PMS D) yielded no detectable coliforms.

Table 2. Total plate count (TPC) and coliform count (CC) of raw, fermented, and pasteurized milk samples

Sample	TPC (CFU/mL)	CC (CFU/mL)
Raw Milk A	TNTC	7.2×10^6
Raw Milk B	1.28×10^7	6.4×10^6
Raw Milk C	1.66×10^7	5.8×10^6
Raw Milk D	TNTC	1.06×10^7
Fermented Milk A	8.7×10^6	1.4×10^6
Fermented Milk B	3.8×10^6	3.2×10^6
Fermented Milk C	5.7×10^6	2.7×10^6
Fermented Milk D	6.4×10^6	2.2×10^6
Pasteurized Milk A	1.9×10^6	4×10^5
Pasteurized Milk B	5.0×10^5	1×10^5
Pasteurized Milk C	1.3×10^6	1×10^5
Pasteurized Milk D	2.4×10^6	ND

TNTC = Too Numerous to Count; ND = Not Detected. CFU/mL = colony forming units per millilitre.

3.2 Isolation of Pathogens on Selective/Differential Media

Table 3 presents the growth profiles of the three target pathogens on their respective selective media. All four raw milk samples (100%) were positive for *Staphylococcus* spp. on MSA, *E. coli* on EMB, and *P. aeruginosa* on cetrimide agar. In the fermented milk group, all four samples were

positive for *Staphylococcus* spp. and *P. aeruginosa*, while *E. coli* was detected in three of four (75%) samples. The pasteurized milk group showed substantially reduced isolation rates: *Staphylococcus* spp. was recovered from one sample (25%), *E. coli* from one sample (25%), and *P. aeruginosa* was not detected in any pasteurized sample (0%).

Table 3. Occurrence and percentage isolation of target pathogens in milk samples by milk type

Milk Type	n	<i>Staphylococcus</i> spp.	<i>E. coli</i>	<i>P. aeruginosa</i>
Raw Milk	4	4 (100%)	4 (100%)	4 (100%)
Fermented Milk	4	4 (100%)	3 (75%)	4 (100%)
Pasteurized Milk	4	1 (25%)	1 (25%)	0 (0%)

3.3 Gram Staining and Biochemical Identification

A total of 25 bacterial isolates were characterised: 9 *Staphylococcus* isolates (36% of total), 8 *E. coli* isolates (32%), and 8 *P. aeruginosa* isolates (32%). Gram staining classified 36% as Gram-positive cocci and 64% as Gram-negative rods. Biochemical test results resolved the 9 *Staphylococcus* isolates into 5 *S. aureus* (coagulase-positive,

catalase-positive, oxidase-negative, citrate-positive, indole-negative) and 4 *S. epidermidis* (coagulase-negative, catalase-positive, oxidase-positive). All *E. coli* isolates were catalase-positive, coagulase-negative, oxidase-negative, indole-positive, and citrate-negative. All *P. aeruginosa* isolates were catalase-positive, coagulase-negative, oxidase-positive, and citrate-positive (Table 4).

Table 4. Summary of Biochemical Characterisation of Bacterial Isolates

Organism	n	Gram Stain	Catalase	Coagulase	Oxidase	Indole	Citrate
<i>S. aureus</i>	5	+ve cocci	+	+	—	—	+
<i>S. epidermidis</i>	4	+ve cocci	+	—	+	—	+
<i>E. coli</i>	8	—ve rods	+	—	—	+	—
<i>P. aeruginosa</i>	8	—ve rods	+	—	+	—	+

3.4 Antimicrobial Susceptibility Profiles

AST results for *Staphylococcus* spp. and *E. coli* isolates are summarised in Tables 5 and 6. Among the nine *Staphylococcus* spp.

isolates, the highest proportion of resistance was observed against ceftriaxone (66.7%), while ceftiofur demonstrated moderate activity (55.6% susceptible). Susceptibility to

amoxicillin–clavulanic acid was comparatively higher (77.8%). Among the eight *E. coli* isolates, susceptibility was highest for ceftriaxone (75.0%) and cefoxitin (62.5%), with lower susceptibility observed for amoxicillin–clavulanic acid (37.5%). Two *E. coli* isolates demonstrated complete absence of inhibition zones (0 mm) against all three β -lactam agents tested.

Although *P. aeruginosa* was isolated from raw and fermented milk samples (Section 3.2), categorical AST interpretation was not performed for this organism. The antibiotics employed in this study (ceftriaxone, cefoxitin, and amoxicillin–clavulanic acid) lacks CLSI-defined disc diffusion breakpoints for *P. aeruginosa* and this limitation is duly mentioned.

Table 5. Antimicrobial susceptibility pattern of *Staphylococcus* spp. isolates (n = 9)

Antibiotic	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
AMC	7 (77.8%)	1 (11.1%)	1 (11.1%)
CRO	3 (33.3%)	—	6 (66.7%)
CFX	5 (55.6%)	—	4 (44.4%)

Table 6. Antimicrobial susceptibility pattern of *E. coli* isolates (n = 8)

Antibiotic	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
AMC	3 (37.5%)	3 (37.5%)	2 (25.0%)
CRO	6 (75.0%)	—	2 (25.0%)
CFX	5 (62.5%)	—	3 (37.5%)

4. DISCUSSION

The TPC values obtained across all three milk categories in this study substantially exceeded the internationally accepted limit of 1×10^5 CFU/mL. Raw milk had the highest mean TPC (1.47×10^7 CFU/mL), consistent with the absence of any antimicrobial

treatment and reflecting contamination from udder surfaces, milking equipment, and the handling environment (Quigley *et al.*, 2013; Martin *et al.*, 2018). These values align with findings from Nigerian raw milk studies reporting high microbial loads in unprocessed milk under informal handling

conditions (Odetokun *et al.*, 2023; Omoshaba *et al.*, 2020; Ghali-Mohammed *et al.*, 2025).

Fermented milk (nono) returned a mean TPC of 6.15×10^6 CFU/mL, reflecting the microbiological complexity introduced by spontaneous fermentation. While the acidic pH generated by lactic acid bacteria may suppress some spoilage organisms, environmental microorganisms from utensils, the milking area, and handlers can enter the product during the fermentation process (Omola *et al.*, 2019). Pasteurized milk, despite having the lowest mean TPC (1.53×10^6 CFU/mL), still exceeded acceptable limits, most plausibly due to the survival of thermotolerant organisms (Sarkar, 2015).

High coliform counts, particularly in raw and fermented milk, indicate fecal contamination and/or poor general hygiene, both of which are widely documented in informal dairy vending contexts across Nigeria (Quigley *et al.*, 2013; WHO, 2015). The near-absence of coliforms in one pasteurized sample demonstrates that appropriate heat treatment can effectively eliminate coliforms; however, the residual contamination in three of four pasteurized samples highlights the

vulnerability of the post-pasteurisation handling chain.

The isolation of *Staphylococcus aureus* from raw and fermented milk at 100% occurrence is consistent with the established association between *S. aureus* and unhygienic milk handling (Martin *et al.*, 2018). *S. aureus* is a leading cause of staphylococcal food poisoning due to heat-stable enterotoxins produced under favorable temperature conditions, and its presence in milk is strongly linked to environmental contamination by handlers (Roy *et al.*, 2024). The detection of *Escherichia coli* in raw milk (100%) and fermented milk (75%) could be associated with indirect faecal contamination during milking or processing (Sarba *et al.*, 2023). The detection of *Pseudomonas aeruginosa* in all raw milk samples and in 100% of fermented milk is a great concern, given that *P. aeruginosa* is an opportunistic pathogen, raising concerns about the safety of the consumption of these milk samples. Its complete absence in pasteurized milk confirms susceptibility to the applied heat treatment.

The AST findings revealed clinically significant resistance patterns. The high resistance of *Staphylococcus* spp. to

ceftriaxone (66.7%) is consistent with established antimicrobial susceptibility frameworks and may reflect β -lactamase production or altered target site affinity (CLSI, 2023). Its high susceptibility to amoxicillin-clavulanic acid (77.8%) aligns with the synergistic role of clavulanic acid in overcoming β -lactamase-mediated resistance in *Staphylococcus* spp (Saini *et al.*, 2025). In contrast, *E. coli* showed varying susceptibility to ceftriaxone and cefoxitin, consistent with the generally higher activity of cephalosporins against certain Gram-negative organisms depending on resistance determinants (Foo *et al.*, 2025). Resistance to amoxicillin-clavulanic acid in these Gram-negative isolates is likely attributable to restricted outer membrane permeability, efflux systems, or production of antibiotic-inactivating enzymes (Uruén *et al.*, 2022; Marathe *et al.*, 2026). The identification of two *E. coli* isolates with zero-zone inhibition against all three antibiotics tested warrants further characterisation for multidrug resistance mechanisms.

These findings demonstrate a pattern of milk contamination in Kaduna Metropolis that poses significant public health risks. The results confirm that pasteurization

substantially reduces, but does not eliminate, microbial burden, and that inadequate post-pasteurisation handling can reintroduce contamination. The detection of antibiotic-resistant strains in food-associated environments reflects a broader One Health concern: the intersection of food safety, human health, and antimicrobial stewardship (Brower *et al.*, 2015). A principal limitation of this study is the small sample size ($n = 4$ per group), which limits the statistical generalizability of the findings; future work should incorporate larger sample sizes, molecular confirmation of species identities, and minimum inhibitory concentration (MIC) determination.

5. CONCLUSION

This study demonstrates that raw milk, fermented milk (nono), and pasteurized milk distributed in Kaduna Metropolis, Nigeria, carry microbiological burdens substantially exceeding permissible regulatory thresholds. Pathogenic bacteria (*S. aureus*, *E. coli*, and *P. aeruginosa*) were prevalent in raw and fermented milk, with reduced but non-zero occurrence in pasteurized milk, consistent with the survival of thermotolerant organisms following LTLT heat treatment.

Antimicrobial susceptibility testing identified resistance to at least one first-line antibiotic in isolates from all three milk types, including multidrug-resistant phenotypes. These findings underscore the urgent need for: (i) enforcement of good hygiene practices throughout the milk supply chain; (ii) consumer education on the importance of pasteurisation and safe milk storage; and (iii) routine microbiological surveillance of milk products in peri-urban Nigerian markets as part of an integrated food safety and AMR monitoring framework.

Study Limitations

This study provides useful baseline information on the microbiological quality of milk in Kaduna Metropolis; however, several limitations should be acknowledged. First, the relatively small sample size ($n = 4$ per milk category) limits the statistical power of the study and restricts the generalisability of the findings to the broader population. Second, the study relied on culture-based and biochemical methods for bacterial identification, which, while standard and widely used, may not detect viable but non-culturable organisms or provide strain-level resolution that molecular techniques could

offer. Third, antimicrobial susceptibility testing was limited to three antibiotics, which may not fully capture the resistance profiles of the isolates; furthermore, the small number of selected discs as well as the fact that the antibiotics employed lack CLSI-defined disc diffusion breakpoints for *P. aeruginosa*, are presented as limitations of this study warranting cautious interpretation of results. Despite these limitations, the study provides meaningful preliminary data and highlights important food safety and public health concerns in the study area.

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Conflicts of Interest

The authors declare no conflicts of interest.

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