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Hygienic Status and Microbial Profile of Locally Produced Fermented Milk in Wukari North-East, Nigeria

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Abstract

Background: Milk and its derivatives are widely consumed for their nutritional benefits; however, their improper handling and processing can lead to contamination with pathogenic microorganisms, posing significant public health risks.

Aim: This study aimed to investigate the microbial quality of locally fermented milk products, sold in Wukari, North-East, Nigeria.

Methods: A total of fifteen (15) fermented milk samples were collected from five different retail locations and analyzed for microbial contamination using standard microbiological techniques.

Results: The results revealed high levels of bacterial contamination across all sampling sites, with total viable counts ranging from 0.3×10^6 to 2.4×10^6 CFU/mL. Coliform bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, were detected, indicating fecal contamination and poor sanitary conditions during processing and distribution. *Staphylococcus aureus*, a major foodborne pathogen, was identified in 27.1% of the total bacterial isolates, highlighting a

potential risk of food poisoning. Other bacterial isolates included *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter spp.*, and *Staphylococcus epidermidis*.

Conclusion: The findings suggest that the unhygienic handling of fermented milk contributes to its microbial contamination, making it unfit for human consumption. This study emphasizes the need for improved hygiene practices, proper pasteurization, and strict regulatory measures to ensure the safety of locally produced dairy products. Public awareness campaigns should be conducted to educate dairy vendors and consumers about the health risks associated with contaminated milk.

Keywords: Milk, Nono, Microorganisms, *Staphylococcus*, Microbial contamination, Wukari

INTRODUCTION

"Nono" or "nunu" is a traditional Hausa dairy drink commonly sold by cattle maidens in Northern Nigeria and much of West Africa [1-3]. It is referred to as "dahi" or "lassi" in the Middle East [3,4]. This dairy product is an excellent source of amino acids, calcium, phosphorus, and vitamins A, C, E, and the B complex [5]. Milk and its derivatives, known for their high biological value and enriched nutritional content, are widely consumed for their health benefits and lack of significant risks when properly processed. Naturally evolved as a complete food for young animals, cow's milk provides essential nutrients, making it not only an ideal food for calves but also highly beneficial for young children and valuable for adults [6]. Additionally, cow's milk has been reported to reduce allergic reactions due to its natural proteins and antibodies [7, 8]. Its consumption spans the Sahara and the West African sub-region.

In Nigeria, the traditional methods used to process and sell cow milk and its products expose them to the risk of microbial contamination by both spoilage and pathogenic microorganisms. While all milk products, except for raw milk, are typically boiled by local cattle handlers before being sold, this boiling is considered a form of pasteurization. However, despite this precaution, the milk can become re-contaminated through contact with the handler, utensils, and other external sources [9]. Both raw and processed milk are well-known to support the growth of various microorganisms, which can lead to spoilage, as well as cause infections and intoxications in consumers [3, 10].

Microbes originate in Nono, Kindrimo, Manshanu, and raw milk are mainly introduced through the water used in making the milk, as well as during handling, storage, processing and packaging [3]. Hayes et al. [11] reported that bacteria in milk can be introduced through colonization of the teat canal or an infected udder (whether from clinical or subclinical mastitis) or may be contaminated at various stages, including from the animal, milker (whether manual or automated), dirt, or unclean water. Mohammed and Biyabra [3] further noted that to increase the volume and improve the color of Nono, Kindrimo, Manshanu, and raw milk, female Fulani hawkers often engage in fraudulent practices, such as adding stream water and the milky white supernatant from soaked baobab tree seeds before selling the milk. This practice can contribute to contamination and spoilage of the milk products.

The presence of contaminant microorganisms, particularly pathogenic bacteria, in milk and milk products is a significant public health concern. Poor hygiene practices by those handling these products often lead to the introduction of harmful microorganisms. Since these products typically do not undergo further processing before consumption, they can pose serious health risks to consumers [3].

Milk and its by-products create favorable conditions for the growth of microorganisms such as fungi, bacteria, rickettsia, and viruses. The extent of microbial contamination is influenced by factors such as the health and hygiene of the cow, the cleanliness of the milking environment, the sanitation of production facilities, and the condition of storage equipment. Insufficient pasteurization or re-contamination of the product have been linked to milk-borne diseases [12].

Foodborne diseases are a prevalent and widespread issue worldwide. Numerous outbreaks have been linked to the consumption of milk that, despite appearing normal, is contaminated with high levels of harmful bacteria [13, 3].

Despite advancements in medical technology, foodborne diseases continue to pose a significant threat. Milk serves as an ideal medium for the growth of *Staphylococcus aureus*, and among foods associated with staphylococcal food poisoning (SFP), milk and dairy products are of particular concern, as enterotoxigenic strains of *S. aureus* have frequently been isolated from them [3]. In Northern Nigeria, raw milk and traditional dairy products such as Nono (fermented milk), Kindrimo, and Manshanu (milk fat) are commonly sold and consumed on the streets. Due to their processing and distribution methods, these products

are often exposed to conditions that facilitate the growth of contaminating organisms, including potential toxin producers [14]. Although milk is recognized as a nutritious food, containing essential nutrients like proteins and vitamins, its unique composition makes it an excellent growth medium for both spoilage and pathogenic microorganisms [15, 3]. Therefore, this work is basically needed and aimed toward examining the advance effect of local fermented milk ("nunu" and "kindrimo") sold in Wukari town.

MATERIALS AND METHODS

Study Area

Wukari Metropolis, located in Taraba State, serves as the headquarters of Wukari Local Government Area. The area is traversed by the River Donga and River Benue [16, 17]. Geographically, Wukari is one of the 16 local government areas in Taraba State, positioned at latitude 7.53'43'N and longitude 9.47'59'E, with a population estimated between 5,000 and 10,000. The location is documented in the Times Comprehensive Atlas of the World on plate 86F [18, 19] and covers an area of 4,308 km² [20]. The Jukun people are the dominant ethnic group in Wukari, which is renowned as the administrative center of the historic Kwararafa Kingdom. The region's vegetation mirrors that of the Savannah zone, characterized by grasslands interspersed with scattered tree species [16]. The main economic activities include farming, fishing, and livestock rearing [19, 16]. The most commonly spoken languages are Jukun, Hausa, Fulani, and Tiv. In addition to agriculture, commerce, and civil service are significant occupations in the area [16].

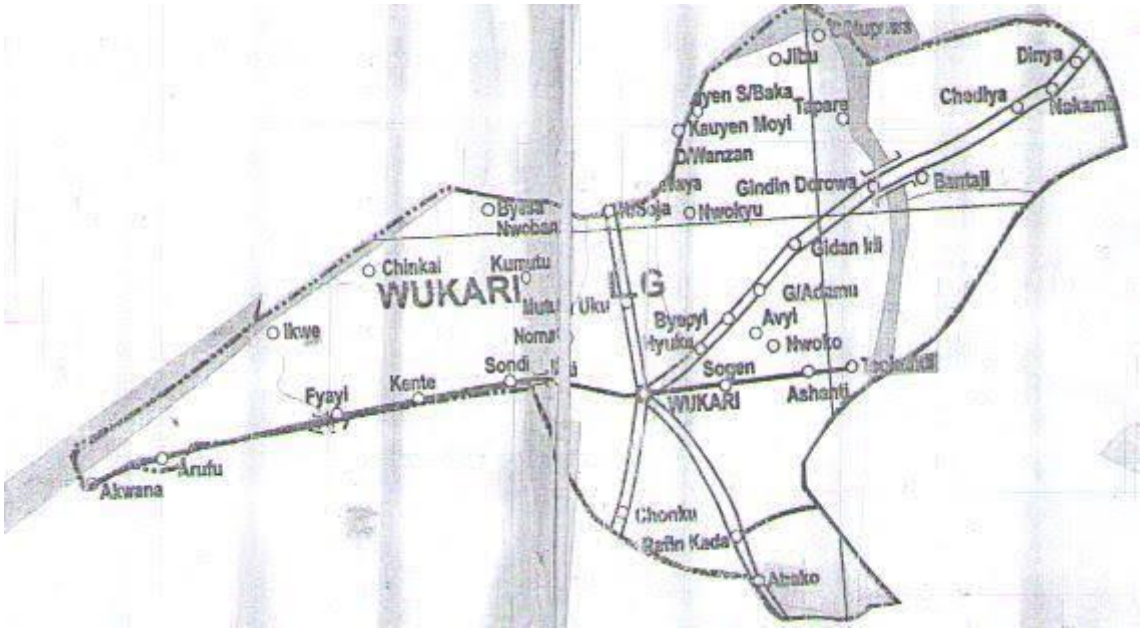

Figure 1: Map showing the study area (Wukari)

Media and Sterilization

Nutrient agar, Mannitol salt agar, and Mueller-Hinton agar were utilized in this study. The media were accurately weighed and prepared following the manufacturers' guidelines, then sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the media were aseptically dispensed into Petri dishes and left to solidify for sample inoculation [3].

Sample Collection

Fifteen (15) samples of locally made milk (nono) were collected from five (5) distinct retail locations in the city. The samples were taken to the Federal University Wukari Department of Microbiology for microbial analysis after being kept in sterile containers inside ice-packed coolers.

Total Aerobic Bacterial Count

Standard plate count method was used to determine the Total Aerobic Colony Counts of bacteria in the samples of the nono. Tenfold serial dilution was prepared by introducing 1ml of nono with a syringe into a test tube. A ten-fold serial dilution was carried out by homogenizing 1ml of the sample into the first test tube that contain 9ml and was labeled as 10^{-1}. A 1 ml aliquot was taken from the first test tube and transferred into 9 ml of diluent in a second test tube, resulting in a dilution factor of 10^{-2}. This serial dilution process was

repeated across subsequent test tubes until the seventh tube, which achieved a dilution factor of 10^{-7}. From this final test tube, 1 ml was withdrawn and discarded. For culturing, 0.1 ml of the 10^{-7} dilution was added to an empty Petri dish, followed by the addition of molten nutrient agar cooled to approximately 45°C. The plates were allowed to solidify and incubated at 37°C for 24 hours [21]. After incubation, the plates were examined for distinct colonies, which were counted to determine the microbial load [18]. The results were expressed as colony-forming units per gram (CFU/g) of the sample [22, 18]. The average bacterial loads of cow milk products from the five locations were determined and reported as Colony Forming Units per milliliter (CFU/ml) [21].

Isolation of the microorganism

Using the spread plate approach, a drop of each sample was inoculated onto Mannitol Salt Agar plates using the 10^{-7} dilution. The bacterial growth was surrounded by yellow zones after the plates were incubated for 24 hours at 37°C. In accordance with Cheesbrough's [23] methodology, the organism was further identified by biochemical confirmation utilizing coagulase and catalase tests.

Identifications of Microorganisms

Following the instructions in Bergey's Manual of Systematic Bacteriology, the isolate was identified using traditional techniques based on its morphological, physiological, and biochemical characteristics, such as Catalase, Coagulase, and Citrate Utilization tests [23].

Standardization of Inoculum

Using a sterile wire loop, a 24-hour colony of the isolates was prepared from each test isolate suspension and placed into a sterile test tube filled with sterile normal saline to create a turbidity that matched the 0.5 scale of McFarland's standard (1.5×10^8 cells/ml), as explained by Coyle [24].

Antibiotic Susceptibility Test

Antibiotic susceptibility test for isolates of *Staphylococcus* species were performed according to the Kirby-Bauer method as cited in the evaluation methods of the clinical and laboratory standards institutes [25]. Diluents of the test organism's equivalent to 0.5 McFarland standards were spread on plates of sterile Mueller-Hinton agar [25] using sterile swabs and isolated plates were left for 15min. The antibiotic sensitivity disc were aseptically positioned (20 mm apart from one another) using the inoculated Mueller Hinton agar plates [21]. The

antibiotic discs were impregnated containing the Gram positive (+ve) multiple susceptibility antibiotic discs, CPX-Ciprofloxacin (10 µg), PEF=Pefloxacin (20 µg), CN-Gentamycin (10 µg), AMX-Amoxicillin (20 µg), AU=Augmentin, (30 µg), SXT=Septrin (20 µg), S=Streptomycin (30 µg), CH-Chloramphenicol (30 µg), APX-Ampiclox (20 µg), and SP=Sparfloxacin (20 µg) were then placed on the inoculated Mueller-Hinton agar plates carefully with the aid of a forceps and incubated at 37°C for 24 hours [25, 21]. Zones of inhibition cleared by each of the antibiotics against the test organisms were measured and the results were interpreted using the guideline from CSLI (Clinical and Laboratory Standard Institute) [25], and all the results were recorded appropriately.

RESULTS AND DISCUSSION

Milk and other food illnesses have been linked to microorganisms. It multiplies in foods like milk and dairy products that are stored at room temperature. Foods that may not exhibit any signs of spoiling, such as a poor odor, may contain hazardous levels of the poisons. Nausea, vomiting, retching, cramping in the stomach, and diarrhea are the symptoms. In more severe cases, there may be changes in blood pressure and pulse rate, headaches, cramping in the muscles, and dehydration [26]. Fifteen samples of fermented milk, 3 samples from different 5 point, and the samples were analyzed microbiologically for incidence of pathogenic microorganisms.

Table 1: Total Microbial Viable Count

Sample area	Samples	Dilution factor	Microbial count	cfu/ml
AMP	Sample A	10^{-5}	09	0.9×10^{6}
	Sample B	10^{-5}	08	0.8×10^{6}
	Sample C	10^{-5}	11	1.1×10^{6}
TJA	Sample A	10^{-5}	18	1.8×10^{6}
	Sample B	10^{-5}	14	1.4×10^{6}
	Sample C	10^{-5}	08	0.8×10^{6}
FUTH	Sample A	10^{-5}	03	0.3×10^{6}
	Sample B	10^{-5}	18	1.8×10^{6}
	Sample C	10^{-5}	06	0.6×10^{6}

FUWG	Sample A	10^{-5}	18	1.8×10^{6}
	Sample B	10^{-5}	09	0.9×10^{6}
	Sample C	10^{-5}	20	2.0×10^{6}
WOM	Sample A	10^{-5}	06	0.6×10^{6}
	Sample B	10^{-5}	24	2.4×10^{6}
	Sample C	10^{-5}	21	2.1×10^{6}

Key: AMP = Atoshi Motor Park, TJA = Takum Junction Area, FUWG = Federal University Wukari Gate, FUTH = Federal University Teaching Hospital, WOM = Wukari Old Market.

Coliform bacteria were detected in all sample areas, with the highest coliform count in WOM Sample B (3.4×10^{6} cfu/ml). The presence of coliforms, particularly in dairy products, suggests fecal contamination and poor sanitary conditions. The Federal University Wukari Gate (FUWG) recorded the lowest coliform count (0.3×10^{6} cfu/ml), indicating relatively better hygiene compared to other locations [27].

The total *S. aureus* count (10^{4}–10^{5} cfu/mL) was characterized as an inadequate level of bacterial quality in the meals in a London microbiological investigation [28]. These results were similar to the count reported by Mohammed and Biyabra [3]. The range of counts they recorded was 0.3 $x10^{6}$ to 3.3 x 10^{6} cfu/ml. The level of bacterial contamination observed in the current study makes the nono samples studied makes the non-unfit for consumption.

Table 2: Total Microbial Coliform Count

Sample area	Samples	Dilution factor	Microbial count	cfu/ml
AMP	Sample A	10^{-5}	18	1.8×10^{6}
	Sample B	10^{-5}	09	0.9×10^{6}
	Sample C	10^{-5}	22	2.2×10^{6}
TJA	Sample A	10^{-5}	12	1.2×10^{6}
	Sample B	10^{-5}	21	2.1×10^{6}
	Sample C	10^{-5}	10	1.0×10^{6}

FUTH	Sample A	10^{-5}	21	2.1×10^{6}
	Sample B	10^{-5}	18	1.8×10^{6}
	Sample C	10^{-5}	13	1.3×10^{6}
FUWG	Sample A	10^{-5}	03	0.3×10^{6}
	Sample B	10^{-5}	03	0.3×10^{6}
	Sample C	10^{-5}	06	0.6×10^{6}
WOM	Sample A	10^{-5}	07	0.7×10^{6}
	Sample B	10^{-5}	34	3.4×10^{6}
	Sample C	10^{-5}	04	0.4×10^{6}

Key: AMP = Atoshi Motor Park, TJA = Takum Junction Area, FUWG = Federal University Wukari Gate, FUTH = Federal University Teaching Hospital, WOM = Wukari Old Market.

The total microbial count across all sample areas shows significant variation. The highest microbial load was recorded in Wukari Old Market (WOM) Sample B (2.4×10^6 cfu/mL), while the lowest was in Federal University Teaching Hospital (FUTH) Sample A (0.3×10^6 cfu/mL). High microbial counts indicate contamination, possibly due to inadequate hygiene, environmental exposure, or poor handling of milk samples. Previous studies have shown that microbial contamination in milk can be influenced by storage conditions, temperature, and handling practices [29].

These numbers were comparable to those that Uzoaga *et al.* [30] reported. The range of counts they recorded was 5.6 ± 1.7 to 7.0 ± 0.4 log10 cfu/ml. Compared to Mohammad and Abdullahi [31], who reported that counts ranged from 2.7 x 107 cfu/ml to 4.l x 107 cfu/ml, the current study's bacterial count was greater. The nono samples examined in this study are unsafe for human consumption due to the degree of bacterial contamination found in it.

Poor handling and hygiene throughout the milking process may be the cause of the high levels of bacterial contamination in nono found in this study. The failure of most of these handlers to pasteurize their milk also results in high total aerobic count because pasteurization lowers the microbial load of food to safer levels. This was also the

conclusion of Uzoaga *et al.* [30], who reported that the vendors who produce and market this locally fermented milk (nono) do not follow any standard hygienic procedures in the preparation of this product [3].

Table 3: Biochemical Tests of Bacteria Isolates

S/N	Morphology	Gram stain	Catalase Test	Oxidase Test	Indole Test	Citrate Test	Glucose	Lactose	Sucrose	Coagulase Test	Isolate
1.	Large, spherical, moist and raised on nutrient agar	-ve Rod	+	-	-	-	+	-	-	-	*Pseudomonas aeruginosa*
2.	Medium, spherical, golden yellow, dried and flat on nutrient agar.	+ve Cocci	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
3.	Milky, medium, Spherical, raised surface on mannitol salt agar.	+ve Rod	+	-	-	+	+	-	+	-	*Bacillus* spp.
4.	Milky, round, large, entire and flat surface on nutrient agar.	+ve Cocco Bacilli	-	-	-	-	+	+	+	-	*Lactobacillus* spp.
5.	Milky, medium, Spherical, raised surface on mannitol salt agar.	+ve Rod	+	-	-	+	+	-	+	-	*Bacillus* spp.
6.	Circular, small, entire and fla colonies on mannitol salt aga	-ve Rod	+	-	-	+	+	+	+	-	*Klebsiella* spp.
7.	Circular, entire, medium, and flat colonies	-ve Rod	+	-	-	+	-	+	-	-	*Klebsiella* spp.
8.	Spherical and raised in colony	+ve Rod	+	-	-	+	+	-	+	-	*Enterobacter* spp
9.	Large, spherical, moist and raised	-ve Rod	+	-	-	-	+	-	-	-	*Pseudomonas aeruginosa*
10.	Medium, spherical, golden yellow, dried and flat.	+ve Cocci	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
11.	Medium, spherical, golden yellow, dried and flat	+ve Cocci	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
12.	Large, whitish, spherical, dried and raised colonies	+ve Cocci	+	-	-	+	+	+	+	-	*Staphylococcus epidermidis*
13.	White, round, large, entire and flat surface on nutrient agar.	-ve Rod	+	-	-	-	+	+	+	+	*E. coli* spp
14.	Milky, medium, Spherical, raised surface on mannitol salt agar.	+ve Rod	+	-	-	+	+	-	+	-	*Bacillus* spp.

The bacterial isolates include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Lactobacillus bulgaricus*, and *Staphylococcus epidermidis*. *Staphylococcus aureus* is a major foodborne pathogen associated with milk contamination and can cause food poisoning [32]. *Escherichia coli* and *Klebsiella pneumoniae* are indicators of fecal contamination and can cause severe gastrointestinal diseases [33]. *Pseudomonas aeruginosa* is known for its ability to spoil dairy products and resist antimicrobial agents [34]. The presence of *Escherichia coli* in some samples further confirms potential fecal contamination, which poses health risks [27].

Table 4: Bacterial isolates with frequency and percentage frequency of occurrence

Isolated Bacteria	Frequency	Percentage Frequency (%)
Enterobacter spp.	3	5.7
Pseudomonas aeruginosa	3	5.7
Klebsiella pneumoniae	3	5.7
Lactobacillus bulgaricus	8	15.4
Staphylococcus aureus	14	27.1
Escherichia coli	3	5.7
Bacillus subtilis	7	13.5
Staphylococcus epidermis	11	21.2
Total	**52**	**100**

Table 5: Bacterial Isolates from the Milk Samples

Organism	Isolates
Bacteria	*Enterobacter* spp. *Pseudomonas aeruginosa*, *Klebsiella* spp., *Lactobacillus* spp., *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Staphylococcus epidermis*

Among the identified bacteria, *Staphylococcus aureus* (27.1%) had the highest frequency, followed by *Staphylococcus epidermidis* (21.2%) and *Lactobacillus bulgaricus* (15.4%). The dominance of *S. aureus* suggests significant contamination from handlers, as this bacterium is commonly found on human skin and can be transmitted through direct contact [35].

The incidence of *Staphylococcus* spp. in locally pasteurized milk in the current study was 30%. The reported incidence of *Staphylococcus aureus* in the current study was higher than the 21.2% reported by Mohammed and Biyabra [3] and Alage Veterinary College Dairy Farm 21.2% [36]. It was also higher than prevalence reported from Uganda 20.3% [37] and China 22.3% [38]. While also higher than prevalence of 25.53% reported by Tibebu *et al.* [28]. But was lower than prevalence reported in Addis Ababa and Hawassa 50% and 51.2% respectively [39]. These discrepancies could be due to the location's distinct management strategy or sample types.

Table 6: Antibiotics susceptibility test of *Staphylococcus* species

Samples	AMX	AU	CN	PEF	SXT	S	SXT	CH	SP	CPX
AMP 3	R	R	R	R	R	R	R	R	R	R
TJA 2	R	S	S	S	S	R	S	S	S	S
FUTH 3	R	S	S	S	S	S	S	S	S	S
FUGW 3	S	S	S	S	S	S	S	S	S	S
WOM 1	I	S	S	S	S	S	S	I	S	S

Key: AMP = Atoshi Motor Park, TJA = Takum Junction Area, FUWG = Federal University Wukari Gate, FUTH = Federal University Teaching Hospital, WOM = Wukari Old Market. S=Sensitive, R=Resistant, I=Intermediate, AMX=Amoxacillin, AU=Augmentin, CN=Gentamycin, PEF=Pefloxacin, SXT=Septrin, S=Streptomycin, SXT=Septrin, CH=Chloramphenicol, SP=Sparfloxacin, CPX=Ciprofloxacin.

The results indicate varying degrees of antibiotic resistance among *Staphylococcus* species: Complete resistance to Amoxicillin (AMX) across all samples suggests widespread resistance to β-lactam antibiotics, which aligns with global trends of antimicrobial resistance in *S. aureus* [40]. High sensitivity to Ciprofloxacin (CPX) and Sparfloxacin (SP) suggests these antibiotics remain effective against *Staphylococcus* spp. Intermediate resistance was observed in WOM samples, indicating a possible gradual adaptation of bacterial strains to certain antibiotics. These findings highlight the growing concern of antimicrobial resistance, emphasizing the need for proper antibiotic stewardship and hygiene practices to reduce the spread of resistant bacteria [41].

The most active antibiotics against *Staphylococcus* spp. in the current study, were Augmentin, Gentamycin, Septrin, Sparfloxacin and Ciprofloxacin followed were Chloramphenicol, Streptomycin and the last is Amoxicillin. This result agrees with the study of Tibebu *et al.* [28] who reported a high level of sensitivity was observed for Gentamicin 100%, Ceftriaxone 100%, Chloramphenicol 96%, Cefoxitin 96% and Ciprofloxacin 96%. The same study corroborates the result of the current study that indicated that *Staphylococcus aureus* isolated in the current were most resistant to Ampiclox (50%) and Rifampicin (83.3%).This high resistance is due to production of β-lactamase by *S. aureus* that inactivates ampicillins and related antibiotics. The beta-lactams are the drugs of choice for

inframammary infections. However, the inappropriate and regular use of these medications has contributed to the emergence of resistant bacteria.

CONCLUSION

This study assessed the microbial profile and hygienic status of locally fermented milk sold in Wukari, North-East, Nigeria. The study reveals significant microbial contamination in milk samples, with *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* spp. posing the greatest health risks. The findings revealed the presence of both beneficial and potentially harmful microorganisms, highlighting the role of fermentation in promoting the growth of probiotic bacteria while also raising concerns about contamination due to poor hygiene practices during production and handling. The presence of coliforms and other pathogenic bacteria suggests the need for improved sanitary measures in the processing, storage, and distribution of these products. Proper hygiene, quality control, and public awareness are crucial to ensuring the safety of fermented milk and minimizing health risks to consumers. The presence of coliforms confirms poor sanitary conditions, and the antibiotic susceptibility profile suggests rising antimicrobial resistance, particularly to β-lactam antibiotics. Further research should focus on identifying critical control points in the production chain, evaluating the antibiotic resistance patterns of the isolates, and exploring improved fermentation techniques to enhance product safety while maintaining its nutritional and probiotic benefits.

Recommendations

1. Strict hygiene measures should be implemented at milk handling points to reduce microbial contamination.
2. Regular microbial screening should be conducted to monitor contamination levels.
3. Antibiotic stewardship programs should be encouraged to prevent the spread of resistant bacterial strains.
4. Public health awareness campaigns should educate milk vendors and consumers on proper milk storage and hygiene practices.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IUM participated in study conception, design and prepared the manuscript. IUM and STCB participated in sample collection and performed the experiments. STCB, and EEO participated in data curation. IUM and STCB performed most of the experiments, and reviewed the manuscript. IUM, STCB, and EEO analyzed results and reviewed the manuscript. IUM and STCB revised the manuscript and coordinated the whole project. All authors read and approved the final manuscript.

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