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Bacteriological Quality of Raw Cow Milk Obtained from Fulanis Within University of Abuja Environment

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Abstract

Milk is a highly nutritious food that can serve as a suitable medium for microbial growth if not properly handled. This study assessed the bacteriological quality of raw cow milk obtained from Fulani herders within the University of Abuja environment with emphasis on the prevalence of bacterial species and their potential public health implications. Twenty raw milk samples were collected and analyzed using standard microbiological techniques, including serial dilution and spread plate methods for enumeration, determination of total bacterial load and total coliform count, Gram staining and biochemical identification. Five bacterial species were isolated; *Bacillus* spp. (34.29%), *Lactobacillus* spp. (28.57%), *Staphylococcus aureus* (17.14%), *Pseudomonas aeruginosa* (11.43%) and *Escherichia coli* (8.57%). The total bacterial load ranged from 5.5×10^8 to 1.15×10^9 CFU/mL, while total coliform counts ranged from 3.35×10^5 to 8.40×10^5 CFU/mL. The predominance of Gram-positive bacteria such as *Bacillus* and *Lactobacillus* reflects the natural microbial flora of raw milk, whereas the presence of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* indicates contamination from handlers, equipment or the environment. These findings reveal significant microbial contamination and associated public health risks linked to raw milk consumption. The study underscores the need for improved hygienic practices during milking, handling, and storage to reduce contamination and ensure consumer safety.

Keywords: Raw milk; Total bacterial load; coliform counts; contamination; safety.

INTRODUCTION

Milk is a nutritionally complete food rich in essential macronutrients and micronutrients required for human growth and development (Chopra and Pathak, 2025). Its high nutrient and water content also make it an ideal medium for the growth of microorganisms (Dubey *et al*., 2022).

Raw milk, defined as milk obtained directly from animals without heat treatment or pasteurization is particularly susceptible to bacterial contamination due to poor hygienic practices during milking, storage and transportation (Ntuli *et al.,* 2023; Ghali-Mohammed *et al*., 2022). Consequently, milk supports the growth of a wide range of spoilage and pathogenic microorganisms (Dash *et al*., 2022; Dubey *et al.,* 2022).

The presence of bacterial contaminants in milk poses significant public health risks, including foodborne illnesses caused by pathogens such as *Escherichia coli*, *Staphylococcus aureus, Listeria monocytogenes* and *Salmonella* spp. (Ntuli *et al*., 2023; Dubey *et al.,* 2022). In developing countries, raw milk consumption remains common, and studies have consistently reported higher microbial loads in raw milk compared to pasteurized milk (Martin *et al*., 2023).

In Nigeria and other developing countries, the continued consumption of raw milk is influenced by cultural practices, weak enforcement of food safety regulations and consumer preference for unprocessed foods perceived as fresher or more nutritious (Ghali-Mohammed *et al.,* 2022). This practice raises serious public health concerns as consumers may be unknowingly exposed to pathogenic microorganisms capable of causing gastrointestinal and systemic infections (Martin *et al*., 2023; Deneke *et al*., 2022).

STUDY AREA

The study was conducted at the Microbiology Laboratory, University of Abuja, located in Gwagwalada, Federal Capital Territory (FCT), Nigeria. Gwagwalada, approximately 35 km west of Abuja city, is a major administrative and residential area in the FCT.

Sample Collection
A total of 20 raw cow milk samples were collected aseptically from local dairy vendors and Fulani settlements within the University of Abuja permanent site. Samples were collected in sterile 50 mL bottles, immediately placed in an ice box, and transported to the laboratory for analysis within 4 hours to maintain sample integrity.

Glassware Washing and Sterilization
All glassware and equipment were thoroughly cleaned with liquid soap, rinsed with clean water and dried. Test tubes were autoclaved at 121°C for 15 minutes and cooled for 20 minutes. Disposable Petri dishes were used once and discarded eliminating the need for sterilization.

Media Preparation and Sterilization
Media were prepared following manufacturer instructions and sterilized by autoclaving at 121°C for 15 minutes. Sampling bottles were also autoclaved to maintain aseptic conditions.

Serial Dilution of Raw Cow Milk Samples
Ten-fold serial dilutions were performed for each milk sample. One milliliter of milk was transferred aseptically to 9 mL sterile water (10^{-1} dilution) and homogenized. Subsequent dilutions were prepared similarly until 10^{-2} dilution was achieved (Terrones-Fernandez *et al* ., 2023)

Determination of Total Bacterial Load
For total viable bacterial count, 0.1 mL from dilutions 10^{-1} to 10^{-8} was aseptically spread on sterile Nutrient Agar plates and incubated at 37°C for 24 hours. Plates with 30–300 colonies were selected for counting. Colony-forming units per mL (CFU/mL) were calculated based on colony count, dilution factor and inoculum volume. Representative colonies were subcultured to obtain pure isolates (Armstrong *et al*., 2023)

Determination of Total Coliform Count
MacConkey Agar, a selective and differential medium, was used to isolate coliform bacteria. After aseptic transfer of 0.1 mL from stock dilutions onto plates, the spread plate method was applied. Plates were incubated at 37°C for 24 hours and pink to red colonies, indicating lactose fermentation were counted on plates with 30–300 colonies. Representative colonies were subcultured for pure isolates (Shah *et al*., 2024).

Identification of Bacterial Isolates
Bacterial colonies were initially characterized based on cultural features such as color, shape, elevation, margin and surface appearance on Nutrient Agar, MacConkey Agar and Mannitol Salt Agar. Pure cultures were further identified using Gram staining and a series of biochemical tests (Patra *et al*., 2020).

Gram staining
Gram staining differentiated bacteria into Gram-positive and Gram-negative groups. Smears were heat-fixed, stained with crystal violet, mordanted with iodine, decolorized with ethanol, and counterstained with safranin. Gram-positive bacteria appeared purple, while Gram-negative appeared pink/red (Rohde, 2019).

Biochemical tests
Biochemical tests were conducted to further identify isolates:

- Indole Test: Detects indole production from tryptophan, red ring indicates positive (Fardami *et al.,* 2022).
- Methyl Red Test: Indicates stable acid production, red = positive, yellow = negative (Patra *et al*., 2020).
- Citrate Utilization Test: Determines ability to use citrate as sole carbon source, blue = positive, green = negative (Patra *et al.,* 2020).
- Catalase Test: Detects catalase enzyme, bubble formation = positive, no bubbles = negative (Patra *et al*., 2020).
- Coagulase Test: Identifies Staphylococcus aureus, clotting within 10 seconds = positive (Patra *et al*., 2020).
- Oxidase Test: Detects cytochrome oxidase, purple-blue color = positive, no color change = negative (Patra *et al*., 2020).
- Voges-Proskauer (VP) Test: Detects acetoin production, pink-red = positive, no color change = negative (Fardami *et al*., 2022).

RESULTS AND DISCUSSIONS

The total bacterial count (TBC) of the raw milk samples ranged from 5.5×10^8 CFU/mL (Sample 1) to 1.15×10^9 CFU/mL (Samples 3 and 13), indicating variability in microbial load among the samples. Some samples exhibited relatively low counts, while others exceeded 10^9 CFU/mL, reflecting differences in contamination levels and handling practices during collection.

Table 1. Total bacterial load of raw cow milk samples.

Samples	Total bacteria load (Cfu/mL)
1	5.5×10^8
2	8.9×10^8
3	1.15×10^9
4	8.0×10^8
5	8.1×10^8
6	1.03×10^9
7	9.0×10^8
8	7.8×10^8
9	1.12×10^9
10	8.6×10^8
11	9.3×10^8
12	1.09×10^9
13	1.15×10^9
14	7.9×10^8

Samples	Total bacteria load (Cfu/mL)
15	1.02×10^9
16	8.8×10^8
17	9.7×10^8
18	1.11×10^9
19	8.4×10^8
20	1.13×10^9

Table 2. Total coliform count of raw cow milk samples.

Samples	Total coliform count (Cfu/mL)
1	7.95×10^5
2	3.80×10^5
3	5.00×10^5
4	3.50×10^5
5	4.30×10^5
6	6.30×10^5
7	4.45×10^5
8	3.35×10^5
9	4.05×10^5
10	4.30×10^5
11	4.80×10^5
12	3.90×10^5
13	4.85×10^5
14	7.05×10^5
15	7.25×10^5
16	8.40×10^5
17	3.95×10^5
18	5.30×10^5
19	7.05×10^5
20	7.90×10^5

Total Coliform Count (TCC) of Raw Cow Milk Samples

Total coliform counts ranged from 3.35×10^5 CFU/mL (Sample 8) to 8.40×10^5 CFU/mL (Sample 16). High coliform loads were observed across most samples, suggesting contamination likely due to inadequate hygienic practices during milking, handling, and storage. Coliform presence indicates potential fecal contamination and public health risk.

Cultural Characteristics of Isolated Bacteria

The bacterial isolates displayed distinct colony characteristics, including color, shape, margin and elevation aiding preliminary identification. Five bacterial species were identified: *Bacillus* spp, *Lactobacillus* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.*

Table 3. Cultural characteristics of bacteria isolated from raw cow milk samples.

Isolate	Colour	Shape	elevation	Margin
1	Creamy/whitish	Irregular	flat	Undulate
2	White/creamy	Circular	flat	Entire (smooth)
3	Golden yellow/creamy	Circular	convex	Entire (smooth)
4	Bluish-green	Irregular	Flat	Undulate
5	Grayish-white/ pink (on maconky)	Circular	convex	Entire (smooth)

Microscopic Morphology, Gram Reaction and Biochemical Profiles

Gram staining and biochemical tests confirmed the presence of both Gram-positive and Gram-negative bacteria. The isolates were identified as *Bacillus* spp, *Lactobacillus* spp, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* based on cellular morphology, Gram reaction and biochemical characteristics.

Table 4. Microscopic morphology, Gram reaction and biochemical test results.

Isolate	Cellular Morphology	Gram Reaction	Cat	Cit	Coa	Met	Oxi	Ind	VP	Inference
1	Rod in chain	+	+	–	–	+	–	–	–	*Bacillus* spp.
2	Rod in chain	+	–	–	–	+	–	–	+	*Lactobacillus* spp.
3	Cocci in clusters	+	+	–	+	–	–	–	+	*Staphylococcus aureus*
4	Rod	–	+	+	–	–	+	–	–	*Pseudomonas aeruginosa*
5	Rod	–	–	–	–	+	–	+	–	*Escherichia coli*

Prevalence of Bacterial Isolates

A total of 35 bacterial isolates were obtained. *Bacillus* spp. was the most prevalent (34.29%), followed by *Lactobacillus* spp. (28.57%). *Staphylococcus aureus* accounted for 17.14%, while *Pseudomonas* spp. and *Escherichia coli* were less common, with prevalence

rates of 11.43% and 8.57%, respectively. The predominance of Gram-positive bacteria (*Bacillus* and *Lactobacillus*) reflects the natural microbial flora of raw milk whereas the detection of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* suggests contamination from handlers, equipment or the environment.

Table 5. Prevalence of bacterial isolates from raw cow milk samples.

Isolate	Number of occurrence	Percentage (%)
Bacillus spp	12	34.29
Lactobacillus spp	10	28.57
Staphylococcus spp	6	17.14
Pseudomonas spp	4	11.43
Escherichia coli	3	8.57
Total	**35**	**100**

Discussion

This study revealed diverse bacterial species in raw cow milk samples obtained from the University of Abuja environment. Five genera were isolated: *Bacillus* spp, *Lactobacillus* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. *Bacillus* spp. was the most prevalent (34.29%), followed by *Lactobacillus* spp. (28.57%), *Staphylococcus aureus* (17.14%), *Pseudomonas aeruginosa* (11.43%), and *Escherichia coli* (8.57%). The predominance of Gram-positive bacteria aligns with previous reports that spore-forming and lactic acid bacteria are commonly associated with raw milk.

The high prevalence of *Bacillus* spp. is likely due to their ability to form resistant spores that survive harsh environmental conditions and milk handling processes. *Lactobacillus* spp are natural inhabitants of the mammary gland and contribute to protection against spoilage and pathogenic microorganisms.

Staphylococcus aureus was also isolated, albeit at lower prevalence likely originating from handlers, mastitis-infected udders or contaminated milking equipment. Its detection is of public health concern due to the potential production of enterotoxins causing food poisoning.

The presence of Pseudomonas aeruginosa, a psychrotrophic bacterium capable of surviving refrigeration and contributing to milk spoilage through lipolytic and proteolytic activity, suggests post-harvest contamination. *Escherichia coli*, detected at 8.57%, indicates possible fecal contamination and inadequate hygiene during milking and handling, serving as an important indicator for enteric pathogens.

Overall, the bacterial profile observed reflects patterns reported in developing countries, where traditional milking practices, poor storage and inadequate hygiene contribute to raw milk contamination. The predominance of spore-forming and lactic acid bacteria highlights the natural milk microbiota, while the detection of *Staphylococcus aureus* and *Escherichia coli* underscores risks from external contamination.

CONCLUSION

The bacteriological analysis of raw cow milk samples revealed total bacterial loads ranging from 5.5×10^8 to 1.15×10^9 CFU/mL, far exceeding the FDA recommended limit of 1×10^5 CFU/mL, indicating that all samples were heavily contaminated. Total coliform counts ranged from 3.35×10^5 to 8.40×10^5 CFU/mL, well above the recommended <100 CFU/mL, reflecting poor hygiene and potential fecal contamination.

Among 35 isolates, *Bacillus* spp. (34.29%) and *Lactobacillus* spp. (28.57%) were predominant, followed by Staphylococcus aureus (17.14%), *Pseudomonas* spp. (11.43%), and *Escherichia coli* (8.57%). The prevalence of *Bacillus* and *Lactobacillus* indicates environmental contamination and possible natural fermentation activity, while *Staphylococcus aureus* suggests contamination from handlers or udder skin flora. The presence of coliforms such as *Escherichia coli* highlights fecal contamination risks and Pseudomonas spp. indicate environmental contamination and potential spoilage.

These findings demonstrate that the raw cow milk studied is microbiologically unsafe for human consumption unless subjected to strict hygienic handling and processing including pasteurization, to protect public health.

RECOMMENDATIONS

Hygienic Milking Practices: Milkers should maintain proper hygiene, including washing hands and using clean gloves. Utensils, containers and the udder should be properly cleaned before and after milking to minimize bacterial transfer, particularly *Staphylococcus aureus.*

Safe Consumption: Consumers should always pasteurize or boil raw milk before consumption to eliminate high bacterial and coliform loads, including *Escherichia coli* and *Pseudomonas aeruginosa*, which pose foodborne illness risks.

Public Health Awareness: Awareness campaigns should sensitize milk producers, vendors and consumers

about the dangers of consuming unpasteurized milk. Training programs should emphasize hygienic milking, safe storage and handling practices to reduce contamination from environmental and skin-associated bacteria such as *Bacillus* spp. and *Lactobacillus* spp.

Preservation: A study on the development of phenolic capped Nano Composite of a plant as source of natural preservative should be conducted against the bacterial isolates from this research to see if we can move from using synthetic preservatives in raw milk to using natural blend using nano particles or composite as it's carrier.

Competing Interests: The authors declare that there are no competing interests.

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