



## **Bacteriological Quality and Antibiotic Residues in Raw Cow Milk at Producer Level and Milk Products at Sale Points in the Northern Region of Ghana**

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### **Authors' contributions**

*This work was carried out in collaboration with all authors. Authors GIM and KKA designed the study. Authors GIM and EKV wrote the protocol and the first draft. Author EKV did the sample collection. Authors EKV and SEKA performed the bacteria culture and isolation. Authors EKV and SOA performed the mycobacteria culture and confirmation. Authors EKV, GIM, PKF, JBZ and CSKS performed the data analysis. Authors PKF and KKA revised the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Objective:** To evaluate the bacteriological quality of raw cow milk and milk products produced and retailed in the Northern Region of Ghana based on total bacteria and coliform count, prevalence of bacteria pathogens and antibiotic residues.

**Methodology:** A cross sectional study was designed where raw milk (n=210) and milk products

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(n=60) comprising (local milk and millet beverage) (burkina), cottage cheese (wagashi) and yoghurt were sampled from cattle kraals and retailers respectively.

Total viable bacterial counts (TVBC) and total coliform counts (TCC) were determined for all samples followed by isolation and identification of common milk-borne bacteria pathogens using normal laboratory identification systems. Antimicrobial residue in raw milk was detected using the Charm ® Blue-Yellow II Test for Beta-lactams and Other Antimicrobial Drugs in Milk.

**Results:** Mean total viable bacteria and coliform counts were  $(2.40 \pm 7.44) \times 10^7$  and  $(1.10 \pm 1.53) \times 10^4$  cfu/ml for raw milk and  $(8.99 \pm 2.24) \times 10^6$  and  $(6.62 \pm 9.54) \times 10^3$  cfu/ml for milk products respectively. *Escherichia coli* (15.6%), *Klebsiella pneumoniae* (14.1%), *Staphylococcus aureus* (10.7%), *Pseudomonas aeruginosa* (4.8%), *Mycobacterium* species, (4.8%), *Salmonella* spp. (4.4%), *Shigella* spp. (2.6%), *Escherichia coli* O157:H7 (1.9%) and *Proteus* spp. (1.5%) were isolated. Antibiotic residues above the EU maximum residue limit (MRL) were detected in 18.1% of raw milk samples.

**Conclusion:** The quality of raw milk sold in the northern region of Ghana is compromised by several bacteria pathogens and antibiotic residues at the farm level. This calls for continuous education on milk pasteurization, hygienic practices and proper antibiotic usage by herdsman.

**Keywords:** Milk; Northern Ghana; total bacteria count; total coliform count; antibiotic residue.

## 1. INTRODUCTION

Marketing of raw cow milk is becoming highly lucrative because of preference for fresh milk and minimally processed dairy products by the populace. This development is weakening the fight against food borne diseases [1]. To eliminate the risk of food borne illness, milk that is meant for human consumption must be free from any contaminant [2]. The contaminants can be microorganisms, chemical agents such as toxins, antimicrobials, hormones, pesticides and physical agents like debris from vegetation, soil etc [3]. Over the years, raw milk and its products have been identified as a major source of food borne diseases in humans [2,4-8]. Most of these cases have been due to contamination of milk with various pathogenic bacteria or spoilage organisms. Shiga toxin-producing *E. coli* (*E. coli* O157:H7), pathogenic species of *Bacillus*, *Brucella*, *Campylobacter*, *Coxiella*, *Listeria*, *Mycobacterium*, *Salmonella*, *Shigella*, *Yersinia* and certain strains of *Staphylococcus aureus* which can produce highly heat-stable toxins have all been isolated from raw milk [8-11]. Milk, therefore, is an efficient vehicle for transmission of food borne zoonosis. To avoid the risk of infection by milk-borne zoonotic organisms, developed countries have enforced standards of hygiene and laws on pasteurization of raw milk. In many developing countries, however, the bulk of milk is sold raw through informal channels where hygienic measures during milking and distribution are ignored [3]. In African countries, cows are the main milk producing animal with a small proportion of milk from camels and goats in the pastoralist areas of some countries. Bacterial contamination of milk primarily originates

internally from infected or sick lactating cows. External factors such as the kraal environment, milking processes and sanitation of equipment can also lead to microbial contamination of raw milk. Ambient temperatures at which the milk is stored until consumption also enhances the rapid multiplication of the bacteria [7,12,13].

Additionally, antimicrobial agents administered to prevent, control or treat infection in animals or as feeding and growth enhancers may remain in raw milk and its products, leading to detectable levels of antimicrobial residues [5,11].

Ghana like many developing countries has a burgeoning informal milk marketing sector with herdsman and their families leading in the production and sale of milk and milk products [14]. Though the marketing and consumption of raw milk or milk products (mainly yoghurt, a local cottage cheese known as “wagashi” and a milk and millet beverage known as “Burkina”) occurs in all regions, the northern part of the country is a significant contributor in this sector. This is because unlike in large parts of Southern Ghana, where raw cow milk and its products are not traditionally consumed by indigenes, consumption levels are higher in northern Ghana, where cattle rearing is a traditional practice [3].

We previously assessed the raw cow milk quality along the entire coastal savanna zone, spanning four regions in the southern part of Ghana and found that the quality was poor, and knowledge of herdsman on milk borne zoonosis and safe handling of milk was very low [5,14,15]. Therefore, the objectives of the present study were to assess the bacteriological quality of raw

cow milk and milk products produced and marketed within the northern region of Ghana and identify the major bacterial pathogens compromising milk safety in order to make evidence-based suggestions for improvement.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The northern region is the largest (in terms of land mass) of 10 administrative regions in Ghana. It is also one of three regions occupying the northernmost part of the country which together account for 75% of all cattle produced in Ghana [16]. Sanga, West African short horn, Ndama and their crosses are the dominate cattle breeds in Ghana.

### 2.2 Ethics

The study did not involve experimental animals or human subjects. As such it was exempted from institutional ethical clearance.

### 2.3 Study Design

The study was cross-sectional. Cattle kraals (n=210) and 60 sale points at the market level in the northern region were sampled between March and September 2017 when milk production was at its peak due to feed availability.

### 2.4 Sampling Method

Seven (7) out of 26 districts namely; Tolon, Central Gonja, East Gonja, Kumbungu, Sanarigu, Savlegu-Nanton and Tamale Metro were purposively selected because of their high milk production and supply to the local community and milk processing assemblies (Fig. 1). With assistance from district veterinarians, 30 kraals in each district were selected based on the size of kraal, accessibility and proximity to market sites.

### 2.5 Milk and Milk Products Sample Collection

From each kraal, 50 mls of raw milk was aseptically withdrawn from the receptacle containing the bulk milk into sterile screw cap falcon tubes in duplicate. Milk products (wagashie, cottage cheese, burkina and fresh yoghurt) were purchased from various sales points. All samples were labeled with permanent markers and kept below 10°C in a cool box with

icepacks. The samples were transported to the Spanish laboratory at the University for Development Studies (UDS) for bacteriological analysis. The samples were processed immediately and within 24 hours of milking and sample collection, while the duplicate samples were frozen at -20°C and later transported to the Pathogen level 3 (P3) laboratory of the Noguchi Memorial Institute for Medical Research (NMIMR) for Mycobacteria culture, isolation and identification.

### 2.6 Bacteriological Analysis

Determination of bacteria load in raw milk involved total viable count (TVBC) and total coliform count (TCC) using standard plate count agar (APHA, Oxoid, UK) and violet red bile (VRB) agar (HiMedia, India), respectively.

### 2.7 Standard Plate Count (SPC)

The TVBC was done using the spread plate method to determine the extent of microbial contamination of milk at the kraal level before any processing was done and the quality of milk products on sale. A tenfold serial dilution ( $10^{-1}$  to  $10^{-10}$ ) of each sample was prepared in sterile buffered peptone broth by adding 1 ml (for milk and liquid milk products) of each sample to 9 ml of buffered peptone broth and homogenized. For solid samples, 10 g was weighed into a stomacher bag and homogenized with 90 ml of phosphate buffered saline (PBS). A 1 ml of each homogenate was used to prepare the tenfold dilution. Sterile duplicate SPC agar plates were labeled according to the dilution index. One ml of each dilution was aseptically withdrawn using a sterile 1 ml Pasteur pipette and delivered into an opened SPC agar plate and then closed. This was repeated till all the dilutions were pipetted into their corresponding plates. This was followed by spreading the sample over the entire surface of each SPC agar using sterile glass spreaders. The plates were inverted and incubated at 32°C for 48 hours.

### 2.8 Coliform Count

Total coliform (TC) determination was done by spreading 1ml of each sample dilution over VRB agar and incubating at 37°C for 24 hours.

### 2.9 Estimating Bacteria Counts

After incubation, plates were examined, and bacterial colonies were counted using the Reichert colony counter. Plates with colonies

between 25 and 250 were selected for Colony forming units (cfu) calculation. The TVBC and TCC were calculated as the weighted mean from two successive dilutions of every sample and were converted into colony forming units per milliliter (cfu/ml) using ISO 7218:2007(E) formula.

### 2.10 Isolation and Identification of Bacteria

100 µl of each sample (diluted by 1in10) was sub-cultured onto MacConkey, Baird parker and Salmonella-Shigella (SS) agar plates and incubated at 37°C for 18-24 hours. Characterization of bacterial isolates was carried out using colonial morphology, microscopic techniques and biochemical tests including gram's reaction, coagulase test, oxides test, Oxidation–Fermentation test, catalase test and 3% KOH tests.

*Escherichia coli* (*E. coli*) was specifically tested using *E. coli* Chromogenic agar (ECC chromo Selective-85927, Sigma-Aldrich, Germany). Dark blue-violet colonies were confirmed as *E. coli* when the colonies turned cherry red colour upon the addition of Kovac's reagent. The confirmed *E. coli* isolates were further tested for the

presence of *E. coli* O157:H7 using the latex slide agglutination kit (DR0120, Oxoid). Coliforms were identified when growths formed salmon-to-red colored colonies.

For isolation of *Salmonella* and *Shigella* spp, distinct pale and colourless colonies on MacConkey and Salmonella-Shigella agar were tested biochemically on Kligler iron agar, urea agar, and Simmons citrate agar (all obtained from (Oxoid® Ltd., Basingstoke, Hampshire, England). The isolates with reaction result typical of *Salmonella* colonies were sub cultured on Xylose Lysine Deoxycholate (XLD) media and incubated at 37°C for 24 hours. Red colonies with black centers after the incubation period were identified as *Salmonella* sp and speciation was done using Oxoid rapid latex agglutination test kit (Oxoid® Ltd., Basingstoke, Hampshire, England).

*Staphylococcus aureus* colonies appeared as black or grey colored colonies on Baird parker agar were then picked and streaked on nutrient agar for coagulase test. Staphylase Test (Oxoid DR0595A), a rapid test kit for the detection of coagulase positive *S. aureus* was used according to manufacturer's instruction.

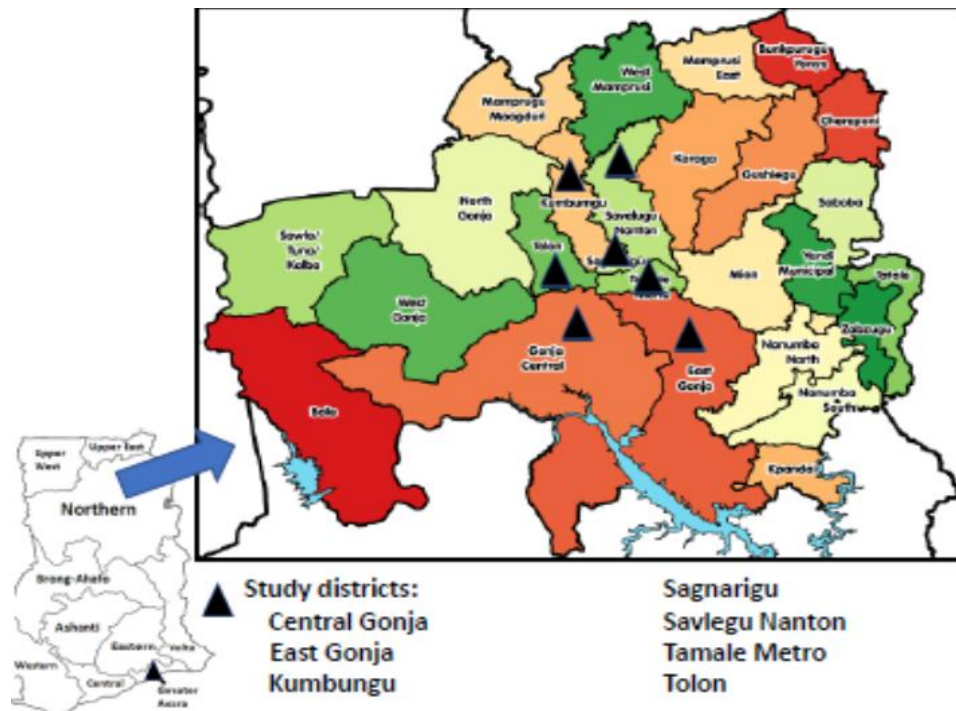


Fig. 1. Distribution of 7 districts selected for the study

### 2.11 Mycobacteria Culture and Identification

Frozen milk samples were thawed, decontaminated using the method described by Kazwala et al, 1998 and cultured on two slopes of Lowenstein Jensen (LJ) media (one containing glycerol and the other pyruvate) for up to 12 weeks. Cultures were observed weekly, and growth suspected to be mycobacteria were confirmed with Ziehl Neelsen staining. Acid fast colonies were characterized as *Mycobacterium tuberculosis* complex (MTBC) or non-tuberculous mycobacteria (NTM) based on growth rate, colonial morphology, pigmentation as well as result of the GenoQuick® MTB (Hain Life science, Nehren, Germany) test.

### 2.12 Detection of Antibiotic Residues

Antibiotic residue detection in the raw milk samples was performed using the Charm Blue-Yellow antibiotic residue test kit (CHARM Sciences Incorporated, MA, USA). The Charm Blue Yellow II Test detects antibiotics in raw commingled and ultra-pasteurized cow milk. Bacteria, cultured in a vial with milk, generate acid and turn a pH indicator from purple to yellow. Milk samples that prevent a color change are considered positive for antibiotics. Antimicrobial drug-free samples yield 90% negative results with 95% confidence. The test is sensitive enough to detect antimicrobial drugs in µg/kg or ppb (parts per billion) compared to EU MRL (Maximum Residue Limit). To avoid false positive results due to presence of non-antibiotic (heat sensitive) inhibitors, all initial positive samples were heated to 82 to 100°C for three minutes and retested. Only samples that remained positive after retesting were recognized as positive for antibiotic residues.

### 2.13 Data Collection and Statistical Analysis

Raw data generated were entered in Microsoft Excel spreadsheet, presented in summary tables and then subjected to statistical analyses. The statistical analyses were performed using STATA (STATA™ 10, Stata Corp., 4905 Lakeway Drive, College Station, Texas 77845 USA). Continuous data such as Bacteria counts (TVBC and TCC) were presented as mean ± standard error (SE) and percentage (%) and compared across sample type (student t test) based on Ghana's food and drugs authority (FDA) microbiological limit (TVBC  $\leq 1 \times 10^5$

CFU/ml and TCC  $\leq 10^3$  CFU/ml) for fresh or processed food meant for consumption (GS 955:2013). Categorical data such as presence of antibiotic residues based on European Union acceptable limits) were compared across districts using Chi-square. Statistical significance was determined at  $P = .05$ .

## 3. RESULTS

The total viable count (TVBC) of the samples ranged from  $1.12 \times 10^3$  to  $9.4 \times 10^8$  cfu/ml with mean count being  $(2.07 \pm 6.68) \times 10^7$  cfu/ml. TVBC was higher than FDA limit of  $1.0 \times 10^5$  for 69.5% (146/210) of raw milk samples and 46.7% (28/60) of milk products (Table 1).

The total coliform count (TCC) for all samples ranged from  $9.09 \times 10^1$  to  $1.95 \times 10^5$  cfu/ml with mean of  $(1.00 \pm 1.43) \times 10^4$  cfu/ml. Of the 270 samples analyzed, about 52.9% (111/210) of raw milk samples recorded TCC values higher than FDA Ghana recommended value of  $\leq 10^3$  cfu/ml compared to 26.7% (16/60) of milk products (Table 2).

Bacteria species isolated include: *Escherichia coli* (15.6%), *Klebsiella pneumoniae* (14.1%), *Staphylococcus aureus* (10.7%), *Pseudomonas aeruginosa* (4.8%), *Salmonella* sp. (4.4%), *Shigella* sp. (2.6%), *Mycobacterium tuberculosis* complex (MTbc) (2.6%), Non-tuberculous mycobacteria (NTM) (2.2%), *E. coli* O157:H7 (1.9%) *Proteus* sp. (1.5%) (Fig. 1). Antibiotic residues were detected in 18.1% (38/210) of the raw milk samples.

## 4. DISCUSSION

Total viable bacterial count (TVBC) is an indicator of the microbial quality of food. In this study, majority (64.4%) of the samples had TVBC above the FDA, Ghana limits. In our previous study, 45% of raw milk samples from kraals within the coastal savannah zone were above the FDA-Ghana limit. Such high contamination at producer level can be attributable to several factors such as milking manually with bare and often unclean hands and the general insanitary conditions under which the milk is pooled into the final storage receptacle at the farm/kraal.

The farmers do not have cold storage facilities, so raw milk is stored and transported under high temperature conditions using motorbikes,

bicycles or on foot. The time lapse after milking and the holding temperature of the milk before purchase promote rapid bacterial growth [5,17].

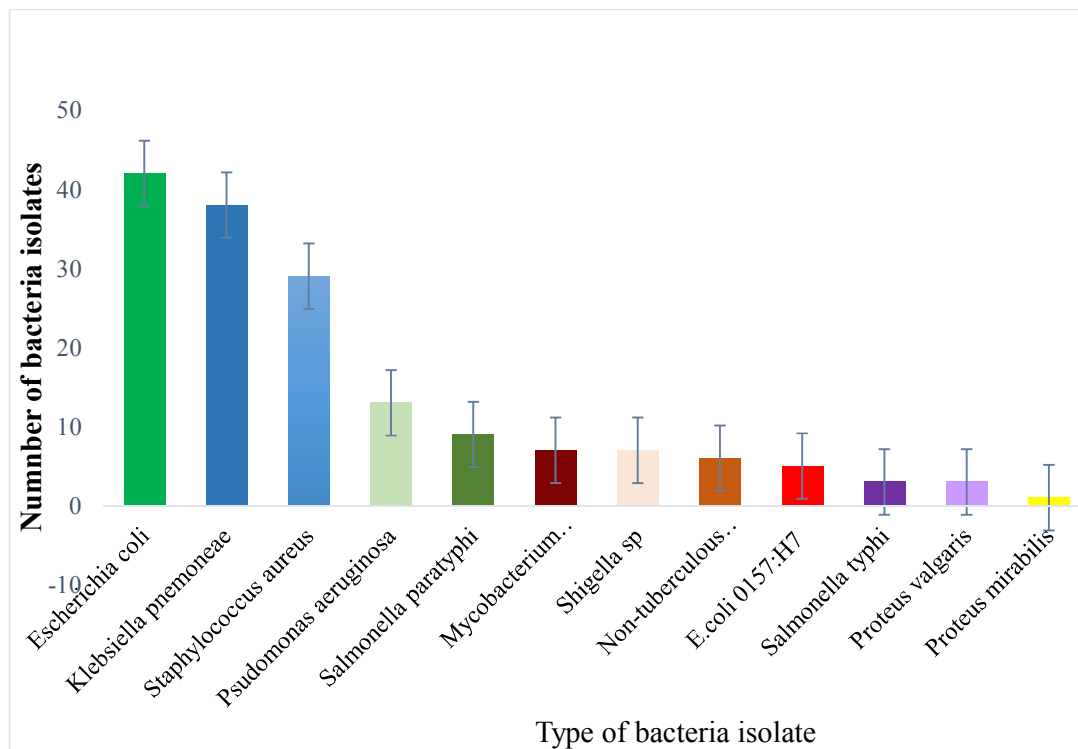
Hence raw milk already highly contaminated from the producer level will eventually get to the processors or retailers in a worse state.

**Table 1. Total viable bacterial count in raw milk and selected milk products**

Type of sample	Number of samples	Mean CFU/ml	Number (%) of samples in the range			
			$\leq 10^5$ CFU/ml		$>10^5$ CFU/ml	
Raw milk	210	$(2.40\pm 7.44) \times 10^7$	64	(30.5)	146	(69.5)
Burkina	15	$(7.32\pm 2.03) \times 10^6$	9	(60.0)	6	(40.0)
Cottage cheese	15	$(1.67\pm 2.87) \times 10^7$	1	(6.7)	14	(93.3)
Fresh yoghurt	15	$(7.51\pm 2.04) \times 10^6$	10	(66.7)	5	(33.3)
Wagashie	15	$(4.43\pm 1.62) \times 10^6$	12	(80)	3	(20.0)
Total dairy samples	270	$(2.07\pm 6.68) \times 10^7$	96	(35.6)	174	(64.4)

**Table 2. Total coliform counts in raw milk and selected milk products**

Type of sample	Number of samples	Mean cfu/ml	Number (%) of samples in range			
			$\leq 10^3$ CFU/ml		$>10^3$ CFU/ml	
Raw milk	210	$(1.10\pm 1.53) \times 10^4$	99	(47.1)	111.0	(52.9)
Burkina	15	$(6.64\pm 6.65) \times 10^3$	10	(66.7)	5.0	(33.3)
Cottage cheese	15	$(7.62\pm 6.08) \times 10^3$	9	(60.0)	6.0	(40.0)
Fresh yoghurt	15	$(6.17\pm 6.77) \times 10^3$	12	(80.0)	3.0	(20.0)
Wagashie	15	$(6.05\pm 1.54) \times 10^4$	13	(86.7)	2.0	(13.3)
Total dairy samples	270	$(1.00\pm 1.43) \times 10^4$	143	(53.0)	127.0	(47.0)



**Fig. 2. Bacteria species isolated from raw cow milk and milk products in the Northern Region of Ghana**

**Table 3. Antimicrobial residues detected in raw milk samples in the northern region of Ghana**

District	N	Number (%) of samples positive for antimicrobial residue	
		n	(%)
Central Gonja	30	10	(33.3)
East Gonja	30	8	(26.7)
Kumbungu	30	6	(20.0)
Sagnarigu	30	2	(6.7)
SaveluguNanton	30	4	(13.3)
Tamale metro	30	2	(6.7)
Tolon	30	6	(20.0)
<b>Total raw milk</b>	<b>210</b>	<b>38</b>	<b>18.1</b>

About half of the raw milk samples had coliform counts above  $10^3$ cfu/ml while majority (60-87%) of the raw milk products had coliform counts below  $10^3$ cfu/ml. Coliform counts are useful hygiene indicators for food as their presence is indicative of possible contamination with enteric bacteria and thus faecal matter. Faecal contaminants in raw milk may originate from the contaminated udder or teats of the animal or from the farm environment [5,18]. The conditions under which milking is done in all the kraals sampled makes fecal contamination unavoidable. The teats which may touch the floor covered with cow dung must be washed very well prior to milking which is not often done by the farmers. Lower coliform contamination of the milk products could be attributed to the processing methods like boiling or frying (cottage cheese/wagashie) which destroy most vegetative bacteria thereby reducing the bacterial load [5]. In the case of yoghurt, the fermentation process decreases the pH making the milk acidic, a condition which is bactericidal. However, post production contamination may occur if products are not handled and stored properly before or during the retail process.

*Escherichia coli* (*E. coli*) was detected in 15.6% of raw milk samples but not in any of the milk products. Some studies have reported presence of *E. coli* in milk and milk products in Ghana with prevalence rates of 11.2% [5] and 12.7% [19] while in Tanzania up to 37.33% has been reported [20]. However, other studies in Ghana and Tanzania have reported very low *E. coli* prevalence of 2.1% [21] and 6.3% [22] respectively. Significantly in this study, *E. coli* O157:H7 was detected in 1.9% of raw milk samples, a possible indication that this highly pathogenic bacterium is emerging among cattle and gaining access to the food chain. This is because previous studies in Ghana did not detect *E. coli* O157:H7 in raw cow milk and dairy

products produced along the coastal savannah zone of Ghana [5] or from the Northern region but rather from 12.7% of cattle faeces [19]. *E. coli* O157:H7, is a shiga toxin-producing *E. coli* (STEC) which can cause severe foodborne illnesses such as diarrhoea and haemolytic uremic syndrome. Another important pathogen isolated was *Salmonella* spp.( $\geq 4.4\%$ ) known to cause food poisoning, typhoid fever, enteric fever and gastroenteritis. The source of the contamination may be related to poor animal housing and poor milking hygiene practices [11,22,23]

*Staphylococcus aureus* was detected in 10.7% of the samples in this study although a lower rate of 2.1% [21] was reported in a similar study in Accra and Kumasi- the two most populous cities in Ghana. The mammary gland of dairy cows and food handlers carrying enterotoxin-producing *S. aureus* in their nostrils or hands may be the source. Improper handling and storage of milk at ambient temperatures permit growth of *S. aureus* leading to the production of the heat resistant enterotoxin [24] that is often the cause of food borne intoxication in humans [9,25,26].

Annually, 10% of people in the developing countries acquire *M. bovis* infection [27], mostly attributed to consumption of unpasteurized milk [28]. The detection of *Mycobacterium* species (4.8%) of which 2.6% were found to be *Mycobacterium tuberculosis* complex (MTBC) presents a threat for zoonotic TB and calls for stricter regulations on milk pasteurization.

Antibiotic use in animal husbandry is a normal practice however, the detection of antibiotic residues in raw milk from all 7 districts (18.1%) indicates that generally, regulations regarding withdrawal period after administration of antibiotic therapy are not being adhered to. Apart

from possible allergic reactions in humans that consume these products, another important problem caused by antimicrobial residue is its interference in fermentation processes [29] because they fail to get deactivated by boiling and pasteurization methods [30,31].

## 5. CONCLUSION

Raw cow milk produced and sold in the Northern region contain high levels of bacterial contamination as well as antibiotic residues at farm/kraal level. Pasteurization to reduce or eliminate potential pathogens in raw milk should be encouraged and milk producers should be educated on hygienic milking, storage, handling of raw milk and proper use of antibiotics.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The study did not involve experimental animals or human subjects. As such it was exempted from institutional ethical clearance.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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