Original Research Article

Bacteriological quality and antibiotic residues in raw cow milk at producer level andmilk products at sale points in the Northern region of Ghana.

Abstract

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

A cross sectional study was designed where raw milk (n=210)and milk products (n=60) comprising (local milk and millet beverage) (burkina), cottage cheese (wagashi) and yoghurt were sampled from cattle kraals and retailers respectively.

Methodology: Total viable bacterial counts (TVBC) and total coliform counts (TCC) were determined for all samples followed by isolation and identification ofcommon milk-borne bacteria pathogens using normal laboratory identification systems.Antimicrobial residue in the raw milk was determined using the Charm ® blue-yellow kit.

Results: Mean total viablebacteria and coliform counts were (2.40±7.44) $x10^7$and(1.10±1.53) $x10^4$ for raw milk and (8.99±2.24) $x10^6$ and (6.62±9.54) $x10^3$for milk productsrespectively. Isolatedbacteria included *Escherichia coli* (15.6%), *Klebsiella pneumoniae* (14.1%), *Staphylococcus aureus* (10.7%), *Pseudomonas aeruginosa* (4.8%), *Mycobacterium tuberculosis*species, (4.8%), *Salmonella* sp. (4.4%), *Shigella* sp. (2.6%)*E. coli*O157:H7 (1.9 %) and *Proteus* sp. (1.5%). 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 for herdsmen.

Keywords:

Milk, Northern Ghana, Total bacteria count, total coliform count, antibiotic residue, kraal.

Introduction

Marketing of raw cow milk has seen a resurgence because of human preference for fresh milk and minimally processed dairy products, a situation that 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 major sources 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 be 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

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developing countries, however, the bulk of milk are 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 contribution 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 environs, 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 used as feeding and growth enhancers may remain in raw milk and 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 all in 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 qualityalong the entire coastal savanna Zone, spanning four regions in the southern part of Ghana and found the quality to be poor and knowledge of herdsmen on milk borne zoonosis and safe handling of milk 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, to isolate and identify the major bacterial pathogens compromising milk safety and to make evidenced-based suggestions for improvement.

Materials and methods

Study area

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

Ethics

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

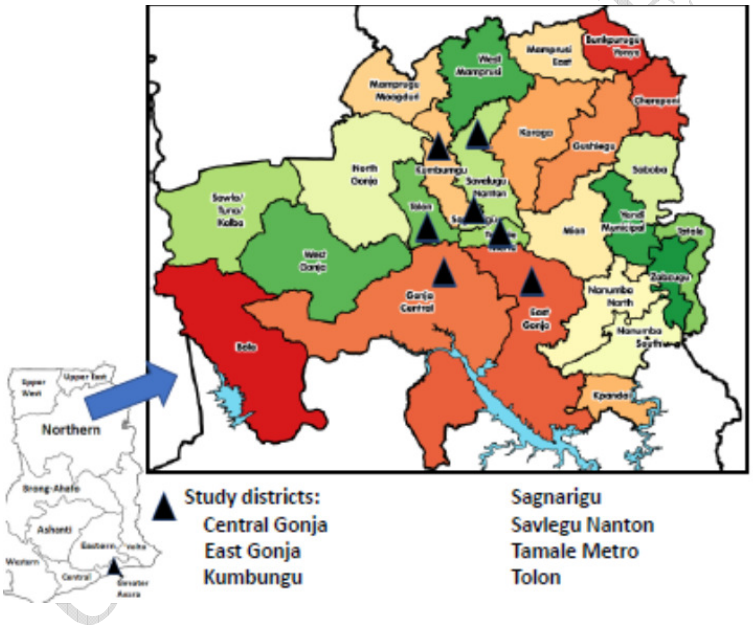
Study design

The study was cross-sectional. Cattle kraals (n=210) and 60sale 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,

Sampling method

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

Figure 1: Distribution of 7 districts selected for the study



Milk and milk products sample collection

From each kraal, 50ml 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 salespoints randomly. All sample were labeled with permanent markers and kept below 10 °C in a cool box with icepacks. The sampleswere transported to the Spanish laboratory at the University for Development Studies (UDS) forbacteriologicalanalysis. The samples were processedimmediately and within 24 hours of milking and sample collection, while the duplicate samples were frozen at -20^{o}C and later transported to the Pathogen level 3 (P3) laboratory of the NoguchiMemorial Institute for Medical Research (NMIMR) for Mycobacteria isolation culture and identification.

Bacteriological analysis

The bacteriological tests considered for determination of the bacteria load in raw milk samples were total viable bacterial count (TVBC) and total coliform count (TCC). For these two procedures, standard plate count agar (APHA, Oxoid, UK) and violet red bile (VRB) agar (HiMedia, India) were used, respectively.

Standard plate count (SPC)

The TVBC was done using the spread plate methodto 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 adding1ml (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 byspreading 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.

Coliform count

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

Estimating bacteria counts

After incubation, plates inoculated with sample dilution yielding between 25 and 250 colonies were counted using the Reichert colony counter.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 anISO 7218:2007(E)formula.

Isolation and identification of Bacteria

100 µl of each sample (diluted by 1in10) was sub-cultured onto MacConkey, blood, Baird parker and Salmonella-Shigella(SS) agar plates and incubated at 37^{o}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.

E. coli was specifically tested for 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*, distinct pale and colourless colonies on MacConkey and on 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 XLD media and incubated at 37ºC for 24 hours. Red colonies with black centers after the incubation period were identified as *Salmonell*a sp and speciation was doneusing 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.

Mycobacteria culture and identification

Frozen milk samples were thawed, decontaminated using the method described by Kazwala et al, 1998 and culturedon two slopes of Lowenstein Jensen (LJ) media (one containing glycerol and the other pyruvate) for up to 12 weeks. Cultures wereobserved

weekly, and growth suspected to be mycobacteria were confirmed with ZiehlNeelsen 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 GinoQuick® MTB (Hain Life science, Nehren, Germany) test.

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).

Data collection and statistical analysis

Raw data generated were entered in Microsoft Excel spreadsheet, presented in summary tables and then subjected to statistical analyses. Thestatistical analyses were performed using STATA (STATA^{TM} 10, StataCorp., 4905 LakewayDrive, 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 onGhana'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 residuesbased on European Union acceptable limits) were compared across districts using Chi-square.Statistical significance was determined at ***P***= .05.

Results

The total viable count (TVBC) of the samples ranged from $1.12x10^3$ to $9.4x10^8$ cfu/ml with mean count being $(2.07\pm6.68)\ x10^7$cfu/ml.TVBC was higher than FDA limitof $1.0x10^5$for69.5% (146/210) of raw milk samples and 46.7% (28/60) of milk products (Table 1).

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***	
			≤ 10^5 CFU/ml	**>10^5 CFU/ml**
Raw milk	210	$(2.40\pm7.44)x10^7$	64 (30.5)	146 (69.5)
Burkina	15	$(7.32\pm2.03)x10^6$	9 (60.0)	6 (40.0)
Cottage cheese	15	$(1.67\pm2.87)x10^7$	1 (6.7)	14 (93.3)
Fresh yoghurt	15	$(7.51\pm2.04)x10^6$	10 (66.7)	5 (33.3)

Wagashie	15	(4.43±1.62)x10^6	12	(80)	3	(20.0)
Total dairy samples	270	(2.07±6.68)x10^7	96	(35.6)	174	(64.4)

The total coliform count (TCC) for all samples ranged from 9.09x10^1 to 1.95x10^5cfu/ml with meanof (1.00±1.43) x10^4cfu/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 ≤10^3 cfu/mlcompared to 26.7%(16/60) of milk products (Table 2).

Table 2. Total coliform countsin raw milk and selected milk products

Type of Sample	Number of samples	Mean cfu/ml	Number (%) of samples in range	
			≤10^3 CFU/ml	>10^3 CFU/ml
Raw milk	210	(1.10±1.53)x104	99 (47.1)	111.0 (52.9)
Burkina	15	(6.64±6.65)x10^3	10 (66.7)	5.0 (33.3)
Cottage cheese	15	(7.62±6.08)x10^3	9 (60.0)	6.0 (40.0)
Fresh yoghurt	15	(6.17±6.77)x10^3	12 (80.0)	3.0 (20.0)
Wagashie	15	(6.05±1.54)x10^4	13 (86.7)	2.0 (13.3)
Total dairy samples	270	(1.00±1.43)x10^4	143 (53.0)	127.0 (47.0)

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%) (Figure 1). Antibiotic residues were detected in 18.1% (38/210) of the raw milk samples.

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

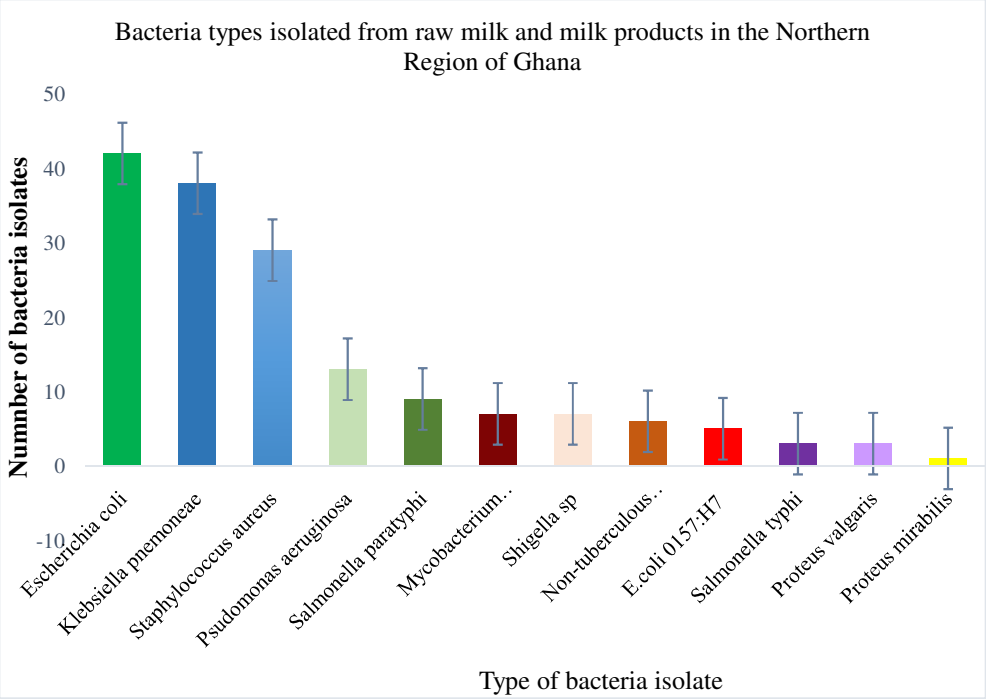


Table 3. Antimicrobial residues detected in raw milk samples in the northern region of Ghana.

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)
Savelugu Nanton	30	4	(13.3)
Tamale metro	30	2	(6.7)
Tolon	30	6	(20.0)
Total raw milk	**210**	**38**	**18.1**

Discussion

Total viable bacterial count (TVBC) is as an indicator of the microbial quality of food. In this study majority (64.4%) of the samples had TVBC above the FDA, Ghana limits.A previous study also found 45%of raw milk samples from kraals within the coastal savannah zone to be above the FDA limits[5].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.

About half of the raw milk samples had coliform counts above 10^3 while majority (60-87 %) of the raw milk products had coliform counts below 10^3. 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 the 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 encounter the floor covered with the cow dung, must be washed very well prior to milking, but this is not often done by the farmers. On the contrary, majority of milk products had lower levels of coliform. This could be attributed to the processing methods like boiling or frying 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. Other studies have reported comparable counts of *E. coli* in milk and milk products in Ghana with contamination rates of 11.2 %[5] and 12.7% [19]while in Tanzania up to 37.33% has been reported[20]. However, previous studies in Ghana and Tanzania reported much lower *E.coli* prevalence of 2.1% [21]and6.3%[22] respectively. Significantly *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 earlier 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 was Salmonella (≥ 4.4%) spp. 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 theheat 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 are affected with *M. bovis* infection [27]mainly 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 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 who 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].

Conclusions

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.

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