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

Objective: To evaluate the bacteriologicalquality 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 (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 ofcommon 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 viablebacteria and coliform counts were (2.40±7.44) $x10^7$and(1.10±1.53) $x10^4$cfu/ml for raw milk and (8.99±2.24) $x10^6$ and (6.62±9.54) $x10^3$cfu/mlfor milk productsrespectively. *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 herdsmen.

Keywords:

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

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 it 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 that the quality was poor, and knowledge of herdsmen 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 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. It is also one of three regions occupying the northernmost part of the country whichtogether 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.

Ethics

The study 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.

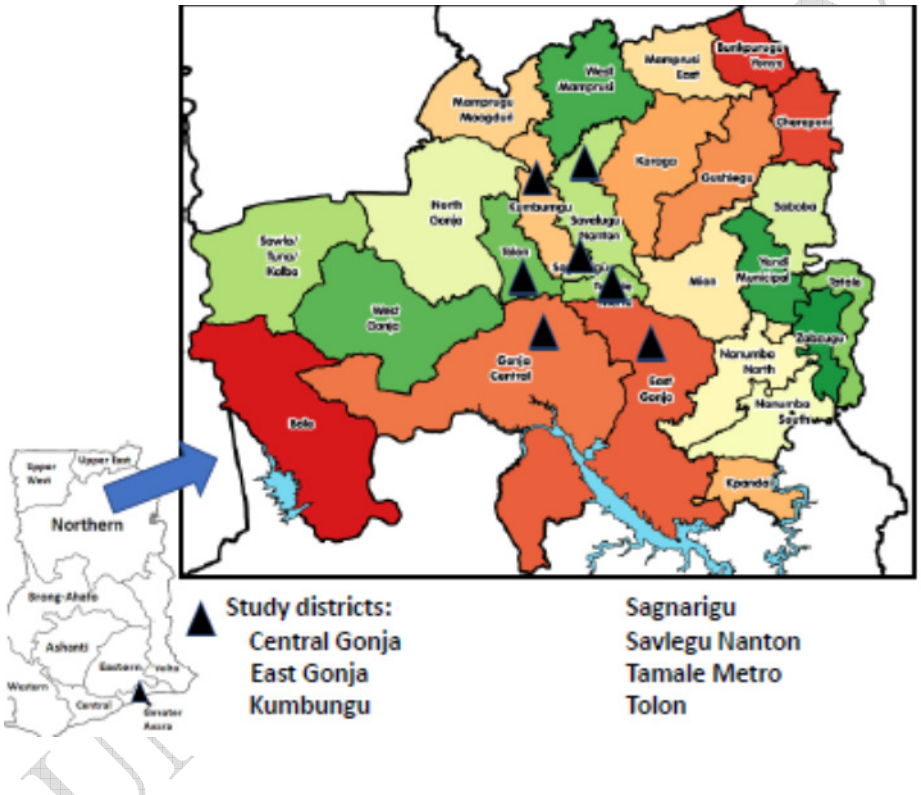

Figure 1: Distribution of 7 districts selected for the study

Milk and milk products sample collection

From each kraal, 50mls 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 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

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.

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 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.

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.

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*spps, 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 onXylose Lysine Deoxycholate (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).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^{0}Cfor three minutes and retested. Only samples that remained positive after retesting were recognized as positive for antibioticresidues.

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±6.68) $x10^7$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±7.44)$x10^7$	64 (30.5)	146 (69.5)
Burkina	15	(7.32±2.03)$x10^6$	9 (60.0)	6 (40.0)
Cottage cheese	15	(1.67±2.87)$x10^7$	1 (6.7)	14 (93.3)
Fresh yoghurt	15	(7.51±2.04)$x10^6$	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 $1.95x10^5$cfu/ml with meanof (1.00±1.43) $x10^4$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/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	
			$\leq 10^3$ 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.

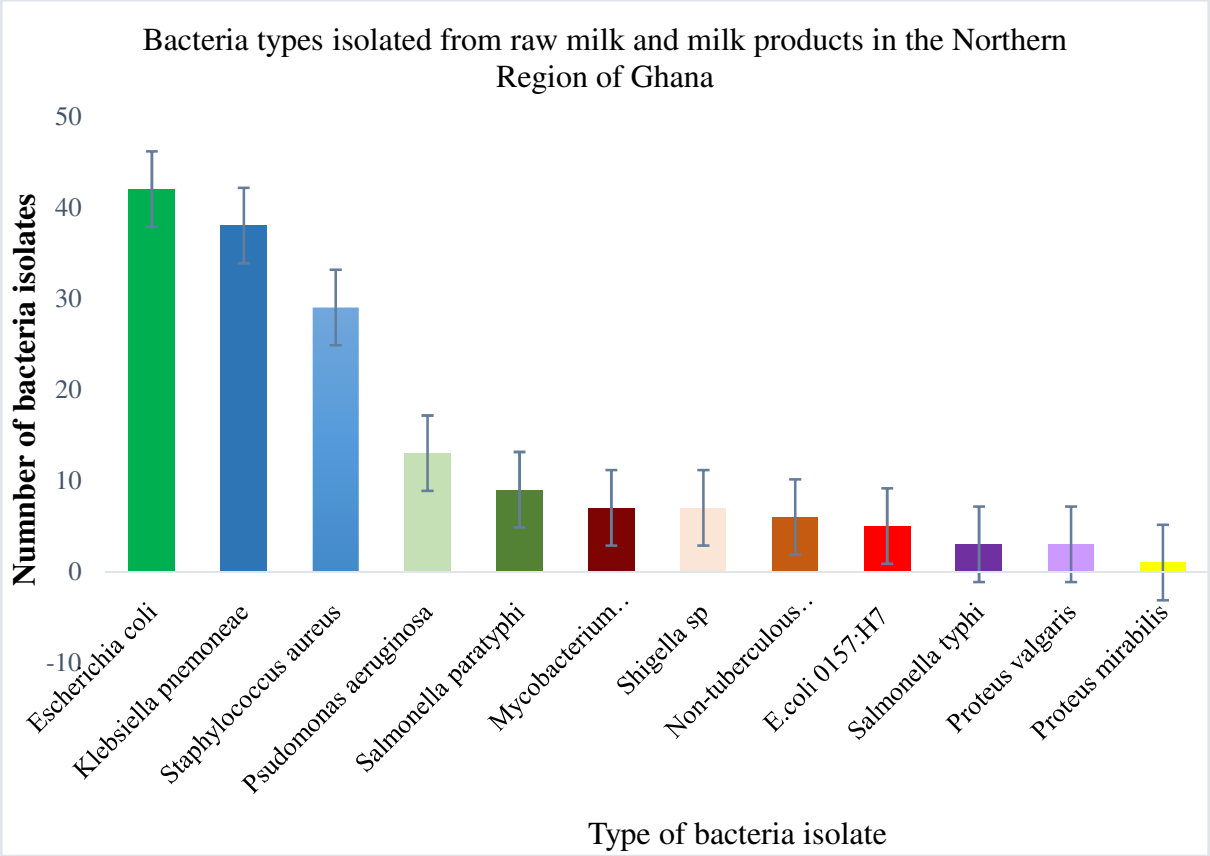

Figure 2. Bacteria species isolated from raw cow milk and milk products in the Northern Region of Ghana

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.In our previous study,45%of raw milk samples from kraals within the coastal savannah zone wereabove 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.

About half of the raw milk samples had coliform counts above 10^3cfu/mlwhile majority (60-87 %) of the raw milk products had coliform counts below 10^3cfu/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]and6.3%[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 Ghanadid 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.(≥ 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 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 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].

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.

REFERENCES

1. Schelin J, Wallin-Carlquist N, Thorup Cohn M, Lindqvist R,Barker GC. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Virulence. 2011; 2(6):580-92.DOI: 10.4161/viru.2.6.18122.

2. Bertu W, Dapar M, Gusi A, Ngulukun S, Leo S,Jw L. Prevalence of *Brucella* antibodies in marketed milk in Jos and environs. Afr. J. Food Sci. 2010; 4(2):062-64.DOI: 10.1155/2018/9378976.

3. Omore A, Staal SJ, Wanyoike F, Osafo EL, Kurwijila L, Barton Det al. Market mechanisms and efficiency in urban dairy products markets in Ghana and Tanzania. in Research Report 19,IRIL (InternationalLivestockResearchInstitute). 2009: 55.

4. Schmidt RH, Goodrich RM, Archer DL, SchneideKR. General Overview of the Causative Agents of Foodborne Illness. Emerging Infectious Diseases. 2009; 17(1):7-15.

5. Addo KK, Mensah GI, Aning KG, Nartey N, Nipah G, Bonsu Cet al. Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. Trop Med Int Health. 2011; 16(2):227-32.DOI: 10.1111/j.1365-3156.2010.02666.x.

6. Frederick A, Courage KSS,Gabriel AT. Antibiotic Susceptibility of *Escherichiacoli* Isolated from Milk and Hands of Milkers in Nyankpala Community of Ghana. Curr. Res. Dairy Sc. 2016(8):6-11.DOI: 10.3923/crds.2016.6.11.

7. Vithanage NR, Dissanayake M, Bolge G, Palombo EA, Yeager TR,Datta N. Microbiological quality of raw milk attributable to prolonged refrigeration conditions. J Dairy Res. 2017; 84(1):92-101.DOI: 10.1017/S0022029916000728.

8. Wanjala G, Mathooko F, Kutima P,Mathara J. Microbiological quality and safety of raw and pasteurized milk marketed in and around Nairobi Region. Afr. J. Food Agric. Nutr. Dev. 2017; 17(1):11518-32.

9. Gohar S. Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated from raw milk and dairy products.Mat. Sc. Med 2017.DOI: 10.26480/msm.01.2017.10.14.

10. Sharma S, Sharma V, Dahiya DK, Khan A, Mathur M,Sharma A. Prevalence, Virulence Potential, and Antibiotic Susceptibility Profile of *Listeria monocytogenes* Isolated From Bovine Raw Milk Samples Obtained From Rajasthan, India.Foodborne Pathog Dis. 2017; 14(3):132-40.DOI: 10.1089/fpd.2016.2118.

11. Sharma D, Sharma PK,Malik A. Prevalence and antimicrobial susceptibility of drug resistant *Staphylococcus aureus* in raw milk of dairy cattle. Int. Res. J. Microbiol. 2011; 2(11):466-70.

12. Wallace RL. Bacteria count in raw milk. Dairy Cattle. Management. 2009:1-4.

13. Tegegne B,Tesfaye S. Bacteriological milk quality: possible hygienic factors and the role of *Staphylococcus aureus* in raw bovine milk in and around Gondar, Ethiopia. Int. j. food contam. 2017; 4(1):1.DOI: 10.1186/s40550-016-0046-2

14. Addo KK, Mensah GI, Nartey N, Nipah GK, Mensah D, Aning Get al. Knowledge, Attitudes and Practices (KAP) of herdsmen in Ghana with respect to milk-borne zoonotic diseases and the safe handling of milk. J. Basic Appl. Sci. Res. 2011; 1(10):1566-62.

15. Mensah GI, Addo KK, Aning KG, Nartey N, Nipah GK,Smits H. *Brucella abortus* antibodies in raw cow milk collected from Kraals within the coastal savannah zone of Ghana. J. Basic Appl. Sci. Res. 2011; 1(8):942-47.

16. Adzitey F. Animal and meat production in Ghana-An overview. J. World's Poult. Res. 2013; 3(1):1-4.

17. Bukuku JN. Awareness of health risks as a result of consumption of raw milk in Arusha City and Meru District, Tanzania. 2013, Sokoine University of Agriculture: Unpublished dissertation for award of MSc. degree 1 - 89.

18. Mahami T, Odonkor S, Yaro M,Adu-Gyamfi A. Prevalence of antibiotic resistant bacteria in milk sold in Accra. Int. Res. J. Microbiol. 2011; 2(4):126-32.DOI: 10.15406/jdvar.2015.02.00052.

19. Saba CKS, Yankey E,Adzitey F. Prevalence of *Escherichia coli* and Shiga Toxin producing *Escherichia coli* in Cattle Faeces and Raw Cow Milk Sold in the Tamale Metropolis, Ghana. j. Dairy Vet Anim Res. 2015; 2(5):53-57.

20. Lubote R, Shahada F,Matemu A. Prevalence of *Salmonella spp. and Escherichia coli* in raw milk value chain in Arusha, Tanzania. ajrc. 2014; 2(9):1-13.

21. Donkor E, Aning K,Quaye J.Bacterial contaminations of informally marketed raw milk in Ghana. Ghana Med J, 2007;41(2):58-61.

22. Karimuribo ED, Kusiluka LJ, Mdegela RH, Kapaga AM, Sindato C,Kambarage DM. Studies on mastitis, milk quality and health risks associated with consumption of milk from pastoral herds in Dodoma and Morogoro regions, Tanzania. J. Vet. Sci. 2005; 6(3):213-21.

23. Durnez L, Sadiki H, Katakweba A, Machang'u R, Kazwala R, Leirs Het al. The prevalence of *Mycobacterium bovis*-infection and atypical mycobacterioses in cattle in and around Morogoro,Tanzania. Tropl Anim Health Prod. 2009; 41(8):1653-59.DOI: 10.1007/s11250-009-9361-4.

24. Aydin A, Sudagidan M, Muratoglu K, Prevalence of staphylococcal enterotoxins, toxin genes and genetic relatedness of food-borne *Staphylococcus aureus* strains isolated in the Marmara region of Turkey.Int J Food Microbiol2011;148**:** 99-106.DOI: 10.1016/j.ijfoodmicro.2011.05.007.

25. Nyenje ME,Ndip N.The challenges of foodborne pathogens and antimicrobial chemotherapy: A global perspective.AJMR.2013; **7**(14):1158-72DOI: 10.5897/AJMRx12.014.

26. Jamali H, Paydar M, Radmehr B, Ismail S,Dadrasnia A et al. Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. Food Control, 2015; 54:383-88. DOI:10.1016/j.foodcont.2015.02.013.

27. EtchechouryI,Echeverrıa Valencia G, Morcillo N, Sequeira MD, Imperiale B,López M.Molecular typing of *Mycobacterium bovis* Isolates in Argentna: First

descripton of a person-to-person transmission case. Zoonoses Public Health, 2010(57):375–81.DOI: htp://dx.doi.org/10.1111/j.1863-2378.2009.01233.x.

28. Katale BZ, Mbugi EV, Kendal S, Fyumagwa RD, Kibiki GS, Godfrey-Faussett Pet al.Bovine tuberculosis at the human-livestock-wildlife interface: Is it a public health problem in Tanzania? A review.J Vet Res, 2012;79(2): 84-97..DOI: 10.4102/ojvr.v79i2.463.

29. Nisha A. Antibiotic residues-a global health hazard. Vet World, 2008;1(12): 375-377.

30. Shitandi A, Sternesjö A. Detection of antimicrobial drug residues in Kenyan milk.J Food Saf, 2001;21(4):205-214.DOI: 10.1111/j.1745-4565.2001.tb00319.x.

31. Kurwijila L, Omore A, Staal S,Mdoe N. *I*nvestigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania.J.FoodProt., 2006:69(10):2487-2492.