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Microbial load in unpasteurized milk obtained from selected milk outlets in Kawempe division of Kampala, Uganda

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Abbreviations: Cfu/g: Colony Forming Units Per Gram; DDA: Dairy Development Authority; E coli: Escherichia Coli; FAO: Food and Agricultural Organization; FDA: Food and Drug Administration; MRT: Milk Ring Test; UHT: Ultra Heat Treatment; UNBS: Uganda National Bureau of Standards; COVAB: College of Veterinary Medicine, Animal Resources and Biosecurity.

Abstract

Background: Milk consumption and demand has increased drastically over the past years in response to expanding human population in Uganda and world at large. Despite this, many residents of Kampala city obtain milk from shops supplied by milk vendors, who in turn, collect it directly from farms. The microbial load of this unpasteurized milk is not known, yet it could expose the population to milk-borne diseases. The purpose of this study was to determine the level of bacterial contamination of unpasteurized milk sold in different milk outlets in Kawempe Division, one of the five divisions of Kampala City.

Methods: This was a cross-sectional study in which 50 milk samples were collected from bulk tanks in different locations of Kawempe Division i.e. Kamwokya, Kalerwe, Mulago, Bwaise and Kawempe. The samples were taken to the Central Diagnostic Lab of the College of Veterinary Medicine, Animal Resources and Biosecurity of Makerere University and cultured using suitable media. Total aerobic counts, *Staphylococcus aureus* counts, coliform as well as *E. coli* and Salmonella counts were determined. The prevalence of *Brucella*in the milk samples was determined using the *Brucella* milk ring (Rose Bengal) test. The bacteriological indices were compared with UNBS and WHO acceptable standards. ANOVA was used to compare levels of contamination in various locations. All differences were considered significant at $p<0.05$.

Results: The study revealed that all milk samples from the different outlets had total aerobic counts, coliform counts and *E. coli* counts above the WHO and Uganda National Bureau of Standards (UNBS) limits. There were significant differences ($p=0.039$, ANOVA) in the mean *Staphylococcus aureus* counts of the milk samples from the different places. Kawempe location had the highest mean counts of *Staphylococcus aureus* within the Division. Furthermore, 52% of the milk samples had Salmonella; however none of the samples contained *Brucella*.

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Conclusions: This study has shown that the raw milk sold in outlets of Kawempe division does not meet the set standards and hence not safe for human consumption. Food safety authorities should be strengthened and more restrictions put on the sale of low quality milk.

Background

Milk and its products are excellent high quality foods providing both culinary and nutritional values. The constituents may vary with breed, type of feed, stage of lactation, season and age of cow among others, and also individuals of the same breed. Thus, it serves as an excellent medium for bacterial growth where under inappropriate conditions it acts as a carrier for disease causing pathogens from cows to humans (zoonosis). Freshly drawn milk from the udder of a healthy cow should be free from micro-organisms. However, contaminations have been reported and this has been attributed to the movement of pathogens up the teat canal and/or their presence at the lower ends of the teats [1,3]. Some studies have also showed that milk contains low numbers of commensal micro-organisms less than 500 bacteria per ml, usually the coagulase negative, nonpathogenic micrococci and streptococci although coliform bacteria are also common [3,4].

Microbial contamination of milk depends on the storage temperature, the time elapsing before collection and the initial microflora [5]. When milk is cooled to <4˚C, this will normally prevent bacterial multiplication for at least 24 hours, and the microflora is similar to that present initially [6]. Therefore, the number and types of micro-organisms present in milk immediately after production (initial microflora) directly reflects the microbial contamination during production. To render milk safe for human consumption, the pasteurization process was developed to particularly eliminate pathogens [7].

Pasteurization is a process which minimizes possible microbial health hazards associated with milk by heat treatment, which is consistent with minimal chemical, physical and organoleptic changes in the product [8]. Pasteurization is only limited to the vegetative micro-organisms and thus non-vegetative ones may not be destroyed for example spores which may serve as reservoirs for disease. However, very few people in Uganda consume well packaged and pasteurized milk, and this could be due to the high prices [9]. Most of milk produced is never pasteurized and sold as a raw product, and this could be a source of disease to the final consumers [10].

There is an increasing demand for quality check in regards to different sources of unpasteurized milk by consumers and yet at the farm level little attention is paid to ensure quality of milk and yet milk is perishable**.** In recent years, food-borne illnesses have continued devastating consumers through contaminated food products, both in Uganda and globally. These illnesses have caused high morbidities and mortalities to the consumers; financial losses through treatments and low productivity. However, not much has been reported about the microbial load in unpasteurized milk in Uganda, and yet it may be a source of infection to the consumers. The purpose of this study was to determine microbial load in unpasteurized milk sold in various outlets in Kawempe Division of Kampala city, Uganda.

Materials and methods

Study design

This was a cross-sectional study in which the level of contamination of unpasteurized milk was determined in randomly collected samples from the different milk collection outlets. Fifty samples were collected from the five selected milk vending outlets in Mulago, Kawempe, Kalerwe, Bwaise, and Kamwokya parishes of Kawempe Division of Kampala city. Total aerobic counts, *Staphylococcus aureus* counts, coliform as well as *E. coli* and Salmonella counts were determined. The prevalence of *Brucella* in the milk samples was determined using theRose Bengal test.

Sample collection and processing

The samples (50 in total) were purchased from the different milk vending outlets in a systematic simple random method. The sample size was determined by the standard formula [11] and was based on the assumption that 50% of milk sold by the vendors is unsuitable. Samples were collected in sample bottles and placed under ice in a cool box and immediately transported to Central Diagnostic Laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity of Makerere University for analysis. Samples were collected in such a way that 500ml of milk were picked from 10 different vending outlets in each parish. Using a sterile pipette tip, the milk was serially diluted by transferring 1ml of milk to a universal tube containing 9ml of buffered peptone water (10^{-1} dilution). Subsequent dilutions were carried out up to 10^{-5} dilution.

Aerobic plate count

Onto sterile petri dishes, 15ml of freshly prepared sterile plate count agar was poured. After the agar had set, the petri dishes were overturned and incubated at 37°C for 18 hours. The plate's were then checked for microbial contamination as a sterility test. With the aid of sterile tips, 100ul of the sample from the selected dilutions (10^{-4} and 10^{-5}) was spread on the surface of the plate count agar and then incubated at 37°C for 24 hours [12]. The colonies that formed on the plate count agar were counted and expressed as aerobic mesophilic counts, cfu/g. The number of colony forming units per ml of unpasteurized milk was calculated by multiplying the number of colonies formed on a particular plate by 10 and the reciprocal of the dilution factor [13].

Isolation and enumeration of coliforms and *E. coli*

This was done using by preparing tenfold dilution using buffered peptone water and 100ul from selected dilutions (i.e. 10^{-4} and 10^{-5}) were picked, inoculated on MacConkey agar (Europharm, Spain) which was then incubated at 37°C for 24 hours. Pink colonies for coliforms whereas red colonies for *E. coli* were observed and counted. Confirmation of *E. coli was* done by the Indole test, methyl red and citrate utilization.

Isolation and enumeration of *Staphylococcus aureus*

Tenfold serial dilution of the samples were made using Buffered peptone water (ISO). Hundred (100) µl of the 10^{-4} and 10^{-5} dilutions were plated on Mannitol Salt Agar (Europharm, Spain) and surface spread. The plates were then incubated at 37°C for 24 hours. Yellow colonies surrounded by yellow zones were seen and counted. 2 of the colonies showing these colony features were selected from each dilution plate sub cultured, gram stained and confirmed by Nitrate reduction, urease production

and coagulase [14].

Detection of *salmonella species*

The procedure was done basing on ISO 6579: 2003 methodologies. Hundred (100) ul of the original sample was inoculated into 9.9ml of Rappaport media (Oxoid limited, England), incubated at 42°C for 24 hours then followed by inoculation onto XLD agar (Mast Group Limited, UK) with further incubation at 37^{0}C for 24 hours. Red yellow colonies with black centers were observed. A colony with the distinct features was then picked and sub-cultured then confirmed using Indole, methyl red, citrate and hydrogen sulphide production [13-15].

Milk ring test (MRT) for brucellosis

The test was carried out by adding 30ul of the *Brucella* antigen to 2ml of the unpasteurized milk then incubated at 37^{0}C for 1 hour. A blue ring above a white milk column indicated positive whereas no ring indicated a negative test [16].

Quality management

The systematic random sampling method was used to collect a representative sample from the milk sold. Aseptic techniques were employed during processing to prevent any possible foreign contamination. Proper labeling and identification was done for the different samples going to be processed. The media was prepared and sterilized by autoclaving at 121ºC for 15 minutes before pouring it on the petri dishes to cool. The samples were processed as soon as they are collected to prevent any disruption to its integrity.

Data analysis

The results were recorded in Microsoft Excel software and exported to SPSS.16.0 for analysis. In this case ANOVA and chi square statistical tests were performed. Then tables were drawn to demonstrate trends and comparisons of the results. The bacteriological indices determined were compared with acceptable levels set by the UNBS and WHO.

Ethical consideration

An ethical clearance was sought from College of Veterinary Medicine, Animal Resources and Biosecurity Ethical Review Committee. In addition, the study was further approved by student's research review committee of the College.

Results

The mean total aerobic counts ranged between 7.3x10^{7}cfu/ml and 1.67x10^{8}cfu/ml. The mean count was highest in samples got from Kalerwe and lowest in samples got from Mulago (Table 1). However there was no significant difference (p= 0.515) in the mean counts of samples from all places as shown in Table 4. The total aerobic counts of all the milk samples obtained from all places did not meet the UNBS standards for milk meant for human consumption.

The mean *Staphylococcus aureus* counts ranged from 1.97x10^5 cfu/ml to 1.97x10^{8}cfu/ml. Results showed that samples obtained from Kawempe had the highest mean counts and those from Mulago had the lowest mean counts. Comparison of mean counts of the samples from the different places showed a significant difference (p=0.039, ANOVA) in *Staphylococcus aureus* counts (Table 4). Mulago area recorded the highest number of samples that passed the UNBS standard for *Staphylococcus aureus* counts (60%), followed by Kawempe (20%), Bwaise (10%), Kamwokya (10%) and lastly Kalerwe (0%) as shown in Table 1.

Discussion

The total aerobic count reported in the study was generally high for all the five study areas with Kalerwe having the highest whereas Mulago having the least this could probably be due to the high numbers of rubbish heaps that originate from the market. The study corroborates with [17] which reported an exceptionally high total aerobic as well as coliform counts. The high total aerobic counts were probably due to poor hygiene and management practices whereby the milk was kept at temperatures higher than 4°C, milking of very dirty cows and in addition the cows could have been suffering from mastitis [18]. Kamwokya had the highest *staphylococcus* counts whereas Mulago had the lowest counts (Table 4) which could probably be due to the fact the milk received in Kamwokya was got from mastitis infected herds.

Staphylococcus aureus is often found in raw milk and its products due to contamination caused by poor hygiene conditions or the origin of the milk, which can come from mastitic cows. According to a study done by the National Research Institute in Poland, 32% the bulk tank milk contained *Staphylococcus aureus*. This prevalence being much lower than that of this study which reported a prevalence of 40%-100% could be due to variation in environmental conditions or better enforcement of milk hygiene standards in developed countries.

The very high counts in Bwaise could probably be due to its location in a swampy area. The high coliform count in the different areas could probably also be due to poor hygiene practices whereby the milk gets into contact with feces of healthy animals, use of equipment that are not cleaned thoroughly, milking of dirty cows or cows washed with contaminated water. Since coliforms are mastitis causative agents it could also be due to milking of cows infected with mastitis. Studies in Tanzania by Swai (2011) reported a coliform count of 3.0x10^6 which is much lower than that reported in this study. This could be due to the good hygiene practices and enforcement exercised in Tanzania. According to Bramley and Mackinnon (1990), coliforms multiply rapidly in the environment; therefore their presence in high numbers in food products is conclusive of improper handling and contamination. *E. coli* is the most prominent fecal coliform and its prevalence is indicative of fecal contamination of the milk. *E. coli* is a reliable parameter for fecal contamination of water sources and hence its presence in milk is not only an indicator for fecal contamination but also an indicator for over all sanitary condition of the dairy farms and the different selling points (bulk tanks). With revolutionary advances in modern medicine, there has been heavy reliance on the availability of effective antibiotics to manage infections and enable invasive surgery due to emergence of antibiotic-resistant bacteria, however novel approaches are necessary to prevent the formation of biofilms on sensitive surfaces such as antimicrobial nanomaterials that are derived from biological polymers or that rely on the incorporation of natural compounds with antimicrobial activity in nanofibers made from synthetic materials is timely, that will help to hinder these micro-organisms [19].

According to studies elsewhere, the prevalence of *E. coli,* especially the Enterohemorrhagic *E.coli* (EHEC), strain is usually very low, less than 1%. For example in July 2007, US Department of Agriculture (USDA) reported that nearly a quarter of the raw milk collected from 861 farms in 21 states contained bacteria

linked to illness. Among the results 3% of the samples had *Salmonella* and 4% had types of *E. coli* that cause diarrhea and gastrointestinal illness. Less than 1% had the most dangerous forms of *E. coli*. However in the presence of unhygienic handling practices of milk and the numerous opportunities for contamination from the producer to the consumer, the potential for their propagation and dissemination is real. According to Grimaud (2008), Enterohemorrhagic *E.coli* (EHEC) was present in raw milk in Kampala city which was in agreement with Grace (2008), who indicated that EHEC exists in Kampala city and hence Kampala milk is unsafe. However according to [20], the prevalence of EHEC was less than 1%.

Kawempe had the highest salmonella counts. Salmonella contamination of milk could probably be due to poor sanitation in terms of equipment which could have got into contact with fecal matter. Salmonella gets into milk majorly through contamination with both human and animal feces; it can also get into milk through handling teats with dirty hands by the milkers. According to studies in Tanzania [21], Salmonella prevalence was reported in 10.1% which is lower than the prevalence of salmonella reported in this study. This could indicate higher levels of poor hygiene and fecal contamination in Kampala.

According to [22], 56% of the samples at the collection points in Tanzania were positive for *Brucella*; a prevalence value much higher than reported in this study. This could probably be due to high levels of vaccination in Uganda compared to Tanzania possibly leading to more *Brucella* free herds.

Conclusion

The study shows that there is a considerable level of bacterial contamination in raw milk especially *E. coli*. Most of the milk samples did not meet the safety criteria recommended by the World Health Organization and the Uganda National Bureau of Standards. These levels may be capable of causing disease to humans which is therefore a health risk to milk consumers. This calls for measures by the dairy farmers, milk transporters and sellers as well as health inspectors to minimize this problem.

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Authors' contributions

JNK, EN, JGN, and MS conceptualized the project, performed most of the laboratory experiments and wrote the manuscript. CA assisted in statistically analysing the data. JGN assisted in drafting and finalizing the manuscript. All authors read and approved the final manuscript.

Tables

Table 1: Conformity of microbial counts of milk samples with UNBS standards

Parameter	Bwaise		Kawempe		Kalerwe		Mulago		Kamwokya	
	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail
Total aerobic counts	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
S. aureus counts	10%	90%	20%	80%	0%	100%	60%	40%	10%	90%
E. coli counts	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
Coliform counts	20%	80%	0%	100%	0%	100%	0%	100%	0%	100%

Key: The mean total coliform counts ranged from $1.9690x10^8$cfu/ml to $4.3120x10^8$cfu/ml with Kawempe having the lowest mean counts and Mulago having the highest value. Comparison of the different parishes showed that there was no significant difference the mean total coliform counts (p=0.700). The mean *E. coli* counts ranged from $1.24x10^8$cfu/mlto $3.63x10^8$cfu/ml. Kawempe had the highest mean counts and Kalerwe had the lowest mean counts. Comparison of mean counts of samples from the different places showed that there was no significant difference (p=0.466) in the mean of *E. coli* (Table 2).

Table 2: Mean microbial counts in milk samples from the different parishes

Parameter(cfu/ml)	Sampling sites					P-value
	Bwaise	Kawempe	Kalerwe	Mulago	Kamwokya	
Total aerobic counts	$1.0320x10^{9\pm}7.51x10^8$	$1.2740x10^{9\pm}7.85x10^8$	$1.6710x10^{9\pm}2.38x10^9$	$7.3800x10^{8\pm}3.59x10^8$	$1.0670x10^{9\pm}4.80x10^8$	0.515
S. aureus counts	$1.9870x10^{6\pm}4.59x10^6$	$1.9690x10^{8\pm}1.76x10^8$	$1.3932x10^{8\pm}3.42x10^8$	$1.1080x10^{6\pm}2.75x10^6$	$2.2760x10^{8\pm}2.42x10^8$	0.039
E. coli counts	$2.9124x10^{8\pm}3.44x10^8$	$3.6300x10^{8\pm}5.52x10^8$	$1.2350x10^{8\pm}6.96x10^7$	$2.3750x10^{8\pm}2.30x10^8$	$1.6500x10^{8\pm}1.59x10^8$	0.466
Coliform counts	$3.2280x10^{8\pm}3.74x10^8$	$1.9690x10^{8\pm}1.76x10^8$	$3.1000x10^{8\pm}3.42x10^8$	$4.3120x10^{8\pm}6.47x10^8$	$2.2760x10^{8\pm}2.42x10^8$	0.700

Key: Out of the 50 analyzed milk samples, 52% contained Salmonella. Samples from Bwaise and Mulago contained the highest percentage of Salmonella (80%) this was followed by Kawempe which had 10%, Kamwokya and Kalerwe samples did not have any Salmonella. There was a significant association (p=0.000, Chi Square) between sampling locations and Salmonella prevalence (Table 3).

Table 3: Salmonella prevalence in milk samples from the different sampling sites

Location	Samples (N)	*Salmonella spps* status	
		Present	Absent
Bwaise	10	80.0% (8)	20% (2)
Kamwokya	10	0% (0)	100.0% (10)
Mulago	10	80.0% (8)	20.0% (2)
Kawempe	10	100.0% (10)	0% (0)
Kalerwe	10	0% (0)	100.0% (10)
TOTAL	50	52.0%	48.0%

Key: X^2: 37.179, P: 0.00: spps: Species, N: Sample Size

The samples from all different collection points did not contain any *Brucella* antibodies as shown in Table 4.

Table 4: *Brucella* antibody prevalence in milk samples from the different parishes

Place	*Brucella* Status		Total
	Present	Absent	
Bwaise	0%	100.0%	100.0%
Kamwokya	0%	100.0%	100.0%
Mulago	0%	100.0%	100.0%
Kawempe	0%	100.0%	100.0%
Kalerwe	0%	100.0%	100.0%
Total	0%	100.0%	100.0%

Key: %:Percentage

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