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Bacteriological Profile and Safety of Soured Milk in Uganda

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Abstract

Background: Soured milk has an outstanding nutritional quality and highly consumed by people in Kampala and Uganda. However, it is an excellent medium for pathogen bacterial growth and an important source of bacterial infections when poorly handled. A cross sectional study was carried out to assess for occurrence of pathological bacterial species in soured milk sold in retail dairy shops in Makindye, Kampala, Uganda. A total of 174 soured milk samples were purposively collected and analyzed using by standard bacteriological methods. 89.1

Index terms— soured milk, bacterial species, food safety, contamination, bacteriological profile.

1 I. Introduction

lobally, 550 million cases and 230,000 deaths happen every year due to consumption of fresh agricultural products and dairy products contaminated by non Typhoidal salmonella, pathogenic Escherichia coli, Campylobacter and Norovirus.

Diarrheal diseases account for more than half of the global burden of food-borne diseases more especially among children below 5 years of age **??**WHO, 2015). Milk has for long been a delicacy for many communities, however, its complex biochemical composition, high water activity and nutritional value makes it an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exist (Parkh and Subhush, 2008).

Humans may be infected with milk-borne pathogens through consumption of infected raw or unpasteurized milk and milk products (Belli et al., 2013).Unpasteurized milk sold to the public enters either directly from producers, via informal markets or through dairy farmer cooperatives and resources are extremely limited, yet retail production is underdeveloped with low levels of hygiene (Knight-jones et al., 2016). Some of the pathogenic microorganisms commonly solated from milk and milk products posing a serious threat to human health include Escherichia coli, staphylococcus aureas, Salmonella species (spp), Listeria monocytogen, Brucella abortus, Mycobacterium spp, campylobacter spp, Lyptospira spp, Clostridium spp, Pseudomonas arginosa and Proteus spp (Kanyeka, 2014). According to a study that was done by Abel, 2016, Escherichia coli were found to be the dominating coliform in soured milk. Specifically, a strain of Escherichia coli (E coli O157:H7) (CDC, 2016).

Cow's udder, barns, milk collection equipment and materials, various ingredients added to the dairy products and dairy farm workers are some of the sources of bacterial contamination in soured milk (Abera et al., 2016). Coliforms which are the target of the present study have been reported to contaminate soured milk mainly as a result of using poorly sanitized utensils during milking, transportation and storage processes **??**Garedew et al., 2012).

In countries with poor milk production and distribution practices, one can expect a higher percentage of bacterial contamination which poses a health hazards such as Typhoid fever by salmonella and bloody diarrhea by E coli **??**Garedew et al., 2012). In the current study area in particular and Uganda in general, there is lack of published information on the extent of soured milk contamination by Salmonella species, and Enterohaemorrhagic Escherichia coli, yet soured milk is favored over fresh milk in this setting (Knight-Jones et al., 2016).

In many of the low income countries, raw milk and its products is found to be contaminated with potentially pathogenic E coli strains which impose a serious public health threat to humans **??**Ombarak et al., 2016). In a

recent study done in Uganda by the Diary Development Authority (DDA) in Kasese district, milk sold to the public was found to be adulterated and contaminated with pathogenic bacteria (DDA, 2017). If nothing is done to improve the quality of dairy products sold in public centers, consumers will continuously be predisposed to potentially pathogenic organisms that are usually present in contaminated milk.

The dairy market in Uganda is mainly dominated by small-holder or retail dairy business holders with low levels of hygiene. The information generated by this study will be used by relevant regulatory bodies such as the Uganda Diary Development Authority to scale up monitoring and evaluation of operations of dairy businesses in Uganda.

In addition, design interventions that can help to improve the quality of dairy products sold out to the public. The study sought to assess for occurrence of selected common bacteria species associated with contamination of soured milk sold out in retail dairy shops in Kampala city, Uganda.

2 II. Methods and Materials

Study Design and Setting: This was a cross sectional study that was carried out during the months of May to July, 2017. The study was conducted in Makindye Division. Makindye division is one of the divisions that make up Kampala capital city of Uganda. Sampling Method: The study population was soured milk and milk retailers. Soured milk sellers in retail daily shops who sell soured milk at the time of sampling, and were residing in Makindye division were included in the study. Sellers who declined informed consent were excluded.

Purposive sampling technique was used. In this case, only retail milk sellers were selected for participation in study until the sample size was reached.

3 a) Data Collection Method and Tools

This study employed quantitative data collection. A Questionnaire and an Observational checklist was developed using some of recommended general principles codex of food hygiene **??**FAO, 2003) for the general hygiene of the milk retailers and premises, washing processes, their knowledge and attitude towards proper handling of soured milk, sources of water used and general upkeep of the soured milk. The organization of the questionnaire and observation check list was in accordance with the specific objectives of the study.

4 b) Sample Collection

This involved collection of soured milk and performing laboratory analysis on the samples. A total of 174 soured milk samples were collected from the diary retail shops in the selected markets and areas of Makindye division.

5 c) Soured Milk Sample Handling

Soured milk was collected into sterile containers, given lab numbers and immediately transferred in a courier cold box with ice before being delivered to the school laboratory at International Health Sciences University. A guided questionnaire/ observation check list was also checked during the process of collecting samples. Samples were then aliquoted into sterile glass containers in the laboratory. Samples were then examined for the presence of selected common bacterial species with main emphasis on Coliforms and specifically Enterohaemorrhagic Escherichia coli O157:H7.

6 d) Microbiological Analysis

Laboratory analyses were carried out at the International Health Sciences University microbiology laboratory. In the Laboratory, serial dilutions were made for each sample for coliform counting on MacConkey agar, and also for identification lactose and non-lactose fermenters.

7 e) Coliform Counting

A total of 10 tubes were dispensed with 9 ml of sterile normal saline. Tenfold serial dilution of the sample from 10-1 to 10 -10 in sterile normal saline solution was done. Then, 1 ml of soured milk sample was added into the 9 ml normal saline (10-1 dilution); the 1 ml of resulting solution was transferred into a second tube containing 9 ml of normal saline (10-2 dilution) and the procedure was repeated for more dilutions as shown in figure **??**. The dilutions were mixed using a vortex mixer for 10 seconds.

0.1ml of each dilution was poured on a MacConkey agar plate, and spread with a sterile glass spreader on the media. Plates were allowed to dry with their lids on and then they were inverted and incubated at 35 0 c under aerobic conditions for 24 hours to allow bacterial growth.

A new sterile pipette was used for each dilution and the procedure was repeated as above with further dilutions up to the last dilution and for the remaining test samples, where consecutive critical dilution steps were chosen for the inoculation on plates. After incubation period, coliforms on MacConkey agar were counted manually and findings were recorded (ISO4833-1:2013).

The cfu/ml of sample was determined using the formula below **??**Ogot, Ochoudho and Machoka, 2015).

8 cfu/ml of Sample = Number of Colonies

Amount Plated x Dilution Factor

9 f) Identification of Bacteria on Different Media

Samples were incubated at 35 o C-37 o C for 24 -48 hrs, and colonies that appeared pink/red on MacConkey were acknowledged to be lactose fermenters while the colorless colons were registered as non-lactose fermenters. Isolated colonies where then sub-cultured on different bio-chemicals. These included the catalase test, coagulase test, triple sugar iron agar (TSI), Cled, Citrate, Indole, BA for identification of Shigella spp, Enterobacter spp, Klebsiella spp, Salmonella spp and E coli.

10 g) Organism Confirmation

This was done in compliance with standard biochemical tests including TSI, Sulfide Indole Motility (SIM), Citrate agar and methyl red Voges Proskauer (MRVP), Urea and Cled agar (Pizarro et al., 2013). The presumptive EHEC colonies were then confirmed using commercial E coli O157:H7 strains anti-sera (Oxiod Basingstoke, UK) based on detection of EHEC antigens. Salmonella species were confirmed by sub culturing them on XLD, where the colonies present with black centers.

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Year 2018 (D D D D) L h) Data Analysis

Samples collected, were given identification numbers, recorded and later stored in both soft and hard copies in the laboratory manual. Data was cleaned, coded and entered into the statistical package for social sciences (SPSS) version 20. All statistical tests were two-tailed and a P-value of less than 0.05 was considered significant.

12 i) Quality Control for Laboratory Methods

Prepared media was sterilized by autoclaving at a temperature of 121°C for 15 minutes. Complete sterilization was confirmed by using autoclave tapes attached to conical flasks containing media for sterilization. Aseptic methods were used during casting of culture media onto petri dishes to minimize contamination and the agar was incubated for 16 to 18 hours to check for cases of contamination. The experiment was carried out in duplicates and positive control strains of Salmonella species and Escherichia coli were obtained from Lancet laboratory Uganda and used.

13 j) Ethical Considerations

The research protocol was cleared by the research and ethics review committee of the Institute of Allied Health Sciences of International Health Science University. Permission was then granted by the Makindye division mayor to proceed with data collection. The research was conducted while upholding the moral, tradition and customary rules and regulations of the community in a manner that did not compromise the scientific inclinations of the research. Scientific standards in the methods employed in the collection and analysis of data were maintained. All study participants provided informed consent.

14 III. Results

A total of 174 samples of soured milk were examined to assess the occurrence of selected common bacteria species and risk factors associated with contamination of soured milk sold out in retail dairy shops in Makindye division, Kampala district-Uganda.

15 a) Common Bacterial Isolates in Soured Milk

Amongst the 174 samples, 89.1% (n=155) showed bacterial growth with total plate counts greater than 25 cfu/ml, the limit acceptable by the East African standards (EACs, 2008). Only 10.9% (n=19) showed no bacterial growth even after 48 hours of incubation. Of the 155 samples that had bacterial growth, the most commonly isolated were E coli (47.1%); 44 samples (28.4%) had Klebsiella spp and 18 (11.6%) had Shigella spp. The other bacterial species that were found in soured milk included Salmonella spp (7.1%) and Enterobacteria faecalis (5.8%).

Notably, Enterohaemorrhagic E coli strains were not found in all the soured milk samples analyzed. Some samples had more than one type of bacterial isolate, however the common ones where E coli and Klebsiella spp.

16 b) Demographic Factors Associated with Contamination of Soured Milk

Majority (41.4%) were in the age group of 21-30 years; 81.6% were female while the rest were male. Kisugu had the highest number of respondents (29.3%) and at least 125 (71.8%) of all respondents had attained secondary education. Majority (98.3%) reported to have had formal training on milk handling mainly through radios

(51.1%) and many of the respondents (85.7%) had an experience of 1 year and above in milk handling (Table **??**).

Age (? 2 =55.3; P value <0.001), level of education (? 2 = 30.9; P value<0.001), sources of information on milk handling (? 2 =4.5; P value=0.001) and work experience (? 2 =21.6; P value <0.001) were significantly associated with microbial contamination of soured milk. There was no statistically significant difference in the level of bacterial contamination of soured milk obtained from female or male retail milk sellers. Also, there was no statistically significant difference in bacterial contamination of soured milk collected from milk sellers who reported having been formerly trained in milk handling and those that never received formal training (? 2 =0.35; P value=0.110) (Table **??**).

Notably, milk sellers of age 21-30 years and those between 31-40 years were 18 and 31 times respectively more likely to have bacterial contamination of soured milk than those of 41 years and above.

With respect to the level of education, retail milk sellers who had reached the primary and secondary levels were 1.6 and 13.5 times respectively more likely to have bacterial contamination of soured milk than those who had reached the university level.

The level of contamination of soured milk collected from milk sellers who obtained knowledge about milk handling from radios and those trained by community health workers was almost similar (OR=1.03; 95% CI=0.3-3.4); while working experience had a significant impact on the level of bacterial contamination of soured milk. In this study, milk sellers who had 1 to 5 years of working experience were 6.5 times more likely to have contamination of soured milk than those whose working experience was more than 5 years (Table **??**).

17 c) Hygienic Related Factors Associated with Contamination of Soured Milk

All respondents had at least a refrigerator for storage of soured milk. The main source of water for soured milk preparation reported by the majority (88.5%) was tap water followed by wells (9.2%). Most of the soured milk was sold off between the second and third day after preparation. Majority of the respondents (89.7%) didn't have hand washing equipment and didn't wear protective gears (97.7%). There was also presence of pests (house flies) in some areas. All in all the general cleanliness was regarded as poor (Table **??**I).

In this study, longevity of soured milk (? 2 =10.2; P value=0.004), availability of hand washing equipment (? 2 =2.3; P value <0.001), presence of pests at facility (? 2 =2.8; P value=0.001) and general cleanliness (? 2 =12.6; P value=0.005) were significantly associated with contamination of soured milk. There was only slight association between wearing protective gears and contamination of soured milk (? 2 =0.5; P value=0.052); whereas no statistically significant differences in the level of contamination of soured milk were observed when tap water or other source of water was used in soured milk preparation (Table **??**I).

Notably, for most of the hygienic related factors under consideration, it was not possible to derive an odds ratio because some of the cells included in the 2x2 contingency tables had zero integers. The only odds ratio that was calculated was for estimating the relation between having pests at the facility and contamination of soured milk. Surprisingly, milk sellers who had pests at the retail facility were less likely than those who did not have pests at the facility to have had contamination of their soured milk (OR=0.38; 95%CI=0.14-2) (Table **??**I).

18 IV. Discussion

80% of milk sold in Uganda goes through informal markets and such milk may pose a health hazard due to contamination with pathogens. Generally, it was found that soured milk was highly contaminated with pathogenic bacteria exceeding the recommended 25 cfu/mL of total aerobic count as per the East Africa community standards **??**EACs, 2008) This calls for an urgent need for improvement of milk handling protocols among the milk distributors and handlers in Uganda. Soured milk is popular delicacy in Uganda. Therefore, the high bacterial contamination pose a considerable threat to public health.

Findings of this study could reflect poor levels of hygiene displayed in preparation and handling of soured milk, since coliforms are mainly of faecal origin. This contamination could occur during harvesting, storage or during distribution of milk to the customers at the diaries. Microbial quality of food is very important measure of the safety of the food for human consumption. The presence of Salmonella spps, E coli, Shigella spps, Entrobacteria, Klebsella pneumonae indicate that the food is hazardous and is not fit for human consumption. On the other hand, presence of non-pathogen bacteria in food doesn't necessitate unfitness for consumption, but may indicate the hygiene status of the preparation and processing. However, certain levels may indicate a serious case of poor hygiene and the food becomes unfit for consumption.

These findings are similar with findings from many others studies that were done in Africa and Uganda that reported exceedingly high bacterial contamination (Knight-Jones et al., 2016; **??**elli et al., 2012;Kanyeka, 2014). Elsewhere, similar studies have also demonstrated that milk products such as soured milk commonly contain similar bacterial isolates to the ones identified in this study **??**Lu et al., 2013). however percentages of bacteria isolated was not given. Only in a longitudinal study that was carried out in Khartoum state in South Sudan during the dry and rainy seasons in April 2008 to February, 2009 where more than half of the milk (51.3%) sold for direct consumption by vendors and shops in Khartoum state conformed to the internationally accepted standards for total coliform counts (<100 cell/ml). The difference in the level of contamination in comparison

to the present study could relate to the season in which samples were collected since samples that were used in the present study were collected during the dry season. High bacterial contamination rates of milk are usually observed more in dry season than in rainy or wet seasons (Adjlane-Kaouche et al., 2014).

Although few studies have investigated the relationship between demographic factors and bacterial contamination of milk, the findings from this study show that age, level of education, source of information on milk handling and work experience were statistically associated with contamination of soured milk. Majority of the soured milk samples were obtained from the informal milk sector that is to say from diaries which employ persons with low education and usually in family businesses. Notably, milk sellers in the age bracket of 21-30 years and 31-40 years were more likely to have contamination of soured milk than those above 40 years. The effect of age on milk contamination may correlate to the level of training and working experience. Usually young people have worked for fewer years and probably have achieved little training on the aspects of their work yet learning and attaining expertise may come with experience. This may explain why more contamination of soured milk was evident in samples that were collected from the young retail milk sellers. This is also in line with the findings from the present study in which milk sellers with fewer years of working experience (1-5 years) reflected more bacterial contamination than those who had working experience of more than five years.

Regarding the level of education, milk sellers of lower academic levels (primary and secondary) were more likely to have contamination of their soured milk than those who had reached university level. Again this observation may relate to the level of knowledge regarding milk handling and hygiene demonstrated by the University retailers as compared to the primary and secondary level milk retailers. More likely, those with lower education levels may not have additionally obtained sufficient training on milk handling, thus the higher chances of milk contamination. Even though majority of the respondents reported to have received some formal training in milk handling and had knowledge about bacterial contamination of milk, given the fact that they elaborated that "they boil the milk to kill of the bacteria after purchasing the milk", the high level of microbial contamination found reflects poor practices of handling milk leading to bacterial contamination. Presumably, the procedure that they use to make soured milk, the utensils and water used could be the source of contamination. This is also in line with a study by Salman and Hamad (2011) in which the lack of knowledge regarding clean milk production, and potable water for cleaning purposes were some of the factors which contributed to the poor hygienic practices that led to contamination of milk. Another study that was done in Mbarara district also reported a significantly high proportion of farmers who had never received formal training on best milk handling practices **??**Kagoro et al., 2016).

In this study, there was no difference in the level of soured milk contamination sold by female or male retail sellers. Even though relationship between sex and contamination of milk in the Mbarara study was not investigated, it was noted that majority of the milk handlers (74.9%) were female and rest being male (Kagoro et al., 2016).

Notably, the fact that the present study noted significant relationships between demographic factors and soured milk contamination, yet there is very limited information on the subject; the present findings highlight the need for thought and further exploration of the influence of demographic factors on soured milk consumption. This may provide more insights into understanding the risk factors of contamination of milk besides the hygienic related factors.

In this study, longevity of soured milk, availability of hand washing equipment, presence of pests at facility and general cleanliness were significantly associated with contamination of soured milk. Unsafe practices such as not washing hands, sell of expired milk and access of pests to containers of soured milk leads to introduction of microorganisms to the milk. Food borne pathogens can be carried by the hands of the milk handlers, pests or they may develop over time as the milk is kept longer in the storage (WHO, 2015).

In the current study, respondents were observed preparing and storing soured milk in Jerri cans and polyethene bags, a similar case that was reported by Tumushabe, (2013). Similar findings were also reported from studies that were done in Gambia, Senegal and Guinea in which raw and soured milk were found to be highly contaminated. In these studies, the high microbial contamination was attributable to the poor hygiene arising from poor handling at the farm, at collection centers, during transportation and at retail points and more often by faeces (of animal or human origin), personnel, water and containers that are not properly sterilized. More often milk is packaged in discarded vegetable oil jerry cans for easy transportation; yet such jerry cans are difficulty to clean since the opening is too small to enter with hands or cleaning tools. Additionally, it was observed that some milk men and milk traders use cloth to strain out the flies and dirt, but those cloths are not thoroughly washed. Usually, milk vendors at the markets use spoons or cups as measuring tool, which also are not cleaned properly during the day but rather simply wiped with some piece of cloth (Hempen et al., 2004), a similar practice seen in the present study.

Additionally, one of the respondents kindly explained that to quicken the process of making soured milk, "a left over soured milk is added to fresh milk so that it can ferment faster". Similar techniques have also been reported by Tumushabe, (2013) in ghee processing in western Uganda. This method is so hazardous since the fresh milk will also be contaminated by bacteria carried from the previous batch of soured milk. In this regards use of artificial acidifier such as citric acid would be recommended.

Also in the recent study done in Kasese by DDA similar observations where reported (DDA, 2017) however they never went into the details to confirm which common pathogen was predominate in milk.

Many of the selling points had a lot of pests in particular house flies which aid in the contamination of soured milk and food in general and of recent, studies demonstrated that adults house flies that feed on food contaminated with various levels of food borne bacteria were able to transmit those pathogens to their eggs and in the process mitigate the spread of food borne pathogens **??**Monica et al., 2015).

Furthermore, other studies in Zimbabwe, Tanzania and Ghana reported that unhygienic practices along the milk value chain predisposed milk to high bacterial load and recently it was noted by Knight-Jones et al., (2016) upon arrival at point of sale, poor hygiene led to high bacterial load with almost all samples culture positive for Stapylococcus aureas and E coli. This is also in line with some other studies that demonstrated poor hygiene practices such hand washing and pests in the milk stores as compared to those at the production levels (Abera et al., 2016). Disassa (2017) observed that in Asosa town Ethiopia milk samples were produced and handled under poor hygienic conditions and Bonaventure et al., **??**2015) found that milk samples had added water to them in a study done in Zambia **??**Bonaventure et al., 2015).

Overall, the risk of food borne diseases is most severe in low-and middle income countries and is linked to preparing food with unsafe water, poor hygiene and inadequate conditions for food production and storage, lower levels of literacy and education and insufficient food safety legislation and implementation of such legislation (WHO, 2015) and in the current study the poor levels of hygiene were found to predispose the soured milk to bacterial contamination; the water used in preparation, the selling site/ residence and storage container among others were shown to have a direct relationship with the bacterial status in the soured milk.

This study had some limitations that need to be mentioned; sampling of milk was not done at farm level, during transportation, from milk collectors and whole sellers and thus the probable bacterial contamination at all these check points was not evaluated. This has a limitation on determining the point of contamination by selected common bacteria spieces, since some of the milk might be contaminated before it reaches the distribution point. The authors recommend routine mandatory assessment of milk quality in order to safeguard the public from milk-borne infections. In addition, periodic surveillance visits to dairy retail shops and health education on milk handling practices is needed to prevent milk contamination. Milk supply chains should be regulated in a way that certified, trained and licensed suppliers by DDA should distribute milk to the retail shops.

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Figure 1: Table 2 :

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.1 Acknowledgement

We would like to thank the Institute of Allied Health of International Health Sciences University for their assistance.

.2 Areas of Further Research

There is limited information regarding assessment of risk factors for soured milk contamination; thus other studies investigating the risk factors here in mentioned in other settings is highly recommended.

.3 Conflicts of Interest

The authors declare that they have no competing interests.

.4 Funding Disclosure

The Authors received no funding for this project.

.5 Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

.6 Practical Application

All the bacteria isolated were coliforms indicating probable faecal contamination of the soured milk as a result of poor hygiene. This indicates that soured milk sold by small retail diary shops is highly contaminated with pathogenic bacteria. There is a need for improvement in soured milk handling so as to ensure that the quality of soured milk sold out to the public is acceptable and safe for human consumption.

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[Table 1: Demographic Information of the Study Respondents Characteristics of the Respondents and Occurrences of Isolated Ba *Table 1: Demographic Information of the Study Respondents Characteristics of the Respondents and Occurrences of Isolated Bacterial Species in Makindye*, Kampala, Uganda.

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