Prevalence of *Salmonella spp.* and *Escherichia coli* in raw milk value chain in Arusha, Tanzania

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Abstract

This study aimed to investigate the prevalence of *Salmonella spp.* and *Escherichia coli* (*E. coli*) in milk value chain in Arusha, Tanzania. A total of 75 raw milk samples were collected from smallholder dairy farmers, street vendors and outlet shops in Arusha and Arumeru districts. A questionnaire survey was also used to assess hygienic practices along the chain. *Salmonella* and *E. coli* were detected in 28/75 (37.33%) and 68/75 (90.67%) samples, respectively. Bangata ward in Arusha showed relatively high prevalence of *Salmonella spp.* (42.11%) while Akeri ward in Arumeru showed relatively low prevalence (31.58%). In milk value chain, the highest prevalence was observed in street vendors (43.75%) while the lowest prevalence was in dairy farms (33.33%). Mean count for *E. coli* from milk producers, vendors and shops were 3.0×10^3, 8×10^3 and 6.6×10^3 cfu/mL, respectively, indicating a significant ($p < 0.05$) increase in *E. coli* load along the chain. Furthermore, confirmatory test showed that *Salmonella* isolates were predominantly identified as *Salmonella enterica* serovar Arizonae. Besides, *Salmonella* and *E. coli*, other enterobacteria detected were *Enterobacter cloacae*, *Klebsiella pneumonia*, and *Serratia marcescens*. Taken together, qualitative and quantitative findings revealed that poor animal husbandry, poor hygienic practices, lack of refrigeration and less awareness of the zoonotic pathogens had a significant impact on the prevalence of detected bacteria, posing a public health risk.

Key words: Raw milk, *Salmonella spp, Escherichia coli*, prevalence, Arusha

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Introduction

Arusha is among the areas in Tanzania which produces sufficient quantities of milk and has its market due to the presence of livestock keepers such as Maasai and Meru people. Milk and milk products provide a wealth of nutritional benefits however; raw milk can harbor dangerous microorganisms which may pose serious health risks to humans. Over 200 known diseases are transmitted through eating food contaminated by a variety of agents including bacteria, parasite, viruses, and fungi (Oliver et al., 2005). In Tanzania, total annual milk production is currently estimated at 1.65 billion liters and 70% of the milk comes from the indigenous cattle kept in rural areas and 30% comes from improved cattle mainly kept by smallholder farmers (Njombe, 2011). In developing countries such as Tanzania, more than 80% of the milk consumed is informally marketed as loose, raw milk (Kilango et al., 2012). Milking and milk handling practices in informal sector are done commonly without observing hygienic practices. It is a common practice to vend milk in inappropriate milk holding and storage equipment. Such practice possesses a threat to public health as chances of consuming unsafe milk are very high. Since there is little or no quality control for milk produced and handled in the informal channels, there is potential risk of contamination by zoonotic pathogens, adulterants and antimicrobial drug residues hence public health risks to consumers (Kurwijila et al., 2006). In Arusha and Arumeru districts most residents prefer to buy raw milk from shop outlets or bicycle vendors who collect milk from farmers during morning and evening hours. Generally, raw milk may be contaminated by food handlers, diseased animal, dirty milking equipment, feed, soil, faeces as well as grasses. Also, in the raw milk value chain, milk can be contaminated at any point, from milk producers to consumers. It is well known that milk-borne diseases are much higher among communities that frequently consume raw milk from communally grazed herds, such as among the Maasai community in East Africa (Arimi et al., 2005), since it's a common practice to drink raw

unpasteurized milk. Most residents prefer drinking raw milk believing that they have advantages and value such as taste and convenience over the pasteurized one (Altalhi and Hassan, 2009; Angulo et al., 2009). Pathogens involved in causing food borne diseases due to the consumption of raw milk include *Escherichia coli, Listeria monocytogenes, Salmonella, Campylobacter, Brucella abortus, Staphylococcus aureus, Bacillus cereus, Mycobacterium spp. and Clostridium botulinum.* If these pathogenic bacteria are present in raw milk, it is a major public health concern, especially for those individuals who drink raw milk frequently (Chye et al., 2004). *Salmonella* food poisoning is one of the most common and widely distributed diseases in the world, estimated to cause 1.3 billion cases of gastroenteritis and three million deaths worldwide (Ohud et al., 2012). *E. coli* is frequently a contaminating organism compared to other microbes and it is a reliable indicator of fecal contamination (Kumar and Prasad, 2010). *E. coli* is mainly abundantly in the intestinal tract of most mammalian species, including humans and cattle. Most *E. coli* are commensals, but some are known to be harmful or pathogenic bacteria, whereby causing severe intestinal and extra intestinal diseases in humans (Kumar and Prasad, 2010). In the raw milk value chain, milk producers, vendors and shop outlets can influence the prevalence of harmful pathogens in milk through poor animal husbandry, adulteration, washing equipment, udder and hands with unsafe water, storing and transportation in unhygienic condition and abuse of storage temperature. Milk contamination by zoonotic pathogens is often natural but can also occur through handling milk in unhygienic conditions (Ali, 2010). Therefore, the objective of this study was to investigate the prevalence of *Salmonella spp* and *E. coli* in raw milk value chain in Arusha region.

Materials and Methods

Study area

The study was conducted in two districts namely Arusha and Arumeru in Arusha region. The study area was selected based on the concentration of animal keepers with production of sufficient milk. The study covered eight villages from four Wards namely Bangata, Midawe (Bangata Ward), Ngiresi and Oldadai (Sokoni II Ward), Akeri and Nduruma (Akeri Ward), and

Kikwe and Nambala (Kikwe Ward). Sampled area lies between Latitude 3'000 – 3'400 and Longitude 360 - 5500 in the Eastern South of Equator.

Sample collection

The milk value chain involves different stakeholders namely milk producers (farmers), vendors and outlet shops. A total of 75 raw milk samples were collected from farmers (*n*=39), vendors (*n*=16) and milk shops (*n*=20). Samples were collected early in the morning around 6.00 to 7.00 am. Approximately 250 mL of raw milk was aseptically sampled into a sterile Scotch bottle and stored in a cool box at less than 4 °C and analyzed within six hours after collection. Peptone water, MacConkey Agar, and Xylose–Lysine Deoxycholate Agar (XLD Agar) were purchased from HiMedia Laboratories, India. API 20E Test (strips) kits were from bioMe´rieux®, SA, 69280 Marcy/Etoile-France. All other chemicals and reagents used in this study were of analytical grade.

Questionnaires survey

A survey was conducted using questionnaires to assess hygienic practices and public awareness on how zoonotic pathogens are transmitted. The questionnaires were administered to smallholder dairy farmers (*n*=39), street vendors (*n*=16), outlet shops (*n*=20) and consumers (*n*=100).

***Salmonella* pre-enrichment**

Salmonella spp pre-enrichment was carried out as previously as described by Amagliani et al., 2012. Briefly, homogenized raw milk sample (25 mL) were added to 225 mL of sterilized buffered peptone water and incubated overnight at 37 °C.

Selective enrichment

Selective enrichment was done according to the method previously described by El-Shamy et al., 2008. Briefly, 10 mL of pre-enrichment were transferred to 100 mL of tetrathionate enrichment broth and 20 mL of iodine solution (Iodine-6g and potassium iodide 5g) and 10 mL of 0.1% brilliant green solution were then added and the bottles were incubated at 42 ºC for 24 h.

Plating on solid selective media

Each selective enrichment broth bottle was well shaken and then a loopful from each was streaked onto plates of XLD Agar and all plates were then aerobically incubated at 37 °C for 24 h as previously described (El-Shamy et al., 2008). The positive colonies which showed red with or without black centers were subcultured to obtain pure colonies.

***Escherichia coli* isolation**

Escherichia coli were determined according to the method previously described by Addo et al., 2011. For each sample, dilutions were made by aseptically withdrawing 1 mL of each sample into 9 mL of 0.1% sterilized buffered peptone water, then serial dilutions were prepared. A 10 µL was drawn from appropriate dilutions and plated on MacConkey Agar. The sterile glass beads were used to spread the sample on agar, and plates were incubated at 37 ºC for 24 h. The positive colonies which showed pink colour were subcultured to obtain pure colonies.

Biochemical identification

Biochemical identification for both *Salmonella spp* and *E. coli* was carried out using API 20E Test (strips) kit (Addo et al., 2011) . An incubation box (tray and lid) was prepared and about 5 mL of distilled water were distributed into the honey-combed wells of the tray to create humid atmosphere. A single well isolated young colony (18 - 24 h) was picked up using sterile disposable pipette and emulsified in 5 mL of API Suspension medium so as to achieve a homogeneous bacterial suspension. Bacterial suspension was distributed into tubes of the strip. For the tests arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), H_2S and URE anaerobiosis was created by overlaying with mineral oil. The incubation box was closed and incubated at 37 °C for 24 h as described by the manufacturer, and the results were determined according to API 20E (Ohud et al., 2012).

Data analysis

All experiments were done in triplicates. Data were analyzed using Statistical Package for Social Sciences (SPSS) Program Version 16.0, and Microsoft Excel (2007). Analysis of Variance (ANOVA) was used to test the significant difference at $p < 0.05$.

Results

Hygienic practices and public awareness

Survey showed that 45% of consumers were aware of the potential milk borne pathogens and concerned with milk safety. Nevertheless, 65% of consumers were not aware that *Salmonella* and *E. coli* can be transmitted from animals to humans through drinking raw milk. Also, survey showed that 35% of milk producers were unaware of the zoonotic potential of the most common bacterial contaminants in milk as depicted in Table 1. Similarly, 78% of milk producers surveyed were using unboiled water to wash equipment, udder, and hands; 89% did not use detergents/disinfectants; 79% did not have milk storage facilities and 82% were not practicing good animal husbandry. Furthermore, 69% of vendors and 66% of milk shops had no good storage facilities. Milk markets for vendors in the chain were reported to be above 6 km distance (Table 1).

Table 1: Assessment of hygienic practices and public awareness

Variable	Milk producers (%) *n*=39		Vendors (%) *n*=16		Shops (%) *n*=20		Consumers (%) *n*=100	
	Yes	No	Yes	No	Yes	No	Yes	No
Use of detergents/disinfectants	11	89	18	82	21	79	-	-
Selling of raw milk	100	0	100	0	40	60	-	-
Use of refrigerators	4	96	7	93	28	72	-	-
Use of boiled water	22	78	22	78	28	72	8	92
Good animal husbandry	18	82	-	-	-	-	-	-
Awareness on risk of getting diseases	65	35	56	44	47	53	45	55
Good storage facilities	21	79	31	69	34	66	-	-
Distance to markets above 6 km	9	91	70	30	0	100	-	-

Prevalence of *Salmonella* and *E. coli*

Prevalence of *Salmonella spp* and *E. coli* are summarized in Table 2. *Salmonella spp* and *E. coli* were detected in 28/75 (37.33 %) and 68/75 (90.67%) raw milk samples respectively, indicating a significant prevalence ($p < 0.05$) in the two districts. The highest *Salmonella spp* prevalence was observed in Bangata (42.11%) followed by Kikwe (38.39%), Sokoni II (36.84%) and Akeri (31.58%). *E. coli* prevalence was high in all wards with Bangata and Sokoni II wards showing 94.73% followed by Kikwe (88.88%) and Akeri (84.21%) wards, respectively. Among the wards, Bangata showed the highest *E. coli* count of 8.0 x 10^3 cfu/mL followed by Sokoni II with 7.2 x 10^3 cfu/mL. Low *E. coli* counts of 3.4 x 10^3 and 3.1 x 10^3 cfu/mL were observed in Kikwe and Akeri wards (Table 2).

Table 3 shows *Salmonella* and *E. coli* prevalence in raw milk value chain. Highest *Salmonella* prevalence was from vendors (43.75%), followed by milk shops (40%) and milk producers (33.33%). Mean count for *E. coli* were 3.0 x 10^3, 8.0 x 10^3 and 6.6 x 10^3 cfu/mL for milk producers, vendors and outlet shops, respectively, indicating a significant ($p < 0.05$) increase in *E. coli* load along the chain. Moreover, highest *E. coli* count of 8.0 x 10^3 cfu/mL was observed from milk vendors.

Table 2: Prevalence of *Salmonella spp* and *E. coli* in Arumeru and Arusha districts

District	Ward	No. of samples	No. of positive *Salmonella spp* (%)	No. of positive *E. coli* (%)	*E. coli* count (cfu/mL)
Meru	Kikwe	18	7 (38.89) *	16 (88.88) *	3.4 x 10^3
	Akeri	19	6 (31.58) *	16 (84.21) *	3.1 x 10^3
Arusha	Bangata	19	8 (42.11) *	18 (94.73) *	8.0 x 10^{3*}
	Sokoni II	19	7 (36.84) *	18 (94.73)	7.2 x 10^{3*}
Total		**75**	**28**	**68**	

***** Asterisk within the same column indicate a significant difference in bacterial load between the wards at $p < 0.05$

Table 3: *Salmonella* and *E. coli* prevalence in raw milk value chain

Group	No. of tested samples	Positive samples *Salmonella spp* (%)	Positive samples *E. coli* (%)	*E. coli* count (cfu/mL)
Farmers	39	13 (33.33) *	35 (89.74) *	$3 \times 10^{3*}$
Vendors	16	7 (43.75) *	15 (93.75) *	$8 \times 10^{3*}$
Shops	20	8 (40.0) *	18 (90.0) *	$6.6 \times 10^{3*}$
Total	**75**	**28**	**68**	**-**

*Asterisk within the same column indicate a significant difference in bacterial load along the milk value chain at $p < 0.05$

A confirmatory test employing 28 *Salmonella* isolates identified *Salmonella enterica* serovar Arizonae as a predominant *Salmonella* serovar. Besides *Salmonella* and *E. coli*, other enterobacteria detected were *Enterobacter cloacae, Klebsiella pneumonia ssp. pneumonia* and *Serratia marcescens*.

Discussion

Raw milk in value chain is commonly distributed locally to consumers with no controlled measures to maintain the safety and quality before it reaches consumers in Tanzania. This study revealed that street vendors and outlet shops collected milk which was already contaminated at the farm level. This could be explained by the facts that 78% of milk producers surveyed were found using unboiled water to wash equipment, udder, and hands; 89% did not use detergents/disinfectants; 79% did not have milk storage facilities and 82% had poor herd structure. Maintenance of healthy dairy herds has been shown to reduce the likelihood that zoonotic pathogens will be introduced into the milk via the mammary gland or from the faeces (Commission, 2004). Contamination of raw milk could originate from surrounding environment especially during milking and milk handling, from water and milking equipment and facilities (Bille et al., 2009). Well-constructed herd structure, milking and pre-storage conditions are also determinants of the quality and safety of raw milk (Bonfoh et al., 2003). The use of safe/boiled

and portable clean water with detergent in washing milking equipment, hands and udder is a good way to remove milk remains including pathogens and, therefore, affecting the microbiological safety of raw milk (Chye et al., 2004).

This study revealed a significant increase ($p < 0.05$) in bacterial contamination along the milk chain. High levels of bacteria counts in raw milk value chain was attributed to lack of cooling facilities, use of unsafe water, inappropriate handling of equipment for milk storage and poor knowledge on good hygienic practices as revealed during the survey. It has been reported that contamination occurs in milk chain because of poor hygienic practices, lack of storage facilities such as refrigerators and long period of transportation (Vahedi et al., 2013). All utensils and equipment must be cleaned and rinsed using boiled water and detergents; and disinfected immediately after use so as to reduce milk contamination (Chye et al., 2004). According to (Njombe, 2011), potential for increased milk supply from rural areas still exists and in order to exploit, it requires improved infrastructure such as milk collection centers, power supply, cooling systems, road networks and transport facilities.

It's noteworthy that previous studies have also reported on prevalence of *Salmonella* in raw milk (Sandgren et al., 2008). Salmonellae cause enteric infection characterized mainly by gastroenteritis on humans and other animals worldwide, and sometimes in severe cases it can result in systemic infection and even death. In general, *Salmonella* prevalence observed in Arusha and Arumeru districts was relatively higher compared to 20% in Ethiopia (Tadesse and Dabassa, 2012) and 8.7% in Nigeria (Karshima et al., 2013). In contrast, the prevalence observed in Arusha and Arumeru districts was relatively lower compared 70% in India (Pant et al., 2013). Incidence of *Salmonella* in raw milk using different methods and frequency of detection was reported to range 0.17 to 28.6% (Kaushik et al., 2014). Nevertheless, *Salmonella* detection and subsequent identification is very complex and has been gone to several changes and controversies. *Salmonella enterica subsp*. Arizonae is a Gram negative bacillus and a member of the family *Enterobacteriaceae*. It was first named as *Salmonella dar-es-salaam* and subsequently reclassified as *Arizona hinshawii*, *Salmonella arizonae*, *Salmonella choleraesuis* subsp. Arizonae and finally *Salmonella enterica* subsp. Arizonae in 2002 (Schneider et al., 2009).

On the other hand, evaluation of *E. coli* in raw milk is important because of isolation simplicity and the fact that the bacterium is used as an indicator organism for faecal contamination. *E. coli* was detected in a majority of raw milk samples followed by far with *Enterobacter cloacae*,

Klebsiella pneumonia and *Serratia marcescens*. Prevalence of *E. coli* (90.67%) observed in this study was higher than 83% reported in Dar es Salaam, Tanzania (Kilango et al., 2012) however, it was relatively lower than 100% reported in Tanga, Tanzania (Swai and Schoonman, 2011). Different studies in Africa reported *E. coli* prevalence of 11.20% in Ghana (Addo et al., 2011) and 23% in Botswana with mean count of 5.4 x 10^3cfu/mL (Aaku et al., 2004). Moreover, *E. coli* count in a range of 3.1 x 10^3 to 8.0 x 10^3 cfu/mL was observed in this study whereas 6.8 x 10^3cfu/mL and 3 to > 1 x 10^3cfu/mL were reported in Malaysia (Chye et al., 2004) and Kuwait (Al-Mazeedi et al., 2013) respectively. Therefore, it is important to ensure microbiological quality of raw milk along chain to minimize prevalence of *Salmonella* and *E. coli* hence lowering risks of zoonotic diseases to the public.

Conclusion

Presence of *Salmonella spp* and *E. coli* in raw milk indicates fecal contamination due to poor animal husbandry and hygienic practices, inappropriate transportation and storage facilities, lack of cooling systems and use of unsafe water. Also, the practice of drinking raw unpasteurized milk is hazardous because it increases risk of acquiring zoonotic diseases. Findings of the present study provide insights into the magnitude and public awareness on the health risks associated with consumption of raw milk. In order to ensure safety of raw milk, regulatory authorities should establish guidelines and/or standards based on research findings to cover the entire milk chain in Tanzania.

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