



Coliforms Contamination and Hygienic Status of Milk Chain in Emerging Economies

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HIGHLIGHTS

- Local marketing of raw milk has been increased in the low and middle income countries.
- Emerging economies are at risk of illnesses from food-borne pathogens due to poor milk handling.
- The regulatory bodies from low and middle income countries are usually underfunded.
- It is necessary for the low and middle income countries to invest in infrastructures and establishments.

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Acronyms and abbreviations

 CFU=Colony Forming Unit
MPN=Most Probable Number

ABSTRACT

Emerging economies have often poor hygiene practices in traditional milk and dairy production all over the world. Therefore, pathogenic bacteria in milk pose major public health concerns especially for those communities who still consume raw milk. *Escherichia coli* and coliforms are often used as indicator microorganisms, so their presence in food implies poor hygiene and sanitary practices. The main purpose of this article is to review information on milk quality and coliform bacteria contamination associated with the production and distribution of raw milk in some of the low and middle income economies around the world. Data reported on coliform counts in milk samples from some countries categorized as low and middle income economies in Africa, South America, Asia, and Europe. Local marketing of raw milk has been increased in the low and middle income countries because of low purchasing power of most native consumers. This population is at risk of illnesses from food-borne pathogens as a result of poor milk handling along the informal milk value chains. The regulatory bodies from these low and middle income countries are usually underfunded; therefore the safety standards of the milk chain cannot be sufficiently provided, endangering public health. On the other hand, there are huge losses of milk due to microbial spoilage in the low and middle income countries. So, it is necessary for the low and middle income countries to invest in infrastructures and establishments such as potable water supply to the dairy actors, increase the electricity connectivity from national grid and off-grid, improve the transport infrastructure, and financial support of the standards regulatory institutions. Paying attention to the mentioned actions can help to improve milk safety and quality and thereby reduce the risk of the food-borne illnesses.

Introduction

Emerging economies often have poor hygiene practices in traditional milk and dairy production all over the world (Georgescu et al., 2014; Nduko et al., 2017). Now, the outlets for the selling of milk and milk products operate

under unhygienic conditions in these economies and are not adequately monitored or regulated by the relevant regulatory agencies. Therefore, under these conditions, the food-borne zoonotic hazards posed by milk and milk products are of great public health concern (Arrigoni et al., 2009; Swai and Schoonman, 2011). These outlets sell raw milk which target the majority of the poor people,

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who do not have the economic capacity to purchase high quality processed milk products like commercial pasteurized milk (Claeys et al., 2013).

Milk has been well documented as a vehicle for the transmission of a variety of bacterial diseases' agents for a very long time (Bhat et al., 2007). Therefore, pathogenic bacteria in milk pose major public health concerns especially for those communities who still consume raw milk (Fuquay et al., 2011; Giacometti et al., 2012) and by extent, milk products made from raw milk. For instance, outbreaks of *Escherichia coli* O157:H7 have been associated with a range of foods such as raw milk. The *E. coli* O157:H7 outbreaks in milk have been majorly reported in advanced economies ranging from mild diarrhea to potentially fatal hemolytic uremic syndrome, hemorrhagic colitis, and thrombotic thrombocytopenic purpura (Coia et al., 2001; Robinson, 2005). There are many reported food-borne illnesses all over the world caused by *E. coli* O157:H7 (Painter, 2013). Apart from *E. coli*, *Salmonella* spp. are also considered as the public health concern since they produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and typhoid fever (Oliver et al., 2005). There has been reported outbreaks of salmonellosis associated with the consumption of raw milk and raw milk products in the United Kingdom (De Buyser et al., 2001). In USA, the majority of food-borne disease outbreaks are related to consumption of raw milk (Langer et al., 2012). However, awareness of food-borne outbreaks as a result of consumption of contaminated raw milk in the low and middle income countries remains to be very scanty (Fuquay et al., 2011).

Coliforms are Gram-negative and non-spore forming bacteria that ferment lactose with the production of acid and gas at 35 °C within 48 h. Coliform bacteria belong to the family Enterobacteriaceae, including the genera of *Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter* (Corry et al., 1996; Dehghan Banadkouki et al., 2017; Hajimohammadi et al., 2017; Jasemizad et al., 2016; Zandi et al., 2017). Fecal coliforms are a subset of coliform bacteria that can grow at higher temperatures of 44.5-45.5 °C and also ferment lactose, resulting in production of acid and gas within 48 h (Robinson, 2005; Ugochukwu et al., 2015). *E. coli*, *K. pneumoniae*, *C. freundii*, and some *Enterobacter* spp. are considered as fecal coliforms. *E. coli* as well as coliforms are often used as indicator microorganisms, so their presence in food implies poor hygiene and sanitary practices (Arafa, 2013; Bakhshi et al., 2017).

Many economies with the low and middle income have regulatory institutions for developing and enforcing acceptable standards for foods, such as milk. As announced by the Uganda National Bureau of Standards, maximum acceptable fecal coliforms count in raw milk is <100 Most Probable Number (MPN)/ml (Fuquay et al., 2011;

Grimaud et al., 2009). While the South African standard for total coliform is 20 Colony Forming Unit (CFU)/ml with no *E. coli* detection (Lues et al., 2010). The Zimbabwean dairy regulation standards for grade A raw milk are <10³ CFU/ml for the total coliforms and <10 CFU/ml for *E. coli* (Gran et al., 2003). In Kenya, acceptable level of total coliform counts in raw milk has been set as <10³ CFU/ml (KEBS, 2007; 2010). Contrary to the standards set by the regulatory institutions in the low and middle income economies, the European Union (EU) limit for coliforms in raw milk is <100 CFU/ml (Jay et al., 2005), which is more stringent. Some countries have no milk microbial limits like India (Lingathurai and Vellathurai, 2010). However, the main challenge of the standards regulatory bodies in ensuring production, distribution, and consumption of good quality and safe milk is the lack of resources in terms of personnel and equipment (Fuquay et al., 2011; Vairamuthu et al., 2010).

The main purpose of the current article is to review information on milk quality and coliform contamination associated with the production and distribution of raw milk in some of the low and middle income economies around the world. Data reported on coliform counts in milk samples from some countries are categorized as low and middle income economies in Africa, South America, Asia, and Europe.

Coliform contamination in milk samples in some emerging economies in the world

Africa

Milk from emerging economies has been reported to contain very high coliform counts that are of public health concern. For instance in Tanzania, mean coliform counts of 3-14×10⁶ CFU/ml and high prevalence of *E. coli* O157:H7 has been reported also in milk that was examined along the informal value chain (Swai and Schoonman, 2011). In Zimbabwe, total coliform counts of 1.56–6.22 log₁₀ CFU/ml and *E. coli* counts of 1.78–2.21 log₁₀ CFU/ml were reported in milk samples (Chimuti et al., 2016). In North Africa, in Egypt, total coliforms and fecal coliforms were detected in 89.5 and 65.8% examined raw milk samples with mean counts of 1.65×10⁶ and 3.69×10⁵ MPN/ml, respectively. *E. coli* was isolated from 52.6% raw milk samples (Arafa, 2013). Similar work reported mean total coliform counts of 3.28×10²-1.4×10³ CFU/ml in Egypt with the dominant isolated coliforms of 8% *E. coli*, 14% *Salmonella* spp., and 15% *Yersinia enterocolitica* (El-Leboudy et al., 2014). Belbachir et al. (2015) stated that the average counts of the total coliforms and fecal coliforms in milk samples of Morocco were as high as 2.6×10³ and

1.9×10^2 CFU/ml, respectively; they also showed that 52% milk samples showed an unsatisfactory quality since the samples exceeded the maximum acceptable counts of fecal coliforms (10^2 CFU/ml). The difference between these results may be due to a difference in awareness of farmers to control hygiene, transport, and storage conditions. Still in Morocco, loads of 1.7×10^4 CFU/ml for total coliforms and 6.8×10^3 CFU/ml for *E. coli* have been reported in raw milk. It has been indicated that probably climate as well as environmental conditions may be involved in these differences; as the Oujda city is situated in dry and an arid climate, whereas Kenitra city has a humid climate (Belbachir et al., 2015). In Ethiopia, the mean fecal coliform counts were reported as 2.66–5.94 \log_{10} CFU/ml by Dan et al. (2008), 1.84 \log_{10} CFU/ml by Franciosi et al. (2009), and 3.48–7.38 \log_{10} CFU/ml by Worku et al. (2012). The most fecal and non-fecal coliforms isolated from milk directly obtained from the udder and bulked containers were *E. coli* (12.89–12.91%), *E. aerogenes* (7.74–8.89%), *E. cloacae* (5.28–6.67%), *E. agglomerans* (5.84–6.56%), *C. freundii* (2.13–2.33%), *C. diversus* (1.57–2.78%), *K. pneumoniae* (6.62–6.67%), *K. oxytoca* (5.33–6.29%), *S. typhi* (6.00–6.85%), *S. typhimurium* (4.6–5.0%), *Shigella dysenteriae* (3.56–4.83%), *S. boydii* (2.56–3.26%), *S. flexneri* (2.78–3.14%), *S. sonnei* (2.33–2.81%), as well as *Yersinia enterocolitica* (1.33–1.91%) (Worku et al., 2012). In Nigeria, it was reported that 88.43% milk had a mean total coliform counts of 20×10^0 – 3×10^7 CFU/ml. The national standard for milk quality is 100 CFU/ml (Oluwafemi and Lawal, 2015). In Cameroon, it has been announced that 87.1% milk had coliforms levels below 3 \log_{10} CFU/ml with a mean load of $3.83 \pm 0.86 \log_{10}$ CFU/ml while contamination by *E. coli* was 79.5% with a mean load of $2.25 \pm 1.44 \log_{10}$ CFU/ml (Belli et al., 2013). Also, in milk samples from South Africa, the mean total coliforms count of 1.9×10^3 CFU/ml and *E. coli* with the mean value of 1.6×10^1 CFU/ml have been reported (Lues et al., 2010).

Asia

In Iran, mean contamination of *E. coli* has been reported between 4.43 to 6.35 \log_{10} CFU/100 ml in raw milk (Yavarmanesh et al., 2015). Other studies on total coliforms and *E. coli* contamination in milk have reported the prevalence of 79 and 69%, respectively (Fadaei, 2014). Some of the dominant coliforms isolated from raw milk samples in Malayer city of Iran were *E. coli* (75%), *Enterobacter* spp. (42%), and *Klebsiella* spp. (36%) with mean counts for coliforms and *E. coli* as 1.4×10^5 and 2.1×10^1 CFU/ml, respectively (Pourhassan and Taravat-Najafabadi, 2011). In India, the mean counts of 10^3 – 10^4 CFU/ml coliforms have been reported in raw milk with

prevalence rate of 70–90% as well as 13.3% for *E. coli* and *Salmonella* spp., respectively (Lingathurai and Vellathurai, 2010). In raw milk, the mean coliform counts of 2.2–2.9 \log_{10} CFU/ml with the prevalence of 45% *E. coli* has also been reported (Parekh and Subhash, 2008). In Malaysia, 90% milk samples were reported to be contaminated by coliform bacteria with a mean count of 1.7×10^5 CFU/ml; also, *E. coli* was isolated from 64.5% milk samples with a mean count of 6.8×10^3 CFU/ml. Moreover, *Salmonella* spp. was isolated from 1.4% milk samples where the most dominant identified serotype were *S. muenchen* (23.1%), *S. agona* (15.4%), and *S. anatum* (15.4%) (Chye et al., 2004).

Europe

In Latvia located in the Eastern Europe, the average number of total coliform counts was the lowest in summer at $4.66 \pm 4.01 \log_{10}$ CFU/ml and the highest in autumn at $5.43 \pm 4.80 \log_{10}$ CFU/ml. The Enterobacteriaceae spp. were isolated from 10.3% raw milk samples, including *E. coli*, *K. oxytoca*, *Serratia marcescens*, *Kluyvera ascorbata*, *Pantoea agglomerans* (Gulbe and Valdovska, 2014). The same effect of season on milk coliform contamination has been reported in Slovakia with the mean counts of 2.1 \log_{10} CFU/ml and the prevalence of 48.2% (Torkar and Teger, 2005). However, these high coliform counts and prevalence reported in Latvia and Slovakia are still lower as compared to other emerging economies from Africa and South East Asia.

South America

In Brazil, the mean contamination levels have been reported in raw milk between 1.25 to 1.78 MPN/ml for total coliforms and between 0.94 to 1.60 MPN/ml for fecal coliforms (Nadia et al., 2012).

Coliform bacteria contamination sources of milk

Sanitary infrastructures and establishments

Studies in emerging economies have shown that the high counts of various microbial contaminants in milk can be mainly attributed to ignorance towards the fundamental sanitation and lack of basic infrastructure like electricity and inadequate water supply. This confirms the relationship between socio-economic status and household hygiene (Lues et al., 2003). Many emerging economies lie in the tropical regions that have a hot and humid climate for much of the year. This offers ideal temperature for growth and multiplication of bacteria after initial contamination. The problem is aggravated further by the lack of basic infrastructure like poor state

of roads especially during the rainy season and lack of refrigeration facilities to curb the bacterial growth (Godefay and Molla, 2000; Wafula et al., 2016). The poor condition of the roads makes time taken to transport milk from the producers who are mostly in rural areas to the consumers who are in urban centers. This increases the number of coliforms in the milk. The milk transportation ways range from motorbikes, buses, trucks to animals such as donkeys, which do not have refrigeration systems. Ideally, during transportation of milk, the cold chain must be maintained and on arrival at milk reception establishment, the temperature of the milk should not be more than 10 °C (Jay et al., 2005; Wafula et al., 2016). Figure 1 shows some unhygienic milk handling practices in Kenya (as an example of emerging economies) which are risk factors for coliforms contamination.

Milking equipments

Microorganisms, including coliforms find their way into milk from the surfaces of handling equipment and cow's udder. This contamination occurs during and/or after milking (Saran, 1995). Teat skin is one of the sources of coliforms in milk, which even when it looks reasonably clean, may harbor a large number of coliforms. A pre-milking treatment of the teats with iodine or other disinfectant greatly reduces the level of microbial contamination of the milk (Fuquay et al., 2011; Miseikiene et al., 2015). Pre-milking teat cleaning protocol, including washing of teats with disinfectant agents followed by drying is the most effective way for control of bacterial contamination (Gibson et al., 2008). Studies have also shown that teats pre-dipping using 0.25% iodine decrease significantly the Gram-negative bacteria (Oliver et al., 1993). Most coliforms are Gram-negative and the use of iodine in the pre-dipping prior to milking can reduce the coliform contaminants from the teat surfaces. However, some evidences from the emerging economy countries show that milk producers do not practice pre and post dipping of the teats (Fuquay et al., 2011). Most of these milk producers either do not wash the teats completely or wash without the use of potable water and wipe with towels. In some cases, farmers may let the calves to suckle before milking instead of teat washing (Robinson, 2005).

Coliform contamination of milk has also been associated with milking equipment in case of hand milking (Bava et al., 2011; Elmoslemany et al., 2009). It has also been established in previous studies that higher levels of bacterial contamination in milk result from hand milking compared to milk obtained by the use of milking machines (Filipoviet and Kokaj, 2009; Millogo et al., 2008). Many smallholder farmers in the emerging economies practice hand milking because machine milking is not econom-

ic for 2–3 heads of cattle in some African countries with low incomes such as Kenya (Fuquay et al., 2011)

Milk containers

Many milk handlers use plastic containers along the informal value chains, including jerry cans and buckets during milk handling practices such as milking, farm bulking, and its distribution (Wafula et al., 2016). The plastic containers in comparison with aluminum cans are cheap; therefore, they have widespread usage by the dairy actors in emerging economic world for milk handling. Secondly, these containers are easier to handle especially transporting by motorbikes, which is the most common mode of milk transportation in emerging economies such as Kenya (Odongo et al., 2016). Studies have found that milk producers who use plastic containers have high coliform counts in their milk (Gemetchu et al., 2015). Plastic jerry cans are difficult to properly clean and this result in unhygienic handling, which contributes to milk quality deterioration compared with the use of aluminum cans that are easy to clean. Using plastic jerry cans for milk handling has been reported in many emerging economies. This is another reason for high coliform counts and prevalence in milk from emerging economies as compared to the advanced economies (Fuquay et al., 2011; Wafula et al., 2016).

Water

Previous results have shown that bovine feces are not an important source for coliforms contamination in raw milk but the water used in sanitation and the milking environments are considered as one of the critical sources (Fuquay et al., 2011; Kagkli et al., 2007). Lack of enough water sources for cleaning the milk handling equipments may result in milk remaining on the surfaces of the equipment, providing nutrients for bacterial growth, and then milk contamination (Van Kessel et al., 2004). Hence, usage of low quality and unhygienic water during sanitation procedures can indirectly contaminate the milk (Robinson, 2005).

Animal mastitis

High coliform contaminations in milk could result from undiagnosed mastitis in milking animal (Torkar and Teger, 2005). Some coliforms like *E. coli* as well as *K. pneumoniae* have been isolated from milk in cases where mastitis has been reported (Jay et al., 2005; Kuang et al., 2009). It is required that complete udder health control and monitoring programs are considered in farms (Schukken et al., 2003). This ensures the appropriate health of cows and thereby reducing the risk of milk contamination with coliform (Fuquay et al., 2011).



A



B



C



D

Figure 1: Unhygienic milk handling practices in Kenya which are risk factors for coliforms contamination (A: Animal hand milking by a Kenyan farmer; B: Milk transportation using donkeys; C: Milk transportation in jerry cans using pickup truck and motorbike; D: Bottle used for milk transportation)

Risk of coliforms contamination in milk consumers

High level of *E. coli* in milk is an indicator of the possibility of the presence of other pathogenic bacteria and viruses in the milk (Robinson, 2005). This could be a major problem to the immune-compromised individuals, for example HIV patients, since some coliforms have been reported to cause a wide range of infections as opportunistic pathogens (Boor et al., 1998; Yavarmanesh et al., 2015).

Coliforms not only have the risk of pathogenicity, but also they are responsible for milk post-harvest losses in the low as well as middle economy countries through qualitative losses. Qualitative losses occur due to spoilage especially due to microorganisms. Coliforms decompose nutrients causing milk spoilage (Ozer and Akdemir-Errendilek, 2014). Also, lactose is broken down to lactic acid through fermentation resulting in undesirable flavor in raw milk (Jackson et al., 2012).

Conclusion

Local marketing of raw milk has been increased in the low and middle income countries because of low purchasing power of most native consumers. This population is at risk of illnesses from food-borne pathogens as a result of poor milk handling along the informal milk value chains. The regulatory bodies from these low and middle income countries are usually underfunded; so the safety standards of the milk chain cannot be sufficiently provided, endangering public health. On the other hand, there are huge losses of milk due to microbial spoilage in the low and middle income countries. So, it is necessary for the low and middle income countries to heavily invest in infrastructures and establishments such as potable water supply to the dairy actors, increase the electricity connectivity from national grid and off-grid, improve the transport infrastructure, and financial support of the standards regulatory institutions. Paying attention to the mentioned actions can help to improve milk safety and quality and thereby reduce the risk of the food-borne illnesses.

Conflicts of interest

All the authors declare that they have no conflict of interests.

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