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Evaluation of Total Coliform Counts in Non-Fermented and Fermented Cow Milk Products (Kindirmo, Mai-Shanu and Nono) in Zaria Metropolis, Nigeria

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SUMMARY

Testing for coliforms has a long history in dairy production, and has helped to identify the unsanitary condition of unpasteurized and pasteurized milk products. The study was performed to evaluate the level of coliform bacterial contamination in raw cow milk and milk products (*kindirmo*, *mai-shanu*, and *nono*). A total of 426 samples comprising 106 raw milk, 106 *kindirmo*, 106 *mai-shanu* and 108 *nono* were obtained from four selected Fulani herds (Damari, Marwa, Tudun-Muntsira, and Wuciciri) and four milk markets (Kasuwan-Mata, Kwangila, Samaru and Tudun-Wada) in Zaria Metropolis, all samples were tested using the bacteriological method. Coliform counts were analyzed using the one-way analysis of variance (ANOVA) using Tukey package and recorded as mean coliform counts (CFU/ML). The overall mean coliform counts for the milk products (raw milk, *kindirmo*, *mai-shanu*, and *nono*) were $98.88 \pm 7.68 \times 10^8$ CFU/ML, $60.19 \pm 5.49 \times 10^8$ CFU/ML, $60.36 \pm 5.50 \times 10^8$ CFU/ML and $73.5 \pm 7.09 \times 10^8$ CFU/ML respectively. The mean coliform counts for raw milk were significantly different ($p \leq 0.05$) from the three products (*kindirmo*, *mai-shanu* and *nono*). The study revealed heavy coliform bacterial load, ranging between $60.19 \pm 5.49 \times 10^8$ CFU/ML and $98.88 \pm 7.68 \times 10^8$ CFU/ML. This study calls for educating farmers and milk retailers at different levels level of production on the hygienic way of handling milk along the value chain to minimize unnecessary contamination of milk and milk products which can be of public health significance.

Keywords: Coliforms, Contamination, Herds, *Kindirmo*, *Mai-shanu*, Markets, *Nono*, Raw milk.

INTRODUCTION

Milk is a fresh liquid, clean, and normal mammary secretion produced by mammals for the nourishment of their young ones (Erickson *et al.*, 2020). It is obtained by milking the udder of a properly fed and well-kept dairy animal (Rumbold *et al.*, 2021). Milk is highly nutritious with micronutrients, macronutrients, and immunoglobulins that make an important part of the diet (Haug *et al.*, 2007; Rani and Maheshwari, 2012; Penhaligan, *et al*., 2022). Milk from dairy animals (cattle, sheep, and goats) are an important food item for over 6 billion people all over the globe, and a major contributor to food security as it alleviates poverty and mitigates malnutrition (Rumbold *et al.,* 2021).

Despite the outstanding nutritional quality and health benefits of milk, it serves also as an excellent vehicle for the transmission of milk-borne pathogens, which may cause serious health risks to consumers (Berhe *et al.,* 2020). Inappropriate handling of the milk may cause an outbreak of diseases to public health and economic losses, thus hygienic vigilance of milk and milk products is essential throughout the entire milk chain starting from producer to consumer (Gizaw 2019).

Nutritionally, non-useful substances like enzymes are present in normal cow milk and some of these enzymes are used as an index in screening for quality control tests (Niamh *et al.*, 2018).

Common coliform genera in raw milk and milk products
include *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella* (Jayarao and Wang, 1999). These originated from a variety of sources in the dairy farm environment, including water, plant materials, equipment, dirt, fecal sources, poor management practices, and storage (Eka and Ohaba, 1977; Kurwijila *et al.,* 2006; Saba *et al.*, 2016). Improper pasteurization and/or post-processing contamination may also be a source due to a little quality control for milk produced and handled in the informal channels (Jay *et al*. 2003). This bacterial contamination has zoonotic potential, and antimicrobial drug residues, hence causing public health risks to consumers (Kagkli *et al*., 2007). Although, coliforms can be used as hygienic indicators for milk and its products (Martin *et al*., 2016).

The safety of dairy products concerning foodborne diseases is of great concern, especially in developing countries where the production of milk and various dairy products takes place predominantly under unsanitary and poor production conditions (Tola *et al.*, 2007; Asaminew and Eyassu, 2011; Negash *et al*., 2012).

Traditionally, cow's milk is consumed raw without being pasteurized or boiled. Dairy products, such as *kindirmo* (fermented milk), *mai-shanu* (butterfat) and *nono* (skimmed milk) are locally made dairy products manufactured at small-scale dairy parlors, where hygienic measures are neither applied nor enforced. Food-borne outbreaks due to the consumption of dairy products constitute a chronic problem facing food hygienists, as milk and dairy products are subjected to different sources of contamination by many pathogens either from endogenous origin or directly and indirectly from the exogenous origin (Garbaj *et al.,* 2016). Thus, this present study aimed to determine the total coliform counts in raw cow milk and some milk products in Zaria Metropolis as a means of measuring the safety of these products for consumption.

MATERIALS AND METHODS

Study area: The study was carried out in Zaria Metropolis, which comprised Zaria, Sabon Gari, and Soba local Government Areas of Kaduna State, Nigeria. Kaduna State lies between latitude 11^0. 00'N to latitude 12^0 12'N and longitude 07^0 33'E to 08^0 03'E with an altitude of 675 meters above sea level.

Sample Collection and Transportation

Twenty milliliters (20 ML) each of raw milk samples were aseptically collected directly from the udder of milking cows into sterile universal bottles collected from four selected Fulani cattle herds (Damari, Marwa, Tudun-muntsira, and Wuciciri). For the milk products, twenty milliliters (20 ML) each of *kindirmo,* and *nono* were collected in a clean sterile universal bottle while twenty-five gram (25 g) of *mai-shanu* were also aseptically collected in clean sterile polythene bags from the Fulani vendors at

Kasuwan-mata, Kwangila, Samaru and Tudun-wada markets respectively. Raw milk samples were collected in the morning between 8:00 and 10:00 am, while milk products were collected between 12: noon and 3:00 pm based on availability, two times a week for six months. A total of 426 samples of milk and milk products were collected, packaged, and labeled appropriately. The samples were placed on ice-packed in a Coleman box to maintain a temperature of 4^0C and transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, for analyses. A non-Probability sampling technique based on convenience was used for the research work.

Media Preparation and Coliform Counts

Duplicate plates of Mac-Conkey agar for each sample were prepared as described by Sanders (2012). A ten-fold serial dilution method was carried out on each of the milk samples (raw milk, *kindirmo, mai-shanu,* and *nono*). 1 ML of milk sample was suspended into 9 ML of normal saline using a sterile graduated pipette, and 0.1 ML of the diluent was taken and inoculated into 9.9 ML of normal saline. Various dilutions were made to 10^3 dilutions. 0.1 ML from 10^3 was placed on a Mac-Conkey agar plate and was spread gently to cover the surface using a glass bent rod (hockey stick) and incubated at 37^0C for 18-24 hours. The lactose fermenting colonies on the Mac-Conkey agar plates were counted and calculated as colony-forming units per milliliter (CFU/ML) of the milk samples.

RESULTS

A total of (426) screened samples, showed high mean values of coliform counts (CFU/ML). Raw milk had the highest mean coliform counts 98.88 ± 7.68 x 10^8 CFU/ML, followed by *nono* (73.5 ± 7.09 x 10^8 CFU/ML), *mai-shanu* (60.36 ± 5.50 x 10^8 CFU/ML) and *kindirmo* (60.19 ± 5.49 x 10^8 CFU/ML). The mean coliform count for all the sample types was significantly different from one another (Table I).

TABLE I: Overall Mean Coliform Counts of Raw Cow Milk and Milk Products (*Kindirmo, Nono* and *Mai-shanu*) in Zaria Metropolis

Sample type	No. of samples	Mean ± SEM (CFU x 10^8 mL^{-1})
Raw milk	106	98.88 ± 7.68*
Kindirmo	106	60.19 ± 5.49
Mai-shanu	106	60.36 ± 5.50
Nono	108	73.5 ± 7.09

*$p \leq 0.05$ (Significantly different between the milk products)

The highest bacterial count was recorded in raw milk at Damari herd (126.20 ± 14.78 x 10^8 CFU/ML), followed by those at Marwa (119.50 ± 19.57 x 10^8 CFU/ML), Tudun-muntsira (78.80 ± 9.916 x 10^8 CFU/ML) and Wuciciri (62.93 ± 12.29 x 10^8 CFU/ML) herds. Mean value sampled from Damari and Wuciciri herds were significantly different ($p \leq 0.05$) (Table II). Coliform bacterial counts (CFU/ML) for the three milk products collected from the four different locations revealed a higher bacterial counts in *kindirmo* milk product at Kasuwan mata (85.65 ± 17.20 x 10^8 CFU/ML), Kwangila (61.28 ± 7.29 x 10^8 CFU/ML), Tudun wada (53.15 ± 7.60 x 10^8 CFU/ML), and Samaru (41.88 ± 8.11 x 10^8 CFU/ML) markets. The highest bacterial counts for *nono* was recorded at Samaru (111.40 ± 17.50 x 10^8 CFU/ML) followed by Kasuwan mata (95.58 ± 18.93 x 10^8 CFU/ML), Tudun wada (50.54 ± 6.00 x 10^8 CFU/ML and Kwangila (43.35 ± 7.74 x 10^8 CFU/ML) markets respectively. While, highest bacterial counts for *mai-shanu* was recorded at Tudun wada (76.92 ± 12.8 x 10^8 CFU/ML), Samaru (66.14 ± 10.5 x 10^8 CFU/ML), Kwangila (50.18 ± 7.81 x 10^8 CFU/ML) and Kasuwan mata (40.42 ± 7.14 x 10^8 CFU/ML) markets respectively. Bacterial counts for all the milk products were not significantly different based on their different locations (Table III).

TABLE II: Mean Coliform Counts of Raw Cow Milk based on Sampling Location in Zaria Metropolis

Sample Location	No. of samples	Mean ± SEM (CFU x 10^8 mL^{-1})
Damari	26	126.20 ± 14.78*
Marwa	26	119.50 ± 19.57*
Tudun Muntsira	26	78.80 ± 9.916
Wuciciri	28	62.93 ± 12.29

*p ≤ 0.05 (Statistically significant)

TABLE III: Mean Coliform Counts of Milk Products (*Kindirmo, Mai-shanu*, and *Nono*) based on Sampling Locations in Zaria Metropolis)

Locations	*Kindirmo* Mean ±SEM (CFU x 10^8 mL^{-1})	*Mai-shanu* Mean ± SEM (CFU x 10^8 mL^{-1})	*Nono* Mean ± SEM (CFU x 10^8 mL^{-1})
Kasuwan-mata	85.65 ± 17.20*	40.42 ± 7.14	95.58 ± 18.93
Kwangila	61.28 ± 7.29	50.18 ± 7.81	43.35 ± 7.74
Samaru	41.88 ± 8.11	66.14 ± 10.5	111.40 ± 17.50*
Tudun-Wada	53.15 ± 7.60	76.92 ± 12.8	50.54 ± 6.00
	p= 0.0083	*p*=0.0005	*p* = 0.0750

*p ≤ 0.05 (Statistically significant)

DISCUSSION

The origin of contamination by food-borne pathogens varies with the type of product and the mode of production and processing (Garbaj *et al*., 2016). This study determined the hygienic status of raw milk and milk products using bacteriological detection of coliforms. The analyses showed that raw milk (98.88 ± 7.68 x 10^8 CFU/ML) and milk products (*kindirmo* 60.19 ± 5.49 x 10^8 CFU/ML, *mai-shanu* 60.36 ± 5.50 x10^8 CFU/ML and *nono* 73.5 ± 7.09 x10^8 CFU/ML) obtained from the selected dairy herds (Damari, Wuciciri, Marwa, and Tudun-muntsira) and markets (Samaru, Tudun-wada, Kwangila, and Kasuwan mata) were generally contaminated with high coliform bacterial counts. Mean coliform counts of raw milk samples were significantly different ($p \leq 0.05$) from the three milk products (*kindirmo*, *mai-shanu* and *nono*), as subjected to one-way analysis of variance (ANOVA) using the Tukey package. The study, therefore, revealed a high level of contamination in raw milk i.e. before pasteurization with mean coliform counts between 60.19 ± 5.49 x 10^8 CFU/ML and 98.88 ± 7.68 x 10^8 CFU/ML of the four sampled herds. Damari and Marwa dairy herds marked a significant difference ($p \leq 0.05$) as compared to Tudun-muntsira and Wuciciri dairy herds. Nyalekwa and Nonga (2018) also reported 95% (8.1 ± 8.2 CFU/ML) average total rates of coliform counts contamination of in raw cow milk collected from 20 different dairy farms in Morogoro Municipality Tanzania, and Dharan

Nepal. Limbu *et al*. (2020) also recorded an average of 14 × 10^4 CFU/ML (95%) total coliform contamination in raw cow milk. This findings also agree with the high mean average value (2.68-log10^3 CFU/ML) of coliform counts in raw milk samples collected from different local small farms and markets in Bangladash as reported by Muzahidul *et al*. (2021). Similarly, Agunbiade *et al.* (2015) reported mean values of 1.42-$\log_{10}$ CFU/ML and 2.78-$\log_{10}$ CFU/ML in raw milk obtained from cleaned and uncleansed teats of cows in Zaria, Nigeria. A similar study was done by Acharya *et al.* (2017) and reported total coliform count range from 2-52% in raw milk. The higher coliform count detected in this study may suggest the possible roles played by different environmental management practices such as udder contamination, poor sanitation of bedding, plants, or fecal contamination during the milking process (Hussein *et al.*, 2003). On the other hand, the rate of cluster washes, and milking unit fall-off during milking also correlate to variations in levels of coliforms in raw milk (Pantoja *et al*., 2011). In addition, milking done in an open environment can also introduce an indirect contact with dust from roadsides, fecal materials and feeds might be a source of contamination (Abid *et al.,* 2009). Some previous studies also reported contamination of raw milk, which can persist in the farm environment for an extended period due to the movement of humans and animals within the farm, livestock excrement, soil, and plants (Kupriyanov *et al.,* 2010).

Comparing the mean coliform counts for the three milk products, *kindirmo* (41.88 ± 8.11 x 10^8 CFU/ML - 85.65 ± 17.20 x 10^8 CFU/ML), *mai-shanu* (40.42 ± 7.14 x 10^8 CFU/ML - 76.92 ± 12.8 x 10^8 CFU/ML), and *nono* (43.35 ± 7.74 x 10^8 CFU/ML - 111.40 ± 17.50 x 10^8 CFU/ML) means were not significantly different (*P* = 0.0083, 0.0005, and 0.0750), as compared with the sampling locations (Kasuwan-mata, Kwangila, Samaru, and Tudun-wada). However, *nono* showed a higher mean coliform values at Samaru (111.40 ± 17.50 x 10^8 CFU/ML) and Kasuwan-Mata (95.58 ± 18.93 x 10^8 CFU/ML). All the mean values for milk products were found to exceed the minimum acceptable values of 3.0-$\log_{10}$ CFU/ML as recommended by W.H.O. (2005). The values in this study were higher but could be comparable to previous work in Zaria, where the mean counts of 3.16 and 3.74-log10 CFU/ML were reported in pasteurized milk reported by Lawan *et al.* (2012). The presence of coliforms in milk products may be due to defective pasteurization, adulteration of pasteurized milk with raw milk, and unsanitary handling (Limbu *et al.,* 2020). The difference in coliform counts for the milk products might range from faulty processing to post-pasteurization contamination following several parameters, such as contamination from unhygienic handling of the milk products during processing and package, contamination from utensils used for processing and storage, flies, specks of dust, contaminated water from the well and/or addition of unclean water from ice and milk storage.

CONCLUSION

The findings recorded heavy contamination of coliforms in the four different milk samples ranging between 60.19 ± 5.49 x 10^8 CFU/ML and 98.88 ± 7.68 x 10^8 CFU/ML, which implies unhygienic practices in the process of milking, handling, and/or storage of milk and milk products in Zaria Metropolis. This calls for the need to educate the farmers and milk retailers at different levels of production on hygienic handling of milk along the value chain to minimize unnecessary contaminations, as well as routine monitoring of dairy products vendors, and to promote awareness, which can be of public health significance.

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