



Research Article

Prevalence of *Escherichia coli* and *Staphylococcus aureus* in raw milk and its products sold for human consumption in Maiduguri, Northeastern Nigeria: A potential public health concern

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ABSTRACT

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Globally, milk represents an important human nutrition. However, it also serves as an important vehicle for transmission of foodborne pathogens some of which may be multidrug resistant. The current study was undertaken with the main aim of assessing the microbiological quality of raw milk and its products consumed in Maiduguri metropolis. Fresh milk was sampled from six different milk source outlets in the metropolis and the samples were subjected to standard microbiological procedures for the detection of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). A cross-sectional study was conducted with a total of 120 milk samples collected and cultured on appropriate growth and differential media and further biochemical tests were carried out in order to determine the presence of *E. coli* and *S. aureus*. A total of 66 (55.0%) of the 120 samples were contaminated with bacteria, while 54 (45.0%) milk samples tested were negative for the contaminants. Contaminating bacteria comprised 51 (42.5%) and 43 (35.8%) of *S. aureus* and *E. coli* respectively. The mean pH of the milk samples ranges from 3.3 to 6.3 respectively in “Kindirmo” and fresh milk with an overall mean pH of 3.9. A high prevalence of both pathogens was detected in “Kindirmo” and “Nono” fermented milk products. Overall, a statistically significant association was found between the detection of *Escherichia coli* ($p = 0.046$) and *Staphylococcus aureus* ($p = 0.013$) with the different types of milk samples examined. Our findings of the high prevalence of these pathogens in readily consumed fresh milk and its products in the study area raise public health concerns. Therefore, we recommended the application of strict quality control and good hygienic standards along the milk value chain by application of hazard analysis and critical control points (HACCP). Public health awareness of the health risks associated with the consumption of contaminated milk or its products is also highly recommended

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INTRODUCTION

Among the food of animal origins, raw milk, processed milk or its products represent good growth medium for pathogens, especially bacterial organisms, because of factors such as high water activity, relatively neutral pH as well as a significant amount of nutrients (Abate and Wolde, 2015; Reta and Addis, 2015; Perin *et al.*, 2018). These factors make milk an important culture medium for many bacterial pathogens; many of which are of public health importance (Uzoaga *et al.*, 2020). When proper hygienic milking procedures and hygienic handling and storage are not applied along the steps of milk production at the farm level (milking and storage), transportation to dairy industries and processing, the result is increased contamination of milk with pathogenic organisms posing a public health hazard to humans (Perin *et al.*, 2018; Rai *et al.*, 2020). Previous studies (Montel *et al.*, 2014; Perin *et al.*, 2017) have documented the presence in raw milk of diverse contaminant microbiota including spoilage microorganisms, pathogenic microorganisms as well as bacteria of significant industrial or technological applications. Factors such as environmental hygiene, personal hygiene of personnel, sanitary conditions of the dairy herds, milking equipment used and others related to the milk value chain were described as directly influencing the diversity of milk microbiota (Murphy *et al.*, 2016). Improper handling, poor hygienic practices and lack of environmental control during milking could lead to bacterial contamination of the raw milk with its subsequent development and multiplication in the milk medium resulting in public health threat to humans (Raza and Kim, 2018; Fusco *et al.*, 2020). In recent years, several studies have documented the presence of pathogenic bacteria in raw milk or milk products that may be resistant to single or multiple antimicrobials (Aliyu *et al.*, 2019; Yakubu *et al.*, 2020; Abdi *et al.*, 2021). By implication, this means milk would serve as a vehicle for the transmission of these antimicrobial-resistant pathogens to humans through the consumption of raw or

processed milk and its products thereby further compounding the public health risks associated with milk consumption. The emergence, rapid global spread and dissemination of antimicrobial-resistant pathogens, such as through the consumption of raw milk or its products, represent a global public health threat. According to the World Health Organization (WHO), about 700,000 people die each year from antimicrobial-resistant infections and by 2030, antimicrobial resistance could force up to 24 million people into extreme poverty (<https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis>).

Escherichia coli and *Staphylococcus aureus* are among the important bacterial pathogens responsible for outbreaks of food poisoning worldwide (Elmonir *et al.*, 2018; Dai *et al.*, 2019). They are common contaminants of raw milk or its processed products thereby posing public health risks. For instance, several foodborne outbreaks of *S. aureus* intoxications associated with the consumption of contaminated milk or its products have been documented worldwide (Weiler *et al.*, 2011; Jöhler *et al.*, 2015). For instance, in 2006 alone, staphylococcal food poisoning resulted in 6 deaths and an estimated 241,148 cases in the United States (Scallan *et al.*, 2011). Under favourable conditions, some strains of *S. aureus* in milk produce food-poisoning enterotoxins that can persist even after the pasteurization of the milk (Elmonir *et al.*, 2018; Dai *et al.*, 2019). At the farm level, sources of contamination include the animal skin, mucosal surfaces, infected glands, milking equipment, the environment (Kayili and Sanlibaba, 2020) or milker's hands/food handlers who are *S. aureus* carriers and poor hygienic practices (Kadariya *et al.*, 2014).

The presence of *E. coli* in foods such as milk may indicate faecal contamination because of its presence in the guts (Gundogan and Avci, 2014). Because some strains such as *E. coli* O157:H7 grouped as enterohaemorrhagic *E. coli* and Shiga (Vero)-toxin-producing *E. coli* are pathogenic

and can cause many life-threatening human infections like haemorrhagic colitis and diarrhoea associated with haemolytic-uremic syndrome (Rahimi *et al.*, 2011). The presence of *E. coli* in foods such as milk is of major public health concern. Recently, several reports documented outbreaks of shiga toxin-producing *E. coli* (Kintz *et al.*, 2019; Kim *et al.*, 2020; Tack *et al.*, 2021). In food-producing animals, particularly dairy animals, *S. aureus* and *E. coli*, are the most frequent causative agents of subclinical and clinical mastitis (Abebe *et al.*, 2016; Hinthong *et al.*, 2017) hence they represent common endogenous contaminants of raw milk (Mhone *et al.*, 2011). In subclinical mastitis, these pathogens are released into the raw milk without any abnormalities in milk consistency or udder shape (Elmonir *et al.*, 2018).

In Nigeria, there is a lot of concern regarding the process of milking, its transportation, storage, distribution and retailing in the milk value chain. This is due largely to the unhygienic practices at pre- and post-milking stages, the poor quality of water used as well as the utensils, and the lack of cold storage facilities and retailing environments (Uzoaga *et al.*, 2020). Hawkers with poor knowledge of quality control and hygienic standards the retail majority of the milk or its products; hence, subjecting milk to contamination with pathogenic bacteria of public health significance. Some studies have attempted to assess the microbiological quality of raw milk or its products in different parts of Nigeria (Aliyu *et al.*, 2019; Uzoaga *et al.*, 2020; Yakubu *et al.*, 2020). To the best of our knowledge, there is a paucity of information regarding this in Northeastern Nigeria, despite the region serving as the largest livestock-producing hub in the country. This is coupled with the need to combat food insecurity induced by a decade-long insurgency by harnessing dairy products. Therefore, it has become imperative to undertake the current research to assess the microbiological quality of readily consumed raw milk and its products sourced from different

milk outlets in Maiduguri, North-eastern Nigeria.

MATERIALS AND METHODS

Sample Size and Sampling Procedure

The sample size for this study was calculated using the web-based Statulator® sample size calculator (Dhand and Khatkar, 2014). For the sample size calculation, a prevalence of 10% was reported by Abike *et al.* (2015), and an allowable error of 5% and a 95% level of confidence were utilized. One hundred and twenty (120) samples were collected aseptically from milk sellers in a sterilized sample container using simple random sampling by balloting, as defined by Moore and George (2006). Twenty (20) samples were collected from each of the six milk outlets in the Maiduguri metropolis.

Collection of Samples

A total of 120 different milk samples comprising Fresh milk = 20, “Kindirmo” = 60, and “Nono” = 40 were collected from six milk outlet stations in Maiduguri metropolis, Nigeria from different market sources including Gidan Madara (n=20), Tashan Baga (n=20), Tashan Bama (n=20), Monday Market (n=20), Bulumkutu (n=20) and Custom area (n=20). To avoid influencing the experiment's outcome, the milk was collected from points of sale and bought without disclosing that it was for research purposes. About 10mL of milk, samples were collected in a sterile sample container from selected milk vendors and aseptically transported in an ice pack container to the Veterinary Public Health and Preventive Medicine Laboratory, Faculty of Veterinary Medicine, University of Maiduguri.

Sample Preparation by Serial Dilutions

One millilitre (1mL) of milk was transferred aseptically into a test tube containing 9mL of sterile peptone water solution and thoroughly mixed to make a 1:10 ratio. To make a 2:10 dilution, 1mL of the previous dilution was transferred to another test tube containing 9mL sterile peptone water. To

make ten dilutions, this process was repeated (1:10 to 1:90).

Isolation of *E. coli*

Using a sterile wire loop 1:90 dilution of the milk sample was picked and streaked onto MacConkey agar plates (differential media for *E. coli* identification). The plates were inverted, labelled with sample identification (ID) and incubated for 24 hours at 37°C. The plates were then checked for typical and atypical colonies after the incubation. On MacConkey agar, typical *E. coli* colonies were dry, medium in size, pink in colour, and appeared singly or in groups (Cooke, 1985). Atypical colonies appear as small red colonies that can be found singly or in groups. To obtain pure *E. coli* colonies, the presumed typical and atypical colonies were sub-cultured in Eosin Methylene Blue (EMB) agar under the same conditions. Following another 24 hours of incubation, a typical, *E. coli*, colonial morphology of greenish-metallic sheen was observed (Cooke, 1985). A well-isolated colony was chosen and subcultured into peptone water for further biochemical confirmation.

Isolation of *S. aureus*

Using an inoculation wire loop, the milk sample was picked and streaked onto Blood agar plates. After that, the plates were inverted, labelled with sample identification (ID) and incubated for 24 hours at 37°C. Both typical and atypical colonies were examined after 24 hours, and the plates that have not yielded growth were re-incubated at 37°C for another 24 hours (48 hrs). A simple zone of haemolysis can be seen in typical staphylococcal colonies grown on Blood agar. A single isolated colony was Gram stained to confirm the microscopic morphology of Staphylococci. An isolated typical colony was then transferred to Mannitol salt agar a selective agar

for *Staphylococcus*. To obtain pure *S. aureus* colonies, the presumed well-isolated typical colonies were sub-cultured in Mannitol salt agar under the same conditions (Han *et al.*, 2007). Following the 24 hours of incubation, a well-isolated colony was chosen and subcultured into Peptone water for biochemical confirmation.

Confirmation of *E. coli* and *S. aureus*

For confirmation of both *Escherichia coli* and *Staphylococcus aureus* in the test samples, Gram staining and biochemical reactions including the Catalase and Coagulase tests were performed on well-isolated colonies from Eosin Methylene Blue (EMB) agar and Mannitol salt agar, respectively. These procedures were carried out as previously described (Uzoaga *et al.*, 2020; Yakubu *et al.*, 2020).

Data Analysis

In the present study, raw data obtained were first entered into Microsoft® Office Excel (version 2010). Initially, descriptive statistics such as percentages and means were determined. Subsequently, Chi-square or Fisher's exact test, as the case may be, was used for the exploratory analysis to determine the effect of either catalase (*E. coli*) or coagulase (*Staphylococcus aureus*) tests on the different types of milk samples. One-way ANOVA was performed to compare the mean pH of the milk on the different types of milk samples. With a significant overall *p-value* of 0.05 of the One-way ANOVA, the Bonferroni post-hoc test for multiple comparisons was further performed to identify which mean pH of the different types of milk differs significantly. All the statistical analyses were performed using IBM SPSS Statistics (IBM, Armonk, NY: IBM Corp.) at the significance level of $\alpha = 5\%$

RESULTS

Sixty-six (55.0%) of the 120 samples are positive for either or both the isolates tested in

this study while 54 (45.0%) milk samples tested were negative for both the isolates tested in this study (Fig 1). Out of the 120 milk samples, 58 yielded growths for *E. coli* on EMB while 65 milk samples yielded growth for *S. aureus* on Mannitol salt agar. Out of the 58 *E. coli* isolates obtained from EMB, 42 (72.4%) were catalase positive while 16 (27.6%) were found to be a catalase-negative variant of *E. coli* (Table 1).

There is a statistically significant association between the two tests ($\chi^2 = 65.3$; $p = 0.0001$). Out of the 65 *S. aureus* obtained from mannitol salt agar 51 (78.5%) were coagulase positive while 14 (21.5%) were coagulase-negative (Table 2). There is a statistically significant association between the two tests (Fisher's exact $p = 0.0001$).

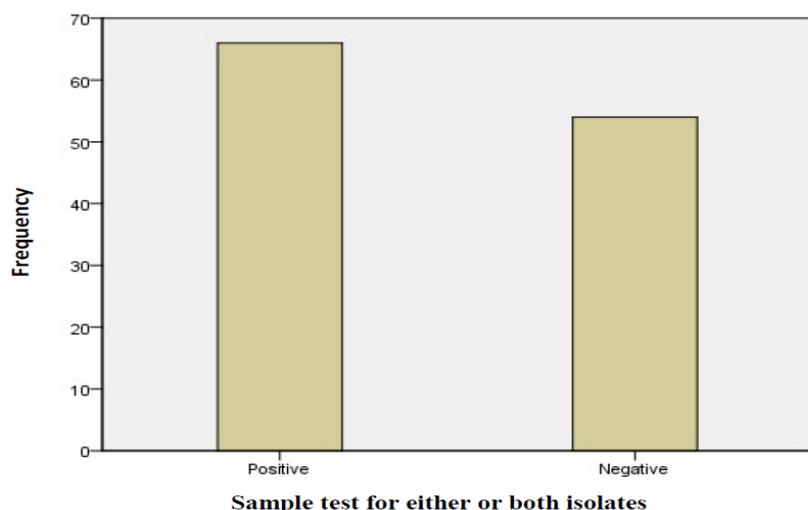


Figure 1: Sample test for either or both isolates

Table 1: Isolation rates of *E. coli* in the different milk samples (n=120) from Maiduguri, North-eastern Nigeria.

EMB	Catalase (%)			χ^2	p-value
	Positive	Negative	Total		
Positive	42 (72.4)	16 (27.6)	58 (100)	65.3	0.0001
Negative	1 (1.6)	61 (98.4)	62 (100)		
Total	43 (35.8)	77 (64.2)	120 (100)		

Table 2: Isolation rates of *Staphylococcus aureus* in the different milk samples (n=120) from Maiduguri, North-eastern Nigeria

Mannitol Salt Agar	Coagulase (%)			p-value*
	Positive	Negative	Total	
Positive	51 (78.5)	14 (21.5)	65 (100)	0.001
Negative	0 (0)	55 (100)	55 (100)	
Total	51 (42.5)	69 (57.5)	120 (100)	

*Fisher's exact test

Of the 20 Milk samples (Nono) collected from the Customs market, 10 (50%) were positive, and of the 20 "Nono" samples collected from the Tashan Bama market, 9 (45%) were positive

for *E. coli*. While lowest isolation rate of *E. coli* 3 (15%) was recorded from Fresh milk sourced from the Gidan Madara milk outlet (Table 3). Twelve (60%) of the Nono samples

collected from the Custom market were positive, while 10 (50%) out of 20 samples each of Nono from Tashan Bama and Kindimo from the Monday market were positive for *S. aureus*.

Similarly, the lowest isolation rate of *S. aureus* was seen from Fresh milk sourced from the Gidan Madara milk outlet (Table 4).

Table 3: Isolation rates of *Escherichia coli* in the different milk samples (n=120) from different locations in Maiduguri, North-eastern Nigeria.

Sources of Milk	Type of milk sample	No. collected	No. of positives	Percentage (%) positive
Gidan Madara	Fresh Milk	20	3.0	15.0
Tashan Baga	Kindirmo	20	6.0	30.0
Tashan Bama	Nono	20	9.0	45.0
Monday Market	Kindirmo	20	7.0	35.0
Bulumkutu	Kindirmo	20	7.0	35.0
Custom	Nono	20	10.0	50.0
Total		120	42	35.0%

Table 4: Occurrence rates of *Staphylococcus aureus* in the different milk samples (n=120) from different locations in Maiduguri, North-eastern Nigeria

Sources of Milk	Type of milk sample	No. collected	No. of positives	Percentage (%) positive
Gidan Madara	Fresh Milk	20	3	15.0
Tashan Baga	Kindirmo	20	9	45.0
Tashan Bama	Nono	20	10	50.0
Monday Market	Kindirmo	20	10	50.0
Bulumkutu	Kindirmo	20	7	35.0
Custom	Nono	20	12	60.0
Total		120	51	42.5%

Tables 5 and 6 explored the association between the presence or absence of *Escherichia coli* and *Staphylococcus aureus* according to the different types of milk samples examined. Overall, statistically significant association was found

between the presence and absence of growth of *Escherichia coli* ($p = 0.046$) and *Staphylococcus aureus* ($p = 0.013$) according to the different types of milk samples examined

Table 5: The distribution of Catalase positive *E. coli* according to the type of milk (n=120) obtained from Maiduguri, Northeastern Nigeria.

Catalase test	Type of Milk (%)				χ^2	p-value
	Fresh milk	Kindirmo	Nono	Total		
Positive	3 (7.0)	21 (48.8)	19 (44.2)	43 (100)	6.161	0.046
Negative	17 (22.1)	39 (50.6)	21 (27.3)	77 (100)		
Total	20 (16.7)	60 (50.0)	40 (33.3)	120 (100)		

Table 6: The Distribution of Coagulase Positive *S. aureus* according to the type of milk (n=120) obtained from Maiduguri, Northeastern Nigeria.

Coagulase test	Type of Milk (%)				χ^2	p-value
	Fresh milk	Kindirmo	Nono	Total		
Positive	3 (5.9)	26 (51.0)	22 (43.1)	51 (100.0)	8.764	0.013
Negative	17 (24.6)	34 (49.3)	18 (26.1)	69 (100.0)		
Total	20 (16.7)	60 (50.0)	40 (33.3)	120 (100.0)		

The mean pH of the milk samples collected in this study ranges from 3.3 in “Kindirmo” to 6.3 in fresh milk as shown in Figure 2 below. The result of the One-way ANOVA revealed that there was statistically significant difference in the mean pH between at least two different groups/types of milk samples (Fresh, Nono or Kindirmo) ($F = 1201.795, p = 0.0001$) (Table

7). The mean difference in pH of milk was statistically different between Fresh milk and Kindirmo samples ($3.0 \pm 0.062, 95\% \text{ CI} = 2.85-3.16, p=0.0001$), Fresh milk and Nono ($2.7 \pm 0.066, 95\% \text{ CI} = 2.52-2.84, p=0.0001$) as well as between Nono and Kindirmo ($0.32 \pm 0.049, 95\% \text{ CI} = 0.20-0.44, p=0.0001$) (Table 7).

Table 7: One-way ANOVA for the effect of milk pH on the type of milk samples

Type of Milk	N	Mean	SD	SE	F	p-value
Fresh milk	20	6.337 ^a	0.111	0.025	1201.795	0.0001
Kindirmo	60	3.331 ^b	0.076	0.010		
Nono	40	3.653 ^b	0.401	0.063		
Total	120	3.939	1.112	0.102		

SD = Standard Deviation; SE = Standard Error, a, b, c = different superscripts denotes statistically significant difference

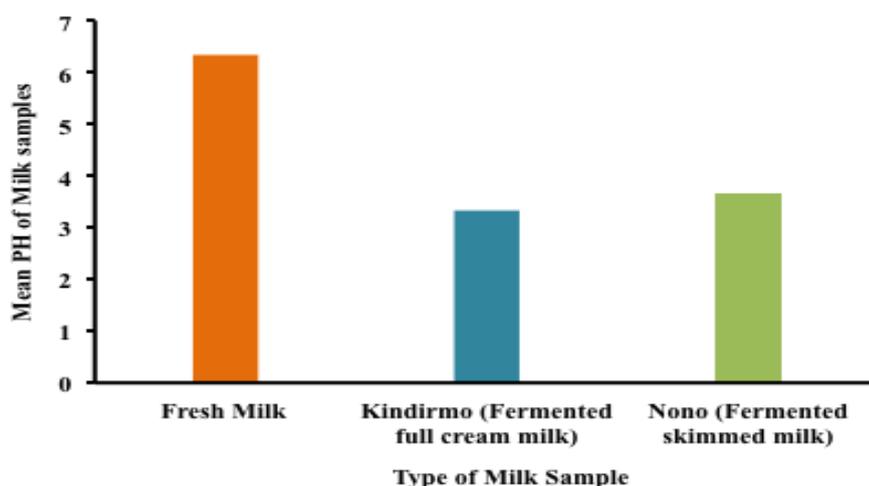


Figure 2: Showing distribution of the mean milk P^H based on the type of milk (n=120) sampled from Maiduguri, Northeastern Nigeria

DISCUSSION

Worldwide, *E. coli* and *S. aureus* are known contaminants of milk or its products. Some strains of these pathogens are the causative agents of mastitis in livestock as well as pathogenic to humans when they consume dairy products that are contaminated by these pathogens. In the present study, both bacterial organisms were detected in the raw milk and its products, suggesting the public health significance of these pathogens. We found that 55.0% (66/120) of the milk samples tested were contaminated with *E. coli* (35.0%) and *S. aureus* (42.5%). Our finding of 55.0% is higher than 28.9% (35/121) and 18.5% (25/135) reported from Egypt and India respectively (Elmonir *et al.*, 2018; Kumar and Prasad, 2010). Similarly, a study (Uzoaga *et al.*, 2020) assessing the bacteriological quality of “Nono” sold in the Federal Capital Territory of Nigeria found a lower prevalence of contamination of 19.3%. The finding of higher prevalence in the current study could be attributed to the lack of milk quality control and low hygienic standards by the milkers, retailers and hawkers along the milk value chain stages including the pre-milking and post-milking stages in the study area.

The prevalence of *E. coli* and *S. aureus* in milk or its products reported elsewhere (Elmonir *et al.*, 2018; Kumar and Prasad, 2010) was lower than what we found. In Nigeria (Olatoye *et al.*, 2018; Uzoaga *et al.*, 2020; Yakubu *et al.*, 2020), the prevalence of *S. aureus* and *E. coli* in milk or its processed products were similarly lower than what we obtained in the present study. In this study, the prevalence of *E. coli* and *S. aureus* in milk samples was higher in the Customs area followed by the Tashan Bama area, while the prevalence found in Gidan Madara and Bulunkutu Market was lower. The high prevalence found in Custom and Tashan Bama areas could be attributed to the fact that the level of contamination was high resulting from poor hygienic practices, or as a result lack of quality control of the milk and proper care along the milk value chain.

The high number of pathogens found in milk in these areas is of public health concern considering the number of people that patronises these markets to buy the milk for different purposes. Furthermore, the high prevalence found in “Nono” compared to other products in the study areas could be a result of poor hygiene in traditional ways of processing this product and poor quality of the raw milk used during the processing of this milk product.

In the present study, we recorded a high prevalence of both the pathogens in “Nono” and “Kindirmo” (Tables 1 and 2). This corroborates with findings from a similar study (Yakubu *et al.*, 2020) in Nassarawa state. In contrast, other studies conducted by Zaria (Usman and Mustapha, 2016) and Kaduna (Umaru *et al.*, 2013) recorded a high prevalence of *S. aureus* in fresh and fermented milk. The high rates of isolation of *S. aureus* and *E. coli* portray the public health importance of these bacterial pathogens as it relates to food safety. Moreover, the fact that many people in the study area consumes “Nono” or “Kindirmo” is a cause for concern. The paucity of information on these pathogens in milk or its products in the study area makes it difficult to make any comparisons.

Our results of the mean pH of the different milk samples range between 3.3 and 6.3 respectively in “Kindirmo” and fresh milk with an overall mean pH of 3.9 (Fig. 1). This result is slightly higher (3.8 – 5.4 in fresh and fermented milk) than those described in Khartoum, Sudan (Bakri and Zubeir, 2008). However, our findings slightly differ (5.5 – 6.29) from a similar study (Adesokan *et al.*, 2011) conducted in Northern Nigeria. The variations in the mean PH obtained from the different scientific reports as compared with those in the present study could be due to factors such as the type of milk sampled, method of milk collection, prevailing environmental temperature, and length of fermentation and preservation status of the milk or its products.

In developing countries such as Nigeria, commercial production as well as processing procedures of milk and its products is commonly undertaken traditionally. In the study area, milking and milk product processing is being carried out by livestock farmers, retailers or hawkers with poor knowledge of food safety and standard hygienic procedures. These practices predispose raw milk and its products such as “kindirmo” and “Nono” to contamination by diverse microbial pathogens that could cause life-threatening human infections. This is because milk is known to be an efficient vehicle for the transmission of disease-causing pathogens to humans through the consumption of contaminated milk or its products (Garedew *et al.*, 2012). Other researchers have reported outbreaks of milk-borne diseases after pasteurization of the milk or its processed products due to either improper pasteurization or product recontamination possibly through improper handling, transportation and storage (Hartman, 1997; Nebedum and Obiakor, 2007).

CONCLUSION

According to the findings of the present study, fresh milk or its products such as “Kindimo and Nono” were found to be contaminated with both *S. aureus* and *E. coli*. These pathogens (some strains) have been implicated in several outbreaks of food intoxications leading to human deaths or hospitalizations. Thus, it could be concluded that the microbiological quality of milk or its products in the study area is unsatisfactory and should be of public health concern. It is recommended that strict hygienic and high-quality control procedures be applied along the milk value chain including creating public health awareness to the milk retailers and hawkers on the public health risks associated with unhygienic practices during pre- and post-milking stages. Lastly, the application of procedures similar to hazard analysis and critical control points (HACCP) along the milk value chain is highly recommended.

CONFLICT OF INTEREST

We declare that there is no conflict of interest

REFERENCES

- Abate, M. and Wolde, T. (2015). Bacteriological Quality and Safety of Raw Cow's Milk in and around Jigjiga City of Somali Region, Eastern Ethiopia. *International Journal of Research Studies*, 3: 48–55.
- Abdi, R. D, Gillespie, B. E, Ivey, S., Pighetti, G. M., Almeida, R. A and Dego, O. K (2021). Antimicrobial resistance of major bacterial pathogens from dairy cows with high somatic cell count and clinical mastitis. *Animals*, 11: 1–14.
- Abebe, R., Hatiya, H., Abera, M., Megersa, B and Asmare, K. (2016). Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, 12.
- Abike, T, Olufunke, O. and Oriade, K. (2015). Prevalence of Multiple Antibiotic Resistant *Escherichia coli* Serotypes in Cow Raw Milk Samples and Traditional Dairy Products in Osun State, Nigeria. *British Microbiology Research Journal*, 5: 117–125.
- Adesokan, I. A, Odetoynbo, B. B, Ekanola, Y.A, Avanrenren, R. E. and Fakorede, S. (2011). Production of Nigerian nono using lactic starter cultures. *Pakistan Journal of Nutrition*, 10: 203–207.
- Aliyu, Y., Abdullahi, I.O, Whong, C.M.Z. and Olayinka, B.O. (2019). Antibiotic resistant phenotypes of *Staphylococcus aureus* isolated from fresh and fermented milk in parts of Nasarawa State, Nigeria. *African Journal of Microbiology Research*, 13: 446–456.
- Bakri, J.M.E. and Zubeir, I.E.M.E. (2008) Chemical and Microbiological Evaluation of Plain and Fruit Yoghurt in Khartoum State, Sudan. *International Journal of Dairy Science*, 4: 1–7.
- Cooke, E.M. (1985). *Escherichia coli*—an overview. *Epidemiology & Infection*, 95(3): 523-530.
- Dai, J., Wu, S., Huang, J., Wu, Q., Zhang, F., Zhang, J., Wang, J., Ding, Y., Zhang, S., Yang, X., Lei, T., Xue, L. and Wu, H. (2019). Prevalence and Characterization of *Staphylococcus aureus* Isolated from

- Pasteurized Milk in China. *Front. Microbiology*, 10.
- Dhand, N.K, and Khatkar, M.S. (2014). Statulator: An online statistical calculator. Sample Size Calculator for Estimating a Single Proportion [WWW Document].
- Elmonir, W., Abo-Remela, E.M. and Sobeih, A. (2018). Public health risks of *Escherichia coli* and *Staphylococcus aureus* in raw bovine milk sold in informal markets in Egypt. *Journal of Infection in Developing Countries*, **12**: 533–541.
- Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A.F., Cho, G.S., Kabisch, J., Böhnlein, C. and Franz, C.M.A.P. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, **19**: 2013–2049.
- Garedew, L., Berhanu, A., Mengesha, D. and Tsegay, G. (2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. *BMC Public Health*, **12**.
- Gundogan, N. and Avci, E. (2014). Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. *International Journal of Dairy Technology*, **67**: 562–569.
- Hartman, P.A. (1997). The Evolution of Food Microbiology, in: Doyle, M.P., Beuchat, L.R. (Eds.), *Food Microbiology: Fundamentals and Frontiers*. ASM Press, Washington DC, USA, Pp. 3–12.
- Hinthong, W., Pumipuntu, N., Santajit, S., Kulpeanprasit, S., Buranasinsup, S., Sookrung, N., Chaicumpa, W., Aiumurai, P. and Indrawattana, N. (2017). Detection and drug resistance profile of *Escherichia coli* from subclinical mastitis cows and water supply in dairy farms in Saraburi Province, Thailand. *Peer Journal*, **5**(6): e3431.
- Johler, S., Weder, D., Bridy, C., Huguenin, M.C., Robert, L., Hummerjohann, J. and Stephan, R. (2015). Outbreak of staphylococcal food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk. *Journal of Dairy Science*, **98**: 2944–2948.
- Kadariya, J., Smith, T.C. and Thapaliya, D. (2014). *Staphylococcus aureus* and *Staphylococcal* Food-Borne Disease: An Ongoing Challenge in Public Health. *Biomedical Research International*, 1-9.
- Kayili, E. and Sanlibaba, P. (2020). Prevalence, characterization and antibiotic resistance of *Staphylococcus aureus* isolated from traditional cheeses in Turkey. *International Journal of Food Properties* **23**: 1441–1451.
- Kim, J.S., Lee, M.S. and Kim, J.H. (2020). Recent Updates on Outbreaks of Shiga Toxin-Producing *Escherichia coli* and Its Potential Reservoirs. *Frontiers in Cellular and Infection Microbiology*, 10.
- Kintz, E., Byrne, L., Jenkins, C., McCarthy, N., Vivancos, R. and Hunter, P. (2019). Outbreaks of Shiga Toxin–Producing *Escherichia coli* Linked to Sprouted Seeds, Salad, and Leafy Greens: A systematic review. *Journal of Food Protection*, **82**(11): 1950 - 1958.
- Kumar, R. and Prasad, A. (2010). Detection of *E.coli* and *Staphylococcus* in milk and milk products in and around Pantnagar. *Veterinary World*, **3**: 495–496.
- Han, Z, Lautenbach, E., Fishman, N. and Nachamkin, I. (2007). Evaluation of mannitol salt agar, CHROMagar *Staph aureus* and CHROMagar MRSA for detection of *Staphylococcus aureus* from nasal swab specimens. *Journal Medical Microbiology*, **56**(1): 43–46.
- Mhone, T. A., Matope, G. and Saidi, P.T. (2011). Aerobic bacterial, coliform, *Escherichia coli* and *Staphylococcus aureus* counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. *International Journal of Food Microbiology*, **151**: 223–228.
- Montel, M.C., Buchin, S., Mallet, A., Delbes-Paus C, Vuitton DA, Desmasures N & Berthier F (2014). Traditional cheeses: Rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology*, **177**: 136 - 154.
- Moore, D.S. and George, P.M. (2006). *Introduction to the Practice of Statistics*, 5th edition, Freeman.

- Murphy, S.C, Martin, N.H., Barbano, D.M. and Wiedmann, M. (2016). Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? *Journal of Dairy Science*, **99**: 10128–10149.
- Nebedum, J.O. and Obiakor, T. (2007). The effects of different preservation methods on the quality of nunu, a locally fermented Nigerian dairy product. *African Journal of Biotechnology*, **6**: 454–458.
- Olatoye, I.O., Uba, U.C. and Akintunde, S.O. (2018). Prevalence of *Staphylococcus aureus* contamination in bovine milk from local dairy herd in Oyo State, Nigeria. *Tropical Veterinarian*, **36**: 87–93.
- Perin, L.M., Pereira, J.G., Bersot, L.S. and Nero, L.A. (2018). The microbiology of raw milk, In: *Raw Milk: Balance Between Hazards and Benefits*. Pp. 45–64.
- Perin, L.M., Savo, Sardaro, M.L., Nero, L.A., Neviani, E. and Gatti, M. (2017). Bacterial ecology of artisanal Minas cheeses assessed by culture-dependent and -independent methods. *Food Microbiology*, **65**: 160–169.
- Rahimi, E., Chaleshtori, S.S. and Parsaei, P. (2011). Prevalence and antimicrobial resistance of *Escherichia coli* O157 isolated from traditional cheese, ice cream and yoghurt in Iran. *African Journal of Microbiology Research*, **5**.
- Rai, S., Karki, B., Humagain, S., Rimal, S., Adhikari, S., Adhikari, S. and Thapa, S. (2020). Antibigram of *Escherichia coli* and *Staphylococcus aureus* isolated from milk sold in Kathmandu district. *Nepal Journal of Biotechnology*, **8**: 82–86.
- Raza, N. and Kim, K.H. (2018). Quantification techniques for important environmental contaminants in milk and dairy products. *Trends in Analytical Chemistry*, **98**: 79–94.
- Reta, M.A. and Addis, A.H. (2015). Microbiological Quality Assessment of Raw and Pasteurized Milk. *International Journal of Food Science and Microbiology*, **2**: 87–91.
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M. (2011). Foodborne illness acquired in the United States-Major pathogens. *Emerging Infectious Disease*, **17**: 7–15.
- Tack, D.M., Kisselburgh, H.M., Richardson, L.C., Geissler, A., Griffin, P.M., Payne, D.C. and Gleason, B.L. (2021). Shiga toxin-producing *Escherichia coli* outbreaks in the United States, 2010–2017. *Microorganisms*, **9**.
- Umaru, A.G., Kabir, J., Umoh, V.J., Bello, M. and Kwaga, J.K.P. (2013). Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Fresh and Fermented Milk in Zaria and Kaduna, Nigeria, *International Journal of Drug Resistance Technology*, **3**(3): 67 - 75.
- Usman, R.Z, and Mustapha, B.M. (2016). Isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk “nono” and yoghurt in Kaduna metropolis. *Food Science and Quality Management*, **2**: 1 – 21.
- Uzoaga, G.O., Umeokonkwo, C.D., Usman, A.B., Kia, G.S. and Okolocha, E.C. (2020). Bacteriological quality of Nono, a milk product sold at retail outlets in Federal Capital Territory, Nigeria. *Journal of Interventional Epidemiology and Public Health.*, **3**.
- Weiler, N., Leotta, G.A., Zarate, M.N., Manfredi, E., Alvarez, M.E. and Rivas, M. (2011). Foodborne outbreak associated with consumption of ultrapasteurized milk in the Republic of Paraguay. *Revista Argentina de Microbiología*, **43**: 33–36.
- Yakubu, A., Abdullahi, I., Whong, C.Z. and Olayinka, B. (2020). Prevalence and antibiotic susceptibility profile of *Staphylococcus aureus* from milk and milk products in Nasarawa State, Nigeria. *Sokoto Journal of Veterinary Science.*, **18**: 1–12.