RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v16i4.6

Maikai & Madaki/*Sokoto Journal of Veterinary Sciences*, **16**(4): 50 - 57.

Enumeration of coliforms in fermented milk product (*nono*) sold in Samaru, Kaduna State, Nigeria

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Publication History:
Received: 11-03-2018
Accepted: 07-09-2018

Abstract

The quality of cow milk is reduced by microbial contamination and this poses a health risk to humans. One hundred fermented milk products (*nono)* sold within Samaru, Zaria were collected to determine the bacterial quality of *nono* using total aerobic plate and coliform counts on Nutrient and MacConkey agar plates respectively, The isolation of *Escherichia coli* was with the use of Eosin Methylene Blue agar. The mean pH of the nono samples from ten retail outlets ranged from 3.2 in Samaru leather research to 3.8 each in Faculty of Veterinary Medicine and ICSA market. Other mean pH values were as follows: Suleiman Hall and Dogon Ichi 3.3 each, Community market, Faculties of Science and Arts, 3.5 each, Samaru market and Hayin Dogo, 3.6 each. The highest total aerobic plate count of 1.390×10^{13} CFU/mL was from site B while the least of 0.789×10^{13} CFU/mL was from site G. The mean coliform count ranged from 2.10×10^{10} to 5.12×10^{10} CFU/mL. Site F had the least of 2.10×10^{10} while site H had the highest count (5.12×10^{10}) closely followed by site E (5.03×10^{10}). Out of 100 samples obtained, 46 (46%) yielded colonies suspected to be *E. coli.* On confirmation, after biochemical characterization, only 27 (58.7%) out of the 46 presumptive samples were positive for *E. coli*. An *E. coli* isolation rate of 27% was recorded out of the 100 *nono* samples, with the highest prevalence of 50% in *nono* samples from site I and the least of 10% from site E. The prevalence of other coliforms in the 100 samples were *Enterobacter* (8%), *Klebsiella* (6%), *Citrobacter* (2%) and *Proteus* (3%) species. The study has shown that fermented milk, *nono* is contaminated and can pose a health hazard to consumers of this product.

Keywords: Coliforms, *Escherichia coli*, Milk, *Nono,* Samaru

Introduction

Fermentation of fresh cow milk by bacterial organisms results in a product called "*Nono",* which is its Hausa name. (Atanda & Ikenebomeh, 1990). *nono* is a healthful liquid food, which is opaque white to milky in colour, and is consumed from the Saharan tribe of West African sub-region up to the Mediterranean region to the Middle East, where it is called "Dahi" or "Lassi" (Nahar *et al*., 2007). *Nono* which forms two (2) major parts of marketable food in Northern part of Nigeria is one of the popular foods consumed in the North. (Egwaikhide *et al*., 2014*)*, and it can be obtained from raw milk of cows, sheep or goats that are hardly pasteurized. Food borne illnesses can result from this raw unpasteurized milk product because the product can contain dangerous bacteria such as *Salmonella* spp, *Escherichia coli*, and *Listeria* spp, (Omotosho *et al*., 2013). *Nono* which is also called *Nunu* in some parts

of Nigeria is rich in amino acids, calcium, phosphorus and vitamins A,C,E and B complex (Nebedum & Obiakor, 2007). It has been widely reported that the presence of pathogenic microorganisms in milk and milk products poses a major threat to human health (Mubarack *et al*., 2010), and consumers of this product are at risk of being infected with milk-borne pathogens since *nono* does not undergo any further processing against pathogenic organisms.

Handlers of *nono* product who do not practice good hygiene can introduce pathogenic microorganisms into the product during processing, which when consumed can affect the health of consumers (Omotosho *et al*., 2013). Zoonotic milk borne diseases in humans are caused by several other pathogenic microorganisms such as leptospirosis, tuberculosis, brucellosis, Q fever, and campylobacteriosis (Rajesh *et al*., 2017), and *Escherichia coli* 0157:H7 which is an emerging pathogenic milk-borne bacterial, has also been isolated in the product (Karmali *et al*., 2010).

The high frequency of milk products in the transmission of human pathogens have been demonstrated (Rajesh *et al*., 2017). The wide distribution of coliforms in nature has been implicated in the contamination of dairy products by coliforms. Irrespective of the method used in the preparation of *nono*, the female Fulani hawkers add stream water and other products like the milky-white soaked baobab tree referred to as "Kuka" to the product to increase its volume and equally improves the taste and colour (Yabaya *et al*., 2012), which may predispose the fermented product to increased coliform contamination.

Coliforms which have been used as indicator organisms for bacteriological quality of milk and its product are normal flora of intestinal tract of humans and animals (Chaltergee *et al*., 2006). Coliforms are often found in raw milk but with good methods of production, the number can be kept very low (Boor *et al*., 1998). Coliform count is used as a definite index of faecal contamination of milk and its product, and it also indicates the possible presence of other enteric pathogens which may constitute health hazards to the consumers. Generally, the most important index of microbiological quality is total bacterial count, coliforms, yeast moulds count and detection of specific pathogens and their toxins (Szita *et al*., 2008).

Microbial examination of milk and milk products is essential since it enable us to ascertain the degree of contamination with indicator organism (Benkerroum *et al*., 2004). The coliform bacteria are organisms that can grow well in a variety of substrates and utilizes a number of carbohydrate and some other organic compounds as food for energy, and their source of nitrogen is from a number of fairly simple nitrogenous compound (Davidson *et al*., 2004). Coliforms are excreted in large number in both human excreta and animal droppings and they may be found in the soil, on vegetables and untreated water (Gebra-Emanuael, 1997). Total coliform, *E. coli* and *S. aureus* are used as hygienic parameters for milk production, as they indicate the conditions of raw milk obtained and during storage, and inadequate handling during the manufacturing process. The presence of pathogenic bacteria in milk appears as main public health concerns, especially for people who still drink raw milk (Fadaei, 2014; Wanjala *et al*., 2018). The coliforms have been used as an indicator organisms for evaluating water for faecal contamination and for identifying unsanitary conditions in dairy products and other foods (Nicole *et al* ., 2016). Hence the aim of this research was to carry out coliform count in fermented milk product (*nono*) sold in Samaru, Kaduna State, Nigeria.

Materials and Methods

Study area

Samaru town in Zaria Local Government Area of Kaduna State was the study area. The area is known for Hausa/Fulani women hawking fermented milk (*nono*) in plastics and calabashes. Zaria, is located between latitude 11º 7' 11º 12' N and longitude 07º 41' Eand it is situated at elevation 644 meters above sea level and operates on the West Africa Time (WAT) Zone. It is a medium sized city with an estimated population of 547,000 and a growth rate 3.5% per annum (Mortimore, 1970). About 50 to 70% of the working populations of Zaria metropolis derive their livelihood from farming. Agriculture practices in Zaria are divided into rainfall (from May to October) and irrigation farming in the dry season (from November to April). Zaria is characterised by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7ºC and an annual rainfall of 1092.8mm. In Zaria, dry season farming is the second most prevalent agricultural activity with vegetables being the common produce, but in some cases fruits are planted alongside cereal crops (Agbogu *et al*., 2006).

Sample collection

One hundred millilitres each of 100 *nono* samples were purchased from ten (10) retail outlets within Samaru town in Zaria, into sterile containers and

sealed immediately. Samples were conveyed in ice-packed cooler to the Bacterial Zoonoses Laboratory of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for analysis. Sample collection and analysis lasted for 10 weeks with 10 samples collected and analysed at each period of analysis per week. The selling points were visited between 12 noon- 3pm during the period of the analysis. Purchased samples that were not analysed immediately were kept in the refrigerator at a temperature of 4^{o}C not later than 3 days pending analysis.

Determination of nono potential hydrogen concentration (pH)

The pH of various samples of *nono* was measured using A pH meter (WPA pH meter) was used to measure the pH of various *nono* samples after initial standardization of the pH meter with buffer 4 and 7.

Microbiological analysis

Preparation of nono samples: According to standard method by APHA (1993), *a* hundred-fold serial dilution (1:100) of 0.1 mL *nono* was added into 9.9mls of sterile physiological saline to make first serial dilution (10^{-2}). A series of up to a total of 5 dilutions (10^{-10}) was prepared by transferring initial dilution (0.1 mL) into test tube containing sterile diluents (0.9 mL) to obtain 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10} in repeated operations.

Total aerobic plate count: The total plate count (TPC) was used for enumeration of total viable bacteria by which 0.1 mL of 10^{-10} dilution was inoculated into nutrient agar plate and spread using sterile glass spreaders. This was then incubated at 37^{o}C for 24 hours after which the plates that exhibited growth within 30-300 colonies were counted. To obtain the TPC, the number of colonies in the dilution was multiplied by the dilution factor. The calculation of the TPC was expressed as the number of organisms of colony forming units per mL (CFU/ml) of samples using the following formula below:

Number of bacteria per mL of serially diluted bacteria:

$$\frac{\text{Number of CFU counted}}{\text{Volume plated (0.1mL) x dilution } (10^{-10}) \text{ used}} = \text{Number of CFU/mL}$$

Total aerobic plate count

Enumeration of bacterial count in the locally fermented milk nono sold within Samaru, is as shown in Table 2. Site B recorded the highest mean colony forming units of 1.390 x 10^{13} CFU/mL, while the least of 0.789 x 10^{13} CFU/mL was at site G.

Total coliforms count:

MacConkey agar was used for enumeration of total coliform bacteria by transferring 0.1 mL of 10^{-8} dilution into the agar plate and spread using sterile glass spreaders. The was incubated at 37^{o}C for 24 hours after which typical pinkish colonies were counted indicative of lactose fermenting bacteria while non-lactose fermenting bacteria appeared colourless. Colony forming units per mL (CFU/ml) was used to depict the counts.

Isolation of *Escheichia coli*:

Eosin-Methylene Blue (EMB) agar was selective plate used for isolation of *E. coli.* A volume of 1 mL of *nono* was transferred into dilution bottles containing 9 mL of Tryptose soya broth and this was incubated at 37^{o}C for 24 hours. The sample was streaked onto EMB agar and it was then incubated at 37^{o}C for 24 hours. *Escherichia coli* colonies were identified as they appeared as greenish metallic sheen. These were then inoculated onto nutrient agar slants and incubated for 24 hours at 37^{o}C and preserved in the refrigerator at 4^{o}C. Prior to biochemical test, the preserved isolates were subcultured onto EMB agar to obtain a pure culture at 37^{o}C for 24 hours. The sub cultured colonies were used for biochemical tests.

Biochemical characterisation: The characteristic colonies were identified on the basis of sugar utilisation IMVIC pattern and production of hydrogen sulphide (H_2S). The suspected *E. coli* colonies were tested for the ability to ferment lactose and sucrose in triple sugar iron (TSI) agar, slants for SIM medium (Sulphide, Indole and Motility), Methyl red and Voges proskaeur reaction (Oxoid Ltd, Basingstoke, UK), citrate utilisation (Oxoid Ltd, Basingstoke, UK) and Urease Production (Difco, Detroit, USA) were used.

Results

Mean pH of nono

The mean pH of the nono samples from the ten (10) retail outlets within Samaru ranged from 3.2 in Samaru leather research neighbourhood to 3.8 each in Faculty of Veterinary Medicine and Icsa market. Other mean pH values were as follows: Suleiman Hall and Dogo Ichi, 3.3 each, Community market, Faculties of Science and Arts, 3.5 each, Samaru market and Hayin Dogo, 3.6 each (Table 1).

Total coliform count

The total coliform count is as shown in Table 3. The mean coliform count ranged from 2.10×10^{10} to 5.12×10^{10} CFU/mL Site F had the least of 2.10×10^{10}, followed by 3.24×10^{10} in site B. *Nono* samples from sites H (5.12×10^{10}) and E (5.03×10^{10}) were higher in coliform count when compared to other sites.

Isolation of E. coli

Out of 100 samples obtained, 46 (46%) yielded colonies suspected to be *E. coli.* Following biochemical characterization, only 27 (58.7%) out of the 46 *nono* samples were *E. coli* positive (Table 4). The overall prevalence of *E. coli* in the 100 *nono* samples was therefore 27%, with the highest prevalence of 50% in site I and the least of 10% in site E.

Isolation of other species of coliforms

Table 5 shows the prevalence of other species of coliforms isolated from the *nono* in this study; Such as *Enterobacter* (8%), *Klebsiella* (6%), *Proteus* (3%) and *Citrobacter* (2%) species.

Discussion

The microbiological quality of fermented milk product (*nono*) in this study gives an overview of how fit the product is for consumption. The study also gives an insight into the overall sanitation level of the whole process and the hygienic status of the product as the acceptable limits of coliform counts in milk should be less than 100 cell/mL (Boor *et al*., 1998; Douglas, 2003; Shojaei & Yadollahi, 2008).

Table 1: Mean pH of locally fermented milk *nono* sold at selected retail outlets in Samaru, Zaria, Kaduna State, Nigeria

Location	Number of samples collected	Mean pH
Faculty of Veterinary Medicine (A)	10	3.824
Faculty of Science (B)	10	3.52
Faculty of Art (C)	10	3.513
Samaru Market (D)	10	3.663
Hayin Dogo (E)	10	3.652
Sulieman Hall (F)	10	3.374
Community Market (G)	10	3.591
Dogon Ichi (H)	10	3.328
Icsa Market (I)	10	3.822
Samaru Leather Research (J)	10	3.251
Total/mean	100	3.5538

Table 2: Total aerobic plate count of locally fermented milk *nono* sold at selected retail outlets in Samaru, Zaria, Kaduna State, Nigeria

Number of samples	Number of bacterial colonies (CFU/mL) in sample sites									
	A	B	C	D	E	F	G	H	I	J
1	7	282	16	182	43	160	49	35	142	153
2	144	16	140	200	64	40	80	30	220	0
3	121	102	122	160	182	184	0	164	174	42
4	162	184	124	0	101	162	103	70	120	37
5	30	74	63	0	0	27	205	28	43	321
6	0	152	10	104	11	21	76	242	56	182
7	193	242	214	172	70	0	42	2	78	57
8	107	122	106	82	124	142	120	200	0	148
9	107	62	80	58	120	43	110	132	144	80
10	72	154	180	232	122	212	4	16	100	15
Total	943	1390	1055	1190	837	991	789	919	1077	1035
Mean (x 10^{13})	0.943	1.39	1.055	1.19	0.837	0.99	0.789	0.919	1.077	1.035

Table 3: Total coliform count of locally fermented milk *nono* sold at selected retail outlets in Samaru, Zaria, Kaduna State, Nigeria

Location	Number of samples collected	Mean TCC (CFU/mL)
Faculty of Veterinary Medicine (A)	10	3.35×10^{10}
Faculty of Science (B)	10	3.24×10^{10}
Faculty of Art (C)	10	4.01×1^{10}
Samaru Market (D)	10	4.82×1^{10}
Hayin Dogo (E)	10	5.03×10^{10}
Sulieman Hall (F)	10	2.10×10^{10}
Community Market (G)	10	3.37×10^{10}
Dogon Ichi (H)	10	5.12×10^{10}
Icsa Market (I)	10	3.65×10^{10}
Samaru Leather Research (J)	10	3.28×10^{10}

Key: TCC = total coliform count

Table 4: Isolation of *E. coli* from locally fermented milk sold at selected retail outlets in Zaria, Kaduna State, Nigeria

Location	Number of samples collected	Number of suspected isolates	Number confirmed *E. coli* (%)
Faculty of Veterinary Medicine (A)	10	5	3 (30)
Faculty of Science (B)	10	3	2 (20)
Faculty of Art (C)	10	5	2 (20)
Samaru Market (D)	10	5	3 (30)
Hayin Dogo (E)	10	3	1 (10)
Sulieman Hall (F)	10	3	2 (20)
Community Market (G)	10	5	3 (30)
Dogon Ichi (H)	10	6	4 (40)
Icsa Market (I)	10	7	5 (50)
Samaru Leather Research (J)	10	4	2 (20)
Total	100	46%	27 (27%)

Table 5: Prevalence of coliforms in 100 fermented milk samples sold at retail outlets in Samaru, Kaduna State, Nigeria

Isolates	Number of isolates	Prevalence (%)
Escherichia coli	27	27
Enterobacter spp.	8	8
Klebsiella spp.	6	6
Proteus spp.	3	3
Citrobacter spp.	2	2
Total	46	46%

Studies have shown that when milk is produced under hygienic conditions from healthy animals, the bacterial count should not be more than 5×10^3 bacterial/mL (Chatterjee *et al*., 2006). Retailed milk products like *nono* stands a high chance of contamination (Omotosho *et al*., 2013) because of the unhygienic environmental (Amusan *et al*., 2005; Uwakwe, 2012) and hot weather conditions in the study area (Anon, 2018). Nigeria is a tropical country where the annual average temperature in Zaria is 25.6°C and the warmest (April) and coolest (January) months of the area have an average temperature of 30°C and 22.8°C, respectively (Yamusa *et al*., 2015). This indicates that milk product like *nono* can only be fit for consumption if processed under hygienic conditions and preserved. Bedding, soil, water and inadequate cooling of milk have been associated with both fecal and environmental contamination by coliforms (Douglas, 2003). The occurrence of coliforms and *E. coli* in milk and its products has received considerable attention because such contamination has been associated with fecal contamination and there is the consequent risk of infection with other more

pathogenic fecal organisms and also because they are associated with milk spoilage as a result of their growth in milk at ambient temperatures (Salman & Hamad, 2011). Sources of contaminations in *nono* include starter cultures, the cow udder, the utensils, and the water used in processing the *nono* (Omotosho *et al*., 2013).

Though the *E. coli* count is a suitable indicator of the microbiological quality of foods, but both the total viable count and faecal coliform counts are also significant in specifying the microbiological assessment of foods as it has been stated that the total viable count should not exceed 102cfu/g or mL (Owanumi, 1997). The high total aerobic plate count is indicative of poor hygiene either during processing or during handling. When the fermentation stage of *nono* production which is its critical control point is not well monitored, the quality of the finished product will be affected, making the *nono* to become sour and acidic especially when fermentation time exceeds 72 hours (Egwaikhide *et al*., 2014). The total aerobic plate count as recoded in this study corroborates with the values obtained by Uzeh *et al*. (2006) and Yabaya *et al*. (2012) but differs from the lower bacterial count obtained by Egwaikhide *et al*. (2014). Aerobic colony count is useful benchmark for studying the overall microbial quality of milk products as it also acts as an indicator for food quality and shelf-life duration (Ogbonna, 2011). The low level of hygiene maintained during the processing and sale of the product may be a pointer towards the high total bacterial count recorded in the study.

Mean pH of the *nono* samples recorded in the present study corroborates with earlier report of Yabaya *et al*., 2012, compared to higher values reported by Obi & Ikenebomeh (2007). Milk pH gives an indication of milk hygiene, hence the keeping quality. Milk pH should be between 6.6 and 6.8 when the milk temperature is at 20ºC (Javaid *et al*., 2009). The low pH values reported in this study may be indicative of poor hygienic procedures of the milk and hence poor keeping quality. The differences in pH in this study from other studies may be attributed factors such as duration of fermentation and use of starter cultures. It has been observed that if the keeping time of *nono* increases prior to consumption, the acidity increases and this consequently affects the number and kind of contaminating organisms that may be present in the milk (Yabaya *et al*., 2012).

Coliform bacteria are indication of some degree of potentially hazardous contamination and in this study, there was high coliform count. The presence of coliforms which is a major contaminant may also indicate that there may be other enteric pathogens (Egwaikhide *et al*., 2014). The result of coliform count as obtained in the present study supports reports previously obtained by Obiekezie *et al*. (2012) and Uzeh *et al*., 2006 but contradicts lower values obtained by Yabaya *et al*. (2012.)

The measure of sanitary quality and index for faecal contamination of *nono* in this study was assessed with the use of *E. coli*. The 27% prevalence of *E. coli* in *nono* which is in its ready-to-eat form may be seen as undesirable because it indicates poor hygienic conditions (Benkeroum *et al*., 2004). Isolation of *E. coli* from milk products indicate the presence of enteropathogenic microorganisms which constitute a very important public health hazard. Enteropathogenic *E. coli* causes severe diarrhoea and vomiting in infants, and also young children (CDC, 2015; Wanjala *et al*., 2018). The detection of other coliforms such as *Enterobacter, Klebsiella, Proteus and Citrobacter* species may not have a significant public health threat but may result in spoilage of the *nono*, which may greatly affect the shelf life of the product and hence may not be fit for consumption.

In conclusion, It is evident from this study that the minimum total aerobic plate and coliform counts obtained from fermented milk (*nono)* exceeded the required standard signifying a high contamination with coliforms and other bacteria. This may also indicate possible feacal contamination and may pose a health hazard to the consumers of this product.

Acknowledgement

Appreciation goes to staff of the Bacterial Zoonoses laboratory of Veterinary Public Health and Preventive Medicine, where the laboratory analysis was carried out, and also to staff of Department of Veterinary Physiology, Ahmadu Bello University, Zaaria, Nigeria for their assistance in taking the pH reading.

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