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Research Article

Incidence of Bacterial Contaminants in Fermented Milk (Nono/Kindirmo) Sold By Fulani Women to Consumers in Wukari Metropolis, Taraba State, Nigeria

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ABSTRACT

A study was conducted to investigate contaminants in fermented milk sold by Fulani women in Wukari, Nigeria. Fifteen milk samples were obtained from five different sales points (A, B, C, D, and E) for analysis. The Standard Plate Count method was employed to detect contaminants, with spread plate techniques isolating bacteria. The total aerobic bacteria count on nutrient agar plates was found on sample 2 of sales point D with a bacteria count of 3.3 x 10^6. The total aerobic bacteria count on Manittol salt agar plates was found on sample 2 of sales point D with a bacteria count of 2.4×10^6. The highest bacterial growth was *staphylococcus aureus* with 73.33%. The least bacterial growths was *Escherichia coli* and *Pseudomonas aeruginosa* with 13.33% respectively. Sample 1 of sales point A, samples 1 and 2 of sales point D and sample 3 of sales point E show bacteria growth on the Mannitol salt agar plate, while the other samples shows no growth. Also on a Nutrient agar plate, sample 1, 2, and 3 of sales point A, sample 3 of sales B, sample 3 of sales point C, and sample 2 of sales point D were negative, while the other samples were positive. It can be concluded that the high incidence of contamination with bacteria *in nono* could be attributed to the unhygienic milking process and post-contamination after pasteurization and during sales.

Keywords: Consumers, Fermented Milk, Fulani Women, Incidence, Sold, *Staphylococcus*

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INTRODUCTION

Nono is a Hausa food drink hawked by the cattle maidens common to the Northern parts of Nigeria and most of the Western part of Africa (Obi and Ikenebomeh, 2007; Adesokan *et al.*, 2011). It ranges from being whitish to milky and is produced from non-pasteurized cow milk collected, and allowed to pass through 24 hours fermentation at room temperature (Olusupo *et al.,* 1996). Cow milk consumption cuts across the Sahara through the West African Sub-region. The food drink (nono) is called 'dahi' or 'lassi' in the Middle East (Nahar *et al.*, 2007). Obande and Azua, (2013) reported that the herdsmen employ the services of handlers and peddlers who hawked the product after fermentation has been completed. Nono is a good source of amino acids, calcium, phosphorous and vitamins A, C, E and B complex (Nebedum and Obiakor, 2007). It has also been reported to reduce allergic reactions as it contains many natural proteins and antibodies (Baars 2013; Hodgkinson *et al.*, 2014).

Food borne diseases are common and widespread global problem. Several outbreaks have been reported as a result of consuming milk that may appear normal but are in fact contaminated with large number of harmful bacteria (Varga, 2007). According to an analysis by Centers for Disease Control and Prevention (CDC), between 1993 and 2006, more than 1500 people in the United States became sick from drinking raw milk or eating cheese made from raw milk. CDC has also reported that

unpasteurized milk is 150 times more likely to cause food borne illness and result in 13 times more hospitalization than illnesses involving pasteurized dairy products (CDC, 2009).

In Nigeria, the traditional method of processing and selling cow milk and its products exposes these products to the danger of microbial contamination from spoilage and pathogenic microorganisms. All the milk products except raw milk processed by the local cattle handlers are often boiled before sales to the public, and this is considered as a form of pasteurization. However, the milk may be cross-contaminated from the handler's, utensils and other external sources (Adesiyun, 1994). Raw milk or processed milk is a well-known good medium that supports the growth of several microorganisms with resultant spoilage of the product or infections and intoxications in consumers (Oliver *et al*., 2005). Microorganisms found in Nono, Kindrimo, Manshanu and Raw milk mostly come from the water used in preparing the milk or handling, storage and processing activities. Hayes *et al.* (2001) reported that bacteria in milk can occur through colonization of the teat canal or an infected udder (clinical or subclinical mastitis) or get contaminated at various stages whether from the animal, milker (manual as well as automated), extraneous dirt or unclean water. Shehu and Adesiyuh (1990), had earlier reported that in order to increase the volume and improve colour of Nono, Kindrimo, Mashanu and Raw milk, the female Fulani hawkers prior to sales often do engage in fraudulent act of adding stream water and milky white supernant of water obtained from soaked baobab tree seeds. This act could further lead to the contamination and spoilage of the cow milk products.

The presence of contaminant microorganisms, especially pathogenic bacteria in milk and milk products is of serious public health concern. Poor hygiene practices by handlers of these products do lead to introduction of pathogenic microorganisms into the products, and since these products do not undergo further processing before consumption, they may pose health risk to the consumers of these products (Adeyemi and Umar, 1994).

Plotter (2002), reported that campylobacteriosis, salmonellosis, tuberculosis, brucellosis, hemorrhagic colitis, brainerd diarrhea, fever, listeriosis, yersiniosis and toxoplasmosis were associated with the consumption of nono. Milk and its by-products provide favorable growth condition for microorganisms to thrive such as fungi, bacteria, rickettsia and viruses. Microbial contamination is dependent on the health status and hygiene of the cow, housing and milking environment, cleaning and production facilities, coupled with storage equipment. Poor or inadequate pasteurization and product re-contamination have been reported to lead to milk-borne diseases (Nebedum and Obiakor, 2007).

Food-borne diseases remain a threat despite advances in medical technology. Milk is a good substrate for *Staphylococcus aureus* growth and among the foods implicated in staphylococcal food poisoning (SFP), milk and dairy products play an important role, since enterotoxigenic strains of *Staphylococcus aureus* have been frequently isolated in them (Normanno *et al.,* 2007). Raw milk, other milk products such as Nono (locally fermented milk), Kindrimo and Manshanu (fat from milk) are often commonly hawked and consumed in many Northern Nigerian streets. These products due to their processing and distribution practices often are exposed to conditions which may permit growth of contaminating organisms and possible toxin producers (Ezeonu and Ezurike, 2007). Pasteurization will reduce these problems and will make fermented milk products safer.

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics. Antibiotics resistance in this organism has occurred in epidemic waves, beginning with the emergence of strain that were resistant to penicillin and progressing to the present pandemic of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Community-associated methicillin-resistant *Staphylococcus* aureus (CA-MRSA) can spread rapidly among healthy individuals (Frank *et al*., 2010).

Milk is considered a nutritious food because it contains several important nutrients including proteins and vitamins. Because of their unique composition and properties, milk and dairy products represent excellent growth media for many spoilage and pathogenic microorganisms (Nada *et al*., 2012*;* Claey*s et al*., 2013). Nigeria like other developing countries has its dairy industry facing major problem of low demand for raw milk, partly because of public health concern over its safety and quality. Presently, it is difficult to ascertain the extent to which these indigenous dairy products contribute to food poisoning outbreaks. However if efforts are made to undergoes good manufacturing practices (GMP), it

would not only protect public health but also stimulate the growth of the dairy industry in Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in Wukari Metropolis Wukari Local Government of Taraba State. Wukari's climate is classified as tropical. In dry season, there is much less rainfall than in rainy season. .This climate is classified as Aw (tropical wet-dry climate).The average annual temperature in Wukari is 28.2°C (82.7°F). Precipitation is about 986mm (38.8 inch) per year. Wukari has a latitude of 7’52’38.4N and a longitude of 9’46’44.48E or 7.877332 and 9.779023 respectively.

Ethical considerations

A written approval was obtained from the Director Primary Health Care Wukari Local Government Area. Before the commencement of the study a familiarization tour was carried out to the study area where the significance, relevance and objectives of the study were discussed for the success of the study.

Sample Collection

A total of fifteen (15) samples of locally prepared milk (nono) were purchased from five different retailers points within Wukari Metropolis. The samples were placed in sterile containers in ice-packed coolers and were taken to Department of Biological Sciences Laboratory Federal University Wukari for microbial analysis.

Media and Sterilization

Nutrient agar (Hi-Media; India), Mannitol salt agar (Hi-Media; India) and Mueller hinton agar were used in this study. The media were weighed correctly and were prepared according to the manufacturers’ instructions before being sterilized in an autoclave at 121^{o}C for 15 minutes. The sterilized media were poured into petri dishes aseptically and allowed to solidify.

Determination of Total Aerobic Bacterial Count in the Nono

Standard plate count method was used to determine the total aerobic colony counts of bacteria in the samples of the cow milk product (Nono) (Sanders, 2012). Tenfold serial dilution was prepared by introducing 1ml of Kindrimo with a sterile syringe into a sterile test tube. A plastic rack was arranged with sterile test tubes containing 9ml of sterile water. A ten-fold serial dilution was carried out by homogenizing 1ml of the sample into the 1st test tube and was labeled as 10^{-l}. It was mixed thoroughly and 1ml was taken again from the 10^{-1} dilution tube and transferred into the next test tube labeled 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and into the seventh tube 10^{-7}. Each test tube was shaken vigorously before each transfer as describe by (Cheesbrough, 2006). From the seventh tube, 1ml was discarded and 0.02 ml was inoculated into a nutrient agar plate with the aid of a pasture pipette. There after incubated for growth at 37^{o}C for 24 hours. The average bacterial loads of cow milk product from the five different locations were obtained and expressed as Colony Forming Units per milliliter [cfu/ml] (Harrigan and McCance, 1976).

Isolation of Staphylococcus Species

Using the 10^{-7} dilution, one drop each were inoculated into plates of mannitol salt agar using spread plate technique and were incubated at 37ºC for 24 hours produce yellow zones surrounding their growth. The organism was confirmed biochemically by catalase and coagulase tests as described previously (Cheesbrough 2006).

Biochemical Test

Catalase Test

A loopful of the 24 hours culture was placed on a clean grease free slide. The culture was emulsify with a loopful of freshly prepared 3% hydrogen peroxide (H_2O_2) on the slide and the reaction was observed immediately for Catalase positive or negative organism as described by (Cheesbrough, 2006).

Coagulase Test

A drop of distilled water was placed on each end of a slide. A colony of the test organism was emulsified in each of the drop to make two thick suspensions. A loopful of plasma was added to one of the suspensions, and mixed gently. Clumping of the suspension within 10 seconds indicates a coagulase positive as described by (Cheesbrough, 2006).

Citrate Utilization Test

The media was prepared and dispensed into clean test tubes. It was sterilized at 121^{o}C for 15 minutes and slopes of about 1 inch were made. The test tubes were kept in a slanted position to set and the surfaces of slopes were inoculated with the test organisms

using sterile wire loop and then stabbed the butt. They were incubated at 35^{o}c for 48 hours and observed for a bright blue colour in the media as described by (Cheesbrough, 2006).

Identifications of Isolates

Identification of the isolates was performed using classical methods based on their morphological, physiological, and biochemical characteristics (Catalase, Coagulase and Citrate Utilization Test) with reference to Bargeys systematic Bacteriology manuals (Cheesbrough, 2006).

Standardization of Inoculum

Dilution from each of the suspension of the test isolates was prepared by picking a 24h colony of the isolates using sterile wire loop into sterile test tube containing sterile normal saline to form turbidity that match with 0.5 scale of McFarland's standard (1.5 × 10^8 cells/ml) as described by (Coyle, 2005).

Data Analysis

Data obtained from this research were subjected to descriptive statistical analysis using percentage in determining rates of contaminants in nono. The number of isolations in the contaminants was determined by microbial count (cfu/ml).

RESULTS AND DISCUSSIONS

Fifteen (15) samples of fermented milk, 3 samples from 5 different points, and the samples were analyzed microbiologically for incidence of Staphylococcus species. Sample 2 of sales point D had the highest incident of the microbial count of 33 which is equivalent to 3.3 x 10^6 on nutrient agar followed by sample 2 of sales point C with the microbial count of 22 which is equivalent to 2.2 x 10^6 on nutrient agar, sample 3 of sales point A had the least microbial count of 03 which is equivalent to 0.3 x 10^6 nutrient agar as shown in Table 1.

Fifteen (15) samples of fermented milk, 3 samples from 5 different points, and the samples were analyzed microbiologically for incidence of Staphylococcus species. Sample 2 of sales point D had the highest incident of microbial count of 24 which is equivalent to 2.4 x 10^6 on mannitol salt agar, followed by sample 2 of sales point E with a microbial count of 20 which is equivalent to 2.0 x 10^6 on mannitol salt agar, sample 1 of sales point A had the least microbial count of 03 which is equivalent to 0.3 x 10^6 on mannitol salt agar as shown in Table 2.

On the biochemical test the bacteria isolates on nutrient agar i.e *E. coli spp* shows positive test to Catalase test, Glucose, Lactase, Sucrose and Coagulate test, while *Staphylococcus aureus* isolates shows positive test to Catalase test, Citrate test, Glucose, Lactase, Sucrose and Coagulate test, while *Bacillus* spp isolates shows positive test to Catalase test, Citrate test, Glucose, and Sucrose, while *Lactobacillus* spp isolates shows positive test to Catalase test, Citrate test, Glucose, Lactase, Sucrose and Coagulate test as shown in Table 3.

The highest percentage frequency of bacterial isolated were *staphylococcus aureus* wit*h* the percentage frequency of occurrence of 73.33%.While the least percentage frequency of bacterial isolated were *Escherichia coli* and *Pseudomonas aeruginosa* with percentage frequency of occurrence of 13.33% respectively as shown in Table 4.

Isolation of *staphylococcus species* from the two different media indicated that sample 1 of sales point A, sample 1 and 2 of sales point D and sample 3 of sales point E did not show growth on Mannitol salt agar plate, while the other samples show bacterial growth. Also on Nutrient agar plate, the sample 1, 2, and 3 of sales point A, sample 3 of sales B, sample 3 of sales point C and sample 2 of sales point D were negative for bacterial growth, while the other samples were positive as shown in Table 5.

Table 1: Total aerobic bacterial count on nutrient agar plates

Sample area	Samples	Microbial count	cfu/ml
Sales Point A	Sample 1 (10^{-6})	06	0.6×10^{6}
	Sample 2 (10^{-6})	06	0.6×10^{6}
	Sample 3 (10^{-6})	03	0.3×10^{6}
Sales Point B	Sample 1 (10^{-6})	21	2.1×10^{6}
	Sample 2 (10^{-6})	12	1.2×10^{6}
	Sample 3 (10^{-6})	18	1.8×10^{6}
Sales Point C	Sample 1 (10^{-6})	10	2.2×10^{6}
	Sample 2 (10^{-6})	22	2.2×10^{6}
	Sample 3 (10^{-6})	07	0.7×10^{6}
Sales Point D	Sample 1 (10^{-6})	09	0.9×10^{6}
	Sample 2 (10^{-6})	33	3.3×10^{6}
	Sample 3 (10^{-6})	05	0.5×10^{6}
Sales Point E	Sample 1 (10^{-6})	20	2.0×10^{6}
	Sample 2(10^{-6})	13	1.3×10^{6}
	Sample 3 (10^{-6})	18	1.8×10^{6}

Table 2: Total aerobic bacterial count on mannitol salt agar plates

Sample area	Samples	Microbial count	cfu/ml
Sales Point A	Sample 1 (10^{-6})	03	0.3×10^{6}
	Sample 2 (10^{-6})	18	1.8×10^{6}
	Sample 3 (10^{-6})	06	0.6×10^{6}
Sales Point B	Sample 1 (10^{-6})	18	1.8×10^{6}
	Sample 2 (10^{-6})	08	0.8×10^{6}
	Sample 3 (10^{-6})	21	2.1×10^{6}
Sales Point C	Sample 1 (10^{-6})	07	0.7×10^{6}
	Sample 2 (10^{-6})	14	1.4×10^{6}
	Sample 3 (10^{-6})	10	1.0×10^{6}
Sales Point D	Sample 1 (10^{-6})	05	0.5×10^{6}
	Sample 2 (10^{-6})	24	2.4×10^{6}
	Sample 3 (10^{-6})	09	0.9×10^{6}
Sales Point E	Sample 1 (10^{-6})	18	1.8×10^{6}
	Sample 2(10^{-6})	09	0.9×10^{6}
	Sample 3 (10^{-6})	20	2.0×10^{6}

Table 3: Biochemical tests of bacteria isolates

S/N	Morphology	Gram stain	Catalase Test	Oxidase Test	Indole Test	Citrate Test	Glucose	Lactose	Sucrose	Coagulase Test	Isolate
1.	White, circular, large, entire and flat surface on nutrient agar.	ve Rod	+	-	-	-	+	+	+	+	*E. coli* spp
2.	Medium, spherical, golden yellow, dried and flat on nutrient agar.	+ve Cocci	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
3.	Creamy, medium, Spherical, raised surface on manitol salt agar.	+ve Rod	+	-	-	+	+	-	+	-	*Bacillus* spp.
4.	Creamy, circular, large and flat surface on nutrient agar.	+ve Cocco Bacilli	-	-	-	-	+	+	+	-	*Lactobacillus* spp.
5.	Large, whitish, spherical, dried and raised colonies	+ve cocci	+	-	-	+	+	+	+	-	*Staphylococcus epidermidis*
6.	Circular, small, and flat colonies on manitol salt agar	- ve rod	+	-	-	+	+	+	+	-	*Klebsiella* spp.
7.	Circular, medium, and flat colonies	-ve rod	+	-	-	+	-	+	-	-	*Klebsiella* spp.
8.	Spherical and raised colony	+ve rod	+	-	-	+	+	-	+	-	*Enterobacter* spp.
9.	Large, spherical, moist and raised	-ve rod	+	-	-	-	+	-	-	-	*Pseudomonas aeruginosa*
10.	Medium, spherical, golden yellow, dried and flat on nutrient agar.	+ve Cocci	+	-	-	+	+	+	+	+	*Staphylococcus aureus*

Table 4: Distribution of bacterial species from the 15 samples collected

Isolated Bacteria	Frequency	Percentage Frequency (%)
E. coli spp.	2	13.33
Pseudomonas aeruginosa	2	13.33
Enterobacter spp.	3	20
Lactobacillus spp.	9	60
Staphylococcus epidermidis	8	53.33
Klebsiella spp.	3	20
Bacillus spp.	5	33.33
Staphylococcus aureus	11	73.33
Total	**43**	**286.65**

Table 5: Isolation of Staphylococcus species from two different media

Sample area	Samples	MSA	NA
Sales Point A	Sample 1	NG	NG
	Sample 2	Staphylococcus spp.	NG
	Sample 3	Staphylococcus spp.	NG
Sales Point B	Sample 1	Staphylococcus spp.	Staphylococcus spp.
	Sample 2	Staphylococcus spp.	Staphylococcus spp.
	Sample 3	Staphylococcus spp.	NG
Sales Point C	Sample 1	Staphylococcus spp.	Staphylococcus spp.
	Sample 2	Staphylococcus spp.	Staphylococcus spp.
	Sample 3	Staphylococcus spp.	NG
Sales Point D	Sample 1	NG	Staphylococcus spp.
	Sample 2	NG	NG
	Sample 3	Staphylococcus spp.	Staphylococcus spp.
Sales Point E	Sample 1	Staphylococcus spp.	Staphylococcus spp.
	Sample 2	Staphylococcus spp.	Staphylococcus spp.
	Sample 3	NG	Staphylococcus spp.

Key: MSA = Mannitol Salt Agar, NA = Nutrient Agar, NG = No growth

The total bacterial load ranged from $4.2 \times 10^6 \pm 0.7$ cfu/ml to $9.4 \times 10^8 \pm 0.5$ cfu/ml. These counts were similar to the counts reported by (Uzoaga *et al*., 2020). They reported a count ranging from 5.6 ± 1.7 - 7.0 ± 0.4 log10 cfu/ml. The bacteria count in the current study was higher than the counts reported by Mohammad and Abdullahi (2015), who reported counts ranged from 2.7 x 10^7cfu/ml to 4.l x 10^7cfu/m. The level of bacterial contamination observed in the current study makes the nono samples unfit for consumption. In microbial study conducted in London described as the total *Staphylococcus aureus* count (10^4–10^5 cfu/mL) was described as unsatisfactory level of bacterial quality in the foods (Tibebu *et al*., 2021).

The incidence of *Staphylococcus spp*. in locally pasteurized milk in the current study was 73.33%. The reported incidence of *Staphylococcus aureus* in the current study was higher than the 25.53% reported by (Tibebu *et al*. 2021), 21.2% reported from Alage Veterinary College Dairy Farm by (Bekele *et al*., 2016), 20.3% reported by (Asiimwe *et al*., 2017) in Uganda.and 22.3% reported by (Liu *et al*., 2018) in China. 13.33% incidence for both *E. coli spp and P. aeruginosa* is lower than the 50% findings of (Beyene *et al*., 2017) in Addis Ababa. 20% incidence for both *Enterobacter* spp.and *Klebsiella* spp was lower than the 51% reported by (Abebe *et al*., 2016) in Hawassa. The research revealed 60% of *Lactobacillus spp* and 33.33% of *Bacillus spp* which is higher than 31.20% and 23.40% reported by (Beyene *et al*., 2017) in Addis Ababa. The research also revealed 53.3% incidence of *Staphylococcus epidermidis* which is higher than 45.70% reported by (Asiimwe *et al*., 2017) in Uganda. These variations might be as a result of differences in management system used by the handlers and types of samples.

CONCLUSION

This study revealed high incidence of *Staphylococcus aureus* and other bacterial species in nono in the study area. This high incidence may be attributed to unhygienic milking, and post contamination after pasteurization and during sales. The high incidence of *Staphylococcus aureus* and other bacterial species, it is crucial that milking and the entire critical points in the production of nono should be entirely checked or monitors to reduce contaminations.

Conflict of Interest

There were not any conflicts of interest between the authors from beginning of the study to the end. Everything went well as design and agrees on the proposal.

Author Contributions:

Conceptualization, M.K. and B.M. I; methodology, M.K.; validation, M.K. and B.M. I.; formal analysis, K.M. and B.M. I.; investigation, B.M.I.; resources, M.K. and M.B.I.; data curation, M.K. and B.M.I.; writing original draft preparation, M.K.; writing review and editing, M.K. and B.M.I.

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