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Evaluation of the Microbial Quality of 'Nono' Sold in Mangu Local Government Area of Plateau State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author GSD wrote the protocol and wrote the first draft of the manuscript. Authors GSD, CCI and ETA managed the analyses and the literature searches. Author BSD designed the study and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the microbial quality and safety or otherwise of 'nono' sold to the public for consumption.
Study Design: A cross sectional study design was employed for the study.
Place and Duration of Study: Mangu Local Government Area, Plateau State is the study area. The study lasted between May 2017 and June 2018.
Methodology: Questionnaire was administered to 300 'nono' sellers and subsequently, 300 'nono' samples were randomly collected (30 samples collected at intervals) from 10 markets and the samples were transported to central diagnostic laboratory of the National Veterinary Research

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Institute (NVRI) Vom, Plateau State for laboratory analyses of the samples using serial dilution and spread-plate technique.

Results: Results showed that majority (86.0%) of the respondents depends on selling 'nono' as the only source of income, and 75.7 % of them had no formal education. An overall mean total bacterial count (TBC) of 6.09 $Log_{10}cfuml^{-1}$ was recorded from all the samples. Majority of the 'nono' samples collected from the different markets had significantly higher bacterial count than the recommended level of 5.0 $Log_{10}cfuml^{-1}$ set by the International Farm Comparison Network (IFCN) for a minimum acceptable level of bacterial count in milk and milk products. Bacteria isolated were Coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* with overall prevalence of 52.7%, 43.0%, 10.0%, 8.3%, 5.0%, and 16.3% which recorded overall mean counts of 4.37, 3.56, 0.83, 0.69, 0.41 and 1.30 $Log_{10}cfuml^{-1}$, respectively. *Aspergillus niger*, *A. flavus*, *Penicillium* spp., *Mucor* spp., *Rhizopus* spp. and *Candida* spp. isolated from the products had an overall prevalence of 25.7 % and overall mean fungal count of 2.13 $Log_{10}cfuml^{-1}$. A statistically significant ($P<0.05$) difference was established among the means of the microbial groups.

Conclusion: Microbiological safety of 'nono' sold in Mangu is not guaranteed as at time of study possibly as a result of unhygienic practices during 'nono' production and product contamination from the vendors.

Keywords: Evaluation; nono; microbial; quality; sold; Nigeria.

1. INTRODUCTION

'Nono' is a Fulani word for cow's milk sold by the Fulani women [1]. Ogbonna [2] described 'nono' as an opaque white to milky coloured liquid food drink gotten from fermented raw milk. Omotosho et al. [3] also considered 'nono' as the Hausa name for fermented milk which is sold along with butter ('Maishanu') a by-product of its production. 'Nono" according to [2] is a healthful food whose consumption transverses the Saharan tribes of West African Sub-region extending to the inhabitants of the Mediterranean region and also the Middle East. It is considered by [4] as a Nigerian milk food similar to yoghurt and other fermented milk products that is traditionally produced and consumed particularly by the Hausa and Fulani of Northern Nigeria. In Nigeria, locally fermented raw cow milk is known as nono [5]. Obande and Azua [6] also view nono as a general name used for locally fermented cow milk which is widely consumed in many African countries, including Nigeria. Nono is Nigerian locally fermented milk product commonly prepared by Hausa/Fulani cattle herders [7].

The traditional method of processing and selling cow milk and its products such as 'Nono' exposes these products to microbial contamination [5]. Uzeh et al. [8] also explained that poor hygiene practiced by handlers of these products, may lead to introduction of pathogenic microorganisms into the products.

Milk quality continues to be a topic of intense debate in the dairy industry and in medical and public health sectors [9]. Production of the best quantities of good quality milk and milk products (nono inclusive) is an important aspect of standard dairy practice [10]. The demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers and manufacturers to produce and market safe milk and milk products [11]. Oliver et al. [9] summarised that high-quality milk contains a low bacteria count, low number of somatic cells, free of human pathogens and antibiotic residues. Bhatia et al. [12] stated that the quality and safety of milk encompasses milk characteristics such as chemical composition, physical properties, microbiological quality, and sensory properties. This implies that 'nono' with adequate microbiological status (absence of pathogenic organisms and other microbial contaminants) can be considered safe and qualitative for human and public consumption. However, pathogenic microorganisms have been claimed to be implicated in 'nono' due to poor hygiene practice by handlers of the product, hence the serious need to always assess and ensure the microbiological safety of this product owing to the fact that the product is produced in homes, especially villages where shelf-life and safety of the product is often ignored. Therefore, the objective of this study is to evaluate the microbial quality and safety of locally fermented cow milk (nono) sold in Mangu Local Government Area of Plateau State.

1.1 Production and Preparation of 'Nono'

Nono is produced mainly by the nomadic Fulani. It is also being prepared predominantly by the nomadic Hausa/Fulani cattle herdsmen [13]. The fresh milk is directly obtained from a cow into a properly washed semi-dried calabash and kept wide open in the sun for approximately two hours to facilitate isolation of the fat layer. Some quantity of overnight fermented milk is added therefore, to serve as a starter culture. The inoculated fresh milk is left overnight at room temperature for fermentation to get sour milk known as "Kindirmo", and the addition of large volume of water to the curdle sour milk which is then stirred with a T-shaped stick to a liquid of fine consistency gives rise to "Nono" [14]. Omotosho et al. [3] also explained that after milking from cow's udder during nono production, the physical hazards are taken care of by sieving the raw milk to remove for example stones, dry leaves, insects, and sand. And that during fermentation, the acid produced reduces the growth of bacteria but may favour the growth of fungi. Omotosho et al. [3] described the 'Nono' production chart with identified critical points (Fig. 1).

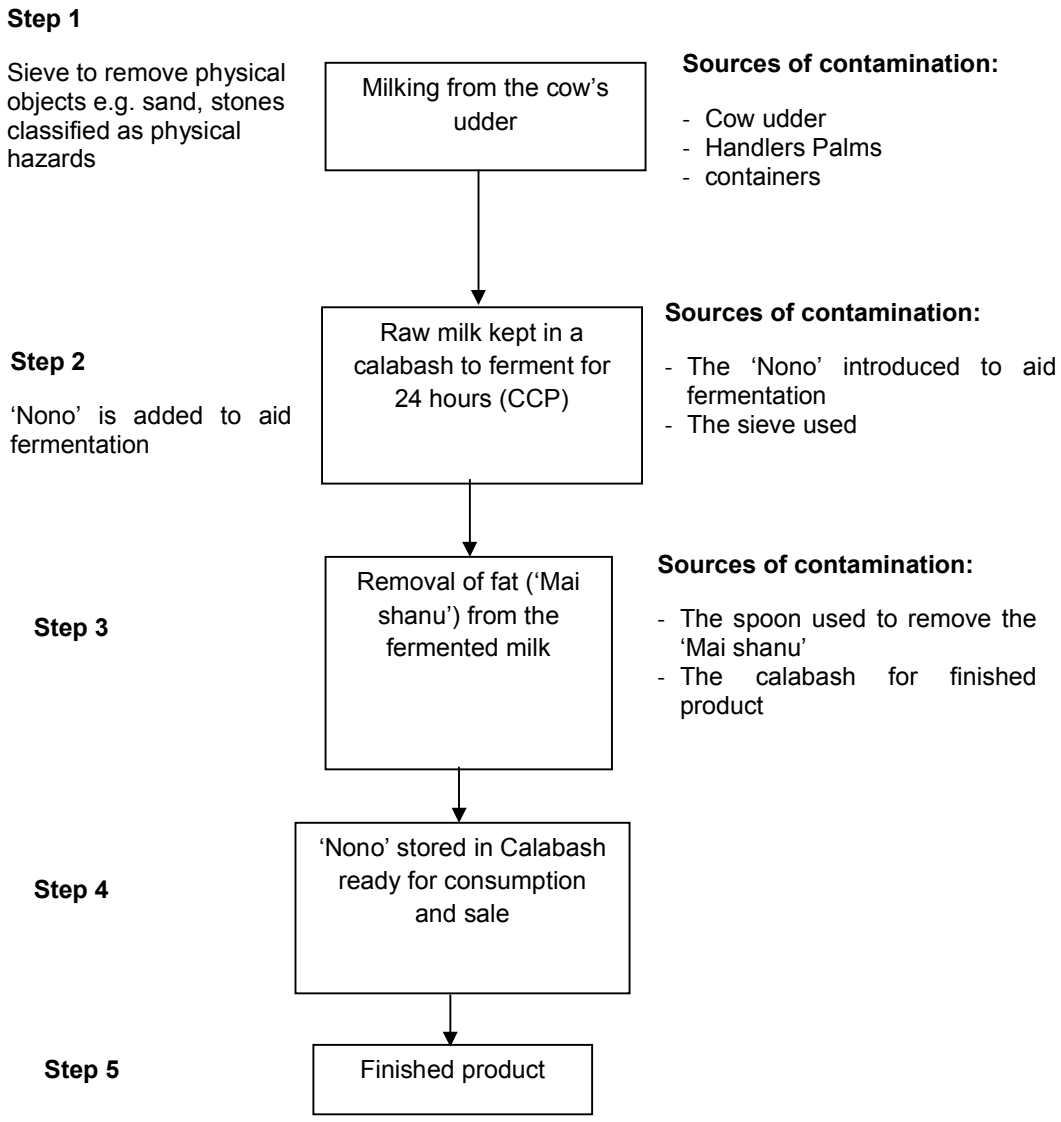

Fig. 1. 'Nono' Production chart with identified critical points

2. MATERIALS AND METHODS

2.1 The Study Area

This research work was carried out in Mangu Local Government Area of Plateau State, Nigeria (Fig. 2). The Local Government is situated in the south Eastern part of the state, and it is one of the local governments that make up the Plateau central senatorial zone. Mangu which lies about 77 kilometers south of Jos, is a semi-urban settlement with a huge farming population. It is located on Latitude 9°31'N and Longitude 9°06'E. The Local Government has nine (9) districts with a population of 294,931 people as at 2006 census, and has a land area of 1,653 square kilometers.

2.2 Selection of Markets

The ten (10) markets were purposively selected, and the selection of these markets was based on cattle population and frequent patronage of nono sellers or hawkers.

2.3 Study Design

The present study employed a cross-sectional study design of [15] to evaluate the microbial quality and safety of nono produced and sold in the study area. The study also involved a laboratory-based investigation. Nono sellers who patronised these markets in the study area were randomly selected for samples collection. Questionnaires were administered to them at the

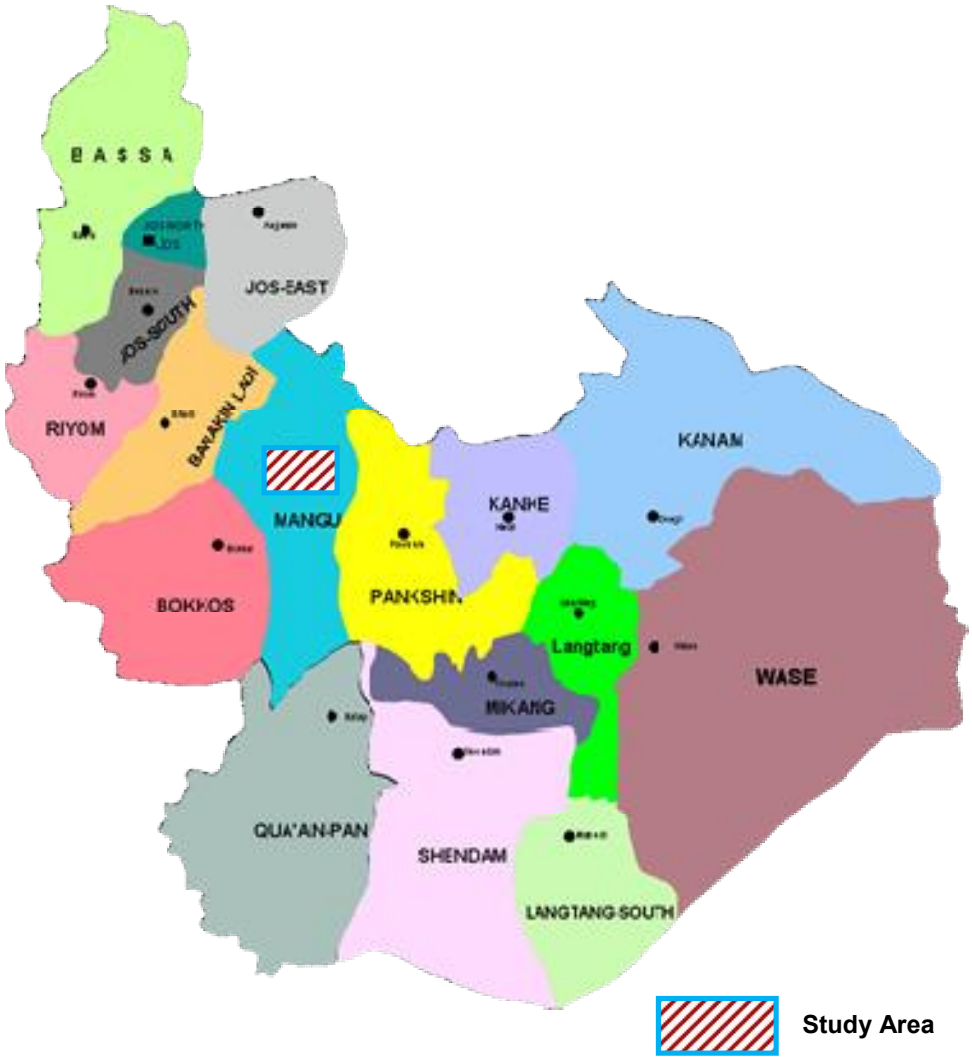

Fig. 2. Map of Plateau State showing the study area (Mangu L.G.A)

same time to assess how the product is being produced and handled, hygienic practices employed in the process line, possible sources of contamination, and other conditions thought to affect the quality and safety of the product.

2.4 Administration of Questionnaires

The questionnaires were administered using the method of Kanyeka [15] in which face to face interview conversation was carried out before administering the questionnaires for them to fill. The questionnaire was used to collect sociological information on possible risk factors for contamination of 'nono'. While administering the questionnaires, direct observation on general cleanliness and hygienic conditions and practices with regard to nono were also done and noted. Upon finishing of the administration of questionnaires, nono samples were collected for laboratory analyses.

2.5 Sample Collection

The sample collection method of Ogbonna [2] was adopted in this study. Nono samples were purchased from ten (10) different markets all within Mangu Local Government Area. Thirty (30) samples were randomly purchased at intervals from nono sellers in each of Mangu market, Pushit market, Kerang market, Ampang market, Panyam market, Gindiri market, Mangun market, Kombun market, Chanso market and Kadunu market. The selection of these markets was based on cattle population, nono hawkers and patronage. The purchased samples were transported to the central diagnostic laboratory of the National Veterinary Research Institute (NVRI) Vom in sterile corked plastic tubes parked in an iced container for microbiological analyses.

2.6 Microbiological Analyses

Microbiological analyses were done as described by Ogbonna [2]. Nono samples collected were used for the isolation and enumeration of the microorganisms. In each isolation protocol, nono sample was shaken and 10 ml of the sample was aseptically introduced into 90 ml of sterile normal saline solution and was homogenised by hand shaking followed by further decimal dilutions to up to 10^{-6} concentrations. A 0.1 ml quantity of appropriately diluted sample was used to inoculate freshly prepared media surfaces using the spread-plate method. The inoculated plates were incubated at 37°C for 24 hours for bacteria and at 35°C for at least 48-72 hours for fungi [6].

2.7 Colony Count

Colony counts were performed on the various selective culture media used. Discrete colonies that appeared on the plates after appropriate inoculation and incubation were counted using digital colony counter and were recorded for each organism. The total viable count (TVC), *Escherichia coli* count (ECC); Coliform count (CC), and fungal count (FC) were obtained on Nutrient Agar, Eosin Methylene Blue Agar, MacConkey Agar and Sabouraud Dextrose Agar, respectively. Other organisms were also counted on their respective selective media. The number of colonies counted was multiplied by the reciprocal of the dilution factor plated, and was divided by the volume of inoculums used to obtained the colony forming unit per milliliter (cfu/ml) of each sample. This is expressed as:

cfu/ml = (Number of colony counted X Reciprocal of dilution factor)/ Volume inoculated

2.8 Characterisation of Isolates from Nono

At intervals, colonies on the incubated plates were picked and purified by repeated sub-culturing by streaking on the desired media with a sterile wire loop. The strategy consisted of picking 1 colony to represent every visibly different morphology on each plate. A maximum of 5 colonies were obtained per samples, which were examined microscopically for Gram's reaction and colony morphology (shape, colour, texture, size) using 24 hour old cultures [16].

2.9 Identification of Bacterial Isolates

Bacterial isolates were identified as described by Mubarack et al. [16] based on growth on selective media, colony morphology, Gram's reaction and biochemical test results. Results were analysed using Cowan and Steel Manual, and other methods for the identification of medical bacteria [17].

2.10 Isolation and Identification of Fungi

This was done using the method for the isolation of fungi as described by Obande and Azua [6] and Bhatia et al. [12]. The isolation and identification of the fungi was done using potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) was used for the colony count. A small portion of the fungal culture was carefully picked using a scalpel and pin. It was prepared

and stained using Lactophenol Cotton Blue Stain, and was examined under the low power and high-dry power objectives. The hyphal structure, shape, spore type and arrangement were noted and applied in the identification of the isolates. Preliminary identification was also done by macroscopic observation of the cultures with regards to colour, shape and appearance of colonies of the culture medium which was compared with the observed microscopic structures [14,17,18].

2.11 Statistical Analysis

The data obtained for the different microbial counts were subjected to analysis of variance (ANOVA) using the Minitab version 17.0 software to determine differences among counts of the microbial species. Significance was determined at 5% probability level. In addition, simple percentage score was calculated for the frequency of responses and was analysed using chi-square test.

3. RESULTS AND DISCUSSION

3.1 Socio-demographic Characteristics of Nono sellers in Mangu L.G.A

The socio-demographic characteristics of respondents which included age, marital status, level of education, location, and selling of nono as the only source of income are shown in Table 1. Majority of the respondents (57.7%) were within the age group of 21-30 years. This was followed by those who were between 15-20 years (23.7%), 31-40 years (14.7%) and those above 40 years (4.0%). A statistically significant difference (P<0.05) was established among the different age groups of the nono sellers in the study area. However, majority of the nono sellers (73.0%) were married women. This was followed by those who were single (19.7%), widows (5.7%) and divorced (1.7%). A significant difference (P<0.05) was also found among these variables. Similarly, majority (75.7%) of the respondents had no formal education. This was followed by those who had been to school; primary, secondary and tertiary education with values of 18.3%, 5.3% and 0.7% respectively. This result also shows a statistically significant difference (P<0.05) among the variables.

The distribution of respondents according to location is also shown in Table 1. Majority of the respondents (93.0%) were from rural areas and only 7.0% were from the urban settlement. Also, 86.0% of the respondents indicated that selling of nono is their only source of income while 14.0% indicated that they have other sources of income apart from selling nono. In addition, a statistically significant difference (P<0.05) was established between the variables in each category.

3.2 Practices at Farm Level that can Predispose Nono to Microbial Contamination

Assessment of microbial contamination predisposing factors at the farm level is summarised in Table 2. Most respondents (68.7%) believe that animal house with the floor covered with manure predisposed milk to contamination compared to earthen floor (26.7%) and concrete floor (4.7%). 89.0% of the nono sellers responded that most of the animal house where the raw milk were bought were observed to be dirty while 11.0% observed cleanliness in some of the animal houses. Also, majority (77.7%) of them agreed that the milkers often wash their hands before milking while few (22.3%) disagreed with it. In relation to the sources of water use in the entire process line of nono which some could also be predisposing factors, majority (70.3%) of the respondent admitted the use of well water, 12.7% used river water, 10.2% used stream water while 6.7% accepted the use of borehole water. However, none of them accepted the use of tap water. 32% of the respondents admitted that some of the milkers milked sick animals. Also, 38.3% accepted that they observed some of milkers milking animals with udder problems. These factors could also predispose nono to microbial contamination. Consequently, a statistically significant difference (P<0.05) was established among all the variables assessed in each category.

3.3 Hygiene Practiced in the production Line of Nono

Table 3 shows the hygiene practices by the respondents in the processing of nono. Most of the respondents (91.0%) admitted that they used to heat the raw cow milk in the first step of the process and only 9.0% admitted not doing so. Majority of the respondents (48.7%) accepted that they used to heat the raw cow milk during nono production for 20 minutes, 25.0% admitted the heating of the raw cow milk for 30 minutes, 47.0% reported that they usually heat the raw cow milk till boiling point, while 10.7% accepted heating of the raw cow milk for only 10 minutes.

Most respondents (95.7%) admitted that they washed their hands and utensils regularly during nono production with only few (4.3%) that did not observed or practiced that. Also, most of the respondents (82.0%) used only water in washing their hands and utensils while 17.3% accepted that they used water with soap. Only 0.7% of them used water with disinfectant and soap for washing of hands and utensils during nono production (Table 3). Majority of the respondents (93.7%) used to cover their nono during storage with only few (6.3%) not used to practice that. Similarly, a statistically significant difference ($P<0.05$) was revealed among all the variables assessed in each category.

3.4 Percentage Occurrence of Microorganisms Isolated from Nono Samples

Out of the total of 300 samples analysed, 158 (52.7%), 129 (43.0%), 49 (16.3%), 15 (5.0%), 30 (10.0%), 25 (8.3%) and 77 (25.7%) were positive for *Coliform*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and fungi respectively (Table 7). Out of the number of samples tested from each market, Mangu market had the highest percentage of samples (66.7%) that were positive for *Coliforms* with Gindiri market having the least (36.7%). For *Escherichia coli*, Pushit market had the highest percentage of occurrence (56.7%) compared to other markets. Mangu, Pushit and Kadunu markets have the highest percentage of samples (23.3% each) that were positive for *Staphylococcus aureus*. For *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and Fungi; Mangu, Kadunu, Ampang and Pushit markets have the highest percentage of occurrence of 20.0%, 23.3%, 23.3%, and 46.7%, respectively (Table 7). The results of the percentage occurrences of these microbial groups show a statistically significant difference at probability level of between (P=0.00) and (P=0.03) at 5% level of significance.

3.5 Mean Values ± Standard Deviation for Microbial Counts of Nono Samples

Mean values of microbial counts ($\log_{10}$ cfuml^{-1}) are shown in table 8. The mean total bacterial counts ranged from 5.27±4.08 to 7.22±2.88 in the different markets with an overall mean of 6.09±3.68. Mean Coliform counts ranged between 3.06±4.09 and 5.53±3.98 with an overall mean of 4.37±4.15. The table also shows that the mean *Escherichia coli* counts were between 2.49±3.86 and 4.66±4.41 with an overall mean of 8.56±4.10. Mean *Salmonella* counts fell between 0.00±0.00 and 1.95±3.59 with an overall mean of 0.83±2.49. Mean *Shigella* counts were between 0.00±0.00 and 1.94±3.58 with an overall mean of 0.69±2.30 while *Pseudomonas aeruginosa*

Table 1. Socio-demographic characteristics of nono sellers in Mangu L.G.A

Demographic information	Frequency (Total=300)	Percentage (%)	χ^2	P-value
Age (years)				
15-20	71	23.7	194.00	0.00*
21-30	173	57.7		
31-40	44	14.7		
>40	12	4.0		
Marital status				
Single	59	19.7	390.08	0.00*
Married	219	73.0		
Widow	17	5.7		
Divorced	5	1.7		
Level of education				
No formal education	227	75.7	430.85	0.00*
Primary school	55	18.3		
Secondary school	16	5.3		
Tertiary education	2	0.7		
Location				
Urban	279	93.0	221.86	0.00*
Rural	21	7.0		
Selling nono as the only source of income				
Yes	258	86.0	155.52	0.00*
No	42	14.0		

Table 2. Responses of nono sellers on practices at farm level that can predispose nono to microbial contamination in Mangu L.G.A

Variable Assessed	Frequency (Total=300)	Percentage (%)	χ^2	P-value
Type of cattle house floor				
Covered with manure	206	68.7	190.32	0.00*
Concrete	14	4.7		
Earthed floor	80	26.7		
Cleanliness of animal house				
Dirty	267	89.0	182.52	0.00*
Clean	33	11.0		
Washing hands by milkers				
Yes	233	77.7	91.85	0.00*
No	67	22.3		
Use of water for cleaning and washing				
Yes	189	63.0	20.28	0.00*
No	111	37.0		
Sources of water used				
Tap water	0	0.0	331.01	0.00*
Borehole water	20	6.7		
Well water	211	70.3		
River	38	12.7		
Stream	31	10.3		
Milking sick animals				
Yes	96	32.0	38.88	0.00*
No	204	68.0		
Milking animals with udder problems				
Yes	115	38.3	16.33	0.00*
No	185	61.7		
Cleaning cow teats before milking				
Cleaning	63	21.0	22.34	0.00*
Not cleaning	237	79.0		
Types of nono storage container				
Calabash	278	92.7	100.92	0.00*
Plastic container	19	6.3		
Metal can	3	1.0		
Glass bottle	0	0.0		

counts were between 0.00±0.00 and 1.64±3.33 with an overall mean of 0.41±1.80. Furthermore, the mean *Staphylococcus aureus* counts were between 0.00±0.00 and 1.95±3.59 with an overall mean of 1.30±3.02 whereas that of fungal counts ranged between 0.83±2.52 and 3.86±4.19 with an overall mean of 2.13±3.64 from the different markets. A statistically significant difference ($P<0.05$) was established among the mean microbial counts from the different markets in the study area.

The nono samples collected and analysed from the different markets were contaminated by bacteria with an overall mean total bacterial count (TBC) of 6.09 $Log_{10}cfuml^{-1}$ (Table 8). The highest mean value (7.22 $Log_{10}cfuml^{-1}$) of the TBC was found in nono samples from Kadunu market, while the lowest mean value (5.27 Log_{10} $cfuml^{-1}$) where found in nono samples collected from Kombun and Chanso markets (Table 8). The mean total bacterial count range (5.27-7.22 $Log_{10}cfuml^{-1}$) is closely comparable to the findings of [19] who found the range of 7.36 - 7.88 $Log_{10}cfuml^{-1}$. Also, the overall mean TBC of 6.09 $Log_{10}cfuml^{-1}$ detected in this study almost agrees with the 7.07 $Log_{10}cfuml^{-1}$ reported by [20]. However, total bacterial counts greater than 5.0 $Log_{10}cfuml^{-1}$ as obtained in this study is higher than the given international standard set for minimum acceptable level of bacterial count in milk and milk products [21]. The implication of this result is that nono from the study area is of poor microbial quality. The observed high TBC in this current study is in line with those done by Schoder et al., Parek et al., Addo et al., [22-24]

who also reported higher bacterial counts above recommended level by standards in most of the samples that were tested. Presence of high bacterial load in milk and milk products indicates contamination possibly from lactating cows, milking equipment, storage containers, unsatisfactory hygiene/sanitation practiced at farm level, unsuitable storage condition, unclean udder and/ or teats, poor quality of water used for cleanliness and dirty hands of milkers. Generally, it further indicates the degree of hygiene practices in the whole milk production process [15,25,26]. Therefore, from the observed practices involved in the whole chain of the nono production, handling, storage and local processing in this study, the observed high TBC was expected.

The overall mean coliforms count obtained in this research was 4.37 $Log_{10}cfuml^{-1}$ (Table 8), and the range of the mean counts was between 3.06 and 5.53 $Log_{10}cfuml^{-1}$. This result is almost in agreement with the range of 4.03 $Log_{10}cfuml^{-1}$ to 6.57 $Log_{10}cfuml^{-1}$ obtained by Asrat et al., Abebe et al. [27,28]. Even though it is not practical to produce milk that is consistently free of coliforms, their presence in raw milk and milk products may therefore be tolerated [29]. However, if present in large numbers, over 100 coliform organisms per milliliter of raw milk and milk products, it means that the milk was produced under unhygienic condition [30]. Fulva [31] also reported that coliform counts regularly in excess of $100cfuml^{-1}$ are considered by some authorities as evidence of unsatisfactory production hygiene. Hence, their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers' health [29].

Escherichia coli had an overall mean count of 3.56 $Log_{10}cfuml^{-1}$ (Table 8). The mean *Escherichia coli* counts ($Log_{10}cfuml^{-1}$) were between 2.49 and 4.67 from the different markets. This result is different from the mean range of 1.37 and 3.29 corresponding to an overall mean of 2.21 $Log_{10}cfuml^{-1}$ obtained by Ogbonna [2] from different markets. From the results, *E. coli* happened to be one of the most frequent isolates with higher counts. Higher counts of different species of enterobacteriaceae were reported with *E. coli* being the most abundantly isolated [32], which is a good indicator of recent fecal contamination [33].

The mean *Salmonella* counts ($Log_{10}cfuml^{-1}$) of 0.83 contradict the 1.17 obtained by Ogbonna [2] and the 1.12 obtained by Abdalla and El-Zubeir [34]. The mean *Salmonella* count was between 0.00 and 1.95 $Log_{10}cfuml^{-1}$. This is not in agreement with the counts range of 0.43 and 2.37 $Log_{10}cfuml^{-1}$ recorded by Ogbonna [2]. *Salmonella* are pathogens that could originate from the animals themselves. Thus, the presence of *Salmonella* in fermented milk is not surprising since they could either be transmitted from the animal before preparation or could have come through cross contamination. However, the organism was not detected from four markets (Pushit, Mangun, Kombun, and Gindiri).

Table 3. Responses of nono sellers on practices in the production line of nono in Mangu L.G.A

Practices in the production line	Frequency (Total=300)	Percentage (%)	χ^2	P-value
Heating raw cow milk before nono production				
Yes	273	91.0	201.72	0.00*
No	27	9.0		
Duration of heating				
10 minutes	32	10.7	102.32	0.00*
20 minutes	146	48.7		
30 minutes	75	25.0		
At boiling point	47	15.7		
Washing of hands and utensils in between time of nono production				
Yes	287	95.7	250.25	0.00*
No	13	4.3		
What was used in the washings				
Water only	246	82.0	332.24	0.00*
Water with soap	52	17.3		
Water with disinfectant and soap	2	0.7		
Covering of nono during storage				
Yes	281	93.7	228.81	0.00*
No	19	6.3		

Table 4. Morphological and cultural characteristics of bacteria isolated from nono samples in Mangu L.G.A

Bacteria	Gram's reaction	Cultural characteristic on selective media
Escherichia coli	Gram-negative rods	Colonies showing metallic sheen
Salmonella spp.	Gram-negative rods	Non-lactose fermenting pale coloured colonies with black centers
Shigella spp.	Gram-negative rods	Non-lactose fermenting pale coloured colonies
Staphylococcus aureus	Gram-positive cocci (in clusters)	Yellow colonies with yellow zones
Pseudomonas aeruginosa	Gram-negative rods	Green colonies

Table 5. Morphological and cultural characteristics of fungi isolated from nono samples in Mangu L.G.A

Fungi	Microscopic Morphology	Cultural characteristics
Aspergillus niger	Septate hyphae with V-shaped branching and long conidiophores	Black colonies
Aspergillus flavus	Septate hyphae with long conidiophores which have a rough texture	Greenish yellow colonies
Mucor spp.	Coarse hyaphae with branched sporangiosphores without rhizoids	Whitish, flat round colonies
Penicillium spp.	Septate hyphae and brush-like conidiophores	Green colonies with powdery surfaces
Candida spp.	Yeast cells and Pseudohyphae	Cream coloured pasty colonies with distinctive yeast smell
Rhizopus spp	Aseptate hyphae with root-like rhizoids extending near the stolon hyphal base with unbranched sporangiosphores	Whitish-brown, fluffy, cotton-candy like colonies.

Table 6. Biochemical characterisation of bacteria isolated from nono samples in Mangu L.G.A

Bacteria	TSIA medium												
	OX	UR	CI	ID	CA	CO	Slope	Butt	H_2S	Gas	G	L	S
Escherichia coli	-	-	-	+	+	-	Y	Y	-	+	+	+	+
Salmonella spp.	-	-	+	-	-	-	R	Y	+	+	+	-	-
Shigella spp.	-	-	-	-	-	-	R	Y	-	-	+	-	-
Staphylococcus aureus	-	-	-	-	+	+	Y	Y	-	-	+	+	+
Pseudomonas aeruginosa	+	-	+	-	-	-	R	R	-	-	-	-	-

OX = Oxidase test; UR=Urease test; CI=Citrate test; ID= Indole test; CA=Catalase test; CO= Coagulase test; TSIA= Triple Sugar Iron Agar test; H_2S = Hydrogen Sulphide Production; GLU= Glucose fermentation; LAC = Lactose Fermentation; SUC= Sucrose fermentation; Y=Yellow colour (acid production); R=Red Colour (Alkaline Production); + = Positive test; - = Negative test

Shigella spp. had an overall mean counts value of 0.69 $Log_{10}cfuml^{-1}$ (Table 8). This almost agrees with the overall mean counts of 0.30 $Log_{10}cfuml^{-1}$ recorded by Ogbonna [2] in Maiduguri. *Shigella* spp. was not detectable in samples of nono obtained from three markets (Pushit, Mangun, and Kombun). This also agrees with the finding of Ogbonna [2] that did not isolate the organism from nono samples collected from three markets in Maiduguri, Borno State. *Shigella* spp. is not an intrinsic flora of the animal; therefore, contamination of the fermented milk with the organism could have arisen from handling [2].

The overall mean *Pseudomonas aeruginosa* count was 0.41 $Log_{10}cfuml^{-1}$ (Table 8). The counts range from 0.00 to 1.64 $Log_{10}cfuml^{-1}$. *Pseudomonas* spp. is also a known causative agent of chronic mastitis in animals and may be shedded in milk. The isolation of *Pseudomonas aeruginosa* in 15(5.0%) nono samples in this study agrees with the findings of Kanyeka [26] that isolated *Pseudomonas* spp. in 9.5% of milk samples, suggesting that they come from mastitic cows.

Table 7. Percentage occurrence of microorganisms isolated from nono samples in Mangu L.G.A

Market n	Number of positive samples (%)								
	Coliform	*E. coli*	*S. aureus*	*P. aeru*	*Sal. spp*	*Shi. spp*	Fungi	χ^2	P-value
Mangu (30)	18(60.0)	16(53.3)	7(23.3)	6(20.0)	6(20.0)	6(20.0)	8(26.7)	16.687	0.01*
Pushit (30)	17(56.7)	17(56.7)	7(23.7)	0(0.0)	0(0.0)	0(0.0)	14(46.7)	18.62	0.00*
Panyam (30)	19(63.3)	13(43.3)	5(16.7)	0(0.0)	4(13.3)	1(3.3)	7(23.3)	39.71	0.00*
Kerang (30)	16(53.3)	15(50.0)	4(13.3)	5(16.7)	3(10.0)	4(13.3)	13(43.3)	23.53	0.00*
Ampang (30)	20(66.7)	13(43.3)	5(16.7)	1(3.3)	6(20.0)	7(23.3)	7(23.3)	27.49	0.00*
Mangun (30)	14(46.7)	11(36.7)	6(20.0)	3(10.0)	0(0.0)	0(0.0)	6(20.0)	29.65	0.00*
Kombun (30)	14(46.7)	11(36.7)	5(16.7)	0(0.0)	0(0.0)	0(0.0)	6(20.0)	37.5	0.00*
Gindiri (30)	11(36.7)	9(30.0)	3(10.0)	0(0.0)	0(0.0)	1(3.3)	3(10.0)	30.29	0.00*
Chanso (30)	14(46.7)	10(33.3)	0(0.0)	0(0.0)	4(13.3)	2(6.7)	5(16.7)	16.77	0.01*
Kadunu (30)	15(50.0)	14(46.7)	7(23.3)	0(0.0)	7(23.3)	3(10.0)	8(26.7)	14.20	0.03*
Total (300)	158(57.7)	129(43.0)	49(16.3)	15(5.0)	30(10.0)	24(8.0)	77(25.7)	266.06	0.00*

n = Number of samples per market, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, P. aeru = Pseudomonas aeruginosa, Sal. spp = Salmonella spp., Shi. spp = Shigella spp.

Table 8. Mean values ± standard deviation for microbial counts (log_{10}cfu ml^{-1}) of nono samples obtained from different markets in Mangu L.G.A

Variables	Markets										Overall means
	MGU (n=30)	PUSH (n=30)	PAN (n=30)	KER (n=30)	AMP (n=30)	MGN (n=30)	KOM (n=30)	GIN (n=30)	CHAN (n=30)	KAD (n=30)	
TBC	6.07^{a}±3.73	6.04^{a}±3.71	6.68^{a}±3.40	6.63^{a}±3.37	6.67^{a}±3.39	5.81^{a}±3.87	5.27a±4.08	5.28^{a}±4.09	5.27^{a}±4.08	7.22^{a}±2.88	6.09±3.68
TCC	4.94^{ab}±4.11	4.69^{a}±4.17	5.27^{ab}±4.08	4.41^{ab}±4.20	5.53^{ab}±3.98	3.87^{ab}±4.21	3.86^{ab}±4.20	3.06^{ab}±4.09	3.89^{ab}±4.23	4.17^{b}±4.24	4.37±4.15
TEcC	4.36^{abc}±4.14	4.66^{a}±4.14	3.61^{bc}±4.20	4.14^{ab}±4.21	3.60^{bc}±4.19	3.05^{bc}±4.07	3.04^{abc}±4.07	2.49^{bc}±3.86	2.78^{bc}±4.00	3.86^{b}±4.20	3.56±4.11
TSalC	1.91^{c}±3.53	0.00^{c}±0.00	1.11^{cd}±2.88	0.83^{d}±2.53	1.65^{cd}±3.35	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.84^{cd}±2.56	1.95^{bc}±3.59	0.83±2.49
TShC	1.64^{c}±3.31	0.00^{c}±0.00	0.56^{d}±2.12	1.12^{cd}±2.90	1.94^{cd}±3.58	0.00^{d}±0.00	0.00^{d}±0.00	0.28^{cd}±1.54	0.56^{cd}±2.12	0.83^{c}±2.54	0.69±2.30
TPaC	1.64^{c}±3.33	0.00^{c}±0.00	0.00^{d}±0.00	1.38^{cd}±3.13	0.28^{d}±1.52	0.84^{cd}±2.55	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{c}±0.00	0.41±1.80
TStaC	1.90^{c}±3.50	1.92^{bc}±3.53	1.41^{cd}±3.20	1.10^{cd}±2.86	1.11^{cd}±2.88	1.67^{bcd}±3.40	1.11^{cd}±2.87	0.82^{cd}±2.51	0.00^{d}±0.00	1.95^{bc}±3.59	1.30±3.02
TFC	2.20^{bc}±3.72	3.86^{ab}±4.19	1.94^{cd}±3.58	3.62^{bc}±4.21	1.94^{cd}±3.58	1.67^{bcd}±3.39	1.66^{bcd}±3.37	0.83^{cd}±2.52	1.40^{cd}±3.18	2.22^{bc}±3.74	2.13±3.64

Means followed by different superscript letters within a column are significantly different (P<0.05) using Tukey pairwise comparisons test.TBC = Total bacterial count, TCC = Total Coliform count, TEcC = Total Escherichia coli count, TSalC = Total Salmonella spp. count, TShc = Total Shigella spp. count, TPaC= Total Pseudomonas aeruginosa count, TStaC = Total Staphylococcus aureus count, TFC = Total Fungal count; MGU=Mangu, PUSH=Pushit market, PAN= Payam market, KER=Kerang market, AMP=Ampang market, MGN=Mangun market, KOM=Kombun market, GIN=Gindiri market, CHAN=Chanso market, KAD=Kadunu market, n = Number of samples per market

The mean *Staphylococcus aureus* count ($Log_{10}cfuml^{-1}$) of 1.30 in this study is in line with the 1.51 $Log_{10}cfuml^{-1}$ obtained by Ogbonna [2]. *S. aureus* counts in the nono samples which varied from 0.00 – 1.95 $Log_{10}cfuml^{-1}$ corresponding to an overall mean concentration of 1.30 $Log_{10}cfuml^{-1}$ contradicts [34] results of 0.00 – 2.90 $Log_{10}cfuml^{-1}$, but almost agrees with the range value of 0.00-2.90 $Log_{10}cfuml^{-1}$ obtained by Ogbonna [2]. Consequently, the result is lower than those reported by Tankoana et al., Abdalla and Ahmed [35,36]. According to the Turkish Food Codex (No. 2009/14) as reported by Fulva [31], the *S. aureus* numbers must not exceed a maximum of $5\times10^{2}cfuml^{-1}$ (≤ $Log_{10}2.70cfuml^{-1}$). Therefore, the mean *S. aureus* counts in the present study is within this recommended level. Anonymous [37] mentioned that the minimum numbers of *S. aureus* required to produce toxicity in human beings is estimated to be in excess of $10^{5}cfuml^{-1}$ (≥ $5Log_{10}cfuml^{-1}$). However, the presence of *S. aureus* in the nono samples corroborates the findings of Tormo et al. [38] who stated that the organism was the dominant bacterial species of milk, and inferior health condition of the animal increased the contamination of milk with the organism. Many studies conducted in different areas implicated *S. aureus* as the common mastitis causing organism in lactating cows [39]. According to Kanyeka [15], consumption of milk contaminated with *S. aureus* can be a health hazard because the main threat is based on the fact that 10% of mastitis Staphylococci are known to be producers of enterotoxins which are heat stable toxins. Some reports have associated *S. aureus* with gastroenteritis through these enterotoxins [25].

The concentration of fungi (yeasts and moulds) in the nono samples ranged from $0.83Log_{10}cfuml^{-1}$ to 3.86 $Log_{10}cfuml^{-1}$ corresponding to the overall mean count of 2.13 $Log_{10}cfuml^{-1}$ (Table 8). Most of the mean concentration values in this study were relatively higher than the 2 $Log_{10}cfuml^{-1}$, the limit recommended by EOSQC [40] for yoghurts and other fermented milk products. However, this finding almost agrees with the mean count of 2.30 $Log_{10}cfuml^{-1}$ reported by Torkar and Teger [41], but higher than the 1.23 $Log_{10}cfuml^{-1}$ recorded by Ogbonna [2]. Also, the result contradicts the no detectable recorded by Ukwuru and Ogbodo [42]. The reason for the contradictions could be linked to the fermentation practiced by many local producers as differences in fungal counts of the same product from different manufacturers had been documented [43]. Yeasts and moulds are common contaminants in food. While yeast does not result in food poisoning, it does cause food to spoil [44]. A very large number of moulds produce toxic substances designated as mycotoxins [31].

4. CONCLUSION

From the findings of this study, it is concluded that due to the presence of some pathogenic microorganisms which exceeded the limit stipulated by the authorities except that of *Staphylococcus aureus*, it does appear that the safety of nono produced and marketed in the study area as at the time of this research cannot be guaranteed for human or public consumption and can be a source of milk-borne infections. It is therefore recommended that relevant authorities on food safety and food safety standards should monitor the production-line of nono sold to the public in order to ensure its safety for public consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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