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Original Article

Bacteriological assessment of locally fermented milk (nono) produced from Maidako, Niger State

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ABSTRACT

Nono is a locally fermented dairy product made from cow's milk, commonly found in northern Nigeria. This study aimed to determine the bacteriological quality of nono produced in Maidako, Niger State. Five samples were collected from the different stages of production (directly from the udder, after mixing the milk form several cows, after sieving the milk, after fermentation, and after churning). The samples were cultured on Nutrient and MacConkey agar for total bacterial and total coliform counts using pour plate method. Results revealed that the total viable count (TVC) in the different stages of 'nono' production ranged from $1.00x10^6 \pm 0.00$ - $9.00x10^6 \pm 0.00$ while the total coliform count (TCC) ranged from $1.00x10^6 \pm 1.00^a$ - $7.50x10^6 \pm 0.50^b$. A total of 34 isolates were recorded. The bacteria isolated were *Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Salmonella enterica, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Citrobacter freundii, Staphylococcus aureus, Kocuria rosea,* and species *of Lactobacillus, Streptococcus, Proteus, Lactococcus, and Shigella. Bacillus subtilis* had the highest frequency of (7) 20.59 % followed by *Pseudomonas aeruginosa* (4) 11.76 % while the lowest frequency of 1 (2.94 %) was recorded for *Klebsiella oxytoca, Citrobacter freundii, Kocuria rosea* and *Streptococcus* sp. Consumption of nono produced in the study area poses a potential public health threat due to the presence of pathogenic organisms therefore precautionary measures such as pasteurization should be practiced.

Keywords: Bacteria, Dairy product, Locally fermented, Nono, Production stages.
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INTRODUCTION

Locally produced dairy products have increasingly emerged as a vital alternative source of protein in developing countries, largely due to their accessibility and affordability [1]. Nono is a traditional fermented milk product widely consumed in northern Nigeria, a region where cattle rearing is commonplace and consumption of fresh and locally processed milk has been part of the local diet [2]. Nono is not only a staple food but also a highly nutritious option, rich in essential amino acids, high-quality protein, various vitamins, and phosphorus, making it an important component of the nutritional landscape. This fermented milk is particularly valued for its distinct flavor profile, which is largely attributed to the presence of diacetyl, a compound that enhances the sensory qualities of fermented products [3]. Despite its nutritional benefits, nono faces significant challenges regarding food safety. The product is highly perishable, making it susceptible to spoilage and contamination. The methods of handling and preparation in local communities often lack the necessary hygiene standards, increasing the risk of foodborne pathogens. These concerns are compounded by the lack of access to potable water sources, which is critical for sanitation and overall food safety [4,5]. This situation highlights the need for improved food safety practices and better infrastructure to ensure that nono can be safely enjoyed as part of the local diet.

Nono continues to be a popular street-vended beverage among middle and low-income earners in northern Nigeria, despite a noticeable lack of proper training in safe food processing and handling techniques among vendors [6]. This has consequently attracted numerous studies on its safety over the years. Several of these studies have confirmed the presence of pathogenic bacteria in retail [7-10]. The various stages of production, including raw material handling, processing, and storage, significantly contribute to the contamination levels. This study focuses on the bacteriological assessment of nono while paying attention to the stages of production that expose the product to microbial contamination.

MATERIALS AND METHODS

Sample Collection and Processing

Nono samples were collected from Fulani settlement, Maidako, in Niger State. The samples were collected at different stages of production of nono (directly from the udder, after mixing the milk form several cows, after sieving the milk, after fermentation, after churning) under aseptic conditions into sterile airtight sampling jars [6]. They were further conveyed to the Microbiology Department laboratory of Federal University of Technology, Minna in an insulated icebox immediately for microbiological analyses. Figure 1 shows the different stages of nono production.

Lactating dairy cows

↓

Manually milking of cow to acquire fresh milk sample

↓

Collection of milk sample from several cows into one container

↓

Adding previously made nono to aid fermentation

↓

Sieving the milk

↓

Keeping raw milk in calabash for fermentation (18-24 hours)

↓

Churning (removal of fat and some whey)

↓

Storage in calabash ready for intake and trade

Figure 1: Stages of nono preparation

Source: [11,12].

Microbial Analysis

Enumeration and Isolation of Bacteria from the Stages of Nono Production

The microbial enumeration was done by determining the total viable counts (TVC) and total coliform count (TCC) using the techniques of [13]. Serial dilution was carried out on the samples, dilution factor 10^6 was aseptically transferred in duplicates onto nutrient agar and MacConkey agar plates for TVC and TCC, respectively, then incubated at 37 ºC for 24-48 hours. Colonies of various colours, shapes and sizes were picked from various plates and sub-cultured repetitively to acquire pure isolated. The pure isolates were stored on agar slants for further characterization and identification.

Characterization and Identification of isolates from Different Stages of Nono Production

Bacterial isolates were identified based on their cultural, morphological and biochemical characteristics as described by [14]. Cultural identification was done by observing the appearance of isolates on the culture media and taking notes of the colour, sizes and shapes of colonies. Bacterial identification was done using the appearance of colonies on culture media, Gram's reaction, biochemical tests (coagulase, indole, catalase, methyl red, Vogues-proskauer, citrate, and carbohydrate fermentation test [14].

Data Analysis

Data generated from this research was subjected to one-way analysis of variance (ANOVA) test followed by a post-hoc DUNCAN ALPHA test to determine its significance. The data were evaluated in duplicate and presented as mean $\pm$ standard error of mean, P-values ≤ 0.05 were considered statistically significant. The data was statistically evaluated using the statistical package for social sciences (SPSS) version 26.

RESULTS

Total bacterial counts from the different stages of nono production

The study revealed that the total viable count (TVC) in the different stages of nono production ranged from $1.00x10^6 \pm 0.00^a$ - $9.00x10^6 \pm 0.00^b$ while the total coliform count (TCC) ranged from $1.00x10^6 \pm 1.00^a$ - $7.50x10^6 \pm 0.50^b$ (Table 1).

Table 1: Total bacterial count for the stages of nono production

Stages	TVC (cfu/mL)	TCC (cfu/mL)	SON limit (cfu/mL)
1	$1.00x10^6 \pm 0.00^a$	$2.00x10^6 \pm 0.00^a$	0
2	$3.50x10^6 \pm 0.50^{ab}$	$1.08x10^6 \pm 0.05^a$	0
3	$2.00x10^6 \pm 2.00^a$	$1.00x10^6 \pm 1.00^a$	0
4	$9.00x10^6 \pm 0.00^b$	$7.50x10^6 \pm 0.50^b$	0
5	$7.50x10^6 \pm 0.50^b$	$1.00x10^6 \pm 0.00^a$	0

Values are in ± mean S.E. (*S. E = Standard error of Mean*). Values bearing the same superscript are not significantly different at the 5% level (P>0.05).

KEY: 1 = samples collected directly from the cow's udder, 2 = samples collected after the milk from different cows has been mixed, 3 = samples collected after sieving the milk, 4 = samples collected before churning, 5 = samples collected after churning, NG = No growth, SON =Standard Organization of Nigeria.,

Frequency of Occurrence of Bacterial Isolates in Nono from the Different Locations

Results from the bacterial analysis revealed eighty-nine (34) isolates which were separated into sixteen (16) genera and species. They include *Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Salmonella enterica, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Citrobacter freundii, Staphylococcus aureus, Kocuria rosea,* and species of *Lactobacillus, Streptococcus, Proteus, Lactococcus,* and *Shigella.* The frequency of occurrence for bacterial isolates revealed that *Bacillus subtilis* had the highest frequency of (10) 20.59 % followed by *Pseudomonas aeruginosa* (4) 11.76 % while the lowest frequency recorded was (1) 1.22 % for *Klebsiella oxytoca, Citrobacter freudii, Kocuria rosea,* and *Streptpcocus* sp. (Table 2).

Table 2: Frequency of Occurrence of Bacterial Isolates from Nono

S/N	Isolates	No of isolates	Percentage (%)
1	*Klebsiella pneumoniae*	2	5.88
2	*Bacillus subtilis*	7	20.59
3	*Pseudomonas aeruginosa*	4	11.76
4	*Micrococcus luteus*	3	8.8
5	*Salmonella enterica*	2	5.88
6	*Escherichia coli*	3	8.82
9	*Klebsiella oxytoca*	1	2.94
10	*Citrobacter freundii*	1	2.94
11	*Staphylococcus aureus*	3	8.82
12	*Kocuria rosea*	1	2.94
13	*Lactobacillus* sp.	2	5.88
14	*Streptococcus* sp.	1	2.94
15	*Lactococcus* sp.	2	5.88
16	*Shigella* sp.	2	5.88
	TOTAL	**34**	**100.00**

DISCUSSION

The total viable counts of $1.00x10^6 \pm 0.00^a$ - $9.00x10^6 \pm 0.00^b$ cfu/mL were recorded in this study. The total coliform counts recorded in this study ranged from $1.00x10^6 \pm 1.00^a$ - $7.50x10^6 \pm 0.50^b$ cfu/mL with the highest recorded in stage 4. The high counts, although lower than the Codex standard of 10^7 cfu/mL, are an indication of contamination, which may have resulted from unhygienic practices such as the use of non-potable water and unsterilized utensils during production.

This result agrees favorably with the work of [9]. who recorded 1.29 - 7.63 x 10^5 cfu/mL. They reported that the high counts might be as a result of product handling, the handler's sanitary or processing conditions, the low level of cleanliness preserved throughout the processing of the product (nono), water quality used, and the tools used in production and storage. Other explanations for the high counts detected could be a result of the length of time in fresh milk stowing used for the making of nono which might permit microbial contaminants to reproduce and increase in number thus prompting the entire viable/bacteria counts and the types of bacteria existing in the bulk fresh milk. Coliforms have been proven and endorsed by public health authorities worldwide as a pointer of post-handling contagion in manufacture [15]. The occurrence of coliform bacteria in high amounts in nono is a suggestion that the milk has been tainted with fecal materials, inadequate cleaning of milking vessels, unhygienic milking environs, filthy udder and teats of lactating cows, unclean water and/or cows with subclinical or clinical mastitis [16]. Therefore, their occurrence in great amounts in dairy products is a sign that the products are possibly harmful to the consumer's health. At stage 1 during collection of samples directly from the udder, the presence of coliform in this stage may be due to contamination by

fecal matter attached to the teats of the animal or the hand of the milking man. There was a decline in coliform counts in stage 2 and stage 3. This may mean that some organisms were not picked during culturing. There was an increase significantly in the coliform counts in stage 4. This contamination might have resulted from the utensils used in this step such as the sieve and the sieving container that could have been contaminated before usage. The significant reduction at stage 5 could mean that some of the contaminating organisms were removed along with the fat/whey that was removed during the churning process.

Fresh milk is a likely source of serious bacterial pathogens. The microorganisms isolated from the different stages of nono production include Gram-negative and Gram-positive bacteria such as *Klebsiella pneumoniae, Salmonella enterica, Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, Citrobacter freundii, Pseudomonas aeruginosa, Bacillus subtilis, Lactobacillus* s*p*, *Micrococcus luteus, Staphylococcus aureus, Micrococcus roseus, Streptococcus, Proteus, Lactococcus,* and *Shigella* species. The result corroborates with the report of [17]. who isolated similar organisms such as *Enterobacter, Proteus*, *Klebsiella,* and *Citrobacter* species from nono in Samaru, Kaduna, Nigeria and the work of [18]. who isolated *Staphylococcus aureus, Salmonella* sp, *Lactobacillus plantarum* and *Escherichia coli*.

The presence of *E. coli* (7.32 %) and other enteric bacteria in the samples is a suggestion that they possibly contain other pathogens, as the incidence of *E. coli* in foods is a sign of contamination of faecal source. The presence of *Salmonella* (6.09 %) and *Shigella* (3.66 %) species agrees with the work of [8]. which could be due to the level of hygienic practices in the area of study and the lack of potable water used in the production processes. The use of water from the streams that are visited by humans and animals may also be a possible reason for this contamination.

The cattle do sit on the same ground where they defecate and their teats are not usually cleaned before milking. This could also be a likely explanation for the presence of these organisms in the samples analyzed. The isolation of *Citrobacter* and *Proteus* species is in concordance with a previous study by [19]. They established that *citrobacter* spp can cause meningitis, septicaemia and pulmonary infections in newborn and young children and has also been found to cause septicaemia in patients with a number of influencing factors. *Proteus* species are opportunistic human disease-causing bacteria which can bring about infections in humans with compromised immunity [19]. Their isolation in this study could be as a result of the presence of house flies and surface run-offs of polluted surfaces into streams and animal houses through rainfalls. The congested poor sanitary practices observed in the research environment messy animal houses and keeping of unhealthy cattle with healthy ones could increase the chance of exposure to disease causing microbes [20]. They may be a source of complicated urinary tract infections.

Bacillus sp with a frequency of 7 (20.59 %) is known to be pathogenic and resistant to environmental stress due to its spore-forming nature, and it can cause emetic syndrome (characterized by nausea and vomiting) and food-borne intoxication [21,22]. The incidence of *Pseudomonas aeruginosa* with frequency (4) 11.76 % in nono is a suggestion of a likely contact with

discharge from lacerations and excretory products of either human or cow, respectively. This agrees with the study of [23]. who confirmed that *Pseudomonas aeruginosa and Klebsiella* species are connected with wounds and body discharge.

CONCLUSION

This study focused on the bacteriological assessment of the different stages involved in nono (a locally fermented dairy product) production that expose the product to microbial contamination. Results revealed the presence of both Gram-negative and Gram-positive pathogenic bacteria including *Klebsiella pneumoniae, Salmonella enterica, Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, Citrobacter freundii, Pseudomonas aeruginosa, Bacillus subtilis, Lactobacillus* s*p*, *Micrococcus luteus, Staphylococcus aureus, Micrococcus roseus, Streptococcus, Proteus, Lactococcus,* and *Shigella* species. The occurrence of these bacteria indicate possible contamination and therefore pose a significant threat to the consumers. Precautionary measures such as pasteurization should be encouraged.

DECLARATION SECTION

Authors Contribution

Conceptualization*: Bala Jeremiah David and Nasiru Usman Adabara.* ***Supervision:*** *Bala Jeremiah David, John Jiya Musa and Julian Chukwuemeka Anuonye.* ***Investigation:*** *Bala Jeremiah David, Ummulkhair Salamah Ilyasu* ***Writing the first draft of article:*** *Ummulkhair Salamah Ilyasu* ***Editing:*** *Garjila Danjuma Gansheya. All authors contributed to the development of the final manuscript and approved its submission.*

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Disclosure of Conflict of Interest

None

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