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Bacteriological Analysis of Cow Milk (Nono) Sold Within Gusau Metropolis, Zamfara State

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ABSTRACT

Fresh cow milk popularly called *Nono* was collected from ten (10) different sellers at different areas within Gusau metropolis and one control was collected directly from a lactating cow and transported immediately to Microbiology Laboratory of Federal University Gusau where they were assessed for their bacteriological quality. The total bacteria viable count of the ten *Nono* samples ranged between 6.6 x10^5 to 2.7 x10^6 cfu/ml, the total coliforms count ranged between 4.2x10^5 to 2.2x10^6cfu/ml, the range of the total Staphylococcal count and total lactic acid bacterial count were 2.5x10^5 to 4.0x10^6 cfu/ml and 5.9x10^5 to 1.7x10^6 cfu/ml respectively. The control sample had total viable count of 5.6 x 10^3 cfu/ml, total staphylococcal count of 3.2 x 10^3 cfu/ml, total lactic acid bacteria count of 4.2 x 10^3 cfu/ml whereas the coliform count gotten was too few to count (TFTC). The isolates were identified based on their morphological characteristics, Gram's reactions and biochemical profiles and a total of seventy-four (74) bacterial isolates belonging to the genus *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Micrococcus*, *Pediococcus*, *Escherichia coli*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Enterobacter* and *Staphylococcus spp*. were identified from the eleven samples analyzed. The presence of Lactic Acid Bacteria in *Nono* is very good as they are one of the most significant groups of probiotic which not only enhance lactose digestion but also stimulate the immune system, However, the presence of *E. coli* and the other *enterobacteriaceae* and *Staphylococcus sp.* is of public health concern because they can cause several diseases therefore it was concluded that the bacterial loads of the *Nono* samples were not satisfactory with the exception of the control which had organisms within the acceptable limit of 30,000/ml for raw milk. The presence of potential pathogenic bacteria in all of the *Nono* samples is an indication that the samples were contaminated and this can potentially cause diseases to the consumers, Hence the need for public enlightenment for handlers and producers of *Nono* to observe good manufacturing practices in production and storage of the product to avoid outbreak of infections associated with the organisms encountered in this study.

1. INTRODUCTION

Nono is a pale liquid produced by the mammary glands of cow. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. It contains many nutrients including protein and lactose. Moreover, milk itself is known as one of the natural habitats of lactic acid bacteria (LAB) [1]. *Nono* is a nutritious food primarily obtained from cows, goats, sheep, buffalo and even camels. It provides protein, vitamins, minerals and fatty acids [2]. *Nono* constitutes nutritional supplements not only for humans, but also to a large extent for the exponential growth of autochthonous and even environment-based microbial populations (allochthonous) that find their way in to the milk either through milking or processing procedures. *Nono* is usually the milk that has been fermented and becomes very thick [3].

Nono serves as an excellent medium for the growth of many bacteria and is contaminated from various sources. Bacteria play an important role in dairy industries. The quality

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and conditions of milk products can be judge by its microbial contents. If bacteria are allowed to grow in milk, they produce chemical changes that make it unpalatable. Pathogenic bacteria can grow very well in milk; therefore, milk may serve as a medium for the dissemination of infectious diseases [4].

Common bacteria reported to be isolated from *nono* include *Lactobacillus*, *Pediococcus*, *Leuconostoc Staphylococcus* sp., *Listeria* sp., *Salmonella* sp., *E. coli*, *Campylobacter* sp., Mycobacterium sp., *Brucella* sp., *Coxiella burnetii*, *Yersinia* sp., *Pseudomonas aeruginosa* and *Corynebacterium ulcerans.* [5]. Others are *Proteus* sp., *Leptospira* sp., *Clostridium* sp., *Streptococcus* sp., *Klebsiella* sp., *Enterobacter* sp. and *Bacillus* sp. [6].

Garba (2023) reported that, in developing countries, such as Nigeria, milk spoilage and low-quality milk are of concern. These countries rely on local small holder farms to supply milk to local consumers. Often, these products are transported with unreliable refrigeration. Breaks in the cold chain allow bacteria to quickly multiply, eventually reaching levels unsafe for consumption. The resulting decrease in milk quality and supply negatively impacts processors and consumers along the dairy value chain. [7]. The current market structure in Nigeria is fragmented, with 85% of milk sold in informal and unregulated markets and 15% sold in the formal, urban market which requires regulatory oversight. However, many small holder dairy farmers lack basic knowledge of sanitation, hygiene, and do not have access to clean water, adequate feed for their animals, and training. The resulting low-quality milk potentially exposes consumers, especially infants, children, pregnant, and nursing women to health challenges [8].

Nono is one of the most frequently consumed foods in the study area due to its nutritional and health benefits. But despite the nutritional advantages of the product to the consumers, one may come down with one or more ailments ranging from mild stomach disturbances, nausea, vomiting and sometimes to severe diarrhoea. The *nono* drink can be contaminated at different stages of production and hence the need to determine the quality of the *nono* sold in Gusau metropolis. Although procedures and equipment used for *nono*-processing are relatively simple, the product may harbour harmful bacteria which may be of health risk to consumers, especially when not hygienically prepared. Despite the fact that *nono* is one of the most popular drink consumed within the study area, there is a paucity of information on their bacteriological quality which can lead to disease outbreak if the products are contaminated. Analysing *nono* allows for the assessment of its microbiological safety, including the presence of pathogenic bacteria or toxins. This information is crucial for ensuring the safety of *nono* consumption and implementing appropriate food safety measures during its production and storage. Hence, in order to ensure proper safety compliance of the *nono* from the producers to consumers, the bacteriological quality of the products sold within the Gusau metropolis was investigated in this study.

2. MATERIAL AND METHODS

2.1 Study Area

The study was conducted in Gusau Zamfara state. Gusau is the capital of Zamfara state located in north-western Nigeria. The area is predominantly a semi-arid savannah with large expanse of dry forest and has a land area of approximately 3364 km^2. It is also a home of two ethnic groups namely, the Hausa and Fulani's. It has a population of 383162 as of the 2006 census. Agriculture is the main stay of the state's economy. The major crops cultivated in the state are: millets, ground nut, guinea corn, beans, rice, etc. Household in the study area are predominantly involved in farming, rearing of animals, petty trading and civil service [9].

2.2 Sample Collection

A total of eleven (11) *Nono* samples were collected randomly from 10 selling points and one animal farm (control) designated as S, D, T, B, G, J, Z, Ts, K, U and C. Aseptic techniques were strictly maintained during the sample collection. The samples were collected in sterile plastic bottles, kept cool in ice boxes and immediately transported to the Microbiology laboratory of Federal University Gusau for processing.

2.3 Media Preparation and Inoculation of Organisms

The media used in this study include: Nutrient agar, MacConkey agar, Mannitol salt agar and deMann Rogosa Sharpe agar. These media were prepared according to the manufacturer's instructions. The mixture prepared was hot plated for a proper and homogenous dissolution of the solution; and sterilized by autoclaving. It was then allowed to cool at a temperature between 45ºC to 50ºC before pouring 20mls of the molten agar into a sterile petri-plate and allowed to solidify.

The total viable count, total coliform count, total Staphylococcus count and total lactic acid bacteria count were determined by sterile standard plate count agar, MacConkey agar, Mannitol salt agar and deMann Rogosa Sharpe agar respectively. One milliliter of raw milk (Nono) sample was added to a sterile test tube containing 9 mL of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-5}. From all diluted tubes, 0.1 mL was taken and spread on sterile 20 mL petri-plates containing various media. The inoculated petri dishes were incubated at 37°C for 24 hours. The plates with colonies ranging from 30–300 colony forming units per milliliter (CFU/mL) of sample were selected for determination of Total bacterial count. TBC was determined as the total number of CFU per milliliter of Nono samples plated.

Colonies identified as discrete on nutrient agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests such as Catalase test, Coagulase test, Citrate utilization test, Motility test, Urease test and Carbohydrate fermentation test. The isolates were identified by comparing their characteristics with those of known taxa, as described by [10].

2.4 Identification of Organisms through Biochemical Testing

Motility test

A sterile needle was used to pick a colony from a 24 hours old culture and was stabbed onto nutrient agar in test tube. The tubes were incubated at 37°C for 24-48 hours. Non-motile bacteria gave a growth confined to the stab line with definite margins without spreading to surrounding area while motile bacteria gave a diffused growth extending from the surface [11].

- Catalase test

A small quantity of 24hour culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas was seen as bubbles which indicates the presence of catalase enzyme [12].

- Coagulase test

The test was carried out using 18-24 hours' culture. A loopful of isolated bacterium was emulsified with normal saline solution on a microscope slide. A drop of undiluted plasma was added to the suspension and stirred for 5 seconds. A coagulase-positive result indicates clumping of colonies together [11].

- Citrate test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. The tubes were inoculated by streaking the organisms on the surface of the media. A change from green to blue indicated the utilization of the citrate [12].

- Indole Test

Each isolate was inoculated and dispensed into 5ml of peptone water in a bottle using a sterile wire loop. It was incubated at 37ºC for 24 hours. There after 3 drops of kovac's reagent was added, development of a red color in the layer above the broth indicated positive reaction. Absence of any observable reaction as obtained with the isolates indicates a negative test.

- Vogues-Proskauer (VP) Test

Isolates were inoculated in the medium and incubated at 37ºC for 48 hours. 3ml of 5% solution of alpha naphtol was added in absolute ethanol. 1ml of 40% potassium hydroxide was added and appearance of bright pink color after five minutes indicates a positive test, while no color change indicates a negative test.

- Methyl Red (MR) Test

The medium was inoculated lightly from a fresh culture of the organism and was then incubated at 35ºC for two days, 5 drops of indicator were added to the culture a red color indicates a positive (acid) reaction.

- Growth Test on Different Salt Concentrations

One colony of bacteria was inoculated into deMann Rogosa Sharpe broth (MRS broth) media with a concentration of 4%, 6.5% and 10% NaCl. The inoculated media were then incubated at 37°C for seven days. The bacterial growth was shown by the sediment formation in the media [13].

- Growth Test at Different Temperatures

One colony of bacteria was inoculated into deMann Rogosa Broth (MRS broth) media and incubated at 15ºC, 25ºC and 37ºC for seven days. The bacterial growth was shown by the turbidity formation in the media [13].

- Triple Sugar Iron (TSI)

Upon cooling, the tubes were inoculated by stabbing the butt and streaking the slant of the tubes. The tubes were incubated at 37ºC for 24hours.Yellow coloration at the slant and butt indicated positive for glucose as well as lactose and sucrose respectively.

The data were statistically analyzed by SPSS version 20 for descriptive statistics, frequency, and percentage for association tested between dependent and independent variables, and an Anova and t-test was used for mean comparison. For all analyses, a 95% confidence level and p value <0.05 were considered statistically significant. The information was presented in Table 1 – Table 7.

3. RESULTS

A total of 10 samples of *nono* denoted S, D, T, J, B, G, Z, K, Ts and U were collected from different selling points and one control denoted C collected from an animal farm, all within Gusau Metropolis were subjected to bacteriological analysis in the Microbiology Laboratory of Federal University Gusau and the results of bacterial distribution in the collected samples are shown below in Table 1.

The total viable count, staphylococcal count, coliform count and latic acid bacteria count from the Nono samples analyzed are shown in Table 1 with Sample J having the highest viable count of $2.7x10^6$ CFU/ml and sample K had the lowest count of $6.6x10^5$ CFU /ml. The highest total coliform count was recorded in sample G as $2.2x10^6$ CFU/ml whereas sample K had the least count of $4.2x10^5$ CFU/ml. Sample Z had the highest Staphylococcal count of $4.0x10^6$ CFU/ml while sample U had the least count of $2.5x10^5$ CFU /ml. Sample G had the highest Lactic acid count of $1.7x10^6$ CFU/ml while the least count was recorded in Sample Ts as $5.9x10^5$ CFU /ml. The control had counts within acceptable limits of 5×10^4 CFU/ml according to [14].

Colonial morphology of the cultures such as color, form, elevation and margin were observed. The colors observed include: pinkish, rose-pink, colorless, creamy-white, off-white, yellowish, etc. The form observed include: round, flat etc. Most of the elevations observed were convex, but flat and raised were also observed. The margin observed was entire for most of the colonies.

In Gram's staining under microscope, smears from deMann Rogosa Sharpe agar (MRS), MacConkey agar (MCA) and Mannitol salt agar (MSA) Media were examined. From MCA, Gram's negative, mostly rod shape organisms were microscopically observed. For Mannitol salt agar, Gram's positive, short cocco-bacilli and cocci were observed within bundles and singly arranged. For MRS, Gram-positive, cocci, bacilli and coccobacilli were observed.

S. aureus, *Lactobacillus* sp. and *E. coli* were recorded to have the highest percentage of occurrence (13.7%), followed by *Pseudomonas* spp species (12.3%), then *Micrococcus* species with a percentage of 10.9%. *Leuconostoc* species was recorded to have the lowest frequency of occurrence with the percentage of 1.4%.

The isolates from the control sample were also identified using the Bergey's manual for bacterial identification and found to belong to the genera *Lactobacillus, Micrococcus, Leuconostos, Klebsiella* and *Pseudomonas.*

Table 1. Total Viable, Staphylococcus, coliforms and lactic acid bacteria count of *nono* samples (CFU/ml)

S/N	Sample code	Total Viable count (cfu/ml)	Total Staphylococcus count (cfu/ml)	Total Coliform Count (cfu/ml)	Total Lactic Acid Bacteria Count (cfu/ml)
1	S	$2.3X10^6$	$9.9X10^5$	$8.6X10^5$	$8.1X10^5$
2	D	$1.5X10^6$	$8.6X10^5$	$1.0X10^6$	$1.0X10^6$
3	T	$2.2X10^6$	$5.9X10^5$	$7.3X10^5$	$7.7X10^5$
4	J	$2.7X10^6$	$8.7X10^5$	$1.8X10^6$	$1.3X10^6$
5	G	$2.3X10^6$	$1.0X10^6$	$2.2X10^6$	$1.7X10^6$
6	Z	$9.2X10^5$	$4.0X10^6$	$6.6X10^5$	$8.6X10^5$
7	K	$6.6X10^5$	$3.3X10^5$	$4.2X10^5$	$9.2X10^5$
8	Ts	$8.9X10^5$	$4.2X10^5$	$5.5X10^5$	$5.9X10^5$
9	B	$8.7X10^5$	$4.1X10^5$	$4.9X10^5$	$7.7X10^5$
10	U	$8.8X10^5$	$2.5X10^5$	$5.7X10^5$	$1.01X10^6$
11	Control	$5.6X10^3$	$3.2X10^3$	TFTC	$4.2X10^3$

Key: S=Sabongida, D= Damba sample, T= T/wada sample, J=Janyau sample, G= Gada biyu samples Z= Zawiyya sample, K= Kasuwar kanawa sample, Ts= Tsohowar kasuwa sample, B= Bebeji sample, U= University sample, CFU = Colony Forming Unit, TFTC = Too few to count.

Table 2. Occurrence of Bacteria in the Various Samples of Nono

Organisms	S	D	T	J	G	Z	Ks	T	L	U
***Lactobacillus* sp.**	+	+	+	+	+	+	+	+	+	+
***Micrococcus* sp.**	+	-	+	+	+	-	+	-	+	+
***Pediococcus* sp.**	-	-	-	-	-	+	-	-	-	-
***Leuconostoc* sp.**	-	-	-	-	-	+	-	-	+	+
***Streptococcus* sp.**	+	+		+	+		+	+	-	+
Escherichia coli	+	+	+	-	+	+	+	+	+	+
***Pseudomonas* sp.**	+	+	+	-	+	+	-	+	+	+
***Proteus* sp.**	-	+	-	+	+	-	+	+	-	-
***Enterobacter* sp.**	-	-	+	-	-	+	-	-	+	+
***Klebsiella* sp.**	+	-	-	+	-	-	+	-	-	-
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+
Coagulase negative Staph	-	+	-	-	+	-	-	-	-	-

Key: + = present, - = absent, S= Sabongida, D= Damba, T= Tudun wada, J= Janyau, G= Gada Biyu, Z= Zawiyya, K= K/Kanawa, Ts= T/Kasuwa, B= Bebeji and U= University.

Table 3. Frequency of occurrence of the Isolates from *nono* Samples

Probable Organisms	Frequency in *nono*	Percentage (%)
Lactobacillus species	10	13.7
Micrococcus species	8	10.9
Pediococcus species	-	0
Leuconostoc species	1	1.4
Streptococcus species	7	9.6
Escherichia coli	10	13.7
Pseudomonas species	9	12.3
Proteus species	5	6.8
Enterobacter species	5	6.8
Klebsiella species	3	4.1
Staphylococcus aureus	10	13.7
Coagulase negative staph.	5	6.8
TOTAL	**73**	**100**

Table 4. Morphological Characteristics of the Colonies and Gram's Reaction of the Isolates on MCA Plates

Sample code	Shape	Size	Elevation	Margin	Color	Gram's reaction
S	Circular, irregular, filamentous	Small, large, large	Convex, raised, raised	Entire	Rosepink, colorless, pink	All negative rods
D	Round, circular, filamentous	Large, large, small	flat, convex, flat	Entire	Colorless, pink, colorless	All negative rods
T	filamentous, circular, circular	Large, large, large	Raised, flat, convex	Entire	Pinkish, colorless, pinkish	All negative rods
J	Circular, circular, circular	Small, large, large	Flat, convex, convex	Entire	Colorless, pinkish, pinkish	All negative rods
B	Irregular, circular, circular	Large, small, large	Raised, convex, flat	Entire	Colorless, pinkish, colorless	All negative rods
G	Round, flat,	Small, small, large	Convex, flat	Entire	Pinkish, colorless, rose pink	All negative rods
Z	Circular, round, circular	Small, large, large	convex	Entire	Rose-pink, colorless	Negative rods
K	Round, circular, circular	Small, large, large	Convex, raised	Entire	Colorless, rose-pink, colorless	Negative rods
TS	Circular, filamentous, round	Large, large, small	Flat, raised, convex	Entire	colorless, pinkish rose pink,	Negative rods
U	Round, circular, round	Large, large, small	convex	Entire	Pinkish, colorless, pinkish	Negative rods

Key: S=Sabongida, D= Damba sample, T= T/wada sample, J= Janyau sample, B=Bebeji sample, G= Gada biyu samples Z= Zawiyya sample, K= K/kanawa sample, Ts= Tsohowar kasuwa sample and U= University sample. MCA= MacConkey Agar.

Table 5. Morphological Characteristics of The Colonies and Gram's Reaction of the Isolates on MRS Plates

Sample code	Shape	Size	Elevation	Margin	Colour	Gram's reaction
S	Circular, irregular, filamentous	Small, large,	Convex, raised,	Entire	Creamy-white	Gram positive rods
D	Round, circular, filamentous	Large, small	flat, convex,	Entire	Off-white	Gram positive cocci
T	filamentous, circular,	Large, small	Raised, flat, convex	Entire	White, off-white	Gram positive rods
J	Circular,	Small, large	Flat, convex,	Entire	Creamy-yellow	Gram positive cocci
B	Irregular, circular,	Large, small,	Raised, convex, flat	Entire	White, creamy	Gram positive rods
G	Round, flat,	Small, large	Convex, flat	Entire	Off-white	Gram positive cocci
Z	Circular, round,	Small, large,	convex	Entire	Creamy-white	Gram positive rods
K	Round, circular,	Small, large,	Convex, raised	Entire	Off-white, white	Gram positive rods
TS	Circular, filamentous, round	Large, small	Flat, raised, convex	Entire	Creamy, off-white	Gram positive cocci
U	Round, circular,	Large, small	convex	Entire	White, creamy-white	Gram positive rods

Key: S=Sabongida, D= Damba sample, T= T/wada sample, J= Janyau sample, B=Bebeji sample, G= Gada biyu samples Z= Zawiyya sample, K= K/kanawa sample, T= T/kasuwa sample, U= University sample, MRS= deMan Ragosa Sharpe Agar.

Table 6. Morphological Characteristics of the Colonies and Gram's Reaction of the Isolates on MSA Plates

Sample code	Shape	Size	Elevation	Margin	Color	Gram's reaction
S	Circular, filamentous	Small, large,	Convex, raised,	Entire	Yellowish, colorless	Gram positive
D	Round, filamentous	Large, small	flat, convex,	Entire	Colorless, yellowish	Gram positive
T	filamentous, circular,	Large, small	Raised, flat, convex	Entire	Yellowish, colorless	Gram positive
J	Circular,	Small, large	Flat, convex,	Entire	Colorless, yellowish	Gram positive
B	Irregular, circular,	Large, small,	Raised, convex, flat	Entire	Yellowish, colorless	Gram positive
G	Round, flat,	Small, large	Convex, flat	Entire	Yellowish, colorless	Gram positive
Z	Circular, round,	Small, large,	convex	Entire	Colorless, yellowish	Gram positive
K	Round, circular,	Small, large,	Convex, raised	Entire	Golden, colorless	Gram positive
TS	Circular, filamentous,	Large, small	Flat, raised, convex	Entire	Golden, colorless	Gram positive
U	Round, circular,	Large, small	convex	Entire	Colorless, golden	Gram positive

Key: S=Sabongida, D= Damba sample, T= T/wada sample, J= Janyau sample, B=Bebeji sample, G= Gada biyu samples Z= Zawiyya sample, K= K/kanawa sample, Ts= Tsohowar kasuwa sample, B= Bebeji sample, U= University sample, MSA= Mannitol Salt Agar

Table 7. Biochemical Profiles of isolates from MCA *Nono* Samples

Sample code	L	MR	VP	I	C	O	M	*P/O*
S1	-	-	-	-	+	+	+	***Pseudomonas spp***
S2	+	-	+	-	+	-	-	***Klebsiella spp***
S3	+	+	-	+	-	-	+	***E.coli***
D1	-	-	-	+	+	-	+	***Proteus spp***
D2	+	+	-	+	-	-	+	***E.coli***
D3	-	-	-	-	+	+	+	***Pseudomonas spp***
T1	+	+	-	+	-	-	+	***E.coli***
T2	+	-	+	-	+	-	+	***Enterobacter spp***
T3	-	-	-	-	+	+	+	***Pseudomonas spp***
J1	-	-	-	+	+	-	+	***Proteus spp***
J2	+	+	-	+	-	-	+	***E.coli***
J3	+	-	+	-	+	-	-	***Klebsiella spp***
B1	+	+	-	+	-	-	+	***E.coli***
B2	-	-	-	-	+	+	+	***Pseudomonas spp***
B3	+	-	+	-	+	-	+	***Enterobacter spp***
G1	-	-	-	-	+	+	+	***Pseudomonas spp***
G2	+	+	-	+	-	-	+	***E.coli***
G3	-	-	-	+	+	-	+	***Proteus spp***
Z1	-	-	-	-	+	+	+	***Pseudomonas spp***
Z2	+	+	-	+	-	-	+	***E.coli***
Z3	+	-	+	-	+	-	+	***Enterobacter spp***
K1	+	+	-	+	-	-	+	***E.coli***
K2	-	-	-	+	+	-	+	***Proteus spp***
K3	+	-	+	-	+	-	-	***Klebsiella spp***
TS1	-	-	-	-	+	+	+	***Pseudomonas spp***
TS2	-	-	-	+	+	-	+	***Proteus spp***
TS3	+	+	-	+	-	-	+	***E.coli***
U1	+	-	+	-	+	-	+	***Enterobacter spp***
U2	+	+	-	+	-	-	+	***E.coli***
U3	-	-	-	-	+	+	+	***Pseudomonas spp***

Key: S=Sabongida, D= Damba sample, T= T/wada sample, B=Bebeji sample, G= Gada biyu samples, Z= Zawiyya sample, K= K/kanawa sample, T= T/kasuwa sample, B= Bebeji sample, U= University sample. L=Lactose, MR=Methyl red, VP=Voges-Proskaeur, I=INDOLE, O=Oxidase, M=Motility, C=Citrate, CL=Catalase, P/O=Possible organism, MCA=MacConkey agar, + indicates Positive reaction and - Indicates negative reaction.

4. DISCUSSIONS

The bacterial analysis of *nono* conducted using different samples collected from different selling points revealed the result that was used to determine the overall safety of the different *Nono* brands. Enumeration of Bacteria from *nono* samples was conducted using Nutrient, MacConkey, Mannitol and deMann Rogosa Sharpe agar which was in agreement with the work Mhone *et al.,* (2011) [15].

This study results revealed that the total viable counts were within the range of $6.6x10^5$ to $2.7x10^6$ CFU/ml corresponding to the findings of Amirul-Hassan *et al.,* (2015) [10]. A similar result ($7.1x10^5 – 3.2x10^6$ CFU/ml) was also reported by George *et al.,* (2020) [16] in Abuja, Nigeria. The average total coliforms count was $3.2x10^6$ CFU/ml which was in contrast to $2.0X10^5$ CFU/ml reported by Amirul-Hassan *et al.,* (2015) [10]. The result of this study on total coliforms count was in agreement with the work of Ogodo *et al.,* (2017) [17] in Wukari, who reported an average total coliforms count of $1.42x10^6$ CFU/ml which were above the acceptable limit of coliform in fresh milk which ideally should be between $1.0x10^2$ to $1.0x10^3$ CFU/ml according to NAFDAC [26].

The average range of total *Staphylococcus* count was $2.5x10^5$ to $4.0x10^6$ CFU/ml which was in agreement to the findings of Mohammed *et al.,* (2020) [18] and Amirul-Hassan *et al.,* (2015) [10], who reported a highest count of 3.7×10^6 CFU/ml. This finding was in contrast to the findings of Alloysius *et al.,* (2018) [19] who reported a highest staphylococcal count of $1.2x10^7$ CFU/ml. *Staphylococcus aureus* is a normal flora of human skin, therefore, this high staphylococcal counts might be attributed to the fact that the processors used their non-disinfected bare hands throughout the process.

Total lactic acid bacteria count ranged from $5.9x10^5$ to $1.7 x10^6$ cfu/ml for the tested samples. This result corresponded to the findings of by Misganaus and Teketay (2016) [1] who reported ranges between $2.2x10^5$ and $1.52x10^6$ CFU/ml. However, this result also corresponded to the findings of Alloysius *et al.,* (2018) [19] in Wukari Taraba state, Nigeria who reported a total lactic acid bacteria range from $2.36x10^5$ to $1.52x10^6$ cfu/ml. The high lactic acid bacteria count could be attributed to the low pH, high content of Nacl, nutritious constituents of the products and oxygen variability of the environment which favoured their growth as well as the fermentation processes [16]. High lactic acid bacteria count had been previously reported by Yabaya *et al.,* (2012) [20] in Zaria Kaduna state, Nigeria. He also reported that the high LAB count is beneficial for their probiotic potential as it aids in fermentation process.

The high bacteria load could be attributed to contamination by the utensils, the water used during processing and hygiene of the producers. It could also be attributed to the inherent bacteria in the raw materials from which this drink is made and contamination through environment [21]. The *nono* might be contaminated right from milking stage either from the hands of the persons in-charge or the teat through contacts to the soil or the faeces of the animals.

Colony morphology of the cultures such as color, form, elevation and margin were observed. The colors observed include: pinkish, rose-pink, colorless, creamy-white, off-white, yellowish, etc. This pigmentation may be as a result of the organism's ability to ferment some certain constituents of the media. In this study, the bacterial isolates identified belonged to the genera *Lactobacillus*, *Lecunostoc*, *Streptococcus*, *Micrococcus*, *Pediococcus*, *Escherichia coli*, *Pseudomonas*, *Proteus*, *klebsiella*, *Enterobacter* and *Staphylococcus* from ten *nono* samples. Isolation of these bacteria were previously reported by [22]; [23] and [10].

The result also showed that lactic acid bacteria, *Staphylococcus* and *E. coli* are present in all the samples. The presence of lactic acid bacteria and *Staphylococcus* in every samples were also reported in Abuja, Birnin Kebbi and Wukari by [16]; [24]; [19] respectively who also in contrast to this study, reported the least occurrence of *E. coli* in the samples. *Pseudomonas* sp., *Micrococcus* sp., *Streptococcus* sp., and *Leuconostoc* sp. were also found in many of the tested samples. This agrees with the report by Achi and Ukwuru (2015) [25] which said apart from *Lactobacillus* sp., *E. coli* and *Staphylococcus aureus; Pseudomonas* sp., *Micrococcus* sp., *Streptococcus* sp. and *Leuconostoc* sp. were also identified. The reasons for the presence of these bacteria may be due to unhygienic production method, poor processing and handling conditions, improper personal hygiene of the workers, being some of them the inherent bacteria of the raw materials etc.

The result showed that *S. aureus*, *Lactobacillus* and *E. coli* were recorded to have the highest percentage of occurrence (13.7%) in Nono samples, followed by *Pseudomonas* (12.3%), then *Micrococcus* with the percentage of 10.9%. *Leuconostoc* was recorded to have the least frequency of occurrence with the percentage of 1.4%. This is in agreement with the findings of [19] in which *Lactobacillus spp* and *staphylococcus aureus* had the highest occurrence but in contrast to that of *E. coli.* The isolates from the control sample were also identified using the Bergey's manual for bacterial identification and found to belong to the genera *Lactobacillus*, *Micrococcus*, *Leuconostos*, *Klebsiella* and *Pseudomonas.* The presence of these bacteria might be attributed to the fact that many are autochthonous in the materials used for the milking process and the wide spread of the others are attributed to environmental contaminant.

The *nono* samples tested were considered contaminated based on NAFDAC standard [26]. NAFDAC stated that the total viable count in *Nono* must not exceed $4.0x10^4$ and $1.0x10^2$ to $1.0x10^3$ CFU/ml for coliform bacteria. Also in comparison to the bacterial load and the type of bacteria isolated from the control, the tested *nono* samples might have been contaminated at any stage of production.

5. CONCLUSION

The study was conducted to enumerate, isolate and identify the bacteria that is present in the samples especially *Enterobacteriaceae, staphylococcal* group and the naturally occurring lactic acid bacteria. With this a total of 73 bacterial isolates (*Enterobacteriaceae*, *Staphylococcal* and *Lactic acid bacteria*) belonging to the genus *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Escherichia coli*, Pseudomonas, *Proteus*, *klebsiella*, *Enterobacter* and *Staphylococcus* were identified from the ten samples. The results obtained from this study demonstrated that there is a diversity of lactic acid bacteria in *Nono*. The presence of LAB in *Nono* enhances bioavailability of nutrients and act as a preservative. However, the Presence of *E. coli* (and the other enterobacteriaceae) and *Staphylococcus spp.* is of public health concern. It was concluded from the study that bacterial loads of *Nono* were not satisfactory. Therefore, it could be assumed that the handler of the products does not maintain good personal hygiene. All most all the samples tested in this study were of low quality based on NAFDAC standards.

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