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Occurrence of Staphylococcus aureus, Escherichia coli and Salmonella spp in 'Nono' Sold in Lafia Metropolis

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ABSTRACT

Nono is a fermented food-drink from cow. It is readily affordable, tasty, and laden with nutritional and health beneficial values. The study aimed to isolate and quantify the presence of Staphylococcus aureus, Escherichia coli and Salmonella sp in nono sold within Lafia metropolis to ascertain their safety for consumption. Four hundred samples were collected randomly from twenty different hawkers at ten locations within the metropolis. Isolation was done using the pour plate technique, while the presence of minerals was also determined. Samples collected in Tudun Gwandara had the highest population of bacteria with 7.8×10^6 cfu/ml. Staphylococcus aureus and Escherichia coli were isolated from the samples but Salmonella sp was not isolated. Staphylococcus aureus had the total occurrence rate of 66.7%, while Escherichia coli obtained 33.3% presence in the samples. Sodium, calcium, potassium, magnesium and copper were minerals found in different proportions in parts per million within the samples. Findings showed that nono consumption in Lafia may not be very safe because bacteria isolated were pathogenic and are potential sources of food borne infections.

Keywords: *food-drink, nono, minerals, pathogenic, infection*

INTRODUCTION

Nono or nunu is a Hausa food drink hawked by the cattle herdsmen 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 in coloration, and is produced from non-pasteurized cow milk collected, and allowed to pass through 24 h fermentation at room temperature (Eka and Ohaba, 1977; Olusupo *et al.*, 1996). Cow milk consumption cuts across the Sahara through the West African Sub-region. The food drink 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 hawk the product after fermentation has been completed. Nono is a good source of amino acids, calcium, phosphorous and vitamins A, C, E and the 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; Ijaz, 2013; Hodgkinson *et al*., 2014).

Challenges and health concerns have continued to trail the problems of; storage and preservation of fermented but unsold nono, safety regarding uncontrolled microbial fermentation, and contamination resulting from the milking procedures and processes (Wouters *et al*., 2002). Milking by peasant herders are mostly carried out under unhygienic conditions which inadvertently introduces pathogens such as *Listeria monocytogenes, Campylobacter jejuni, Salmonella* sp., and *E. coli* from feaces, soil, grass, milking apparatus (Coorevits *et al*., 2008; Vasavada, 1988).

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 provides 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). The study aimed to determine bacterial load of nono sold in Lafia, Nasarawa State, Nigeria;isolate and quantify *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp in the fermented milk; and identify minerals in the milk.

MATERIALS AND METHODS

Sample Collection and Study Locations

Nono samples were purchased from different points in Lafia, Nasarawa state. The milk is sold by street peddlers who are majorly Fulani women.

Four hundred samples were collected from different hawkers at different locations (Table 1) in sterile polythene bags.

Five hundred millilitres of the sample was collected from each hawker between 7 and 9 am in the morning because nono milk is termed 'fresh' in the morning as the fermentation occurs overnight. The points of collection include:

Table1. *Description of collection points.*

S/N	Collection Point	Description of Environment
1	Lafia Roundabout	Untidy with refuse dump
2	Bukan-Sidi	Untidy environment with litters
3	College of Agric	Clean environment
4	Mararaba	Untidy and very busy environment
5	Makurdi road	Untidy environment with refuse dump
6	Lafia Market	Very busy and Untidy
7	Tudun Gwandara	littered environment
8	Cow Market	Very untidy and busy environment with lots of animal dung around
9	Lafia East	Clean environment
10	Nasarawa State Polytechnic	Clean environment

Isolation and Identification of Bacteria

Innoculation was done using the pour plate technique as described by Orole and Adejumo, (2011). One milliliter aliquot of the diluted samples at dilution 10^{-6} were transferred using sterile blow out pipette to Mannitol Salt Agar (MSA) and Salmonella Shigella Agar (SSA), Nutrient Agar (NA) and Eosin Methylene Blue Agar (EMBA). The mixture was gently swirled for homogeneity.

Cultural characteristics like: opacity, elevation, edge and color were observed and recorded for the plates. Biochemical tests: Gram staining, motility, catalase and coagulase tests, sugar fermentation and MR-VP test (Methyl Red, Voges-Proskauer reaction) were done and additional characteristics described by Balows *et al.* (1992) and Bergey's Manual of Systematic Bacteriology (Krieg *et al.*, 1984) were used for identification of the isolates.

Test for Minerals

Nono was dried in the oven at 105 ^{0}C for 30 min to remove moisture, and dry ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550 ^{0}C, then left to ash at this temperature for 6 h. The samples were removed from the furnace and two drops of concentrated hydrochloric acid (HCl) added and returned to the muffle furnace to ensure that the sample is totally ashed. The crucibles were removed after 45 min and put in the desiccator to cool.

The ashed samples were digested in 10 ml 10% HCl and filtered into a 50 ml volumetric flask using Whatmann #1 filter paper. The filtrate was then diluted with distilled water, made up to mark in a 50 ml flask, and transferred into a sample bottle for further analysis. All samples were prepared in triplicates.

Analysis of the minerals present in the nono using atomic absorption spectroscopy (AAS) was carried out using Buck model scientific 210 VGP Atomic absorption spectrophotometer at Sustainable Laboratory Services Ltd Akure, Ondo State.

RESULTS

Total Bacterial Count

As shown in Table 2, Tudun Gwandara with 7.8×10^6 cfu/ml had the highest bacterial count, while Lafia East with 1.0×10^6 cfu/ml obtained the lowest count. Samples collected in cow market had thehighest coliform count of 3.9×10^6 cfu/ml, while Lafia roundabout had the lowest coliform count with 3.2×10^5 cfu/ml.

Table2. *Total viable and coliform count from the different collection points.*

Collection sites	Total viable count(cfu/ml)	Total coliform count (cfu/ml)
College of Agric	1.1	9.4
Lafia Market	2.6	3.4
Mararaba	4.1	1.0
Makurdi Road	4.9	1.2
Lafia Roundabout	7.2	3.2
Bukan Sidi	7.5	2.3
Lafia East	1.0	2.3
Cow Market	2.6	3.9
Tudun Gwandara	7.8	3.3
Nasarawa State Polytechnic	1.4	8.0

Total Count of Isolated Bacterial Species

The result of identified bacterial species revealed the presence of *Staphylococcus aureus* and *Escherichia coli* in cfu/ml as shown in Table 3. Samples from Tudun Gwandara with contained 9.8×10^5 cfu/ml of *S. aureus* as the highest count obtained from the collection points. *E.coli* had highest counts in Bukan Sidi and Lafia East with loads of 8.2×10^5 cfu/ml respectively.

Table3. *Total bacterial isolates' count from the different collection point*

Collection sites	*S. aureus*($\times 10^5$cfu/ml)	*Escherichia coli*($\times 10^5$ cfu/ml)	*Salmonellasp*($\times 10^5$cfu/ml)
College of Agric	4.9	5.2	0
Lafia Market	7.1	4.8	0
Mararaba	3.2	3.8	0
Makurdi road	8.1	4.1	0
Lafia Roundabout	3.4	5.2	0
Bukan sidi	6.8	8.2	0
Lafia East	6.3	8.2	0
Cow Market	9.1	4.2	0
Tudun Gwandara	9.8	4.2	0
Nasarawa State Polytechnic	8.6	5.8	0

Percentage Occurrence of Isolates In Nono

Table 4 reports the percentage occurrence of the isolates generally, and from the different locations where samples were collected. *Salmonella* spp being totally absent obtained a zero percentage, *Staphylococcus aureus* and *Escherichia coli* obtained 34.1% and 65.9% respectively.

Tudun Gwandara with 58.7% had the highest percentage for *S. aureus* load, while Mararaba with 89.2% contained the highest percentage of *E. coli* contamination.

Table4. *Occurrence of isolated bacteria in nono samples.*

Collection sites	*S. aureus*	*E. coli*	*Salmonella. spp.*	Total
College of Agric	127 (43.8%)	163 (56.2%)	0	290
Lafia market	98 (33.4%)	195 (66.6%)	0	293
Mararaba	44 (10.8%)	362 (89.2%)	0	406
Makurdi road	155 (16.0%)	810 (84.0%)	0	965
Lafia Roundabout	12 (4.5%)	185 (60.9%)	0	267
Bukan Sidi	155 (14.9%)	881 (85.1%)	0	1036
Lafia East	119 (39.1%)	881 (88.1%)	0	1000
Cow market	498 (53.6%)	430 (46.4%)	0	928
Tudun Gwandara	618 (58.7%)	435 (41.3%)	0	1053
Nasarawa State Polytechnic	159 (57.8%)	116 (42.2%)	0	275

Mineral Content of Nono

Five collection sites were randomly chosen and samples from such location sent for mineral analysis. Samples from Nasarawa State Polytechnic with 0.50 ppm had the highest amount of calcium, while Bukan Sidi samples obtained 0.29 ppm making it the lowest. Tudun Gwandara with 180 ppm had the highest amount

of potassium, while Cow market with 148 ppm had the lowest amount of potassium.Nasarawa state polytechnic recorded the highest amount of magnesium (3.76 ppm), and Bukan Sidi had the lowest with values 3.04 ppm. Tudun Gwandara obtained the highest sodium content in nono with 92.10 ppm, while cow market had the lowest amount of sodium with 47.80 ppm. Nasarawa State Polytechnic obtained the lowest amount of copper with 0.29 ppm, while Bukan sidi with 0.50 ppm had the highest amount of copper content.

Table5. *Mineral Analysis*

Collection sites	Na(ppm)	Ca(ppm)	Kppm)	Mg(ppm)	Cu(ppm)
Cow Market	47.80	24.00	148.00	3.16	0.32
Bukan Sidi	36.10	16.00	153.00	3.04	0.50
Tudun Gwandara	92.10	25.00	180.00	3.07	0.39
Lafia Market	82.60	22.50	175.00	3.08	0.33
Nasarawa State Polytechnic	77.30	47.00	158.00	3.76	0.29

DISCUSSION

The bacterial loads of nono samples sold within Lafia metropolis were relatively high. Milk and milk products are nutrient rich foods that can serve as very good medium for the growth of all kinds of microorganisms. Total coliform count was carried out to ascertain the presence of coliforms present in the samples. Coliforms belong to the family Enterobacteriaceae, and their presence indicates contamination from feacal origin. Total coliform count were also relatively high and this is because nono contains high amount of lactose making it possible for lactose fermenters (Coliforms) to ferment it. This corroborate the earlier report by Ruegg and Reinemann (2002) that milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. Lafia roundabout had the least number of coliforms with 3.2×10^5 cfu/ml and cow market had the highest coliform count with 3.9×10^6 cfu/ml.

High load of bacteria from the cow market is probably because cow market as the name implies is where cows are reared for sale, resulting in fecal contamination. The environment was unclean as at the time of sample collection, having animal dung littering everywhere. Lafia roundabout had dirty environment with refuse dump, but it had little animal dung lying around. The milking procedure, handling and sale is still entirely based on the traditional milking systems which could provide favorable environment for bacterial contamination. Unclean hands of workers, milk from sick cow, unhygienic conditions of the other processing units and unclean water supplied for washing and cooking could be sources of bacterial contamination of nono beside the post manufacturing contamination (Elmahmood and Doughari, 2007). The environment where nono is sold could be a contributing factor to the presence of organisms as air could easily deposit them on open nono surfaces. Two bacterial genera were isolated from the samples: *Escherichia coli* and *Staphylococcus aureus. S. aureus* was the most predominantly isolated bacteria with 66.7% occurrence rate. *E. coli* on the other hand is an indicator of pollution from fecal sources and was found present in all the nono samples gotten from strategic areas in Lafia metropolis. Consumption of nono sold in Lafia could result in public health concern like Salmonellosis, staphylococcal poisoning and diarrhea.

Staphylococcus aureus is a Gram positive bacterium which is a normal flora of the human skin, and may colonize the nasopharyngeal region. The presence of this species of microorganisms may be from the human handlers. It releases a toxic chemical, enterotoxin with as little as 1.0 µg of the toxin in contaminated food producing symptoms of illness. The bacteria causes diseases in human, it is responsible for a long list of different disease such as folliculitis, furuncles, carbuncles, erysipelas, , cellulites, scalded skin syndrome, impetigo, pneumonia, Osteoporosis, toxic shock syndrome (toxemia) meningitis and staphylococcus food poisoning (Marjorie and Kathleen 2006). Several strains of *Salmonella* spp are known to be pathogenic to humans and their absence indicated a little amount of safety in the consumption of nono. This study conforms to the work of Makwin *et al*., (2014) who reported the presence of *E. coli, Salmonella* species, *Staphylococcus aureus* and *Streptococcus* species in raw milk and nono, and suggested that consumption of nono has potential health risks to the consumers. The study showed that pathogenic microorganisms are present in nono and may be

potential sources of food borne infection for the consumers of this product in and around the sampling areas.

Enumeration of total bacterial load found in the sample showed potentially disturbing high counts which could be as a result of Lafia being an open, busy, and dusty environment having refuse dump around in most places. The total viable bacteria counts in the samples analyzed were beyond standard set by National Agency for Food and Drug Administration and Control (NAFDAC). NAFDAC (2009) gave a limit that the microbial load for total viable colony count should not exceed 1.0 x 10^2 cfu/ml and *Escherichia coli* should not be present in such samples. The presence of *E. coli* invalidates the safety of the samples collected in Lafia. *Salmonella* spp being a strict pathogen had no habitat other than human or animal bodies. Sources of contamination could be human or animals (carriers) of the pathogen when passed in the faeces or urine and transmitted by food or water then ingested by another host. It can cause any one of these three (3) types of infections; bacterial food poisoning, enteric fever and systemic fever (septicemia) (Stewart and Besorick, 1997).

Mineral elements occur in milk and dairy products as inorganic ions and salts, as well as part of organic molecules, such as proteins, fats, carbohydrates and nucleic acids. The chemical form of mineral elements is important because it determines their absorption in the intestine and their biological utilization. The mineral composition of milk varies as result of lactation phase, geographical location, cow specie, and nutritional status of the animal, health of the animal and environmental and genetic factors. Milk is an excellent source of calcium which is essential for healthy growth and maintenance of teeth and bones. It is also important for normal blood coagulation, normal energy yielding metabolism, normal muscle and nerve functions, normal digestive function and normal regulation of cell division and differentiation. Potassium aids in maintaining normal blood pressure, muscular, and neurological functions. Magnesium plays an important role in many physiological processes, such as metabolism of proteins and nucleic acids, neuromuscular transmission and muscle contraction, bone growth and blood pressure regulation. Magnesium is also a co-factor of many enzymes. On the other hand, magnesium deficiency may also cause osteoporosis.

Sodium concentration in milk decreases to average levels as day passes though, its concentration in milk does not depend on its dietary intake. It is higher at the end of the lactation period, when the quantity of milk is reduced. Sodium is the major cation in the extracellular fluids and is an important regulator of osmotic pressure, acid-base balance and cellular membrane potential. It is also important for the active transportation of substances through the cellular membrane. The contribution of cow milk to daily sodium intake in human nutrition is low, but cheese and some cream products which contain added quantities of salt, can provide significant sources of sodium. Copper is an essential trace mineral necessary for survival. It helps with the formation of collagen, increases the absorption of iron and plays a role in energy production. The above metals are all vital and good for human consumption for one body activity or another. Their presence connotes that nono is healthy and good for consumption.

CONCLUSION

From the result of this study it can be concluded that locally prepared nono contains pathogenic and spoilage bacteria. The presence of pathogenic organisms indicates unhygienic handling during production. Nono sold in Lafia contain pathogens that are harmful to humans and considered unsafe for consumption which agrees with the work of Musliu and Aliyu Garba, (2012). Milk should be pasteurized and handled hygienically before consumption and the results presented in this study therefore indicated that strict preventive measures should be adopted to ensure contamination free nono for the good health of all consumers. Consciousness and care of the product is required from the point of generation to the point of consumption of this widely distributed and consumed milk product.

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