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Microbiological Analyses and Safety Evaluation of Nono: A Fermented Milk Product Consumed in Most Parts of Northern Nigeria

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ABSTRACT

Microbiological analyses and safety evaluation of nono was investigated by assaying for the presence of some microorganisms frequently implicated in food safety problems and spoilages including *E. coli*, *S. aureus*, *Shigella* and *Salmonella* sp. Others included aerobic mesophilic, anaerobic mesophilic, psychrotrophic microorganisms and yeasts and mould counts. *E. coli*, *S. aureus*, *Shigella*, *Salmonella*, aerobic mesophilic, anaerobic mesophilic, psychrotrophic microorganisms and yeasts and moulds were isolated in varying degrees with a range of 7.89, 6.60, 6.00, 6.50, 8.90, 6.30, 4.30 and 4.30 log₁₀ cfu mL⁻¹, respectively. Overall mean values of these counts were also 2.21, 1.51, 0.30, 1.17, 3.76, 1.76, 0.76 and 1.23 log₁₀ cfu mL⁻¹, respectively. Present Laboratory prepared nono did not contain any of the assayed organisms. There was statistically significant (p<0.05) difference between the means of the microbial groups at 95% confidence interval. Of the market nono samples analyzed, 86 (43%), 56 (28%) and 16 (8%) were positive for *E. coli*, *S. aureus* and *Shigella* sp., respectively. 48 (24%) harboured *Salmonella* sp. *Shigella* sp. was not detectable in three areas under study while *E. coli* approximately had the highest number of occurrence. Nono consumed in the said area has potential health risks to consumers. Proper hygiene in the process-line of nono was recommended.

Key words: Nono, bacterial count, yeasts and mould, food safety, microbiology

INTRODUCTION

'Nono' (Hausa) is an opaque white to milky coloured liquid food drink got from fermented raw milk. It 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. In the Middle East, it is called 'dahi' or 'lassi' (Nahar *et al.*, 2007). Nono also called nunu by some tribes in Nigeria contains good quantities of amino acids, calcium, phosphorous and vitamins A, C, E and the B complex (Nebedum and Obiakor, 2007).

Predominantly, nono is being prepared and hawked by the nomadic Hausa/Fulani cattle herdsman, who control over 80% of Nigerian's cattle production. Consumption of nono was limited to Fulani/Hausa indigenous (Obi and Ikenebomeh, 2007; Adesokan *et al.*, 2011) since most non indigenous see their preparation as apparently unhygienic and since it has poor shelf life (Yahuza, 2001) it is gaining wider acceptance nowadays.

Raw milk has low keeping quality and at room temperature, spontaneous microbial spoilage occurs turning the product sour few some days. This is brought about by the activity of lactic acid bacteria (Wouters *et al.*, 2002). Depending on its preservation and process-line, microorganisms

other than lactic acid bacteria could be found in the nono. Milk's nutritional composition makes it not only suitable for human nutrition but also ideal for microbial life. The growth of microorganisms in food could make the food grossly unwholesome and harmful to consumers. Outbreaks of milk-borne diseases have occurred despite pasteurisation, as a result of either improper pasteurisation or product recontamination (Altekruse *et al.*, 1998; Da Silva *et al.*, 1998; Hartman, 1997; Nebedum and Obiakor, 2007).

It is noticed that raw milk often contains microorganisms which may likely cause foodborne diseases (Adesiyun *et al.*, 1995; Headrick *et al.*, 1998; Steele *et al.*, 1997). Even when the milk is fermented, the fermentation process with the attendant drop in pH may not rid the product of these organisms and may be carried to consumers. *Salmonellae* and other microorganisms have been known to underscore the importance of milk and milk products as vehicle for human infections. For these reasons, McEwes *et al.* (1988) reasoned that the presence of *Salmonellae* and other human pathogens in unpasteurized milk is a public health hazard. Food safety guideline for food banks recommends that milk and milk products including cream and cream products, ice cream, frozen desserts, yoghurt and similar foods must be pasteurized, held and distributed in their original unopened containers.

Different categories of microorganisms including fungi, bacteria, rickettsia and viruses could be found in milk since the udder of the animal could harbour organisms while others come as contaminants due to poor handling. Most handlers or sellers of nono are street peddlers. Often, not all nono sent to the market by peddlers is sold the same day and in most developing countries, such unsold ones have to go back to market with no special attention to preservation or safety. Thus pathogenic organisms that might have gained access into the product have enough time to multiply and/or produce harmful metabolites. The present work was aimed at investigating the microbiological quality and safety of nono consumed in Maiduguri, Nigeria.

MATERIALS AND METHODS

Sample collection: Nono samples were purchased from five different markets namely: Bulunkutu, Gwange, Monday, Bama Road Motor Park and Unimaid Motor Park Markets all within Maiduguri metropolis. Forty samples were randomly purchased from each of the markets. Purchased samples were transported to the University of Maiduguri Microbiology Laboratory in sterile corked plastic tubes packed in iced container. Purchases were done between 9.00 am and 11.00 am each day lasting from September 2009 to February 2010. Our laboratory prepared nono got from fresh cow milk (purchased at Fulani Farm in Maiduguri) was also analysed.

Nono preparation and consumption: It is produced from cow milk collected in calabash and allowed to ferment naturally for 24h (Eka and Ohaba, 1977; Olasupo *et al.*, 1996).

Microbiological analyses: Nono samples approximately 40 mL each contained in pre-autoclaved containers 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 homogenized by 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 and surface-plated. Media employed for the isolation and enumeration of the organisms included: Baird Parker Medium (BPM) (Lab M. Ltd, Bury Lancashire BL9 6As, United Kingdom) for *S. aureus*; Eosine Methylene Blue Agar (EMBA) (Himedia

Laboratories Pot Ltd, India) for *E. coli* and Desoxycholate Citrate Agar (DCA) (Park Scientific Limited, Moulton Park, Northampton) for *Salmonella* and *Shigella* spp. Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) was used for aerobic mesophilic, anaerobic mesophilic and psychrotrophic microorganisms' counts. Yeasts and mould counts were carried out on a Potato Dextrose Agar (PDA) (Lab M. Ltd, Bury Lancashire BL9 6As, United Kingdom) plate. Media were sterilized by autoclaving at 121°C for 15 min except DCA which involved only boiling over gauze. In all cases of colony counts, the resulting colonies following inoculation and incubation were counted using digital colony counter (Labtech, New Delhi, India).

Aerobic mesophilic counts (AM): Inoculated NA plates were incubated at 30°C for 48 h.

Anaerobic mesophilic counts (AAM): Inoculated NA plates were incubated in anaerobic jars (Equotron) containing heated iron nail and candle as recommended by Cheesbrough (2004) at 30°C for 48 h.

Psychrotrophic microorganisms' counts: Inoculated NA plates were incubated at 6.5°C for 24-48 h.

Yeasts and moulds counts: PDA plates containing Chloramphenicol were inoculated as above. Incubation was at 28°C for 5 days. Colonies were thereafter counted.

Isolation and enumeration of *E. coli*: EMBA plates were inoculated as described previously and incubated at 37°C for 48 h after which typical colonies with greenish metallic sheen were subjected to biochemical tests for *E. coli*.

Isolation and enumeration of *S. aureus*: BPM were inoculated as above and incubated at 37°C for 48 h. Greyish-black or black colonies with or without a halo were presumptively identified as Staphylococci as recommended by Macfaddin (1977) and coagulase test was carried out to further characterize *S. aureus*.

Isolation and enumeration of *Salmonella* and *Shigella* sp: Isolation and enumeration of *Salmonella* and *Shigella* sp. was as recommended by Macfaddin (1977).

Biochemical identification of the isolates: The biochemical tests for the identification of the isolates were the citrate utilization, indole, methyl-red, Voges-proskauer, Triple Sugar Iron (TSI), urease, oxidase, coagulase and catalase tests. Cowan and Steel (1965) and Cheesbrough (2004) procedures were used for these biochemical tests.

The sample pH: The pH of various samples was measured using a pH meter (WPA pH Meter, India) after standardization with pH 4, 10 and 7 buffers (BDH England).

Statistical analysis: Data were analyzed by Multiple-sample comparison using STATGRAPHICS Centurion XVI Version 16.1.05 (32-bit). A one-way analysis of variance was performed and when the F-test in the ANOVA was significantly ($p \leq 0.05$) different between the means, Multiple range tests were conducted to tell which means were significantly different from others.

Table 1: Mean microbial counts (counts were expressed as \log_{10} cfu mL⁻¹) and mean pH of samples of nono from different markets in Maiduguri

Market	Ec	Sa	Sg	Ss	Am	AAm	Pm	Ym	pH
Bulunkutu	2.80	2.98	0.92*	2.37	4.66*	4.09	0.90	1.34	5.40
Gwange	1.37	1.82	0.57*	1.48	3.75*	1.03	0.93	1.61	4.00
Monday	1.92	0.76	0.00*	0.66	2.53*	0.69	6.45	0.94	3.80
Bama	1.69	0.85	0.00*	0.43	2.96*	0.90	0.64	0.81	5.30
Unimaid	3.29	1.15	0.00*	0.88	4.89*	2.09	0.86	1.44	4.50
Overall means	2.21	1.51	0.30	1.17	3.76	1.76	0.76	1.23	4.60

NB: Ec: *E. coli*, Sa: *S. aureus*, Sg: *Shigella*, Ss: *Salmonella* counts, Am: Aerobic mesophilic, AAm: Anaerobic mesophilic counts, Pm: Psychrotrophic microorganism, Ym: Yeast and mould counts. *Indicate statistically significant difference ($p < 0.05$) between organisms in the column and other organisms in the same row

Table 2: Minimum and maximum values of count of microorganism isolated from different markets in Maiduguri

Market	Ec	Sa	Sg	Ss	Am	AAm	Pm	Ym
Bulunkutu	0-5.78	0-6.00	0-4.90	0-6.06	1-6.00	0-5.70	0-2.85	0-5.00
Gwange	0-5.00	0-6.60	0-6.00	0-6.30	0-8.90	0-3.30	0-3.00	0-4.45
Monday	0-5.90	0-5.48	0-0	0-5.48	0-8.00	0-5.00	0-2.30	0-4.00
Bama	0-7.89	0-5.85	0-0	0-5.54	0-7.30	0-4.45	0-3.70	0-3.30
Unimaid.	0-6.30	0-5.90	0-0	0-6.50	0-8.00	0-6.30	0-4.30	0-5.00

Counts were expressed as \log_{10} cfu mL⁻¹. Total No. of samples = 40 from each market. NB: Ec: *E. coli*, Sa: *S. aureus*, Sg: *Shigella*, Ss: *Salmonella* counts, Am: Aerobic mesophilic, AAm: Anaerobic mesophilic counts, Pm: Psychrotrophic microorganism, Ym: Yeast and mould counts

RESULTS

***E. coli* count (\log_{10} cfu mL⁻¹):** The mean *E. coli* counts (\log_{10} cfu mL⁻¹) were between 1.37 and 3.29 from the different markets (Table 1) with an overall mean of 2.21 \log_{10} cfu mL⁻¹. The mean *E. coli* count of Unimaid was significantly higher ($p \leq 0.05$) than Monday, Gwange and Bama but homogeneous with Bulunkutu market. *E. coli* count from different markets ranged from 0.0 to 7.89 \log_{10} cfu mL⁻¹ (Table 2).

***S. aureus* count (\log_{10} cfu mL⁻¹):** The mean *S. aureus* counts fell between 2.98 and 0.76 with an overall mean of 1.51 \log_{10} cfu mL⁻¹ (Table 1). The mean *S. aureus* count of Bulunkutu market was significantly higher ($p \leq 0.05$) than that got from other markets. The *S. aureus* count of the samples ranged from 0.0 to 6.60 \log_{10} cfu mL⁻¹ (Table 2).

***Shigella* sp count (\log_{10} cfu mL⁻¹):** The mean *Shigella* count was between 0.0 and 0.92 and had an overall mean of 0.30 \log_{10} cfu mL⁻¹. There was no significant ($p \leq 0.05$) difference between the means of *Shigella* from the markets. The range values of count of *Shigella* fell between 0.0 and 6.0 and percentage distribution between 0.0 and 12.0. *Shigella* was not isolated from three markets under study (Table 1-3).

***Salmonella* count (\log_{10} cfu mL⁻¹):** The mean *Salmonella* count was between 0.43 and 2.37 and the overall mean was 1.17 \log_{10} cfu mL⁻¹ (Table 1). Bulunkutu market value was significantly higher ($p = 0.05$) than that of other markets. The range value of counts of *Salmonella* was between 0.0 and 6.5 and the distribution and percentage frequency of occurrence between 4.0 and 22.0 (Table 3).

Table 3: Distribution and (%) frequency of occurrence of different microorganisms isolated from nono samples

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Shigella</i>	<i>Salmonella</i>
Bulunkutu	24 (60)	26 (65)	12 (30)	22 (55)
Gwange	12 (30)	12 (30)	4 (10)	10 (25)
Monday	14 (35)	4 (10)	0 (0)	6 (15)
Bama	12 (30)	6 (15)	0 (0)	4 (10)
Unimaid	24 (60)	8 (20)	0 (0)	6 (15)
Overall	86 (43)	56 (28)	16 (8)	48 (24)

Values in parenthesis are individual market (%) frequency. In each market, 40 nono samples were analysed (n = 40)

Aerobic mesophilic count (\log_{10} cfu mL⁻¹) (AMC): The mean AMC from the different markets fell between 2.53 and 4.89. The overall mean AMC was 3.76 \log_{10} cfu mL⁻¹ (Table 1). There was no significant difference between the means of AMC (Table 2). The range values of AMC fell between 0.0 and 8.90.

Anaerobic mesophilic counts (\log_{10} cfu mL⁻¹) (AAMC): The mean AAMC was between 0.69 and 4.09 with an overall mean of 1.76 \log_{10} cfu mL⁻¹ (Table 1). There was a significant ($p \leq 0.05$) difference between the means obtained from Bulunkutu and other markets analysed. The range values of AAMC were between 0.0-6.30 (Table 1).

Psychrotrophic microbial count (PMC) (\log_{10} cfu mL⁻¹): The mean PMC (\log_{10} cfu mL⁻¹) was between 0.86 and 6.45 from the different markets. The overall market mean for PMC was 0.76 \log_{10} cfu mL⁻¹ (Table 1). There was no significant difference between the means obtained from the markets. The range values of PMC fell between 0.0 and 4.3 (Table 2).

Yeast and mould count (YMC) (\log_{10} cfu mL⁻¹): The mean YMC fell between 0.81 and 1.61. The overall mean YMC was 1.23 \log_{10} cfu mL⁻¹ (Table 1). There was no significant difference between the mean values obtained from the markets. The range values of YMC from the markets were between 0.0 and 5.00 (Table 2).

The sample pH: The pH range of the nono samples from the various markets was between 3.8 and 5.4 (Table 1).

DISCUSSION

The presence of *E. coli* (43%), *S. aureus* (28%), *Shigella* (8%) and *Salmonella* (24%) in nono sold within Maiduguri Metropolis is quite high to attract public health attention. From the results, *E. coli* was the most frequent isolate (Table 1-3). Results obtained for % *E. coli* content of the samples fell within the range of 15-65 which supports that obtained by Soomro *et al.* (2002) but lower than not detectable to 20% reported by Ekici *et al.* (2004) and 2.5% by Mohamed and El-Zubeir (2007). The mean *E. coli* count of $\log 2.21$ cfu mL⁻¹ supports that obtained by Abdalla and El-Zubeir (2006). While originally *E. coli* had been associated with the contamination of milk and milk products (Kulshrestha, 1990), researches have linked the presence of *E. coli* with the presence of other enteric pathogens (Adesiyun *et al.*, 1995; Norwegian Food Control Authority, 1994; Smoot and Pierson, 1997; Van den Berg, 1988).

The mean *S. aureus* counts (\log_{10} cfu mL⁻¹) of 1.51 contradicts a lower mean of 0.48 obtained by Abdalla and El-Zubeir (2006) from yoghurt samples and the % distribution in samples of 28 was

lower than 75% obtained by Ekici *et al.* (2004) from cow milk and 60% from goat milk Tormo *et al.* (2011). The range value of count of *S. aureus* between 0.0 and 6.60 also contradicts Abdalla and El-Zubeir (2006) results of 0.00-2.90 log cfu mL⁻¹. Processing operations and fermentation parameters could have brought about the discrepancy. The presence of *S. aureus* in the milk samples corroborates the finding of Tormo *et al.* (2011) that *Staphylococcus* was the dominant bacterial sp. of milk and inferior health condition of the animal increased the contamination of milk with Staphylococci. Jablonski and Bohach (1997) noted that *S. aureus* could be spread from humans to food by direct contact, indirectly by skin fragments, or through respiratory tract droplet nuclei. Mastitis udder also harbours *S. aureus* (Wellenberg *et al.*, 2002). Consequently, milk from mastitis cows could be another reservoir for *S. aureus* (Gran *et al.*, 2002). The range of *S. aureus* in this study exceeded the estimated minimum for the organism to produce intoxication and also, the pH (3.8-5.4), temperature (30-45°C) and the holding time conditions agreed with the requirement for *S. aureus* to produce sufficient enterotoxin (Rorvik and Granum, 1999).

Shigella sp. was not detectable in three areas under study. This organism is not an intrinsic flora of the animal; therefore, contamination of the fermented milk with the organism could have arisen from handling. Twenty-four percent of the assayed samples showed the presence of *Salmonella* spp. The mean *Salmonella* counts (log cfu mL⁻¹) of 5.9 contradicts 1.12 obtained by Abdalla and El-Zubeir, (2006) and % distribution of 24 contrasts not detectable reported by Ekici *et al.* (2004) and Mohamed and El-Zubeir, (2007). *Salmonella* are pathogens that could originate from the animals themselves. Thus, presence of *Salmonella* in fermented milk is not surprising since they could either be transmitted from the animal before preparation or could have come via cross contamination. Since, these organisms including *E. coli* and *S. aureus* were not isolated from our laboratory prepared nono, it is possible to get pathogen-free nono via general hygiene of the process line thereby saving consumers from the potential health risks.

The aerobic mesophilic count (AM) ranged from not detectable 0 to 8.9 log₁₀ cfu mL⁻¹ and the mean AM counts was 3.76 log₁₀ cfu mL⁻¹ (Table 2). The mean aerobic mesophilic count (log cfu mL⁻¹) of 3.76 agreed closely with 3.6 average bacterial counts of Tormo *et al.* (2011). Aerobic colony count is useful for indicating the overall microbial quality of food product. It generally does not relate to food safety hazards but acts as an indicator for food quality and shelf-life duration (Pianetti *et al.*, 2008). Except for Bulunkutu, anaerobic organisms were generally very low throughout the study. The reason for this could be their absence during nono preparation and distribution. The mean Psychrotrophic microbial counts (log cfu mL⁻¹) of nono were low throughout the counts with ranges between 0 and 4.3 log₁₀ cfu mL⁻¹. It stands to reason that storage of the product at such temperature could increase its shelf life.

The mean YMC of 1.23 log₁₀ cfu mL⁻¹ was higher than not detectable recorded by Ukwuru and Ogbodo (2011) from fermented tigernut- a milk product but however contradicts higher value of 3.48 reported by Awad *et al.* (2006) from similar product. Range yeast and mould count of 0.81-1.61 was partly in support of 1.44-2.06 obtained by Nahar *et al.* (2007) but lower than 1.83×10³-3.7×10⁶ obtained by Savadogo *et al.* (2004). The reason for the contradictions could be linked to the chance fermentation practiced by many local producers as differences in YMC of the same product from different manufacturers had been documented (El-Bakri and El-Zubeir, 2009). Yeasts are known to cause spoilage in products such as yoghurt and sour milk; however they are also important because they produce desirable favours as in cheese ripening (Fleet, 1990; Rohm *et al.*, 1992; Jakobsen and Narvhus, 1996). The mean YMC of 1.23 log₁₀ cfu mL⁻¹ (Table 1) was lower than 10⁵-10⁶ cells g⁻¹ reported to cause pronounced spoilage (Fleet, 1990).

The pH range of samples between 3.8 and 5.4 supports that obtained by El-Bakri and El-Zubeir (2009) but however contradicts 5.51-6.29 reported by Adesokan *et al.* (2011) and 5.7 by Obi and Ikenebomeh (2007). The differences in pH could be as a result of some factors including; length of fermentation and the starter culture. If the keeping time of nono increases prior to consumption, the acidity increases and this determines the number and kind of contaminating organisms.

CONCLUSION

The significant ($p \leq 0.05$) difference between the means of the microbial groups in this study could be due to lack of standardized method of nono preparation, or the health status of the milk producing animal or other environmental variables. The recommendation of the food safety guideline for the pasteurization of food like milk and distribution in unopened form for food safety could account for the many microorganisms isolated from the nono. The practice of preparation and distribution of nono in open calabash contravenes such principles. Proper hygiene in the process-line of nono is recommended since the markets selected were the major nono selling points in Maiduguri and since the laboratory prepared nono had no detectable pathogen.

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