Extent of microbial contamination of nono, fresh cow milk and yoghurt sold in Makurdi, Benue State, Nigeria

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ABSTRACT

Nono, fresh cow milk and yoghurt, sold in Makurdi town, were analyzed to ascertain their microbiological safety and compare the level of contamination of the products, by following standard bacteriological and mycological methods. A total of 30 samples of the three dairy products were collected. Nono samples were obtained from both hawkers and farmsteads, fresh cow milk from farmsteads only, and yoghurt samples from super markets. Findings were subjected to X²-test to ascertain the extent of microbial contamination for each product. A total of ten (10) bacteria, six (6) moulds and two (2) yeasts were isolated with an average total viable count of 1.63×10⁷ cfu/ml and 1.26×10⁷ cfu/ml for nono obtained from hawkers and farmsteads respectively. Yoghurt and fresh cow milk had an average total viable count of 1.42×10⁷ cfu/ml and 2.60×10⁶ cfu/ml respectively. Average enterobacteria counts obtained were 7.45×10⁶ cfu/ml and 7.25×10⁶ cfu/ml for hawked nono and yoghurt respectively, 1.26×10⁶ cfu/ml for fresh cow milk and 4.25×10⁶ cfu/ml for nono, both collected from farmsteads. Average fungal counts for hawked nono, yoghurt, fresh cow milk and nono from the farmsteads were 9.20×10⁶ cfu/ml, 1.07×10⁶ cfu/ml, 1.14×10⁶ cfu/ml and 8.02×10⁶ cfu/ml respectively. Significant differences (p<0.05) in the level of contamination of the three products were established. The study revealed that appropriate microbiological standards were not applied in the processing, packaging and retailing of the dairy products and they are thus, not fit for human consumption. It was recommended that appropriate measures should be taken to reduce microbial contamination of these products.

Key words: Microbial contamination, dairy products, fresh cow milk, nono, yoghurt, farmstead.

INTRODUCTION

Milk is an essential first food for man, forming an important part of man’s diet both for infants and adults. The majority of milk consumed throughout the world is bovine milk, although in some regions, milk from goat, ewe or mare may be more commonly used. Milk contains carbohydrate in form of lactose, fat, vitamins and chloride, inorganic phosphate and citrate [9].

Nono is a general name used for locally fermented cow milk and it is widely consumed in many African countries, including Nigeria [19]. It is an opaque white to milky coloured liquid food drink got from fermented raw milk. It is a healthy 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’ [12]. Predominantly, nono is prepared and hawked by the nomadic Hausa/Fulani cattle herdsman, who control over 80% of Nigerian’s cattle production [14]. Fresh cow milk is unfermented, raw cow milk collected fresh, from the cow’s udder. Consumers have a strong preference for this traditionally produced and processed milk products due to
the satisfying nature and also its high protein content [5]. It could also be due to its affordability in comparison with imported, processed milk products whose prices are higher.

Yoghurt is made by the controlled thermoduric fermentation of pasteurized non-fat or low-fat milk, carried out around 45°C [17]. It is probably the most popular fermented milk product in the United States and in Nigeria too [13] [17]. It is produced both commercially and by individuals, using yoghurt-making kits. Commercial starter culture consisting of Lactobacillus bulgaricus and Streptococcus thermophilus are used in the fermentation process of yoghurt production [17]. The consumers’ expectation of processed milk is that it should have the typical appearance of milk, be free from extraneous matter, and should have a clean and slightly sweet taste, with no abnormal odours or taints. Also, apart from the microbiological quality of raw milk being of great importance in regard to product and food safety, raw milk should be unadulterated and free of taints, antibiotics, blood and visible sediment. Since nono and fresh cow milk are produced by essentially illiterate Fulani’s in villages with poor knowledge of shelf-life, product safety, sanitation and aseptic milking techniques, handlers of these products may unknowingly introduce pathogenic microorganisms into the products. Since these products do not undergo further processing before being sold for consumption, these foods may become potential sources of illness to their consumers [19].

Many dairy products, even those made from pasteurized milk, have been associated with food-borne diseases [16]. This is partly because milk and milk products provide a favourable environment for microbial growth. 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 [14]. Most handlers or sellers of nono are street peddlers. Often, not all nono brought to the market by peddlers are sold the same day and in most developing countries, such unsold ones are reintroduced into the market with no further treatment or proper preservation. Thus, pathogenic organisms that might have gained access into the product have enough time to multiply and/or produce harmful metabolites [14].

Since dairy products constitute an integral part of the Nigerian diet, attention ought to be paid more to the hygienic aspects of handling and distribution of such foods. This study was undertaken to ascertain and compare the microbial quality of nono, fresh cow milk and yoghurt sold in Makurdi Benue State, so as to determining their safety or otherwise, for consumption.

MATERIALS AND METHODS

Study Area
Makurdi Local Government was created in 1970, eventually giving birth to Gwer, Guma and Gwer-West Local Government Areas. Makurdi serves as both the Local Government headquarters and the Benue State Capital. It has an area of 16 Km radius and comprises of two constituencies namely Makurdi North and Makurdi South. It consists of 11 council wards and is located on Latitude 7°41’N and Longitude 8°28’E [11]. Situated on both banks of River Benue, it has a total population of 297,393 inhabitants (2006 census). Makurdi is bordered on the West and North by Lafia, Keana and Domba Local Governments of Nassarawa State, on the East by Guma Local Government, and on the South by both Gwer and Gwer-West Local Government [11]. It experiences two distinct seasons: the wet or rainy season and the dry season, with an annual rainfall of 150mm. The dry season comes between November and March, within which cold (harmattan) and heat are experienced. Harmattan comes with cool and chilly weather between December to early February, while the heat period comes with hot weather and high temperature between February and April. The rainy season comes between May and early October [11]. Makurdi belongs to the Guinea Savannah belt of Nigeria and has farming and rearing of animals as the major occupation of its native inhabitants. A good number of the inhabitants are also fishermen, owning to the passage of River Benue (the second largest river in Nigeria) through Makurdi, the state capital.

Sample Collection
A total of thirty (30) samples were collected, out of which ten (10) were from two different Yoghurt products collected from different batches. Ten (10) Nono samples were collected from Fulani hawkers in North Bank, Wurukum, High Level, Wadata and Ankpa Ward. The other ten (10) samples consisting of five (5) Nono and five (5) fresh cow milk samples were collected from Fulani farmsteads in Tse-Atungu (North Bank) and Abinsi. The samples were maintained in an ice-packed container immediately after collection and transported to the Laboratory where analysis was carried out within 1-2 hours of collection.
Identification of Bacteria Isolates

In the laboratory, tenfold serial dilution was done for each sample using sterile distilled water. One milliliter (1ml) of sample was dispensed into 9ml of the distilled water in a sterile test tube using a sterile pipette, and mixed properly. One milliliter (1ml) of the mixture was then transferred into a second test tube already containing 9ml of the distilled water and mixed also, to obtain a 1:100 dilution. This was repeated further to obtain dilutions of 1:1000, 1:10000 and 1:100000 respectively. The pour plate method was used. One milliliter (1ml) of the serially diluted sample was aseptically transferred from the fourth and fifth dilutions into sterile petri dishes using sterile pipettes. Liquid agar medium cooled to about 45°C was then added to the petri dishes containing the sample, and swirled to allow for proper mixing. The plates were allowed to solidify and then incubated in an inverted position in an incubator. Bacteria cultures were incubated for 18-24 hours at 37°C, while fungal cultures were incubated at 35°C for at least 48-72 hours [17]. For further identification of bacteria isolates, each typical colony was further sub-cultured to obtain discrete colonies.

Sub-cultured colonies on Nutrient Agar were also Gram-stained [6]. A small portion of twenty-four hours (24 hours) old colony was emulsified in a drop of water on a clean glass slide and fixed by passing the smeared slide rapidly over flame. The smear was covered with crystal violet for about 30-60 Seconds, rinsed with water and flooded with iodine for 30-60 seconds. The smear was rinsed, rapidly decolorized with acetone-alcohol mixture and then counter-stained with Safaranine for about 30-60 seconds. The slide was dried and viewed using the oil immersion (X100) objective, under a light microscope for Gram positive and Gram negative organisms.

Motility test was also carried out [6]. A suspension of the test organism was prepared on a clean slide and covered with a cover slip. The edges were sealed with wax and the suspension was viewed using the X10 and X40 objectives. Bacteria motility was observed in the same direction for some, and different directions for others. The diaphragm was sufficiently closed to give good contrast.

Catalase and Coagulase tests were carried out on some of the isolates according to the methods described by [6]. A portion of colony from an 18-24 hours old Nutrient Agar culture was placed in a drop of hydrogen peroxide on a clean glass slide. An immediate, rapid bubbling was read as a positive reaction for the production of the enzyme catalase by the organism. The absence of bubbles was read as a negative result. To check for the presence or absence of coagulase, a suspension was made on a slide by emulsifying an 18-24 hours old colony portion of the test organism in a drop of distilled water on a clean glass slide. A drop of plasma was thereafter added to the suspension and mixed. Clumping of the organism within 10 seconds implied a positive result. The absence of clumping after 10 seconds indicated a negative result.

Identification of Fungal Isolates

Identification of fungal isolates was done using the method described by [7] and [10]. A small portion of the fungal culture was picked carefully, using a scalpel and pin. This was stained with Lactophenol Cotton Blue Stain and viewed under the low power and high-dry objectives. The hyphal structure, spore type, shape and arrangement were noted and applied in the identification of the isolates. Preliminary identification was done by macroscopic observation of the cultures, with regard to colour and appearance of colonies on the agar medium.

Colony Count

Colony count was performed on the various culture media used. Discrete colonies appearing on the plate after appropriate incubation were counted and recorded. The Total Viable Count, Enterobacteria Count and Fungal Count were obtained by counting discrete colonies on Nutrient Agar, MacConkey agar and Potato Dextrose Agar respectively. The number of colonies counted was multiplied by the reciprocal of the dilution factor plated, and divided by the volume of inoculum used, to obtain the Colony Forming Unit per milliliter (cfu/ml) of each sample.

Statistical Analysis

Statistical analysis was carried out using Genstat SP 18 package. The procedures used include summary statistics and X²-test.
RESULTS

A total of ten (10) bacteria species were isolated. The occurrence of bacteria species in nono, fresh cow milk and yoghurt samples sold in Makurdi metropolis, Benue State is represented in Table 1. Salmonella spp. was found in nono and yoghurt, but absent in fresh cow milk while Staphylococcus aureus was found in all three products. Pseudomonas spp. occurred only in nono, but was absent in fresh cow milk and yoghurt.

A total of eight (8) fungi, six (6) molds (Rhizopus spp., Candida spp., Fusarium spp., Penicillium spp., Aspergillus spp. and Alternaria spp.) and two (2) yeasts (Candida spp. and Saccharomyces spp.) were isolated. Alternaria spp., Fusarium spp., and Penicillium spp. had the least occurrence in all the three products analyzed (Table 2). The mould Rhizopus spp. was present in all the three products analyzed. The yeast, Candida spp. Fusarium spp. and Penicillium spp. were both absent in nono.

The mean microbial count is presented in Table 3 as the average of five (5) samples each. Nono samples from hawkers had the highest Mean Total Viable Count, Enterobacteria and Fungal Counts (1.63x10^7 cfu/ml, 7.45x10^6 cfu/ml and 9.20x10^6 cfu/ml respectively). The lowest Mean Total Viable and Enterobacteria Counts of 2.60 × 10^6 cfu/ml and 1.26 × 10^6 cfu/ml respectively were recorded from fresh cow milk samples obtained from the farmsteads, while yoghurt samples recorded the lowest Mean Fungal Count of 1.07x10^6cfu/ml. The occurrence of bacteria isolates in the sampled products is presented in Figure 1. Lactobacillus spp. had the highest occurrence of 83.3% while Pseudomonas spp. and Proteus spp. had the lowest occurrence of 3.3%.

The occurrence of fungal isolates in all the three products is presented in Figure 2. Saccharomyces spp. had the highest occurrence of 63.6% while Penicillium spp. had the lowest occurrence (6.3%). Significant differences (p<0.05) in the level of contamination of the three products were established.

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>NONO</th>
<th>FRESH COW MILK</th>
<th>YOGHURT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus spp</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lactobacillus spp</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Pseudomonas spp</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Proteus spp</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
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<tr>
<td>Streptococcus spp</td>
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<td>+</td>
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</tr>
</tbody>
</table>

+ = Present  - = Absent

<table>
<thead>
<tr>
<th>FUNGI</th>
<th>NONO</th>
<th>FRESH COW MILK</th>
<th>YOGHURT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus spp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alternaria spp</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Candida spp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium spp</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mucor spp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhizopus spp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saccharomyces spp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present  - = Absent
Figure 1: Occurrence of Bacteria isolates in nono, Fresh cow milk and yoghurt sold in Makurdi, Benue State
Figure 2: Occurrence of Fungal isolates in nono, Fresh cow milk and yoghurt sold in Makurdi, Benue State
Findings revealed that the dairy products analyzed were all contaminated with microorganisms. This can be seen from the high microbial counts obtained in the products. Nono samples collected from hawkers had very high average total viable, enterobacteria and fungi counts as compared to those collected from the farmsteads. This may be due to the continuous exposure of the hawked product to contamination from dust and the utensils used by the hawkers. Counts slightly higher than those obtained in this study have been documented [4]. They reported mean bacteria, enterobacteria and fungi counts of 3.53×10^7 cfu/ml, 1.7×10^6 cfu/ml and 4.60×10^5 cfu/ml respectively for hawked nono. The high counts obtained in hawked nono are indicative of poor production and handling hygiene [15]. Contamination could have been through the animal’s udder or skin, the immediate environment, the milking process, milk handler and handling equipment [1]. The presence of Staphylococcus spp. and Escherichia coli could be as a result of mastitis in the cows milked [1]. Poor hygienic practices such as using unwashed hands, containers and utensils for milking and sales of the product, as well as exposure of the product to flies and the environment also might have contributed a lot to its contamination. Also, since larger populations of the fulanis who produce and sell nono in Makurdi metropolis live on the bank of the River Benue, water obtained directly from the river might be a major source of water used for processing of the products and washing of nono processing containers. Milking of the cows is also done with bare hands where the cows are kept and this can easily facilitate interaction between the product, sand, faecal matter, contaminated air and animal feed stuff. It has also been suggested that milk’s high water activity, moderate pH (6.4-6.6) and ample supply of nutrients makes it an excellent medium for microbial growth. Thus, the microbes grow rapidly and increase in number to the detriment of its quality and safety [1]. This explains the low counts of bacteria and fungi in fresh cow milk, as compared with those in nono, where the organisms have had enough time to grow and multiply.

The counts obtained from commercial yoghurt were lower than those for nono, but are high enough to raise health concerns. This is indicative of poor handling during processing and packaging of the product. A total viable count ranging between 7.79-7.92 Log cfu/g in an earlier study on commercial yoghurt [5] have been reported; whereas mean viable counts ranging from 1.4×10^7 to 2.2×10^7 cfu/ml was reported in yoghurt sold in Enugu State, Nigeria [13]. These results agree with the range obtained for yoghurt in this study.

The high occurrence of Lactobacillus spp. (83.3%) and Streptococcus spp. (53.3%) in these dairy products is not surprising since they are involved in the fermentation of milk [3] [13]. Escherichia coli (30.0%) is an indication of possible faecal contamination. The presence of Salmonella spp. could be attributed to asymptomatic carriers in the production and packaging process [8]. These bacteria have also been isolated in other studies [14] [2]. Some of the bacteria isolates are normal flora of milk products and animal skin, while others are spoilage and pathogenic species. Pseudomonas spp., Lactobacillus spp., Bacillus spp. and Proteus spp. have all been implicated in the spoilage of milk and milk products [18]. Bacillus spp. is known to be pathogenic and resistant to environmental stress due to its sporing nature, and can cause an emetic syndrome and food-borne intoxication that leads to diarrhea [?]. Its high frequency of occurrence (46.7%) is therefore hazardous to health. It could have come from the soil, water or the gut flora of humans [1]. E. coli, also isolated from this study is a normal flora of the gut and can survive in water. It is of public health importance since it can cause diseases such as urinary tract infections, neonatal meningitis and gastroenteritis [5]. The presence of Salmonella spp. (23.3%) in the products is of importance to public health because it causes gastroenteritis, leading to diarrhoea and enteric fever (typhoid fever) in humans. Its source could have been from faecal contaminated materials by asymptomatic carriers in the course of processing and packaging.

**Table 3: Mean microbial count as an average of five samples of each product (nono, fresh cow milk and yoghurt)**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>MEAN TVC cfu/ml</th>
<th>MEAN EBC cfu/ml</th>
<th>MEAN FC cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt</td>
<td>1.42×10⁷</td>
<td>7.25×10⁶</td>
<td>1.07×10⁶</td>
</tr>
<tr>
<td>Nono from hawkers</td>
<td>1.63×10⁷</td>
<td>7.45×10⁶</td>
<td>9.20×10⁶</td>
</tr>
<tr>
<td>Fresh Cow Milk</td>
<td>2.60×10⁶</td>
<td>1.26×10⁶</td>
<td>1.14×10⁶</td>
</tr>
<tr>
<td>Nono from farmsteads</td>
<td>1.26×10⁷</td>
<td>4.52×10⁶</td>
<td>8.02×10⁶</td>
</tr>
</tbody>
</table>

**TVC = Total Viable Count  EBC = Enterobacteria Count  FC = Fungal Count**

**DISCUSSION**

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of the products. Its presence in milk and dairy products has been mentioned [17] [1]. *Staphylococcus aureus* has its reservoir in man and is a pointer to unhygienic handling of these products. *Staphylococcus aureus* however, has been reported to be pathogenic, causing food-borne intoxication. Its high percentage (50.0%) occurrence therefore poses a great health risk. However, *Proteus* spp cause disease only in host with impaired resistance. The source of *Klebsiella* spp. could have been partly through air (talking, sneezing, laughing or singing) since it inhabits the upper respiratory tract of man. The health implications of the presence of *E. coli* and *Klebsiella* spp. in dairy products have been reported [5].

The fungal count obtained in this study is slightly higher than the range of 1.0 x 10^4 to 2.9 x 10^5 cfu/ml reported in an earlier study [2]. Fungal isolates are known as spore formers that can easily contaminate dairy products exposed during processing, storage and hawking. They are major spoilage organisms of carbohydrate foods [19]. However, their growth can result in the production and accumulation of mycotoxins which are of public health and economic importance [19]. Among the fungal isolates, *Saccharomyces* spp. occurred most (63.3%), followed by *Mucor* spp. (50.0%) and *Candida* spp. (43.3%). *Saccharomyces* spp is majorly a fermentative organism (yeast) and is not known to be pathogenic [19] [4] [5]. *Saccharomyces* spp had earlier been isolated in a research work on nono and fura de nono which is also a local milk drink in Nigeria [2]. Significant differences (p<0.05) in the level of contamination of the analyzed three products were established.

**CONCLUSION**

The result of this study reveals that the dairy products analyzed were all contaminated with microorganisms showing to the fact that appropriate microbiological standards might have not been applied in the processing, packaging and retailing of the analyzed yoghurt, nono and fresh cow milk samples, though the latter is less contaminated. The studied products are therefore dangerous for direct human consumption unless appropriate measures are taken to reduce microbial contamination of the products, since the dairy products have very high nutritional value.

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**REFERENCES**


