MICROBIAL CONTAMINANTS ASSOCIATED WITH FERMENTED MILK "NUNU" SOLD IN MAKURDI METROPOLIS, BENUE STATE OF NIGERIA

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ABSTRACT

Nunu is a fermented milk product consumed as a drink in parts of West Africa.To assess the microbial contamination of "nunu",a total of sixty(60) samples of 'nunu' was collected from five different market locations in Makurdi metropolis. This included samples from both sources of production and vendors. Samples were cultured on nutrient agar, MacConkey agar, Mannitol salt agar and Sabouraud dextrose agar. The microorganism isolated includes Escherichia coli, Salmonella spp, Proteus spp, Klebsiella spp, Candida spp, Saccharomyces spp, Trichosporum spp, Cryptococcus spp, Fusarium spp, Alternaria spp, Moraxella spp, Bacillus spp, and Mucor Spp. e.t.c. From the results 'nunu' collected from the source of production had higher mean microbial total viable count, enterobacterial and fungal counts of 2.58×10^6cfu/ml, 1.56×10^6 cfu/ml and 2.08×10^6 cfu/ml respectively as compared to samples collected from vendors which had mean microbial total viable counts of 1.13×10^6cfu/ml enterobacterial count of 1.22×10^6cfu/ml and fungal count of 1.53×10^6cfu/ml. The sources of contamination could be from the producing animal(s), the milker as well as the water used for 'nunu' processing. Efforts should be intensified on improving the sanitary condition of this product as this could lead to fatal health hazards.

Key words: fermented milk, "nunu", mannitol salt agar, macConkey agar.

INTRODUCTION

Milk is often described as a complete food because it contains proteins in the form of casein and whey, carbohydrate in the form of lactose, fat in the form of butter fat, vitamins and mineral (Gaman and Sherington, 1990). Milk is an essential first food for man, for countless of generations, it has formed an important part of man's diet not only for the infant, but in many societies throughout life. (Komorowski and Early, 1992). Milk drawn aseptically from the healthy udder is not sterile, but often contains low numbers of mirrorganisms known as "udder comensals". These microorganisms are predominantly micrococci and streptococci, although corynebacterium bacteria are also faiply common (Ozer, 1999). Local milk production in Nigeria is mainly carried out by the Fulanis who lives mainly in the Northen part of the country. The men do the milking of the cows and distribute the raw milk to women in the farmstead, who then process it into various products like cheese(maishanu)," fura de nunu" and fermented milk "nunu" itself (Belewu and Aina , 2000). Milk and milk product provide a favourable environment for microbial growth and thus gets contaminated easily. A dirty environment harbours flies which can contaminate milk with soil microorganism that has previously been contaminated with faecal materials thus serving as a source of enteric

pathogenic bacteria (Norman and Gravani, 2006). Pathogenic organisms cause diseases. Some of which are very serious. George and Pamplona-Roger, (2006), listed out the microorganisms associated with milk and the diseases they cause as follows.

Some Diseases causes by Microorganisms in Milk

Microorganism	Disease
Mycobacterium bovis	Human and borialtuberculosis
Brucella abortus	Brucellosis
Salmonella typhi	Typhoid fever
Escherichia coli	Colitis
Corynebacterium diphtheria	Diphtheria
Streptococcus pyogenes	Scarlet fever
Vibrio cholerae	Cholera
Campylobacter Jejuni	Gastroenteritis gastroduodenal ulcer
Listeria monocytogenes	Listeriosis

George and Pamplona – Roger (2006)

MATERIALS AND METHODS

This research study covered North Bank I, Mission. Ankpa-wadata, Modern Market, and Walaimayo council wards political areas all situated in the heart of Makurdi town.

Media and Reagents: Nutrient agar, MacConkey agar, peptone water, safranin, normal saline, Lugol's iodine and hydrogen peroxide (H_2O_2).

Glass wares: Beakers, conical flasks, test tubes, Petri-dishes, pipettes and Bijou bottles were thoroughly washed with detergent and rinsed with distilled water, allowed to drain off and air-dry. Thereafter, they were sterilized in a hot air over at temperature of 100^0C for 1 hour.

Sample Collection: A total of 60 samples, twelve from each of North Bank I, Mission, Ankpa-wadata, Modern market and Walaimayo areas within Makurdi metropolis was collected from the Fulani women between the hours of 8:30-9.30am and transported in iced bags to the laboratory.

Prepared Media Used: Peptone water, nutrient agar, mannitol salt agar, MacConkey agar were prepared according to methods outlined by (Cheesbrough, 2000) while sabouraud dextrose agar was prepared according to manufactures instructions.

Peptone Water: This was used as diluents. 35.5g of 0.1% peptone water was weighed and poured into a 500ml of distilled water in conical flask and swayed thoroughly to dissolve. The conical flask was covered with an aluminum foil and left at room temperature on the table.

Nutrient Agar: Fourteen gram (14g) of nutrient agar was weighed and poured into 500ml distilled water in a conical flask and allowed to soak for 15 minutes. It was swirled to obtain a homogenous mixture and thereafter sealed with an aluminum foil and left on the table at room temperature.

MacConkey Agar: This media was used for isolation of *Escherichia Coli, Salmonella spp, Proteus* and *Yersinia.* 26g MacConkey agar was weighed and poured into 500ml of distilled water in a conical flask and allowed to soak for 15minutes. Thereafter it was swirled to obtain a uniform mixture and later covered with an aluminum foil and allowed on the table at room temperature.

Mannitol Salt Agar: This was used to support the growth of *Staphylococci, Escherichia Coli* and other Gram negative organisms. 555g of mannitol salt agar was weighed and poured into 500ml of distilled water in a conical flask and allowed to soak for 15minutes. This was later swirled to obtain a homogenous mixture and covered with an aluminum foil and left on the temperature at room temperature.

Sabouraud Dextrose Agar: This medium supports the growth of fungi. 15g of sabouraud dextrose agar (SDA) was weighed and poured into 500ml of distilled water in a conical flask and allowed to soak for 15 minutes. It was swirled to obtain a homogenous mixture and covered with an aluminum foil and left on the table at room temperature.

Inoculation of the Media: Nutrient agar, mannitol salt agar, and MacConkey agar were cooled at 50^0C while sabouraud dextrose agar was cooled at 55^0C. 9ml each of peptone water (diluent) was poured into a test tubes and 1ml each of the "nunu" samples was transferred in the prepared peptone water which was a stock prepared as diluent.

Serial dilution of 10^{-1} of the diluents was used from which subsequent dilutions of up to 10^{-4} were made (four folds dilutions). The aerobic bacterial count was carried out using 0.1ml of appropriate serially diluted sample in nutrient agar during pouring of plates. For the staphylococci counting, mannitol salt agar during pouring of plates. The same was used on sabouraud dextrose agar during pouring of plates for fungi count. For counting of coliforms, the same 0.1ml was used on MacConkey agar during pouring of plates. The media were poured separately into their respective Petri-dishes that have been sterilized at 160^0c for 30 minutes in the hot air oven. The inoculum was swirled during and after each pour to ensure homogenous mixture of the diluent. The Petri-dishes used for total bacterial and fungi count were incubated at room temperature of 25^0c for 48 hours to enable colonies formation, while those for staphylococci and coliforms count were incubated at 36^0c for 24 hours in an

incubator for development of colonies. Colonies of aerobic bacteria, fungi, *Staphylococci* and Escherichia coli of the " nunu" samples were thus analyzed.

Gram Stain procedure: A thin smear of a colony of the test organism was made on a cleaned glass slide. It was allowed to air-dry and then heat-fixed by passing glass slide through the flame for three times. The fixed smear was then covered with 3 drops of crystal violet for 60 seconds and rinsed with clean water; Lugol's iodine was added for 60 seconds and rinsed with clean water. It was decolorized with 95% acetone and rinsed immediately with clean water, the smear was covered with safranin for 30 second before it was rinsed. The back of the slide was wiped clean and kept in draining rack to air-dry. It was viewed with oil immersion under the microscope by the use of 100x objective lens. **Biochemical Tests** Methods according to Monica Cheesborough (2000) was adopted in carrying out these tests.

Coagulase test: Two drops of physiological saline, was placed on a cleaned glass slide 2cm apart. One colony was gently mixed in each drop of saline suspension one on each side and mixed. The slide was held up and tilted back and forth for 60 seconds, clumping of cells showed that it was coagulase positive organisms. Coagulase positive are highly pathogenic than coagulase negative organisms.

Catalase Test: This test was done to differentiate between *Staphylococci* which are positive from *streptococci spp* which are negative in a culture. The test indicates the ability of the test organism to produce catalase in the presence of hydrogen peroxide. Three (3) drops of hydrogen peroxide were placed in a cleaned slide and a loopful of the test organism grown on the culture was examined after a few minutes. Presence of bubbling and frothing indicated a positive test while absence of bubbles and froths indicated a negative test

RESULTS

The occurrence of bacterial and fungal species isolated from "nunu" samples is presented in Table 1, from the results, *Lactobacillus spp, Bacillus spp* and *Mucor spp* were isolated from all samples collected from the different locations, thus recording the highest frequency of occurrence, while Moraxella spp, Aspergillus spp, Fusarium spp and Alternaria spp had the least frequency of occurrence. Values of the mean count is presented in Table 2, samples collected from North bank 1 area recordeed the highest total viable count of 4.58×10^6cfu/ml, while samples collected from source of production in modern market area had the least total viable count of 3.79×10^6cfu/ml. The samples collected from source of production in walaimayo area recorded the highest enterobacterial count of 2.93×10^6cfu/ml while the least enterobacterial count of 1.39×10^6cfu/ml was obtained from samples collected from vendors in modern market area. The mean microbial count of samples collected from sources of production recorded the highest total viable count of 2.58×10^6cfu/ml while fungal count of 2.08×10^6cfu/ml were recorded as shown in Table 3. The samples from vendors had relatively low mean counts when compared to those from sources of production.

Table 1: Occurrence of bacterial and fungal species in samples from the various locations.

Organism	**Location**				
Organism	North bank 1 Mission		Wailamayo	Ankpa-Wadata	Modern Market
Escherichia Coli	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	-
Lactobacillus spp	+	+	+	+	+
Streptococcus spp	+	+	+	+	-
Salmonella spp	+	+	+	+	+
Bacillus spp	+	+	+	+	+
Streptococcus pnuemoniae	_	_	+	_	_
Enterococcus spp	_	_	+	+	+
Kliebsiella spp	_	_	+	_	_
Moraxella spp	_	_	_	_	+
Proteus spp	_	_	+	_	_
Pseudomonas spp	_	_	_	+	_
Rhizopus spp	++	++	++	_ _	++
Candida spp	++	++	++	_ _	_ _
Mucor spp	++	++	++	++	++
Saccharomyces spp	++	++	_ _	++	++
Trichosporum spp	_ _	++	_ _	++	_ _
Aspergillus spp	_ _	_ _	++	_ _	_ _
Cryptococcus spp	_ _	_ _	++	_ _	++
Fusarium spp	_ _	_ _	++	_ _	_ _
Alternaria spp	_ _	_ _	++	_ _	_ _

KEY

+ = Bacterial Organisms Present

- = Bacterial Organism absent

++ = Fungal organisms present

- - = Fungal Organism absent

Table 2: Mean Count for nunu samples collected from the different locations.

Ward	Point of Collection	Mean TVC Cfu/ml	Mean EBC Cfu/ml	Mean FC cfu/ml
North Bank1	Source	4.58×10^6	2.20×10^6	3.89×10^6
	Vendors	2.35×10^6	1.47×10^6	2.69×10^6
Mission	Source	4.43×10^6	2.44×10^6	3.98×10^6
	Vendors	2.23×10^6	1.66×10^6	2.75×10^6
Walaimayo	Source	34×10^6	2.93×10^6	3.76×10^6
	Vendors	2.52×10^6	35×10^6	2.74×10^6
Ankpa- Wadata	Source	36×10^6	2.78×10^6	3.85×10^6
	Vendors	2.25×10^6	2.02×10^6	2.78×10^6
Modern –Market	Source	3.79×10^6	2.61×10^6	1.83×10^6
	Vendors	1.85×10^6	1.39×10^6	1.61×10^6

Key

Cfu/ml = Colony Forming Unit per Milliliter
EBC = Enterobacterial Count
FC = Fungal Count
TVC = Total Viable Count

TABLE 3: Mean microbial count as an average of the two types of nunu samples collected from the different locations.

Sample	Mean TVC cfu/ml	Mean EBC cfu/ml	Mean FC cfu/ml
Sources	2.58×10^6	1.56×10^6	2.08×10^6
Vendors	1.13×10^6	1.22×10^6	1.53×10^6

KEY:

Cfu/ml= Colony forming unit per Mililiter
EBC = Enterobacterial Count
FC = Fungal Count
TVC = Total viable Count

DISCUSSION

A 2001 research by a group of micro biologists who conducted experiments on the beverage also lends credence to the caution being taken by some consumers as to the hygienic condition of its preparation. It found that the, "poor handling of fura da nono during processing and marketing exposes it to microbial contamination, thereby making it a "source of microbial food poisoning". Houseflies are always found in large numbers at the production

sites and at sale outlets." The health journal quoted another researcher as saying that female hawkers, prior to sale, in order to increase volume and improve colour of nunu, "engage in the fraudulent act of adding stream water and milky white supernatant watersoaked baobab tree seeds. "The local Fulanis who are the major producers should be educated on sanitary practices during milking of cows and further processing. The use of portable water, where available, should also be encouraged. Raw milk drawn from a healthy udder normally will contain only a few hundred to a few thousands of bacteria per milliliter, mostly from the genus *Micrococcus*. From the results, nunu samples collected from the source of production recorded a high total viable count of 4. 8×10^6cfu/ml as compared to samples collected from vendors that recorded 2.44×10^6cfu/ml thus reflecting that nunu samples from the source of production are more contaminated than samples collected from the vendors. An observation from the current study shows that most of the producers do not employ good milking practice to minimize contamination of the product. For instance it was observed that they did not wash their hands before milking, containers like buckets were not properly washed. Lack of potable water was a major challenge and thus a major factor leading to contamination. Many producers also did not clean the udder of the cows before milking. According to Galton *et al*., 1989, pre milking udder preparation plays an important role in milk contamination, this have accounted for the isolation of a variety of organisms in this study.

The microbiological assessment of nunu samples revealed the dominance of *Lactobacillus spp, Bacillus spp* and *Mucor spp* from all samplescollected from the different locations under study. *Lactobacilli* counts in nunu samples conforms with reports of Obodai and Dodd, 2005 who reported counts of 8 and 10logcfu/ml in a fermented milk product in Ghana. This high numbers of Lactobacillus spp , coupled with the high acidity for the sourness in taste of the final product. It is reported that fermented milk in regions with low temperatures supports the growth of mesophillic bacteria such as Lactococcus and Leuconostoc spp, while areas having high temperatures (like Makurdi, the study area) favors the growth of thermophillic bacteria like Lactobacillus and Streptococcus. (Savadogo *et al.*, 2004). This might probably account for the reason why thermophillic bacteria were frequently isolated in this study. The presence of *Staphylococcus* spp and *Escherichia coli* could be an indication of mastitis infection in the cows (Adams and Moss, 1999). The presence of coliform bacteria like *Enterococcus* and *Escherichia coli* is indicative of faecal contamination which may have originated from the stream where inhabitants defaecate by the bank.Bacillus spp was isolated fom nunu samples, this organism is pathogenic and can resist environmental stress during its spore form causing emetic syndrome and food borne intoxication leading to diarrhea and subsequently dehydration as suggested by Adams and Moss, 1995. A number of fungal organisms were isolated from this study, however their growth can result from the production and accumulation of mycotoxins posing a public health concern. (Uzeh *et al. ,* 2006).

CONCLUSION

This study has established microbial contamination of nunu sold in Makurdi metropolis. Therefore, concerted efforts should be geared towards improving the sanitary condition of this product to prevent the occurrence of the incident in Zamfara in 2008, as reported by the National Mirror 2011 in which seven family members died after drinking the beverage.

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