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Microbiological Quality of Dairy Cattle Products

Okeke Kingsley Susan[1*], Abdullahi Isa Obansa[2] and Makun Hussaini Anthony[3*]

[1]Department of Nutrition and Dietetics, Federal Polytechnic Bida, Niger State, Nigeria.
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OKS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AIO and MHA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the chemical properties (pH, titratable acidity) and microbiological qualities of fresh cow milk and traditional cultured skimmed (defatted) milk (*nono*) and full fat or partially skimmed cultured milk (*kindirmo)* in Bida local government area of Niger State, Nigeria.
Study Design: To assess the microbial load of dairy cattle products.
Place and Duration of Study: Samples were collected from local farmers in Madobia and Project quarters in Bida Local Government, Nigeria. Analyze at laboratories of Microbiology Department of Federal University of Technology, Minna and Federal Polytechnic, Bida between September 2011 and December 2012.
Methodology: Ninety samples of fresh milk, *nono* and *kindirmo* obtained from two areas in Bida Local Government Area were analyzed to determine their pH, titratable acidity, microbial properties (Total viable count, Fungal count, Staphylococcal count, Coliform count) and antibiogram of pathogenic organisms isolated from the samples. Results obtained were subjected to statistical analysis.
Results: The results obtained showed that the pH of *nono* was more acidic than other milk products. The total viable count ranged log106.02-6.36cfu/ml, coliform count $\log_{10}$ 6.02-6.57cfu/ml, staphylococcal count $\log_{10}$ 6.10-6.57cfu/ml; fungal count $\log_{10}$ 4.49-5.10cfu/ml respectively. The microorganism isolated included *Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Aspergillus flavus* and *Aspergillus niger*.

**Corresponding author: Email: tochimum@yahoo.com; susanokeke@gmail.com;*

Notably *S. aureus* and *A. flavus* were frequently isolated (60.1% and 44% respectively). The antibiogram of pathogenic organisms isolated from the dairy cattle products showed that *E. faecalis and S. aureus* were sensitive to gentamicin (10µg) and streptomycin (30µg).

Conclusion: The growth of these pathogenic organisms in local dairy cattle products is a reflection of poor sanitary practices in the production of fresh milk and its products. This high microbial load in cow milk and its product may pose a great public health concern and therefore calls for public awareness campaign.

Keywords: Microbial assessment; chemical properties; Kindirmo; Nono; Nigeria.

1. INTRODUCTION

Dairy cattle products are traditionally staple food commodities for the nomadic population of Northern Nigeria and many other part of Africa. These products are important part of the national economy and serve as major source of family income and greater potential in improving public health. Cow milk is utilized in the production of at least 400 different fermented products all over the world [1]. *Kindirmo* and *Nono* are fermented milk products mostly consumed by the Hausas (Fulanis) in northern Nigeria. *Nono* is a crude cultured whole milk whose fermentation may be brought about by a number of bacterial species from various sources that contaminate the fresh milk while *kindirmo* is a full fat or partially skimmed cultured milk. According to previous report [2], fermentation of milk during *nono* production reduces pH from 6.5 to 3.8 due to the production of organic acid.

In Nigeria about 90% of the dairy cattle belong to the Fulani agro-pasteuralist and their women strictly control the processing and marketing of their milk [3]. Most of them are not literate hence do not monitor the safety of milk and its products. The poor handling of dairy cattle products during processing and marketing exposes it to microbial contamination. The lapses in hygienic practices could result in milk borne disease such as tuberculosis, diphtheria, listeriosis, brucellosis, and staphylococcal food poisoning [4], especially among urban residents who drink fresh milk sold by the Fulani women. Animal milk are the main sources of nutrition for infants whose vulnerability due to undeveloped immune system is obvious therefore contaminated cow milk products pose serious health concern as such they can no longer be ignored as they are among the main entry routes of microbial contamination into the human dietary system in Africa [5].

This research was therefore conducted to investigate the microbiological qualities of fresh cow milk and traditional cultured milk (*nono* and *kindirmo*) in Bida local government area of Niger State, Nigeria

2. MATERIALS AND METHODS

2.1 Production of *Nono* and *kindirmo*

Kindirmo was produced by boiling fresh milk for about 20min.This was allowed to cool and ferment over night by spontaneous fermentation in a local calabash. After fermentation well water which usually is their source of water was added to the product to dilute it and also maximize profit. However, *nono* is produced by defatting fresh milk for 30-45min and then left to ferment over night by a number of bacterial species from various sources that

contaminate it. Also, some left over *nono* (back slopping) from previous sale was added to aid fermentation process.

2.2 Sampling

Ninety samples of fresh milk and its products (*nono* and *kindirmo*) produced by two local dairy farmers were collected from Bida and its environs in sterile sampling bottles and immediately transported in ice packed box within temperature range of 4-6ºC to the laboratory of Microbiology Department of Federal University of Technology, Minna , Nigeria for analysis.

2.3 Chemical Analysis

2.3.1 pH

The pH of the samples was determined using a pH meter. The electrode of the pH meter was standardized by dipping it into sterile water after which two different buffers (4.0 and 7.0) were used. The set electrode was then used for the various samples and readings were recorded [6].

2.3.2 Titratable acidity (TTA)

Thirty millilitres of each sample: (fresh milk, *nono* and *kindirmo*) were boiled on hot plate to remove carbon dioxide. These were allowed to cool and the initial volume was restored by adding sterile distilled water. Aliquot (10ml) of diluted samples were transferred into a conical flask and a drop of phenolphthalein indicator was added and titrated with 0.05M NaOH until a pink colour appeared [6]. The titratable acidity was then calculated.

2.4 Microbiological Analysis

2.4.1 Serial dilution

Twenty five (25) millilitres out of each sample (raw milk, *nono*, *kindirmo*) were aseptically transferred by means of sterile pipette into 225ml of sterile diluents (0.1% peptone water). Serial dilutions were prepared up to 10^{-5} which was used for fungal count 10^{-4} and for total bacterial count 10^{-5} as described subsequently.

2.4.2 Isolation of microbes associated with fresh milk and its products

One millilitre of the diluted samples was pour plated in triplicate plates on Nutrient agar for total bacterial count (TBC), Oxoid Mac Conkey agar No 3 (CM115) was used for coliform counts which presented colonies with an intense violet red colour. Pathogenic Staphylococcus was enumerated using Mannitol Salt agar followed by biochemical test (coagulase test). Potato dextrose agar (PDA) was used for the enumeration of fungal count. 4mg of chloramphenicol per 100ml of medium prior to autoclaving was incorporated into the PDA to prevent bacterial growth [7-9]. All plates were incubated for 48hrs at 37ºC. The colonies differing in size, shape and colour were selected from the plates and sub-cultured repeatedly on nutrient agar for bacterial and PDA for fungi to obtain pure isolates. The pure isolates were maintained on the corresponding agar slants for further characterization and identification [7,8].

2.4.3 Identification of the microbial isolates

Identification of isolated microbes was carried out using growth on diagnostic media, microscopic appearance, morphological and biochemical test as described previously [9].The microbiological tests were Gram stain, motility, presence of spores and cell shape while biochemical tests included catalase test, coagulase test, methyl red test, Voges Proskauer test, gelatin hydrolysis test, urease test, nitrate reduction test, citrate utilization test, hydrogen sulphide production test, Indole test and fermentation of sugars (glucose, sucrose, manitol, fructose, lactose and maltose). The isolates were identified by comparing their characteristics with known taxa as described [10]. A wet mount of the fungal isolates was done using lactophenol cotton blue and observed under the microscopes. Following the examination of characteristics as well as the back view of the plate culture, the molds where identified as described by Harrigan & McCance [9].

2.5 Antibiogram of Milk and Its Products

The antibiogram was done by adopting Kirby-Bauer disk sensitivity method [11,12]. The adjusting density of microbial suspension according to the MacFarland standard No 0, 5 was employed in standardizing the test organisms. Four morphologically similar colonies were transferred aseptically from an agar plate culture into a tube containing 5ml of nutrient broth. The broth was mixed and incubated at 37ºC for 18hrs. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain turbidity that was optically comparable to that of the 0.5 MacFarland standards. This resulted in a suspension containing approximately 1.0 to 2.0×10^8cfu/ml [11]. A sterile swab was used to collect some standardized test organism and streaked on Mueller Hinton agar plates. The antibiotic disc with known disc potency was impregnated on Mueller Hinton agar surface using sterile forceps and the plates were incubated in inverted manner at 35ºC for 18 hrs. A clear zone of inhibition indicated the sensitivity of isolates to the antibiotic. The diameter of the zone of inhibition was measured and compared with the report of Clinical Laboratory Standard Institute guidelines [11,12].

2.6 Statistical Analysis

The results obtained were subjected to analysis of variance using one- ANOVA. Analysis of Variance (ANOVA) was carried out for the pH, titratable acidity and microbial counts, The mean scores were computed and significant differences among the mean was determined using 2006 Statistical Packages for Social Sciences (SPSS) For Windows version 15.0 [13].

3. RESULTS

3.1 Chemical Properties of Dairy Cattle Milk Products

The results shown in Table 1 revealed that the mean pH values of the three milk products collected from the two dairy producers ranged from 3.7-6.5. The mean titratable acidity of the fresh milk, *nono* and *kindirmo* products was 0.1, 0.9 and 0.9 respectively.

3.2 Microbiological Quality of Milk and Milk Products

The total viable count of (fresh milk, *nono* and *kindirmo*) collected from the two sites ranged from 6.0-6.4 $\log_{10}$ cfu/ml (Table 2). The coliform count ($\log_{10}$ cfu/ml) ranged from 6.1-6.4.

There were no significant differences (p.05) of the microbial load between the different milk. Again, there were no significant differences (p>.05) amongst the means of the dairy products in either of the two settlement (Table 2). Table 2 shows the staphylococcal count (log_{10}cfu/ml) of milk products ranged from 6.1-6.6. There were no significant differences (p>.05) amongst their means. The fungal counts (log_{10} cfu/ml) in Table 2 ranged from 4.5-5.1 but these did not differ significantly (p>.05). The microorganisms isolated from the three dairy products are shown in Tables 3. The identified bacteria included *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis*, *and Lactobacillus delbrueckii subsp, bulgaricus.*

The results in Table 4 revealed the probable fungi isolates including *Aspergillus niger, Aspergillus flavus, Fusarium spp, Penicillum spp, Rhizopus spp* and *Mucor spp*.

3.3 Antibiogram of Pathogenic Bacterial Isolates

The antibacterial sensitivity test of pathogenic bacteria isolates *Streptococcus faecalis,* and *S. aureus* are shown in Table 5. These organisms were all sensitive to streptomycin (30µg) and gentamicin (10µg). *Staphylococcus aureus* was resistance to (µg) Ampiclox (30), Zinnacef (20), Rocephin (25), Ciprofloxacin (10), Cotrimoxazole (30) and Pefloxacin (30).*Staphylococcus aureus* and *Enterococcus faecalis* were both resistance to (µg) Ampiclox (30), Zinnacef (20), Amoxacillin (30), Septrin (30) and Pefloxacin (30).

Table 1. Chemical properties of dairy cattle products [1,2]

Milk products	pH	Titratable acidity
Fresh milk	6.49±0.06[a]	0.13±0.02[b]
Nono	3.74±0.11[c]	0.91±0.85[a]
Kindirmo	4.14±0.04[b]	0.87±0.12[a]

[1]Each value is the mean ± S.D of 3 determinants
[2]The different letters within column shows significant differences at (p<.05)

Table 2. Microbial count of in dairy cattle milk products

Milk products/ Number of microorganisms (log10 of cfu/ml)			
Sample site/Analysis	**Freshmilk**	**Nono**	**Kindirmo**
1. Madobia			
Total viable count	6.02±5.94[a]	6.36±5.97[a]	6.34±6.01[a]
Coliform count	6.57±6.39[a]	6.22±6.14[a]	6.02±6.05[a]
Stapphylococcal count	6.40±6.10[a]	6.57±6.21[a]	6.23±5.67[a]
Fungi count	4.76±4.81[a]	5.10±5.15[a]	4.57±4.95[a]
2. Project Quarters			
Total bacterial count	6.10±5.80[a]	6.36±6.10[a]	6.19±6.12[a]
Coliform count	6.44±6.37[a]	6.13±5.87[a]	6.16±6.09[a]
Stapphylococcal count	6.30±5.99[a]	6.57±6.06[a]	6.14±5.73[a]
Fungi count	4.59±4.56[a]	4.49±4.41[a]	4.49±4.41[a]

[1]Each value is the mean ± S.D of 3 determinants
[2]The different letters within row shows significant differences at (P>.05)

Table 3. The frequency of occurrence of bacterial isolates from milk products

Isolates	Madobia	(%)	Project quarters	(%)
Staphylococcus aureus	10	22.3	17	37.8
Bacillus subtilis	5	11.1	3	6.7
Enterococcus faecalis	4	8.9	3	6.7
Lactobacillus delbrueckii subsp. bulgaricus	15	33.3	10	22.2
Micrococcus spp.	5	11.1	4	8.9
Streptococcus lactis	6	13.3	4	8
Total isolates	45		41	

Table 4. The frequency of occurrence of fungal isolates from milk products

Isolates	Madobia	%	Project quarters	%
Fusarium spp.	8	17.8	10	22.3
Aspergillus flavus	10	22.3	10	22.3
Mucor spp.	4	8.9	8	17.8
Penicillium spp.	3	6.7	5	11.1
Rhizopus spp.	5	11.1	6	13.3
Aspergillus niger	13	28.9	6	13.3
Total isolates	43		45	

Table 5. Antibiogram of pathogenic bacteria isolated from milk products

Antibiotics/Disc potency (µg)	IS	*E. facealis*		*S. aureus*	
		ZH(mm)	Sensitivity	ZH(mm)	Sensitivity
Ampiclox (30)	≥15	16	R	0	R
Zinnacef (20)	≥20	0	R	0	R
Amoxacillin (30)	≥17	0	R	0	R
Rocephin (25)	≥19	19	S	0	R
Ciprofloxacin (10)	≥21	18	I	16	R
Streptomycin (30)	≥15	20	S	16	S
Cotrimoxazole (30)	≥16	0	R	0	R
Erythromycin (10)	≥23	0	R	18	I
Pefloxacin (30)	≥19	0	R	16	R
Gentimicin (10)	≥15	18	S	17	S

S-sensitive, R-resistant, I-intermediate, 0=no zone of inhibition, ZH=Zones of inhibition, IS=Interpretive standards for dilution and risk diffusion susceptibility testing (CLSI, 2006)

4. **DISCUSSION**

The chemical contents of dairy cattle milk products (fresh milk, *nono* and *kindirmo*) collected from the two Sites in this study revealed that the pH and titratable acidity of milk and its products were at variance. Significant differences ($p<.05$) were noted amongst the cow milk products obtained from Madobia and Project Quarters. The pH of fresh milk as recorded in this study 6.5 is within the range 6.4-6.8 as reported previously [14]. The low pH of *nono* (3.74) is not surprised as *nono* is a fermented product and may be due to the activities of the lactic acid bacteria which were isolated from the *nono.*

The microbiological quality of fresh milk *nono* and *kindirmo* as shown in Table 2 revealed that the total viable count, coliform count, staphylococcal count and fungal count were not in conformity with the standard as recommended [15]. Grade A raw milk ($<10^5$cfu/ml) and Grade B (milk from local producers) ($<10^6$cfu/ml). The high total count for fresh milk (Log_{10}6.1cfu/ml) *nono* (Log_{10} 6.4cfu/ml) and *kindirmo* milk (Log_{10}6.3 cfu/ml) obtained in this study showed that these values are high for raw milk. [8] Reported that counts greater than 10^3cfu/ml for raw milk indicates a serious fault in hygiene during the production line. Other workers reported total bacterial count in fresh milk to be $3.5x10^3$ [8] and $12.5x10^6$cfu/ml [16] in Pakistan and India.

The coliform count of this study is higher than log 3.72cfu/ml [17] and log 4.78cfu/ml [18] as reported in Maiduguri and Egypt respectively. The high count obtained in this study could be a result of poor hygienic method of milking usually practiced by the local producers. The possible sources of contaminating organisms associated with the products in this study, could be the use of previously fermented *nono* as starter culture (back slopping) and the use of well water for processing. The contaminating organisms could also be introduced via micro flora adhering to the calabash, spoons, and bowls used by the producers. Also the high microbial contamination of the samples used in this study could be due to the health state of the milked animals. High microbial contamination in milk may be due to clinical and subclinical state of the cattle as organisms of milk and milk products may get into milk either directly from udder and or indirectly through infected body discharge which may splash into the milk [19].

The organisms identified included *Staphylococcus aureus, Enterococcus faecalis, Micrococcus spp, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus lactis and Bacillus subtilis*. The occurrence of *S. aureus* from the two farm settlements was quite high and was predominant in the entire samples*. S. aureus* has been implicated in food poisoning outbreak in milk and therefore of great public health importance. A similar observation had been reported by other workers [20,21]. This organism probably got into the milk through the crude methods of milking the cow. *Staphylococcus aureus* and *Streptococcus. agalactiae* are said to be commonly associated with contagious mastitis. *E. faecalis* are more related to environmental mastitis [18]. Streptococci have been implicated in pharyngitis, tonsillitis, sinusitis, otitis media, arthritis, bone infection, bacterial pneumonia, and rheumatic fever.

The fungal isolates from fresh milk, *nono* and *kindirmo* included *Aspergillus flavus, Aspergillus niger* and species of *Mucor Penicillum Rhizopus* and *Fusarium* (Table 4). The presence of fungi in milk products might be attributed to contamination from air, earthenware, or lack of observance of proper hygiene by the local producers. Contaminated cattle feed could be a source of fungi dissemination. The presence of *A. flavus* in the cow milk products might probably make its consumption hazardous to public health. Some strains of *Aspergillus flavus* produce aflatoxin, a potent toxin that has been implicated in hepatoxicity and cancer in mammals including man [5,21].

The antibiogram of pathogenic organisms isolated from the milk products in the present study (Table 4) showed that *E. faecalis* and *S. aureus* were sensitive to gentamicin and streptomycin but resistant to amoxicillin, pefloxacin and erythromycin. The antibiotic sensitivity of these organisms indicated that ampiclox, pefloxacin and erythromycin are not the drugs of choice in the case of outbreak of food infections or food poisoning caused by these organisms. The high level of resistance of the above antibiotics of the isolates is probably related to the wide use of this class of antibiotics in livestock production. This could

enhance the spread of bacterial resistance among people who may consume these products.

5. CONCLUSION

The results showed that all milk samples analyzed had a high viable bacterial count $\log_{10}$ of 6.02-6.36cfu/ml and fungal count of $\log_{10}$ 4.49-5.10cfu/ml and was higher than the regulated standard as recommended [6]. Some of the organisms isolated included: *S. aureus*, *S. faecalis* and *Aspergillus* spp. The growth of these pathogenic organisms and their toxins in local dairy cattle products is a reflection of poor sanitary practices in the production of fresh milk and its products. It is however noted that the types of organisms and their density in the dairy cattle milk products from the two studied sites should be of great concern to the health authorities as these pose serious public health problems to consumers. Safety of food consumers is of utmost importance all hand must be on deck to have this assured all the time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Robinson RK, Tamime AY. Types of fermented milks. In fermented milks, ed. AY Tamime. pp1-10. Hoboken NJ: Wiley; 2006.
2. Atanda OO, Ikenebomeh MJ. Microbiological quality of nono. World Journal of Microbiology. 2006;1(17):89-91.
3. Chukwuma M. Furors over animal milk products rage. World J. Microbiol and Biotech. 2009;5(6):23-30.
4. Ijah JJ, Odagboyi G, Uwabujo AE. Microbiological screening of furan da nono. Nig J. Sci Tech and Math Edu. 2002;5(1):9-16.
5. Okeke KS, Abdullahi IO, Makun HO, Mailafiya SC. A Preliminary survey of Aflatoxin M1 in Dairy cattle products in Bida, Niger State, Nigeria. Afri. J. Food Sci and Tech. 2012;3(10):273-276.
6. Field M C. Laboratory Manual in Food Preservation. AVI Pub. Co., Inc. Westport. 1977;2-10.
7. Collins CH, Lyne MP, Grange JM. Microbiological Methods. Butterworths and Company Ltd., London. 1987;331-345.
8. Abid H, Waqas M, Anwar Y, Ali J, Ullah J. Microbial quality assessment study of branded and unbranded milk. Pak. J. Nut. 2009;8(5):704-309.
9. Harrigan WF, McCance ME. Laboratory Methods in Foods and Diary Microbiology. Academic Press, London. 1976;9-88.
10. Sneath PH, Matr NS, Sharp ME and Holt JG. Bergey's manuel of systemic Bacteriology. Baltimore William and Wilkins. 1986;11.
11. CLSI (Clinical Laboratory standard Institute). Performance standards for antimicrobial disk susceptibility test; Approved standard (9th Ed.).CLSI document M.2-Aa 26:1.Clinical Laboratory Standard Institute, Wayne, PA; 2006.
12. Allouc FA, Hela LB, Laraba A. Antimicrobial thermophilic lactobacilli in milk industry. Nature Et Technologies. 2010;3:13-20.
13. SPSS (Statistical Package for Social Sciences) for Windows (version 15.0); 2006. Available: http://www.spss.com.

14. Rahman ZU, Salaria AM. Effect of storage conditions on the nutritional quality of UHT processed buffalo milk. Journal of Chemical Society Pakistan. 2005;27(1):73-76.
15. APHA (American Public Health Association). Compendium of Methods for the Microbiological Examination of Foods (4th ed.). D.C. Downes FL, Ito, K. (eds). Washington. 2001;483-495.
16. Lingathurai S, Vellathural l. Bacteriological quality and safety of raw cow milk in Madurai South India. Web Med Cent Microbio. 2010;1(10):MWC1029.
17. Ogbonna lO, David AB, Waba JT, Eze PC. Microbiological quality assessment of biradin, kesham and kindirmo (milk product) sold in Maiduguri Nigeria. Int J. of Dairy Sci. 2012;7(1):11-19.
18. Abeer AA, Dardir HA. Hygienic quality of local traditional fermented skimmed milk sold in Egypt. World J. Diary and Food Control Sciences. 2009;4(2):205-209.
19. Egbere OJ. Principles and Practice of Food Microbiology. Deka Publications, Jos. 2008;110-103.
20. Abdullahi IO, Danda I, Tonak A. Microbiological profile of *nono* and *kindrmo* as sold in Samaru, Zaria, Nigeria. J. of Biotech. 2001;12(1):69-73.
21. Makun HA, Arijorin ST, Moronfoye B, Adejo FO, Afolabi OA. Fagbayibo G. Fungal and aflatoxin contamination of some human food commodities in Niger; 2010.

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