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Hygienic Status of Cow Milk and Wara from Local Fulani Herdsmen in two Western States of Nigeria

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Authors' contributions

This work was carried out in collaborations between all authors. Author FO designed the experiment, supervised the execution of the project, wrote the first draft and managed literature searches. Author SL carried out data collection, laboratory experiments and managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Microorganisms in milk and milk products are considered to pose certain hygienic risks for human health. The study aim was to assess the microbial load of cows' milk from free-grazing cattle and local cheeses (wara) produced from milk.
Study Design: Samples of milk and soft cheese (wara) were randomly collected from farms within Oyo and Ogun states.
Place and Duration of Study: Milk samples were collected from Fulani herdsmen in Oyo and Ogun states during the summer months of May and June, 2012.
Methodology: One hundred and twenty-one samples of cows' milk were collected from local Fulani herdsmen in Abeokuta and Ibadan, cities south west of Nigeria. Local cheeses called wara, processed by the wives of the pastoralists were obtained for pH and microbial analysis using standard microbiological techniques such as total aerobic count, *Staphylococcus aureus* count, *Enterobacteriaceae* count and fungal counts.
Results: pH of milk and wara were 5.8 - 6.8 and 5.0 - 6.5. Total viable count of bacteria in milk and wara were 2×10^2 - 2.6×10^7 and 3.3×10^3 - 3×10^7cfu/ml. Fourteen samples had no coliforms. Coliform positive samples were 20 cfu - 3×10^7 and 5.5×10^2- 2.5×10^6 for milk and wara. Sixteen samples had no *Staphylococcus aureus* while positive samples had 21 cfu – 3×10^7 and 1000cfu –

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6.4 x 106 for milk and wara respectively. 26 samples had no fungal growth. Fungal positive samples had2.6 x 10^2 -5.7 x 10^6cfu/ml and 4 x 10^4 - 4.3 x 10^6 for milk and wara. Aflatoxin M1 in positive samples range between 3000 – 7000 ng/L.

Conclusion: Results indicated that some of the milk and wara samples were low in microbiological quality. Routine surveillance test and basic education is recommended for herdsmen and their wives to avoid possible food-borne illness.

Keywords: Microbial quality; cow milk; wara*; pH; aflatoxin M1; basic education.*

1. INTRODUCTION

The Fulanis produce milk locally in Nigeria and the excess milk is processed into wara a local soft cheese [1]. The quality and safety of milk depends on many factors varying from the cow milker, extraneous dirt, the environment or unclean water [2]. The safety of dairy products with respect to food-borne diseases is of great concern around the world. This is true in developing countries where production of milk and milk products take place under unsanitary conditions and poor practices [3]. The microbial load of milk is a major factor in determining its quality. It indicates the hygienic level exercised during milking [4]. Milk produced under hygienic conditions from healthy animals should not contain more than 5 x 10^5 bacteria/ml [5]. The number and types of microorganisms present in milk and dairy products depend on the microbial quality of milk used, heat treatment of milk, the conditions in which the products are manufactured, the temperatures and duration of storage, feeding of the animals, season, area, general sanitation in the plant, quality of starter cultures, occurrence of phages, quality of rinsing water etc [6].

During production the air of processing areas can contaminate foods with pathogenic or spoilage microorganisms. The greatest aerosol sources in dairy plants are personnel, floor drainage, and ventilation system and water [7]. A dirty environment harbours flies which can contaminate milk with soil microorganisms that had previously been contaminated with faecal materials thus serving as source of enteric pathogenic bacteria [8]. Pathogenic microorganisms cause diseases, some of which are very serious. According to Lingathurai and Vellathurai [9], there are 21 milk borne pathogens currently being recognized. Food-borne infections caused by milk and its products range from salmonellosis, listeriosis, diarrhoea, gastroenteritis, brucellosis to fatal haemorrhagic colitis. The pattern of acute poisoning may be different even within the region of a country [10]. Many milk-borne epidemics of human diseases have been spread by contamination of milk by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies [11].

It has been reported that 90% of all reported dairy related illnesses are of bacterial origin [12], the remaining 10% may come from fungi metabolites such as aflatoxin. Milk and dairy products are fundamental components in the human diet and may be the principal way for entrance of aflatoxin into the human body. Aflatoxins are toxic metabolites generally produced by *Aspergillus flavus, A. parasiticus, and A. nomius* [12]. They have immunosuppressive, mutagenic, teratogenic, and carcinogenic effect especially in the liver [13]. Aflatoxin M1 (AFM1) is a hydroxylated metabolite of Aflatoxin B1 and can be detected in milk and dairy products from dairy cattle that have ingested feed contaminated with AF B1. Its parent molecule has been categorized as Class 1 human carcinogen while AF M1 has a carcinogenicity of 2-10% [14]. The result of their toxicity ranges from gastroenteritis to cancer. The presence of mycotoxins in food and feeds depends on many biological factors such as region, season, humidity, and temperature as well as the conditions under which crops are harvested, stored, and processed. The presence of AFM1 in milk and milk products is considered undesirable due to toxic and carcinogenic properties [15]. Regulatory limits throughout the world are influenced by economic considerations and may vary from one country to another. The European community and Codex Alimentarius have prescribed the maximum limit of AF M1 in liquid milk and dried or processed milk products is 50ng/kg [16]. According to U.S.A. regulations, the level of AFM1 in milk should not be larger than 500ng/kg. In Egypt, the ministry of health established that fluid milk and dairy products should be free from AFM1 [17]. In Nigeria, the regulatory agency for food has stated the maximum dose of 500ng/ml. In order to reduce poverty among the rural poor, the wives of the herdsmen engage in processing of raw milk into local soft cheese called wara. The hygienic status of such milk and milk products coupled with the high temperatures encountered in this climatic zone, dirty processing equipment with unavailability of potable water has necessitated the need for this study. The main objective of this study was to assess the hygienic status of milk produced by local Fulani herdsmen.

2. MATERIALS AND METHODS

2.1 Collection of Samples

A total of 121 raw milk and local cheeses (wara) samples were collected from 7 locations in two south western states (Ogun and Oyo) of Nigeria and were brought to the laboratory in a sterile ice-packed cooler.

2.2 Determination of pH

pH of both raw milk and processed milk was determined using a pH meter (Mettler Delta 340).

2.3 Isolation of Microorganisms

One milliliter of each sample was serially diluted and 1 ml of an appropriate dilution was inoculated on nutrient agar plates in triplicates for total viable counts; On Mac Con key agar for coliforms; On De Man Rogosa Sharpe agar for lactic acid bacteria and on Potato Dextrose Agar for moulds.

2.4 Characterization and Identification of Bacterial Isolates

This was done according to standard methods described by Olutiola et al. [18]. The methods involved Gram staining, morphological, cultural and biochemical characteristics (catalase, coagulase, oxidase, methyl red, VogesProskauer, citrate reduction, indole and sugar fermentations test).

2.5 Characterization and Identification of Fungi Isolates

Two drops of lactophenol blue was put on a clean glass slide containing mycelia growth. The mould isolates were examined macroscopically and microscopically using the schemes of Klich [19].

2.6 Extraction of Aflatoxin M1 from Milk

Twenty millilitres of milk were centrifuged at 3500g/10℃ for 5 min. The fatty layer was removed and 100ul of defatted milk was applied directly to the Enzyme-Linked Immuno Sorbent Assay (ELISA).

2.7 Enzyme-linked Immunosorbent Assay

The dilution strips were placed in a microwell strip holder. Equal number of antibody coated microwell strips were plated in a microwell strip holder. Two hundred microlitre of conjugate was dispensed into each dilution well. One hundred microlitre of each standard extract was added using a micropipette. One hundred microlitre of the contents in the dilution well was transferred into a corresponding antibody coated microwell. The samples were incubated at room temperature for fifteen minutes. The contents of the microwell strips were emptied and themicrowells were washed three times. One hundred microlitres of stop solution was added into each microwell strip and the colour formation noted. The strips were read with a microwell reader using a 450nm filter.

2.8 Statistical Analysis

All data were analysed using ANOVA, SPSS version 14 and Duncan Multiple range test.

3. RESULTS AND DISCUSSION

The normal pH of cow milk is usually between 6.4 - 6.8 and 6.5 - 6.7 [20]. Most milk samples fell within this approved range (Table 1). However, few samples from the fifth location had pH values lower than 6.0.

Table 1. pH of raw milk and wara from free-ranging cows in Oyo and Ogun States

Location	Raw milk n=73	Wara n =48
1	6.0 – 6.6	5.5 -6.4
2	6.3 – 6.8	4.5- 5.0
3	6.7 – 6.8	5.9 -6.5
4	6.4 – 6.8	4.5 – 5.0
5	5.8 – 6.2	5.0 – 5.1

Key 1. Gaaseriki village, Oyo State, 2. Alabata village, Ogun State, 3. Ibarapa North, Oyo State, 4. Abeokuta South, Ogun State, 5. Kotopo village, Ogun State

The approved EU total bacterial count is 105cfu/ml. It was found that a total of 53% of samples of raw milk had bacterial count less than or equal to the EU approved 105 cfu/ml. Thirty-four percent of samples had less than or equal to 1000cfu/ml. In location 1, two samples had no coliforms; Location 3, one sample; location 5, three samples totalling 7 samples with no coliforms (Table 2).

A total of 8 samples had no *Staphylococcus aureus* while the range of contaminated samples was between 103-106 cfu/ml (Table 2).

A total of 13 samples were free of fungal growth while fungi contaminated samples range between 102-106cfu/ml (Table 2). Some milk samples analysed for aflatoxin M1during the rainy season months of May and June gave a range of 3000ng/L – 7000ng/L.

The frequency distribution of microorganisms is shown in Table 3 where 34 samples exceeded a count of 100,000 cfu/ml [21].

The correlation between pH and bacteria count became evident as bacterial count increased with decrease in pH. This is as a result of microbial activity in the milk that lowered the pH due to formation of lactic acid. All wara samples had low pH. This might be due to the addition of sodom apple (*Calotropis procera*) which has an acidic pH of 4.5 [22].

The microbial safety of any food produce is determined by its level of microbial load [23]. Poor handling of milk during processing and especially marketing exposes it to microbial contamination thereby making it a source of microbial food poisoning at the production sites and sales outlets. Microbial count in raw milk can be as low as 103cfu/ml. However this number can increase due to several factors such as mastitis, keeping raw milk at elevated temperatures, unhygienic practices of uneducated food processors, lack of portable water etc. [24,25,9,26].

The range of total bacterial count obtained in this study was similar to counts in some Asian countries as reported by Rizwan et al. [26]; Lingathurai and Vellathurai, [9]; Dan et al. [24] and Khaliq et al. [25]. For instance it was observed in some locations that the local Fulani herdsmen did not wash their hands before milking. Also, containers like buckets were not properly washed; Udders of the cows were not cleaned before milking. According to Aernan et al. [27], pre milking udder preparation plays an important role in milk contamination.

The total bacterial count of processed milk locally called wara was higher than the raw milk. The use of contaminated water, unclean utensils and poor hygienic environment in which the milk is being extracted and processed into wara, with little or no attention paid to good manufacturing and storage procedures by the Fulani men can independently or collectively be responsible for this high count. Wara is produced manually with little or no serious consideration given to sanitary practices, therefore hazards in form of microbial contamination and ultimately food-borne illness will occur while consuming these products.

Coliforms are almost found in milk but with good methods of production, numbers of this group can be kept low as seen in some locations where some samples had no coliforms. The presence of these organisms in milk and milk products is an indication of unsanitary production and/or improper handling of either milk or milk utensils [28].

Table 2. Range of bacterial/fungal count of milk and warafrom free-ranging cows in Oyo and Ogun states

Location	**Total viable count**		**Coliform**		**S. aureus**		**Fungi**	
	Milk	**Wara**	**Milk**	**Wara**	**Milk**	**Wara**	**Milk**	**Wara**
1	2×10^2	3.3×10^3	20cfu	550	21cfu	10^3	2.6×10^2	2×10^5
	1×10^4	2.5×10^6	2.3×10^3	2.5 x 104	30×10^3	4.2×10^7	3.4×10^3	6.2×10^5
2	1.3×10^6	1.2×10^6	52×10^4	83×10^4	6×10^4	3.2x105	26×10^4	3x106
	1.9×10^6	1.7×10^6	1.8×10^6	1×10^6	21×10^4	8.4x105	4×10^4	4.6 x106
3	2.9×10^6	3.4×10^3	2.2×10^5	4×10^5	2.2×10^5	1.6×10^5	1.2×10^6	3x106
	8×10^6	1.5×10^6	1.8×10^6	1×10^6	3.2×10^5	8.4×10^5	1.3×10^6	4.6 x106
4	7×10^5	6×10^5	6×10^5	1.5×10^6	4.1×10^6	Nil	2×10^4	Nil
	6×10^6	6×10^6	6×10^6	2.5×10^6	5.7×10^6	5.9×10^6	1.4×10^5	4×10^4
5	2×10^6	3.2×10^6	3.1×10^6	3.1×10^4	1.9×10^6	8.7 x106	2.9×10^6	3.9×10^6
	2.6×10^7	3×10^7	3×10^7	1.5×10^5	2.8×10^6	6.4 x106	5.7×10^6	4.3×10^6

Key: 1. Gaaseriki village, Oyo State, 2. Alabata village, Ogun State, 3. Ibarapa North, Oyo State, 4. Abeokuta South, Ogun State, 5. Kotopo village, Ogun State

Milking udder with sub-clinical mastitis and wet environment lead to contamination of bulk tank milk and hence raw milk reaches the consumers with elevated coliform count [29,30]. In Table 1 some coliform counts were slightly higher than the acceptable 100cfu/ml while some are above 500cfu/ml indicating poor hygiene [31].

Bulk milk coliform bacteria are used as indicator of hygienic condition during handling and processing of milk and milk products [21].

Table 3. Frequency distribution of total viable count of bacteria in 73 raw milk samples

Total count	Sample numbers
10^1	3
10^2	7
10^3	15
10^4	6
10^5	8
10^6	30
10^7	4

Staphylococcus aureus is one of the most common bacterial pathogens by which raw milk is contaminated. *S. aureus* causes one of the most common types of chronic mastitis and life threatening endocarditis and toxic shock syndrome [32]. Raw milk is recommended to be pasteurized prior to consumption.

Fungal infection and fungal intoxication can both occur in livestock. One of the common fungal infection causes cutaneous disease. The economic damages of dermatophytosis in animals are very high and besides skin injuries, it causes stress to the animals, decrease skin quality, causes weight loss and decreases milk production. Two species of Trichophyton have been implicated [33]. In this study we investigated fungal intoxication of milk from free-ranging cows.

The presence of AF M1 in milk and milk products is considered undesirable due to toxic and carcinogenic properties [15]. Regulatory limits throughout the world are influenced by economic considerations and may vary from one country to another. The European Community and Codex Alimentarius has prescribed that the maximum limit of AF M1 in liquid milk and dried or processed milk products is 50ng/kg [21]. According to U.S.A regulations, the level of AF M1 in milk should not be larger than 500ng/kg. In Egypt, the Ministry of Health established that fluid milk and dairy products should be free from AFM1 [17]. In Nigeria, the regulatory agency called the National Agency for Food, Drug Administration and Control (NAFDAC) has stipulated a maximum level of AFM1 at 500ng/kg [34].

The values obtained in this study were above values of all regulatory agencies stated above. It was observed that the local herdsmen feed their cattle from grasses as they graze through farmlands. During the grazing period, especially during the rainy season, these animals also feed on mouldy crops in the farms. This might probably be the source of aflatoxin contamination in these milk samples. In Senegal, a partnership was established between Laiterie du Berger and local herders where the company supplies quality feed free to the herders and herders in turn sells quality milk to the company at a price fixed by the company [35]. This will contribute in no small way in reducing microbial contamination of milk. Aflatoxin M1 (AFM1) is a hydroxylated metabolite of Aflatoxin B1 and can be detected in milk and dairy products from dairy cattle that have ingested feed contaminated with AFB1. Its parent molecule has been categorised as Class 1 human carcinogen while AF M1 has a carcinogenicity of 2-10% [14].

4. CONCLUSION

This study has established microbial contamination of raw milk and local soft cheese processed in Oyo and Ogun states of Nigeria. Therefore, concerted effort should be geared towards improving the sanitary conditions of these products in order to prevent the occurrence of foodborne diseases. The local herdsmen should be educated on sanitary measures and practices during milking of cows. Use of portable water where available should be encouraged, clean and sterile milking buckets should be used. Hand washing should be encouraged before and after milking processes and cows' udders should be cleaned before and after milking.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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