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Raw Milk as a Potential Source of Food Poisoning Outbreaks

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ABSTRACT

In Africa, the use of poor and unhygienic methods for animal milking and milk processing that leads to spoilage of milk by microbes affects the production of milk and dairy products, especially in the small scale and local processing plants. This study was conducted to analyze the quality and safety of raw milk collected from six different towns in Kwami local government area of Gombe State, Nigeria. The samples were serially diluted using ten-fold dilution and used aliquot 1 ml to inoculate the appropriate media using pour plate technique. The total viable count for bacteria in CFU/ml on plate count agar (PCA) was highest in sample E from U/Anchau with an average of total viable count of $3.8x10^4$ CFU/ml, followed by $3.0x10^4$ CFU/ml in sample B (Dirri), then $2.8x10^4$ CFU/ml in sample D from Burakosuma, $2.5x10^4$ CFU/ml in sample F from Dun urji, $2.3x10^4$ CFU/ml in sample C from Zanbe with least count from sample A at Bele as $1.8x10^4$ CFU/ml. Five (5) bacterial species of public health importance were isolated and identified using biochemical tests namely; *Enterobacter sp., Yersinia enterocolitica, Escherichia coli, Staphylococcus aureus and Salmonella sp.*. Out of the organisms isolated, *Enterobacter sp.* had the highest occurrence of 93% (n=280), then *Yersinia enterocolitica* 90% (n=270), *E. coli* 70% (n=210), *S. aureus* 57% (n=170), and finally *Salmonella sp.* 23% (n=70). Based on the microbiological outcomes, preventive measures for milking and processing that focus on training of farmers and dairy employees for the improvement of the hygiene of local milk and dairy production chain should be defined.

INTRODUCTION

Unsafe foods remain a global concern particularly in the developing countries of Africa [1]. Microbial contamination is the major risk associated with unsafe foods, and microbial foodborne infections are its major public health concern. In fact, documented incidences of foodborne diseases have significantly increased over the few decades in most countries with case-reporting systems [2]. Raw milk is a protein, fat, sugars, vitamins and minerals rich food. Raw milk is sterile upon secretion into the udder. However, microbial contamination occurs during handling, storage and other processing. There are specialized cells responsible for the synthesis of milk and it is secreted as sterile into the alveoli of the udder. It gets contaminated by microorganisms in three main ways; in the udder, outside the udder and from the surface of the equipment used in milk handling and storage [3, 4].

One of the requirements of the World Health Organization is the urgent compliance with the principles of Codex Alimentarius principles [5] by small scale dairy processing plants in the developing countries. Additionally, Good Manufacturing Practices (GMP) and Hazard Analysis of Critical Control Points (HACCP) are also among the recommendations of the WHO for

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such small units of production [5]. Few countries in Africa have legislated system of surveillance for foodborne diseases; in Nigeria the regulations governing dairy products hygiene control have been issued but are rarely or not enforced, thus the milk chain hygiene condition has not been adequately managed [6].

Milk contamination can be derived from the cow itself, from the environment and from human procedures [1]. Various pathogens and commensal organisms could be harboured in the udder particularly where cases of subclinical or clinical mastitis are involved. Another risk factor is the procedure of traditional milking because it is done manually outdoors; not in places particularly designed for it. Prior to milking, calf suckling is performed, and this promotes oxytocin production and without previous sanitization of the container, the hands of the milking personnel and the teats. When milking outdoor, there are many factors involved in the spoilage of milk including faecal contamination of the skins of animals, the usage of unsafe water to rinse the udder, equipment, presence of dust and faeces and the hands of the milking staff [7, 8].

As a result of this, there is need to strictly adhere to good hygiene practices in the collection, transportation and processing to avoid the milk spoilage, particularly in local small scale dairy processing plants. This study aimed to evaluate the microbial contamination of raw milk from farms in a local setting of Bojude town in Gombe state, Nigeria which will serve as a determining factor for the potential of food poisoning outbreaks due to the presence of bacterial pathogens.

MATERIALS AND METHODS

Sample Collection

A total of 30 samples were collected from six different localities namely, Bele, Dirri, Zanbe, Burakosuma, Unguwan Anchau and Dun urji from farms labeled A, B, C, D, E and F respectively; 5 from each town. Each sample was collected directly after milking in sterile sampling bottles in sterile ice-packed cooler and transported to the laboratory for analysis.

Microbiological Analysis of Raw Milk

Isolation of bacteria was performed using ten-fold serial dilution. One millitre of raw milk sample was dispensed in 9 ml Peptone water. This was marked as 10^{-1}. One milliltre (1ml) from 10^{-1} dilution was further transferred to another test tube containing 9 ml peptone water to give a concentration of 10^{-2}. Further dilution of up to 10^{-5} was obtained in this manner. Following the serial dilution, aliquots were dispensed each in petri dishes by pour plate technique; 1ml of the diluted sample was dispensed into Plate count agar (PCA) for total aerobic mesophilic count, and on other selective or differential media which include; Mannitol Salt Agar (MSA), Eosine Methylene Blue (EMB) agar, MacConkey Agar (MA), and Salmonella-Shigella Agar (SSA) for selective isolation of *Staphylococcus*, *E. coli*, coliform bacteria and *Salmonella Shigella* respectively. The plates were placed in an incubator at 37°C for 24 hours. The bacterial populations in colony forming units (CFU/ml) were obtained following incubation using digital illuminated colony counter.

Identification of the Isolates

Following isolation of the organisms in their respective selective media, they were subcultured on nutrient agar medium and then subjected to Gram staining and subjected to biochemical tests such as coagulase, catalase, mannitol fermentation, urease, citrate utilization, motility, methyl red, Voges-Proskauer, indole production, H_2S production and gas production. This was conducted to confirm their identities.

Statistical Analysis

All data were analyzed using One-way ANOVA by Minitab version 18. Statistically significant values were identified based on P-values.

RESULTS

The results obtained in this study are tabulated. The mean total viable counts of the 30 raw milk obtained from the six different locations namely, Bele, Dirri, Zanbe, Burakosuma, Unguwan Anchau and Dun urji are shown in Table 1. All counts were in multiples of 10^4. In location A (Bele), the five samples had values of 1.0, 1.4, 3.2, 2.5 and 1.1 x 10^4 CFU/ml respectively. In location B (Dirri) there were 5.1, 2.6, and 1.2 x 10^4 CFU/ml in samples 1, 2 and 3 respectively while there was no organism recorded in sample 4. Sample 5 had 3.1 x 10^4 CFU/ml. There no organism isolated in sample 1 at location C (Zanbe) while samples 2-5 had 3.5, 2.2, 2.5 and 1.0 x 10^4 CFU/ml respectively. However, in location D (Burakosuma) the counts were obtained in samples 1-4 as 3.3, 2.6, 1.5 and 3.7 10^4 CFU/ml respectively, but the count in sample 5 was recorded as Nil, indicating no colony count observed. All the five samples in location E (Unguwan Anchau) recorded considerable counts of viable organisms as 3.7, 4.4, 2.5, 3.2, and 5.2 x 10^4 CFU/ml respectively. In location F (Dun Urji), all the samples were found to have the mean total viable counts as 3.2, 2.5, 1.7, 1.9 and 3.3 x 10^4 CFU/ml respectively.

The average for the mean aerobic mesophilic counts of the six locations surveyed for microbiological quality of raw milk shows that location E had the highest with count of 3.8 x 10^4 CFU/ml. This is followed by location B with 3.0 x 10^4 CFU/ml, location D with 2.8 x 10^4 CFU/ml, location F with 2.5 x 10^4 CFU/ml, then location C with 2.3 x 10^4 CFU/ml and finally location A with the least value of 1.8 x 10^4 CFU/ml (**Table 1**).

Table 1. Mean total viable counts (CFU/ml) in the raw milk samples.

Location /Samples	A	B	C	D	E	F
1	1.0 x10^4	5.1x10^4	TFTC	3.3 x10^4	3.7x10^4	3.2x10^4
2	1.4 x10^4	2.6x10^4	3.5 x10^4	2.6 x10^4	4.4x10^4	2.5x10^4
3	3.2 x10^4	1.2x10^4	2.2 x10^4	1.5 x10^4	2.5x10^4	1.7x10^4
4	2.5 x10^4	TFTC	2.5 x10^4	3.7 x10^4	3.2x10^4	1.9x10
5	1.1 x10^4	3.1x10^4	1.0 x10^4	TFTC	5.2x10^4	3.3x10^4
Total	1.8 x10^4	3.0 x10^4	2.3 x10^4	2.8 x10^4	3.8 x10^4	2.5 x10^4

Key: A-Bele, B-Dirri, C-Zanbe, D-Burakosuma, E-Unguwan anchau, F-Dun urji, nil-no bacterial growth, TFTC-too few to count

The results for frequency of occurrence of each organism isolated from the six locations are depicted in **Table 2**. It shows that *Enterobacter sp.* had the highest frequency (n=28) with percentage occurrence of 93%. This is followed by that of *Yersinia enterocolitica* (n=27) with 27% occurrence, followed by *E. coli* (n=21) with 70% occurrence, *S. aureus* (n=17) with 57% occurrence and finally *Salmonella sp.* (n=7) making 23% occurrence. The percentage of each organism is based on a total of 30 samples obtained from all the locations. For instance, *E. coli* was detected in all the five samples of location A, not detected in all the samples of location B, in three of the five samples from locations C and D, in all the five samples of locations E and F making a total of 21 samples of the 30 obtained (21%). This was similarly obtained for all the organisms; *Enterobacter* sp., *S. aureus,* Salmonella sp. and *Y. enterocolitica.*

Table 2. Frequency of occurrence of bacterial isolates from the different sampling locations.

Organisms/ Locations	A	B	C	D	E	F	Total	Percentage
E. coli	05	00	03	03	05	05	21	70
Enterobacter sp.	05	05	05	04	04	05	28	93
S. aureus	04	00	03	00	05	05	17	57
Salmonella sp.	00	04	00	00	03	00	07	23
Y. enterocolitica	05	04	04	04	05	05	27	90

Key: A-Bele, B-Dirri, C-Zanbe, D-Burakosuma, E-Unguwan anchau, F-Dun urji,)

DISCUSSION

Pathogenic bacteria have been a major global public health concern. Milk contains a variety of nutrient that makes it a good place for survival and viability of various microbes; both saprophytes and the pathogens. The application of the microorganisms into the milk may be due to several sources such as animal skin, udders that are infected or dirty udder, the hands of the milking personnel, utensil and faeces, stressed on hygienic handling of milk and milk products in order to prevent dangers linked to contamination by microorganisms [4].

From the result, the mean total viable count for bacteria in CFU/ml on PCA was highest in sample E from U/Anchau with an average of $3.8X10^4$, this may be attributed to the use of unsanitary utensils, rearing of cattle in contaminated environment and milking from dirty or non-disinfected udder, followed by $3.0X10^4$ in sample B (Dirri), then $2.8X10^4$ in sample D from Burakosuma, $2.8X10^4$ in Burakosuma (D), $2.5X10^4$ in sample F from Dun urji, $2.3X10^4$ in sample C from Zanbe and $1.8X10^4$ in A from Bele being the least [6]. The results show that the raw milk samples had contamination by several microbial species that include *Staphylococcus aureus, Yersinia enterocolitica, Salmonella* sp., *Enterobacter* sp., and *Escherichia coli.*

In this study, five bacterial genera had been isolated; *S. aureus, Salmonella sp., Enterobacter* sp., *Y. enterocolitica* and *E. coli.* The *Enterobacter* sp. was the most prevalent, 93% (n=280), followed by *Yersinia enterocolitica* 90% (n=270), then *Escherichia coli* 70% (n=210), then *S. aureus* 57% (n=170) and the least was *Salmonella* sp. 23% (n=70). This observation confirms the finding of Oladipo et al. [9] who reported that the growth of these organisms in raw milk can affect its storage qualities. The result shows there is a significant difference ($P<0.05$) in the occurrence of the five isolates with respect to the locations where raw milk samples were collected.

The presence of these organisms indicates the degree of contamination of the milk by contaminating agents such as the animals, environments and the milking utensils. The bacterial counts are below the limit set by the European Council (EC) Regulation (No. 853, 2004) of the European Parliament and of the Council (EC) which sets down the hygienic limit as ≤ 100,000CFU/ml of milk for the total bacteria count (TBC) in cow's raw milk. TBC is among the major hygiene quality indicators of cow raw milk. This is also employed as a measure for the milk purchasing price [10]. It was also reported by Jayarao and Henning [11] that the conditions for operation when failed to be observed based on the regulations of milking hygiene contribute largely to the weakened microbial quality of bulk samples of cow raw milk.

Variations in the incidence (Table 2) are indication of contamination level in the analyzed samples. Percentage of *S. aureus* based on this finding agrees with the findings of Bonfoh *et al.* [12] who discovered high loads of *S. aureus* in the milk samples. The presence of *Staphylococcus aureus* in raw milk is linked with mastitis; the commonest fatal infection of the farm animals that bedevils the dairy industry. It is a communicable disease that is characterized by inflammation of the bovine udder [13]. Prevalence of *S. aureus* in the milk is also connected to it being normal microbiota of the humans and animals. It has also been connected to nosocomial infections [14] and was considered a cause of clinical and sub-clinical mastitis in cows [15]. *S. aureus* causes foodborne intoxication that is mostly not severe, usually self-limited. Its associated symptom is vomiting, sometime involving diarrhea [16]. Presence of *S. aureus* in raw milk can present potential risks for the health of the consumers as result of enterotoxin production [17].

The presence of coliforms such as *Enterobacter* sp. and *Escherichia coli* is an indication of poor level of hygiene of the milker's utensils, water and the environments where milking is conducted. This agrees with Reta et al. [18, 19] that investigated the sources of *E. coli* contaminating raw milk as manure, soil, faeces of humans and unsanitary equipment. The prevalence of *E. coli* in the raw milk reveals the presence of other pathogenic enterobacteria in the sample. *E. coli* in the raw milk sample can be risky because the isolated strains could be toxigenic or enteropathogenic, inducing major public health hazards. Some strains of *E. coli* are linked with several foodborne outbreaks. They are also responsible for bloody diarrhea that is often associated with dairy producing cattle. Raw milk and soft cheeses upon contamination by microbes such as *E. coli* can lead to infections. In addition, the act of drinking milk by rural dwellers can cause a serious health concern as a result of the presence of *E. coli* [8].

Salmonella sp. was not found in all the analyzed milk samples. The study is in comparison with that of Mennane *et* al. [20] who observed nearly similar result in their attempt to determine the hygienic quality of raw cow's milk feeding from domestic waste in two regions in Morocco. This shows that the prevalence of Salmonella in raw milks from the area is low, thus *Salmonella* sp. is not considered a potential danger to the consumers health. The evidence of Salmonellosis was especially found in developed nations such as Wales and England in which Salmonellosis associated with raw milk and milk product consumptions was the cause of frequent reports of outbreaks [17].

CONCLUSION

This study showed that raw milk sold in Bojude had been contaminated with pathogenic (*S. aureus, E. coli, Enterobacter* sp., *Salmonella* sp.) and microaerophilic (*Yersinia enterocolitica*) bacteria. Their occurrence signifies poor hygienic levels of the raw milk implying that raw milk consumers in Bojude stand a high risk of exposure to foodborne pathogens. Finally, there is a significant difference between isolated bacteria in the raw milk with regard to the different locations of Kwami local government area of the state.

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