



Bacteriological Profile and Proximate Composition of Raw Milk from Different Cow Breeds in Amansea Cow Market, Anambra State, Nigeria

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Abstract

Raw cow milk, once it is produced from the udder is vulnerable to contamination from spoilage bacteria and food-borne diseases. This study was carried out to evaluate the bacteriological profile and proximate composition of raw milk from different cow breeds in Amansea Cow Market, Anambra State. A total of 27 raw cow milk samples from three different breeds of cow which include Bokolo, Chekpe, and Turuku breeds were collected for this study and were subjected to standard Microbiological and laboratory procedures to analyze the samples. The results revealed that the highest mean for Total Heterotrophic Bacterial Counts was observed in the Turuku breed as $8.06 \pm 1.9 \times 10^6$ cfu/ml, while the lowest mean for Total Heterotrophic Bacterial Counts was observed in the Chekpe breed as $5.46 \pm 0.12 \times 10^6$ cfu/ml. Total Coliform Count had the highest value recorded for the Bukulo breed as $6.73 \pm 1.11 \times 10^6$ cfu/ml, while the lowest Total Coliform Count was recorded for the Chekpe breed as $4.43 \pm 0.25 \times 10^6$ cfu/ml. The Feecal Coliform Count had the highest value recorded for the Turuku breed as $17.0 \pm 3.9 \times 10^4$ cfu/ml, while the lowest mean for Feecal Coliform Count was recorded for the Chekpe breed with a value of $15.58 \pm 4.70 \times 10^4$ cfu/ml. Four (4) bacterial isolates were identified based on morphological and biochemical characterization, which include *Staphylococcus aureus* (48.1%), *Escherichia coli* (48.1%), *Klebsiella* spp. (44.4%) and *Bacillus* spp. (18.5%). The identification of these bacterial isolates indicates microbiological contamination and the likely presence of pathogens that are harmful to human health. Results of the proximate analysis showed that parameters such as moisture content ranged from 83.72% to 85.22%, 1.85% to 2.47% for fat content, 6.43% to 7.95% for carbohydrate content, 4.62% to 5.19% for crude protein and 1.01% to 1.21% for ash content. The bacteriological analysis of the raw milk samples showed a high bacterial load in the various breeds which was higher than the regulatory standards for milk consumption. The proximate composition of the raw milk samples was within the acceptable limit. The prevalence of these bacterial species that have the potential to cause food-borne infections are quite concerning for public health. This research demonstrated that direct consumption of raw milk is not safe as a result of bacterial contamination and thus can lead to health challenges. Since the consumption of raw milk is in high demand, handlers should be trained and educated on proper milking procedures and storage to reduce the risk of milk contamination.

Keywords: Bacterial Isolates; Raw Cow Milk; Bokolo Breed; Chekpe Breed; Turuku Breed; Proximate Composition

Introduction

Raw milk and other dairy products have always been an important part of making traditional African dishes, and this is

likely to increase, as the world's population grows [1]. In Nigeria, the production of milk increased from 220,000 thousand tons in 1971 to 524,733 thousand tons in 2020, growing at an average annual rate of 1.94% [2]. Milk is a large source of goods with a wide

range of proximate compositions, and the composition of raw milk varies from species to species [3]. Quantitatively, the four most abundant components are water (moisture content), fat, protein, and lactose (carbohydrate), with minerals, enzymes, vitamins, and dissolved gases rounding out the equation [4]. As a result of their vital role in maintaining human life, the exogenous nutrient supply in the human diet is essential. Milk is considered the most exceptional and optimal type of food because it provides for all of the body's nutritional requirements in one convenient serving [5].

Milk is considered a fertile breeding ground for numerous types of bacteria and zoonotic diseases that can cause food poisoning [6]. At the point of milking, milk from a healthy cow should have a microbiological purity suitable for human consumption [7]. However, once milk is produced from the udder, it is vulnerable to contamination from spoilage bacteria and food-borne diseases. These contaminants can come from animal dung, air, food, water, machinery, animal hides, and humans [8]. The incidence of disease-causing and spoilage bacteria in milk and dairy products is affected by a wide range of variables. The state of the dairy herd, the cleanliness of the dairy farm, milking and pre-storage procedures, storage capacities, technological advances, farm management practices, geographical location, and time of year are some of the variables that could affect the quality of the milk produced [9,10]. When processed and distributed using Nigeria's traditional method, cow milk, and its products are vulnerable to deterioration and microbial contamination [10].

According to [11] the food safety risks associated with milk and dairy products can be mitigated through excellent farming methods and on-farm controls, good manufacturing and hygiene procedures, consumer safety knowledge, and the proper application of food safety management systems. The dangers of consuming raw milk and dairy products have greatly diminished in developed countries [12]. Smallholder farmers and processors dominate the dairy business in developing nations, in contrast to the large-scale operations that dominate the developed world [13]. In most African countries' milk and dairy products are handled by the unregulated informal sector, where unpasteurized milk is marketed through small-scale channels without a cold chain and no oversight [14]. This study, therefore, aims to evaluate the bacteriological profile and proximate composition of raw milk from different cow breeds in Amansea cow market, Anambra State, Nigeria.

Materials and Methods

Study area

The study was conducted in Amansea town in Awka North Local Government Area of Anambra State, Nigeria. The study area is a cow market in Amansea, where indigenes are mostly crop farmers, fishermen, and herdsmen from Northern Nigeria who migrated to Amansea both for cattle trading and grazing. The coordinates of the sample location have latitude 6°15'9.545" N and longitude 7°8'41.032" E.

Sample collection

A total of 27 raw cow milk samples were collected from three different breeds of cows (Bokolo, Chekpe, and Turuku breeds), and 9 samples were collected from different cow vendors in Amansea Cow Market, Anambra State, Nigeria. The samples were put in sterile universal sample bottles, properly labeled, and transported in an icebox to the Microbiology Laboratory at Rivers State University for further processing and Microbiological analysis.

Enumeration, isolation, and identification

Respective differential media were used for the enumeration and classification: Nutrient Agar (NA), MacConkey Agar (MAC), Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA), and De Man, Rogosa, and Sharpe Agar (MRS). All media were prepared according to the manufacturer's instructions.

Bacteriological analysis

Tenfold serial dilution of the raw cow milk samples were carried out; this was accomplished by transferring 1 ml of each of the milk samples into 9 ml of sterile normal saline under aseptic conditions. After the dilution, an aliquot of 0.1 ml was spread out in triplicate on the various media. The total heterotrophic bacterial counts (THBC) were estimated using the colonies that developed on Nutrient Agar; total coliform count (TCC) for MacConkey Agar, and fecal coliform counts (FCC) for EMB Agar [15]. Colonial features were examined and reported. Pure cultures of bacteria were produced by streaking representative separate colonies of various morphological types that grew on the culture plates onto newly prepared nutrient agar plates in an aseptic manner and then incubating the plates at 37°C for 24 hours. The colonies that developed on the respective plates were counted and subcultured on freshly prepared nutrient plates until pure cultures were obtained. The isolates were stored on

nutrient slants and refrigerated at 4°C until required for further use [16].

Biochemical characterization of bacterial isolates

The morphological and biochemical testing of isolates were done, including colony colour, shape, size, elevation, margin, texture, and opacity. Isolates were characterized using Gram staining and some biochemical tests such as Oxidase test, Catalase test, Indole test, Voges- Proskauer test, Methyl red test, Citrate test, and coagulase tests were carried out [17].

Determination of the proximate composition of the raw cow milk sample

Parameters such as protein content, fat content, moisture content, ash content, and carbohydrate content were determined using the 2005 AOAC methods [18].

Statistical analysis

A one-way analysis of variance (ANOVA) was used to check for significant differences between each of the different breeds of raw cow milk samples. The mean separation was analyzed using Tukey’s High Significant Difference (HSD).

Results

Bacterial population of the various milk samples

The results of the Total Heterotrophic Bacterial Counts, had the least count of $5.5 \pm 0.12 \times 10^6$ cfu/ml from the Chekpe breed, followed by the Bukolo breed with a value of $6.7 \pm 1.3 \times 10^6$ cfu/ml, while the Turuku breed had the highest counts, of $8.1 \pm 1.9 \times 10^6$ cfu/ml.

The results indicated that for Total Coliform Counts, the lowest count of $4.4 \pm 0.25 \times 10^6$ cfu/ml was from the Chekpe breed, followed by Turuku with $5.9 \pm 0.50 \times 10^6$ cfu/ml, while the highest count was obtained from Bukolo breed with the value of $6.7 \pm 1.11 \times 10^6$ cfu/ml.

Feecal Coliform counts showed that the bacteria had the lowest count from the Chekpe breed at $15.6 \pm 4.70 \times 10^3$ cfu/ml, followed by the Bukolo breed with a value of $16.3 \pm 2.5 \times 10^4$ cfu/ml, and in Turuku with the highest value obtained as $17.0 \pm 3.9 \times 10^4$ cfu/ml. The statistical analysis revealed that there was no significant difference in Total Heterotrophic Bacterial Counts (THBC), Feecal

Coliform Counts, and Total Coliform Counts obtained from milk samples from the various breeds of the cow ($p > 0.05$) (Table 1).

Samples	THBC ($\times 10^6$ cfu/ml)	FCC ($\times 10^3$ cfu/ml)	TCC ($\times 10^4$ cfu/ml)
Bokolo Breed	6.66 ± 1.3^a	16.3 ± 2.5^a	6.73 ± 1.11^a
Chekpe Breed	5.46 ± 0.12^a	15.6 ± 4.70^a	4.43 ± 0.25^a
Turuku Breed	8.06 ± 1.9^a	17.0 ± 3.9^a	5.97 ± 0.50^a
P-value	0.8795	0.6517	0.6979

Table 1: Mean of Bacterial Population of the Various Milk Samples.

*Means with the same superscript along the column are not significantly different ($p > 0.05$).

The organisms identified from the three different breeds were suspected to be; *E. coli*, *Staphylococcus aureus*, *Lactobacillus* spp., and *Klebsiella* spp. The isolates were categorized according to their colour, elevation, texture, shape, and Gram reaction for cultural features.

The prevalence of the bacterial isolates is shown in figure 1, it was observed that *Staphylococcus aureus* and *Escherichia coli* had the highest prevalence of 48.1%, which occurred in 13 samples, followed by *Klebsiella* spp. 44.4% and occurring in 12 samples, while the least prevalent is *Lactobacillus* spp. having 18.5% and occurring in 5 samples.

Results of the bacterial abundance in the samples revealed that the majority of the samples contained two or more different bacterial species and were mostly *Staphylococcus aureus* and *Escherichia coli* occurring in 13 samples, followed by *Klebsiella* spp. in 12 samples, and the least *Lactobacillus* spp. in 5 samples.

Comparing among breeds, the Bukolo breed had *Staphylococcus aureus* in 5 samples, *Klebsiella* spp., and *Escherichia coli* occurred in 4 samples each, while *Lactobacillus* spp. occurred in 2 samples. For the Chekpe breed, *Staphylococcus aureus* and *Escherichia coli* occurred in 5 samples, *Klebsiella* spp. occurred in 3 samples, while *Lactobacillus* spp. occurred in 2 samples each. In the occurrence of bacteria in the Turku breed, *Klebsiella* spp. occurred in 5 samples,

Staphylococcus aureus and *Escherichia coli* occurred in 3 samples, while *Lactobacillus* spp. occurred in 1 sample.

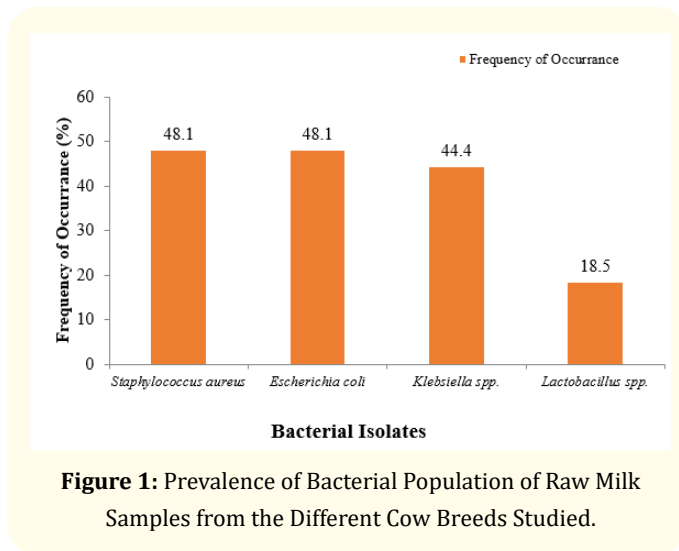


Figure 1: Prevalence of Bacterial Population of Raw Milk Samples from the Different Cow Breeds Studied.

Proximate composition results compared among three different breeds

Results of the proximate composition as presented in table 2 comparing the parameters among the three different breeds of cows (Bokolo, Chekpe, and Turuku breeds) for three milk samples each showed that the lowest moisture content of $83.72 \pm 1.70\%$ was recorded from the Chekpe breed, followed by Bokolo as 84.76

$\pm 1.17\%$, while the highest moisture content of $85.22 \pm 1.02\%$ was obtained from Turuku breed. Statistical analysis revealed that there was no significant difference between the set of data ($p > 0.05$).

The Ash composition varied having the lowest as the Turuku breed at $1.01 \pm 0.14\%$, followed by Bokolo at $1.21 \pm 0.07\%$, and Chekpe breed as the highest at $1.23 \pm 0.14\%$ and there was no significant difference between the data sets ($p < 0.05$).

Results of Fat composition showed that the lowest $1.85 \pm 1.04\%$ was obtained from the Turuku breed, followed by $2.43 \pm 0.35\%$ from Bokolo, while the highest value $2.47 \pm 1.23\%$ was obtained from the Chekpe breed, there was no statistically significant difference ($p > 0.05$) in the values obtained.

The least composition of Crude protein $4.62 \pm 0.55\%$ was obtained from the Chekpe breed, followed by the Turuku breed with $4.76 \pm 0.70\%$, while the highest Crude protein of $5.18 \pm 0.81\%$ was obtained from the Bokolo breed, no significant difference ($p > 0.05$) too among the values obtained.

Results of the proximate composition for carbohydrates had the least value of $6.43 \pm 1.56\%$ from the Bokolo breed, followed by Turuku with a value of $7.17 \pm 0.79\%$, while the highest value from the Chekpe breed $7.95 \pm 0.79\%$. The statistical analysis also revealed that there was no significant difference between the set of data ($p > 0.05$).

Sample	Moisture Content (%)	Ash (%)	Fat (%)	Crude Protein (%)	Carbohydrate (%)
Bokolo Breed	84.76 ± 1.17^a	1.21 ± 0.07^a	2.43 ± 0.35^a	5.18 ± 0.81^a	6.43 ± 1.56^a
Chekpe Breed	83.72 ± 1.70^a	1.23 ± 0.14^a	2.47 ± 1.23^a	4.62 ± 0.55^a	7.95 ± 0.79^a
Turuku Breed	85.22 ± 1.02^a	1.01 ± 0.14^b	1.85 ± 1.04^a	4.76 ± 0.70^a	7.17 ± 0.79^a
P-value	0.1681	0.0119	0.4696	0.3725	0.0916

Table 2: Mean Proximate Composition Of The Milk Samples from Three Different Breeds of Cow Studied.

*Means with the same superscript along the column are not significantly different ($p > 0.05$).

Discussion

Total Heterotrophic Bacterial Counts showed that the highest value was obtained in the Turuku breed at $8.1 \pm 1.9 \times 10^6$ cfu/ml, while the Chekpe breed on the other hand had the least counts of $5.5 \pm 0.12 \times 10^6$ cfu/ml. An increase in the value of Total Heterotrophic Bacterial Counts may suggest that milking and handling procedures

were not carried out with the utmost cleanliness. This suggests that milk may have been contaminated with microbes due to poor production and storage hygiene. Similar counts were reported by [19] for Bunaji and Bokooloji breeds averaging 5.71×10^6 cfu/ml and 5.77×10^6 cfu/ml, respectively; both of these values were five times higher than the 1.0×10^6 cfu/ml threshold recommended for

hygienic milk by milk centers participating in the price incentive program [19,20]. When milk is transported over long distances without being refrigerated, ambient temperature and relative humidity conditions that favour the growth of bacteria, may also contribute to the high THBC [18]. The THBC is a useful indication for checking the cleanliness of raw milk's production, collection, and handling processes [21].

The results of Total Coliform Counts showed that the highest values were obtained from the Bukolo breed at $6.7 \pm 1.11 \times 10^6$ cfu/ml, while the Chekpe breed also had the least counts at $3.4 \pm 0.25 \times 10^6$ cfu/ml. According to [22], the mean Total Coliform Counts of raw milk products reported ranged from 2.10×10^{10} to 5.12×10^{10} cfu/ml, thus were higher compared to the results from this study. The results obtained were higher than the acceptable standard for coliform counts safe for raw milk consumption at 1.0×10^3 cfu/ml [23].

Coliform counts may point to fecal contamination or contamination from poorly cleaned and sanitized equipment. Results of Fecal Coliform Counts showed that the highest value was obtained from the Turuku breed as $1.7 \pm 3.9 \times 10^4$ cfu/ml, while the Chekpe breed had the lowest value of $5.6 \pm 4.70 \times 10^3$ cfu/ml. These counts were observed to be higher than the acceptable counts for coliforms in safe raw milk consumed set by the [23] at 1.0×10^3 cfu/ml, which is in line with the counts obtained by [24] with a mean value of $4.13 \pm 0.757 \log_{10}$ cfu/ml for milk samples collected from dairy farms at Dire Dawa town. On the other hand, the mean fecal coliform counts obtained from this study were higher than the above research works.

There was no significant difference between Total Heterotrophic Bacterial, Total Coliform and Fecal Coliform Counts obtained from the milk samples studied ($p > 0.05$). This statistic value indicates that raw milk sources had similar levels of microbial contamination. The health and hygiene of the cow, the environment or "cowshed" in which the cow is housed and milked, as well as the storage location and state of the raw milk, can all have an impact on the level of microbial contamination [25].

The morphological and biochemical characterization revealed a variety of potential pathogens that, when present in high doses, can cause food poisoning. These isolates were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., and *Klebsiella* spp. in varied

proportions, which could be attributed to the milker's hand, the udders of animals, the milking environment, and other related factors [26].

Major isolates from the samples investigated by [6] were *E. coli*, *Staphylococcus aureus*, and *Klebsiella* spp. *E. coli*, *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Yersinia* spp. appeared to be present in milk samples obtained from the udder and storage containers, suggesting environmental contamination. Fasae and Ogunekun [18] also reported that *Bacillus* spp., *E. coli*, *S. aureus*, *S. epidermis*, and *M. luteus* were the five bacterial species isolated and identified. Identification of isolates using microscopic analysis and phenotypic features (particularly biochemical properties and sugar fermentation ability) has been proven to be very useful and is still the most widely recognized method [27].

In this study, four bacterial species were isolated from the various breeds of cow milk samples which include *Staphylococcus aureus*, *Klebsiella* spp., *Bacillus* spp., and *Escherichia coli*. Based on the raw milk samples studied, the prevalence of the bacterial isolates observed showed that *Staphylococcus* spp. had the highest prevalence, followed by *Klebsiella* spp. and *Bacillus* spp., while the least prevalence level was for *Escherichia coli*.

Based on the results observed from this study, it is observed that some previous research findings are similar to this study's results. Results of the prevalence rate obtained for *Staphylococcus* spp. in this study is however slightly higher than the prevalence value reported by [28] based on the total samples analyzed from a subset of dairy farms. In other studies, similar observations were reported by [29], where *Staphylococcus* spp. and *Escherichia coli* were isolated, with *Escherichia coli* having a higher prevalence rate compared to *Staphylococcus* spp. The prevalence of these pathogens found in these raw milk samples calls for public health concerns as humans can get infected through direct consumption of raw milk, which is likely to establish challenging health conditions.

From the results of the proximate analysis, it was observed that there were high levels of moisture content in the milk samples; Chekpe breed at 83.72%, Bokolo breed at 84.76%, and Turuku breed at 85.22%, and there was no significant difference in the set of data ($p < 0.05$). The shelf life of the milk sample is shortened as a direct result of the high moisture content, which is directly

linked to high water activity. This, in turn, encourages the growth of microorganisms, which further shortens the shelf life. On the other hand, low moisture content means low water activity, which leads to a reduction in microbial development and, as a result, a longer shelf life for the milk sample. The findings in this study are consistent with the information collected by other researchers [30,31]. Shiv, *et al.* [30] found that the proximate composition of the milk samples showed that the moisture content of the milk samples were 86.24%, 87.35%, and 83.92%, which fell within the range of 81% to 89.5% that the International Dairy Foods Association has approved for the proximate composition of milk.

Negash, *et al.* [32] reported that crude protein was between 3.42 and 3.50%. The current study found that crude protein was between 4.62 and 5.18%, which was within the acceptable range for cow milk protein, which was between 2.9 and 5% [33]. Negash, *et al.* [32] reveals that, low protein levels in raw milk could be caused by a high number of microorganisms, a lack of protein supplements for the animal, or other things related to the animal's environment or genes.

The current study demonstrated a significant ($p < 0.05$) difference in the ash content of cow milk samples from different breeds of cows, ranging from 1.01% to 1.37%. Changes in the amount of ash in milk may be related to environmental and genetic factors, including the availability of mineral licks and supplements in the area [34].

The overall proximate composition of cow milk varies from breed to breed, but generally, fat accounts for around 3–4% of the solid content. Fasaie and Ogunekun [18] reported 4.60% of fat content for the Bokolo breed in their research, which also linked closely with the results obtained from this study as fat content amounts to 2%-3%.

According to [35], cow's milk contains about 4.9–9% carbohydrate content, [36] also reported 4.9% carbohydrate content in their study. The carbohydrate content in this study ranged from 4.9% to 8.82%, which correlated with the report from other researchers. Generally, the composition of milk varies based on factors such as the species, breed, diet, and stage of lactation of the animal producing it [35].

Conclusion

This study has revealed that the bacteriological analysis of the raw cow milk samples were higher than that of the regulatory standard for milk consumption. The prevalence of these bacterial species that have the potential to cause food-borne infections is of public health concern. This research demonstrated that *Staphylococcus aureus*, *E. coli*, *Bacillus* spp., and *Klebsiella* spp., were present in the raw milk samples examined. The proximate composition including moisture, fat, ash, carbohydrate and crude protein content of the milk samples were within the acceptable limit. Consuming raw milk without the proper sanitary procedures of extraction and proper microbiological examination of possible contamination puts the lives of consumers at high risk of infection.

It is recommended that Cow breeders/handlers should be trained to maintain proper hygiene at all times, especially during milking. Proper environmental sanitation and sterilization of materials used for milking should be advocated. To prevent bacterial growth in raw milk, a variety of raw milk preservation and treatment options should be evaluated.

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