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Microbial Evaluation of Raw Milk from Dairy Farms in Udi L.G.A Enugu State, Nigeria

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Abstract: *Milk is a food that inherently favours microbial growth and due to its characteristics several precautions must be taken to prevent contamination in its production, processing and consumption, which are routinely subject to changes. This study was carried out to evaluate microbial quality of raw cow milk taken at different sampling points from Amaokwe, Obioma and obinagu, all in Udi L.G.A of Enugu State between Septembers to November 2014. The samples were serially diluted and inoculated using the streaking technique. Overall mean heterotrophic count, coliform count; yeast and mould count of milk produced in the study area were 1.25 x 10^3 - 8.0 3 x 10^2 cfu/ml, 3.17 x 10^3 - 2.87 x 10^3cfu/ml, and 1.87 x 10^3 -0.90 x 10^2cfu/ml, respectively. Amongst the organisms isolated, Bacillus Spp, Escherichia coli and Staphylococcus Spp had the highest frequency of occurrence (3). Only one species of Rhizopus was isolated. Raw milk collected from the dairy farm at Obinagu had the highest number of isolates (6). High heterotrophic, coliform and yeast and mould count obtained in this work is an indication of poor sanitary condition and this call for scrupulous hygienic measures during the handling of milk and its product.*

Keywords: *Milk, Contamination, Microbial Evaluation, Fungi, Coliform and Heterotrophic.*

I. Introduction

Milk, being a complex mixture, nutritious, with a high level of water and a pH close to neutral, is highly perishable (Maldaner*et al.,* 2012). It is a product highly conducive to microbial growth, especially bacterial pathogens (Chye*et al.*, 2004). Milk from a healthy udder contains very few numbers of bacteria (<3x10^4cfu/ml) but may become contaminated by microorganisms from the surrounding environment during milking and milk handling, from water and milk equipment (Cousins and Bramley, 1981) Thus the number of bacteria in milk directly influences the quality and safety of dairy products (Arcuri*et al.,* 2006). Due to its characteristics, milk deserves special attention in its production, processing, marketing and consumption. Several factors, such as the health of the herd, sterility of the cleaning equipment and utensils used to obtain it, the health conditions of the milking place, the excretion from the udder of an infected animal and quality of water used on the farm, may influence the microbiological quality of milk products (Amaral*et al*., 2003). However, despite the fact that presently production is growing using the modern technology, many milk producers still use non-specialized methods, resulting in raw milk of poor quality (Correa, *et al.*, 2009). The milk contaminated by high levels of bacteria usually be-comes unsuitable for further processing since it does not meet the consumer's expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes) (Nanu*et al.*, 2007). The globalization of markets, including the availability of a variety of imported dairy products, has made consumers to become more demanding about the quality and safety of the products offered (Nada *et al.,* 2012). The dairy industry has undergone major transformations in an attempt to become more competitive, with benefits to the producer in terms of quality (Gonzalez *et al.,* 2004). The parameters physico-chemical, microbiological, hygienic, and sanitation measures have been deployed by the industry to test and verify the quality of milk (Guerreiro*et al.,* 2005).

Dairy products quality defects have been attributed to poor microbiological quality of raw milk and heat-resistant enzymes (Marshall, 1982; Muir *et al*., 1986). The production of high quality milk should therefore be priority for good quality end products of long shelf life and for marketing of value added products. This is generally not easy to achieve in developing countries due to factors such as poor hygiene and sanitation during milking and milk handling, unclean water, high ambient temperatures, lack of cooling facilities and inadequate infrastructures for milk transportation to the processing facilities [Berg, 1986; Bille*et al*., 2000].

The objective of this study were to determine the microbiological quality of raw milk produced in Udi of Enugu State for value addition, safety, shelf- life and dairy herd management.

II. Materials And Methods

Study Area and Sample Collection

The study was conducted atUdi L.G.A of Enugu State, South-East Nigeria from September to November, 2014.The collection points of raw milk wereObinagu, Amokwe, and Obioma from the Fulani cattle settlement farms. The raw milk sample was directly collected from the milking bucket using sterile container with cover. The time of collection was between 6.30am – 8.00am (during milking) there after the milk was conveyed to the laboratory for analysis.

Media and Instruments

Media used includes; MacConkey Agar for enumeration of coliforms, Nutrient Agar (NA) for purification of cultures and for storage in slants, de Man Rogosa and Sharpe Agar MRS for selective isolation of *Lactobacililus* sp., Eosin Methylene Blue Agar (EMB) for selective isolation of *Escherichiacoli*, Mannitol Salt Agar (MSA) for isolation of *Staphylococcusaureus, Salmonella Shigella*Agar for selective isolation of *Salmonella* and *Shigella* and Sabrouard Dextrose Agar (SDA) for isolation of fungi. They were all prepared according to the manufactures specifications. They were sterilized by autoclaving at 121°C for 15 Minutes at 15 Psi. They were allowed to cool at about 45°C before been poured into sterile petri dishes. Materials used includes glass petri dishes, conical flasks, scalpel, wire loops were all sterilized in hot air oven at 160°C and allowed to cool before use. The benches were sterilized with 95% ethanol to reduce the microbial load on the work bench as described by Cheesebrough, (2003).

Samples inoculation

One (1) ml of each of the raw milk samples was measured into a sterile small beaker. The raw milk in each beaker was mixed with 9ml of sterile distilled water. This was stirred very well using sterile glass rod. Ten (10) fold serial dilutions were carried out. From the 1st tube, 1ml of the sample was collected and discarded into the second test tube; from the 2nd test tube 1ml was collected and discarded into the 3rd test tube following microbiological methods. This was done till the 10th dilution and 1ml remaining was discarded. Then from a suitable dilution, 0.1ml of each dilution was uniformly spread over the surface of prepared media plates; Nutrient Agar (NA), de Man Rogosa and Sharpe Agar MRS, Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA), *Salmonella Shigella*Agar (SSA) and Sabrouard Dextrose Agar (SDA). The plates were prepared and inoculated in duplicates. They were incubated for bacteria growth at 37°C for 24 hours while Sabrouard Dextrose Agar (SDA) was kept at Room temperature for 5-7 Days for fugal growth. After the expiry of the incubation period, the plates were checked and observed for bacterial and fungal growths. The number of colonies in each plate was recorded. The culture plates with number of colonies less than 300, and its duplicate, for each sample, was selected. Any count obtained was multiplied by the dilution factor and expressed as colony forming unit (CFU) per milliliter of the original sample.

The Total Viable, total coliform and Total fungal counts were all calculated suing this formula below;

TVC (cfu/ml) = 1/v x N x D

V = Volume of innoculum

N = Number of colonies counted

D = Dilution factor

Identification of Bacteria and Fungi

Identification and characterization of bacterial isolates were carried out using standard methods as described by Cheesebrough, (2005). The shape and arrangement of the cells were observed by the use of electron microscope. They were future identified following biochemical analysis which includes; Gram's staining, Indole test, Catalase test, Oxidase test, coagulase and sugar fermentation.

Identification ofFungi

Fungal isolates were identified macroscopically and microscopically using lactophenol cotton blue. A portion of the isolated fungi was collected using sterile needle and placed on the microscope slide. A few drops of lactophenol cotton blue (LCB) was added on it, followed by teasing using needle so as the LCB will penetrate into the cells of the fungi. Later it was covered with cover slips and viewed under X40 and X100.

III. Results

Table 1. shows the total microbial population found in the different samples of the raw milk accessed such as raw milk fromObinagu-Udi as sample M_1, raw milk from Obioma-Udi as sample M_2 and raw milk from Amokwe-udi as sample M_3. The highest mean total heterotrophic count was observed in sample 1 which is 1.25 x 10^3cfu/ml followed by 9.93 x 10^2cfu/ml in sample 2 while sample 3 had the least total heterotrophic count of 8.03 x 10^2cfu/ml.The highest mean total yeast and mould count of 1.87 x 10^3cfu/ml was observed in sample 2

followed by 1.80 x 10^3cfu/ml in sample 1 while sample 3 had the least total yeast and mould count of 0.9 x 10^2cfu/ml. All the milk samples had total coliform count of which sample 2 was the highest with 3.17 x 10^3cfu/ml, followed by sample 1 with 3.3 x 10^3cfu/ml while sample 3 had the least count of 2.87 x 10^3cfu/ml.

Table 1: Microbial population in the raw milk samples

Colony counts log_{10}cfu/ml	**M_1**	**M_2**	**M_3**
Total coliform counts	3.3 x 10^3	3.17 x 10^3	2.87 x 10^3
Total heterotrophic count	1.25 x 10^3	9.93 x 10^2	8.03 x 10^2
Total yeast and mould counts	1.80 x 10^3	1.87 x 10^3	0.9 x 10^2

Key = M_1 = Raw milk from Obinagu – Udi
M_2 = Raw milk from Obioma – Udi
M_3 = Raw milk from Amokwe – Udi

Table 2. shows the biochemical characteristics of bacterial isolates from the raw milk samples of which the probable organisms identified in sample 1 are *Staphylococcus* species, *Klebsiella* species, *Bacillus* species, *Streptococcus* species and *Escherichia coli*. *Staphylococcus* species which have positive gram reactions, clustered cocciin shape, catalase positive and indolenegative, in the sugar fermentation test, glucose, fructose and sucrose had a colour change which showed the presence of acid and there was no air space which showed the absence of gas. *Klebsiella* species which had a gram negative rods with a sugar fermentation that showed the presence of acid and gas, citrate positive. And a gram negative rods with a sugar fermentation that showed the presence of acid and gas. An indolepositive with oxidase negative which showed the presence of *Escherichia coli.* In sample 2, there was a positive gram reaction, presence of short rods, catalase positive,indolenegative, then in the sugar fermentation, there was a colour change and an air space which showed the presence of acid and gas in the sugars used. And the probable organism identified was *Bacillus* species, alsoin sample 2, *Escherichia coli* was also probably identified. And a gram positive , cocci in chain shape, catalase negative , oxidase negative , citrate negative and there was a presence of acid and gas which showed a probable organism of the species *Streptococcus*also in sample 2. And in sample 3 the probable organisms identified are *Escherichia coli*, *Staphylococcus* species,*Klebsiella* species and *Bacillus* species.

Table 2: Biochemical characteristics of bacterial isolates from raw milk samples

Sample	**Gram Reaction**	**Shape**	**Catalase**	**Indole**	**Oxidase**	**Citrate**	**Glucose**	**Fructose**	**Surcose**	**Probable organism identified**
M_1	+	Clustered cocci	+	-	-	-	A	A	A	*Staphylococcus* species
	-	Rods	-	-	-	+	A/G	A/G	A/G	*Klebsiella*species
	-	Rods	-	+	-	-	A/G	A/G	A/G	*Escherichia coli*
	+	Short rod	+	-	-	-	A/G	A/G	A/G	*Bacillus* species
	+	Cocci in chain	-	-	-	-	A/G	A/G	A/G	*Streptococcus* species
M_2	+	Short rod	+	-	-	-	A/G	A/G	A/G	*Bacillus* species
	-	Rods	-	+	-	-	A/G	A/G	A/G	*Escherichia coli*
	+	Cocci in chain	-	-	-	-	A/G	A/G	A/G	*Streptococcus* species
	+	Clustered cocci	+	-	-	-	A	A	A	*Staphylococcus* species
M_3	-	Rod	-	+	-	-	A/G	A/G	A/G	*Escherichia coli*
	+	Clustered cocci	+	-	-	-	A	A	A	*Staphylococcus* species
	-	Rods	-	-	-	+	A/G	A/G	A/G	*Klebsiella*species
	+	Short rod	+	-	-	-	A/G	A/G	A/G	*Bacillus* species

Keys = + = Positive - = Negative - = Nil A = Acid G = Gas

Table 3.Shows the biochemical characteristics of fungi isolates from the raw milk samples. Sample 1 and 2 have a gram positive reactions which are cocci in shape, they have clustered conidiophores when viewed under the microspore with the lactophenol cotton blue and the probable organismsidentified were the *Fusarium*species while in sample 3, the gram reaction was negative with a shape of cocci hyphae, when viewed under the microscope with lactophenol cotton blue sporangin and columella were seenwhich the probable organisms identified was *Rhizopus* species.

Table 3: Biochemical characteristics of fungi isolates from raw milk samples

Samples	Lactopherical cotton blue	Probable Organism Identified
M_1	Clustered conidiophores	*Fusarium* species
M_2	Clustered conidiophores	*Fusarium* species
M_3	Sporangia and columella	*Rhizopus* species

Keys = + = positive - =negative
$M_{1=}$ Raw milk from Obinagu-Udi
M_2 = Raw milk from Obioma-Udi
M_3 = Raw milk from Amokwe-Udi

Table 4.Shows the specific isolation of isolates from the raw milk samples, of which *Bacillus* species, *Escherichiacoli*, *Staphylococcus* species were present in all the raw milk samples. *Klebsiella* species were present in sample 1 and 3, *Streptococcus* species were present in sample 1 and 2, *Fusarium* species were present in sample 1 and 2 and *Rhizopus* species were present in sample 3.

Table 4: Specific isolation of isolates from the raw milk samples

Microbial isolates	M_1	M_2	M_3
Bacillus species	1	1	1
Escherichia coli	1	1	1
*Klebsiella*species	1	0	1
Staphylococcus species	1	1	1
Streptococcus species	1	1	0
*Rhizopus*species	0	0	1
*Fusarium*species	1	1	0
Total	**6**	**5**	**5**

Keys:
M_1 =Raw milk from Obinagu- UdiM_2 = Raw milk from Obioma- Udi
M_3= Raw milk from Amokwe-Udi

Table 5.sixteen (16) microbial isolates were found associated with the raw milk samples of which *Bacillus* species occurred by 18.75% in all the samples, *Escherichia coli* occurred by 18.75% in all the samples, *Klebsiella* species occurred by 12.5% in sample 1 and 3, *Staphylococcus* species occurred by 18.75% in all the samples, *Streptococcus* occurred by 12.5% in sample 1 and 2, *Fusarium* species occurred by 12.5% in sample 1 and 2, and *Rhizopus* species occurred by 6.25% in sample 3.

Table 5: Percentage of occurrence of the different microbial isolates in the raw milk samples

Microbial isolates	Number of isolates	Percentage of occurrence (%)
Bacillus species	3	18.75
Escherichia coli	3	18.75
*Klebsiella*species	2	12.5
Staphylococcus species	3	18.75
Streptococcus species	2	12.5
*Rhizopus*species	1	6.25
*Fusarium*species	2	12.5
Total	16	100

IV. Discussion

Temperature, chemical composition and total aerobic counts are usually used to determine the overall hygiene, sanitation, quality and storage conditions of raw milk.Sample 3 had the least of the total heterotrophic count, total yeast and mould count and the coliform bacteria count. This could be because the cows are reared in a more hygienic environment, unlike the sample 2 which had the highest count of coliform, yeast and mould, and high heterotrophic count because of the poor facilities and unhygienic farm settlement under which the cows are been reared. The result of the total viable bacterial count reported in this study is in agreement with those reported by Farhan and Salik (2007),slightly higher than those reported by Afif*et al.* (2008) and higher than those reported by Bille*et al.* (2009). The presence of high numbers of coliforms in milk provides an index of hygienic standard used in the production of milk. The isolation of these organisms from milk could be attributed to the contamination of udder and teats from the soil, feed, personnel or water. S. aureus was present in the milk sample analyzed, but in a consistence proportion with regard to microbiological criteria. Chye*et al*. (2004) and Afif *et al*. (2008) reported that the presence of S. aureus in milk samples is related to environmental conditions. Although S. aureus microbial load in the samples obtained in this study is below the accepted microbiological criteria, appropriate arrangements must be made to counteract this contamination, because the presence of *S, aureus* in food presents potential risk to consumer health due to its production of enterotoxin

(Cenci – Goga*et al.*, 2003). The presence of *Escherichia coli* in sample 3 does not necessarily indicate a direct faecal contamination of milk, but it is considered to be an indicator of poor hygiene and sanitation during milking and post manipulation. The presence of these bacteria in milk can also be linked to contamination by cows excrements, land and water used (Chye*et al.*, 2004). Microbial load in E. coli obtained in this study is comparable to that obtained by Farhan and Salik (2007).The presence of *Klebsiella*species in the raw milk is not surprisingly as it is a normal flora of the intestine of the cow, although it naturally occurs in the soil, water and vegetables (grasses) (Ryan and Ray, 2004). This is likely to be as a result of the water or grass they are feed with. According to Amaral, *et al.* (2003), water used in production has great influence on the contamination of the milk, and being a vehicle for transmission of pathogen, must have characteristics of portability. The *Fusarium* species found in sample 1 and 2 could be as a result of the environment where the cows are reared;this is because*Fusarium* is widely distributed in soil and is associated with plants and are relatively abundant members of soil microbial community. Furthermore, Polyak and Myasinikova (1993) have also shown the presence of other pathogens like *Conuellaburunetti* and *Streptococcus agalactiae* in raw milk aseptically drawn from the udder. The presence of Staphylococcus and Streptococcus in raw milk have been noted, likewise the presence of Pseudomonas species and coliform in raw milk have been elucidated by Kleter (1984).

The highest yeast and mould count was observed from raw milk from sample 2. As observed during the collection of the milk, the environment was quite unkempt. There were heaps of old hays onto which the cows have defecated, urinated and even poured water. This condition of the old hays made them habour microbes especially fungi which eventually get into the animal either during feeding, lying down etc. Sample 3 site was quite kept, no wonder the low fungi growth recorded against it. The pathogens that have been involved in foodborne out-breaks associated with the consumption of milk include *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, *Staphylococcus aureus*, *B. cereus* and *Costridiumbotulinum*and thermo tolerant coliforms, especially *Escherichia coli* that is the most common contaminant of raw and processed milk (Chye*et al.*, 2004,Mhone*et al.*, 2011). According to Mhone*et al.*, 2011, the total count of bacteria also became one of the criteria to evaluate the classification and processing of dairy products.The production of high quality milk should therefore be priority for good quality end products of long shelf life and for marketing of value added products. This is generally not easy to achieve in developing countries due to factors such as poor hygiene and sanitation during milking and milk handling, unclean water, high ambient temperatures, lack of cooling facilities and inadequate infrastructures for milk transportation to the processing facilities (Bekele and Bayileyegn, 2000). Therefore, there is need to evaluate microbes associated with milk and determine factors that necessitate the contamination of our milk and milk products.

V. Conclusion

In conclusion, high bacterial count obtained in this work is an indication of poor sanitary condition. Moreover, the microbial load of raw milk collected from the milking bucket aseptically drawn reveals that the milking condition (bucket, milk maids and milking environment) is an important source of milk contamination.

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