The Bacteriological Quality of Raw cow milk obtained from Herdsmen in Okigwe,Imo State,Nigeria

ABSTRACT

This study was conducted to examine the bacteriological quality of cow's raw milk obtained from herdsmen at Okigwe, Imo States. Standard microbiological procedure was used to carry out this research. A total number of five samples were gotten from different streets in Okigwe metropolis in sterile specimen containers. Ten fold serial dilution was carried with each of the sample with the 4th tube to 6^{th} tube being inoculated on Nutrient agar, Salmonella- Shigella agar and MacConkey agar by spread plate method and incubated at 37^{o}C for 24 hours.The microorganisms were identified were *Staphylococcus aureus*, *Corynebacterium* sp, *Salmonella typhi*, *Streptococcus* sp, *E.coli*, *Lactobacillus* sp and *Salmonella typhi*.The total viable count for raw cow milk ranged from 6.6 $x10^5$ CFU/ml to 1.01 x 10^6CFU/ml while total coliform count was from $6.2x10^6$ CFU/ml to $1.7x10^6$ CFU/ml. They frequency of occurrence of Lactobacillus sp was highest with a value 85 and the lowest frequency was *Salmonella typhi* II with a value of 2.The raw cow milk was observed to harbor some pathogenic microorganisms which can cause infections when ingested.

Keywords: *Lactobacillus* sp, Nutritious, Digestible, inoculation

1.0 . INTRODUCTION

Milk is a yellowish-white non transparent liquid secreted by the mammary gland of all mammals. It's the primary source of nutrition and also sole food for off spring of mammals before they are able to eat and digest other types of food. It contains all the balanced form of the entire necessary and digestible element for building and maintaining the human and animal body (Panday and Voskul, 2011).Milk, being a complex biological food, nutritious with a high level of water and a pH close to neutral (6.4 to 6.8), is highly perishable. It is a product highly conducive to microbial growth, especially bacterial pathogen (Chye, *et al.,* 2004). Because of its specific production, it is impossible to avoid contamination of milk with microorganism. Bacterial contamination of raw milk can originate from different sources such as air, milking equipments, feed, soil, grasses (Coorevits *et al.,* 2008). Due to its characteristics, milk deserves special attention in its production, processing, marketing and consumption. Therefore, the bacteriological quality of milk is a major factor in determining its safety for consumption (Nanu *et al.,* 2007). The condition during storage and transport in refrigerated tanks cause the raw milk micro-biota from predominantly gram's negative bacteria organisms as they grow. Gram's negative bacteria usually account for more than 90% of the bacterial population in cold raw milk that has been stored. The gram's negative flora is composed mainly of psychotropic species of

Pseudomonas, Achromobacter, Aeromonas, Serratia, Alcaligenes, Chromobacterium, Flauobacterium And Enterobacter (Nada *et al.,* 2012). Organisms unable to grow at refrigeration temperature remains at low number, implying that temperature is an important factor contributing to the prevalence and proliferation of specific organisms in milk. Pasteurization in raw milk is effective in eliminating all but not thermometric organisms of the general mycobacterium, micrococcus, streptococcus, lactobacillus, bacillus, clostridium, the coryne forms and occasionally, some gram's negative rods (Mhone *et al.,* 2011). Psychographs can grow at refrigeration temperature below 7^{o}C, produce enzymes, toxins and other metabolites. Most of these bacteria produce extra cellular photolytic and lipolytic enzymes are not inactivated by pasteurizing at 72^{o}C for 15 seconds or by ultra-high temperature treatment (Giacomehi, *et al.,* 2014). Pasteurization cannot guarantee the absence of microorganism when present in large number in raw milk or due to pasteurization contamination (Farhan and Salik, 2007). The contamination of milk and some milk products are due to the production of enzymes/toxins by some bacteria by some bacteria like *Staphylococcus aureus and Lactobacillus spp* (Cenci *et al.,* 2003).The aim was to assess the bacteria quality of cow's raw milk obtained from Hawkers in Okigwe and to evaluate the degree of bacterial contamination of cow's raw milk.

MATERIALS AND METHODS

COLLECTION OF SAMPLE

The samples of cow's fresh raw milk were collected from five different locations in a sterile screw capped containers from Hausa hawkers at different milking location in Okigwe metropolis and transported in ice-cubed container to Science Laboratory Technology of Federal Polytechnic, Unwana, Ebonyi State for Microbiological examination.

INOCULATION, INCUBATION AND ISOLATION OF SAMPLES OF CULTURE MEDIA

Ten folds serial dilution was done on each sample of the cow's fresh raw milk. Exactly 0.1 ml of each dilution of the samples were aseptically taken with a micro pipette and inoculated on Nutrient agar, Mannitol salt agar and MacConkey respectively by spread plate method. The inoculated agar plates were inverted after 15 minutes of inoculation and then incubated at 37^{o}C for 24 hours. All colonies formed were counted, recorded and further purified by subsequently streaking on nutrient agar plates in order to obtain pure isolates. The pure culture colonies obtained were aseptically preserved on nutrient agar slant in bijou bottles for further identification.

IDENTIFICATION OF ISOLATES

The isolates were identified by their morphological and biochemical characteristics followed by Bergey's Manual of determinative bacteriology.

RESULTS

IDENTIFICATION OF THE ISOLATES

The results of the colonial morphological characteristics of the isolates are presented in table 1. The results showed tentative identification of seven bacterial isolates which include: *Staphylococcus aureus, Corynebacterium spp., Salmonella typhi, Lactococcus spp., Streptococcus sp., Salmonella typhimurium* and *Escherichia coli.*

Table 1: Colonial Morphology and Biochemical Characteristics of the Isolates

Isolate	Shape	Opacity	Elevation	CNA	CMAC	CSSA	Gram	Goag.	Cat.	Oxi.	Ure.	VP	MR	Cit.	Glu.	Suc.	Lact.	Ind.	TSIA Slope	TSIA Butt	TSIA H2S	TSIA Gas	Tentative Organism
A	Circular	Transparent	Flat	Light yellow	Colorless	Black centre	-ve rod	NA	+	-	-	-	+	-	+	-	-	-	R	Y	Weak +	-	*Salmonella typhi*
B.	Circular	Translucent	Convex	Whitish	Pink	-	-ve rod	NA	+	-	-	-	+	-	+	+	+	+	Y	Y	-	+	*Escherichia coli*
C	Circular	Opaque	Raised	yellowish	-	-	+ve cocci	+	+	-	+	-	+	-	+	+	+	-	NA	NA	NA	NA	*Staphylococcus aureus*
D	Circular	Opaque	Convex	Grayish	-	-	+ve rod	-	+	+	-	-	+	-	+	-	-	-	NA	NA	NA	NA	*Corynebacterium* spp.
E	Circular	Opaque	Raised	Yellowish	-	-	+ve cocci	-	-	-	+	NA	NA	-	-	+	-	NA	NA	NA	NA	NA	*Staphylococcus* sp
F	Circular	Translucent	Raised	Grayish	-	-	+ve Rod	-	-	-	-	-	+	+	+	+	+	-	NA	NA	NA	NA	*Lactobacillus* sp
G	Circular	Opaque	Raised	yellowish	-	-	+ve cocci	+	+	-	+	-	+	-	+	+	+	-	NA	NA	NA	NA	*Staphylococcus aureus*
H	Circular	Transparent	Flat	Light yellow	Colorless	Black	-ve rod	NA	+	-	-	-	+	+	+	+	-	-	R	B	Strong +	+	*Salmonella typhimurium*

Key: CNA = Color on Nutrient Agar,CMAC = Color on MacConkey Agar,CSSA = Color on Salmonella Shigella Agar

KEY: Gram = gram reaction, Coag. = Coagulase test, Cat. = Catalase test, Oxi. = Oxidase test, Ure. = Urease test, VP = Voges –Proskauer test, MR.= Methyl red test, Cit. = Citrate test, Glu.= Glucose, Suc. = Sucrose, Lact. = Lactose, Ind. = Indole, TSIA = Tripple sugae iron agar, - = negative, + = positive, NA = Not applicable, R = red, Y = yellow, B = Balack

Table 2: Total Viable Count:

	SAMPLES	NO. OF COLONY	CFU/ML
A	Oboumulolo	91	9.1×10^5
B	Ihube	101	1.01×10^6
C	Umuchima	77	7.7×10^5
D	Ikpaeze	70	7.0×10^5
E	Ndiakwaeke	60	6.6×10^5

TOTAL VIABLE COUNT

The result of the colony counting is presented in table 2. The result shows that the colony forming unit (CFU/ml) of the isolates from the milk samples analyzed ranged from 6.0×10^5 to 1.01×10^6 and sample B had the highest load of bacteria (1.01×10^6) while sample E had the lowest load (6.0×10^5).

Table 3: Percentage Occurrence of the Isolates

	TYPES OF BACTERIA ISOLATE	FREQUENCY	PERCENTAGE
A	*Escherichia coli*	65	31.1%
B	*Staphylococcus aureus*	29	13.9%
C	*Salmonella typhi*	17	8.1%
D	*Corynebacterium spp.*	6	2.9%
E	*Lactobacillus spp*	85	40.7%
F	*Streptococcus spp.*	5	2.4%
G	*Salmonella typhimurium*	2	0.9%
	Total	209	100%

PRECENTAGE OCCURENCE OF THE ISOLATES

Table 3 presented the result of frequency occurrence of bacteria isolated in the raw cow milk samples. It showed that *Lactobacillus spp* had the highest frequency occurrence (40.7% compared to other bacteria counter parts; *Escherichia coli* (31.1%), *staphylococcus aureus* (13.9%), *Salmonella typhi* (8.1%), *Corynebacterium* (2.9%), *Streptococcus spp.* (2.4% and *Salmonella typhimurium* (0.9%).

Table 4: Total Coliform Count

	SAMPLES LOCATION	TOTAL COLI FORM COUNT	CFU/ML
A	Oboumulolo	36	3.6×10^5
B	Ihube	62	6.2×10^4
C	Umuchima	44	4.4×10^5
D	Ikpaeze	17	1.7×10^6
E	Ndiakwaeke	24	2.4×10^5

TOTAL COLI FORM COUNT

Table 4 shows the total coliform counts. The results of the coli form count shows that the (Cfu/ml) ranged from 1.7 x 10^6 to 6.2 x10^4. Sample B (6.2 x10^4) had the highest count while sample D had the lowest (1.7 x 10^6).

DISCUSSION

The total bacteria count and total coliform count obtained from the raw cow milk samples ranged from 6.6x10^5 – 1.01x10^6 CFU/g and 6.2x10^4 to 1.7x10^6 CFU/g respectively as shown in table 2 and 4. Some species of bacteria were isolated from the raw cow milk samples which were examined. They were characterized and identified as *Staphylococcus aureus, Streptococcus spp, Lactococcus spp., Corynebacterium spp., Escherichia coli, Salmonella typhi and Salmonella typhimurium* (table 3). The presence of these bacteria in raw cow milk suggested contamination from various sources such as animals, humans, environment and utensils. This corroborates the findings of Kumar *et al.,* (2006), who reported to have isolated a strain of *Staphylococcus aureus* and pathogenic strain of *Escherichia coli* from milk and milk products. *Lactobacillus spp* had the highest microbial count and they can apparently affect the keeping quality of the milk product as shown in table 3. Among the food poisoning organisms *Staphylococcus aureus, Escherichia coli and Salmonella spp* were isolated and this may be due to unhygienic handling, storage, environment and failure of Good Extraction Practice (GEP). This corroborates the work of Jeykumar, *et al*., 2013 who also reported the presence of *S. aureus* in milk which may be from mastitic animals or human sources. In table 3, the percentage frequency of occurrence of *Lactobacillus* sp was highest followed by *E.coli*. The *Lactobacillus* was the major fermenting organism, while the *E. coli* was the major pathogenic organism in the milk. *Escherichia and Salmonella spp* are pathogenic organisms whose presence in food causes spoilage due to fecal contamination of that food product as found in the raw milk analyzed (table 3).This agrees with the work of Oliver *et al*., 2005.The total viable count of milk from Oboumulolo has a highest value of 9.1x10^5 CFU/ml and the lowest was recorded in Ndiakwaeke with a value of 6.6x10^5CFU/ml (table 2).The overall contamination of very high number of milk samples especially at Oboumulolo could be due to failing to wash the udders before milking, insanitary farms, no mastitis investigates, lengthy delivery time, lack of education, unhealthy milking vessels and poor staff's hygiene. The total viable count in Ikpaeze recorded the highest with 1.7x10^6CFU/ml, followed by Umuchima, 4.4x10^5CFU/ml and the least was recorded in Ihube as 6.2 x 10^4 CFU/ml. This could be as a result of unhygienic practices in these locations and the presence of faecal matters within the whole environment. This result agreed with the findings of Wehr, (2004) who reported that improper handling, processing and packaging could lead to the presence of fecal coliform bacteria.

CONCLUSION

Milk for human consumption must be free from pathogens and must if conditions permit, contains no or few bacteria. Clean milk could only be obtained if effective sanitary measures are taken starting from the point of milk withdrawn from the cow until it reaches the consumers. Milk produced by small holder dairy cow owners in Okigwe districts' zone were of poor quality, risky for human consumption and can be a potential source of milk-borne infections. Poor milking procedures, milk handling practice including the surrounding environment and treatment

practices has greater influence on the bacterial contamination of raw milk and contributes to zoonotic pathogens. Based on the level of counts found in the milk ready for consumption, one may suppose that this milk may pose a public health risk and this suggest the need for more strict preventive measures which will raise public health concern about safety of milk to consumer.

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