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A PRELIMINARY STUDY OF THE BACTERIOLOGICAL EVALUATION OF RAW MILK FROM KOGI STATE UNIVERSITY DAIRY FARM

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ABSTRACTS
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. Raw milk is a complex mixture which is highly nutritious, contain high level of water (85%) and a pH close to neutral which makes it highly perishable and a suitable medium for the growth and multiplication of microorganisms. This study was carried out to evaluate the bacteria associated with raw milk from a dairy farm. A total of seven (7) raw milk samples were gotten from different lactating cows. The samples were examined for pH, organoleptic property, and turbidity. Isolation of bacteria was done using the pour plate method, gram staining and various biochemical test were conducted to identify the organisms. The pH value of the samples ranged from 6.5 to 6.9. The total bacterial count ranged from $4.3x10^6$ to $9.0x10^4$ CFU/ml, and total coliform count ranges from $5.1x10^6$ to $4.0x10^4$ the bacteria isolated were Bacillus spp Escherichia coli, Staphylococcus aureus, Klebsiella spp and Streptococcus spp. The high bacteria count obtained in this study is an indication of poor sanitary condition and this calls for strict hygienic measure during handling of raw milk and its products.

INTRODUCTION

Milk is a yellowish-white, non-transparent liquid secreted by the mammary glands of all mammals. It is the primary source of nutrition and sole food for offspring of mammals before they are able to eat and digest other types of food. It contains a balanced form of all the necessary and digestible elements for building and maintaining the human and animal body. The main composition of milk is water (87 – 88%) the remaining part is total milk solids which include carbohydrates, fat, proteins and ash or minerals (Pandey and Voskuil, 2011). Milk as a food of high nutritional value is highly associated with microorganisms. Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans. Being a highly perishable and highly nutritious food, raw milk serves as an ideal medium for the growth and multiplication of various microorganisms if not properly sterilized and consumed directly or used for the production of milk products, causes disease to man and contribute to the spoilage of milk and milk products (Addo *et al.,* 2011).

Milk being a complex mixture with a high level of water and a pH close to neutral, is highly perishable Almost 87% of milk is composed of water and the remaining part comprises total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements for building and maintaining the human and animal body (Pandey and Viskuil, 2011). Other milk ingredients include immunoglobulin which protect the newly born against a number of diseases. Milk has a complex biochemical composition and its high water activity and nutritional value serves as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exists (Maldaner *et al.,* 2012).

Milk meant for human consumption must be free from any pathogenic organisms. Microbial contamination of milk may cause milk-borne diseases to humans while others are known to cause milk spoilage. The bulky nature of milk and its nutritious characteristics attract microbes which contribute largely to the perishability of milk (Orregård, 2013)

Clean milk production depends on milking environment, the milker hygiene, cleanliness of udder, teats and containers that are used to store the milk. Contamination of milk after leaving the farm gate is largely due to poor milk handling practices and milk adulteration (Street and Bogor, 2013).

There is a need to make sure that milk is kept clean by avoiding all sources of contamination. This can be achieved by maintaining a high level of hygiene on milk handling (Debela, 2015).

Bacterial contamination of raw milk can originate from different sources; air, milking equipment, feed, soil, feces and grass. The number and types of microorganisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health. It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk. Rinsing water for milking machine and milking equipment. Washing also involve some of the reasons for the presence of a higher number of microorganisms including pathogens in raw milk (Fadaei, 2014).

MATERIALS AND METHODS

Materials used

Testubes, wireloop, conical flask, beaker, nutrient agar, macconkey agar, manitol salt agar, salmonella shigella agar, Eosin methelene blue agar, testube rack, measuring cylinder, weighing balance, hot air oven, autoclave glass slides, Bunsen burner, petri dishes, glass slide.

Collection of milk samples

Milk samples were collected directly from the udder of seven different lactating cow from the dairy farm. Approximately 10ml of milk were collected and put into a sterile screw capped tubes. The samples were drawn from pooled containers containing milk that were milked on that particular day which was either consumed at household level or sold to the public. All samples were labelled for identification and stored in a cool box with ice packs during field work. Thereafter, the samples were transported to the laboratory and stored at -20°C.

Determination of pH of raw milk sample

The pH meter was calibrated in a pH buffer solution (7.0) and the electrode was washed with distilled water and dried by light blotting. The electrode was dipped into the beaker containing the sample and the scale reading was recorded. The electrode was washed with distilled water before dipping into the next sample and the readings for each samples was recorded. (Bille *et al* 2009)

Serial Dilution of Milk Sample

A total of 6 testubes were dispensed with 9 ml of sterile distilled water to perform the Six-fold serial dilution of the sample from 10^{-1} to 10^{-6}. Then 1ml of the milk sample was added into the 9 ml of distilled water in the first tube (10^{-1} dilution), 1ml of the resulting solution was transferred into a second tube containing 9 ml distilled water to get 10^{-2} dilution the procedure was repeated for more dilutions till 10^{-6}. And in the last dilution 1 ml of inoculums was discard (Cheesbrough, 2000)

Nutrient Agar (NA)

The media nutrient agar was prepared according to the manufacturer's instructions whereby 23 g of the powdered medium was suspended into 1000ml of distilled water, mixed well and left on the bench to stand until the mixture is uniform. Then the mixed solution was heated with gentle agitation to dissolve. The medium solution was sterilized in an autoclave at 121°C for 15 minutes then allowed to cool to 45°C.

Inoculation

After the serial dilutions, 1 ml of the diluted milk samples (dilution 10^{-4} and 10^{-5}) was added into a sterile Petri dish using the pour plate technique. Then approximately 22 ml of molten agar (45ºC) was poured into inoculated Petri dish. The inoculums and the medium were carefully mixed by gentle shaking and allowed to solidify. After complete solidification, all the Petri dishes were inverted and placed in the incubator at 37°C for 24 hours to allow for bacterial growth (Cheesbrough 2000).

Total Bacterial Count

After the incubation period, bacterial colonies on the culture plates were countered manually. Two critical dilutions per each sample was counted. A plate were divided into quarters using a marker-pen and colony forming units were counted on at least two critical dilution plates with the aid of colony counter. The number of colony forming units was count. The cultural characteristics such as shape, size, colour, elevation, edge, form, and texture of the colonies were observed and recorded. The number of colonies were expressed in colony forming units per ml (Cheesbrough 2000).

Coliform Count

MacConkey agar was prepared by dissolving 51.53grams of the Agar in 1litre of distilled water. The solution was pre-heated to dissolve and was autoclaved at 121°C for 15 minutes. It was cooled to 45°C. One ml of each dilution 10-4 and 10-5 were pipetted into separate, labelled empty petri dishes, this was done near the Bunsen burner and 15-20 ml of the MacConkey Agar was dispensed into the petri dish. Which were gently swirled to mix the inoculum with the Agar and were allowed to solidify. The petri dishes were inverted and incubated at37°C for 24 hours (ISO 2000). A blank was prepared using the Agar but without the sample. After incubation, all dishes with colonies between 15-150 were counted.

Sub-culturing of presumed colonies

Each presumed colonies were subcultured on suitable selective media. Presumed *Escherichia coli* colony were subcultured on Eosin-methelene Blue Agar for to confirm the green metallic sheen characteristics of *E coli.* Manitol salt agar was used to subculture presumed Staphylococcus colonies to confirm the yellow lactose fermenting characteristics. Well-isolated colony was selected and sub-cultured further onto Nutrient agar (NA) to get pure culture for biochemical confirmation (Cheesbrough 2000). has the highest TBC count while sample C has the lowest TBC. For TCC, sample A has the highest while sample E has the lowest. Table 3 shows the biochemical characteristics of various isolate. Table 4 shows the specified isolates from the raw milk samples. Table 5 shows the percentage occurrence of different isolates in the raw milk samples.

Table 1 shows the pH, organoleptic properties and turbidity of raw milk samples. The pH value the samples are A(6.93), B(6.67), C(6.66), D(66.80), E(6.60), F(6.65) and G(6.73)respectively. All the tested samples had white colouration. sample D and F had sour taste while sample A,B, C, D, E and G had normal taste . All the samples tested were turbid it was observed that sample D and F were more turbid than A, B, C, E and G.

Table 1: pH, Organoleptic Properties and Turbidity of Raw Milk Sample

MILK SAMPLE	PH	COLOUR	TATSE	TURBIDITY
A	6.93	white	normal	turbid
B	6.67	white	normal	turbid
C	6.66	white	normal	turbid
D	6.80	white	sour	more turbid
E	6.60	white	normal	turbid
F	4.45	white	sour	more turbid
G	4.54	white	normal	turbid

Table 2 shows the total bacteria and total coliform count (CFU/ml) the total bacterial counts are as follows: A($2.9X10^5$), B($6.3x10^5$), C($9.0x10^5$), D($3.1x10^5$), E($1.9x10^5$), F($4.3x10^5$) G ($3.2x10^5$). Sample C had the highest bacterial count ($9.0x10^5$), followed by B ($6.3x10^5$), F($4.3x10^5$), G($3.2x10^5$).), D ($3.1x10^5$), A ($2.9x10^5$), while E had the lowest bacterial count (1.9x105), respectively. The total coliform counts are as follows: A ($5.1x10^5$), B ($4.9x10^5$), C ($1\text{-}5x10^5$), D ($4.5x10^5$), E ($4.0x10^5$), F($1.3x10^5$), G($1.5x10^5$). Sample A had the highest coliform count ($5.1x10^5$) while E had the lowest bacterial count ($4.0x10^5$).

TABLE 2: Total Colony Count for Raw Milk Sample (CFU/ml)

Sample	Total bacteria count (TBC)	Total coliform count (TCC)
A	$2.9x10^5$	$5.1x10^5$
B	$6.3x10^5$	$4.9x10^5$
C	$9.0x10^5$	$1.5x10^5$
D	$3.1x10^5$	$4.5x10^5$
E	$1.9x10^5$	$4.0x10^5$
F	$4.3x10^5$	$1.3x10^5$
G	$3.2x10^5$	$1.5x10^5$

KEY: NG= no growth

Table 3 shows the biochemical characteristics of bacterial isolates from the raw milk samples of which the probable organisms identified are *Staphylococcus aureus, Klebsiella* species *Bacillus* species, *Streptococcus* species and *Escherichia coli. Staphylococcus aureus* which have positive gram reactions, clustered cocci in shape, catalase positive and indole negative. *Klebsiella* species which had a gram negative rod, citrate positive. Gram negative rods, indole positive 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, indole negative, and the probable organism identified was *Bacillus* species, also *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.*

Table 3: Biochemical characterization of Bacteria Isolates

Isolates	CAT	IT	CUT	UT	MT	CT	MRT	OXI	Probable Organisms
A	+	-	-	+	-	+	+	-	*Staphylococcus aureus*
B	+	+	-	-	+	-	+	-	*Escherichia coli*
C	+	-	-	+	-	+	+	-	*Staphylococcus aureus*
D	-	-	+	-	-	-	-	+	*Lactobacillus* sp
E	-	-	+	+	-	-	-	-	*Streptococcus* sp
F	+	+	+	-	-	+	+	-	*Klebsiella* sp
G	+	-	-	+	-	+	+	-	*Staphylococcus aureus*
H	+	+	+	-	-	+	+	-	*Klebsiella* sp

KEY: CAT=Catalase, IT=Indole test, CUT=Citrate utilization test, UT=Urease test, MT=Mortility test, CT= Coagulase test, MRT=Methyl red test, OXI=Oxidase test, -=Negative, += Positive

Table 4: Shows the specific isolation of isolates from the raw milk samples, of which *Staphylococcus aureus* was present in 6 samples, *Escherichia coli* was present in 5 samples, which Bacillus species in 4 samples, Klebsiella species were present in sample 3 samples and Streptococcus species were present in 1 sample.

Table 4: Specific isolation of Isolates from Raw Milk Samples

Bacterial isolates	SA	SB	SC	SD	SE	SF	SG
Lactobacillus sp	1	1	0	0	0	1	1
E. coli	1	1	1	1`	0	1	0
S. aureus	1	1	0	1	1	1	1
Klebsiella	0	1	0	1	0	0	0
Streptococcus sp	0	1	0	0	0	0	0
TOTAL	**3**	**5**	**1**	**3**	**1**	**3**	**2**

Key: sp **=** specie, S= sample

Table 5 shows the microbial isolates were found associated with the raw milk samples of which Lactobacillus species occurred by 22.22%, *Escherichia coli* occurred by 27.78% in all the samples Klebsiella species occurred by 11% Staphylococcus aureus occurred by 33.33% and Streptococcus occurred by 5.55%

TABLE 5: Percentage Occurrence of Different Bacteria Isolate in Raw Milk Samples

Bacteria isolate	Number of isolates	Percentage of .
Lactobacillus sp	4	22.22
Escherichia coli	5	27.78
Staphylococcus aureus	6	33.33
Klebsiella sp	2	11.11
Streptococcus sp	1	5.55
Total	**18**	**100**

Key: sp=species

DISCUSSION

The purpose of this study was to evaluate the bacteria associated with raw cow milk from a dairy farm. This is due to the fact that milk produced in Nigeria by the informal sector is not regulated by any agency and such milk may pose a health hazard due to contamination with pathogens. Generally, findings showed that there are several practices undertaken at farm level such as type of animal house floor, not washing hands and udder/teats before milking, milking sick animals and those with udder problems, animal diseases like mastitis, water used for cleaning hands and milk equipment, type of storage containers used and milk storage duration under room temperature predispose raw milk to microbial contaminations which increases the microbial load and shorten shelf life of raw milk and milk products.

The pH value as shown in table 1 revealed the acidic nature of the high demanding commodity from the various raw milk samples studied. The pH values of the samples analyze ranges from 6.6 to 6.9 which is acidic and helps to check contamination, Acidity according to some authors is one of the good attributes of milk. The taste of the milk samples was noticed to be normal except for sample D and F which has a sour taste this could be due microbial activities or poor storage condition of the sample.

Bacteriologically, high number of milk samples handled had higher TBC than the maximum recommended level given by EAC standards which implied that the total bacteria count TBC < $2x10^6$ and total coliform count <$5.0x10^4$ the raw cow milk examined had poor microbiological quality. The bacteria isolated were *Staphylococcus aureus 33%, Escherichia coli 27%, Lactobacillus* sp 22% and *Klebsiella* (11%), and *Streptococci* sp(5.5%) species were isolated some of these isolates were also isolated from raw milk samples by (Nwakwo *et al.,* 2015). In their study on the microbiological evaluation of raw milk from a dairy farm in Udi, Enugu State

Sample 3 had the least of the total bacteria count and the coliform bacteria count. This could be because the cows are reared in a more hygienic environment, unlike sample 4 which had the highest count of bacteria. 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). The presence of high numbers of coliforms in milk provides an index of hygienic standard because Coliforms are known to be indicators of some degree of potentially hazardous contamination. *Staphylococcus aureus* was present in the milk sample analyzed (Chye *et al.* (2004) reported that the presence of *Staphylococcus 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 is a potential risk to consumer health due to its production of enterotoxin. 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) this is likely to be as a result of the water or grass they are feed on. Water used in production has great influence on the contamination of the milk, and being a vehicle for transmission of pathogen the water to be used must have characteristics of portability. The pathogens that have been involved in foodborne out-breaks associated with the consumption of milk include *Salmonella*, *Staphylococcus aureus, B. cereus* and thermo tolerant coliforms, especially *Escherichia coli* that is the most common contaminant of raw and processed milk 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

CONCLUSION

From the findings of this study it is concluded that milk sample examined are of poor quality, hazardous for human consumption and can be a potential source of milk-borne infections. Poor milking procedures, milk handling practices including the surrounding environment and treatment practices has greater influence on the microbial contamination of raw milk and contributes to zoonotic pathogens. Consumption of raw milk and milk products made from raw milk can result into health problems. This is supported by evidence of pathogenic bacteria isolated in this study and it raises a public health concern about safety of milk to consumers.

RECOMMENDATIONS

Routine assessment of milk quality produced by livestock keepers and consumed by the general public has to be mandatory in order to safeguard the public from milk-borne zoonotic infections which may radiate through consumption of unsafe milk and milk products. Strict hygienic measures should be applied during milking and milk handling practices, achievable by educating small-scale livestock keepers especially pastoralist and agro-pastoralist communities on good animal husbandry practices.

The behaviour of consuming raw milk and milk products made from raw milk should be discouraged. Milk stakeholders have to play their roles in educating the general public on likely public health consequences associated with such behaviour. Veterinary and extension officers and associated stakeholders have to make periodic surveillance visit to livestock keepers and create awareness, advice or conduct training on good animal health and management systems. More research work has to be conducted in different parts of Nigeria with the aim of improving the quality of raw milk from dairy farm.

Conflict of Interest

The authors declared that they have no conflict of interest

Authors Contribution

Azeez, Z.- Conceived the project, supervised and corrected the manuscript

Yakubu, E.H.- Conducted the research, wrote the initial draft of the manuscript

Enemuor, S.C.- Co-supervised the research

Momoh, S.J- Contributed to the quality of the manuscript

All authors approved the manuscript

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