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Microbial Examination of Pathogenic Bacteria Associated With Raw and Pasteurized Milk Samples in Shendam L.G.A Plateau State, Nigeria

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***Abstract*— This research was aimed at examining the pathogenic bacteria in both pasteurized and raw milk in Shendam L.G.A. Plateau State, Nigeria. A total number of thirty (30) samples 25ml each of both raw and pasteurized milk were obtained randomly and examined using white side test, dye reduction test and biochemical test such as oxidase, catalase, coagulase, indole and sugar fermentation test for confirmation. The results show that 24 (86.7%) were contaminated with Staphylococcus aureus when tested with the White Side Test. Based on the quality of the milk, Shimankar and Ndyak had the poorest handling practice. The bacteria load, showed highest count of 140 X 103 in Shimankar's vendor and lowest count was recorded in raw milk in Kalong. The morphological and biochemical identification revealed that fivemicroorganisms namely; Staphylococcus aureus, Salmonellaspp,Klebsiellaspp.,Shigella dysentriaeand Streptococcusspp. Salmonellaspp. was the highest with 14(46.7%) while the least was Streptococcusspp. with 2(6.6%). This study showed that pathogenic bacteria were associated with both raw and pasteurized milk in the studied area. The prevalence of these pathogens in milk sampled could be associated with the occurrence of diseases such as mastitis, poor storage, poor hygiene of milkers and retailers. Goodhealth condition of cow,processing and hygiene practices are hereby recommended.**

***Index Terms*— Pathogenic, Bacteria, Pasteurized, Raw, Milk and Vendor.**

I. INTRODUCTION

Raw milk is an opaque white liquid produced by the mammary gland of mammals. It provides the primary source of nutrition for young mammals before they are able to digest other type of food. The early lactation milk is known as colostrum. (Williams, 2009). Raw milk which is also known as Madara, in Hausa is a Nigerian milk food that is traditionally produced and consumed particularly in Northern Nigeria. It's sometimes consumed on its own or used in the preparation of cereal based milk and sold in the open-market un-refrigerated or refrigerated and its acceptability depends on the texture, flavor and taste which are in turndependent upon the inherent microbial constituents. (Obi and Ikenebomeh, 2007).

Milk carries the mother's antibodies to the baby. it can reduce the risk of many diseases in the body and it contains significant amounts of saturated fat, protein and calcium as well as vitamin C. Cow milk has the pH ranging from 6.4 to 6.8 making it slightly acidic (Williams, 2009). Madara has been claimed to be highly contaminated with organisms like Staphylococcus aureus, Escherichia coli, Bacillus cereus and Pseudomonasaeruginosa. During production, microbial contamination generally occurs from three main sources; from within the udder, exterior of the udder and the surface of milk, handling and storage equipment which cause undulant fever, dysentery, salmonellosis and tuberculosis. (Feresu and Muzoncho, 1990). As an agricultural product, milk is extracted from mammals during or soon after pregnancy. It can also be gotten from some plants such as soya beans, groundnut, tiger nuts, coconuts etc. Milk and milk products constitute important nutritional components for all age group. Good quality milk meets the nutritional needs of the body better than any single food as it contains the entire essential food constituent (Sharma and Joshi, 2002).Beside the beneficial effects on nutrition, milk can also act as vehicle for thetransmission of disease of bacterial, viral and parasitological effect on nutrition. Milk is an excellent medium for certain micro-organism particularly bacteria pathogens whose multiplication depend mainly on temperature, competing micro-organism and their metabolic products. When milk is produced under poor hygienicconditions, and is not cooled, the main contaminants are usually lactic acid pathogenic bacteria. This cause rapid souring in the milk, lactic acid has inhibitory effect on pathogenic bacteria but this cannot be depended on to provide a safe milk product. The number of micro-organisms present during milking has been reported to range between several hundreds and thousands per millimeter. Micro¬organisms found in milk can be classified based on their biochemical types, temperature response and ability to cause infection. (Marie. Caroline and Januel, 2006). This research therefore investigated the pathogenic bacteria in both raw and pasteurized milk in Shendam L.G.A. of Plateau State, Nigeria.

II. MATERIALS AND METHODS

Study Area and Population

The study areas were Shimankar, Kalong and Ndyak in Shendam Local Government Area of Plateau State, Nigeria. Its Headquarters are in the town of Shendam at 8°53'00"N 9032'00'TE. It has an area of 2,477km2 and a population of 208,017 at the 2006 Census. The postal code of the area is

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940. Shimankar and Ndyak are in Dorok District and located at Shendam L.G.A of Plateau state with a code 940104.While Kalong district is located at the centre of the region with the geographic maps around 8°33'33"N, 9° 25'30"E.

Sample Size The total number of thirty (30) milk samples were collected from the udder of the cows(raw milk) and from vendors (pasteurized milk).

Collection of Samples

Twenty-five milliliter (25mls) each of the samples was collected randomly from the udder of 15 cows and 15 vendors from three locations into sterile bottles labeled with their initials and packed in a transport cooler containing ice blocked. The samples packed in a cooler containing ice block were immediately transported to the laboratory for analysis within one hour. (Thomas, 2006).

III. ANALYSIS OF SAMPLES

White side test (WST)

This test was used for diagnosis of mastitis by observing the presence of flakes (Clot) and also based on the development of viscosity in the milk when sodium hydroxide was added. Cows with purulent or muco-purulent discharge or containing white side test reacted positively and were considered positive mastitis. (Kumar et al, 2015).Four percent (4%) sodium hydroxide solution was added to 2 drops of the milk placed on a clean grease free slide and mixed using a sterilized wire loop. It was then allowed to stay for one minute before observing under low power magnification. (Dunn,2015).

Dye Reduction Test (MBDRT)

This was used to assess the overall microbial quality of raw and pasteurized milk. This test is based on the fact that the blue colouris decolourized whenever the oxygen present in the milk gets exhausted due to microbial activity.For each sample of raw milk collected, 10ml were poured into sterile test-tubeand one drop of the dye (methylene blue) was added, themixture was mixed and incubated at 37°C for the dye to react with milk. During incubation, colour changes were observed at regular intervals. Thetime taken for complete reduction of dye is an indication of the density ofmicrobial contamination of the milk sample. Grading of raw and pasteurized milk based on (MBDRT)

Complete reduction in 5hours and above; Very good

Complete reduction in 3 to 4 hours; Good

Complete reduction in 1 to 2 hours; Fair

Complete reduction in less than ½ hours; Poor (Atherton and Newlander,1977). Sterilization of Materials All the materials and apparatus required were sterilized using autoclave at temperature of 121°C for 15minutes (Khuntia, 2013).

Preparation of Media/Pour Plate Method

The required media were prepared according to the manufacturers information and sterilized using (Khuntia, 2013) method. The sterilized media were then poured into the required petri-dishes according to (Robert and Thomas, 1999). Serial Dilution Serial dilution method was employed to reduce the bacterial load of milk sample. One (1ml) of each milk sample was dissolved -in 9ml of distilled water to form a stock solution. Serial dilutions of up to 5-fold dilution were employed. (Rossenthal, 2007). Inoculation of Sample/Incubation One (1ml) each of the sample dilution was dispensed into an already prepared agar and allowed to spread and solidified. The plates were incubated at 37°C for 24hours.

Bacterial Viable Counts Colonies were counted and colony forming unit (cfu/ml) was calculated by given formula

$$\frac{no\ of\ colonies\ X\ dilution\ factor}{inoculum\ volume}$$

Numbers of colonies were given by average of 10^{-2} and 10^{-3} average. The colonies were counted and the organisms were sub-cultured into another / fresh media for differentiating microorganisms that are present in culture media and obtained a pure culture of organism. The sub-cultured media were incubated at 37°C for 24 hours and each isolate was gram stained. (Rosenthal, 2007). The media used for the isolation of microorganisms from samples of milk collected were nutrient agar, blood agar, CLED and Salmonella-Shigella agar. The identities of the bacterial cultures were confirmed based on their morphological, physiological and biochemical characteristics using Bergeys manual of Systematic bacteriology as reference (Sneath, 1984).

IV. RESULTS

Table 1: Result of White Side Test

Table 1 shows result of white side test of both raw and pasteurized milk to detect the presence of *Staphylococcus aureus* which infect the udder of the cow with a disease (mastitis).

SAMPLE	WST	REMARK
CSA	+	MP
CSB	+	MP
CSC	+	MP
CSD	+	MA
CSE	-	MA
VSA	-	MA
VSB	-	MA
VSC	-	MP
VSD	+	MP
VSE	+	MP
CKA	-	MP
CKB	+	MP
CKC	+	MP
CKD	+	MP
CKE	+	MP
VKA	+	MP
VKB	+	MP
VKC	+	MP
VKD	-	MA
VKE	+	MA
CNA	+	MA
CNB	+	MA
CNC	+	MA
CND	+	MA
CNE	+	MA
VNA	+	MA
VNB	+	MA

VNC	+	MA
VND	+	MA
VNE	+	MP

Key
CS (A-E) Cow Shimankar (A_E)
VS (A-E) Vendor Shimankar (A_E)
CK (A-E) Cow Kalong (A_E)
VK (A-E) Vendor Kalong (A_E)
CN (A-E) Cow Ndyak (A_E)
VK (A-E) Vendor Ndyak (A_E)
WST White Side Test + Present - Absent MP Mastitis present
MA Mastitis Absent

Table 2: Result of Dye Reduction Test

Sample	Shimankar MBDRT			Kalong MBDRT			Ndyak MBDRT		
	Tt	Colour	Remark	Tt	Colour	Remark	Tt	Colour	Remark
CA	30 min	g	P. milk	15min	y	P. milk	15 min	y	P. milk
CB	15 min	y	P. milk	4 hrs	b	G. milk	15 min	y	P. milk
CC	4 hrs	b	G. milk	2hrs 30min	y	F. milk	30 min	y	P. milk
CD	30 min	w	P. milk	4 hrs	b	G. milk	30 min	g	P. milk
CE	15 min	y	P. milk	6 hrs	b	V.G. milk	30 min	g	P. milk
VA	15 min	y	P. milk	6 hrs	b	V.G. milk	30 min	y	P. milk
VB	30 min	y	P. milk	2 hrs	y	F. milk	45 min	y	P. milk
VC	45 min	y	P. milk	6 hrs	b	V.G. milk	1 hr	y	F. milk
VD	15 min	y	P. milk	4 hrs	b	G. milk	1 hr	y	F. milk
VE	45 min	y	P. milk	6 hrs	b	V.G. milk	45 min	y	P. milk

Table 2 shows result of Dye Reduction test for assessing the overall handling quality of the milk by analyzing with methylene Blue Dye reduction test. Kalong had a good handling quality while Shimankar had poor quality.

Key
MBDRT = Methylene Blue Dye Reduction Test
CA – E = Cow A-E
VA – E = Vendor A-E
P. milk = Poor milk
F. milk = Fair milk
G. milk = Good milk
V.G. milk = Very Good milk
y = yellowish, g = greenish, w = whitish, b= No colour change Tt = Time taken

Table 3: Bacteria Viable Count for Each Milk Samples (CFU/ML) in log 10

Serial Dilution	Shimankar	Kalong	Ndyak
CA	80×10^3	33×10^3	55×10^3
CB	50×10^3	41×10^3	35×10^3
CC	90×10^3	38×10^3	105×10^3
CD	45×10^3	44×10^3	55×10^3
CE	120×10^3	21×10^3	26×10^3
VA	140×10^3	40×10^3	85×10^3
VB	80×10^3	70×10^3	49×10^3
VC	60×10^3	50×10^3	50×10^3
VD	40×10^3	135×10^3	87×10^3
VE	100×10^3	90×10^3	70×10^3

Table 3 shows result of pour plate that is, viable count and the highest count was 140 X 10^3 in Shimankar vendor A while the least is 21 X 10^3 in Kalong cow.

Key
CA – CE = Cow A-E
VA – VE = Vendor A- E

Table 4: Morphological and Biochemical Identification of Bacterial Isolates

Isolate	Gram	Shape	Catalase	Coagulase	Oxidase	Indole	SFT glucose	Lactose	Sucrose	Fructose	Maltose	Mannitol	Galactose	Probable Organisms
1	-	Rod	-	+	-	+	AG	-	_	+	+	+	+	*Salmonella spp*
2	-	Rod	+	+	-	+	-	-	_	_	AG	+	_	*Shigella dysentriae*
3	+	Rod	_	+	-	-	+	+	+	_	_	_	_	*Streptococcus spp*
4	-	Rod	+	+	-	-	+	+	+	_	+	_	+	*Klebsiela spp*
5	+	Rod	+	+	-	+	AG	A	A	A	A	A	A	*Staphylococcus aureus,*

KEYS
+ Positive
- Negative
A Acid production
AG Acid and Gas production

Table 5: Bacterial Isolates associated with milk samples

Samples	Isolates
CSA	5.4
CSB	1
CSC	2
CSD	5
CSE	1
VSA	2
VSB	5
VSC	3
VSD	1
VSE	1
CKA	3
CKB	1
CKC	2
CKD	4
CKE	1,2
VKB	1
VKC	1
VKD	2
CNC	1
CND	1
CNE	1
VNA	4,1
VNB	2
VNC	2
VND	1
VNE	2

Key 1 *Salmonella spp*
2 *Shigelladysentriae*
3 *Streptococcus* spp
4 *Klebsiella* spp5 *Staphylococcus* spp
CS (A-E) Cow Shimankar (A_E)
VS (A-E) Vendor Shimankar (A_E)
CK (A-E) Cow Kalong (A_E)
VK (A-E) Vendor Kalong (A_E)
CN (A-E) Cow Ndyak (A_E)
VK (A-E) Vendor Ndyak (A_E)

Table 6 shows result of frequency and percentage occurrence of both milk samples, Salmonella spp. had the highest percentage occurrence with 46.7% while *Streptococcus* spp. had the least with 6.6%.

Table 6: Frequency and Percentage Occurrence of Bacterial Isolate in Different Milk Samples

Bacteria isolated	Cow Milk	Vendor Milk	Total	Percentage (%)
Salmonella Spp.	8	6	14	46.7
Shigelladysenteriae	5	3	8	26.7
Streptococcus Spp.	1	1	2	6.6
*Klebsiella*Spp.	1	2	3	10.0
Staphylococcus aureus	2	1	3	10.0
Total	**17**	**13**	**30**	

V. DISCUSSION

In this study, two types of samples were observed: Pasteurized milk collected randomly from different vendors in different locations within Shendam (Shimankar, Kalong and Ndyak). Unpasteurized raw milk collected randomly from different cows in settlements at Shimankar, Kalong and Ndyak within Shendam Local Government Area of Plateau State. Research has shown that the presence of just one enteric bacterium in anyfood substance is enough to initiate human infection (Bruch, 2008).Out of the 30 slides examined under the microscope for the presence of flakes, 24 were positive representing 80% while 6 were negativerepresenting 20%. Table 1 Unpasteurized milk (the cow milk) has the highest percentage occurrence of 86.7% positive which

indicates the presences of flakes while the pasteurized milk (Vendor milk) has the low percentage occurrence of 73.3%. The formation of flakes on addition of 4%NaOH indicates the presence of *Staphylococcus aureus*which means that the udder of the cow is diseased or infected with mastitis. This work is in agreement with a similar research conducted by Kvist *et al,* (2007), noted that the formation of flakes or clumping of cells in milk sample on the addition of 4% NaOH solution indicates the presence of Mastitis and the degree of the flake formation further indicates the level of the disease.Table2 shows result of Dye Reduction Test (DRT) which indicates the handling quality of the milk, out of the 30 samples analyzed by the methylene blue dye test. The 30 samples showed various level of time taken for the dye to re-act completely with the milk. The highest time taken was 6hours while the lowest time taken was15minutes indicating the quality of the milk as very good, good, fair and poor respectively. From the table, milk from Shimankar vendors and cows has the highest poor handling quality while a good handling quality was recorded from Kalongvendors and cows. The handling quality of the milk is categorized as follows:

Very Good, decolorized in 5 to 6hours
Good, decolourized in 3 to 4hours
Fair, decolourized in 1 to 2hours
Poor decolourized in less than ½ hour.

This work is in agreement with a similar research conductedby Henry and Newlander 1997. Table 3 is the result for pour plate. Out of the 30 plates inoculated in SSA (*Salmonella Shigella* Agar), Nutrient Agar, Blood Agar and CLED (Cystine Lactose Electrolyte Deficient agar), various colonies were formed indicating the level of contamination. The highest count was 140 x 10^3while the lowest was 21 x 10^3. Table 4 and Table 5 show almost all the samples analyzed indicated that the udder of the cows was infected with mastitis. And these work is in disagreement with a similar work conducted by Bergdoll 2000. In his work *Staphylococcus* were present with 50% in unpasteurized milk because it's a common bacterium found in the nose, hand, finger-tip hair and skin of most individual. *Streptococcus*spp occurs with 6.6% and is usually found in hair and do not form spores. *Streptococcus* spp was recognized as a major cause of pneumonia. They colonized the respiratory tract, the nasal cavity of both human and cattle and could contaminate milk through sneezing, coughing and via respiratory droplet. Table 6 is the result for frequency and percentage occurrence of bacterial isolated in different milk samples. The prevalence of these pathogens in the milk was associated with the occurrence of diseases in the animal including cow mastitis. From the work done, it was found that all milk examined had high contamination of different bacteria with the highest recorded from the cow milk due to its unpasteurized nature. Therefore, the most encountered organism in this research work is *Salmonella*spp with 46.7% occurrence. Salmonella outbreaks are commonly associated with raw or undercooked eggs, raw milk, contaminated water and raw or undercooked meats. (Akinjogunla*et al.,* 2009). The highest occurrence of *Salmonella* spp in the milk samples are as a result of faecal contaminated hands, water, utensils and this can occur through poor handling of milk by milkers, use of contaminated water to wash milking utensils and storage equipment or even duct contamination with cow faeces. In agreement with (Bergdoll, 2009) analysis conducted on zoonotic *Salmonella*spp in fresh cow milk and fresh skimmed pasteurized market milk (nono). *Streptococcus*spp was the least or lowest encountered bacteria with 6.6% while other bacteria encountered are*Shigelladysenteriae* with 26.7%,*Klebsiellia*spp 10.0% and *Staphylococcus aureus* 10.0%.

VI. CONCLUSION

This study shows that pasteurization helps in inhibiting the activity of microorganisms. However, when it is not properly carried out, it can still lead to contamination of the milk. The pathogenic bacteria isolated from the milk samples can be as a result of post contamination through poor storage, milking equipment and utensils, from the udder of the cow, improper pasteurization, storage facilities, from poor hygiene of the retailers and also the environment. Since these microorganisms could contaminate pasteurized and unpasteurized milk, pasteurization of raw milk should be done properly so as to remove the pathogenic bacteria. It can also be concluded that good hygiene of milkers and retailers toward handling of milk will reduce the rate of contamination and also the health condition of the cow.

VII. RECOMMENDATION

In light with the above contaminations and dangers, this may pose to the health of consumers; therefore, the following recommendations are proposed to reduce the level of contamination of this pasteurized and unpasteurized milk: Education of farmers on proper processing and handling of milk during milking and marketing should be encouraged. Farmers should be encouraged on routine health checkup of animals to reduce the risk of contamination and improve their health condition. Proper pasteurization should be done on milk before distributing to the general public. Packaging of milk- should be done in a hygienic environment with proper precaution taken to avoid contamination

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