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Bacteriological and Physicochemical Evaluation of Raw Milk from some Dairy Farms within Kano Metropolis

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Abstract
Raw milk is most commonly obtained from the Fulani community in Nigeria. Due to inadequate handling and processing, there is need to evaluate the safety of milk produce sold by dairy farmers in Kano metropolitan. For this purpose, this study focuses on the bacteriological evaluation and physicochemical assessment of raw milk collected from some dairy farms within Kano metropolitan. The raw milk sample was directly collected aseptically from the milking bucket using sterile container with cover. The samples were transported to the Bayero University Kano, New Microbiology laboratory and Biochemistry Laboratory for analysis. Bacteriological analyses carried out were Total bacterial count, Total coliforms count, cultural identification and gram staining. The physicochemical parameters such as organoleptic test, temperature, pH, titratable acidity, moisture content, total dissolved solid, viscosity, electrical conductivity, density, turbidity were determined by standard methods. This study recorded high mean number of total bacterial count (5.07415±0.575 log cfu/ml) and total coliform count (234.717 ± 411.3 log cfu/ml) in the study areas which exceeded the standard limit stipulated by authorities. The bacteria isolated from the study wards were identified to be E. coli 16(13.22%), Staphylococcus aureus 40(33.05%), Salmonella sp. 9(7.43%), Shigella sp. 26(21.48%) and Klebsiella sp. 30(24.79). The result of physicochemical parameters varied among different sample analyzed. pH (7.9225±0.71) and Temperature (^oC) (28.4±5.23) of the milk samples from all the study wards comply with the standard limit, while the overall mean Density(g/m^3) (1.02956±0.57), Electric conductivity ($\mu S/cm$) (4.062±954.2), Titratable acidity (0.13395±0.05), Viscosity (cP) (28.05±5.29), Turbidity (NTU) (76.2±25.0), Total dissolved solid (ppm) (2641.3±593.6) and Moisture content (%) (86.274±3.464) values were higher than the recommended standard. It is concluded that there is good condition for the growth of microorganisms, higher level of disease causing pathogenic organisms, and early spoilage in the milk samples.

***Keywords**: Bacteriological, Physicochemical, Dairy farms, Evaluation, Raw Milk, Metropolitan*

Introduction

One of the dominant diets not only in every culture of the world but at every stage of human and other mammalian growth and development from the infant stage to adulthood is milk. Milk is a white secretion of mammary gland of female animals and it contains approximately 87.2% water, 3.7% fats, 3.5% proteins, 4.9% lactose, 0.7% ash and has a pH of about 6.8. Owing to its unique high nutritional content, water activity and complex biochemical composition, it serves as an excellent medium for the growth of different kind of microorganisms when appropriate growth factors are maintained (Parekh and Subhash, 2008). A few number of bacteria ($< 3\times10^4$ cfu/ml) are found in milk from a healthy udder but might become contaminated by microorganism from the surrounding during milking and milking handling, from water and milk equipment's. Microbial contamination of cow milk might result to milk-borne diseases to humans, while others caused milk spoilage (Donkor *et al.*, 2007). The sources of milk microbial contamination include primary contamination from infected lactating animal. Contamination during milking by milkers, milk handlers, unsanitary utensils or milking equipment and water supplies used in sanitary activities are secondary causes of microbial contamination. Microbial contamination during milk handling, transportation and storage are other sources of contamination. The occurrence of tertiary microbial contaminations is due to re-contamination of milk after being processed due to unhygienic condition, poor handling and storage of milk during consumption (Parekh and Subhash, 2008).

Bacteria isolates commonly reported from cow milk include *Staphylococcus species*, *Listeria species*, *Salmonella species*, *Escherichia coli*, *Campylobacter species*, *Mycobacterium species*, *Brucella species*, *Coxiella bunetii*, *Yersinia species*, *Pseudomonas aeruginosa*, and *Corynebacterium ulcerous*. Others are *Proteus species*, *Leptosira species*, *Clostridium species*, *Streptococcus species*, *Enterobacter species* and *Bacillus species* (Al-Tahiri, 2005; Donkor *et al.*, 2007; Parekh and Subash, 2008). Common milk-borne diseases which pose risk to public health, can be transmitted to consumer include bovine Tuberculosis, Brucellosis, Anthrax, Listeriosis, Salmonellosis, Leptospirosis, Campylobacteriosis and *E. coli* 0157:H7 which is an emerged new milk-borne bacterial pathogen as reported recently with a very serious health effects (Sivapalsingams *et al*., 2004).

Raw milk is moderately consumed among the Fulani community in Nigeria. Due to inadequate handling and processing, there is need to evaluate the safety of milk produce sold by dairy farmers in Kano metropolis. For this purpose, this study focuses on the bacteriological evaluation and physicochemical assessment of raw milk collected from some dairy farms within Kano metropolis.

Materials and Methods

Area of Study

The study was conducted in Kano metropolis, Kano State which lies between latitude $11^0$55'0" N to $12^0$0'0" N and longitude $8^0$25'0" E to $8^0$35'0" E. Kano metropolitan is made up of six local government Area (Dala, Fagge, Gwale, Municipal, Nassarawa and Tarauni) and some parts of Kumbotso, Ungogo, and Tofa local government areas. Kano metropolitan has an estimated population of over 4 million people with male-female ratio of about 1 to 1.32 (Maigari, 2014).

Study Population

The numbers of sample collected were obtained as described in a formula for finite population by Kothari (2004). Based on the above formula, 10 samples were collected from each dairy farm above thus giving a total number of 40 samples.

Sample Collection

The milk samples were agitated thoroughly before taken. This was to allow the samples to be a homogenous representative. Agitation was not vigorously because air bubble might have dispersed in milk and changes its physical properties. A plunger was used to take the samples of required amount into the sample bottles. The sample bottles were well closed and turned upside down to ensure it was closed well. The samples were carefully agitated before analysis commenced in the laboratory (Graham, 2004).

Bacteriological Evaluation

Standard Plate Count

The milk samples were well homogenized by inverting the sample bottle multiple times. One ml of the milk sample was aseptically transfers directly in a test tube containing nine ml of peptone water. This tube was then labeled as 10^{-1} dilution. Subsequently other dilutions such as 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were made by transferring the one ml of the previous dilution into the next dilution. One ml of each dilution was inoculated in a sterile and empty petri dish and each petri dish was labeled according to the value of their dilution factor. Plating was done in duplicate (i.e. each dilution was inoculated in two petri dishes). About fifteen ml of sterile prepared nutrient agar was poured in each labeled inoculated plates and each plate were mixed gently along the flat surface in circular motion, 8-10 times clockwise and 8-10 times counterclockwise to allow evenly distribution of the inoculum and the nutrient agar. The plates were allowed to cool and solidified before incubating in an inverted position at a temperature of 37^0 C for 24 hours (Brown and Smith, 2015).

Multiple Tube Method

Presumptive stage

The milk samples were well-homogenized by inverting the sample container multiple times. One ml of milk sample was aseptically transferred into a test-tube labeled 10^{-1} containing nine ml of sterile peptone water. Two subsequent dilutions (i.e. 10^{-2}, 10^{-3}) were inoculated by transferring one ml of the previous dilution into the next dilutions. Each dilution (10^{-1}, 10^{-2}, and 10^{-3}) was aseptically inoculated into three test tubes containing sterile lactose broth with inverted Durham tube thus making a total number of nine test tubes. Each set of three tubes were also labeled according to their dilution factor. The set of test tubes were incubated at temperature of 37^0 C for 24 hours (Swanson *et al.,* 2001).

Confirmed stage

All positive tubes from presumptive stage were passed to confirmatory stage. A set of test tube containing nine ml of prepared sterile Brilliant Green Lactose Bile broth (BGLB) was labeled as the sample code and dilution factor of the positive presumptive tube. The positive presumptive tubes were inoculated aseptically using a wire loop into the BGLB broth. These inoculated tubes were incubated at 35^0 C for 48hrs (Swanson *et al.,* 2001).

Detection of Enteric Bacteria

A loop was inoculated aseptically from the milk samples with the proximate of the Bunsen burner flame and transferred onto a point on the surface of the prepared selective media (i.e. Eosin Methylene Blue agar, *Salmonella-Shigella* Agar, Mannitol Salt Agar) in the different petri dishes. The inoculated plates were labeled by the sample inoculated and media it contains. The plates were incubated at a temperature of 37^0 C for 24hrs. Isolates were gram stained and biochemically established based on their indole production, methyl red, Voges- Proskauer, citrate utilization (IMViC) pattern, hydrogen sulphide production, urease production, catalase test along with different carbohydrate (lactose, sucrose, glucose, dextrose) metabolism (Brown and Smith, 2012).

Physicochemical Investigation

The milk from samples were analyzed for temperature, density, colour, turbidity, pH, total dissolve solid, electric conductivity, titrable acidity, viscosity and moisture content as described by Association of Official Analytical Chemists (2005).

Method of data analysis

The bacterial counts were converted to logarithm of the number of colony forming units per ml of raw milk sample (log cfu/ml). Descriptive statistical data such as means; standard derivation were computed using statistical package for social science (SPSS) computer software. The data obtained from the study were subjected to analysis of variances (ANOVA) to determine the significance difference between the means (Mukhutar, 2013).

Result

Table 1 shows the total bacterial count found in the different samples assessed i.e. raw milk from Sallari, Naibawa, Dan Bare and Zawachiki which were labeled as ward S, N, D and Z respectively. The results collected were converted to log cfu/ml, Ward Z shows the highest bacteria count of 5.10540±0.367282 log cfu/ml followed by ward D (5.1054±0.239124 log cfu/ml and then ward S (5.09240±0.578135 log cfu/ml) while Ward N shows the least bacterial count of 4.93360±0.935871 log cfu/ml, Though The result of total bacterial and coliform count of the different study wards show significant difference ($p<0.05$). From Table 1, the total coliform count was expressed in cfu/ml. Ward D shows a significant means value of 506.51 cfu/ml, followed by samples from Ward S which shows a means value of 241.64 cfu/ml. The least means values were 169.1 and 21.62 cfu/ml, obtained from Z and N respectively the study shows an overall coliform count of 234.71750 cfu/ml across the 40 samples. Comparison of the total coliform count among the study groups shows significant difference ($p<0.05$).

Table 1: Total Bacterial and Coliform Count of Raw Milk Samples

Wards	Bacterial Count (log cfu/ml) (Mean±SD)	Coliform Count (cfu/ml) (Mean±SD)
Ward Z	5.1652±0.367	169.1±333.8
Ward D	5.1054±0.2239	506.57±528.3
Ward S	5.0924±0.578	241.64±452.2
Ward N	4.9336±0.9358	21.62±17.93
WHO Standard	1.0 x 10^2 (cfu/ml)	0 per 100ml
Total Means	5.07415±0.575	234.717±411.3

Result presented from table 2 and 3 show five bacterial isolates were identified in the raw milk sample from the study areas, the bacteria identified and their isolate rate were *E. coli* 16(13.22%), *Staphylococcus aureus* 40(33.05%), *Salmonella sp*. 9(7.43%), *Shigella sp.* 26(21.48%) *and Klebsiella sp.* 30(24.79) (Table 2).

Table 2: Frequency of Occurrence (%) of Bacterial Isolates in Raw Milk Samples

Isolates	Ward Z	Ward D	Ward S	Ward N	Total (n)	Percentage (%)
E. coli	5(4.13%)	5(4.13%)	4(3.30%)	2(1.65%)	16	13.22
Staphylococcus	10(8.26%)	10(8.26%)	10(8.26%)	10(8.26%)	40	33.05
Salmonella	4(3.30%)	1 (0.83%)	2(1.65%)	2(1.65%)	9	7.43
Shigella	7(5.78%)	7(5.78%)	5(4.13%)	7(5.78%)	26	21.48
Klebsiella	9(7.43%)	7(5.78%)	8(6.61%)	6(4.95%)	30	24.79
Total	35	30	29	27	121	

Table 3: Morphological and Cultural Characteristics of Bacteria Isolated from Raw Milk In The Study Areas.

Bacteria Isolates	Gram Reaction	Cultural Characteristic
E. coli	Gram negative rods	Colonies showing metallic sheen
Salmonella sp.	Gram negative rods	Non-lactose fermenting pale coloured colonies with black center.
Shigella sp	Gram negative rods	Non-lactose fermenting pale coloured
Staphylococcus sp.	Gram positive cocci	Yellow colonies with yellow zones
Klebsiella sp.	Gram negative rods	Colonies showing mucoid and shin pink

The results of the physicochemical analysis were obtained by averaging the result of the 40 samples taken within 2 weeks of interval (Table 4). The result shows the means of the temperature of the samples collected from dairy farms in the distinct wards; ward N (29.6±5.68^{0}C), and S (29.4±6.88^{0}C), has the highest mean temperature while ward Z (26.8±4.39^{0}C) and D (67.8±3.67 ^{0}C) recorded the least mean temperature value. The difference in the mean temperature among study sample was significant ($p<0.05$). The mean pH concentration of the samples collected from the dairy farms in the selected wards. Ward D shows the highest pH values of 8.042±0.565 followed by samples in ward S which was 8.040±0.506. Both wards N and Z show the least means pH values of 7.99±0.466 and 7.614±1.11 respectively. The pH values of the distinct wards indicate significant difference ($P<0.05$). The mean density of the raw milk samples in the dairy farms in ward Z, D, S and N were 1.0442±0.028g/ml, 1.0458±0.110428g/ml, 1.01804±0.008399 g/ml and 1.01020±0.11833 g/ml respectively. The result points ward D samples to possess the highest density and sample from ward N as the least. The overall mean of the total samples was 1.02956±0.057437. However, there is statistically significant difference of the average density among the study areas ($p<0.05$). The result obtained in the study for electric conductivity and total dissolved solid shows an overall maximum value of 4.062±1.21 and 2641.3±593.6 ppm respectively while the samples from ward Z shows a least mean value of 3.194±0.95 and 2410.4± ppm, for electric conductivity and total dissolved Solid .The highest means of both electric conductivity (4.568±226.3) and total dissolved solid (3083±99.1) were observed in ward D and N respectively. Insignificant statistical difference was observed in the mean electric conductivity and

total dissolved solid value within study zone (P>0.05). Titratable acidity of the samples in the different ward Z, D, S and N in the study were 0.164, 0.113, 0.122 and 0.138 respectively. The highest obtained value was 0.164 in ward Z and the least mean value of 0.113 in Ward D. from the result, the statistical data analysis of the mean titratable acidity between the study cluster record significant different at p <0.05. Mean viscosity values obtained in the study for the sample in ward Z, D, N and S were 27±5.37 cP, 28.05±5.29 Cp, 27.6±4.599 Cp and 29±6.99 respectively. The highest viscosity mean value was recorded in ward S (29±6.99) while the least viscosity mean value was recorded in ward Z. When the mean viscosity value of the study locations was analyze a significant difference was reported (P<0.05). The study samples collected from respective Ward D, Z, S and N were (85.4 ±30.62NTU), (67.2 ±16.25NTU), (67.4±18.94 NTU), and 84.8 ± 28.42 NTU). Ward D has the highest means turbidity values (85.4±30.62) and Ward S shows the least means values (67.4±18.94NTU). Though the difference in the mean turbidity among the study group show statistically significant difference (p<0.05). From the data obtained, the moisture content of raw samples from the ward Z, D, S and N were (86.20±3.53), (89.26±4.90), (85.93±2.23) and (86.92±1.48) respectively. The mean value for moisture content in the study was (86.92±3.46). The highest and lowest means are found in ward D (89.26±4.90) and ward S (85.93±2.23) respectively. The mean moisture content value of the distinct wards shows significant difference (p<0.05).

Table 4: Physiochemical Parameters of Raw Milk Samples

Parameters	Ward Z	Ward D	Ward S	Ward N	Mean	WHO	NAFDAC	NSDWQ
Temperature^{0}C	26.8±4.39	27.8±3.67	29.40±6.88	29.6±5.61	28.4±5.23	40	-	Ambient
pH	7614±1.11	8.042±0.56	8.04±0.50	7.99±0.46	7.9225±0.71	6.5-9.2	6.5-8.5	6.5-8.5
Density (g/ml)	1.0442±0.28	1.0458±0.11	1.018±0.0084	1.0102±0.11	1.02956±0.57	-	-	-
E/Conductivity (µS/cm)	3.1954±956.9	4.558±282.6	3.925±1219.1	4.456±226.3	4.062±954.2	-	NS	25
Titratable Acidity	0.164±0.11	0.1138±0.017	0.1228±0.136	0.138±0.17	0.13395±0.05	-	-	-
Viscosity (cP)	27±5.37	28±4.45	29±6.99	27±4.59	28.05±5.29	1% (0.1cp)	-	-
Turbidity(NTU)	67.2±16.2	85.4±30.6	67.4±18.9	84.8±28.4	76.2±25.0	25	-	5
Total Dissolved Solid (ppm)	2410.4±458.3	2451±681.8	2620.4±724.8	3083.4±99.17	2641.3±593.6	1500	500	500
Moisture Content (%)	86.20±3.53	89.26±4.90	85.938±2.2	86.274±1.485	86.274±3.464	80	-	-

Key: WHO = World Health Organization; NAFDAC = national agency for food and drug administration and control. NSDWQ = Nigeria Standard for Drinking Water Quality

Discussion

The total bacteria count and total coliform count of the raw milk samples were generally high, they exceeded the standard limit recommended by NAFDAC, WHO and NSDWQ which indicates the insufficient hygienic and contamination of raw milk from the study areas (NAFDAC, 2001; WHO, 2001; NSDWQ, 2007). The mean total bacteria count of all the samples was 5.07415 log cfu/ml. This was lower compared to what was reported by Olatunji *et al*., (2012) 7.74 log cfu/ml and Aaku *et al.,* (2004) 6.74 log cfu/ml and higher to what was reported by Shamsuddeen (2019) 4.06 log cfu/ml. This indicates that slight sufficient hygienic practices were carried out during milking and milking processes in the dairy farms within the study wards.

The mean total coliform count of the samples was 234.717 cfu/ml. This was higher compared to what was reported by Abdoul-latif *et al.,* (2017) 3.91 cfu/ml, Adiele *et al.,* (2015) 3.17 cfu/ml. This indicates fecal and environmental contamination such as bedding, soil and water. All the raw milk samples analyzed in this study have unobjectionable color which is in agreement with the standard color of 6 TCU by WHO and 5 TCU by both NAFDAC and NSDWQ (NAFDAC, 2001; WHO, 2001; NSDWQ, 2007). High coliform counts indicate vividly that raw milks from the study areas were contaminated. All the bacteria species (*Staphylococcus spp., Escherichia Coli, Salmonella spp., Shigella spp. and Klebsiella spp)* found in the study wards are pathogenic to man and domestic animals. The presence of these pathogens in the milk samples were considered fatal to the health of the consumers.

The result of the physicochemical analyses of raw milk samples shows that the pH of milk samples from Zawachiki, Sallari, Dan bare and Naibawa comply with standard requirement. The pH values are within the limit recommended by WHO and NAFDAC (7.0-8.5) (WHO, 2001; NAFDAC, 2001). Although pH has no direct effect on human health, its indirect action on physiological process cannot be over emphasized (Adekunle *et al.,* 2004; NSDWQ, 2007). The mean pH of the distinct ward was 7.923. This is higher compared to what was reported by Shamsuddeen, (2019) 4.890 and Bukar *et al.,* (2019) 5.36. This prevents the growth of pathogenic organisms and spoilage. The temperature of the milk samples is within the recommended standard by WHO (WHO 2001). The overall mean of temperature in the study cluster was 28.4°C. This is lower compared to what was reported by Isa *et al.,* (2012) 30. Total dissolved solid (TDS) of the milk sample from all the wards exceeded the standard limit recommended by WHO, NAFDAC, and NSDWQ (WHO, 2001; NAFDAC, 2001; and NSDWQ, 2007). The TDS is the term used to describe the inorganic salt and amount of organic matter present in solution of milk. This indicates the high level of disease –causing microorganism such as bacteria and other parasites (Shittu *et al*., 2008). The mean total dissolved solid of all the sample was 2641.3±593.6 ppm. This is higher compared to what was obtain by Isa *et al.,* (2013) 247ppm and Shamsuddeen (2019) 19.27ppm.

All the milk samples analyzed in the study have unobjectionable color which is in agreement with the WHO standard. Higher conductivity was observed in the milk samples collected although, there is no disease or disorder associated with conductivity of raw milk (NSDWQ, 2007). The Turbidity of raw milk samples from all the zones exceeded the standard limit recommended by WHO (5-25 NTU) (WHO, 2001). All milk samples analyzed in the study except those from Ward Z were within the recommended standard limit by FAO (≤0.16%) for Titratable acidity. The mean titratable acidity of the samples was 0.13395±0.05. This is higher compared to what was obtained by Shamsuddeen, (2019) 0.315 and Bukar *et al.,* (2019) 2.17. The

density of the raw milk sample from ward Z and D were within the WHO recommended standard range of (1.026-1.032g/ml) (WHO, 2006). The overall mean of density, electric conductivity and turbidity of the samples was 1.029 g/ml, 4.062 µS/cm and 76.2 NTU. These are lower compared to what was obtained by Isa *et al.,* (2013) 0.98Gg/ml, 1098 µS/cm and 1.08 NTU for density, electric conductivity and turbidity respectively.

Higher viscosity was recorded in the milk sample which exceeded the standard limit recommended by WHO (1% 0.1cP) (WHO, 2006). Furthermore, the moisture content exceed the standard limit recommended by NAFDAC (80%) (NAFDAC, 2001). The mean viscosity of all the sample was 28.05 cP, the value is higher to that obtained by Bukar *et al.,* (2019) 10.14 cP while total mean moisture content of all the sample was 86.274 %. This is lower compared to what was obtain by Shamsuddeen (2019) 92.090%.

Conclusion

Identification of these five bacterial isolates; *E. coli*, *Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp. and *Klebsiella* sp. in the raw milk samples all across sampling areas signifies not only contamination but potential threat to consumer health as most of them are known enteric pathogens. However, some of the physico-chemical parameters appeared within approved standard. Higher mean density 1.02956±0.57 g/ml, electric conductivity 4.062±954.2 µS/cm, titratable acidity 0.13395±0.05, viscosity 28.05±5.29 cP, turbidity 76.2±25.0 NTU, total dissolved solid 2641.3±593.6 ppm and moisture content 86.274±3.464 % values testifies to the good conditions provided for the growth of both pathogenic and spoilage microorganisms. Raw milk obtained from all these sources is contaminated with pathogens and therefore need to be sterilized before consumption. It is recommended that there is a need to create awareness about the state of local vendor dairy products as well as to enlighten the dairy farmers and the public on the danger associated with the vendor and consumption of contaminated dairy products.

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