



Assessment of Goat Milk from Some Dairy Farms Within Kaduna North LGA, Kaduna, Nigeria

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ABSTRACT

Bacteria as food borne pathogens in quality evaluation of goat milk from selected dairy farms within Kaduna North Local Government Area (LGA), Kaduna were investigated as a measure of food safety. A total of ten (10) samples of milk were collected in sterile sample bottles and screened for the presence of food-borne pathogens. The milk samples were analyzed with preference to proximate analysis of selected milk samples, total bacteria plate/ coliform counts, most probable number and antimicrobial susceptibility test using standard techniques. The results revealed the mean average proximate composition of goat milk to be 81.1, 0.7, 2.7, 3.1, 9.0 and 3.3 for percentage (%) moisture, ash, crude protein, crude fat, total carbohydrate contents respectively. The mean value of bacterial count and coliform count were 1.7×10^6 - 2.6×10^7 CFU/mL and 1.2×10^6 – 2.4×10^7 estimated colonies respectively. The bacteria isolates were identified as *Staphylococcus aureus* (SA1), *Salmonella* sp (S1), *Shigella*. Sp (SS1), *Eschericia coli* (EC1) and *Klebsiella*. sp.(K1). All the bacteria isolates showed multi-drug resistance to more than two antibiotic drugs used, the isolates showed resistance to chloramphenicol, amoxicillin and sparfloxacin at concentrations of 30µg, 30µg and 10µg respectively. This study concludes that the quality of goat milk analyzed were poor and this could be attributed to unhygienic practices and poor animal husbandry at the farm level and these could predispose farmers, consumers and the public to risk of contracting milk-borne infections and associated bacterial resistances. It is therefore recommended that veterinarians, extension officers and all stakeholders should play their roles properly in order to ensure safe quality milk delivery to consumers.

Keywords: Milk, proximate, evaluation, bacteria, antibiotic resistance

INTRODUCTION

Goat is considered as the poor man's cow, milk and can be described as a healthy diet food due to the essential nutrients contained in it, and these essential nutrients are being required by the body in appropriate proportions (Karishma *et al.*, 2013).

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 (Pandey and Voskuil, 2011). 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.

This composition is not constant, the average percentages of milk components vary with species and breeds of animal, season, feeds, stage of lactation and health and physiological status of a particular animal (Pandey and Voskuil, 2011). Sometimes the composition might even change from day to day, depending on feeding and climate, but also during milking the first milk differs from the last milk drops (Pandey and Voskuil, 2011). Moreover, milk is an excellent source of high quality protein, vitamins, minerals such as calcium and phosphorus. Fresh milk has a pleasant soft and sweet taste and carries hardly any smell. Goat

Received 30 May, 2020

Accepted 30 June, 2020

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milk contains numerous vitamins and minerals consisting of proteins, fats and carbohydrates (Ogboluet *et al.*, 2008). Goat milk is comparable to cow milk as it contains an average nutrient composition of 3.8% fat, 4.1% lactose, and 0.8% ash content. Goat milk is less allergenic and has better digestibility. In addition, goat milk has a higher content of medium chain fatty acids (C6:0, C8:0, C10:0), these medium chain fatty acids are known to be antibacterial, antiviral, inhibit and dissolve cholesterol deposits (Silanikove *et al.*, 2010). Goat milk is a traditional food for some nomadic population in Northern part of Nigeria and many other parts of Africa (Okeke *et al.*, 2014). Goat milk can be contaminated with various pathogens or spoilage bacteria during storage or several stages of processing from farm up to table. Goats have been reported to play a special role in the life of smallholder farmers. Their small size makes it possible for farmers to keep a large herd in a small area. They are known to thrive in virtually any climatic zone and in every imaginable production system, they have also been found to be biologically and economically efficient. As a result of their great contribution to the health and nutrition of the landless and rural poor, they have been referred to as the "poor man's cow" (Suguna *et al.*, 2011). In Nigeria about 90% of the dairy goats belong to Hausa Fulani, and their women strictly control the processing of the milk (Chukwuma, 2009). As a result of low educational status of these women, the goat milk is poorly handled during processing by exposing the milk to bacterial contamination which leads to milk-borne diseases such as tuberculosis, diphtheria, listeriosis and brucellosis especially among the urban-rural residents who consume goat milk (Chukwuma, 2009). However goat milk can serve as a vehicle for transmitting diseases by several pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. (Rallet *et al.*, 2008). There are difficulties in managing the safety of milk derived from the various sources of contamination. Undesirable organisms may get into milk either through the body (endogenously) or from some external source (exogenously) after milk has been drawn. It has become increasingly clear internationally that disease in dairy animals, production and handling of milk under poor hygienic conditions can lead to widespread outbreaks of human diseases. Routinely, World over, for food safety

reasons, microbiological analysis is usually carried out to monitor and evaluate the level of pathogenic and spoilage microorganisms. Up to today no available detailed reports on the microbiological quality of goat milk. Goat milk can serve as an important vehicle for transmitting milk-borne pathogens to humans which are easily being contaminated during milking and handling. And these milk-borne pathogens causes milk-borne diseases such as tuberculosis, diphtheria, listeriosis and brucellosis. Goat milk is being exposed to a wide range of pathogens, Bacteria in particular due to a poor educational status of these goat handlers and a poor hygienic environment in which these animals are being raised. As a result of these, goat milk can be considered as a vehicle for transmitting pathogenic bacteria to humans when they consume it. There is a wide range of microorganisms that goat milk could be exposed to, but this study focused on the pathogenic bacteria that are being suspected to be found in goat milk. There is the need to employ effective control measures and improve hygiene handling of goat milk to protect public health and also create awareness programs for these goat owners by the Ministry of Health. This research aimed at evaluation the bacteriological quality of goat milk in selected dairy farms within Kaduna North Local Government of Kaduna State.

METHODOLOGY

Study Area

The study areas include; RafinGuza (A) in Kaduna North. The geographical location of RafinGuza is longitude 10.5917° N and latitude 7.4665° E and between altitudes 550-650m above the mean sea level. It has an area of 72km² and a population of 357,694 at the 2006 census. UnguwanDosa (B) in Kaduna North. The geographical location of UnguwanDosa is longitude 10.5683° N and latitude 7.4517° E. It has an area of 541km² and a population of 146,259 at the 2006 Census,

Collection of Samples

A total of ten (10) samples of milk were collected from lactating goats with five (5) samples each from targeted area within Kaduna North. Samples were collected from goats that are in their second to fourth month of lactation during their morning milking time (7am-9am) in sterile sample bottles and were placed in an ice

box, then transported to the Laboratory of Microbiology Department Kaduna State University and preserved in the refrigerator with a temperature of 4°C until the analysis.

Proximate Analysis of Goat Milk

The goat milk samples were analyzed for percentage moisture, fat content, crude protein, ash content were determined according to the AOAC (2005) Protocols.

Preparation of Media

The media used were prepared according to their manufacturer's instructions

Enumeration of Bacterial Count in Goat Milk

A tenfold serial dilution of the goat milk was prepared aseptically by dispensing 25ml of each milk sample into 225ml of sterile water to form the inoculum for each sample respectively. From the inoculum, 1 ml of each of the milk sample was transferred into the test tubes containing 9 ml sterile distilled/sterile water and homogenized by shaking followed by further decimal dilutions to up to 10^{-5} concentrations. From dilutions 10^{-4} and 10^{-5} , 1 ml was dispensed into appropriately labeled sterile Petri dishes in duplicates and sterile liquid Mueller Hinton agar was poured into each plate and swirl gently for proper mixture of the medium and the sample. The plates were allowed to solidify before being incubated at 37°C for 24 hours. Bacterial count was determined using direct plate count method as described by Egwikhide *et al.* (2014).

Enumeration of Coliform Count in Goat Milk using MPN

This was carried out according to the method described by Atlas (2008). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tube, was autoclaved to expel air and to be sterilized. Inoculation was made from the serially diluted samples as followed from the 1:10 dilution, 1ml of inoculum was transferred to each of the first three test tubes containing 9ml of lactose broth. The procedure was then repeated for 1:100 and 1:1000 dilutions respectively. All 9 test tubes were incubated at 37°C for 24 hours. Following 24hours of incubation, the tubes were observed for gas production and the number of gas positive tubes were compared with the most probable number (MPN) table to estimate the most probable number of coliforms per gram of sample. A loop

full of the inoculum was streaked on Eosine methyl blue (EMB) agar plate and incubated at 37°C for 24hours. After incubation, colonies that formed bluish black color with green sheen reddish were noted and isolated on agar slant this is the confirmatory test. The colonies were sub-cultured in to tubes of lactose broth and incubated at 37°C. The tube were incubated for 24hours for gas production, this is the complete test for fecal coliforms.

Isolation of Bacteria from Goat milk

After Incubation, discrete colonies from agar plates were picked with a flamed wire loop and sub-cultured onto a newly prepared nutrient agar plate, all plates were incubated appropriately. Purified colonies were transferred into slants and stored properly for further characterization (Mbajiuka, *et al.*, 2014).

Characterization and Identification of Bacterial Isolates from Goat Milk

The characterization of bacteria isolates from goat milk were based on colony morphology. Grams staining and selected biochemical tests which include Catalase test, Indole production test, Voges-Proskauer (VP), Methyl red test, Citrate, Coagulase test as described by Cheesbrough (2008).

Antibiotic Susceptibility Pattern of Bacteria Isolates from Goat Milk

Disk diffusion method was adopted for the antibiotic susceptibility test also known as Kirby-Bauer disk method. Using a sterilized wireloop, a loopful of the test organism was picked and streaked unto the surface of a prepared plate of Mueller Hinton agar. A filter disk impregnated with an antibiotic was applied unto the surface of the agar plate containing the organism to be tested and the plates were incubated at 37°C for 48hours. After incubation, the effectiveness of a particular antibiotic was determined by the presence of zones of inhibition around the colonies.

RESULTS

Table 1 shows the mean average proximate composition values of the parameters of goat milk. The result revealed that the milk samples A and B had slight variations in the percentage proximate compositions obtained. The morphology and biochemical characteristics of bacterial isolates from the targeted locations (A and B) are shown in Table 2. The cultural morphology of the bacteria isolates shows that

some were mucoid, dried, creamy, raised, smooth, round brownish and yellowish in colour. The Gram reaction and biochemical results revealed that some were positive while others were negative to the various test carried out and the bacteria isolates were identified probably as *Salmonella* sp, *Klebsiella* sp. *Staphylococcus aureus*, *Eschericia coli* and *Shigella* sp. From milk samples. The total coliform and bacteria count in goat milk samples are shown in Table 3 were a high and moderate numbers of aerobic mesophilic count were obtained at different dilution factor. The coliform count in goat milk samples for A and B are in Table 4. The result revealed that total number of estimated colonies ranged from 120 to 2400 for samples B and A respectively with milk sample A having the highest estimated bacteria colonies. Table 5 shows the results of the complete test for the Most Probable Number (MPN) of the milk samples from A and B locations. The milk samples analyzed were all positive. The antibiotic susceptibility pattern of Gram negative bacteria isolates (*Salmonella* sp, *Klebsiella* sp., *Eschericia coli* and *Shigella* sp) in goat milk samples are shown in Table 6 which revealed multi-drug resistance to more than two

antibiotics by the isolates at different concentrations, though most of the bacteria isolates were resistant to many of the antibiotics. The antibiotic susceptibility pattern of Gram positive bacteria isolate from goat milk is shown in Table 7 which shows multi-drug resistance to more than two antibiotics by the test isolate (*S. aureus*) at different concentrations. The results also showed that *S. aureus* was resistant to most of the antibiotics at different concentrations.

Table 1. Mean Average Proximate Composition of Goat Milk

Parameters (%)	Goat Milk Samples	
	A	B
Moisture	81.10	81.01
Ash	0.73	0.69
Crude Protein	2.74	2.70
Crude Fat	3.12	3.06
Total solid (TS)	9.01	9.06
Lactose	3.30	3.48

A= Sample from RafinGuza, B= Sample from UnguwanDosa

Table 2. Morphology and Biochemical Characteristics of Bacteria Isolates from Goat Milk

Sample	Morphology	GS	MR	VP	IN	CT	Probable Organisms
A1	Milky with black dots, round mucoid colonies	G-ve	+	-	-	-	<i>Salmonella</i> sp.
A2	Pink-purple, smooth, colonies	G-ve	-	+	-	+	<i>Klebsiella</i> sp.
A3	Brownish-yellow, smooth, round & mucoid colonies	G-ve	+	-	-	-	<i>Shigella</i> sp.
A4	Yellow, smooth, round & non mucoid colonies	G+ve	+	+	-	+	<i>S. aureus</i>
A5	Yellow, smooth, round & non mucoid colonies	G+ve	-	+	-	+	<i>S. aureus</i>
B1	Pink-purple, smooth, round and mucoid colonies	G-ve	-	+	-	+	<i>Klebsiella</i> sp.
B2	Green sheen, smooth, round and mucoid colonies	G-ve	+	+	+	-	<i>E. coli</i>
B3	Pink-purple, smooth, round and mucoid colonies	G-ve	-	+	-	+	<i>Klebsiella</i> sp.
B4	Milky with black dots, smooth, round, & brownish-yellow mucoid colonies	G-ve	+	-	-	-	<i>Salmonella</i> sp. <i>Shigella</i> sp.
B5	Yellow, smooth, round & non mucoid colonies	G+ve	+	+	-	+	<i>S. aureus</i>

A= Rafin Guza B= UnguwanDosa GS= Gram's Stain MR= MethyleRed VP= Voges Prauskaeur IN= Indole CT= Citrate + = Positive & - = Negative

Table 3. Viable Mesophilic count from Goat milk

Samples	(CFU/mL) 10 ⁻⁴	(CFU/mL) 10 ⁻⁵	Average
A1	2.0×10 ⁶	1.4×10 ⁶	1.7×10 ⁶
A2	2.2×10 ⁷	3.0×10 ⁶	1.3×10 ⁷
A3	1.2×10 ⁶	4.0×10 ⁷	2.6×10 ⁷
A4	3.0×10 ⁶	2.8×10 ⁷	1.5×10 ⁷
A5	2.3×10 ⁶	1.6×10 ⁶	1.9×10 ⁶
B1	2.8×10 ⁶	2.1×10 ⁷	1.2×10 ⁷
B2	2.6×10 ⁶	1.2×10 ⁷	7.3×10 ⁶
B3	2.5×10 ⁶	1.5×10 ⁶	2.0×10 ⁶
B4	2.1×10 ⁶	1.6×10 ⁶	1.9×10 ⁶
B5	2.7×10 ⁶	2.0×10 ⁷	1.1×10 ⁷

A= Samples from RafinGuza, B= Samples from UnguwanDosa, CFU/ML= Colony Forming Unit/ Milliliter

Table 4 Most Probable Number and of Coliform (Presumptive) in Goat Milk

Sample	Dilutions			Total Number of estimated colonies
	1:10	1:100	1:1000	
A1	3	3	3	2400
A2	3	3	3	2400
A3	1	3	3	460
A4	3	3	3	2400
A5	3	3	3	2400
B1	3	3	3	2400
B2	3	3	3	2400
B3	3	1	2	120
B4	3	3	3	2400
B5	3	2	3	1100

A= RafinGuza, B=UnguwanDosa

Table 5 Most Probable Number of Coliform (Complete test) in Goat Milk

Sample	1 st set of tubes	2 nd set of tubes	3 rd set of tubes
A1	+++	+++	+++
A2	+++	+++	+++
A3	+++	+++	+++
A4	+++	+++	+++
A5	+++	+++	+++
B1	+++	+++	+++
B2	+++	+++	+++
B3	+++	+++	+++
B4	+++	+++	+++
B5	+++	+++	+++

A= RafinGuza, B= UnguwanDosa

Table 6 Antibiotic Susceptibility pattern of Gram negative bacteria isolates

S/N	Antibiotic	Concentration In (µg)	Zone of inhibition in (mm)			
			<i>Eschericia coli</i>	<i>Shigella</i> sp	<i>Salmonella</i> sp	<i>Klebsiella</i> sp
1	Septtrin	30 µg	14	NZ	NZ	NZ
2	Chloramphenicol	30 µg	NZ	NZ	NZ	NZ
3	Sparfloxacin	10 µg	NZ	NZ	NZ	NZ
4	Ciprofloxacin	30 µg	11	11	NZ	NZ
5	Augmentin	10 µg	NZ	NZ	NZ	NZ
6	Amoxicillin	30 µg	NZ	NZ	NZ	NZ
7	Gentamycin	30 µg	14	11	12	13
8	Pefloxacin	30 µg	12	NZ	11	NZ
9	Tarivid	10 µg	10	NZ	14	NZ
10	Streptomycin	30 µg	18	NZ	16	NZ

NZ= No zone of inhibition

Table 7: Antibiotic Susceptibility Pattern of Gram-Positive Bacteria Isolates (*Staphylococcus aureus*)

S/N	Antibiotic	Concentration (µg)	Zone of inhibition (mm)
1	Pefloxacin	10 µg	NZ
2	Ampiclox	30 µg	NZ
3	Zinnacef	20 µg	NZ
4	Amoxacilin	30 µg	NZ
5	Rocephin	25 µg	NZ
6	Ciprofloxacin	10 µg	12
7	Streptomycin	30 µg	14
8	Septin	30 µg	NZ
9	Erythromycin	10 µg	10
10	Gentamycin	10 µg	15

NZ= No Zone of inhibition

DISCUSSION

The results of mean average percentage proximate composition of goat milk obtained were in slight variations and this could be attributed to the same species of the goat used in this study. This findings is in disagreement with the report of Marielli *et al.* (2015) who reported a high moisture and fat content which maybe due to the variation in species or geographical location of the goats.

There were variations in total aerobic mesophilic count in milk samples analyzed. This might have been influenced by extrinsic factors such as poor hygienic condition during handling of milk, season and geographical location of the dairy farms as reported earlier by Seguna *et al.* (2011). And it might be as a result of contamination of the milk by the hands of the milkers during milking as the milk are obtained by using the bare hands. Contamination by *Salmonella* sp and *E.coli* can be attributed directly to the surrounding environment as well as presence of feces or organic matter as reported by Kousta *et al.* (2010). The possible contamination by *Staphylococcus aureus* in goat milk might occur from infected mammary gland. In the present study, the milking process was performed by hand (by the farmers) and hence the transmission of this pathogen through contaminated hands to mammary glands of the goat might have occurred, presence of this pathogenic bacterium in milk could produce toxin at 6.0 log CFU/mL and hence anything

above this limit is unacceptable. Outbreak of *salmonellosis* is considered to be uncommon in dairy farms. However, dairy products are recognized as one of the main source of *Salmonella* contamination, which can occur from either feces or unhygienic environment encountered in the vicinity of the farm. Their presence even in threshold levels is not acceptable, considering the effects rendered on consumers as reported by Lanzaset *al.* (2010). The presence of coliform in goat milk samples collected from both farms in this study could be of fecal origin. According to food regulation acts (1985), coliform count should not exceed 1.7 log CFU/mL and *E. coli* should not be present in one (1) mL of milk sample. However feces alone might not be the cause of contamination, where other contributing factors such as poor hygiene and poor sanitary practice at farms can also play a significant role, as dairy farms have complex surroundings, coliform might be present in feces, manure and soil, which enables easy dispersal of pathogens through out the farm as reported by Linghathuri *et al.* (2010). The bacterial isolates were resistant to most of the commonly used antibiotics, this finding potentially implies a serious problem, the possible reason for this high prevalence of bacterial resistant could be due to indiscriminate uses of antibiotics by farmers/milkers. Penicillin, penicillin-streptomycin, ampicillin and oxytetracycline for example were observed to be frequently used antibiotics in the sampled farms. This indiscriminate use of antibiotics might be linked with low knowledge on animal health and husbandry, limited extension services and rampant uncontrolled availability of antibiotics even in livestock markets. Other reasons for development of resistant bacteria to antibiotic could be inappropriate use of the antibiotics in cattle, wrong dosage and routes of administration, arbitrary drug combinations and the acquisition of mobile genetic characteristics as reported by Katakweba *et al.* (2012).

Furthermore, multi-antibiotic resistance was also observed in several bacteria isolates where most of the bacterial isolates showed multi-antibiotic resistance patterns. This means that many of the antimicrobial agents that are used in livestock production are not suitable any more. There are several factors which might account for the observed multi-antibiotic resistance, this include antibiotic concentration, long-term

exposure, organism type, antibiotic type and host's immune status. Nevertheless low-level, long-term exposures to these antibiotics remain to have a greater selective potential for development of resistance than short-term, full-dose therapeutic use as reported by Shitandi (2004). In conclusion, the mean average composition of goat milk from A and B were in variations. Goat milk samples were found to be contaminated with food borne pathogens which has possible chance of posing serious health risk to the local populations as well as in the surrounding regions of the country. The study observed high resistance rate shown by some bacteria to commonly used antibiotics in livestock which was likely due to indiscriminate uses. It is therefore recommended that adequate steps need to be taken by the local Governing bodies to minimize the risks associated, by:

1. providing necessary training to the farmers as well as educating health conscious consumers. This will surely ensure long term benefits to the local dairy industries considering the local demand as well as export market of goat milk.
2. employment of hygienic milk processing and handling by the farmers.
3. implementation of task force by the local governing bodies to make sure the environment in which these animals are kept is well hygienic and maintained in that manner.

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