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RAD conceptualized idea of the research, participated in the laboratory work and drafting the initial manuscript. Authors ML, RSUW and AY have all supervised collection of the raw milk samples of camels and impacted on the decision to publish. SU immensely assisted with literature search and presentation of the manuscript. All authors have proofread and approved the final manuscript.

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Microbial Assessment of Raw Milk of Some Female Camels (*Camelus dromedarius*) in Sokoto, Nigeria

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Abstract:

In this research aliquots from gallon-full samples of raw camel milk (M1-M5) that were obtained from five domesticated female camels (*Camelus dromedarius*) at Kwalkwalawa village of Sokoto, Sokoto State, Nigeria were analysed for their microbial content using standard methods. The samples were obtained by hand milking of female camel udders after its thorough cleaning and sterilization at early morning time. Milk samples were inoculated on General Purpose Media, (GMP) and selective media to investigate their microbial presence. Results of performed microbial investigation established the presence of *Escherichia coli* sub spp 0157:H7 and *Salmonella* spp. in (M1). In the remaining milk samples (M2, M3, M4 and M5), only *Staphylococcus aureus* was detected. Thus, conclusively quality of the studied milk samples showed microbial contamination contracted possibly during processing and or its transportation to the laboratory. Raw camel milk therefore, should be used with caution.

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INTRODUCTION

In both African and Asian countries camel milk served as a staple food in arid and semi-arid regions (Abera *et al*., 2016). It is one of the most beneficial food sources for people in arid and semi-arid regions and is becoming more and more popular in many nations in Europe and North America (Abubaker and Wedda, 2019). Particularly, aside being a primary source of food, and nutrition it also provides income security all year round for some pastoralists in the region (Kebede *et al*., 2015). Given that camel milk provides practically all of the minerals needed in desert climates, it is one of the most significant nutritional sources and a remedy for the people in many arid areas (Kaskous, 2019).

The primary product from camel is milk, which is a complete meal that enables nomadic desert people maintain a healthy diet despite their difficult living conditions (Bassuony *et al*., 2014; Khan *et al*., 2016). Milk is an ideal habitat that can provide a high nutritive and favorable media for the growth and proliferation of microorganisms due to its nutritional composition which comprise protein, carbohydrate, mineral and vitamins (Abubaker and Wedda, 2019). It is a remarkable culture medium for the survival of microorganisms, with the rate of multiplication being primarily influenced by a number of variables, such as storage temperature and duration, nutrient content, and handling circumstances (Abera *et al*., 2016).

Contamination of raw camel milk with microorganisms can most likely occur along this value chain line from point of its production to the end users and as such from public health point of view its consumption should therefore be handled with caution, care and is of major concern (Musinga *et al*., 2008). Pathogenic bacteria may be present in raw milk as a direct result of udder disease. The microbes present in raw milk may be pathogenic for humans and have origins inside or outside the udder (Bassuony *et al*., 2014). The risk of camel raw milk contamination increases if the udder health is not observed, if plastic containers are used for milking and storage, if no sanitary precautions were taken during the milking, or if there is no water available for cleaning the milker or the udder before the milking (Mulwa *et al*., 2011).

Camel milk is usually consumed in its raw form (Serda *et al*., 2018), or as an unpasteurized variant of a naturally fermented food (Abera *et al*., 2016; Mwangi *et al*., 2016) without being subjected to any sort of treatment by pastoral societies in developing countries (Siboukeur, 2007). This is because it is believed that raw camel milk and its byproducts have nutritional and medicinal advantages as well as better flavor over the pasteurized milk (Hassen and Amentie, 2022). However, such practices put customers at risk for dangerous milk-borne illnesses including typhoid, paratyphoid, TB, dysentery, gastroenteritis, and others (Mohammed *et al*., 2016).

In addition to its nutritional qualities, camel milk has been linked to a number of possible health advantages, including angiotensin I-converting enzyme inhibition, hypocholesterolemic, hypoglycemic, antibacterial, and hypoallergenic effects (Al Haj and Al Kanhal, 2010). From some past time and recent immediate conducted several studies on some samples of raw camel milk among others; there were established prevalence of some bacterial pathogens in some studied samples (Matofari *et al*., 2007; Adjaine and Amiri, 2013), its chemical composition (Khalil *et al*., 2011), its physicochemical characteristics (Al Haj and Al Kanhal, 2010), its functionality (Rahli *et al*., 2013) and its microbiological quality (El-Ziney and Al-Turki, 2007; Omer and Eltinay, 2008).

Irrespective of the aforementioned facts there is still need for more data on camel milk microbial quality in order to have better characterization of camel resource products and to create its national quality norms and standards in order to promote its applications as a functional food.

Further to having more microbial quality data on raw camel milk outlined above, this study was conducted to determine microbial content of raw milk of some domesticated female camels (*Camelus dromedarius*) within vicinity of permanent site of Usmanu Danfodiyo University Sokoto, Sokoto, Nigeria which hitherto was not

done and also in consideration of an upward surge in raw camel milk's consumption owing to some purported and claimed medicinal uses, to deepen knowledge and information on its hygienic status.

MATERIALS AND METHODS

Collection of raw camel milk (CM) samples

Five (5) healthy domesticated lactating female camels (*Camelus dromedarius*), aged between 5-10 years were tracked at Kwakwalawa village along main road to permanent site of Usmanu Danfodiyo University, Sokoto and randomly selected for provision of raw milk sampling that was collected from July to September, 2021. Using aseptic technique separately gallon-full (5 litres) each of their raw milk was obtained. Milk was collected via hand milking by a skilled and experienced camel attendant after camels' udders were cleaned and sterilized. The collected CM samples were placed directly into sterilized plastic containers before being transferred into suitable sterile glass bottles (vials). These samples labelled A-E, were immediately transported via cold chain medium to the Research laboratory of the Usmanu Danfodiyo University, Sokoto and refrigerated at -20°C until required for further subsequent use.

Microbial analyses of raw camel milk

CM samples collected were further processed in accordance to the standard methods used for microbiological assays for determination of food-borne pathogens adopted by American Public Health Association, Compendium of methods for microbiological examination of foods, 3rd edn (APHA, 1992). Tests commenced within 3 hours' time of aliquot samples collection and arrival at Microbiology Laboratory to determine presence or absence of microbial content.

(i) Colonial morphology and cultural characteristics of the milk samples

Using sterile nickel-chromium wire loop of diameter 2 mm (0.002ml), a loopful each of raw camel milk (A-E) sample was separately inoculated onto three solid media and one liquid selective medium for each. The media consist of Eosin Methylene Blue (EMB) agar, Columbia Blood Agar (CBA), Mannitol Salt Agar (MSA) and selenite broth in accordance to method used by Weimer *et al*. (2011). The EMB plates and the broth were incubated at 37°C in an aerobic incubator, while the CBA and MSA plates were incubated at 42°C in a microaerophilic atmosphere with 10% CO_2 for 48 hrs. Following this incubation a sub culture of the broth was also made onto Salmonella Shigella Agar (SSA) and incubated at 37°C for 24 hrs (Cheesbrough, 2000).

A little portion of the colony was collected using a bacteriological loop, spread on a glass slide, and fixed using gentle heat. After two minutes of staining with crystal violet solution, the smear was cleaned off with running water. For one minute, Lugol's iodine was added to serve as a mordant before being cleaned once more under running water. For five seconds, acetone alcohol was added to serve as a decolorizer. Safranine was introduced as a counter stain and left to stain for two minutes after being washed with water. The slide was cleaned with water, blotted, and dried in the air before being viewed with high power (100X) objectives using immersion oil (Merchant and Packer, 1967).

(ii) Biochemical confirmatory tests of the isolates

The colonies of isolates formed from cultural growth were identified and characterized according to standard biochemical methods (Harley, 2002; Iqbal *et al.,* 2015; Mohammad *et al.,* 2021).

Baird Parker Agar (Difco labs, Detroit, Michigan, USA) (37°C/48 h) was used to identify staphylococci. The coagulase test (IDF, 1997) and the presence of thermonuclease activity on Toluidine blue O-DNA agar (sigma, St Louis, MO, USA) (IDF, 1998) verified that the presumed *Staphylococcus aureus* colonies on this medium belonged to that species.

Salmonella was identified using potassium tetrathionate broth (Biokar diagnostics, Beauvais, France) for selective enrichment and

buffered peptone water (Biokar diagnostics, Beauvais, France) for pre-enrichment. Selective enrichment medium cultures were streaked onto Hektoen Agar plates from Difco Laboratories in Detroit, Michigan, and cultured for 24 hours at 35°C. Streaks were made on nutrient agar from typical colonies on both mediums. Traditional biochemical assays and the API20E micro-identification system (BioMerieux Sa, Marcy l'etoile, France) were used to validate the identities of the isolates produced. Additionally, serological testing was used to identify the *Salmonella* suspect isolates (IDF, 2001).

All the media used were prepared based on manufacturer's instructions (Cheesbrough, 2000). All strains isolated were sub cultured onto nutrient slant agar and stored at -60°C (VT 307 A/S Vestfrost DK-6705 Esbjerg Ø, Denmark) for further testing.

RESULTS

Obtained results from morphological characteristics peculiar to the isolated microbes have pointed that *Escherichia coli* and *Salmonella* spp were found in M1 sample while the remaining samples of raw camel milk were typical of *Staphylococcus aureus* which were subjected to further biochemical confirmatory tests. The colonial/morphological characteristic after sub culturing of the picked milk samples is presented in Table 1. The results of biochemical confirmatory tests for microbes found in sub cultured growth are shown in Table 2. Summary of the isolated microbes is presented in Table 3.

Table 1. Colonial and morphological characteristics after growth of sub culturing milk samples.

Samples	EMB	SSA	MSA	Citrate
M1	Green Metallic Sheen (GMS)	Dark centered colourless transparent colour	-ve (-)	-ve (-)
M2	-ve (-)	Golden yellowish background	+ve (+)	-ve (-)
M3	-ve (-)		+ve (+)	-ve (-)
M4	-ve (-)		+ve (+)	-ve (-)
M5	-ve (-)		+ve (+)	-ve (-)

Key (-)/ (-ve):-negative, no microbial presence or growth observed, (+)/ (+ve):- there is indicated microbial presence or growth against culture medium it is incubated. M (1-5):-raw camel milk samples. EMB:-Eosin Methylene Blue, SSA:-Salmonella Shigella Agar, MSA:-Mannitol Salt Agar

Table 2. Biochemical confirmatory tests used for bacterial identification.

Sample	Coagulase	Catalase	Urease	Sucrose/lactose	Glucose	H_2S	Organism found
M1	NA	NA	ve (-)	-ve (-)	+ve (+)	+ve (+)	*E. coli* 0157:H7, *Salmonella spp*
M2	-ve(-)	-ve (-)	-ve (-)	-ve (-)			*S. aureus*
M3	-ve (-)	-ve (-)	-ve (-)	-ve (-)			*S. aureus*
M4	NA	Nil	-ve (-)	+ve (+)			*S. aureus*
M5	-ve (-)	+ve(+)	-ve (-)	+ve (+)			*S. aureus*

Key:-(-)/(-ve):-negative, no such reaction; (+)/ (+ve):-there is reaction against tested biochemical. NA:-Not applicable, M (1-5):-raw camel milk samples. *E. coli:-Escherichia coli; S. aureus:-Staphylococcus aureus.*

Table 3. Incidence of bacteria in raw camel milk samples.

Sample	*E.coli 0157:H7*	*S. aureus*	*Salmonella spp*	*Citrobacter pneurelli*
M1	+	-	+	-
M2	-	+	-	-
M3	-	+	-	-
M4	-	+	-	-
M5	-	+	-	-

Key:-:-M1-5:- raw camel milk samples 1-5; *E.coli:-Escherichia coli; S. aureus:-Staphylococcus aureus;* (+):-present, (-):-absent.

DISCUSSION

The result of the present conducted study has established presence of *Salmonella* spp and *E.coli* sub specie *0157:H7* in one of the sample of raw camel milk analysed (M1). This finding was partially consistent with Al All et al. (2012) previous studies which also found 5 *Salmonella* spp*.,* 12 *E. coli* and 2 *Listeria monocytogenes* from total of 185 camel's milk samples collected from Sinai, Aswan and Sharqia Governorates, in Egypt. *Listeria monocytogenes* was however not detected in this study. *E. coli* in milk products indicates the existence of enteropathogenic germs, which pose a risk to the health of consumers. Similarly this finding of *Salmonella* spp*.* and *E. coli* in this study had agreed partially with Ayyash et al. (2017) that equally found *Salmonella* spp*., E. coli* sub specie 0157:H7 and *S aureus* in some screened milk samples. Since camel raw milk is often consumed fresh or as a naturally fermented product, it is not pasteurized and is therefore susceptible to contamination at any stage of the milk production and processing process, lowering its quality and safety level (Kaskous, 2018). Camel's raw milk can get contaminated at any point throughout the milking process. One indicator of this is the predominance of udder inflammation in clinical and subclinical mastitis (Wanjohi *et al*., 2013; Niasari-Naslaji *et al*., 2016; Kaskous, 2019) or by unhygienic hand milking (Kaskous, 2019) or even after its harvesting in its value chain as raw milk is very good suitable liquid for microbial growth (Kaskous, 2019). Similarly, result of the M1 sample finding in this study is also in agreement with Adjaine and Amiri (2013) studies. Contrariwise, however *Salmonella* was not found in some other raw camel milk samples screened elsewhere in some studies notably (Konuspayeva *et al*., 2007).

On another aspect of this study, it disagrees with Al All et al. (2012) and Ayyash et al. (2017) studies that found *Listeria monocytogenes* (*L. monocytogenes*) which was not found and reported in this study. Moreover, *Staphylococcus aureus* too that was also detected in addition in the remaining raw camel milk samples (M2-M5) of this study; was another commonly encountered food especially milk-borne pathogen. This finding of *S. aureus* in some milk samples and the earlier *Salmonella spp* and *E. coli 0157:H7* agrees *in toto* with El Demerdash and Al-Otaibi, (2013) studies where *Staphylococcus aureus, Salmonella* and *E. coli 0157:H7* were all found from the collected and analyzed fifty samples of raw camel milk samples from different zones of eastern area of Kingdom of Saudi Arabia, (KSA) for their microbiological quality. It differs with it slightly in not finding *Bacillus cereus,* and *Listeria monocytogenes.*

Escherichia coli serotypes, *Salmonella* spp, and *Staphylococcus aureus* findings in this study is also in agreement with Elhaj *et al*., (2013) that similarly reported such microbial presence in addition to *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Micrococcus* spp. and *Streptococcus* spp. in a study on aerobic bacteria and fungi associated with raw camel's milk. In their study, they concluded that raw camel milk contains a variety of microorganisms that may be harmful to human health. Bacterial contamination of camel raw milk can occur at four different levels: within the udder, following harvest, from the surface of milk processing equipment, and during storage and transit

(Kaskous, 2018), thus most likely the microbial contamination observed from this study could have possibly occurred from any of the likely listed points along the chain as documented in other investigations (Kaskous, 2019). Many food poisoning outbreaks could be caused by the use of milk from sick animals or food produced in contaminated environments or by workers who have bacterial infections.

CONCLUSION

Microbial analyses done on samples of collected raw camels' milk had showed poor raw camel milk quality. This could partly be as a result of contamination from many possible sources along its chain. If the camels' udders are disease-free then after milking or during milking down to transportation and storage or it's processing before or after reaching the microbiology laboratory. Thus, the investigated camel milk samples were contaminated with some of the commonly encountered food-borne pathogens associated with especially dairy product which can flourish very well in milk medium. It is also advised to appropriately heat treat raw camel milk before consumption because there may be a possible health risk linked with consuming it due to the presence of microorganisms. As a result of the study's findings, the value chain for raw camel milk must adopt sufficient hygienic practices. The cleanliness and hygiene of the workforce, the quality of the water, the cooling equipment, and the prevention of contamination of raw camel milk are all crucial. Contamination of raw camel milk is quite possible because these are seldom exercised at the herd level.

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CONFLICT OF INTEREST

The authors hereby declare that they had no competing or conflict of interest.

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