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RAD conceptualized idea of the research, have participated in the laboratory work as well as drafting the initial manuscript. Authors ML, RSUW and AY have all supervised the collection of raw urine samples of camels and impacted on the decision to publish. IS 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 Urine of Some Female Camels (*Camelus dromedarius*) in Sokoto, Nigeria

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Abstract:

Five (5) litres each of raw urine obtained from apparently five healthy domesticated female camels (*Camelus dromedarius*) at Kwakwalawa village of Sokoto, Sokoto State, Nigeria were analysed for their microbial content using standard methods. The urine samples (U1-U5) were obtained overnight whenever the female camels were urinating by placing open plastic bowls to trap the urine with the aid of skilled camel attendants. Bacteriological culture and biochemical identification of the isolates found was done in accordance with standard protocols as described by UK Standards for Microbiological Investigation (SMI), 2017. Result of the microbial investigation showed *E coli* sub spp 0157:H7 and *Salmonella spp* found in U1 sample while detected in U2 sample were *Citrobacter pneurelli* and *S. aureus* only. There was no any microbial presence found in samples U3, U4 and U5. In conclusion, the few studied raw urine samples of camels' revealed minimal picture of microbial contamination of the collected samples, even though very few.

INTRODUCTION

There has been a greater focus on camel research since it has been shown that this animal can survive droughts more effectively than any other species (Sumia *et al*., 2016). Camels normally drink water only once during the winter and four times during the summer; a feat very noteworthy. The two types of camels are dromedary camels (*Camelus dromedarius*, an Arabian species having one hump) and Bactrian camels (*Camelus bactrianus*, a Central Asian species having two humps) (Patel, 2018; Abdel-Aziz and El-Meghanawy, 2016).

In times of scarcity of food and water, the fat stored in the camel humps may be metabolized. Camels are thought to be excellent sources of milk and meat (Ismaili *et al*., 2016; Khan *et al.,* 2016). For thousands of years, camels have been employed as a mode of transportation since they can support up to 500 pounds on their backs. The primary source of meat, milk, and occasionally even leather or wool items comes from domestic camels. They weigh 400 to 600 kg and stand taller than 6 feet. When there is water available, they may each consume 113 litres at once. In the absence of food and water, camels may live for up to six months (Patel, 2018).

Animal urine was widely used as a cure for worms, dropsy, abdominal enlargements, flatulence, colic, anaemia, abdominal tumour, lack of appetite, TB, poison, hemorrhoids, amenorrhea, leukoderma, leprosy, aggravation of kappa, and various other mental illnesses (Thakur, 2004).

Magnesium, potassium, and albumin are abundant in camel urine, which helps camels retain sodium in their bodies to avoid the expulsion of huge volumes of water and to maintain it inside for extended periods of time (Abdelzaher *et al*., 2020). In folk medicine, camel's urine has been used to cure a wide range of ailments. The early Arabs boiled camel urine and drank it to treat certain internal health issues, like fasciolosis, as well as to treat various disorders in general, like hepatitis, liver swelling, and abscesses (Ahmed *et al.,* 2008; El-Shahawy *et al*., 2010).

Drinking camel milk and urine has a number of documented health advantages that have been supported by contemporary scientific studies from the early days of medical science (Al-Abdalall, 2010). Although urine is a waste product of the body, it is employed both inside and outside for medicinal purposes due to its various medical applications (Al-Abdalall, 2010). *Lactobacilli* bacteria; a probiotic microbe was isolated from buffalo milk, camel milk and camel urine and it was all shown to have demonstrated varying levels of antibacterial activity against pathogenic microbes (Abdou *et al*., 2021). Eventhough the data presented in that research have indicated that the least antibacterial activity isolated was from camel urine; compared to milk from both buffaloes and camels, but the antibacterial, antifungal and antiviral activity of both camel milk and urine were earlier reported in some studies (Al-Bashan, 2011; Humaid, 2016; Hu *et al*., 2017). Camel urine is frequently used to treat cancer and respiratory tract diseases in traditional medicine (Al-Kabarity *et al*., 1988). There are no degenerative symptoms seen in the liver or kidney tissues after using camel milk or urine (Khalifa *et al*., 2005; El-Elyani and Khalifa, 2006).

This study was carried out to assess microbial quality of raw urine from some domesticated female camels (*Camelus dromedarius*) around permanent site of Usmanu Danfodiyo University, Sokoto, Nigeria in view of the upward surge in its oral consumption albeit with a motive for its purported medicinal uses, to get enlightened on raw camel urine microbial content because of its wide uses in alternative medical practice.

MATERIALS AND METHODS

Raw camel urine (CU) sample collection

Five (5) healthy domesticated lactating female camels (*Camelus dromedarius*) of 5-10 years age were tracked at Kwakwalawa village along main road to permanent site of Usmanu Danfodiyo University, Sokoto, Sokoto State,

Nigeria between the months of July to September, 2021 and they were used to provide the needed urine samples (CU) overnight. Using aseptic technique separately gallon-full (5litres) each of CU from the tracked camels were obtained. CU was collected by trapping and placing open bowl (plastic) containers; whenever any of those camel urinates with help of experienced camel attendant. The quantity of CU obtained was later transferred into suitable sterile glass vials. These samples were labelled A-E.

Samples (A-E) were immediately transported via frozen ice pack cold chain medium in big vaccine container to University Central Research Laboratory at main campus within (<4 hours) of their collection and were immediately refrigerated at -20°C until required for further subsequent use.

Equipments, materials and reagents

Microscope, sterile bottles, glass slides, chemicals, reagents and all other equipments used were of analytical grade.

Microbial analyses of the CU samples

Exactly 40mls (aliquot) of each samples (A-E) were taken in clean sterile bottles made for microbiological assays and processed further according to standard microbiological methods for determination of especially food-borne pathogens adopted by American Public Health Association, Compendium of methods for microbiological examination of foods, 3rd edn (APHA, 1992). Tests commenced within 8 hours 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 isolates from urine samples

The bacteriological loop was used to take up a tiny colony, which was then spread out on a glass slide and gently heated to fix it. Following the application of crystal violet solution, the stain was left on the smear for two minutes before being removed with running water. After adding Lugol's iodine as a mordant for one minute, the items were once again rinsed under running water. After that, decolorizer acetone alcohol was applied for 5 seconds. Safranine was introduced as a counter stain and left to stain for two minutes after being washed with water. After being cleaned with water, blotted, and dried in the air, the slide was inspected using immersion oil and high power objectives (100X) under a microscope (Merchant and Packer, 1967).

(ii) Biochemical confirmatory tests of the isolates formed

Using a sterile nickel-chromium wire loop of diameter 2 mm (0.002ml) a loopful each of the raw camel urine samples (A-E) and was 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 F broth (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).

The colonies of isolates formed from cultural growth were further identified and characterized according to standard biochemical methods which included catalase, urease, hydrogen sulphide production, citrate utilization, Triple Sugar Iron (TSI) Agar, sugar utilization tests and microscopy of Gram stained isolates as described in detail below. In addition, other set of biochemical tests were also used to detect presence of microbial organisms (Iqbal *et al.,* 2015). This includes carbohydrate fermentation, ß galactosidase activity, gelatin hydrolysis, amino acids and enzyme activity for coagulase (performed in this assay), lysine, ornithine decarboxylase and phenylalanine deamination test (Harley, 2002).

Staphylococci were detected on Baird Parker Agar (Difco laboratories, Detroit, Michigan, USA) by incubation at 37°C for 48 h. The identity of

presumptive *S. aureus* colonies on this medium was established by the coagulase test (IDF, 1997) and the manifestation of thermonuclease activity on Toluidine blue O-DNA agar (sigma, St Louis, MO, USA) (IDF, 1998).

Salmonella was identified using buffered peptone water as the pre-enrichment medium and potassium tetrathionate broth as the selective enrichment media (both from Biokar Diagnostics, Beauvais, France). Hektoen Agar plates (Difco labs, Detroit, Michigan, USA) were streaked with cultures from the selective enrichment medium, and the cultures were then cultured for 24 hours at 35°C. On nutrient agar, typical colonies on both mediums were streaked. By using both conventional biochemical assays and the API20E micro-identification system (BioMerieux Sa, Marcy l'etoile, France), the identities of isolates were further verified. The *Salmonella* presumed isolates were also characterized serologically (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

The results of the microbiological study revealed that *E. coli* subsp. 0157:H7 and *Salmonella* spp. were detected in U1 sample, but *Citrobacter pneurelli* and *S. aureus* were the only microorganisms found in U2 sample. In samples U3, U4, and U5, no microbiological presence was detected.

The colonial morphological characteristics of the smeared urine samples are given in Table 1. The results of biochemical confirmatory test are presented in Table 2. Summary of the isolated microbes is presented in Table 3.

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

Samples	EMB	SSA	MSA	Citrate
U1	Green Metallic Sheen (GMS)	-ve (-)	Golden yellowish background	-ve (-)
U2	Green Metallic Sheen (GMS)	-ve (-)	-ve (-)	+ve (+)
U3	-ve (-)	-ve (-)	-ve (-)	-ve (-)
U4	-ve (-)	-ve (-)	-ve (-)	-ve (-)
U5	-ve (-)	-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. U (1-5):-raw camel urine sample; EMB:-Eosin Methylene Blue, SSA:-Salmonella Shigella Agar, MSA:-Mannitol Salt Agar

Table 2. Biochemical confirmatory tests.

Sample	Coagulase	Catalase	Urease	Sucrose/lactose	Glucose	H_2S	Organism found
U1	+ve (+)	+ve (+)	Weak but +ve (+)	+ve (+)	+ve (+)	-ve (-)	*Escherichia coli* 0157:H7 *Salmonella spp*
U2	NA	NA	-ve (-)	+ve (+)	+ve (+) +ve (+)		*S. aureus, Citrobacter pneurelli*
U3	-ve(-)	-ve (-)	-ve (-)	-ve (-)	-ve (-)	-ve	Nil
U4	-ve (-)	-ve (-)	-ve (-)	-ve (-)	-ve (-)	-ve	Nil
U5	-ve (-)	-ve(-)	-ve (-)	-ve (-)	-ve (-)	-ve	Nil

Key:-(-)/(-ve):-negative, no such reaction; (+)/ (+ve):-there is reaction against tested biochemical. NA:-Not applicable, U (1-5):-raw camel urine sample, *S. aureus:-Staphylococcus aureus.*

Table 3. Summary of isolated microorganisms after biochemical tests.

Sample	*E.coli 0157:H7*	*S. aureus*	*Salmonella spp*	*Citrobacter pneurelli*
U1	+	+	+	-
U2	-	+	-	+
U3	-	-	-	-
U4	-	-	-	-
U5	-	-	-	-

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

DISCUSSION

Results of this study; that is microbiological screening of samples of raw urine of some domesticated female camels (*Camelus dromedarius*) have found presence of *E. coli* 0157:H7, *Salmonella spp.* and *S. aureus* in one of the five samples of raw urine; (U1) analyzed. Thus, this *Salmonella spp* isolated from some of the samples of camel's raw urine in this study has partially agreed with Hamza *et al*., 2012 research that similarly isolated *Salmonella spp* in a random sample studies but with human urine samples among some patients that have urinary tract infection in Iraq. Camel urine and some samples of buffalo and camel milk were all found to contain some probiotic bacteria which have been recognized for their beneficial health effects in humans and animals (Abdou *et al*., 2021). Although pee had previously been believed to be sterile, sequencing techniques have revealed that urine is also colonized by natural flora, including *Lactobacillus* and *Streptococcus* (Akgul and Karakan, 2018). Urine microbial content found in the present study was not consistent with findings of Abdou *et al*., 2021.

Moreover, although some available data from camel's urine showed that it have significant antimicrobial activities against some pathogenic microbes such as *S. aureus, P. aeruginosa, E. coli* and other pathogenic microbes (Kabbashi and Omer, 2016), this present research, however differs with the just mentioned fact in that the studied camel urine here do contained such microbes without presence of *P aeruginosa,* the only microbe which made the two studies to differ in terms of their contamination and contrariwise activity. In addition, *Citrobacter pneurelli* and *S. aureus* were also detected in this study in another screened sample of raw camel urine, thus

further establishing contamination level of the commonly consumed camel urine commodity.

CONCLUSION

The microbial analysis revealed poor raw camel urine quality as evidenced from two of the samples which are U1 which contained two detected microorganisms, namely *E. coli*: 0157:H7, and *Salmonella spp* while U2 contained *C. pneurelli* and *S. aureus*. Poor sanitary practices while handling the raw urine after it was obtained or during its storage and transportation could partially be ascribed to this observed anomaly. Improved personal hygiene of the personnel handling samples along storage and transportation chain might change the ugly occurrence in view of the widely practice of its raw oral consumption among many cultures without the necessary involvement of any decontamination process.

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CONFLICT OF INTEREST

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

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