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Isolation and Molecular Characterization of Camel (*Camelus dromedarius*) Urine Microbiota and its Response to Different Grazing

Ibtesam Alshamrani[1*], Turki M.Dawoud[2], Ahmed M.Al-Hejin[3] and, Ihap Moussa[4]

[1,2]Department of Botany and Microbiology, Science College, King Saud University, Riyadh KSA
[3]Department of Biology, Science College, King Abdulaziz University, Jeddah KSA
[4]Department of Botany and Microbiology, Science College, King Saud University, Riyadh KSA

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***Corresponding author:** Ibtesam Alshamrani
Department of Botany and Microbiology, Science College, King Saud University, Riyadh KSA

Abstract **Original Research Article**

Background: Camels' urine has been utilized for therapeutic purposes and anecdotally hailed as a treatment for a variety of ailments for millennia. The purported medicinal effects of camel urine, on the other hand, have yet to be thoroughly investigated and it has not been verified whether it is free of bacteria dangerous to human health. The common practice of using camel urine as a therapeutic agent in the Taif region of Saudi Arabia may pose health risks. This study examines the distribution of urine microbiota in camels and effect of the type of nutrition on microbiota. ***Material and methods:*** Therefore, 80 camel urine samples were collected from 6 different stations in this region. Urine was collected from healthy male and female (pregnant ,mother) camels aged 1–8 years .The camels were divided into two groups with a different natural feeding pattern G1:which grazes naturally on wild plants (wild feeding)&G2: which grazes naturally on wild plants, fodder and barley(mixed feeding). The 16S rRNA gene of the bacteria will be sequenced to the identify of bacteria isolated from camel urine. Finding a relationship between the urine microbiota and the mode of nutrition by profiling the bacterial species in both groups. ***Results:*** 16S rRNA gene sequence analysis showed that most of the isolates were *Bacillus velezensis* and *Bacillus tropicus*, constituting 42 % and 15 % of the samples, respectively. Surprisingly, these bacterial species have an antimicrobial effect. This may explain the role of camel urine as an antimicrobial. The comparative 16SrRNA Sequencing revealed significant differences in the urine microbiota between the two groups. This indicates the effect of the camel's diet on the microbiota of camel urine.

Keywords: Camel urine; pathogens; urine microbiota; 16S rRNA.

INTRODUCTION

The camel, often known as the "ship of the desert," is renowned for its endurance and ability to travel great miles without food or water in the desert (Fahmy, 2015). Camels(*Camelus dromedarius*)have traditionally been an important part of the lives of people living in arid regions, such as the African, Asian, and Arabian deserts.

To the people of the Arabian Gulf region, camels are an economically important and sustainable livestock species owing to their unique characteristics of being the desert animals most adapted to hot and arid environments, desertification, and scarce natural resources. Additionally, due to their unique genetic makeup, camels are naturally resistant to most diseases that commonly affect livestock (Ali *et al*., 2019 & Ismail *et al*., 2014).

Since they are a multipurpose livestock species of great economic importance, camel rearing and breeding for food (meat and dairy), transport, and leisure have been an integral part of the lives of people living in arid areas. Additionally, there is a growing body of evidence on the therapeutic potential of camel-derived products, such as their milk, antibodies, and urine (Majid, 2011&Fedlelmula *et al*., 2016).

Camel urine has been considered a “miraculous” Prophetic Medicine since ancient times and was commonly used in the pre-Islamic era (O'haj *et al*., 1993). In particular, owing to their antifungal and antimicrobial potential (Al-Haider *et al*., 2011& Sumia *et al*., 2016), the milk and urine of camels are used as tonic beverages for the treatment of common ailments.

Patients have been administered camel urine concoction (approximately 100 mL/day) either alone or mixed with milk for the treatment of fasciolosis, a parasitic worm infection that causes symptoms such as hepatitis, swollen liver, and abscesses (Ahmed *et al*., 2008; ELShahawy *et al*., 2010). A study has indicated that the antimicrobial activity of camel urine can protect the liver from carbon tetrachloride-induced damage (AlBashan, 2011). In addition, gastroprotective and ulcer-healing effects of camel urine have been reported (Hu *et al*., 2017).

Furthermore, camel urine contains metabolites that exhibit beneficial pharmacological properties similar to those of antibacterial, antifungal (Al-Bashan, 2011; Alzahrani and Alharbi, 2011), antiviral, and anticancer agents (Al-Yousef *et al.,* 2012). Among the Arabian population, camel urine has been used to treat diabetic neuropathy and enhance the luster of women's hair (Alhaidar *et al*., 2011; Al-Awadi and Al-Judaibi, 2014).

The use of camel urine as a therapeutic agent against diabetic neuropathy among the Asian population has also been reported (Agarwal *et al*., 2009).

Camel urine has an unusual and unique biochemical composition. Unlike that of humans and other animals, camel urine is devoid of bad odor and toxicity due to low traces of urea and the lack of ammonia. However, studies have shown that creatine and creatinine are present in camel urine. Compared to human urine, camel urine contains about 10-fold more mineral salts. Furthermore, human urine is weakly acidic or basic, whereas camel urine is basic, with a pH ≥7.8 (Read, 1925; Mostafa and Dwedar, 2016).

Sumia, *et al.* (2016)., found the antimicrobial effects of camel urine samples on *Staphylococcus aureus, Escherichia coli, Salmonella spp., Pseudomonas aeruginosa*, and *Enterobacter cloacae*. Other studies have investigated the antimicrobial activity of camel urine against pathogenic microorganisms, including fungi, such as *Aspergillus niger, A. flavus, Fusarium oxysporum, Rhizoctonia solani, Aschochyta spp., Pythium aphanidermatum, Sclerotinia sclerotiorum,* and *Candida albicans*, as well as bacteria, such as *S. aureus, Streptococci, E. coli, P. aeruginosa, and Klebsiella pneumoniae*, camel urine exhibited high antimicrobial activity against various common pathogenic microorganisms. Al-Abdalall, 2010, Al-Bashan, 2011). concentrated camel urine exerted the highest inhibitory effect on the growth of bacteria. Mostafa and Dwedar, 2016) found that all concentrations of camel urine completely inhibited the growth of four C. albicans, one non-albicans Candida, and 10 multidrug-resistant coagulase-negative staphylococci isolates. Camel urine at 10%, 7.5%, and 5% concentrations completely inhibited the growth of methicillin-resistant *S. aureus* (MRSA), *Enterococcus spp*., and ESBL- and carbapenemase-producing gram-negative bacilli. However, all these isolates exhibited significant growth when treated with camel urine at a concentration of 2.5%.

Cancer is a leading health problem worldwide. Found that camel urine plays an important role as an anticancer (Al-Yousef *et al*., 2012 ; Rood *et al*., 2004 and. Romli *et al*., 2017)

Ahamad *et al*. (2017) examined 250 camel urine samples from different areas in Sudan and isolated 11 gram-negative bacterial strains, namely one isolate each of *Staphylococcus hominis, S. hyicus, S. capitis, S. haemolyticus, Corynebacterium xerosis, C. striatum, C. pseudodiphtheriticum, Bacillus cereus, and Mannheimia haemolytica*, and two isolates of *Micrococcus* spp.

Al-Zahrani *et al*. (2011) confirmed that, the high inhibition potential of both fresh and preserved camel urine against the growth of MRSA. Untreated camel urine samples showed the greatest inhibition of bacterial growth. Samples of camel urine that were stored for one year at 5 °C exhibited higher inhibitory effect on the growth of MRSA, especially in a liquid culture medium. Furthermore, an isolate of *E. coli*, which exhibited inhibitory effects on 3 isolates of MRSA that harbored the *mecA* gene, and an isolate of lactic acid bacteria (LAB) that inhibited the growth of MRSA isolates were isolated from camel urine.

Salipante *et al*. (2013) employed 16S rRNA next-generation sequencing to rapidly identify the bacterial composition in polymicrobial infection specimens. Therefore, to overcome the limitations of conventional clinical methodology, this metagenomics approach could be used to accurately profile bacterial species in mixed-infection specimens. 16S rRNA next-generation sequencing has been used to detect the presence of pathogens that are not detectable using standard clinical culture method, particularly the bacterial strains most commonly associated with respiratory tract infection in patients with cystic fibrosis, and fastidious bacteria, such as the *Burkholderia cepacia* complex and *Mycobacterium avium* complex.

He *et al.* (2018) investigated the bacterial communities in 8 different segments (rumen, reticulum, abomasum, duodenum, ileum, jejunum, cecum, and colon) of the gastrointestinal tracts (GITs) and feces of 11 Bactrian camels using 16S rRNA gene amplicon sequencing. Twenty-seven phyla of bacteria were found in the GIT; the fecal microbiome was dominated by Firmicutes, Bacteroidetes, and Verrucomicrobia. There were significant differences in microbial community composition between different segments of the GIT. He *et al*. [28] employed 16S rRNA gene sequencing to characterize fecal microbiota in 2-month-old Bactrian camels and found that Firmicutes, Proteobacteria, and Actinobacteria were the most abundant bacteria. The

fecal microbiota in camels that were 1–3 years of age comprised predominantly Firmicutes, Verrucomicrobia, and Bacteroidetes. In addition, the stability and diversity of gut microbiota increased with age, whereas genes associated with immune system diseases were markedly enriched at 2 months of age. In a recent study, LAB, such as *Enterococcus mediterraneensis*, *Lactobacillus fermentum*, and *Streptococcus lutetiensis*, were isolated from feed ingredients and dairy and meat products. Using 16S rRNA gene sequencing and *in vitro* characterization of probiotic potential, Ahmed *et al.* (2021) discovered a new species of probiotic bacteria, *E. mediterraneensis*.

Rafig Gurbanov, *et al*., (2022)discover the variations in the intestinal microbiota of rats living in urban and rural environments. By using the 16S rRNA next-generation sequencing technology, the taxonomic alterations in the gut microbiota of wild rats belonging to the Rattus rattus species were captured in urban and rural areas of Western Anatolian (Bilecik province) were compared. As reference animals, laboratory rats were used.

The alpha diversities were found to be lower in rural rats compared to urban rats, with laboratory rats having the highest alpha diversity. When comparing rural and laboratory rats to urban rats, the lower Firmicutes to Bacteroidetes ratios (F/B ratio) were found. When compared to urban rats, the Proteobacteria to Actinobacteria ratio (P/A ratio) was lower in rural rats but higher in laboratory rats. At the species and genus levels, heatmap analyses of taxonomic units in each group's microbiota revealed distinct patterns.

The goal of this study was to use 16S rRNA amplicon and shotgun metagenomics to establish the rumen microbial profile of an Indian camel. The camels were fed three different diets, each with a different source of roughage. The comparative metagenomic study revealed that significant differences between two fractions of rumen content were found in larger proportions, followed by diet-related differences. There were also significant differences in the rumen bacteria gathered at different time periods during the feeding phase (Hinsu *et al.*, 2021).

This study was the first to detect the composition of urine microbiota in Bactrian camels of different ages, and our findings provide a baseline for future camel microbiology research.

RESULTS

2.1. 16S rRNA Gene Sequencing of Isolates

Bacterial strains were isolated from 54 (67%) out of the 80 camel urine samples collected. 16S rRNA gene sequence analysis showed that most of the isolates were *Bacillus velezensis* and *B. tropicus*, constituting 42% and 15% of the samples, respectively (Table 1). Ten isolates were identified as *Desemzia incerta*, five as *Exiguobacterium acetylicum*, three each as *Pseudescherichia vulneris* and *Aerococcus urinaeequi*, two each as *Enterococcus mundtii*, *Acinetobacter radioresistens*, *Pisciglobus halotolerans*, *Bacillus haynesii*, *B. subtilis* spp., and *B. albus*, and one each as *Bacillus amyloliquefaciens*, *B. nitratireducens*, *Planococcus maitriensis*, *Bacillus sonorensis*, *B. aryabhattai*, *Aerococcus urinaehominis Enterococcus faecalis*, *Staphylococcus warneri*, *Bhargavaea beijingensis*, *Bhargavaea cecembensis*, *Lysinibacillus macroides*, *Corynebacterium stationis*, and *Microbacterium oleivorans*.

A total of 114 bacterial isolates, comprising 15 distinctive genera, were identified from camel urine samples. Among the isolates, Bacillus (n = 13) was the most abundant genus, whereas the other 14 genera included *Desemzia*, *Exiguobacterium*, *Pseudescherichia*, *Aerococcus*, *Enterococcus*, *Acinetobacter*, *Pisciglobus*, *Planococcus*, *Staphylococcus*, *Bhargavaea*, *Paenibacillus*, *Lysinibacillus*, *Corynebacterium*, and *Microbacterium*.

The most abundant species found in more than 64% of the samples were the members of the genus *Bacillus*, namely, *B. velezensis*, *B. tropicus*, *B. haynesii*, *B. subtilis*, *B. albus*, *B. amyloliquefaciens terium*, *B. sonorensis*, *B. aryabhattai*, *B. amyloliquefaciens*, *B. flexus*, *B. inaquosorum*, and *B. nitratireducens*. Other genera that were widely distributed included *Desemzia* and *Exiguobacterium* respectively.

2.2. Pathogenic Strains Isolated From Camel Urine Samples

Surprisingly, 16S rRNA sequence analysis showed that many isolates shared a 16S rRNA gene sequence similarity of 99% with that of the bacterial strains commonly detected in the region. Additionally, pathogenic strains were detected in more than 2.6% of the urine samples, e.g., *Pseudescherichia vulneris.* causing complicated diarrhoea and sepsis in an infant.*(Jain et al.,2016) E. faecalis*, can cause different nosocomial infections, especially urinary tract infection (UTI) Hashem, *et al*., (2021), where it were isolated from female and mothers camel who mixed feeding . One pathogenic strain isolated from of the urine samples included *B. flexus* which was isolated from a pregnant camel urine and mixed feeding. Those female were more than 6 years old.

Table-1: Number of bacterial strains isolated from camel urine

Strain putative name (16S rRNA)	No.
Bacillus velezensis	48
Desemzia incerta	10
Bacillus subtilis spp.	2
Bacillus sonorensis	1
Bacillus haynesii	2
Bacillus aryabhattai	1
Bacillus amyloliquefaciens	1
Bacillus tropicus	18
Bacillus nitratireducens	1
Bacillus flexus	*1*
Bacillus inaquosorum	1
Bacillus albus	2
Aerococcus urinaeequi	3
Exiguobacterium acetylicum	5
Aerococcus urinaehominis	1
Enterococcus mundtii	2
Enterococcus faecalis	1
Acinetobacter radioresistens	2
Staphylococcus warneri	1
Bhargavaea beijingensis	1
Bhargavaea cecembensis	1
Lysinibacillus macroides	1
Corynebacterium stationis	1
Pisciglobus halotolerans	2
Pseudescherichia vulneris	2
Planococcus maitriensis	1
Microbacterium oleivorans	1
Number of species	27
Total	114

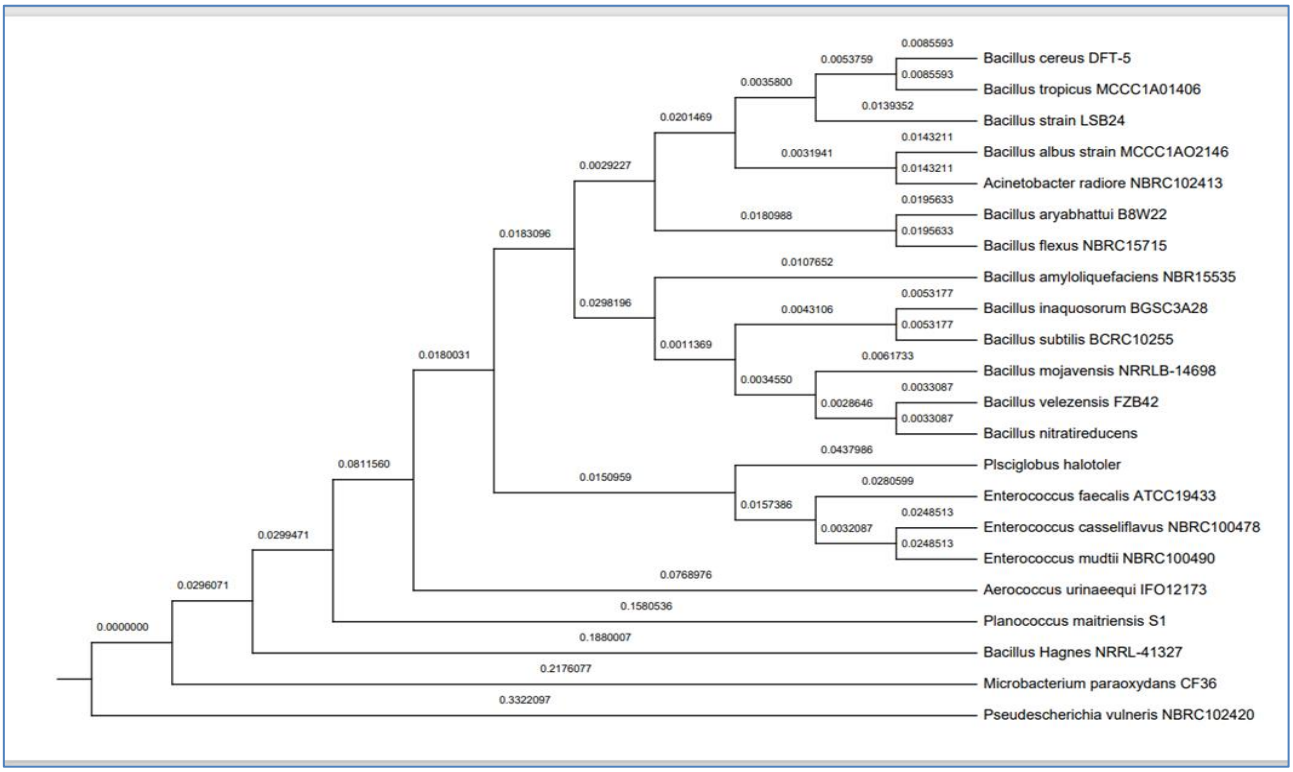


Fig-1: The phylogentic position of some important pathogenic isolates obtained from camel urine samples.

2.3. Relationship between Sex, Age, and Number of Bacterial Species

To analyze the relationship between the sex, age, and number of bacterial species of camels, we first studied the relationship between the sex and the number of bacterial species, followed by that between the age and the number of bacterial species; in the third level, we determined the relationship by considering all 3 variables together.

2.3.1. Relationship between sex and number of bacterial species

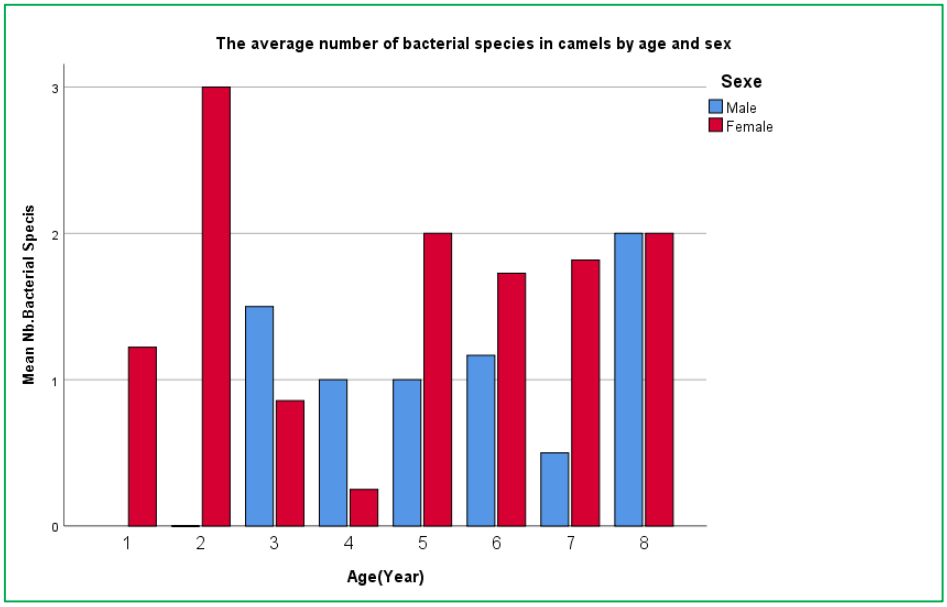
Table-2: Descriptive statistics of number of bacterial species according to sex

Descriptors					
	Sex			**Statistic**	**Std. Error**
No. of Bacterial types	Male	Mean		1.00	0.254
		95% Confidence Interval for Mean	Lower Limit	0.47	
			Upper Limit	1.53	
		Median		1.00	
		Variance		1.222	
		Std. Deviation		1.106	
		Minimum		0	
		Maximum		3	
	Female	Mean		1.62	0.181
		95% Confidence Interval for Mean	Lower Limit	1.26	
			Upper Limit	1.99	
		Median		1.00	
		Variance		2.005	
		Std. Deviation		1.416	
		Minimum		0	
		Maximum		5	

The number of bacterial species was slightly higher in females than in males; on average, females had 1.26 species. Females could have at most 5 species of bacteria, whereas the maximum number of bacterial species in males did not exceed 3. The boxplot below clearly shows this difference.

2.3.2. Relationship between age and number of bacterial species

Graph 1 shows the distribution of the average number of bacterial species according to age and sex; the bar graph shows that the number of bacterial species in females is uniformly distributed over all ages. Females that were 2 and 8 years old had 4 species of bacteria, whereas the number of bacterial species for males increased with age. Moreover, for the majority of ages represented here, the average number of bacteria in females exceeded that in males, except for certain ages, such as 3 and 4 years, where the average number in males was higher than that in females.



Graph-1: Bar graph on average number of bacterial species with respect to age and sex of camel

In summary, females contain bacterial species at all ages (from 2 to 8 years) and more in average number than males (maximum number of species is 3 for males, and this small number is mainly concentrated in male dromedaries that are between 3 and 6 years old).

2.4. Relationship between Status of Camels and Number of Bacterial Species

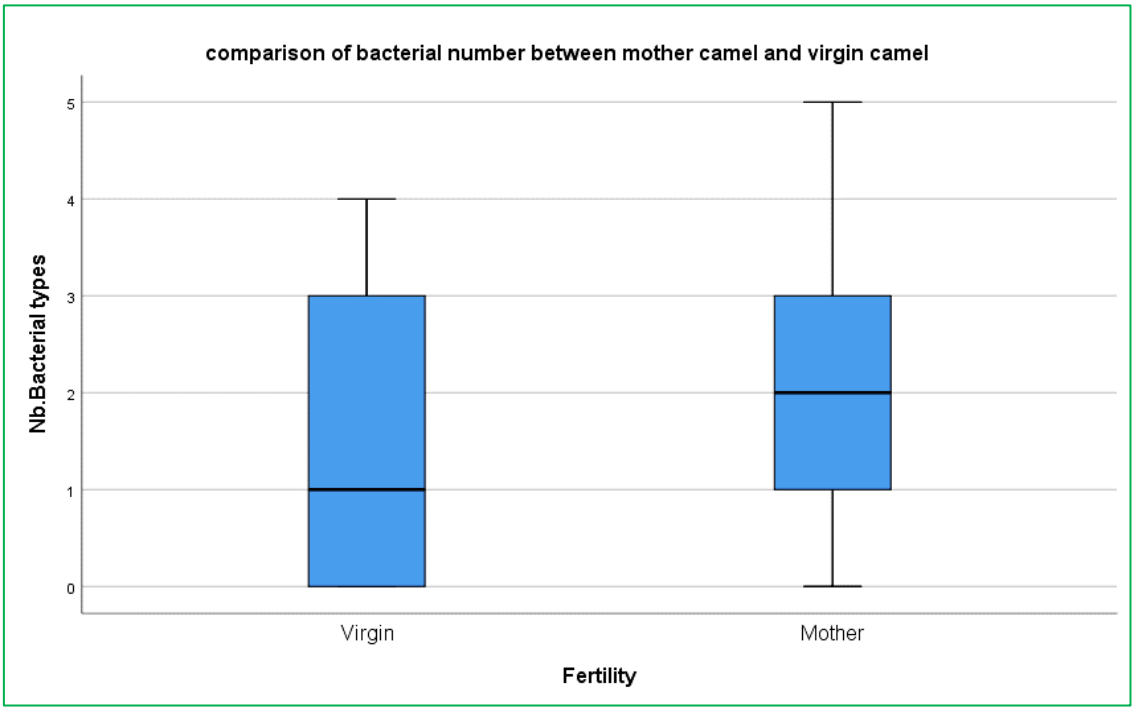
In this part of the analysis, we focused more on female camels.

Table-3: Descriptive statistics of number of bacterial species by status

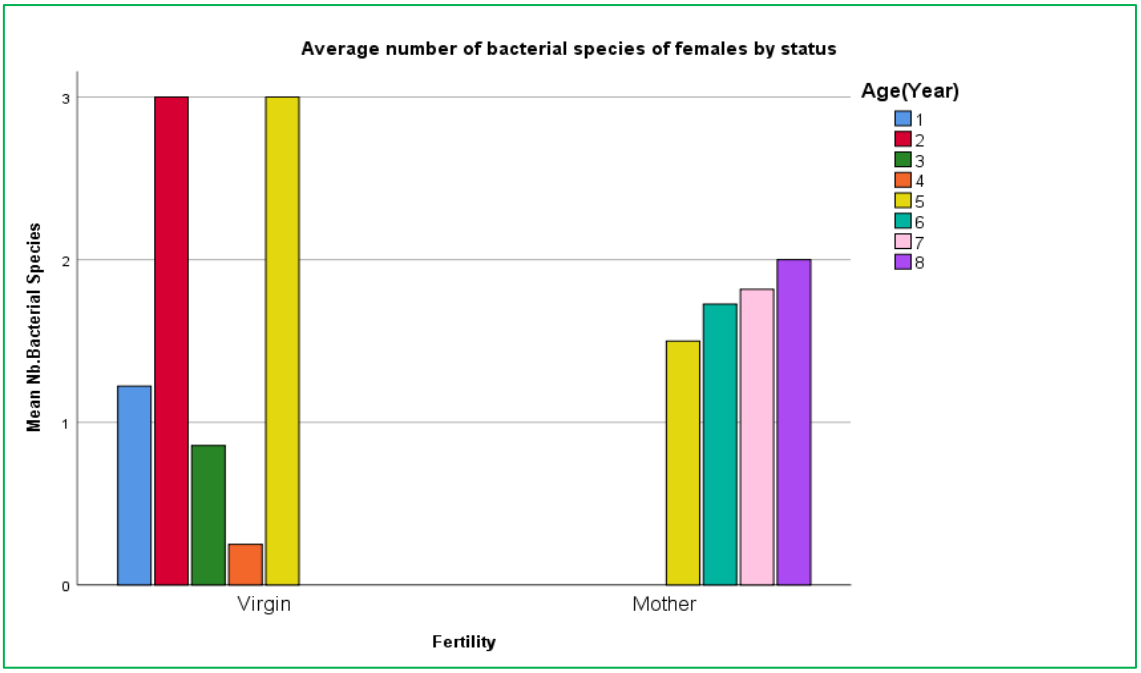
Descriptors					
	Status			Statistic	Std. Error
No. of Bacterial Species	Virgin	Mean		1.38	0.289
		95% Confidence Interval for Mean	Lower Limit	0.79	
			Upper Limit	1.98	
		Median		1.00	
		Variance		2.166	
		Std. Deviation		1.472	
		Minimum		0	
		Maximum		4	
	Mother	Mean		1.80	0.231
		95% Confidence Interval for Mean	Lower Limit	1.33	
			Upper Limit	2.27	
		Median		2.00	
		Variance		1.871	
		Std. Deviation		1.368	
		Minimum		0	
		Maximum		5	

On average, mother camels contain more bacterial species (mean = 1.80) than virgins (mean = 1.38); the maximum number of bacterial species is 5 in mothers, and there are none with no species. The maximum number of species for virgins is 4; 50% of mother camels have more than 2 bacterial species (median = 2), whereas 50% of female virgins have at most 1 bacterial species (median = 1).

The graph below (Graph 2: boxplot) illustrates this difference in the number of bacterial species among female virgins and mothers.

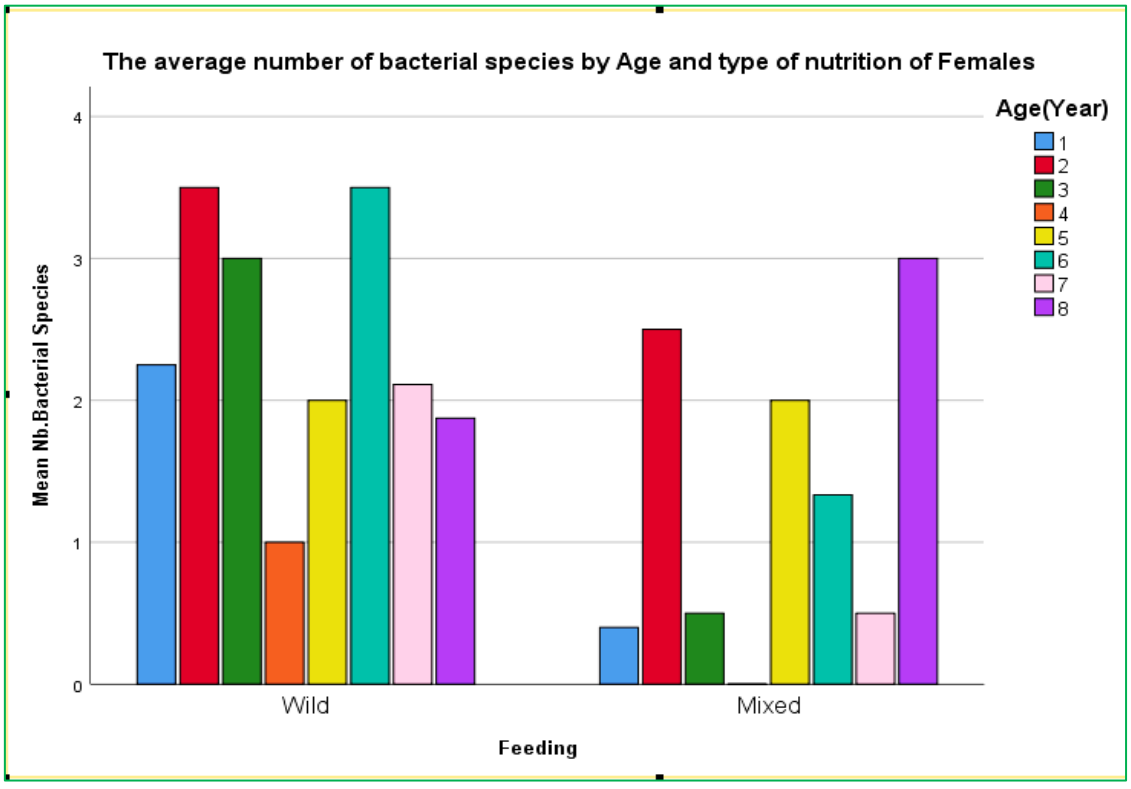


Graph-2: Boxplot on average number of bacterial species with respect to status of female camels



Graph-3: Boxplot on average number of bacterial species with respect to status of female camels

Graph 3 shows that the average number of bacterial species distributed more evenly with an increase in age, whereas that for virgins distributed differently according to age; hence, there is another factor that influences the disposition of bacterial species for virgins. Graph 4 introduces the type of nutrition as a discriminating factor for the identification of bacteria in females; females fed on wild nutrition had bacteria in all age categories, whereas those fed on mixed foods had low numbers of species and not at all ages.



Graph-4: Bar graph on average number of bacterial species with respect to age and nutrition type of female camels

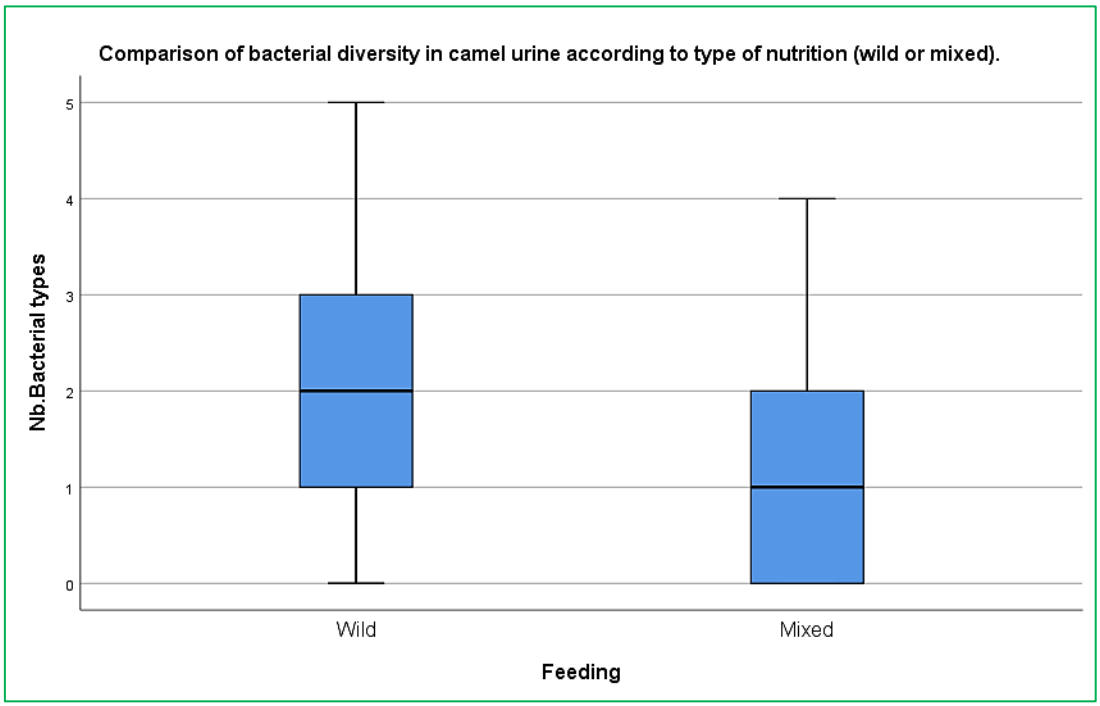
2.5. Effect of Nutrition (Wild or Mixed) on Bacterial Diversity

Table-4: Descriptive statistics of number of bacterial species by type of nutrition

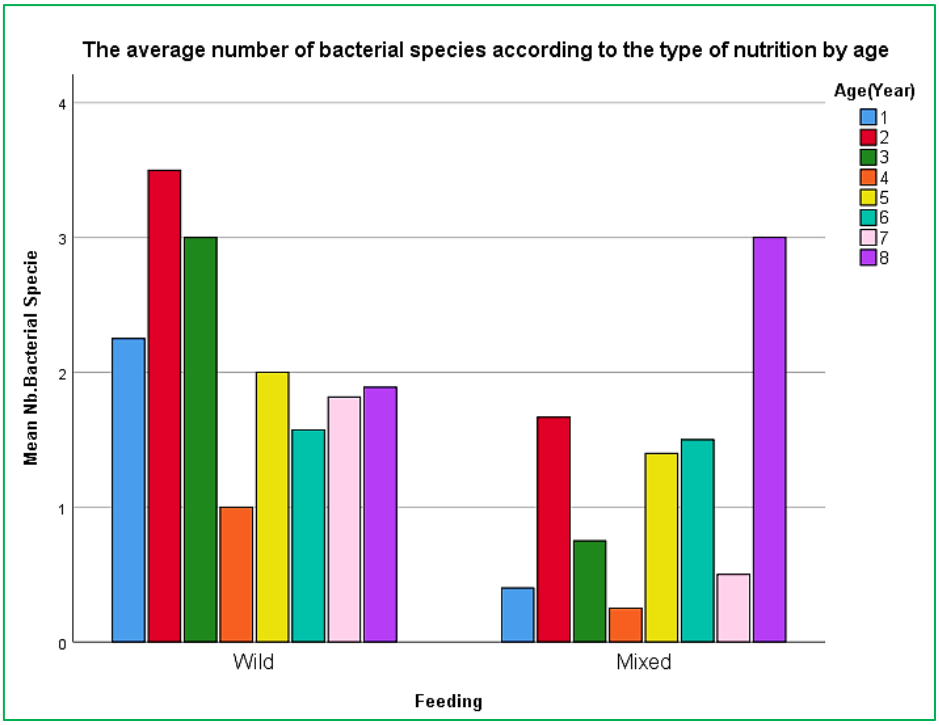
Descriptors					
	Feeding			Statistic	Std. Error
No. of Bacterial types	Wild	Mean		1.93	0.225
		95% Confidence Interval for Mean	Lower Limit	1.47	
			Upper Limit	2.38	
		Median		2.00	
		Variance		2.020	
		Std. Deviation		1.421	
		Minimum		0	
		Maximum		5	
	Mixed	Mean		1.03	0.184
		95% Confidence Interval for Mean	Lower Limit	0.65	
			Upper Limit	1.40	
		Median		1.00	
		Variance		1.358	
		Std. Deviation		1.165	
		Minimum		0	
		Maximum		4	

The table of descriptive statistics shows that the camels fed on wild nutrition are endowed with more bacterial species than those fed on mixed nutrition; 50% of camels fed on wild food have more than 2 species (median = 2) of bacteria, whereas 50% of those fed on mixed feed have less than 1 species (i.e., 20 camels in the mixed feed category have 0 or only 1 species).

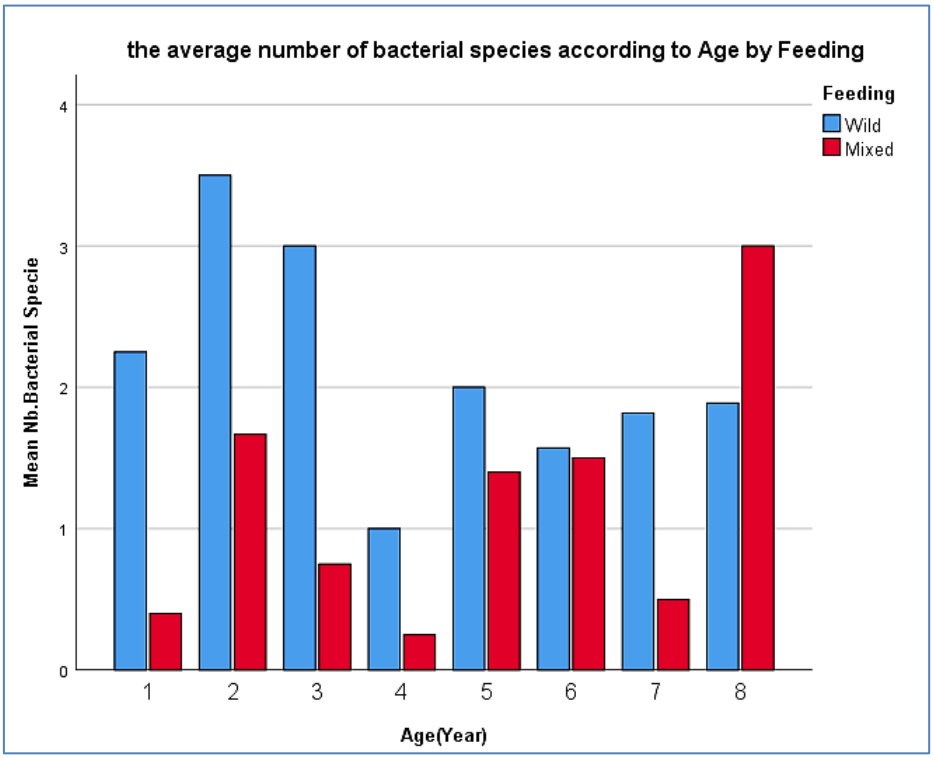
The graphs below (Graphs 5–7) illustrate the effect of different nutrition on bacterial diversity in camel urine. In general (Graph 5), the camels fed on wild food have and produce more bacterial species than those fed on mixed feed; more than 50% of the camels (wild food) have 2 species and 25% have more than 3 species, with 5 species as the maximum, whereas camels fed on mixed food have a maximum number of bacteria of only 4; moreover, 50% of them have at most 1 bacterial species. If age factors (Graphs 6 and 7) are introduced, bacterial species can be found at different ages in camels fed on wild food, whereas for the other nutrition type, bacteria can be found only at specific ages.



Graph-5: Boxplot on average number of bacterial species with respect to age and nutrition type of female camels



Graph-6: Bar graph on average number of bacterial species with respect to age and nutrition type of camels



Graph-7: Bar graph on average number of bacterial species with respect to age and nutrition type of camels

3. DISCUSSION

The great benefits of camel urine have been proven by many studies (Majid, 2011; Fadlelmula, *et al*., 2016).

The rumen ecosystem is rich in a diverse range of bacteria that play a number of tasks. It's thought that a diverse group of microorganisms ferments the incoming feed particles, and then another group of microbes acts on the fermented feed (Hinsu, *et al.*, 2020& Brulc, *et al.*, 2009).

Urine was formerly thought to be sterile based on the results of conventional clinical culture testing. However, since the breakthrough of high-throughput DNA sequencing, bacteria can now be discovered in

urine samples of even culture-negative healthy individuals, urine is no longer considered sterile (Lewis, *et al*., 2013 & Nelson, *et al*., 2010).

In addition, changes in the urine microbiota and the environment have been seen in a variety of urologic diseases. Previous research has linked the features of urine microbiota to an increase in episodes of urge urinary incontinence (UUI) in adult women using 16S rRNA gene sequencing. Although traditional urine culture is negative, the results obtained by 16S rRNA sequencing were also confirmed and supplemented by advanced urine culture methods. Brubaker, *et al*. 2014 & Pearce, *et al*. 2015)

However, camel urine is sometimes contaminated with pathogenic bacteria. We noticed that 25% of camel urine samples did not contain bacteria under the conditions of this study, whereas environmental bacteria could be isolated from the others.

16S rRNA gene sequence analysis showed that the most abundant species in more than 70% of the samples were members of the genus *Bacillus.*

Spore or vegetative forms of *Bacillus* spp. have been used as probiotics, and they exhibit high stability under surrounding atmospheric conditions of heat, gastric environments, and moisture (Lee, *et al*., 2091). *Bacillus velezensis* was dominant and constituted the highest percentage (42.5%) of bacterial isolates; a vast number of biosynthetic gene clusters encoding gene products for the generation of secondary metabolites have been discovered in various B. velezensis isolates. These chemically varied bioactive metabolites could be used as a drug development library. Recent whole-genome sequencing research on B. velezensis have found a large number of biosynthetic gene groups that encode enzymes for the manufacture of a variety of antibacterial chemicalst(Liu,*et al*.,2020;Rabbee &Baek,2020)**.**

In addition, *B. cereus* and *B. subtilis*, which are commercial Bacillus probiotic strains, were present in a ratio between 1% and 2%. Antimicrobial, anticancer, antioxidant, and vitamin-producing characteristics are all present in these strains. Bacillus probiotics, on the other hand, can create toxins and biogenic amines, as well as transfer antibiotic resistance genes; therefore their safety is a worry. It is worth to noting that, Cattle, goats, and sheep graze on a variety of grasses, whereas camels graze on a different variety of grasses thorny bushes, halophytes, and a variety of other plants and aromatic species. that are avoided by those animals (Iqbal &Khan, 2001)**.**

4. MATERIALS AND METHODS

4.1. Sample Collection Site

Urine samples were collected from 80 camels (*Camelus dromedarius*) in 6 sites in Taif City, namely, Alsharafiah, Alsail assaqeer, Oshairah, Alsail alkabeer, Alhaweiah, and Alhada. The 80 camels were aged between 1 and 8 years (53 females and 27 males).

4.2. Sample Collection

Urine samples were aseptically collected from camels that were grazing in open fields in Taif. After scrubbing the urinary bladder surface with 70% alcohol, urine samples were taken with the help of camel attendants.

The urine samples were collected in sterile 50 mL falcon tubes and transferred to the laboratory in a cooler box at 2–8 °C within 4 h. Finally, all urine samples were immediately subjected to biochemical and microbiological analyses.

4.3. Period of Study

All samples were studied between the months of November 2019 and June 2020.

4.4. Isolation of Microorganisms

Serial dilution of urine samples was performed by adding 1 mL of each sample to a tube containing 9 mL sterile distilled water.

Serial dilutions (10^{-1} to 10^{-5}) were prepared for each sample, and 0.1 mL of each diluted sample was inoculated onto nutrient agar, MacConkey agar, and sheep blood agar using the spread plate technique (Eze, *et al*., 2009; Khan&Hiraishi, 2002). The inoculated plates were incubated at 37 °C for 24 h. The number of colonies formed and the morphology (shape, color, edge, elevation, etc.) on the plates were recorded carefully prior to the isolation of pure cultures using the streak plate technique (Khan & Hiraishi, 2002).

4.5. Genotypic Identification Using 16S Ribosomal RNA

Genomic DNA of bacteria isolates was extracted according to the method described by Khan & Hiraishi (2002). The primers for PCR amplification of the 16S rRNA gene were 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) with an expected PCR product size of 1500 bp. A master mix was used following the manufacturer's guidelines. Amplification was done in a thermocycler (Mastercycler® Gradient, Eppendorf, Hamburg, Germany) at 94 ° C for 5 min, followed by 32 cycles of 45 s at 94 °C, 45 s at 57 °C, and 90 s at 72 °C, with a final extension at 72 °C for 10 min.

4.6. Analysis of Amplified PCR Products

A 3 µL aliquot of each PCR amplicon was electrophoresed on a 1% agarose gel containing ethidium bromide in 1× Tris-Acetate-EDTA buffer at 120 V for 40 min and visualized under a UV transilluminator (BioDoc-IT system, Japan).

4.7. Amplified PCR Amplicon Sequencing

Amplified products were purified using a QIAquick PCR purification kit (Promega, Madison, WI, USA) and sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Forster City, CA, USA) on an ABI Prism® 310 Genetic Analyzer (Applied Biosystems).

4.8. Phylogenetic Analysis

Sequences were edited using SnapGene Viewer software version 3.3.3 manually and then compared with the GenBank database of NCBI (http://www.ncbi.nlm.nih.gov) using BLASTN search; reference sequences were retrieved to perform phylogenetic analyses. Phylogenetic trees were constructed using MEGA (available on the NCBI website).

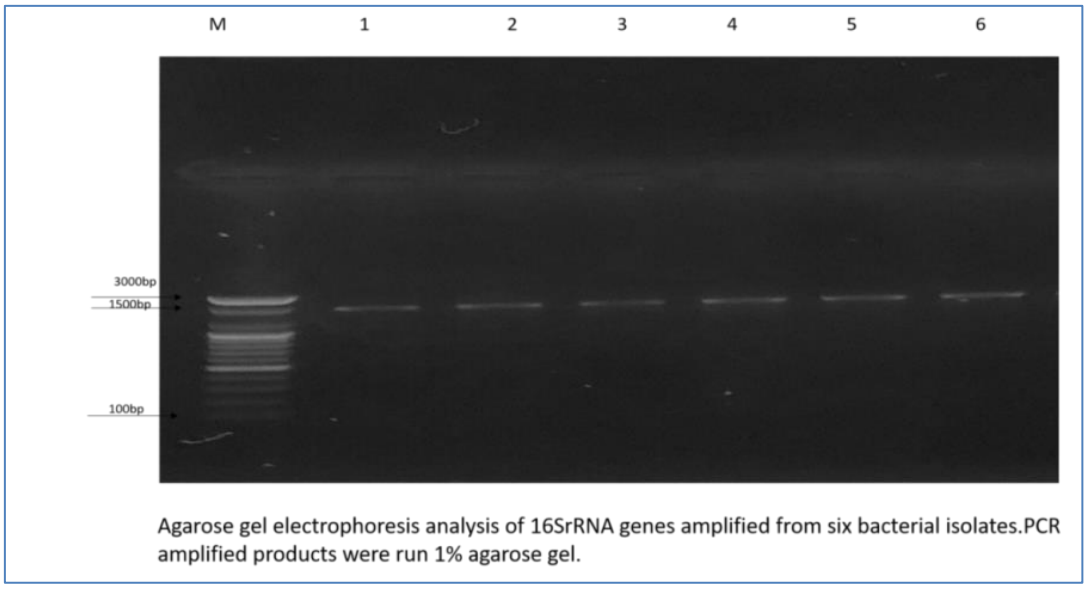


Fig-2: PCR detection of bacterial strains isolated from camel urine samples.

Author Contributions

Conceptualization, I.M.S and A.H.; methodology, T.M.D., and I.M.S.; software, I.M.S., and I.M.; validation, I.M., and I.M.S.; formal analysis, A.H. and I.M.S.and T.M.D.; investigation, I.M.; A.H. and T.M.D.; resources, I.M.S.; data curation, I.M.S.; writing—original draft preparation, I.M.S.; writing—review and editing, I.M.S.; visualization, I.M.S., and A.H.; supervision, I.M.; A.H. and T.M.D.; project administration, I.M.S.; funding acquisition, I.M.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

There are no conflicts of interest declared by the authors.

CONCLUSION

Overall, we present a comprehensive analysis of camel urine microbiota under the influence of various diets. The differences between the two different feed roughages, age and sex were noticed. Further studies are required to detect more bacterial species present in camel urine under the influence of different temperatures and by using other techniques such as metagenomics.

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