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Antimicrobial Activity of Camel's Urine and Its Effect on Multidrug Resistant Clinical Bacterial and Fungal Isolates

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Authors' contributions

This work was carried out in collaboration between both authors. Author MSM designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author RAD managed the experimental process, analyses of the study and revision of the final version of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this work is to study the *In vitro* antimicrobial effect of camel's urine on a variety of multi-drug resistant bacterial and fungal isolates.
Methodology: Agar dilution method was used to determine the effect of different concentrations of camel's urine (10%, 7.5%, 5% and 2.5%) on 50 clinical bacterial isolates including: 10 methicillin-resistant *Staphylococcus aureus* (MRSA), 10 multi-drug resistant coagulase negative staphylococci (CoNS), 10 multi-drug resistant *Enterococcus* spp., 10 extended spectrum β-lactamase (ESBL)–producing Gram-negative bacilli and 10 carbapenemase-producing Gram-negative bacilli. In addition, the antifungal effect of camel's urine on four *Candida albicans* and one candida non albicans was also assessed.
Results: All used concentrations of camel's urine produced complete inhibition of the growth of the four *Candida albicans*, Candida non albicans and the 10 CoNS isolates. The growth of MRSA, *Enterococcus* spp., ESBL-producing and carbapenemase-producing Gram-negative bacilli was completely inhibited by camel's urine at concentrations 10%, 7.5% and 5%. However, these

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bacterial isolates showed significant growth at 2.5% camel's urine concentration.
Conclusion: The present study provides clear evidence that camel's urine has a strong antifungal and antibacterial effect against multi-drug resistant bacteria.

Keywords: Camel's urine; multi-drug resistance; ESBL; carbapenemase; Candida albicans; antimicrobial susceptibility testing.

1. INTRODUCTION

Among the natural products in the Arabic peninsula that are used for the treatment of various diseases is camel's urine. Patients used to drink camel's urine (about 100 mL/day) either alone or mixed with milk for treatment of diseases such as cancer [1].

Camel's urine has an unusual and unique biochemical composition. The chemical constituents of camel's *(Camelus bactrinus)* urine were described by Read [2], who reported that unlike all the other animals, including humans, camels excrete no ammonia and only very slight trace of urea, and these molecules are responsible for the bad smell and toxicity of urine. However, an amount of creatine and creatinine was detected. Camel's urine contains about 10 folds more mineral salts than human's urine. Furthermore, while human's urine is acidic, camel's urine is basic with a pH≥7.8 [2].

Several studies were conducted to assess the value of camel's urine in various clinical conditions. It has been shown that camel's urine has a potent anti-platelet activity [3]. Moreover, it has also shown specific and efficient anti-cancer and potent immune-modulator properties *In vitro.* It has produced cytotoxicity against various, but not all, human cancer cell lines, with only marginal effect on non-tumorigenic epithelial and normal fibroblast cells. This specific anti-cancer effect was not observed when cells were exposed to rat's urine, which killed both cancer as well as normal cells with similar effect. Interestingly, lyophilized camel's urine has inhibited cell proliferation and triggered more than 80% of apoptosis in different cancer cells, including breast carcinomas and medulloblastomas. Apoptosis was induced in these cells through the intrinsic pathway via Bcl-2 decrease [1]. Moreover, all types of camel's urines have significantly inhibited the induction of *Cyp1a1*, a cancer activating gene, expression at both transcriptional and post-transcriptional levels through an aryl hydrocarbon receptor (AhR)-dependent mechanism [4].

It is proposed that camel's urine has similar effect on bacterial cells as that shown on cancer cells. Rabbit liver tissue infected with *Escherichia coli* has recovered with no histopathological effects after treatment with camel's urine of concentrations up to 100% [5]. Other studies have tested the antimicrobial activity of camel's urine against pathogenic microorganisms including fungi such as *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Rhizoctonia solani, Aschocayta* spp*., Pythium aphanidermatum, Sclerotinia sclerotiorum* and *Candida albicans*, as well as bacteria such as *Staphylococcus aureus,* Streptococci, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The results of these studies have shown high antimicrobial activity against the tested microorganisms [6,7].

Antimicrobial activity of camel's urine is due to factors such as high salt concentrations, alkalinity, as well as natural bioactive compounds from plants consumed by camels, together with the resident bacteria, and excreted antimicrobial agents. Compared to other animals, camel's urine is alkaline due to high concentrations of potassium, magnesium and albuminous proteins, and low concentrations of uric acid, sodium and creatine [8].

The aim of the present study is to assess the antibacterial activity of camel's urine against a range of multi-drug resistant bacteria of clinical significance including MRSA, multi-drug resistant coagulase negative staphylococci (CoNS), multi-drug resistant *Enterococcus* spp., extended spectrum β-lactamase (ESBL)–producing Gram-negative bacilli and carbapenemase-producing Gram-negative bacilli. We also evaluated the antifungal effect of camel's urine on four *C. albicans* and one candida non albicans clinical isolates.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates and Phenotypic Identification

A total of 55 clinical isolates were included in this study; 10 Methicillin-resistant *Staphylococcus aureus* (MRSA), 10 multi-drug resistant

coagulase negative staphylococci (CoNS), 10 ESBL–producing Gram-negative bacilli, 10 carbapenemase-producing Gram-negative bacilli, 10 multi-drug resistant *Enterococcus* spp., four *Candida albicans* and one candida non albicans isolates. All bacterial isolates were considered as multi-drug resistant by being resistant to three or more antimicrobial classes. All isolates were obtained from the Strain Bank in the department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University. The isolates were originally collected from patients admitted to Kasr Al-Aini Hospital. They were identified morphologically and biochemically by standard laboratory methods [9,10]. The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method, as per CLSI guidelines [11]. Phenotypic detection of ESBL and carbapenemase production was performed [11] and confirmed genotypically using multiplex PCR.

2.2 Detection of MRSA

Detection of MRSA was performed using cefoxitin (30 µg) discs. Isolates with a zone of inhibition ≤ 21 mm were considered methicillin resistant while those with a zone of inhibition ≥ 22 mm were considered susceptible to cefoxitin and subsequently reported as Methicillin Sensitive *Staphylococcus aureus* [11].

2.3 Testing for ESBL Production

Ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) discs were used for phenotypic detection of ESBL production according to CLSI guidelines. An increase in the zone diameter by 5 mm or more for ceftazidime/clavulanic acid disc compared to ceftazidime alone indicated that the strain is an ESBL producer [11].

ESBL production was confirmed by molecular testing to detect ESBL-encoding genes. A multiplex PCR assay was used to identify blaCTX-M, blaSHV, blaOXA and blaTEM genes as described previously [12]. The primer sequences used are shown in Table 1.

2.4 Testing for Carbapenemase Production

Modified Hodge test was used for detecting carbapenemase production. A 0.5 McFarland's suspension of ATCC *Escherichia coli* 25922 was diluted 1:10 in sterile saline and inoculated onto Mueller Hinton agar plates. The plates were left to dry for 5 minutes and a Meropenem 10 µg disc was placed in the centre of the agar. Colonies of the test organism were inoculated in straight lines from the edge of the disc up to a distance of at least 20 mm. The plates were incubated at 37℃ overnight and then examined. The presence of an enhanced growth around the test organism at the intersection of the streak indicated carbapenemase production [11].

Table 1. Primer sequences for the tested ESBL genes

Tested genes	Primer sequences (5'-3')
CTX-M1	CTT CCA GAA TAA GGA ATC
	CCG TTT CCG CTA TTA CAA
TEM-1	ATG AGT ATT CAA CAT TTC CG
	CTG ACA GTT ACC AAT GCT TA
SHV-1	GGT TAT GCG TTA TAT TCG CC
	TTA GCG TTG CCA GTG CTC
OXA-1	ACA CAA TAC ATA TCA ACT TCG C
	AGT GTG TTT AGA ATG GTG ATC

Multiplex PCR was used to confirm carbapenemases production by detecting carbapenemase-encoding genes; bla OXA-48, NDM -1, VIM, IMP and KPC genes using specific primers described in previous studies [13,14], the used primers are shown in Table 2.

Table 2. Primer sequences for the tested carbapenemase genes

Tested genes	Primer sequences (5'-3')
NDM-1	GCATAAGTCGCAATCCCCG
	CTTCCTATCTCGACATGCCG
VIM	GTTTGGTCGCATATCGCAAC
	AATGCGCAGCACCAGGATAG
IMP	GAAGGCGTTTATGTTCATAC
	GTAAGTTTCAAGAGTGATGC
KPC	TCGAACAGGACTTTGGCG
	GGAACCAGCGCATTTTTGC
OXA-48	TTGGTGGCATCGATTATCGG
	GAGCACTTCTTTTGTGATGGC

2.5 Testing Antimicrobial Activity of Camel's Urine

Samples of *Camelus dromedarius* urine were collected from animals grown in local farms. Samples were collected in sterile bottles and stored at 4℃ for less than one week until used in the study. Four concentrations of camel's urine (100%, 75%, 50% and 25%) were prepared by adding 100, 75, 50, 25 mL urine to 0, 25, 50, 75

mL distilled water, respectively. One tenth dilutions were prepared by adding 100 mL of each dilution of camel's urine to 900 mL of molten Mueller-Hinton agar medium at 45℃ to obtain final concentrations of 10%, 7.5%, 5%, and 2.5%. The media containing the different camel's urine concentrations were shaken well, poured into Petri dishes and left to solidify under sterile conditions. Mueller-Hinton agar plates without adding urine were used as controls. McFarland 0.5 standard dilutions (1-2 $x10^8$ CFU/mL) of fresh cultures of each isolate were prepared and 10 µl of each of the diluted bacterial isolates was inoculated onto Mueller-Hinton agar plates with different urine concentrations as well as the control plate as recommended by the CLSI [15]. The plates were incubated aerobically at 37℃ for 48 hours. After incubation, the plates were examined carefully for > 1 colony or light film of growth [15]. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed during collection of camel's urine. The study was approved by the local ethical committee of the Department of Medical Microbiology and Immunology.

3. RESULTS

Ten Gram-negative bacilli were confirmed to be ESBL-producers by multiplex PCR; they included four *E. coli*, four *Klebsiella* spp., one *Enterobacter* spp. and one *Acinetobacter baumannii*. ESBL types detected are shown in Table 3.

Ten carbapenemase-producing Gram-negative bacilli were detected. They included six *K. pneumoniae,* two *P. aeruginoasa,* one *Proteus mirabilis* and one *Citrobacter* spp. The detected carbapenemase types are shown in Table 4.

All the Mueller Hinton agar plates were examined after 24 and 48 hours of incubation. Regarding antibacterial activity of the different concentrations of camel's urine against the tested isolates, the growth of the four *C. albicans* isolates, candida non albicans, and the 10 CoNS isolates was completely inhibited by the four used concentrations of camel's urine.

The growth of the remaining bacterial isolates including MRSA, multi-drug resistant *Enterococcus* spp., and both ESBL- and carbapenemase-producing Gram-negative bacilli has been completely inhibited on the Mueller Hinton agar plates containing 10%, 7.5%, and 5% camel's urine concentrations. However, on the plates with 2.5% camel's urine, these bacterial isolates have shown obvious growth.

4. DISCUSSION

In the present study, camel's urine has shown a strong *In vitro* antibacterial effect against clinically important multi-drug resistant bacteria as well as a strong antifungal effect against *C. albicans* and candida non albicans. In concordance to our results, Al-Awadi and Al-Judaibi [16] reported a highly effective antifungal effect of camel's urine for treating human and plant fungal diseases. Furthermore, they have shown that the antimicrobial activity of camel's urine increases after storage and heating up to 100℃, which completely inhibited the growth of *C. albicans, A. niger and F. oxysporum.* Heating may increase the concentration of active compounds in urine [16]. Low concentrations of camel's urine (0.5, 1, 2 and 3%) have no significant effect on mycelia growth of *A. niger, A. flavus, Fusarium sp., Pythium aphanidermatum, Aschocayta sp.,* and *S. sclerotiorum* in liquid

Table 3. ESBL-producing Gram-negative bacilli included in the study

	CTX-M	TEM	SHV	CTX-M + TEM	CTX-M + SHV
E. coli	2	1	-	1	-
Klebsiella spp.	1	-	2	-	1
Enterobacter spp.	-	-	-	1	-
Acinetobacter baumannii	-	1	-	-	-

Table 4. Carbapenemase-producing Gram-negative bacilli included in the study

	Bla OXA-48	***Bla KPC***	***BlaVIM***	***Bla NDM-1***	***IMP***
K. pneumonia	2	2	1	1	-
P. aeruginosa	1	-	-	-	1
P. mirabilis	-	1	-	-	-
Citrobacter spp.	-	-	1	-	-

medium. Similarly, camel's urine at concentrations (3, 5, 7 and 10%) has no antifungal effect on the mycelia growth on solid medium. After using concentrations of camel's urine (25, 50%), a significant decrease in fungal growth was recorded [6].

To our knowledge, this is the first study that shows a strong *In vitro* antibacterial activity of camel's urine against multi-drug resistant bacteria including CoNS, MRSA, as well as ESBL-producing and carbapenemase-producing Gram-negative bacilli. The significant antimicrobial action of camel's urine may be caused by its high alkalinity produced by high concentrations of K, Mg, Ca and proteins, and low concentrations of carbohydrate and cellulose [8]. It is worth to mention that camels have different feeding behavior than that of cattle, goat and sheep, as camels graze on a variety of plants including thorny shrubs, halophytes and aromatic species that are avoided by those animals [17]. In addition, camel's urine has no cytotoxic effect against peripheral blood mononuclear cells and has strong immuno-inducer activity through inducing the main Th1 cytokine IFN-γ and a great inhibitory effect on the production of the Th2 cytokines IL-4, IL-6 and IL-10 which has immunosuppressive functions [1].

5. CONCLUSION

The present study provides clear evidence that camel's urine has an antifungal and antibacterial effect even against multi-drug resistant bacteria. Further studies are required to indicate the active antimicrobial components of camel's urine and to study its effect on other bacterial and fungal pathogens in a preliminary step to introduce camel's urine or its active components into local and systemic antimicrobial pharmaceutical preparations.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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