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# *In vitro* assessment of antibacterial property of camel's urine against some isolated *Salmonella* strains

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## Abstract

The study was conducted to assess the antibacterial property of camel's urine against some *Salmonella* strains. Twenty camel's urine samples were collected from different areas (females and males) and examined for their antibacterial activities against the *Salmonella* strains. Results indicated that all concentrations of urine used (100, 75, 50 and 25%) inhibited the growth of *Salmonella* strains. After the neutralization of camel's urine, the results showed the same inhibition effect against *Salmonella* strains. Results showed that the camel's urine was more sensitive compared with some antibiotics sensitivity. The minimum Inhibitory Concentration was determined against *Salmonella* isolates gave the result at low concentration. Results also showed that the camel's urine incubation for 9 days, in different temperatures showed no bacterial growth up the end of 6th day of incubation. The most microbes detected in this day and in the 9th days were mainly: *Staphylococci, Streptococci, Micrococci, Diplococci* and few of *Bacilli, Actinomycetes* and yeasts.

**Keywords:** Camel's urine; Strains; Antibiotics sensitivity; Minimum Inhibition Concentration; Khartoum State; Sudan

## 1. Introduction

The camel is mentioned in the Holy Quran as particularly important animal and is referred to by other names such as al ibil, al-ngah, al-jamal, al-ishar and al-him [1]. Camels urine is considered a "miraculous" drug used in Prophetic Medicine since the Pre-Islamic era[2], which has been used as traditional and folk medicine for women's hair; gums and teeth; skin injuries; snake bites; stomach pain; tumors; the common cold; diarrhea and nausea; diabetes jaundice; scabies and eye, skin, liver and nail infections [3, 4,5]. Camel's urine is also commonly used against cancer and respiratory tract infections in alternative medicine [6].

Camel's urine has been proven to be effective as an antimicrobial agent and may not have any side effects for human [7]. Data available show, however, significant antimicrobial activities against some pathogenic microbes infected human such as *Staphylococcus aureus*, *Pseudomonae aeruginosa, Escherichia coli* and other pathogenic microbes [8]. Camels urine can use to treatment of fungal infection such as ringworm, tinea [9].

Antimicrobial activity of camel's urine is due to factor such as high salt concentrations, alkalinity, and natural bioactive compounds from the plants camels eat, resident bacteria and excreted antimicrobial agents. Compared with other cattle, camel's urine is alkaline due to high concentrations of potassium, magnesium and aluminous proteins and low concentrations of uric acid, sodium and creatine [10, 11]. The different composition of camel's urine compared to other cattle and goats is due to the type of plants they consume and their feeding habits, camels prefer browse with high concentrations of minerals that decline more slowly when they dry instead of other types of forage such as grasses [12,

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13]. Therefore, the present study was mainly designed: to investigate the antibacterial activity of camel's urine against some isolated *Salmonella* strains.

## 2. Material and methods

### 2.1. Camel's urine sample collection

During the period of October to December in the year 2015, a sum of 20 urine samples were collected from 20 apparently healthy males and females camels (*Camelus dromedarius*) from Almoilihan (Omdurm) and Elkabashi (Bahri) with ages ranging from 6 months to 4 years of one breed.

All samples were transferred to the laboratory in sterile screw-capped bottles. On arrival at the laboratory, the samples were immediately subjected to micro-biological processing.

### 2.2. Clinical isolates collection

Twenty pathogenic strains of *Salmonella spp*. (8: *S. typhi*, 7: *S. Paratyphi* and 5: *S. typhimurium)* were reviving. The sources of all strains are human, from Albolok Children Hospital, Omdurman, Sudan.

### 2.3. Antibiotics

Antibiotics powders were obtained from General Medicine Company, Ltd. The antibiotics used were: Ciprofloxacin, Gatifloxacin, Levofloxacin, Amoxillin, Co-trimoxazole (Septrin), Ampicillin, Chloramphenicol, Gentamycin, Naledixic acid, Tetra cyclin, and Cefotaxim.

### 2.4. Sampling methods

The camel's urine samples were collected by Tashweel technique which was done by touching the abdominal side of the camel near the hide of the back leg [14].

### 2.5. Identification of clinical isolates

Purified isolates were identified by microscopic examination [16] and biochemical tests [17].

### 2.6. Determination of antibacterial activity

Antibacterial susceptibility tests of the isolated organisms was done by the disk diffusion method using the Kibry-Bauer technique [18] and as recommended by National Clinical and Laboratory Standards Institute (NCLSI) [19].

### 2.7. Camel's urine sensitivity test

It was done by disk diffusion method to screen for antibacterial activity on plates that contain Muller Hinton Agar (MHA) medium. The sterile discs of 6mm in diameter from filter paper (Whatman No. 2) were used. The discs were soaked with 20µl of 25, 50, 75 and 100% of camel's urine concentrations with sterile distilled water, then the disk were dried at 37$^{o}$C for 30 minutes, then placed on the surface of plates contain MHA medium and incubated at 37$^{o}$C for 48 hours. The diameter of zone was measured, averaged and the values were tabulated. The test was repeated with neutral camel's urine after adding HCl to alkaline camel's urine [18].

### 2.8. Preparation of antibiotics solution (stock solution)

The eleven antibiotics (Ciprofloxacin, Gatifloxacin, Levofloxacin, Amoxicillin, Co-trimoxazole (Septrin), Ampicillin, Chlorampheinicol, Gentamycin, Naledixic acid, Tetracycline, Cefotaxim) powder with potency 99.7, 89, 96, 86.1, 62.1, 85.8, 98.9, 90, 75, 62.6 and 103, respectively. The powder was weighted and dissolved in appropriate diluents distilled water to yield the required concentration of antibiotic solutions expressed in µg/ml was based on the potency per disk prescribed by NCLS[19]. The following formula was used in determine the amount of antibiotic powder to be used:

$$\text{Vol. (Ml)} \times \text{desired conc. (µg/ml)}. \text{Weight (mg)} = \text{potency (µg/ml)}.$$

The diameter of zone was measured. The organism either to be R= resident, I= intermediate or S= sensitive.

Determination of Minimum Inhibitory Concentration (MIC) according to [18]. Preparation of MC Forland and 0.5 turbidity standard:

0.5ml of 0.048 mol/L ($Bacl_2$, $2H_2o$) were added to 99.5mlof 0.18mol/L (0.36 N) $H_2so_4$ (1% v/v) and mixed thoroughly. The tubes were sealed and stored in the dark at room temperature. The standard was mixed thoroughly by using vertex mixer immediately before use Standards were renewed and their absorbencies were checked after storage for 6 months.

### 2.9. Microscopic examination of camel's urine

In the course of 9 days, determination of antimicrobial resistance of camel' urine was conducted as part of microbiological study at temperature of, 1-4, 20-25, 37 and 40-45 ℃ consecutively. Microbiological determination procedures were applied according to the technique recommended by [16].

## 3. Results and discussion

Table (1) showed the inhibition zone diameter for camel's urine against the identified specious. As it can be seen the inhibition zone of *S. typhi* at 25% concentration was (7-8mm) then (13-14mm), (16mm) and 20mm at urine concentration of 50%, 75% and 100% respectively. Almost similar trend was observed with the other species (*S. paratyphi* and *S. typhimurium*). The inhibition zone for *S. paratyphi* at concentration 25% was (7-8mm), at 50% was (12-13mm), at 75% were (16-17mm) at 100% was (19mm), however, *S. typhimurium* expressed (8-9mm) at concentration 25%, (13-14mm), 17-18mm) and (20-21mm) at concentration of 50%, 75% and 100% respectively. Results showed that there was no difference whether the camel's urine was alkaline or neutral. Similar results were reported by Muna *et al.* (2008), Muna (2003) and Raheem (2016), but the results of this study was in contrast with Al-Bashan (2011) who reported that camel's urine has no effect against *Salmonellas spp.*

**Table 1** Antibacterial activity of camel's urine against *Salmonella* isolates at four concentrations

| Conc. (%) | The mean diameter of growth inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *S. typhi* | | *S. paratyphi* | | *S. tyhimurium* | |
| | A | N | A | N | A | N |
| 25 | 7 | 8 | 7 | 8 | 8 | 9 |
| 50 | 13 | 14 | 13 | 12 | 13 | 14 |
| 75 | 16 | 16 | 17 | 16 | 17 | 18 |
| 100 | 20 | 20 | 19 | 19 | 20 | 21 |

Con. = Concentration; A = Alkaline; N = Natural; mm= millimeter

The sensitivity of clinical isolates against 11 antibiotics was shown in Table (2). All isolates were highly sensitive to Ciproflaxacin, Gataifloxacin, Levofloxacin and cefotaxime, but moderate sensitive to Co-tri (Septrin) and Amoxcillin. However *S. typhi* and *S. paratyphi* were moderate sensitive to Ampicillin and chloramphenicol, but *S. typhimurium* was resistant. On the other hand most of isolates were resistant to Ampicillin, Choraphenicol, Gentamycine, Naledixic acid and Tetracydine. In connection to this, Ahmed *et al.* (2000) reported that the percentage of *Salmonella* isolates resistant to Naledixic acid and Ciprofloxacin in Sudan has increased from zero percent to 22.00 and 8-9% respectively. The wide resistance Naledixic acid has been associated with decrease in susceptibility to four quinolones including Ciprofloxacia, which are used for treat of Salmonellesis in humans. As it can be seen the prevalence of antibiotics resistance may be attributed to some factors, one of the most important is the deliberate self-administration of antibiotics by patients themselves, the wide use of antibiotics due to the high prevalence of infectious diseases, lack of laboratory support in rural areas and selective prescribing due to the cost constrains.

The minimum inhibitory concentration (MIC) of camel's urine showed value of 0.15ul for 2 isolates, 0.31ul for 4 isolates and 0.62ul for 2 isolates for *S. typhi* (Table 3). However, *S. paratyphi* have MIC value 0.07 for one isolate, 0. 15 for three isolates, 0.31 for one and 0. 62 for two isolate. The total of 5 *S. typhimurium* have MIC value of 0.15ul for one isolate, 0.31 for one and 0.62ul for three isolates.

Table (4) showed the microbiological examination in the period of 9 days under different temperature conditions. Results revealed that no bacterial growth was detected till the sixth day of urine incubation outside temperature. The most microbes detected in the 6$^{th}$ and 9th day were: *Staphylococci, Streptococci, Micrococci, Diplococcaci* and a few *Bacilli, Actinomycetes* and yeasts. These results were in agreement with Al-Bashan (2011), but in contrast with Muna (2003) and Raheem (2016), who reported that the bacteria was found naturally in camel's urine.

**Table 2** Antibacterial activity of antibiotics against *Salmonella* isolates

| ***Salmonella* isolates** | **Antibiotics** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Cip,** | **Gat.** | **Lev.** | **Amo** | **Co-Tri-** | **Amp,** | **Chlor.** | **Gent.** | **Nale** | **Tet** | **Cef.** |
| ***S. typhi* (8)** | | | | | | | | | | | |
| Sensitive S | 7 | 4 | 7 | 4 | 5 | 2 | 1 | 0 | 1 | 2 | 6 |
| Intermediate I | 1 | 2 | 0 | 1 | 2 | 1 | 3 | 2 | 2 | 2 | 2 |
| Resistant R | 0 | 2 | 1 | 3 | 0 | 5 | 4 | 6 | 5 | 4 | 0 |
| ***S. paralyphi* (7)** | | | | | | | | | | | |
| Sensitive S | 6 | 5 | 5 | 4 | 3 | 1 | 1 | 0 | 1 | 1 | 4 |
| Intermediate 1 | 1 | 1 | 0 | 1 | 3 | 2 | 2 | 2 | 1 | 2 | 2 |
| Resistant R | 0 | 1 | 2 | 2 | 1 | 4 | 4 | 5 | 5 | 4 | 0 |
| ***S. tyhimurium* (5)** | | | | | | | | | | | |
| Sensitive S | 4 | 3 | 4 | 4 | 4 | 0 | 0 | 1 | 0 | 0 | 4 |
| Intermediate 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Resistant R | 0 | 1 | 1 | 1 | 0 | 5 | 4 | 3 | 4 | 4 | 0 |

S= Sensitive (Z18mm); I = Intermediate (14-18mm); R= Resistant (≤14mm); mm= millimeter ; Cip= Ciprofloxacin; Gat= Gatifloxacin; Lev.= Levoflox a cin; Amo.= Amoxillin; Co-tri= Co-trimoxazole; Amp.= Ampicillin; Chlor. = Chloramphenicol; Gent/= Gentamycine; Nale.= Naledixic acid; Tet.= Tetracycline; Cef. = Cetotaxime

**Table 3** Minimum inhibitory concentration (MIC) µl of camel's urine against *Salmonella* isolates

| **Camel's urine concentration (µl)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***Salmonella* isolates** | **0.07** | **0.15** | **0.31** | **0.62** | **1.25** | **2.5** | **5** | **10** | **20** |
| *A. typhi (8)* | 0 | 2 | 4 | 2 | 0 | 0 | 0 | 0 | 0 |
| *S. paratyhi (7)* | 1 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| *S. tyhimurium (5)* | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 0 |

**Table 4** Microbial finding observed in camel's urine in the period of 9 days in the different temperature conditions

| **Period of days** | **Temperature conditions** | | | | |
|---|---|---|---|---|---|
| | **Freezer T** | **Refrigeration T** | **Room T** | **Incubation T** | **Outside T** |
| 1 | Nil | Nil | Nil | Nil | Nil |
| 3 | Nil | Nil | Nil | Nil | Nil |
| 6 | Nil | Nil | Nil | *Staphylococci, Streptococci Micrococci, Diplococci* and *yeast* | Yeasts, *Micrococci,* a few of Bacilli |
| 9 | Nil | Yeast | *Micrococci, Diplococcic, Staphylococci,* A few of *Bacilli & Actinomycetes* | *Diplococcic, Micrococci, Long chain of Bacilli, Staphylococci & streptococci* | *Diplococci, Micrococci,* Long chain of *Bacilli Staphylococci, Streptococci* and *actinomycetes* |

## 4. Conclusion

- The study showed that camel's urine inhibited the growth of *Salmonella* isolates at four concentrations (25, 50, 75 and 100%) and gave a large inhibition zone diameter to all *Salmonella* isolates.
- Neutralization of camel's urine gave the same result as alkaline camel's urine or a little more.
- The microbes grow in camel's urine at the 6th day of incubation and outside temperature.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors have declared that no competing interests exist.

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