Studies on Sudanese Camel Urine

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1995
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A THESIS SUBMITTED TO THE UNIVERSITY OF
KHARTOUM IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF
VETERINARY MEDICINE

DEPARTMENT OF MICROBIOLOGY
FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF KHARTOUM
December 2003
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References
First of all, I render my thanks and praise to almighty god Allah, who gave me the health and strength to accomplish this work.

I am greatly indebted to my supervisor, prof. Abdu El-dawi Abdallh, Department of Microbiology, faculty of Veterinary Medicine, University of Khartoum for his guidance and support.

I am grateful to D. Hadia El Jack Ahmed, Department of Bacteriology, Central Veterinary Research Laboratories for her help and encouragement.

My thanks also goes to Mrs. Rawda, Technician, Department of Biochemistry, Central Veterinary Research Laboratories. I would like to express my deep appreciation to Mohamed Nageeb Ahmed Elsidig, Department of Pathology, Central Veterinary Research Laboratories for his help.

No body has been involved more closely than my family, husband, sisters and brother whom I thank for assistance and encouragement.
A total of 250 samples were collected from different areas of Sudan: Elgadarif state, North Kordofan state, Elgazera state. Physical and chemical analysis of urine samples were done for potassium, sodium, calcium, urea, uric acid, creatinine, creatine and total protein and it was found that the maximum percentage was the potassium, sodium and calcium respectively.

Isolation and Identification of bacteria in urine samples was carried out. The Gram - positive bacteria isolated were: Staphylococcus hyicus, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus haemolyticus, Corynebacterium striatum, Corynebacterium xerosis, Corynebacterium pseudodiphtericum and Bacillus cereus.

The Gram - negative bacteria was Mannheimia haemolytica. And it was important isolate because it causes shipping fever and gangrenous mastitis in sheep.

Susceptibility of some isolates to antibiotics was observed. They were sensitive to Gentamycin (30 µg) and Erythromycin (30 µg) and resistant to Cloxacillin (5 µg) and Ampicillin (10 µg).

Effect of camel urine to ten species of different bacteria was carried out and it was found that it had inhibitory effect on Staphylococcus aureus, E. Coli, salmonella sp and pseudomonas sp, but streptococcus sp, Proteus mirabilis, Klebsiella pneumoneae, neissiria sp and Brucella abortus were not affected by camel urine.

Sensitivity of the same bacteria to camel urine after it was neutralized to pH 7.1 was done and it was found that the zones of inhibition were wider.

Histopathological examination of the camel bladders from which bacteria were isolated was carried out, muscular
degeneration bacterial accumulation, edema and congestion were noticed.
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.ولوجاً دُأبَّتْ عُجْدَيمًا، كَذَكَرْيَ عَدَاٰ السَّحِيد
INTRODUCTION

From the down of history, camel owner used this animal urine for treatment of various diseases; early Arab boiled it and drank it to cure some internal disease problems (Ali, 1993). People in Yemen used to graze their camels on a certain grass and dry the voided urine and used it topically to treat burns, soft bloody injuries (AlZahrawi, Ibn-Elbitar, 1090).

Uses of camel milk and urine are included in two verses of the Holy Quran (1) Sura V Maida or The Table Spread Verse 36 The punishment of those who wage war against God and His Apostle and Strive with might and main for mischief through the land is; excution or crusifixation.” The story of “musul” of this verse is mentioned later.

(2)(Sura XXXVI Yasin: Verse 73”And they have (other) profits from them (besides), and they get (milk) to drink. Will they not then be grateful” (The Holy Quran, translated, Ali, 1975) (Tafsir Ibn Kathir mentions milk and urine.
It was also well known to scholar of Dutch knowledge that processed urine of a camel was a miracle to cure a person in critical condition (Kawazoe, 1996).

**The objectives of the study included:**

1. Physical property of camel urine samples.
2. Chemical analysis of camel urine samples.
3. Isolation and Identification of bacteria in camel urine.
4. Sensitivity test of bacterial isolates from camel urine to antibiotics.
5. Study of *(in vitro)* effect of camel urine on the growth of *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *salmonella* sp., *streptococcus* sp., *Niesseria mucosa*, *Corynebacterium bovis* and *Brucella abortus*.
CHAPTER ONE
LITERATURE REVIEW

*Distribution and economic potential of the camel:*

The camel (*Camelus dromedarius*), or one-humped camel, is an animal uniquely adapted to hot and arid environments. It produces meat, milk, wool and hides. It is also used for riding, and as a draft animal in agriculture (Schwartz, Dioli, 1992). It is also used as a transport animal, carrying fuel, wood, trade goods and water for household consumption in arid zones of the Sudan.

It inhabits the arid area of Africa particularly in East Africa i.e. in Somalia, Sudan, Ethiopia, Kenya and Djibouti and many countries of the Middle East.

Estimates of the camel population in Sudan vary between 2.7 millions (National Statistic, 1989) and 3.1 million (FAO, 1988). Camel is raised mainly in Kordofan, Darfur and Butana, i.e. in the northern dry lands of the Sudan.

Generally camels have a significant role in economic sector and this is for so many reasons for example, the unique ability to survive during dry seasons in climatically harsh areas, their adaptability to high temperatures and reduced dependence on water supplies, their mobility and ability to browse and graze a wide range of plant species and their relative resistance to diseases.
The biology of the camel:

**Evolution and general biology origins and present distribution**

| Name |  
|------|---|
| Class | Mamalia |
| Order | Artiodactle |
| Family | Camelidae |
| Genus | *Camelus droemdarius* |

- **Weight**: 300 to 690kg (661-1521 lb)
- **Size**: 1.8 to 2.3m tall at shoulder
- **Habitat**: Dry region of the Middle East and North Africa
- **Diet**: Throns, leaves, and almost any other vegetation
- **Domestication**: Early Asians
- **Uses**: Pack, riding and racing animal, wool, meat, milk and hide,
- **Life span**: 40-50 years
- **Sexual maternity**: Male at 5 years, female 3-4 year.
- **Gestation**: 12-13 months
- **Defense**: Spitting, biting and kicking.

The *Camelidae* are divided into two genera. The genus *Camelus* (true or Old-world camel) and the genus *Lama* (new-world camel).
The Camilidae are spread from North America to South America and via Asia to Africa.

There are today about 17-18 millions true camel and 4-5 millions new-world camels. The true camels include the one-humped or Arabian camel (*Camelus dromedarius*), commonly called dromedary, and the two-humped or bacterian camels (*Camelus bacterianus*). It is doubtful whether one should accept the general assumption that these are two different species since both types can be crossed and hybrids are fertile.

There are about 15-16 millions dromedaries in Africa, the Middle East and the Indian subcontinent (Madani, 1996). The dromedary (Arabian) camel has a wooly coat and is caramel in color. Its nostrils can be closed against flying dust, in order to survive for long periods of time without drinking. A camel has its hump which stores fat. When the fat is broken down, it turn into hydrogen. Water is formed when this hydrogen is mixed with inhaled oxygen. If a gram of fat is broken down during metabolism, it produce more than a gram of water (Hill and Wyse, 1989). If a camel is thirsty it can consume as much as 30 gallons of water in just 10 minutes (Schwartz, Dioli, 1992).

**Breeds:**

The Republic of the Sudan has the second largest camel population in the world estimated to a number close to three million head (Salih, 1988), and the Country is the home to some
of the most well-known camel nomads such as the Kababishi, Shukri, Hadandawi types and others.

Camel in Sudan:

The type of camel in Sudan include:

1. Sudanese pack camel
   1.1 Rashidi
   1.2 Arabic Kabbashi

The last include:

1.2.1 Light type
1.2.2 Heavy type
1.2.3 Large massive type.

2: Sudanese riding camel:

2.1 Anafi camel
2.2 Bishari camel

The majority of camel in Sudan is pack animal and these are

1.1 Rashaidi camel:

The Rashaida tribe breeds this type of camel in East Sudan. It is light in weight, short, and dark red in color (Madani, 1996).

1.2 Arabic Kabbashi:

These represent the majority of camel in Sudan west and east of River Nile. It has three types:

1.2.1. Lightweight camel in East Sudan where the tribe of Hadandawa and Bani Amer live.
1.2.2. Large sized camel in Butana area where tribes of Shukria and Bataheen live.
1.2.3. Large massive camel is found in desert and semi-desert where the tribes of Kabbabish and Hawaweer live. It has large head and eye, large neck and short hair. The color is grey to white to dark red and some time black.

2. **Riding camel:**

   They represent 10% of camel in Sudan; it found between River Nile and Red Sea.

2.1. **Anafi:**

   Found near Kassala Town and is characterized by long legs and small head, the colour is white or grey and small ear directed forward.

2.2. **Bashari:**

   It is stronger than Anafi, has small head and body, big eyes, long legs, smooth hair, light in movement, can run for long distance, the colour is grey to white (Medani, 1996).
Diseases in camel in general:
Morbidity and mortality rates in camel population due to age factor was found to be higher in young camels under six months (Abbas and Agab, 1993). Diseases such as the former, helmenthiosis are of major importance. Trypanosomiosis is considered to be the most important disease due to its worldwide spread among camel population. It is severe and fatal in its acute form (Rutter, 1967); in its chronic form, it causes drop in milk and meat production (Richard, 1979). It also causes abortion, infertility, inability to feed, immuno-depression and anaemia. Camel disease which are known to nomads by local names as Eldobab, Elnicato, Elhabobe, Elgodda, Elnihaze, Elkassara…etc are needed to be studied and their etiology be specifically defined (Darosa, 2000).

General biology:
Although Camilidae are ruminant animals, they are not classified as ruminatia. They differ from true ruminants in that they walk on the pads of the two last digits instead of on the sole of the hoof; they have no horns and they have completely different stomach system that contain three complex compartments but it is more efficient at feed conversion than ruminants in extracting protein and energy from poor quality forage (Graham, 1996). They are classified as rumanoids.

The general characteristics of ruminant and microbial digestion of fibrous feeds in large and compartmented stomach system have developed independently in camelids and ruminants
and this resulted in marked differences in morphology, histology and motility of the stomach system (Shwartz, Dioli, 1992).

**Adaptation to hot, arid environment:**

The ability of Camilids, and particularly the dromedary to adapt to extreme aridity of the habitat is unique amongst large herbivores. The most significant aspect of this adaptation is the economic use of water in almost all metabolic functions. These fall into two major categories; the intermediary metabolism and the maintenance of body temperature. In the usual habitat of the dromedary, this generally means cooling.

When kept under complete water deprivation and at high environmental temperatures, camel lose about 1.2% of their body weight compared to 7-8% daily loss in taurine cattle under the same conditions. Death by dehydration occurs in mammals inevitably at body weight losses of 25-30%. Thus camel can survive 15-20 days without any water, cattle only 3-4 days; zebo cattle are slightly more tolerant than taurine cattle and the small ruminant take an intermediate position between cattle and camels (Shwartz, Dioli, 1992).

**Body temperature of camel and its relation to water economy:**

The rectal temperature of normal healthy camel at rest may vary from about 34°C to more than 40°C. Diurnal variations in winter are usually in the order of 2°C; in summer the diurnal variation in camel deprived of drinking water may exceed 6°C.
The variation in temperature are of great significance in water conservation in two ways.

a- The increase in body temperature mean that heat is stored in the body instead of being dissipated by evaporation of water.

b- The high temperature of the body means that heat gain from the hot environment is reduced because the temperature heat gradient is reduced (Knut Schmidt-Nielsen, Bodil Schmidt-Nielsen, et al., 1957).

Sweating in camel:

Although it has been stated by an authority on camel that these animals have no sweat gland, this is not true. Camels have sweat glands distributed over the entire body surface. One characteristic of sweating of the camel in the dry desert area is that there is no copious flow of sweat or conspicuous wetting of the fur. This may explain the erroneous reports that the camel does not sweat (Schwartz, Dioli, 1992).

Numerous factors contribute to the superior water economy of the camel. Camel can take a very large amount of water than in other animals. This would result in severe osmotic problems. Camel can do this because water is absorbed very slowly from their stomach and intestine. Further more, their erythrocytes can swell to 240% of normal size without bursting (other animal can only go to 150%) (Graham, 1996). Water losses through urine are minimized by concentrating urine, by reducing renal urine flow.
and by retaining metabolites in the body fluids. Faecal water loss in camel is likewise comparatively low due to the efficient reabsorption of water in the colon (Medani, 1996).

Urea is reabsorbed from the intestines and transferred back to the stomach (Wilson, 1984). The salivary urea is found to be proportion to urea concentration in the blood (Bailey and Balch, 1961). It pass to the rumen through the epithelium (Houpt, 1965). It can also pass through the wall of the rumen (Houpt, 1968).

Urea may be hydrolyzed in the rumen and used in microbial protein synthesis (Yagil, *et al*., 1984; and Schmidt-Nielsen, *et al*., 1947). Urea synthesis in the body was greater in the camel than in the sheep and goats (Mousa, Ali, Hume, 1983). It was observed that urea excretion was markedly reduced in camel on low nitrogen intake. At high environmental temperature, up to 80% of the total daily water loss may be accounted for by heat dissipation through evaporative cooling in camel and about 95% of the evaporative heat are lost by sweating.

The necessity for sweating, however is much reduced in camel by efficient short wave heat reflection from the light and smooth hair coat, by equally efficient large wave heat re-radiation from dark skin, but first and foremost by the fact that camel can tolerate fluctuations at the deep body temperature from 34-42°C. They can store considerable amounts of heat during the day and can dissipate this by non-evaporative mechanisms/radiation, conduction and convection, during the cool hours of might. The
highest deep body temperature are usually reached during the early afternoon, when they produce the additional beneficial effect of lowering the temperature gradient environmental heat gain (Schwartz, Dioli, 1992).

**Urination, defecation and salivation**

Camels frequently urinate and defecate specially after rising. Daily output of urine ranges between 0.5-5.0 litre/day depending to a large extent on the animals status of hydration or dehydration. The colour is usually light yellow but can turn to dark brown. In the early rainy season faeces may take light green colour, become less well formed or even take a liquid consistency, depending on the water content of the available forage. Excessive salivation in camel is usual, increases in nervous disorders such as rabies and in male during the rutting season. Excessive salivation is an essential part of the mating behaviour.

Urine collection is best attempted by free catch. Female camels can be catheterized, but their urethral opening is quite small and sub-urethral diverticulum just in front of urethral opening, makes insertion of catheter quite difficult. Male camels can not be catheterized due to the presence of a urethral recess at the ischiatic arch (Schwartz, Dioli, 1992).

Body fluid and excretions such as urine, saliva and faeces can change in colour, volume, frequency of excretion, consistency and smell, which often points to specific disorder.
A dark reddish to dark brownish discolouration of urine indicate the presence of blood, myoglobin or haemoglobin. This is a serious clinical finding and many East African pastoralists claim that they can diagnose acute phases of trypanosomiasis by the smell of the animal urine. In a survey conducted by Wilson and Schwartz (1983) such diagnosis could be confirmed at a rate of about 75-80% of all infected cases in two herds.

Watering:

All livestock including camels usually find all the drinking water they require. In addition to that, if fresh green forage is available it gives camel almost all water they need. It has frequently been observed that camels go without any drinking for several weeks during such time. In areas where water points are numerous, well spaced and easily operated, the usual watering interval for camels is 3-4 days. Watering interval of 5-7 days are still considered normal; interval longer then 7 days already constitute a stress on the animals.

The water content of camel body (Macfarlane et al., 1963) is similar to that of other ruminants in which 15% -18% of the body weight may be made up of fluid in the alimentary tract. Water requirements of camels although low in comparison to other livestock species, are still quite high in absolute time of the of dry season. When ambient temperature is high and the vegetation is dry, an adult camel requires approximately 80-100 litres of water
every 5-8 days. Requirement will also increase in lactating animals (Schwartz, Dioli, 1992).

The ability to move long distance and finding green forage minimize the camel need for water (Gebrehiwet, 1997).

Urine:

Urine is water which contains soluble waste products. It is filtered from blood during the passage through the kidney. It contains waste products concentrated in the blood and must be lowered so the kidney can reabsorb substance which are needed to maintain the constant composition of the blood (Ibrahim, 1989).

Properties of urine:

Normal urine of most animals is clear, watery fluid usually yellowish in colour.

In the horse it is viscous due to presence of mucoprotein secreted from the kidney and in chickens is creamy and thick due to presence of uric acid which is excreted with faeces from the cloaca (Ibrahim, 1989).

The pH of urine:

The pH of urine in herbivorous animals is alkaline and in carnivores is acidic and this is due to food they eat.

The odour of camel urine:-

The odour of camel urine is unpleasant, but it changes in *Typanosoma evansi* infected camel. In this case the odour change to acacia-flower-like odour (Hussein and Gunid, 1993) or fruity-aroma-like-odour (Kleiner and Orten, 1962) and this is due to presence of large amount of acetone in infected camel urine.

Urinary system in camel:
The kidney of the camel have adaptive function. It can excrete a very concentrated urine and this is of importance in desert condition to conserve water during periods of dehydration (MacFarlone, Morris, and Howards, 1963). Unlike other animals camel can produce urine with salt concentration almost twice that of sea water (Ibrahim, 1989). If camel is fed on low protein diet it can produce urine very low in urea (0.3 g of urea nitrogen/day) (Schmidt-Nielsen, et al., 1957).

**Camel's urine therapy:-**

Urine of one-humped camel, *Camelus dromedarius* is medically used for centuries in different part of Arab countries. It is mostly used for chronic problems and it has been recommended by Prophet Mohammed (peace be upon him) for treatment of some diseases.

Some sick people come to Prophet Mohammed (peace be upon him). They were pale faced and had abdominal distention. They asked for treatment and the Prophet (peace be upon him) advised them to use camel’s urine and milk (Elbukhary, undated) also Prophet Mohammed (peace be upon him) advised people with abdominal disorder to use camel’s urine (Elbukhary) (The story is mentioned in Sura V Maida).

**Practical use of camel’s urine:**

Ancient Arabs used to boil camel urine to treat their patients in Yemen they used to dry it under the sun and press it in shape of tablets. These tablets were used in treatment of bad burns and wet bloody injuries (Ibn-Elbitar, undated), also Ibnsina mentioned the use of camel urine in treatment of acities and achieved successful result (Alrazi, printed, 1958), (O’haj, 1998).
Recently, camel urine has been used for treatment of leukemia and digestive system cancer in Kuwait (O’haj, 1993).

Also it is being used as hair (shampoo) detergent in Morocco, some part of Sudan, Saudi Arabia and Somalia (Ibrahim, 1989; O’haj, 1993; Algedabi, 2000). O’haj (1993) mentioned the use of camel urine for treatment of fever or malaria.

Studies on camel urine:

Kabariti, et al. (1988) reported that in Arabian desert, people used to give the urine of young camels mixed with milk, to overcome the bad odour and taste, every morning as treatment of cancer patients and leukemia cases.

Ibrahim (1989) reported that ladies in Morocco use camel urine as hair detergent.

O’haj (1993) made a survey among five nomadic tribes in eastern Sudan. He reported that 72% used camel urine for treatment of internal problems in general, while 52%, 33% 20% and 33% used it for malaria, acities, dental problem and as hair shampoo respectively.

O’haj (1998) did a clinical trial for treatment of acities by giving daily doses of 150ml of camel urine for a period of two weeks. The control groups were treated with furesimide (40mg) injection twice a day for two weeks. He found that camel urine worked as slow diuretic and good laxative. The control group cured rapidly but they reported earlier (less then two month) with
return of the acities while the camel urine study group took larger period of time before they reported back with ascities.

Animal urine therapy:

Ox-urine was used to treat ear infection (IbnSina, Ibn Elbitar, undated) and cow urine used to cure ulcer.

In Southern Sudan people drink cow urine (O’haj, 1993) for fever.

Donkey urine was used for treatment of nephritis (IbnSina, undated). Ibn Elbitar (undated) mentioned the use of a mixture of all animal urine for injuries and ulcers.

Goats urine had been used for fever (IbnSina, undated). Alrazi (printed, 1958) mixed it with medicinal plants for treatment of jaundice and ascites.

O’haj (1998) found that goat urine inhibited the in vitro growth of Staph. aureus (120%), (100%) and (100%) respectively on nutrient agar, MacConkey agar, (C.L.E.D. agar respectively, while cow urine inhibited the growth of E. coli only on C.L.E.D. agar and Staph. aureus on nutrient agar.
CHAPTER TWO
Material and Methods

Collection of urine samples:-

A total of 250 samples of camel urine were collected during August 2000 to August 2002 from different areas of Sudan: Tampool, EIObied, EIGadarif and ElShowak slaughterhouses. These samples were collected aseptically by taking the whole urinary bladder; the neck being tided tightly swabbing the surface with 70% alcohol, then using sterile syringes, 5ml of urine was transferred aseptically into sterile bijou’s bottles and centrifuged immediately at 6000 rpm for 5 minutes then primary tests were carried out.

Physical analysis of camel urine:-

Immediately after collection of the samples, the following were observed temperature, colour, odour and pH was done by using the yellow litmus paper (Sigma).

Preparation of Media: (Barrow and Feltham, 1993)
1). Blood Agar (Oxoid):

a) Ingredient:

Defibrinated blood 50ml
Nutrient agar 950 ml
Distilled water 1000 ml
The nutrient agar was melted, cooled to 50°C then sheep blood was added aseptically, mixed and distributed in 20ml amount in petri dishes.

**b) Ingredient**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agar Base No.2</td>
<td>40 g</td>
</tr>
<tr>
<td>Defibrinated blood</td>
<td>10 %</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000</td>
</tr>
</tbody>
</table>

An amount of 40g of the base was dissolved completely by boiling in distilled water, mixed and sterilized by autoclaving at 121°C for 15, minutes, then cooled to 45-50°C and sterile blood was added at rate of 10% mixed gently and poured in 20ml amounts in petri dishes.

**Preparation of sheep blood:**

The blood was prepared in a flask which contained many small glass beads. It was closed tightly with a rubber bung with a hole in which was inserted a glass tube open on both sides. The glass tube on the outer sides was connected to a needle through a rubber tube. The needle was wrapped in aluminum foil and the blood collection flask with its accessories was sterilized by autoclaving at 121°C for 20 min.

Aseptically the needle was inserted in the jugular vein of the sheep and the blood was let down. During this operation the flask was shaked gently to prevent coagulation. The unclotted blood was added to blood agar base in the ratio of 10%.
2) Nutrient Broth (Oxoid):

Ingredients:

Meat (beef) extract 10g.
Peptone 10g.
Nacl 5g
D.W. 1000ml

The ingredients were dissolved by heating in boiling water bath. The pH was adjusted to 8.0-8.4 with 10 N-NaoH and boiled for 10 minutes. The solution was filtered, while hot, through thick filter paper to remove phosphates which were precipitated in alkaline solution. The pH was readjusted to 7.2-7.4 and the medium sterilized at 115°C for 20min.

3) Nutrient agar: (Oxoid)

Nutrient broth (A2.1.1) gelled by the addition of 2% agar.

4). MacConkey agar (Oxoid):

Peptone 20g
Nacl 5g
Sodium taurocholate 5g
Neutral red 1% of soln. 10ml
Water 1000ml

Peptone, Nacl and bile salt were dissolved in water by heating. The pH was adjusted to 8.0, boiled for 20min, cooled and filtered. The agar was added and dissolved by boiling, then the pH was readjusted to 7.4. Lactose and indicator solution were added and mixed, sterilized at 115°C for 20min.
5). Mueller-Hinton agar (Oxoid)

Ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat infusion</td>
<td>6g</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
<td>17.5g</td>
</tr>
<tr>
<td>Starch</td>
<td>1.5g</td>
</tr>
<tr>
<td>Agar</td>
<td>10g</td>
</tr>
<tr>
<td>Water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

The ingredients were dissolved by boiling. The pH was adjusted to 7.4, then sterilized at 121°C for 15min.

6). Peptone sugars (Oxoid):

Ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Meat extract</td>
<td>3g</td>
</tr>
<tr>
<td>Nacl</td>
<td>5g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>Andrade, indicator</td>
<td>10ml</td>
</tr>
</tbody>
</table>

The solids were dissolved in boiling water. The indicator was added and the pH adjusted to 7.1-7.2; the medium was sterilized at 115°C for 20 minutes. Then 0.5-1% of the appropriate carbohydrate was added mixed, then distributed in sterile tubes in amounts of 5-7ml, containing inverted inner tube (Durham tube) and then sterilized at 110°C for a period of 5 minutes.

7). Motility test Medium: (Oxoid)

Ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10g</td>
</tr>
</tbody>
</table>
N a c l    5g  
Meat extract  3g  
Gelatin  80g  
Agar  4g  
Distilled water  1000ml  

The gelatin was soaked in water for 30 min. and then added to the other ingredients. The medium was sterilized at 115°C for 20 minutes.

8). Nitrate Broth Medium (Oxoid)  

Ingredients:  
KNo₃  1g.  
Nutrient broth  1000ml  
KNo₃ was dissolved in the broth, distributed into tubes in amount of 5-7 ml. and sterilized at 115°C for 20 min.

9). Urease agar base (Oxoid):  

Ingredients:  
Peptone  1g  
Dextrose  1g  

NaCl  5g  
Disodium phosphate  1.2g  
Potassium dihydrogen phosphate 0.8g  
Phenol red  1.2g  
Agar No. 3.  15g  
D.W.  1000g  

pH  6.8 (approx.)
An amount of 2.4g were suspended in 95ml of distilled water. The medium was dissolved by boiling, then sterilized by autoclaving at 115°C for 20 minutes. After the medium was cooled to 50°C, 1 ml of sterile 40% urea solution was added aseptically. The medium was distributed into 10ml amounts in sterile MacCartney bottles and allowed to set in the slope position.

10). Glucose broth:

*Ingredients:*

- Glucose 20% aq. soln 50ml
- Nutrient broth 950ml

The glucose solution was sterilized by filtration and added aseptically to the nutrient broth. Then mixed and distributed aseptically in 10ml, amount in sterile bottles.

11). Hugh and Leifson’s medium (Hugh and Leifson, 1953).

*Ingredients*

- Peptone 2g
- Nacl 5g
- K₂HPO₄ 0.3g
- Agar 3g
- D. W. 1000ml
- Bromothymol blue 0.2% aqu. soln. 15ml.

The solids were dissolved by heating in water path. The pH was adjusted to 7.1, the medium was filtered through and then the indicator was added.
The medium was sterilized at 115°C for 20 minutes. Sterile glucose solution was added to give final concentration of 1%. The medium was mixed and distributed aseptically in 10ml amounts into sterile tubes of not more than 16mm. in diameter.

12). Peptone water (Oxoid):

**Ingredients:**

- Peptone: 10g
- Nacl: 5g
- Water: 1000ml

The contents were dissolved by heating, pH was adjusted to 80-8.4, then the solution was boiled for 10 min, filtered and the pH readjusted to 7.2-7.4 then sterilized at 115°C for 20 min.

13). VP test medium (Oxoid)

**Ingredients:**

- Peptone: 10g
- Glucose: 5g
- Distilled water: 1000ml

The contents were mixed gently by heating in a boiling water bath, filtered; the pH adjusted to 7.6 then distributed into tubes in amount of 5-7ml, sterilized at 115°C for 10 min.

14). Tryptose agar (Difco)

Forty-one gram of tryptose agar were added to one titre of distilled water and dissolved by boiling, then sterilized by
autoclaving at 121°C for 15 min. The pH was adjusted to 7.2. The medium was distributed 15-20ml amount in petri dishes.

**Reagents:**

**Hydrogen peroxide:**

This was obtained from Agropharm Limited (Buckingham, U.K.) in 3% aqueous solution for the catalase test.

a) **Alpha-naphthol solution:**

Alpha-naphthol was manufactured by BDH (British Drug house, London). The test solution was prepared according to Cowan (1974) as 5% solution and used for Voges-Proskauer test.

b) **Tetraethyl-p-phenylenediamine dihydrochloride:**

This was obtained form BDH, it was prepared as 1% aqueous solution and used for oxidase test. The reagent was protected from light.

d) **Potassium hydroxide:**

This was obtained from BDH. It was prepared according to Cowan (1974) as 40% solution and used for Voges Proskauer test.

e) **Nitrate test reagent:**

**Solution A**

Solution A, 0.33% sulphanilic acid in 5N-acetic acid (dissolved by gentle heating).

**Solution B.**

Solution B, 0.6% dimethyl –α- naphthylamine in 5N-acetic acid.
Zinc dust, 10% dust suspended in 1% methyl-cellulose solution.

**Kovacs reagent:**

**Content**

- p-dimethylamino benzaldehyde 5g
- Amyl alcohol 75ml
- conc. Hcl 25ml

The aldehyde was dissolved in alcohol by warming in water bath (50°-55°C) then cooled and was added to the acid.

**Indicators:**

**Andrade’s indicator:**

It was prepared according to Barrow and Feltham (1993) by dissolving 5g of acid fuchsin in one litre distilled water, 150ml of alkali solution was then added.

**Bromothymol Blue:**

This was supplied by BDH chemicals; prepared by dissolving 0.2g powder in 100 ml distilled water.

**Phenol red:**

Was supplied by Hopkin and William Ltd., London.

**Sterilization:**

Inoculating wire, points of forceps were sterilized by holding them near to vertical as possible in Bunsen flame until they were red hot.

Test tubes, petridishes, flasks, pipettes and forceps were sterilized in hot air oven at 160°C for one hour.
The most important sterilization apparatus is the autoclave which is a metal cylinder designed to contain steam under pressure. It was used for sterilization of media, bottles with rub-lined covers, etc..

**Isolation techniques:**

Blood agar plates were used for isolation. A loopful of the deposit was streaked on the medium and then incubated aerobically at 37°C for 24 hrs. Plates that didn’t show growth were incubated for a week before they were considered negative. Separated colonies were subcultured several times onto other sterile plates of non-selective medium (nutrient agar) or (blood agar) to ensure pure culture. Identification was done according to Barrow and Feltham, 1993.

Pure cultures were also subcultured on blood agar slants which were then stored at 4°C for later use.

**Gram’s staining method:**

a) Crystal violet was applied for 2 min.
b) Washed with clean water.
c) Lugols iodine solution was applied for 2 min.
d) Washed with clean water.
e) Decolorized with few drops of acetone for no time.
f) Washed with clean water.
g) Carbol fuchsin (10%) was applied for ½ min.
h) Washed and slide put in the standing position to drain and dried, examined under 100 x lens (oil immersion)

Biological tests:

1. Coagulase test:

1.1 Slide method:-

Part of a colony was emulsified in a drop of saline on a slide. Then a drop of citrated human plasma was added and mixed with the suspension. A positive result was indicated by a definite clot formation.

1.2 Standard method:

An amount of 0.1ml of 18-24 hours broth culture of the isolated organism was added to 0.5 ml of 1/10 dilution of human plasma in saline, incubated at 37°C then examined after 24 hours. A positive result was indicated by definite clot formation.

2. Motility test:-

2.1 Hanging drop:

A drop of broth culture of the organism that was incubated at 37°C for less than 24 hours, was examined in hanging drop preparations under microscope (10 x dry lens).

2.2 Graige tube method:-

Graige tubes were inoculated on the inside by stabbing using a straight loop dipped in the culture, then incubated at 37°C. Motile organisms migrated throughout the medium which became
turbid. Growth of non-motile organism was confined to the stab line.

3. Oxidation / fermentation of carbohydrate: (O/F Test)

Duplicate tubes of Hugh and Leifson’s medium were inoculated with straight wire loop. To one of the tubes, a layer of sterile melted soft paraffin oil was added to depth of 3cm above the medium. The tubes were examined daily for up to 14 days. If the colour was yellow in the open tube only; this indicated oxidation of glucose, if the colour was yellow in both tubes, it showed fermentation and if the colour in the open tube was blue or green and the sealed tube was green, it indicated production of alkali.

4. Oxidase test:

On a piece of filter paper in a petri dish, 2-3 drops of 1% tetramethyl-p-phenylenediamine dihydrochloride were added. The test organism was removed with glass rod and smeared across the surface of the impregnated paper. A positive reaction was shown by the development of a dark purple colour within 10 seconds.

5. Catalase test:

On clean slide a drop of 3% aqueous solution of hydrogen peroxide was placed. Part of a colony of the tested organisms, on nutrient agar, was picked and put in the drop of hydrogen peroxide. Appearance of bubbles indicated that the organism produced catalase enzyme.
6. **Nitrate reduction:**

Nitrate broth was inoculated and incubated for 48 hours. One ml of reagent A was added followed by 1ml of reagent B. Red colour indicated the presence of nitrite and indicated that nitrate has been reduced.

To tubes not showing a red colour within 5 minutes, zinc powder (up to 5mg/ml of culture) was added. Red colour indicated the presence of nitrite in the medium (not reduced by organism). Absence of red colour indicated that nitrate absence in the medium (reduced by organism to nitrite, which in turn was reduced.

7. **Urease activity:**

A slope of Christensen’s urea medium was inoculated and examined after 4 hours incubation and daily for 5 days. Change of the colour of the medium to red indicated a positive reaction.

8. **Voges-Proskauer reaction:**

Glucose phosphate medium was inoculated at 37°C for two days, then 0.6ml of 5% a-napthol solution was added followed by 0.2ml of 40% KOH aqueous solution. The tubes were then shaken and sloped (to increase the surface area). A positive reaction was indicated by strong red colour after 15min or one hour.

9. **Indole production:**

Peptone water was inoculated with the test organism and incubated at 37°C for 48 hours, then 0.5ml of Kovacs reagent was
added, shaken well and examined after 1 min. Production of indole was indicated by red colour in the reagent layer.

10. Hydrogen sulphide (H₂S) production:-

Nutrient broth was inoculated with test culture. Filter paper impregnated in 10% lead acetate solution and dried was placed in the neck of the tube and incubated at 37°C for 48 hours. A positive reaction was indicated by a brown or black colour of the paper.

Chemical analysis:

1. Determination of calcium:

Calcium in urine was determined by mixing 2.5ml oxalic acid, 2.5g ammonium sulphate and 4ml glacial acetic acid; completed to 100ml with distilled water. Equal volumes of the above reagent and urine were mixed. Turbidity indicated the precipitation of urinary calcium oxalate (O’haj, 1998).

2. Determination of sodium and potassium

The potassium and sodium were estimated by flame emission spectrophotometer (FES) (Jenway). The amount of 0.2ml urine sample was completed to 20ml (1 to 100) with distilled water and directly read on the FES. Two separate standard curves were prepared ranging from 100 to 150 ME Equ/L and two to ten mEqu/L for sodium and potassium respectively (Mousa, 1978).

3. Determination of protein by biuret method:
A violet coloured complex was produced by reacting the urine protein with an alkaline solution. Other non-protein nitrogen compounds such as creatinine and urea do not give reaction with this method.

**Procedure:**

One-ml urine was completed to 100ml with distilled water. Five ml of Biuret reagent was added to three ml diluted urine; three ml protein standard (0.5 of bovine serum albumin in 100 ml (D.W.) and three ml distilled water (used as blank) were treated by the same method. All the tubes were placed in water bath at 37°C for ten minutes. The absorbency was measured after adjusting the colorimeter (Unicom (8625) to zero point at 540 nm (Reinhold, 1953).

**Determination of non-protein nitrogen:**

4. **Determination of urea:**

Urea was determined according to the method of Evans (1968) using diacetylmonoxime (DAM) and thiosemicarbazide (TCA) as colour reagents. An amount of 0.1ml urine was added to 0.9ml distilled water and one ml of 10% TCA. All components were mixed thoroughly and centrifuged for five minutes at 3000 rpm. From the clear supernatant, 0.5ml was taken and added to 5 ml of colour reagent. The standard was prepared by dissolving 10mg urea in 100ml water. From this, the working standard was prepared by taking 10ml of the above solution and 50ml of 10% TCA and made to 100 ml with distilled water. From the working
standard solution, 0.5ml was taken and added to 5ml of colour reagent. The blank, for adjusting zero point, was prepared by adding 0.5ml of 5% TCA to 5ml ml colour reagent. The three tubes were put in a boiling water bath for 5 minutes and then removed from the bath and cooled. The optical density of the standard and tested urine were read after adjusting the colorimeter to zero point, using filter 624.

5. **Determination of creatinine:**

It was determined by the method of Bonsnes and Taussky (1945). Urine was diluted (1ml up to 50ml) then 2ml of the diluted urine was added to 2ml distilled water and 2ml of trichloro acetic acid. The standard was prepared by adding 2ml of creatinine standard to 2ml distilled water and 2ml of trichloroacidtic acid.

The blank for adjusting zero point was prepared by adding 4ml distilled water to 2ml trichloracetic acid. All the three tubes were mixed well separately, and left to stand at room temperature for 10 minuets, then centrifuged at 3000 for 10 minutes and supernatant fluid was filtered. Three ml of the filtrate was pipetted into clean dry tube. Then one ml of picr acid was added, mixed, followed by addition of sodium hydroxide. The absorption was read after 20 to 45 minutes at 540 nm by using colorimeter.
6. Determination of creatine:-

The creatine in the urine was transferred to creatinine by heating urine sample with picric acid. The total creatinine was determined according to the above method of Bonsnes and Taussky (1945) the only difference is the heating of urine with picric acid before adding the NaOH. So the difference between the total creatinine and creatinine will give the amount of creatine in the sample.

7. Determination of uric acid:

Urine sample was diluted 1:100 one ml of diluted urine was added to 7ml of distilled water, then one ml of 10% sodium tungostate and to one ml of $\frac{2}{3}$ NH$_2$SO$_4$ was added, mixed and centrifuged. Then 5ml of the supernatant was taken in a test tube, 5ml of working standard solution and 5ml of distilled water was used as blank. To all tubes 1ml of 10% sodium carbonate and 1ml of diluted phosphotungestic acid was added. Then read at 680nm (Varley, Gowen, Bell, 1980) by using a colorimeter (Unicam 8625 u/v/vis spectrophotometer).

8. Determination of Magnesium:

An amount of 0.22ml of urine samples were added to 2.8ml D.W. The standard was made by adding 0.1ml of working standard to 2.0ml D.W.; for the blank 0.3ml D.W. was used. To all tubes 0.5 ml polyvinyl alcohol reagent, 0.5ml titan yellow and one ml (7.5%, W/V) sodium hydroxide were added in the above
stated order with mixing after each addition. All tubes were allowed to stand for 5 min. before being read at 540nm and after the zero absorbance was set by the blank (Spare, 1962).

**Antibiotic sensitivity test:**

Sensitivity to antimicrobial antibiotics drug for *Staph. hyicus, S. capitis, S. heamolyticus, S. hominis, Corynebacterium xerosis, C. stratium, C. pseudodiphthericum, Micrococcus, Bacillus. cereus* and *Mannheimia haemolytica*, which were isolated from camel urine was determined.

**Standard disc diffusion method was used** (Buxton and Fraser, 1977). Plates of blood agar were dried in the incubator for 30 min. before incubation. They were then soaked with 18 hours nutrient broth culture. Excess fluid was aspirated and the plates were again dried for 30 min.

The antibiotic discs were applied onto the surface with sterile forceps and the plates incubated at 37°C for 24 hours. A ruler was used to measure the zones of growth inhibition around the discs.

Antibiotics used were: Lincomycin (10µg), Ampicillin (10µg), Furazolidone (100 µg), Erythromycin (30 µg), Cloxacillin (5 µg), Gentamycin (30 µg), and Chloramphenicol (30 µg).

Sensitivity of 10 bacterial species were tested on Mueller-Hinton agar against 6 samples of camel urine. These species were *S. aureus* (isolated from human and another isolated from animal), *E. coli, Klebsilla pneumoniae, Pseudomonas aeruginosa, Proteus*
mirabilis, Salmonella sp., Streptococcus sp. Niessina bovis and Brucella abortus.

Discs of 6mm in diameter from sheet of thick filter paper were punched out using paper drill. They were sterilized in hot air oven at 160°C for 1 hour. Then they were impregnated with the test urine and dried at 37°C for ½ hour.

Overnight broth cultures in nutrient broth of the test organisms were used. Culture were diluted with nutrient broth to a density visually equivalent to that of the standard prepared by adding 0.5ml of 1 percent Bacl₂ to 99.5of 1 percent H₂SO₄ approximately (Kirby, et al., 1966).

The dried plates were flooded with the diluted cultures and the excess fluid was removed. Discs containing camel urine were placed on the agar with forceps and gently pressed down to insure contact.

Plates were incubated overnight at 37°C. Zones that showed growth inhibition were measured.

**Histopathological method:**

Representative portions from collected camel’s bladders were fixed in 10 percent formal saline. Dehydrated in 10%, 85% 95% and absolute alcohol they cleared two changes in xylene. Using automatic tissue processor. Then tissues were embedded in paraffin wax and sectioned using microtome. Sections 5um thick were stained with Haematoxylin and eosin and examined using light microscope as described (Clayden, 1971).
CHAPTER THREE
RESULTS

Twelve bacterial isolates were obtained from 250 specimens of camel urine. Eleven isolates were Gram-positive bacteria and one was Gram-negative.

The Gram-positive bacteria were: *Staph. haemolyticus*, *S. capitis*, *S. hominis*, *S. hyicus*, *Corynebacterium striatum*, *Corynebacterium xerosis*, *Corynebacterium pseudodiphtericum*, *Microscoccus spp.* and *Bacillus cereus*.

The Gram-negative bacterium was *Mannheimia haemolytica*. Identification of these bacteria was carried out according to Barrow and Feltham (1993).

**Gram positive organisms:**

**Staphylococcus:**

Colonies on blood agar plates were circular, glistening and convex. The Gram-stained smear prepared from 24 hours cultures on blood agar showed cocci, non-sporing, found singly, in pairs, or clusters. The isolates grew best under aerobic condition. The organism was non-motile. Characters are shown in Table (1)
Table (1) Biochemical tests of *Staphylococcus* species isolated from camel urine.

<table>
<thead>
<tr>
<th>Test</th>
<th><em>S. hyicus</em></th>
<th><em>S. haemolyticus</em></th>
<th><em>S. capitis</em></th>
<th><em>S. hominis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Catalase</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Glucose</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>O/F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Urease</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Lactose</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Maltose</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

S = sensitive  
F = fermentative  
+ve = Positive result  
-ve = Negative result
Micrococcus:-

Micrococcus isolated were Gram-positive non motile cocci, arranged in pairs, tetrads and small clusters. They had uniform size, and were aerobic. Other characters are shown in Table (2).

Table (2) Biochemical tests for Micrococi sp Isolated from camel Urine

<table>
<thead>
<tr>
<th>Test</th>
<th>Micrococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth in air</td>
<td>+ve</td>
</tr>
<tr>
<td>Calatase</td>
<td>+ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+ve</td>
</tr>
<tr>
<td>O/F</td>
<td>O</td>
</tr>
<tr>
<td>V.P.</td>
<td>-ve</td>
</tr>
<tr>
<td>Nitrate</td>
<td>-ve</td>
</tr>
</tbody>
</table>

V.P. (Voges Proskauer test)

O = Oxidative
Corynebacterium:

Colonies on blood agar were small, dry and white. They were Gram-positive non motile rods. Other character are shown in Table (3)

<table>
<thead>
<tr>
<th>Test</th>
<th><strong>C. xerosis</strong></th>
<th><strong>C. striatum</strong></th>
<th><strong>C. pseudodiphtricum</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram/shape</td>
<td>Metachromic granule</td>
<td>Metachromic granule</td>
<td>No Metachromic granules.</td>
</tr>
<tr>
<td>Catalase</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Glucose</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>O/F</td>
<td>F</td>
<td>F</td>
<td>-ve</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Urease</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Maltose</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>sucrose</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

Table (3) Biochamical tests of Corynebacterium species isolated from camel urine.
Bacillus:
Gram-positive motile bacilli in young culture, motile, sporeformers.
Other characters shown in Table (4).

Table (4) Shows tests of bacillus isolated from camel urine

<table>
<thead>
<tr>
<th>Character</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram/shape</td>
<td>+ve rods</td>
</tr>
<tr>
<td>Arrangement of cells</td>
<td>chains</td>
</tr>
<tr>
<td>spore</td>
<td>Central/sub terminal</td>
</tr>
<tr>
<td>Glucose</td>
<td>+ve</td>
</tr>
<tr>
<td>Haemolysis in Sheep B.A.</td>
<td>+ve</td>
</tr>
<tr>
<td>Urease</td>
<td>+ve</td>
</tr>
<tr>
<td>Indole</td>
<td>-ve</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+ve</td>
</tr>
</tbody>
</table>

B.A. = Blood agar
Mannheimia haemolytica:

Gram-negative non motile rods (coccobacilli), showing bipolar staining in some cells. Calatase positive, oxidase positive and attacked sugars by fermentation Table (5).

Table (5) Shows tests of *Mannheimia haemolytica* isolated from camel urine.

<table>
<thead>
<tr>
<th>Test</th>
<th>Mannheimia haemolytica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram/shape haemolysis</td>
<td>-ve rods</td>
</tr>
<tr>
<td>Calatase</td>
<td>+ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+ve</td>
</tr>
<tr>
<td><strong>Haemolysis</strong></td>
<td>Haemolytic</td>
</tr>
<tr>
<td>Growth on MacConky agar</td>
<td>+ve</td>
</tr>
<tr>
<td>Indole</td>
<td>-ve</td>
</tr>
<tr>
<td>Urease</td>
<td>-ve</td>
</tr>
<tr>
<td>(H_2S)</td>
<td>-ve</td>
</tr>
<tr>
<td>Salicin</td>
<td>-ve</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-ve</td>
</tr>
<tr>
<td>Xylose</td>
<td>+ve</td>
</tr>
</tbody>
</table>
Physical properties of camel urine:-

The colour of fresh camel urine varied from slight to dark yellow and some times red in bloody samples and colourless in young camel. The odour was unpleasant.

The pH was determined immediately after collection of sample and it was varied from 7.5 to 9.5 in female and 8 to 9 male.

Chemical properties of camel urine:-

Table (3) , (4) and (5) show electrolytes in camel urine, end product of nitrgn metabolism and total protein respectively.
Table (8) Shows total protein in 16 male and 20 female camel urine.

<table>
<thead>
<tr>
<th>No.</th>
<th>Male g/L</th>
<th>Female g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.8</td>
<td>10.1</td>
</tr>
<tr>
<td>2</td>
<td>8.5</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>10.2</td>
<td>10.6</td>
</tr>
<tr>
<td>4</td>
<td>7.8</td>
<td>9.3</td>
</tr>
<tr>
<td>5</td>
<td>8.6</td>
<td>10.9</td>
</tr>
<tr>
<td>6</td>
<td>8.1</td>
<td>7.5</td>
</tr>
<tr>
<td>7</td>
<td>10.9</td>
<td>8.5</td>
</tr>
<tr>
<td>8</td>
<td>5.9</td>
<td>7.8</td>
</tr>
<tr>
<td>9</td>
<td>8.8</td>
<td>7.8</td>
</tr>
<tr>
<td>10</td>
<td>7.5</td>
<td>13.6</td>
</tr>
<tr>
<td>11</td>
<td>8.8</td>
<td>6.6</td>
</tr>
<tr>
<td>12</td>
<td>7.3</td>
<td>8.0</td>
</tr>
<tr>
<td>13</td>
<td>7.6</td>
<td>10.7</td>
</tr>
<tr>
<td>14</td>
<td>6.6</td>
<td>7.5</td>
</tr>
<tr>
<td>15</td>
<td>6.8</td>
<td>7.1</td>
</tr>
<tr>
<td>16</td>
<td>7.5</td>
<td>7.1</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>13.8</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>10.1</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>Mean</td>
<td>8.4</td>
<td>8.9</td>
</tr>
<tr>
<td>Se</td>
<td>±2(1.99)</td>
<td>±2.2</td>
</tr>
</tbody>
</table>
**Sensitivity of some bacterial isolates from camel urine.**

In this part, seven antibiotics were used: Lincomycin (10µg), Ampicillin (10µg), Furazolidane (100µg), Erythromycin (30µg), Cloxacillin (5µg), Gentamycin (30µg) and Kanamycin (30µg).

The test was done according to standard diffusion method (Buxton and Frazer, 1977).

Isolates used were: *S.hyicus, Micrococcus sp., Mannheimia haemlytica* and *Bacillus cereus*. They were resistant to Ampicillin, Cloxacillin and sensitive to Gentamycin and Erythromycin.
In this part 10 isolated bacteria were chosen: *S. aureus*, *E. coli*, *Salmonella sp.*, *Pseudomonas aeruginosa*, *Proteus mucosa*, *klebsiella pneumoniae*, *streptococcus sp.* *Niesseria mucosa*, *Corynebacteria bovis* and *Brucella abortus*. It was found that camel urine inhibited the growth of *Staph. aureus*, *E. coli*, *Samonella sp* and *Pseudomonas aeruginosa*. Other organisms were not affected by it.
Neutralization of camel urine:

The pH of three samples of camel urine was measured by pH meter and they were determined as alkaline, then neutralization was done by adding HCl (10%) until it was 7.1. It was observed that the zones of inhibition were more wider than before.
The effect of camel urine to some bacteria:

Camel urine inhibited the growth of *Staph. aureus*, *Salmonella*, *E. coli* and *Pseudomonas* (with some resistant colonies in the zone).

*Corynebacterium bovis*, *Proteus mirabilis*, *Salmonella*, *Streptococcus porcinus*, *Klebsilla pnemonae*, *Niessiria mucosa* and *Brucella abortus* were not affected by camel urine.

The type of effect of camel urine on *Staph. aureus* was bactericidal and on *Salmonella*, *E. coli* and on *Pseudomonas sp.* was *Bacteriostatic*.

Table (10) Shows the difference between zones of inhibition in neutralized and normal camel urine.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition by camel urine</th>
<th>Inhibition by neutralized camel urine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>8mm</td>
<td>9mm</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>13mm</td>
<td>14 mm</td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td>9mm</td>
<td>15mm</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>7mm</td>
<td>15mm</td>
</tr>
</tbody>
</table>
Histopathological lesions in camel bladder showing changes and from which two bacteria were isolated:

The urinary bladder from which *Bacillus cereus* was isolated showed, muscular degeneration and tissue necrosis, generalized hyperaemia, congestion and Oedema.

Urinary bladder from which *Staph. hyicus* was isolated showed, muscular degeneration and tissue necrosis, generalized hyperaemia and Dialatation of vein

In both bladders there was bacterial accumulation between tissues.
DISCUSSION

There are some human and animal diseases which are difficult to treat. Camel urine may have an important role in this aspect, for example treatment of leukaemia.

The present investigation was carried out to isolate and identify aerobic bacteria from camel urine. Eleven bacteria was isolated from 250 samples of camel urine.

The physical properties included the pH of camel urine which varied from 7.5 to 9.5 in female and 8 to 9 in male. This finding approximately agrees with Wisal (2002) who found that the pH was 8 in male and 8-9 in female but it seems to be lower than the finding of O’haj (1998) who reported 9 in male and 10 in female. It was also lower than the finding of Amer et al. (1996) who reported that the pH of 14 samples of male camels were slightly acidic (6.5) while 6 other samples were alkaline (8.7). All this variation may refer to different nutritional plants consumed by camel in different areas.

The potassium was high and this appear to agree with O’haj (1998) and Wisal (2002) and that might play a role in electrolytes balance in ascitic patients as well as the low sodium content of camel urine.

The camel urine also contained a high level of magnesium, which may play some role in the curing properties. The important end product of nitrogen metabolism was urea which was
concentrated in camel urine and this explain the high osmolarity in it. The average urea level (13.6 g/L) and (13g/L) for male and female respectively appeared to be in agreement with Wisal (2002) and O’haj (1998) and was in disagreement with finding of Amer et al. (1996) who found the value of 1.95g/L. and Read (1925) who found no urea in camel urine.

In this study the value of uric acid was 215.9 mg/L for male and 208.9 mg/L for female. This finding was higher than that mentioned by Wisal (2002) who found 193.5 mg/L in male and 203.1 mg/L in female and lower than that of O’haj (1998) 245.2mg/L and 260.9 mg/L in male and female respectively.

The excretion of uric acid found to be lowest in camel urine in contrast with cattle 401.7 mg/L in male, 341.5 mg/L in female, goat 636.5mg/L in male 73.4mg/L in female and human 365.2 mg/L in male, 427.8 in female O’haj (1993).

Creatinine was 126.7 mg/L in male and 119.5 mg/L in female. This result approximately agreed with O’haj (1998) 108.1 mg/L in male, 113 mg/L in female and Wisal (2002) 119.2 mg/L in male and 116.4 mg/L in female.

Creatinine of camel urine was 14.9 mg/L in male and 19.6 mg/L in female and this agreed with Wisal (2002) (18.5 in male, 20mg/L in female) and O’haj (1998) (17.7 mg/L in male and disagree in female (39.3mg/L).

The total protein was 8.4 g/L in male and 8.9 g/L in female. This finding appear to be lower than that reported by O’haj (1998) who found 9.5 g/L in male and 10.2 g/L in female. In contrast
with two other animals, the high value of total protein was in camel urine; in cattle 5.3 g/L in male and 6.0 g/L in female, in human 1.5 g/L in male, 0.9 g/L in female.

The high concentration of salts and protein in camel urine lead to high osmolarity.

The result of this investigation include identification of these bacteria by conventional bacteriological methods. The isolated bacteria were *S. hyicus*, *S. capitis*, *S. hominis*, *S. haemolyticus*, *Corynebacterium xerosis*, *Corynebacterium striatum*, *Corynebactium pseudodiphtricum*, *Micrococcus sp.*, *Bacillus cereus* and *Mannheimia haemolytica*.

There was difference in a biochemical test when compared to that mentioned by Barrow and Feltham, 1993), for example *S. hyicus* is usually coagulase positive but in the isolate from camel urine it was negative. In Bergys Manual of Systemic Bacteriology (1986) coagulase was produced by some (24-56%) strains of *S. hyicus*.

Isolation of *Mannheimia haemolytica* was significant. This organism usually cause ovine pneumonic pasteurelosis (shipping fever) and in sheep. It is also a causative agent of pneumonia and gangrenous mastitis (Karim, Markey et al., 2002). This organism may cause disease in camel put under stress in Kordofan region where the urine sample was obtained. Sheep and camel reared together and this bacterium might have originated in sheep. If this assumption is true camel urine can constitute a reservoir of this organism and a true hazard.

* Camel urine inhibited the growth of *Staph. aureus* and *E. Coli*, this finding agree with Wisal (2002) and O’haj (1998). Camel urine is also inhibited the growth of *salmonella* and *pseudemonas* with zone of 15 mm for each.
The inhibition of *salmonella* growth may explain the use of camel urine for abdominal disorders. The effect on *Pseudomonas* was important because this bacteria is resistant for so many antibiotics.

After neutralization of camel urine, the zone of inhibition was 14mm, 9mm, 15mm, 15mm in *S. aureus, E. coli, salmonella sp. pseudomonas* instead of 13 mm, 8mm, 9mm, 7mm respectively. This mean that the zones of inhibition were wider than before and this may indicate that the alkalinity of camel urine had an adverse effect of the urine because when alkalinity was removed the camel urine had more inhibitory effect.

In histopathological studies *S. hyicus* and *B. cersus* were observed accumulation of these bacteria between the tissues of inflamed bladder. This finding indicates that under certain conditions the above organisms can assume pathogenic potential. This might be a new finding.

In table (1) the isolates were resistant to Cloxacillin (5µg) and Ampicillin (10µg) and sensitive to Gentamycin (30µg) and Erythromycin (30µg).

*B. cereus* isolated from camel urine was more sensitive to Gentamycin (xxxxxx) in comparison to the same species isolated from sheep lung (xxxx) by Manal Ismail (1998).

**Conclusion**

1- Camel urine contain some Gram – positive and Gram-negative bacteria.

2- Camel urine when neutralized has a better effect.
3- Bacteria isolated from camel urine were sensitive to some antibiotics while others were resistant.
4- Camel urine when taken per os for treatment should be sterilized.
REFERENCES


Kirby (1966). Department of microbiology and medicine, university of Washington, school of medicine, seattle, Washington 98/05


