

Bacterial Isolation and Identification from Feces of Healthy Camel in Mosul Province

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Abstract

Forty fecal samples were collected from healthy camels in Mosul province in period from December 2013 to April 2014. Bacterial culture technique, biochemical tests and API 20 E system were used for bacterial isolation and identification. The result showed that bacteria isolated in rate of 87.5% (35/40), *Escherichia coli* isolated in rate of 15/35(31.4%), followed *Klebsiella pneumonia* 7/35(20%), *Pseudomonas aeruginosa* 6/35(17.1%), *Proteus vulgris* 4/35 (11.4%) and the lowest percentage was for *Staphylococcus aureus* 3/35 (8.5%). Antibacterial profile of bacterial isolates from camel feces according to antibiotic disc diffusion method were applied to each of kanamycin, ampicillin, chloramphenicol, tobramycin, tetracycline, amoxicillin, ciprofloxacin, all the isolates resistance proportion for tetracycline is 100%.

Key words: Bacteria, Camel, feces.

Introduction

Camels is an important multipurpose animals in arid and semi-arid areas of the world, could be infected with different

infectious diseases, since knowledge of diseases that affected camels and how to treated and prevented them as well as general health monitoring remains limited in camels world (1), Camel (*Camelus dromedarius*) is a wonder of creation of almighty and most

famous domesticated animal in Asia , mostly in middle and eastern part (2). To get a clearer picture about the organism under study, an obvious simplified classification is presented to avoid confusion with other species within the Camelidae family. The family of Camelidae comprises two major subfamilies, namely Camelinae (Old World Camelids) and Laminae (New World Camelids). The old world camelids include two domesticated species; the dromedary or one humped camel (*C. dromedarius*) and the two humped camel or bactrian camel (*C. bactrianus*). Both species are referred to as large camelids and distributed into different regions of the world. Arabian camel (*C. dromedarius*) is located mainly in the hot areas of Middle East and Africa whereas *C. bactrianus* inhabit the cold zones of Central Asia and China , The new world camelids comprise four main species located in South America and are commonly known as small camelids. Yet, two species the llama (*Lama glama*) and the alpaca (*Vicugna pacos*) have been domesticated whereas the other two species, namely the guanaco (*L. guanicoe*) and the vicuna (*V. vicugna*) are wild species, A schematic classification and map distribution of members of the camelidae family is shown in (Figure 1) (3). The Bactrian camel is a very hardy animal which can live in deserts or semi-deserts. It can adapt to the harsh environments, such as arid, poor grazing, hot and cold. Camels are a means of conveyance and producers of milk, meat. Research has shown that Bactrian camels are an ideal model for describing desert adaptations because of their ability to tolerate harsh desert ecological conditions(4). Bactrian camels have ability to adapt to low quality diet. It can eat salt-tolerant vegetation such

as *Chenopodiaceae*, *Compositae* and *Leguminosae* plants. they also have capacity to ingest virtually any kind of vegetation including shrubs and trees)(4). Characterization of the Bactrian camel microbiota is therefore important. Recently, the microbiota in camel rumen and faeces have been detected (5). The study aims to isolate and identify bacterial species from fecal samples were collected from healthy camels.

Material and Method

1- Sample Collection:

Forty fecal samples were collected from healthy camels in Mosul province in period from December 2013 to April 2014. The faces sample have been collected in clean sterile tubes and saved at 4°C in a cold box for transportation to the laboratory to run the experiment directly (6 and 7).

2- Isolation and Characterization :

Fecal samples have inoculated in nutrient agar and incubated at 37°C for 24h. Typical bacteria colonies were randomly selected examined microscopically for their morphology and recultured to obtain pure culture. Different types of culture media used in the isolation of these bacteria (8), All samples were subculture in sheep blood agar, eosine methylene blue agar, brain heart agar , mannitol salt agar, macConkey agar and nutrient agar. Macroscopic and microscopic

morphology tests have been performed after incubation in order to classify the genus level, the isolates from the culture plates were identified according to standard microbiological procedures using Gram staining, morphological character of colony,

catalase, coagulase, oxidase and API 20 E system (9), Shown Figure (2).

Antibiotic Sensitivity Test :

All isolates were subjected to the susceptibility testing by standard methods for

the Antibiotic disk (oxid) used kanamycin(5mg), ampicillin(10mg), chloramphenicol (30mg), tobramycin (10mg), tetracycline (10mg), amoxicillin (25mg) ciprofloxacin (5mg) (10). A standard reference procedure has been described by (11).

Statistical analysis : For statistical analysis, a Chi square test (X^2) was performed to assess the independence of the variables, with SPSS Statistics software, version 27. Values less than or equal to 0.05 were considered statistically significant (12).

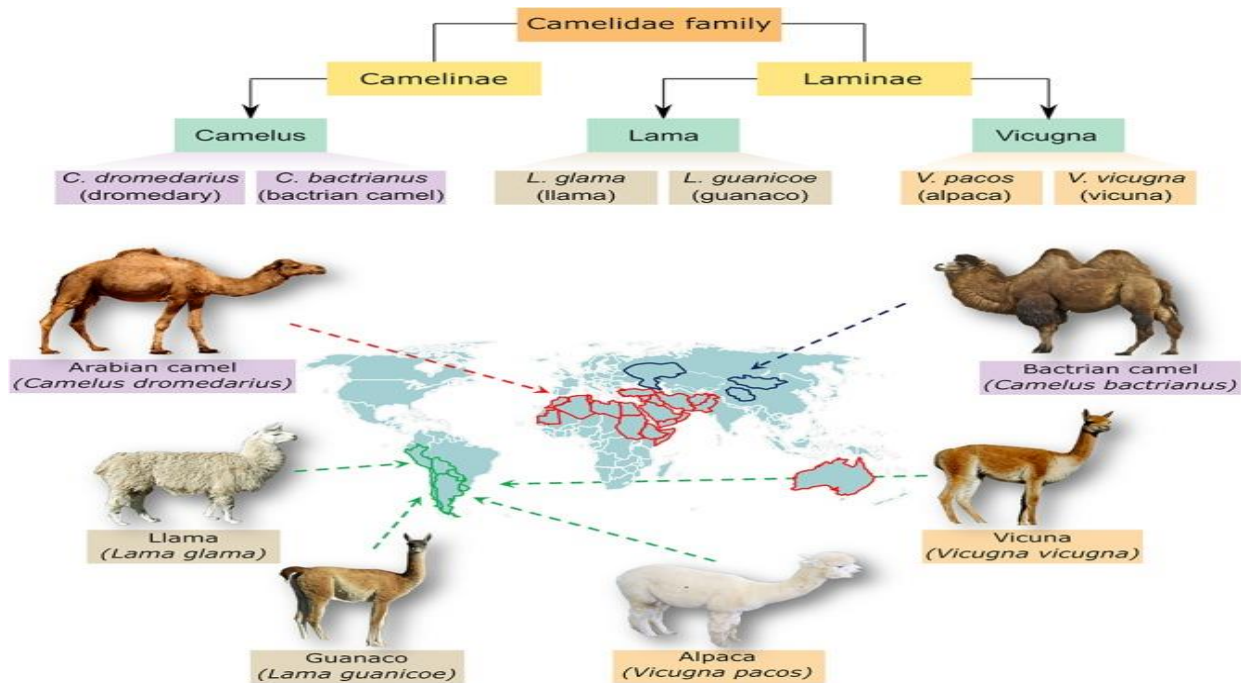


Figure (1) : Shown a schematic classification and map distribution of members of the camelidae family.

Results

1- Isolation and Identification of Bacterial Isolates

Thirty five (87.5%) bacterial isolates out of 40 feces samples were obtained from apparently healthy camel based on cultural, morphological and commercially API 20 E system (Bio Mèrieux, France) were carried out

in various setting phenotyping identification tests for bacteria, the highest rate were belongs *Escherichia coli* 15/35(31.4%), followed *Klebsiella pneumonia* 7/35(20%), *Pseudomonas aeruginosa* 6/35(17.1%), *Proteus vulgris* 4/35 (11.4%) and the lowest percentage was for *Staphylococcus aureus* 3/35 (8.5%), table (1).

Table (1) : Bacterial Isolates From Healthy Camel Feces.

| No | Name of bacteria | NO | % |
|----|-------------------------------|----|--------|
| 1 | <i>Escherichia coli</i> | 15 | 31.4 |
| 2 | <i>Klebsiella pneumonia</i> | 7 | 20 |
| 3 | <i>Pseudomonas aeruginosa</i> | 6 | 17.1 |
| 4 | <i>Proteus vulgris</i> | 4 | 11.4 |
| 6 | <i>Staphylococcus aureus</i> | 3 | 8.5 |
| | Total | 35 | 87.5 |
| | X ² | | 16.07* |
| | P value | | 0.003 |

* Significant difference at P<0.05

2- Antibiotic Susceptibility of Isolated Bacteria

According to antibiotic disks diffusion method the sensitivity test was applied for whole bacterial isolates, all isolates were tested for their sensitivity kanamycin, ampicillin, chloramphenicol, tobramycin,

tetracycline, amoxicillin and ciprofloxacin, table(2), results were interpreted by measure the zone around the discs and compared with the break points of CLSI (clinical laboratory institute 2015) these zone was translated in term of sensitive (S) and resistant (R), shown in Figure (3).

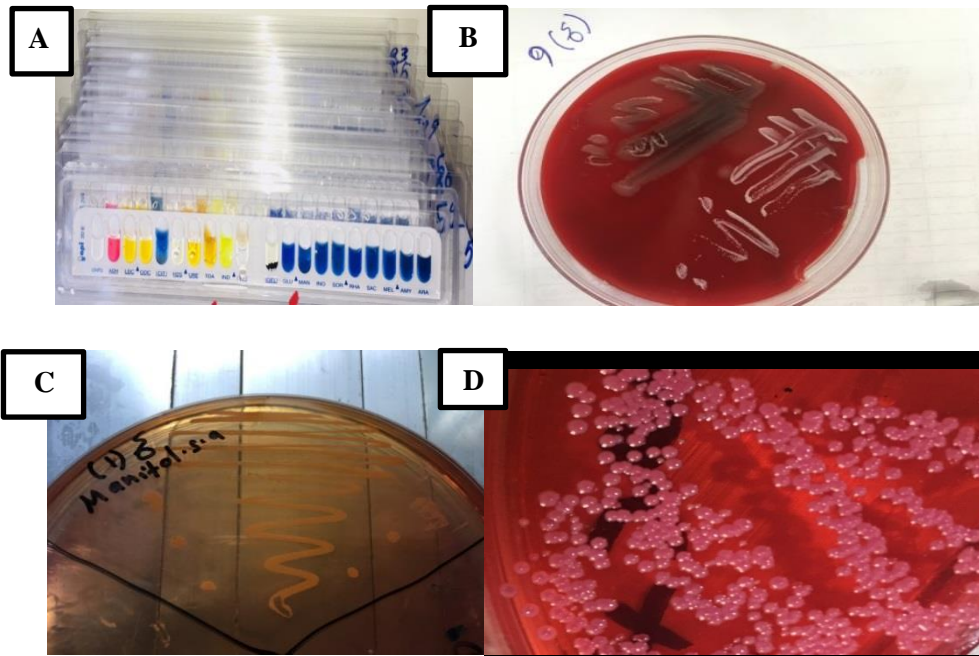


Figure (2): A: API kit *Pseudomonas*, B: *Pseudomonas*, C: *Staphylococcus*, D : *E. coli*

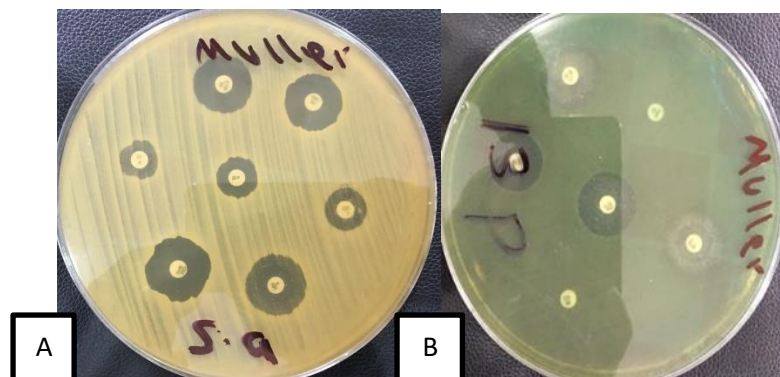


Figure 3: Show Antibiotic susceptibility test A- *Staphylococcus aureus*, B- *Pseudomonas aeruginosa*.

Table(2)- Antibiotic susceptibility of isolated bacteria

| Type of antibiotic | Type of bacteria | | | | | | | | | | | |
|-------------------------|------------------|----------|---------------------|---------|---------------------|---------|------------------|--------|------------------|---------|----------------|---------|
| | <i>E. coli</i> | | <i>K. pneumonia</i> | | <i>P.aeruginosa</i> | | <i>P.vulgris</i> | | <i>S. aureus</i> | | X ² | P value |
| | No. (15) (%) | | No. (7) (%) | | No. (6) (%) | | No. (4) (%) | | No. (3) (%) | | | |
| R | S | R | S | R | S | R | S | R | S | | | |
| Kanamycin(15 mg) | 8(53.3) | 7(46.6) | 2(28.5) | 5(71.4) | 3(50) | 3(50) | 1(25) | 3(75) | 1(33.3) | 2(66.6) | 2.01 | 0.73 |
| Ampicillin (10 mg) | 5(33.3) | 10(66.6) | 3(42.8) | 4(57.1) | 1(16.6) | 5(83.3) | 0(0) | 4(100) | 0(0) | 3(100) | 4.21 | 0.37 |
| Chloramphenicol (30 mg) | 3(20) | 12(80) | 6(85.7) | 1(14.2) | 2(33.3) | 4(66.6) | 1(25) | 3(75) | 3(100) | 0(0) | 13.19* | 0.01 |
| Tobramycin(10 mg) | 5(33.3) | 10(66.6) | 7(100) | 0(0) | 4(66.6) | 2(33.3) | 2(50) | 2(50) | 1(33.3) | 2(66.6) | 9.47 | 0.05 |
| Tetracycline (10mg) | 15(100) | 0(0) | 7(100) | 0(0) | 6(100) | 0(0) | 4(100) | 0(0) | 3(100) | 0(0) | 0 | 1 |
| Amoxicillin (25 mg) | 7(46.6) | 8(53.3) | 3(42.8) | 4(57.1) | 1(16.6) | 5(83.3) | 4(100) | 0(0) | 1(33.3) | 2(66.6) | 7.004 | 0.136 |
| Ciprofloxacin (5 mg) | 5(33.3) | 10(66.6) | 6(85.7) | 1(14.2) | 4(66.6) | 2(33.3) | 0(0) | 4(100) | 1(33.3) | 2(66.6) | 10.05* | 0.04 |
| X ² | 24.94* | | 18.06* | | 13.33* | | 17.79* | | 10.3 | | | |
| P value | 0 | | 0.006 | | 0.038 | | 0.007 | | 0.112 | | | |

() concentration of antibiotic of mg R=resistance, S=sensitive. * Significant difference at P<0.05

Discussion

Complex GIT microbial communities are believed to provide benefits to their host, and are receiving increasing attention. However, the characteristics and distribution of the microbial community in the Bactrian camel GIT remains unclear.

In the present study, the percentage result of the isolated *Escherichia coli* (31.4%) are compatible than these obtained in *Al-Qadisiyah/Iraq* by Mohammed H. Abdulkadhim, 2011 who isolated *Escherichia coli* (39.6%) isolate & identify some of enteric pathogens in camel fecal samples by using analytical profile index. But it is not concomitant with these results obtained by other studies done in Bangladesh by Dutta (2010), who isolated *Escherichia coli* (46%) and bacillus (18%), Iraq by Hamzal (2013) nill isolated bacteria (0%). The geographical variation may be considered one of the reasons of discrepancy in distribution of species, or due to the number of included samples or may due to the differences in the methods used for diagnosis.

The present study is disagree with this done by Jing (2018), in China we describe the bacterial communities from eight different GIT segments (rumen, reticulum, abomasum, duodenum, ileum, jejunum, caecum, colon) and faeces determined from 11 Bactrian

camels using 16S rRNA gene amplicon sequencing. Twenty-seven bacterial phyla were found in the GIT, with Firmicutes, Verrucomicrobia and Bacteroidetes predominating. However, there were significant differences in microbial community composition between segments of the GIT. In particular, a greater proportion of *Akkermansia* and Unclassified Ruminococcaceae were found in the large intestine and faecal samples, while more Unclassified Clostridiales and Unclassified Bacteroidales were present in the in forestomach and small intestine by employing molecular technique in bacteria identification PCR and sequencing and this discrepancy in the results may comes from that in the current study the percentage was higher because the technique PCR were applied on isolates.

The antibiotic Susceptibility Testing:

Antibiotic profile for isolates from camel according to antibiotic disc diffusion method. The sensitivity test was applied for all 35 isolates of bacteria *Escherichia coli* (15), *Klebsiella pneumonia* (7), *Pseudomonas aeruginosa* (6), *Proteus vulgris* (4) and *Staphylococcus aureus* (3). These isolates were tested for their sensitivity to kanamycin, ampicillin, chloramphenicol , tobramycin, tetracycline, amoxicillin and ciprofloxacin.

In the current results of some isolates sensitivity to for ciprofloxacin 100% and which agree with a study (2) were highly sensitive 100% to Ciprofloxacin. In (Iraq), (13) he proved all isolates *E.coli* resistance against tetracycline and most isolates intermediate resistance against chloramphenicol were in agreement with result this study.

Conclusion

The present study shows that six different types of bacteria are present in the fecal sample apparently healthy camel of Mosul province/Iraq. The study suggested that, these organisms may cause clinical diseases in different body system of camel. As such, further epidemiological and pathological study is essential.

Conflict of Interest: The authors report no conflicts of interest.

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العزل البكتيري والتعرف عليها من براز الإبل السليمة في محافظة الموصل

هيفاء حسين ، سيماء فيصل حسب الله

جمعت أربعون عينة براز من الإبل السليمة في محافظة الموصل في الفترة من كانون الأول 2013 إلى نيسان 2014. تم استخدام تقنية الاستزراع البكتيري والاختبارات البيوكيميائية ونظام API 20 E لعزل وتعريف البكتيريا. أظهرت النتائج عزل البكتيريا بنسبة 87.5% (40/35). عزل *Escherichia coli* بمعدل 35/15 (31.4%) ، تليها *Klebsiella pneumonia* 35/7 (20%) ، *Pseudomonas aeruginosa* 35/6 (17.1%) ، *Proteus vulgaris* 35/4 (11.4%) وأقل نسبة كانت *Staphylococcus aureus* 35/3 (8.5%).

تم تطبيق فحص الحساسية المضادة للعزلات البكتيرية من براز الإبل حسب طريقة انتشار قرص المضادات الحيوية على كل من كاناميسين ، أمبيسلين ، كلورامفينيكول ، توبراميسين ، تتراسيكلين ، أموكسيسيلين ، سيبروفلوكساسين ، جميع العزلات المقاومة للتتراسيكلين هي 100%.