Journal of Genetic and Environmental Resources Conservation, 2016, 4(1):46-50.
www.jgerc.com

Prevalence of *Escherichia coli* O157:H7 in camels fecal samples

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Abstract

This study was conducted to isolate *Escherichia coli* O157:H7 from camels, fecal samples were collected from 100 apparently healthy camels from Badia Al-Najaf province in middle of Iraq. The isolation depended upon the cultural, biochemical and serological characteristics of isolates, the morphological properties were diagnosed and identified by culturing on eosin methylene blue agar and sub-culturing on chrom agar, incubated aerobically at 37°C for 24hrs then confirmed by using gram staining and different biochemical tests, serotyping was done by using Latex agglutination test. The results showed that *E. coli* O157:H7 were isolated in 19 out of 100(19%) camels fecal samples. The percentage of isolation nearly between males and females, it appeared (12 and 7%) respectively. there were no significant differences($P<0.05$) in the gender between females and males, also the percentage of isolation according to age was nearly and there was no significant differences ($P<0.05$). The results also revealed resistance of isolated *E. coli* 157:7 to the all antibiotics used in this study except to trimethoprim. This is the first study were isolated of *E. coli* 157:7 from camels suggesting that camels reservoirs to *E. coli* 157:7 and may be play a role in infection and transmission of *E. coli* 157:7 in Iraq.

Keywords: *E. coli* O157:H7, Camel, Feces, Iraq.

Introduction

Escherichia coli O157:H7 is an important food and waterborne zoonotic pathogen because of its widespread diffusion, peculiar tolerance to some physical and chemical treatments, severity of illness and low dose infectiveness (Beneduce *et al.,* 2003), *E. coli* O157:H7 is considered one of the most important food-borne pathogens among shiga toxin producing *E. coli* (STEC) strains. It causes diarrhea that may result in life-threatening conditions ranging from hemorrhagic colitis (HC) to hemolytic-uremic syndrome(HUS) (Mead *et al*., 1999; Meng *et al*., 2001). Gastrointestinal tracts of ruminants especially cattle and sheep have been shown to act as a reservoir of *E. coli* O157:H7 (Kudva *et al*., 1996; Kudva *et al*., 1997; Shere *et al*., 1998).

Epidemiological investigations have clearly associated *E. coli* O157:H7 human infections to the consumption of contaminated raw or undercooked ground beef and products with feces during slaughterhouse processing (Beutin *et al*., 1993; Paiba, *et al*., 2002). *E. coli* 157:7 was isolated from minced beef of camels (Hajian *et al.*, 2011). From camels hid Bosileva *et al*. (2014), Tanzifi, *et al.,*(2015) also isolated *E. coli*157:7/NM from camel milk in Iran.

Antimicrobial resistance of food borne bacteria should not necessarily be considered distinct from that in isolates from humans, food animals, or other niches. When food animals, as carriers of asymptomatic *E. coli* O157:H7, are exposed to antimicrobial agents, they may become the reservoir of this antimicrobial-resistant bacteria. So it becomes important to determine whether the bacteria develop resistance to antimicrobials during food animal production. It is controversial to use antibiotic treatment in humans to prevent HUS due to lysis of the bacteria and increased releasing of the expression of the shiga toxins in the intestinal tract (Takahashi *et al*., 1997; Wong *et al*., 2000). However, it is has been reported that using some antimicrobials in the early stage of infection may be protective against HUS progression (Fukushima *et al*., 1999; Ikeda *et al.,* 1999).

Materials and Methods

Isolation of *E. coli* 157:H7: One hundred fecal samples were collected from apparently healthy camels. *E. coli* O157: H7 was isolated by using conventional methods, which were based on culturing, serological, and biochemical properties of *E. coli* O157: H7 according to Chow *et al.* (2006). All samples were homogenized with normal saline 0.85% and cultured on Eosin Methylene blue agar and incubated aerobically at 37°C for 24hrs. A metallic sheen colonies were picked by loop and cultured on selective chrom agar O157:H7 and

incubated at 37°C for 24hrs, *E. coli* O157:H7 development mauve color colonies.

Latex agglutination test for *E. coli* O157:H7: This test was used for serotyping of *E. coli* O157:H7 by using commercial kit (Wellcolex *E. coli* O157:H7, Remel) to detect both the somatic antigen O157 and the flagellar antigen H7, This test was done according to the manufacturer company.

Antimicrobial sensitive tests: Series of each sample of positive E. coli is selected for sensitivity test to bacteria, the test was conducted by Kirby–Bauer disc diffusion method using the Kirby-Bauer method, performance standards for antimicrobial disk susceptibility tests were used for this experiment: Doxycycline 30 (Do30), Cephalexin 30 (CL30), Erythromycin (E15), Clarithromycin 15 (CLR15), Ceftriaxone 30 (CRO30), Ampicillin 10 (AM10) and Cloxacillin (CX1), Trimethoprim 5 (TMP), Rifampin 5 (RA5), and Carbencillin 100 (py100) .

Results and Discussion

Healthy ruminants such as cattle, sheep and goats are natural reservoirs of EHEC especially *E. coli* O157:H7 in their feces (Beutin *et al*., 1997; Blanco *et al*., 2003; Blanco *et al*., 2004; Rey *et al*., 2003), also other domestic animals such as pigs, cats and dogs, can also harbor these bacteria (Beutin *et al*., 1993; Beutin *et al*., 1995).

The results of bacteriological culturing revealed a green metallic sheen colonies on eosin methylene blue agar, moreover, this colonies showed mauve color on ChromagarO157:H7 agar (Figure 1). The biochemical tests of the isolated bacteria gave different results, the isolates gave negative results for simmon citrate and urease tests, while indol, MR and motility tests gave positive results. Triple sugar iron test showed yellow/yellow with gas production this features indicated that the bacterial colonies belonged to *E. coli* O157:H7. Also the isolates were confirm by serotyping test which give positive agglutination reaction (Figure 2).

The results revealed isolate of 19 out of 100 samples (19%), The percentage of isolation in the current study was nearly between males and females, it appeared (12 and 7%) respectively. there were no significant differences($P<0.05$) in the gender between females and males (Table 1). The percentage of isolation according to age also was nearly and there was no significant differences ($P<0.05$) (Table 2).

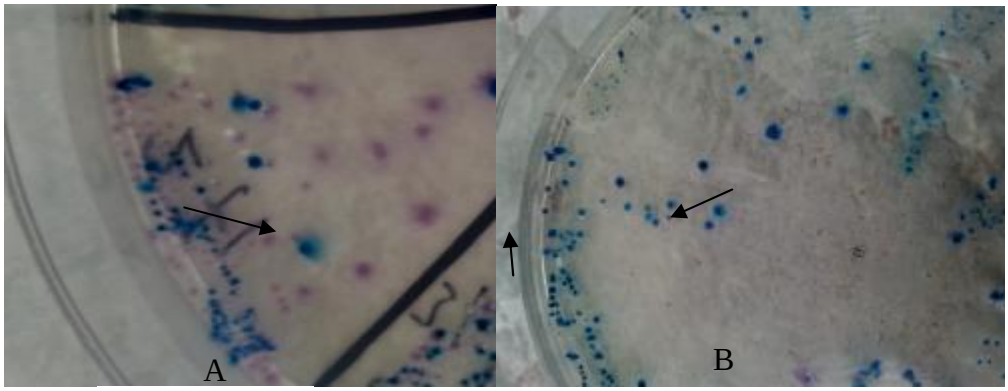

Figure (1): A and B, *E. coli* O157:H7 colonies on chrom agar (mauve color).

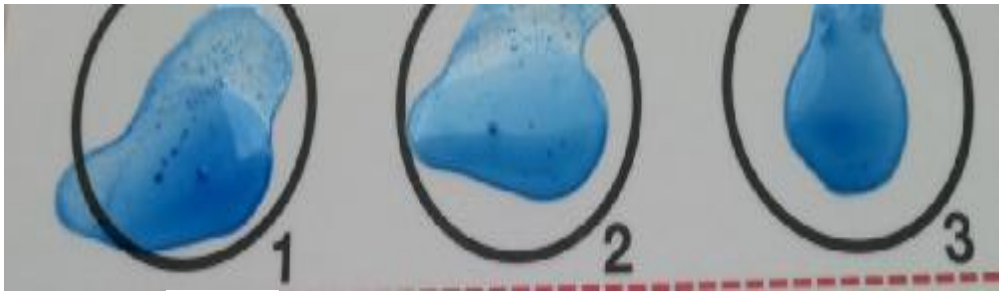

Figure (2): Agglutination reaction of isolated *E. coli* O157: H7

Table(1): Number and percentage of *E. coli* O157:H7 isolates according to the gender.

Gender	No. of samples	No. of isolates	Percentage (%)
Male	67	12	12
Female	33	7	7
Sum	100	19	19

Table(2): Number and percentage of *E. coli* O157:H7 isolates according to the age.

Age(years)	No. of samples	No. of isolates	Percentage (%)
1-2	42	7	16.7
3-5	24	6	25
6-8	25	5	20
9-12	9	1	11.11
Sum	100	19	19

The prevalence of *E. coli* O157 from camels has not been widely studied, Moore *et al*. (2002) failed to identify *E. coli* in feces of racing camel calves in the United Arab Emirates, El-Sayed *et al*. (2008) also failed to detect any positive *E. coli* O157 among 400 camel fecal samples collected from Egypt, Somalia, Djibouti, Kenya, and Sudan, and in Iran Rahimi *et al*. (2012). In contrast El-Hewairy *et al.* (2009) recovered serovars O157 from diarrheic contact camel calves were percentages of 17.9 but not isolated from apparently healthy in-contact camel calves, suggesting that the reason by shedding of *E. coli* in feces with low number which is not sufficient for isolation and/or the bacteria are dead as a result of antibiotic administration.

Sami and Adeli (2013) isolate only one (0.66%) typical (*E. coli* O157:H7) while 2 isolates (1.33%) were atypical O157, the prevalence of *E. coli* O157:H7 in camel feces was lower than that those reported in cattle and suggested that the low presence of verotoxigenic *E. coli* O157:H7 infection in camels, might be attributed to factors related to the STEC, environment and or to the camels themselves and Camel fecal samples may have been infected by non-verotoxigenic *E. coli* O157 serotype. Bosileva *et al*. (2014) isolate 2.4% from camel feces in the same percentage in sheep fecal samples in Riyadh.

The results also revealed resistance of isolated *E. coli* 157:7 to the all antibiotics used in this study except to trimethoprim. Antibiotics have saved millions existences of people and using of them have helped to improve the health of animals and human. Using antibiotics in food-producing animals affected making healthier animals, reducing diseases and mortality (Oliver *et al*., 2011). Also producing more quantities and qualities food stuff and reducing prices of food for human are other advantages of antibiotics. On the other hand, there are the main concerns utilizing antibiotics for public health and producing healthy food in food-producing animals. Over the past two decades, the development of antimicrobial resistance applied in agriculture which can be affected in human's treatments has become the main concern in public health (Paton and Paton.1998). many studies have reported that there has been a rise in the antimicrobial resistance patterns of *E. coli* O157:H7 (Galland *et al*., 2001; Schroeder *et al*., 2002; Schroeder *et al*., 2004; Goncuoglu *et al*., 2010).

Tanzifi *et al*. (2015) indicated the resistance of *E. coli* 157:7/NM which isolated from camel milk to most antibiotics such (ampicillin, erythromycin, gentamycin, nalidixic acid, doxycyclin, streptomycin, kanamycin, tetracycline, chloramphenicol and amoxicillin and sensitiveness to cefuroxime. Goncuoglu *et al.* (2010) found susceptible of *E. coli* 157:7 to trimethoprim isolated from cattle and sheep feces.

This is the first report of antibiotic resistance patterns of *E. coli* O157:H7 strains isolated from camels in Iraq. It is concluded that the overall prevalence of antibiotic resistance of *E. coli* O157:H7 isolates recovered from camels tested in this study is very low. However, longitudinal studies should be performed to monitor and detect any changing in antibiotic resistance profiles of this bacterium in the future.

Conclusions

This is the first study isolated of *E. Coli* 157:7 from camels suggesting that camels may be play a role in infection and transmission of *E. coli* 157:7 in Iraq. resistance of isolated *E. coli* 157:7 to the antibiotics make the camels reservoirs to *E. coli* 157:7.

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