Isolation and Identification of pathogenic *E. coli* Serotype O157:H7 from the common sandpiper (*Actitis hypoleucos*) in Qurna city

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SUMMARY

The study was conducted to isolation and identification of pathogenic *E coli* from the common sandpiper (***actitis hypoleucos)*** birds from north of Basra province (Qurna city), and determine antibiotic susceptibility of *Escherichia coli* isolates from dropping samples of such birds. Through bacterial media, serological and chemical tests show that eight bacterial isolates were positive (6.6%) as *E. coli* O157 H7 from 120 dropping samples and 80 bacterial isolates (66.6%) were belong to other pathogenic *E. coli* serotypes while the rest of the samples (32) were belong to non pathogenic *E. coli* serotypes. Isolation and identification of *E. coli* were done by using enrichment media, selective media, and biochemical tests. Antimicrobial susceptibility testing by the disc diffusion method was conducted for Ampicillin, gentamicin, erythromycin, tetracycline, cephotaxime, cephalothin, and ciprofloxacin. In general, *E. coli* isolates from drooping samples showed resistance to the most of mentioned antibiotics in different rates.

Introduction

Wild birds carry a diversity of micro-organisms that are pathogenic to humans, may be transmitted over long distances during migrations, and are potentially transmissible to people who handle and ring birds. High-profile diseases that are associated with carriage by birds include avian influenza, West Nile fever, and Lyme disease. Also potentially important is the existence of an avian reservoir of bacteria that are enteric human pathogens; for example species of *Campylobacter* and *Salmonella*, and toxin-producing strains of *Escherichia coli*. Wild birds have been implicated in the transfer of these enteric pathogens to human. The subject of wild birds as potential reservoirs of pathogen that may be transmitted to humans in the context of ringing and migration is reviewed and it is recommended that appropriate precautions to minimize risk should be taken during and subsequent to the handling of wild birds. Wild birds are usually regarded as visible indicators of diverse and healthy environments. However, from a public health perspective, this positive view is not always valid (Jones, 2005). In some contexts wild birds are responsible for faecal pollution, for example waterfowl at amenity ponds (Abulreesh *et al*, 2007; Abulreesh, 2005), and they can carry a wide range of viral, bacterial, fungal and protozoan zoonotic agents (pathogens that may be transmitted to humans), either being themselves diseased or being seemingly healthy carriers, or the hosts of infected vectors (Hubálek, 2004). Although *Escherichia coli* are part of the normal flora of the intestinal tract of vertebrates, nevertheless, virulent and sometimes lethal toxin-producing pathogenic strains do exist (Hunter, 2003). Studies that have addressed the incidence of such

strains in the intestinal tract of wild birds suggest that there is an avian reservoir (Hubálek 2004). The vero cytotoxin-producing strain *E. coli* O157, that causes enterohaemorrhagic infections in humans (Hunter, 2003), and other pathogenic serogroups have been recovered from wild birds (Wallace *et al;* 1997, Kullas *et a;l* 2002, Wani *et al;* 2004, Sonntag *et al;* 2005, Ejidokun *et al;* 2006, Foster *et al;* 2006) .

Materials and methods

Collection of samples:

Dropping samples were collected from wild birds with diarrhea and from apparently normal birds from north of Basra city from November 2010 to April 2011 .Dropping samples were collected directely from cloaca by sterile stick and transported to lab transportation media (tryptic soy broth). According to (Sanderson *et al*; 1995) they incubated at 37° for 24 hour then cultured on Sorbitol MaCconkey agar and Eosin-Methyline blue, the plates were incubated at 37 for 24 hour. The Biochemical tests were done for bacterial identification.

Identification of *E coli*

All samples were taken by loop and inculated on Sorbitol MacConkey and Eosin Mythline blue and the plates were incubated at 37° for 24 hour (Beutin *et al*; 1993).

Gram staining

The bacterial cultures were stained with Gram stain and examined by the light microscope.

Biochemical test

The biochemical tests were included indol, mthyle red, vogus-proskaur and simon's citrate (IMVIC) Triple sugar iron (TSI) and cellobiose test.

Serological tests

E. coli latex agglutination test:

This test was performed to identify *E. coli* O157:H7 by commercial kit (wellcolex *E. coli* O157:H7 Remel) to determine somatic O157 and flagellar H7 antigens.

Antibiotic susceptibility test

This test was performed by disc diffusion method to identify the resistant isolates to seven antibiotics. The bacterial colonies were taken from every bacterial isolate by loop on Mullar-Hinton agar and then the antibiotic discs was putted on the plates, this plates was incubated at 37° For 24 hour and after that inhibition zone was measured to determine if the bacterial isolates are sensitive or resistant to antibiotics (.Bauer *et al*., 1966).

Results

Eight bacterial isolates (6.6%) were positive as *E. coli* O157: H7 from 120 dropping samples, while 80 bacterial isolates (66.6%) were belong to other pathogenic *E. coli* serotypes (table 1).

TOTAL SAMPLES	E. COLI O157:H7 POSITIVE SAMPLES	%	OTHER PATHOGENIC E.COLI SEROTYPES	%
120	8	6.6	80	66.6

Cultural characteristic

Colonies of *E coli* on Sorbitol MacConkey agar were small; circle and colorless while on E.M.B give colonies with metallic sheen appearance.

Biochemical test

All isolates showed positive IMVIC pattern to *E. coli* (++--) and positive results to TSI and cellobios.

Serological tests

Eight bacterial isolates were positive to latex agglutination test for O157 somatic antigen H7 flagellar antigen.

Antibiotic susceptibility test

This test was performed on bacterial isolates of E coli which shown positive results to E. coli O157:H7 latex agglutination test (table 2).

ANTIBIOTIC	SENSITIVE %	INTERMEDIATE %	RESISTANT %
Ampicillin	0	0	100
Erythromycin	25.2	15	59.8
Gentamycin	87	13	0
Tetracycline	16.2	16.8	69.6
Ciprofloxacin	75	13.2	11.8
Cephotaxime	62	18.2	19.8
Cephalothin	0	0	100

Discussion

The obtained results show eight samples (6.6%) were *E coli* O157:H7.this rate is higher than that obtained from rock pigeons in Saudi Arabia (abulreesh, 2011), while another study in Tokyo showed that from 447 samples taken from different wild birds 113 isolates (25%) were pathogenic *E. coli* strains, but none of them were O157:H7 (kobaysashi, 2009). In other hand, 80 samples from 120 samples (66.6%) belong to divers of pathogenic *E coli* strains. This rate is similar to that of obtained from pigeons 66.9% in Germany (Grossmann *et al*; 2005) while in other study isolation rate from gulls was 40% in Finland kobayashi *et al*; 2002, A study in Italy showed low isolation rate 10.8 from pigeons (Morabito *et al*; 2001). These differences in isolation rates are may be related to the differences in the environmental conditions where each study conducted and differences in birds' species susceptibility to *E. coli* to form a potential host. Antibiotics susceptibility test showed that all isolates were highly resistant to ampicillin (100%), cephalothin (100%) and tetracycline (69.6%). In other hand isolates were sensetive to gentamycin (87%) and ciprofloxacin (75%). Similar result obtained by Galland *et al*; 2001 and it was 98% and 100% for gentamycin and ciprofloxacin. Guenther *et al*; 2010 also show pattern of resistance to ampicillin (60%), cephalothin (47%) and tetracycline (47%) and sensitivity to gentamycin (86.6%).The data above suggests that the common sandpiper seems to represent a carrier of multiresistant *E. coli* and participates in the transmission of antimicrobial resistant *E. coli* in the environment especially rural areas where it is nesting and may represents health hazard.

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عزل وتشخيص جرثومة الأشيريشيا القولونية الممرضة النمط المصلي O157:H7 من طيور الطيطوى (*actitis hypoleucos*) في مدينة القرنة

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الخلاصة

في هذه الدراسة تم عزل وتشخيص جرثومة الأشيريشيا القولونية الممرضة من طيور ***actitis hypoleucos*** المعروفة محلياً ب (الطيطوى) في شمال محافظة البصرة (مدينة القرنة). وتم أختبار الحساسية الدوائية لعزلات الأشيريشيا القولونية المأخوذة من براز هذه الطيور. من خلال الأوساط البكتيرية والأختبارات المصلية والكيميائية أظهرت أن 8 عينات (6.6%) من 120 عينة أعطت نتيجة موجبة لجرثومة الأشيريشيا القولونية العترة O157:H7 بينما 80 عينة (66.6%) كانت تنتمي لعتر ممرضة أخرى من الأشيريشيا القولونية في حين كانت العينات المتبقية وهي 32 عينة (26,6%) منتمية لعتر غير ممرضة. تم أستخدام الأوساط الزرعية المعززة والأختيارية والأختيارات الكيميائية في عزل وتشخيص الأشيريشيا القولونية. تم أجراء أختبار الحساسية الدوائية للمضادات الحيوية: الأمبسيلين , الجنتامايسين , الأيريثرومايسين , التتراسايكلين , السيفوتاكسيم , السيفالوثين و السايبروفلوكساسين. أظهرت العزلات مقاومة لمعظم المضادات الحيوية المستخدمة وبنسب متفاوتة.