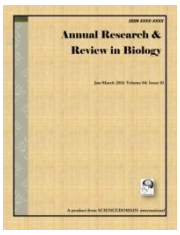

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Faecal Shedding of Antibiotic Resistant *Escherichia coli* Serogroups in Pigeons with Special Reference to *E. coli* O157

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Author's contribution

This Whole work was carried out by author HHA.

Original Research Article

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ABSTRACT

Aims: The aim of this work was to investigate the carriage and antimicrobial susceptibility of *E. coli* serogroups and the prevalence of toxigenic *E. coli* O157 by rock pigeon in western Saudi Arabia.
Place and Duration of Study: A total of 600 faecal droppings of rock pigeons were collected from February 2012 to January 2013, from parks, playgrounds, houses roof tops and yards in Makkah city, samples were collected fortnightly.
Methodology: *E. coli* was detected by spread plate technique on sorbitol MacConkey agar. Confirmation of *E. coli* isolates was carried out by biochemical tests and PCR. Determination of *E. coli* serogroups was performed by latex agglutination kits. Disk diffusion method was used for antimicrobial susceptibility testing.
Results: The results showed low incidence (2.83%, n = 600) of toxigenic *E. coli* O157; and complete absence of O26; O91; O103; O111 and O145 serogroups in pigeon faecal droppings. Multidrug resistance was observed in O157 and non-O157 *E. coli* isolates from pigeons.
Conclusion: Free-living pigeons in western Saudi Arabia may not play an important role in the epidemiology of shiga toxin-producing *E. coli*. However, they could, in part, have an important role in the environmental contamination and distribution of multidrug-resistant *E. coli*.

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1. INTRODUCTION

Free living wild birds are a major reservoir of pathogenic zoonotic agents, which are potentially transmissible to humans through either, the handling of these birds, or contaminated food and water [1]. Large numbers of free living rock pigeons (*Columba livia*) are found in major cities worldwide and frequently live in close proximity to humans. The presence of these large flocks of pigeons may pose public health threats since they carry viral, bacterial and fungal zoonotic agents. Regulation programs to control and assess pigeon hazards had started in some cities in Europe [2].

The carriage of enteric bacterial pathogens by healthy and/or infected free living pigeons that inhibit urban areas is well documented. Pathogenic *Escherichia coli* strains, particularly *E. coli* O157 (known as Shiga toxin-producing *E. coli*, or enterohaemorrhagic *E. coli* [EHEC]), were found in faecal droppings and/or cloacal swabs of pigeons that live in urban and rural areas around the world [2,3,4,5], in addition, other potentially pathogenic serogroups may also present in the faeces of wild birds including pigeons [6,7]. Pigeons can shed pathogenic *E. coli* into the environment when they are ill or without any symptoms. Thus, may play a role in the dissemination of these pathogens in the environment.

Makkah city, western Saudi Arabia, is inhabitant by abundant flocks of rock pigeon and often found in public parks, rooftops of houses, near drinking water reservoirs, and sometime near dining places and food outlets. In an earlier study, the incidence of antibiotic resistant *E. coli* O157 in pigeon faeces in Makkah city was found to be low [8], however it was not determined if those strains were toxigenic or not, and the incidence of other serogroups was not investigated. Thus the aim of this work was to investigate the carriage and antimicrobial susceptibility of *E. coli* serogroups and the prevalence of toxigenic *E. coli* O157 by rock pigeon in Makkah city, western Saudi Arabia.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 600 fresh (still moist) faecal droppings of rock pigeon were collected from parks, playgrounds, houses roof tops and yards in Makkah city in a period of twelve months. Samples were collected fortnightly from February 2012 to January 2013. Faecal samples were collected using sterile forceps and each sample was transferred into sterile 30-ml universal bottle. Samples were packed in ice during transportation and microbiological processing was begun within six hours on the same sampling day.

2.2 Detection of *E. coli* in Pigeon Faecal Droppings

Samples were screened for *E. coli* O157 and other serogroups by selective plating as described by Nye et al. [9]. Briefly, subsample of fresh faeces (0.5 g) was emulsified in 2.5 ml of Maximal Recovery Diluent (Oxoid, Basingstoke, UK). A 3.0-ml pastette was used to inoculate 45 µl of faecal suspension on to Sorbitol MacConkey agar (SMAC) by spread plate technique for obtaining single colonies of *E. coli* O157 and other serogroups [10]. All plates were incubated aerobically at 37°C for 24-48 h. The *E. coli* ATCC 8739 (Oxoid) was used a positive control throughout the study.

2.3 Confirmation of *E. coli* isolates

All non-sorbitol fermenting colonies on sorbitol MacConkey agar (colorless colonies) were considered as presumptive *E. coli* O157 isolates while all lactose fermenting colonies with intense pink to red color on the same medium were considered as presumptive non-O157 *E. coli*. Biochemical confirmation of all presumptive O157 and randomly selected non-O157 *E. coli* isolates was done by indole, methyl red, Voges-Proskauer, and Simmons citrate tests to determine their identity as *E. coli* [11]. All presumptive O157 and randomly selected non-O157 *E. coli* isolates were further confirmed by Fluorocult LMX broth (Merck, Germany) that simultaneously detects *E. coli* β-D-galactosidase under visible light and β-D-glucuronidase under UV transillumination (366 nm) as described by Ossmer [12]. *E. coli* O157 can be distinguished from other *E. coli* serogroups by being β-D-glucuronidase negative [10], thus, no fluorescence will be observed under UV light. Amplification of a fragment of the *E. coli* 16S rRNA by PCR using primers RW01 ('5-AACTGGAGGAAGGTGGGGAT-3') and DG74 ('5-AGGAGGTGATCCAAGCA-3') (Invitrogen, Paisley, UK) [13], was also performed as a method of confirmation of all presumptive *E. coli* isolates that expected to produce a 371 bp PCR product (Fig. 1). PCR protocol was carried out as described by Abulreesh [14].

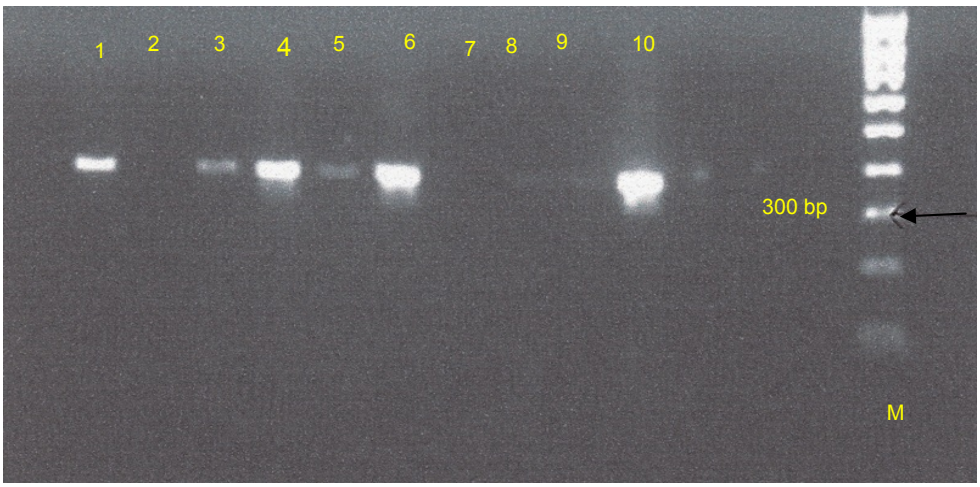

Fig. 1. Results of PCR confirmation of *E. coli* isolates

PCR amplification of 371 bp fragment of the E. coli 16S rRNA using primers RW01 and DG74. Lane M: DNA ladder (Promega, Madison, WI, USA). Lane 1: E. coli ATCC8739 (positive control). Lanes: 3, 4,5,6 and 10 positive PCR amplification of presumptive E. coli isolated from pigeons faeces. Lanes" 2, 7, 8 and 9 negative PCR amplification of presumptive E. coli recovered from pigeon faces. All presumptive E. coli isolates that were confirmed by PCR were further examined by serogroup determination, toxin detection and antimicrobial susceptibility testing

2.4 Determination of *E. coli* Serogroups

Escherichia coli O157 serogroup was determined by latex agglutination using the Dryspot *E. coli* O157 test kit (Oxoid) that detects the *E. coli* O157 antigens [15]. Other *E. coli* serogroups were determined by latex agglutination using the *E. coli* Dryspot Seroscreen test kit (Oxoid) that detects six non-O157 serotype; these include *E. coli* O26, O91, O103, O111,

and O145 serogroups. The procedure was carried out according to manufacturers' instructions.

2.5 Detection of Verocytotoxin

All confirmed O157 and non-O157 *E. coli* isolates were tested for the production of verocytotoxins VT1 and VT2 by the reverse passive latex agglutination test kit (Oxoid). The Oxoid VETC-RPLA test kit detects the toxins themselves, providing clear identification of the VT1 and VT2 [16]. This kit can be used with isolates originate from food and faecal samples and can be tested on O157- and non-O157 *E. coli* strains. The procedure was carried out according to manufacturers' instructions.

2.6 Antimicrobial Susceptibility

Antimicrobial susceptibility tests were carried out using the disk diffusion method. Müeller-Hinton agar plates (Oxoid) were incubated aerobically at 37 °C for 18-22 hours. The interpretation of values of disk diffusion technique was performed according to the guidelines of the Clinical and Laboratory Standard Institute [17]. Eight commercially antimicrobial sensitivity disks (BBL, Cockeysville, USA) were used: Penicillin G (10 µg ml^{-1}), Streptomycin (10 µg ml^{-1}), Cephalothin (30 µg ml^{-1}), Cefazolin (30 µg ml^{-1}), Gentamicin (10 µg ml^{-1}), Ampicillin (10 µg ml^{-1}), Erythromycin (15 µg ml^{-1}), and Tetracycline (30 µg ml^{-1}).

3. RESULTS AND DISCUSSION

A total of 600 fresh faecal droppings of rock pigeon were collected between February 2012 and January 2013. Of these 600 samples only seventeen (2.83%) were positive for *E. coli* O157, and none (0.0%) of the samples were positive for other serogroups (Table 1). *E. coli* O157 was recovered throughout the year, with no marked differences between seasons.

Of the 600 faecal droppings examined in this study, very low (2.83%) incidence of *E. coli* O157 was observed (Table 1). Although shiga toxin-producing *E. coli* were frequently recovered from pigeon faeces [3,4,5,16], the prevalence of species belonging to serogroup O157 were found to be very low. The results reported by Wani et al. [4] showed low prevalence of *E. coli* O157 (4%) in pigeon faeces (n = 25) in India. Moreover, Morabito et al. [18] and Tanaka et al. [19] reported complete absence of *E. coli* O157 isolates from samples collected from 487 pigeons in Italy and from 108 pigeon faecal droppings in Japan respectively. In another study from Japan, the prevalence of shiga toxin-producing *E. coli* in pigeon faeces was found to be 7.5% (n = 67), yet none of these isolates were belonging to O157 serogroup [7]. Thus, the low incidence of *E. coli* O157 in pigeon faeces reported in this study is in consistence with that reported elsewhere.

Several other potentially pathogenic non-O157 serogroups were implicated in human diseases worldwide; these serogroups were found to be in animal reservoir, mainly livestock [20]. Some of these serogroups were also detected in pigeon faeces with various degrees of frequency; O132 and O45 [18], O9; O18; O25; O60; O77; O168 and O169 [4], O2 and O132 [7]. However the most frequently non-O157 serogroups implicated in human diseases (e.g. O26; 091; O103; O111 and O145) [20] were not detected in this study (Table 1), and their incidence in pigeon faecal droppings was less frequently reported elsewhere [5].

Table 1. Isolation of *E. coli* O157 and other serogroups from pigeon faecal droppings

	E. coli O157	*E. coli* O26	*E. coli* O91	*E. coli* O103	*E. coli* O111	*E. coli* O145
	N/P (%)	N/P (%)	N/P (%)	N/P (%)	N/P (%)	N/P (%)
Summer	150/6.0(4.0)	150/0.0(0.0)	150/0.0(0.0)	150/0.0(0.00)	150/0.0(0.0)	150/0.0 (0.0)
Autumn	150/4.0(2.6)	150/0.0(0.0)	150/0.0(0.0)	150/0.0(0.0)	150/0.0(0.0)	15 /0.0 (0.0)
Winter	150/2.0(1.33)	150/0.0(0.0)	150/0.0(0.0)	150/0.0 (0.0)	150/0.0(0.0)	150/0.0 (0.0)
Spring	150/5.0(3.33)	150/0.0(0.0)	150/0.0(0.0)	150/0.0 (0.0)	150/0.0(0.0)	150/0.0 (0.0)
Total	600/17(2.83)	600/0.0(0.0)	600/0.0(0.0)	600/0.0(0.0)	600/0.0(0.0)	600/0.0 (0.0)

N = total number of samples, P = number of positive samples, (%) = Percentage of positive samples

Using the Oxoid VTEC-RPLA toxin detection kit, it was revealed that 41.1% (n = 17) of the O157 serogroup isolates were producing VT1 and VT2 toxin (Table 2). Verocytotoxin producing *E. coli* (O157 and other serogroups) were detected in pigeon faecal material by conventional and molecular methods [4,16,18]. It was found that shiga toxin-producing *E. coli* O128 isolated from pigeons shares similar antigens and eae toxin genes with shiga toxin-producing *E. coli* from a patient with diarrhea, and suggests that the human strain may originate from pigeons, however the authors did not carry out an epidemiological investigation to confirm their assumption [4]. It can be concluded that free-living pigeons may constitute a natural reservoir for shiga toxin-producing *E. coli* serotypes, however it is difficult to establish if these serotypes may represent a potential health hazards to humans [3,18].

Table 2. Shiga toxin-producing isolates among O157 serogroup strains recovered from pigeon faecal droppings

Season	*E. coli* O157	VTEC-RPLA† positive
Summer	6.0	2.0
Fall	4.0	2.0
Winter	2.0	0.0
Spring	5.0	3.0
Total	17	7.0

† = Determined by Oxoid reverse passive latex agglutination kit

In this study the isolates belonging to serogroup O157 showed low resistance to antimicrobial agents targeting *E. coli* (e.g. tetracycline) and non-targeting *E. coli* (e.g. penicillin G and erythromycin) that are commonly used to treat feedlot animals (Table 3). All O157 serogroups isolates that were recovered in this study were susceptible to antimicrobial agents targeting *E. coli* (e.g. cephalothing and cefazolin) but not approved for use with animals (Table 3). Low antibiotic resistance patterns to similar drugs used in this study were observed with *E. coli* O157 recovered from cattle, humans and food elsewhere [21,22]. All other 100 *E. coli* isolates that gave no positive reaction with *E. coli* Dryspot Seroscreen (i.e. not confirmed as O26; O91; O103; O111 and O145) were also examined for their antimicrobial resistance patterns (Table 3). High level of resistance was observed with penicillin G (98%) followed by erythromycin (90%). While the isolates exhibited low resistance to gentamicin (10%) and cefazolin (4.0%) (Table 3). Similar antibiotic resistance patterns with *E. coli* isolated from pigeons and other free-living birds were reported elsewhere [23,24,25,26]. Pigeons may play a role in disseminating multidrug-resistant *Escherichia coli* in the environment, by contaminating drinking water supplies, or spreading antibiotic resistant strains in farm environments [27].

Table 3. Prevalence of antimicrobial resistance in *E. coli* O175 and other serogroups isolated from pigeon faecal droppings

Antimicrobial agent	*E. coli* O157 (n=17)			Other serogroups† (n=100)		
	R N (%)	IR N (%)	S N (%)	R N (%)	IR N (%)	S N (%)
Penicillin G	2 (11.76)	0.0(0.0)	15(88.23)	98(98)	2.0(2.0)	0.0(0.0)
Streptomycin	2 (11.76)	0.0(0.0)	15(88.23)	30(30)	44(44)	26(26)
Cephalothin	0.0 (0.0)	0.0(0.0)	17(100)	5.0(5.0)	77(77)	18(18)
Cefazolin	0.0 (0.0)	0.0(0.0)	17(100)	10(10)	13(13)	77(77)
Gentamicin	0.0 (0.0)	0.0(0.0)	17(100)	4.0(4.0)	0.0(0.0)	96(96)
Ampicillin	0.0 (0.0)	0.0(0.0)	17(100)	15(15)	0.0(0.0)	85(85)
Erythromycin	2 (11.76)	0.0(0.0)	15(88.23)	90(90)	7.0(7.0)	3.0(3.0)
Tetracycline	3 (17.64)	0.0(0.0)	14(82.35)	55(55)	12(12)	33(33)

R = Resistant, IR = Intermediate resistance, S = Sensitive, N = total number of isolates tested for antimicrobial susceptibility, † = These isolates gave negative reaction with E. coli Dryspot Seroscreen (Oxoid), thus they do not belong to O26; O91; O103; O111 and O145

4. CONCLUSION

The results reported in this study clearly showed that free-living pigeons in western Saudi Arabia probably constitute an environmental reservoir of antibiotic resistant strains of shiga toxin- and non-shiga toxin-producing *E. coli* that could be of risk to other birds, livestock and humans. However, since no available evidences to support the implication of pigeons in *E. coli* O157 infections in humans, and the relatively low incidence of *E. coli* O157 in pigeon faecal droppings may suggest that free-living pigeons in western Saudi Arabia may not play an important role in the epidemiology of shiga toxin-producing *E. coli*. However, pigeons could play an important role in environmental contamination of multidrug-resistant *E. coli*

COMPETING INTERESTS

Author has declared that no competing interests exist.

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