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Free Living Rock Pigeon (*Columba livia*) as an Environmental Reservoir of Enteric Bacterial Pathogens Resistant to Antimicrobial Drugs in Saudi Arabia

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ABSTRACT
The aim of this study was to investigate the carriage of antibiotic resistant strains of pathogenic *E. coli* and *Salmonella* sp. by pigeons in Saudi Arabia. A total of 400 fresh faecal samples of rock pigeons were screened for the presence of *E. coli* O157 and *Salmonella*. Selective plating technique was used to isolate the bacteria and disk diffusion method was used to assess their susceptibility patterns to eight antibiotics. Of the 400 faecal samples, 2.5% were positive for shiga toxin-producing *E. coli* and 2.0% were positive for *Salmonella*. *E. coli* O157 showed low resistance patterns (30%) to one or more of the antibiotics used, whereas *Salmonella* isolates exhibited higher resistance (62.5%) to one antimicrobial agent. The results reported in this study clearly showed that free-living pigeons in Makkah, western Saudi Arabia may constitute a reservoir of antibiotic resistant strains of shiga toxin-producing *E. coli* and *Salmonella* that could be of risk to other birds, feedlot animals and humans. However, given the relatively low incidence of these pathogens suggests that free-living pigeons may not play an important role in the infections occurred in the community due to these pathogens.

Key words: Antibiotic resistance, *E. coli*, environment, rock pigeon, *Salmonella*

INTRODUCTION
Wild birds are considered as a major reservoir of pathogenic zoonotic agents, which are potentially transmissible to humans through either, the handling of these birds, or through contaminated food and water (Abulreesh *et al*., 2005, 2007). High 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 (Haag-Wackernagel and Moch, 2004). The carriage of enteric bacterial pathogens by healthy and/or infected free living pigeons that inhibit large cities is well documented. Shiga toxin-producing *Escherichia coli* and *Salmonella* species were found in faecal droppings and/or cloacal swabs of pigeons that live in urban and rural areas around the world (Dovc *et al*., 2004; Haag-Wackernagel and Moch, 2004; Morabito *et al*., 2001; Kobayashi *et al*., 2007, 2009; Wani *et al*., 2004; Pedersen *et al*., 2006). Pigeons can shed these pathogens into the environment when they are either ill or without any symptoms. Thus may play a role in the dissemination of these pathogens in the environment.

In Makkah city, western Saudi Arabia, rock pigeons are abundant and often found in public parks, roof of houses, near drinking water reservoir, farms and sometime near dining places food outlets. Although the reports concerning the distribution of *Salmonella* serotypes within farm environment and poultry in Saudi Arabia are available (Al-Nakhli *et al*., 1999) published data about the distribution of *Salmonella* and shiga toxin-producing *E. coli* in the environment, particularly in pigeon faeces are lacking in Saudi Arabia. The aim of this study is to investigate the carriage and antimicrobial susceptibility of *E. coli* O157 and *Salmonella* species by rock pigeon in Makkah city, western Saudi Arabia.

MATERIALS AND METHODS

A total of 400 fresh (still moist) feacal droppings of rock pigeons were collected from parks, playgrounds, houses roof tops and yards in Makkah city in a period of twelve months, samples were collected twice a month from June 2009 to May 2010. 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 later on the sampling day. Sampling was carried out twice a month (six sampling batches, 100 faecal samples per season).

Samples were screened for *E. coli* O157 and *Salmonella* species by selective plating as described by Nye *et al*. (2001). Briefly, subsample of fresh faeces (0.5 g) was emulsified in 2.5 mL of Maximal Recovery Diluent (Oxoid, Basingstoke, UK). A 3-mL pastette was used to inoculate 45 µL of faecal suspension on to Sorbitol MacConkey agar (SMAC) (Oxoid), Xylose Lysine Desoxycholate agar (XLD) (Oxoid) and *Salmonella*-Shigella agar (S-S agar) (Oxoid) and streaked for obtaining single colonies for *E. coli* O157 and *Salmonella*, respectively. Plates were incubated aerobically at 37°C for 24 h.

Non-sorbitol fermenting colonies on SMAC agar were tested by agglutination using the Dryspot *E. coli* O157 test kit (Oxoid), Presumptive salmonellae colonies on XLD and S-S agar were subcultured onto *Salmonella* Chromogenic agar (Oxoid). Typical salmonellae raised, magneta colonies on chromogenic agar were further confirmed by incubation on lysine iron agar slopes (Oxoid) and in urea broth (Oxoid); incubation at 37°C for 14-18 h.

Antimicrobial susceptibility tests were carried out using the disk diffusion method. Mueller-Hinton agar plates (Oxoid) were incubated aerobically at 37°C for 18-22 h. The interpretation of values of disk diffusion technique was performed according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2009). 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}).

RESULTS AND DISCUSSION

A total of 400 fresh faecal droppings of rock pigeons were collected between June 2009 and May 2010. Of these 400 samples only ten (2.5 %) were positive for *E. coli* O157 and eight (2.0 %) were positive for *Salmonella* (Table 1). *E. coli* O157 was recovered all year round, recovery rates were higher in summer comparing to winter. However, no significant statistical differences between seasons for the carriage of *E. coli* O157 by pigeons ($p = 0.5998$). With regard to salmonellae, it was only recovered from faeces during summer and spring. Similar to *E. coli* O157, Kruskal-Wallis, a non-parametric test showed that the prevalence of *Salmonellae* in pigeons did not significantly differ between seasons ($p = 0.1441$) (Table 2).

Only 30% of shiga toxin-producing *E. coli* isolates were resistant to Penicillin G, erythromycin and tetracycline. All ten *E. coli* isolates were susceptible (100%) to cephalothin and cefazolin

Table 1: Isolation of *E. coli* O157 and *Salmonella* from pigeon faeces in Makkah, Saudi Arabia

	E. coli O157		*Salmonella*	
Seasons	N/P	%	N/P	%
Summer	100/6	6	100/7	7
Autumn	100/2	2	100/0	0
Winter	100/1	1	100/0	0
Spring	100/1	1	100/1	1
Total	400/10	2.5	400/8	2.0

N: Total No. of samples, P: No. of positive samples, %: Percentage of positive samples

Table 2: Seasonal variation of *E. coli* O157 and *Salmonella* carried by pigeons in Makkah, Saudi Arabia

	Summer			Autumn			Winter			Spring			
Strains	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	P
E. coli O157	1	0-3	6	0.33	0-2	6	0.17	0-1	6	0.17	0-1	6	NS
Salmonella	1.17	0-3	6	0	0	6	0	0	6	0.17	0-1	6	NS

P is the probability that there is no seasonal difference in the carriage of *E. coli* O175 and *Salmonella* by pigeons (Kruskal-Wallis test).
N: Number of sampling patches per season

Table 3: Prevalence of antimicrobial resistance in *E. coli* O175 and *Salmonella* isolates from pigeons in Saudi Arabia determined by disk diffusion method

	No. of resistance (%)	
Antimicrobial	*E. coli* O157 (n = 10)	*Salmonella* (n = 8)
Penicillin G	3 (30)	4 (50)
Streptomycin	2 (20)	1 (12.5)
Cephalothin	0 (0)	2 (25)
Cefazolin	0 (0)	1 (12.5)
Gentamicin	1 (10)	0 (0)
Ampicillin	1 (10)	0 (0)
Erythromycin	3 (30)	4 (50)
Tetracycline	3 (30)	5 (62.5)

N: Total No. of isolates tested for antimicrobial susceptibility

(Table 3). With regard to the eight *Salmonella* isolates, five (62.5%) of these isolates were resistant to tetracycline and 50% of the recovered *Salmonellae* were resistant to erythromycin and penicillin G, respectively. All eight salmonella's isolates were susceptible to ampicillin and gentamicin.

Of the 400 faecal droppings examined in this study, 2.5% were positive for *E. coli* O157 (Table 1). Although shiga toxin-producing *E. coli* were frequently recovered from pigeon faeces (Schmidt *et al*., 2000; Morabito *et al*., 2001; Wani *et al*., 2004; Sonntag *et al*., 2005; Pedersen *et al*., 2006; Kobayashi *et al*., 2009). The prevalence of species that belong to sero-group O157 were found to be very low in comparison with other sero-groups such as O132 and O45 (Morabito *et al*., 2001; Pedersen *et al*., 2006). The results of Wani *et al*. (2004) reported the prevalence of *E. coli* O157 in 4% of pigeon faeces (n = 25) from India. Moreover, Tanaka *et al*. (2005) reported complete absence of *E. coli* O157 isolates from 108 pigeon faeces in Japan. In another study from Japan, the prevalence of shiga toxin-producing *E. coli* in pigeons was found to be 7.5% (n = 67), yet none of these isolates were belonging to O157 sero-group (Kobayashi *et al*., 2009). Thus, the low incidence of *E. coli* O157 in pigeon faeces reported in this study is in consistence with that reported elsewhere.

Wild birds are common environmental reservoir of *Salmonellae*, but the incidence of the organism in wild birds in general tends to be very low (Kirk *et al*., 2002; Refsum *et al*., 2002; Reche *et al*., 2003; Abulreesh *et al*., 2007). In this study eight samples of pigeons faeces out of 400 (2.0%) were positive for *Salmonella* (Table 1). This result is similar to those reported worldwide and showed that *Salmonella* species are either completely absent or exhibit very low prevalence in pigeons (Cizek *et al*., 1994; Casanovas *et al*., 1995; Hubalek *et al*., 1995; Kirk *et al*., 2002; Refsum *et al*., 2002; Reche *et al*., 2003; Vlahovic *et al*., 2004; Dovc *et al*., 2004; Tanaka *et al*., 2005; Lillehaug *et al*., 2005; Pedersen *et al*., 2006; Kobayashi *et al*., 2007).

The current study was carried out in twelve months. Sampling for pigeon's faeces was performed twice a month (i.e., six times per season). *E. coli* O157 was recovered from faeces all year round, yet samples collected during warmer months yielded more isolates than those collected during colder months. Similar observation was also noted with the recovery of *Salmonella* (Table 1). In order to test the hypothesis that there is no seasonal variation of the carriage of these pathogens by pigeons, Kruskal-Wallis a non-parametric test was used. The results showed that the carriage of *E. coli* O157 ($p = 0.5998$) and *Salmonella* ($p = 0.1441$) by pigeons did not differ significantly between seasons (Table 2). Pedersen *et al*. (2006) found that shiga toxin-producing *E. coli* was statistically abundant in pigeons during summer but not in winter, while the prevalence of *Salmonella* in pigeon faeces did not show any significant variation between seasons.

There is little information exists with regard to antimicrobial susceptible patterns of shiga toxin-producing *E. coli* carried by pigeons. In this study, only 30% of shiga toxin-producing *E. coli* isolates were resistant to one or more of antibiotics used (Table 3). These isolates showed low resistance to antimicrobial targeting *E. coli* (e.g., tetracycline and ampicillin) and non-targeting *E. coli* (e.g., penicillin G and erythromycin) that are commonly used to treat feedlot animals (Table 3). All shiga toxin-producing *E. coli* recovered in this study were susceptible to antimicrobial targeting *E. coli* (e.g., cephalothing and cefazolin) but not approved for use with feedlot animals (Table 3). Low antibiotic resistance patterns to similar drugs used in this study were noted with shiga toxin-producing *E. coli* recovered from cattle, humans and food (Galland *et al*., 2001; Schroeder *et al*., 2002).

Over all, *Salmonella* isolates from pigeons in this study also exhibited low antimicrobial resistance patterns, with the exception of tetracycline, erythromycin and penicillin G, when 62.5 and 50% of the isolates showed resistance to these three antibiotics, respectively (Table 3). Similar results were noted with *Salmonellae* isolated from waterfowl and gulls (Fallacara *et al*., 2004; Cizek *et al*., 2007). Ampicillin is a drug that usually used to treat infections caused by *Salmonellae*, pigeon isolates reported here were 100% susceptible to this antibiotic (Table 3).

The results reported in this study clearly showed that free-living pigeons in Makkah, western Saudi Arabia may constitute an environmental reservoir of antibiotic resistant strains of shiga toxin-producing *E. coli* and *Salmonella* that could be of risk to other birds, feedlot animals and humans. However, the lack of evidence that supports transmission of these pathogens from pigeons to humans (Haag-Wackernagel and Moch, 2004) and the relatively low incidence of these pathogens reported in this study suggests that free-living pigeons in Makkah may not play an important role in the infections occurred in the community due to these pathogens. To the author best knowledge, this is the first account that reports the incidence of antibiotic resistant pathogenic *E. coli* and *Salmonella* species in pigeons in western Saudi Arabia.

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