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Short communication

Captive and free-living urban pigeons (*Columba livia*) from Brazil as carriers of multidrug-resistant pathogenic *Escherichia coli*

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ABSTRACT

Thirty *Escherichia coli* isolates from captive and free-living pigeons in Brazil were characterised. Virulence-associated genes identified in pigeons included those which occur relatively frequently in avian pathogenic *E. coli* (APEC) from commercial poultry worldwide. Eleven of 30 *E. coli* isolates from pigeons, belonging mainly to B1 and B2 phylogenetic groups, had high or intermediate pathogenicity for 1-day-old chicks. The frequency of multi-drug resistant (MDR) *E. coli* in captive pigeons was relatively high and included one isolate positive for the extended-spectrum β-lactamase (ESBL) gene $bla_{CTX\text{-}M\text{-}8}$. *Pulsed field gel electrophoresis* (PFGE) showed high heterogeneity among isolates. There is potential for pigeons to transmit antibiotic resistant pathogenic *E. coli* to other species through environmental contamination or direct contact.

Urban pigeons (*Columba livia*) can be a reservoir for pathogenic microorganisms, including avian pathogenic *Escherichia coli* (APEC) (Haag-Wackernagel and Moch, 2004). The aim of this study was to determine whether urban pigeons act as carriers of antibiotic resistant APEC to help in assessing the risk associated with the transmission of these strains from pigeons to other species.

Oropharyngeal and cloacal samples were collected from 100 free-living pigeons captured at São Paulo State University (UNESP), Brazil, from February to April 2012. Oropharyngeal and cloacal samples were also collected from 11 captive pigeons from one flock at the Wildlife Veterinary Hospital at UNESP in November 2011. In addition, samples of liver and intestine were collected from two of these captive pigeons; these two birds died following ingestion of pesticide, whereas the other nine pigeons were unaffected. All samples were collected using sterile swabs and inoculated into tubes containing 5 mL brain heart infusion broth. Isolates were recovered according to Borges et al. (2012).

DNA extraction was performed by thermal lysis[1] and isolates screened for 20 virulence-associated genes (VAGs) (Borges et al., 2017; see Appendix: Supplementary Table S1). Isolates were assigned to phylogenetic groups (A, B1, B2 or D) according to Clermont et al. (2000) (see Appendix: Supplementary Table S1). Each isolate was inoculated into ten 1-day-old chicks to determine if it was a high (HP), medium (MP) or low (LP) pathogenic isolate, or if it was non-pathogenic (NP) (Guastalli et al., 2013). Isolates were subtyped using a standardised rapid *pulsed field gel electrophoresis* (PFGE) protocol (PulseNet[2]). Isolates were analysed for O and H antigens at the *E. coli* Reference Center, Pennsylvania State University, University Park, PA, USA, and multilocus sequence typing (MLST) was performed following Achtmans's scheme.[3] Antimicrobial sensitivity was determined using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2014). ESBL genes $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM} were amplified as described by Dallenne et al. (2010) (see Appendix: Supplementary Table S1). Sequencing was performed at the DNA Sequencing Facility of the University of California, Berkeley, USA. The study was approved by Animal Experimentation Ethics Committee (CEUA) of São Paulo State University (protocol number 22.222/10; date of approval 22 October 2010). The frequencies of VAGs and the percentage of resistance isolates were compared by Fisher's exact test using Prism for Windows

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[1] See: http://www.apzec.ca/en/APZEC/Protocols/pdfs/ECL_PCR_Protocol.pdf (accessed 9 September 2016).

[2] See: http://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-mlst-protocol-508c.pdf (accessed 9 September 2016).

[3] See: http://mlst.ucc.ie/mlst/dbs/Ecoli (accessed 9 September 2016).

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Table 1
Serotypes, pathogenicity, phylogenetic groups, virulence genes, sequence types, extended-spectrum β-lactamase (ESBL) genes and antimicrobial profiles of *Escherichia coli* isolates from captive and free-living urban pigeons in Brazil.

Isolate	Serotype	Pathogenicity	Phylogeny	Virulence profile	Sequence type	ESBL	Antimicrobial resistance
Captive urban pigeons							
16i	O153:H51	H	B1	*iss+ iroN+ ompT+ hlyF+ sitA+ traT+ tsh+ fimH+*	155		NAL TET NIT CIP NOR AMP
16T	ONT:HNT	I	B2	*iss+ iroN+ ompT+ hlyF+ sitA+ traT+ fimH+*	Untypable		NAL TET NIT AMP FOX AMC CTX
17i	ONT:H51	I	B1	*iroN+ sitA+ traT+ irp2+ fyuA+ fimH+*	155		NAL TET CIP NOR
17C	O54:H5	L	B2	*iss+ sitA+ irp2+ fyuA+ fimH+*	Untypable		NAL TET CIP NOR
17T	O153:H51	I	B1	*iss+ iroN+ ompT+ sitA+ traT+ fimH+*	155		NAL TET CIP NOR
18C	ONT:HNT	H	B1	*iss+ iroN+ ompT+ iutA+ hlyF+ cvaC+ iucC+ sitA+ iucD+ irp2+ fyuA+ fimH+*	359	$bla_{CTX-M-8}$+ bla_{TEM-1}	NAL SXT TET NIT CIP AMP NOR AMC CAZ CTX FOX
18T	ONT:H51	H	B1	*iss+ iroN+ ompT+ hlyF+ sitA+ traT+ fimH+*	155		NAL SXT TET CIP NOR AMP FOX
19C	O97:HNT	NP	B2	*iroN+ sitA+ irp2+ fyuA+ fimH+*	1170		NAL SXT AMP CIP FOX AMC
19T	ONT:H9	NP	A	*iss+ iroN+ ompT+ hlyF+ sitA+ traT+ fimH+*	4542		NAL SXT TET NIT AMP NOR FOX AMC CTX
20T	ONT:H51	L	B1	*iss+ iroN+ ompT+ hlyF+ sitA+ traT+ fimH+*	155		NAL TET CIP AMP NOR FOX AMC CTX
21C	O153:H51	L	B1	*iss+ iroN+ ompT+ sitA+ traT+ fimH+*	155		NAL SXT TET CIP NOR AMP FOX
Free-living urban pigeons							
22C1	O7:H40	H	A	*iss+ iroN+ ompT+ hlyF+ sitA+ traT+ tsh+ fimH+*	93		SXT TET AMP CIP FOX NAL
22C2	O106:HNT	L	D	*iss+ fimH+*			SXT
23C	O166:H15	NP	D	*iss+ fimH+*			No resistance
24C	O100:H40	H	A	*iss+ iroN+ ompT+ sitA+ fimH+*	93		SXT TET AMP
25C	O51:HNT	NP	D	*iss+ traT+ fimH+*	Untypable		SXT AMP NAL
26C	O83:H6	L	B2	*iss+ sitA+ traT+ tsh+ irp2+ fyuA+ fimH+*			AMP
27C	O166:H15	NP	D	*iss+ fimH+*			No resistance
28C	O68:H45	NP	D	*iss+ traT+ fimH+*			TET
29C	O68:HNT	NP	D	*iss+ sitA+ traT+ fimH+*			No resistance
30C	O78:H34	L	D	*iss+ fimH+*			No resistance
31C	O11:HNT	NP	A	*iss+ iroN+ ompT+ hlyF+ sitA+ fimH+*			No resistance
32C	ONT:H40	NP	A	*iss+ iroN+ ompT+ iutA+ iucC+ sitA+ traT+ fimH+*	93		AMP FOX NIT
33T	O83:H6	I	B2	*iss+ iutA+ hlyF+ sitA+ traT+ tsh+ fimH+ irp2+ fyuA+ fimH+*			No resistance
34C	O73:H45	L	D	*iss+ fimH+*			No resistance
35C	O68:H45	I	D	*iss+ fimH+*			No resistance
36C	O166:HNT	NP	D	*iss+ fimH+*			No resistance
37C	O166:H6	L	D	*iss+ fimH+*			No resistance
38C	O83:HNT	I	B2	*iss+ sitA+ traT+ tsh+ irp2+ fyuA+ fimH+*			NIT
39C	ONT:H8	NP	B2	*iss+ sitA+ traT+ irp2+ fyuA+ fimH+*			No resistance

Bird number and site of isolation: i, intestine; c, cloaca; t, oropharynx.
NT, non-typable; H, high; I, intermediate; L, low; NP, non-pathogenic; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FOX, cefoxitin; NAL, nalidixic acid; NIT, nitrofurantoin; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

version 6.01 (GraphPad Software). Statistical significance was declared at $P < 0.05$.

Eleven *E. coli* isolates were recovered from six captive pigeons, including three isolates from three body sites of one bird, two isolates from two body sites of each of three birds and one body site from each of two birds (Table 1). Nineteen *E. coli* isolates were recovered from 18 free-living pigeons, including two isolates from one bird and one isolate from each of 17 birds. One isolate (18C) from a captive pigeon was positive for the extended-spectrum β-lactamase (ESBL) gene $bla_{CTX-M-8}$. VAGs with a frequency ≥50% were *fimH*, *iss*, *sitA* and *traT* (Table 1). Pathogenicity testing revealed that 5/30 (16.6%) isolates were HP, 6/30 (20.0%) were MP, 8/30t (26.6%) were LP and 11/30 (36.6%) were NP. Phylogenetic typing showed that 8/11 (72.7%) HP and MP isolates belonged to groups B1 and B2, while 10/19 (52.6%) LP and NP isolates belonged to group D (Table 1). Of 20 VAGS tested, three (15.0%) were present in HP and MP isolates; these three genes were related to toxicity (*hlyF*), adhesion (*tsh*) and iron acquisition (*sitA*).Table 2 shows the VAG frequencies among HP, MP, LP and NP strains. Serotypes O153:H51 and ONT:H51 were detected in more than one strain from different birds (Table 1).

Multidrug resistance, which was defined as resistance against three or more classes of antimicrobial agents, was found in 72.7% isolates from captive pigeons and in 21.0% isolates from free-living pigeons. Nine of 19 (16.7%) antimicrobial agents were significantly associated with captive pigeons (Tables 1 and 3), and MDR isolates were also positively associated with captive pigeons. Of the 30 isolates, 26 distinct restriction patterns were revealed by PFGE, while two were untypable, demonstrating a high degree of heterogeneity among the avian *E. coli* examined (see Appendix: Supplementary Fig. S1). MLST performed on all isolates from captive pigeons and on the MDR isolates from free-living pigeons revealed five distinct sequence types (STs) (Table 1).

Table 2
Frequency (%) of virulence-associated genes (VAGs) according to the pathogenicity of *Escherichia coli* isolated from pigeons in Brazil.

VAG	HP/MP	LP/NP
Adhesins		
fimH	11 (100.0%)	19 (100.0%)
sfa	0	0
tsh	4 (36.4%)*	1 (5.3%)
papC	0	0
papGI	0	0
papGII	0	0
papGIII	0	0
Iron acquisition		
iroN	8 (72.7%)	6 (31.6%)
irp2	4 (34.4%)	4 (21.0%)
fyuA	4 (34.4%)	4 (21.0%)
iutA	2 (18.2%)	1 (5.3%)
iucC	1 (9.1%)	1 (5.3%)
iucD	1 (9.1%)	0
sitA	10 (90.9%)*	10 (52.6%)
Serum resistance		
iss	10 (90.9%)	18 (94.7%)
traT	8 (72.7%)	9 (47.4%)
ompT	7 (63.6%)	7 (36.8%)
Toxin		
cnf1	0	0
hlyF	6 (54.5%)*	3 (15.8%)
Other		
cvaC	1 (9.1%)	0

HP, high pathogenicity; MP, medium pathogenicity; LP, low pathogenicity; NP, non-pathogenic.
* $P < 0.05$.

The VAGs identified in the present study are those reported to be frequent in APEC from commercial poultry worldwide (Guastalli et al., 2013; Maluta et al., 2014; Cordoni et al., 2016; Dou et al., 2016). In a previous study in Brazil, *tsh* and *hlyF* were associated with APEC from poultry (Maluta et al., 2014); these same genes were associated with HP and MP isolates in the present study, suggesting that *E. coli* causing extra-intestinal disease could be transmitted between pigeons and poultry. VAGs detected in pigeons in the present work have also been identified in human extra-intestinal pathogenic *E. coli* (ExPEC) (Maluta et al., 2014); it is possible that pathogenic *E. coli* could also be transmitted between pigeons and human beings.

Table 3
Frequency (%) of resistance to antimicrobial agents of *Escherichia coli* isolated from captive and free-living pigeons in Brazil.

Antimicrobial agents[a]	Captive pigeons ($n = 11$)		Free-living pigeons ($n = 19$)	
	n	%	*n*	%
CTX	4	36.4*	0	0
FOX	7	63.6*	2	10.5
CAZ	1	9.1	0	0
AMC	5	45.5*	0	0
AMP	8	72.7*	5	26.3
CIP	9	81.8*	1	5.3
NOR	9	81.8*	0	0
NAL	11	100*	2	10.5
NIT	4	36.4*	1	5.3
SXT	5	45.5	4	21.0
TET	10	90.9*	3	15.8

AMC, amoxicillin-clavulanic acid; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FOX, cefoxitin; NAL, nalidixic acid; NIT, nitrofurantoin; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.
[a] All strains were susceptible to aztreonam, amikacin, cefepime, gentamicin, kanamycin, ertapenem, imipenem and meropenem.
* $P < 0.05$.

Furthermore, isolates from pigeons in the present study belonged to serogroups and STs that have been isolated from poultry with colibacillosis, including O78, which is classically linked to APEC (Maluta et al., 2014; Dou et al., 2016).

The frequency of MDR *E. coli* was higher among captive pigeons than in free-living pigeons. It is uncertain if this is related to a higher level of exposure to antimicrobial agents. PFGE demonstrated high heterogeneity among the *E. coli* isolates in this study, indicating that no one *E. coli* clone is associated with pigeons in Brazil. Although the number of pigeons with *E. coli* carrying VAGs linked to APEC and human ExPEC is not necessarily high, the potential for these birds to transmit MDR pathogenic *E. coli* to poultry and human beings, either via environmental contamination or direct contact, should be considered.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2016.12.015.

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