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First Characterization of *Escherichia coli* Strains Isolated from Wildlife Griffon Vulture (*Gyps fulvus*) in the Southeast of Spain

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Abstract

The aim of the present study was to characterize a collection of *Escherichia coli* strains isolated from asymptomatic griffon vulture (*Gyps fulvus*) during a reintroduction program in the southeast of Spain, in order to establish if griffon vulture could play a role in the spread of resistant or potentially pathogenic *E. coli* strains. For this purpose, 14 *E. coli* strains obtained from 10 griffon vulture were studied to establish their serotypes, phylogroups, virulence-gene profiles and antimicrobial resistances. High heterogeneity was observed within the 14 strains isolated which belonged to three phylogroups (A, B1 and D), 8 serogroups (O2, O21, O29, O60, 073, O78, O103 and O141) and 13 different serotypes. Out of 34 genes screened, we have detected eight virulence genes that are typical of extraintestinal pathogenic *E. coli* (ExPEC) (*fimH*, *fimAv*$_{MT78}$, *iroN*, *iucD*, *cvaC*, *iss*, *traT* and *tsh*); however, none of the studied strains showed the ExPEC status. The 14 strains were also analyzed for the production of extended-spectrum beta-lactamases (ESBLs) and for antimicrobial resistances. None of the 14 strains were ESBL-producing *E. coli*, but high resistance-prevalences to ampicillin and cotrimoxazole were detected. To our knowledge, this is the first characterization of *E. coli* strains isolated from griffon vulture and although they did not show high virulence-gene scores, they showed cotrimoxazole resistance.

Keywords

Antibiotic Resistance, APEC, *Escherichia coli*, ExPEC, Griffon Vulture

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1. Introduction

Griffon vulture (*Gyps fulvus*) is a large-size bird of prey that greatly relies on food found at *muladares* and other refuse dumps. Few studies have been carried out on the role of vultures in the spread of disease [1] [2], and none in relation to *Escherichia coli*, a ubiquitous microorganism of the *Enterobacteriaceae* family. *E. coli* comprises different intestinal and extraintestinal pathogenic (ExPEC) groups for animals and humans [3]. Avian pathogenic *E. coli* (APEC) is included in the group of extraintestinal pathogenic *E. coli* (ExPEC) and is considered an outstanding pathogen for the poultry industry [4]. A wide number of serogroups have been identified within APEC, many of which have also been implicated in human extraintestinal infections [4]. Moreover, certain important clonal groups have been reported as responsible for human and animal extraintestinal *E. coli* infections during the last years [5].

The emergence of multiresistant bacteria of human and veterinary origin is nowadays a great health concern. Wildlife, normally not exposed to clinically useful antimicrobial agents, can acquire resistant bacteria mainly through water polluted from feces of human and farm activity. Current data reveal that carriage of multiresistant strains is widespread in, at least, some wild populations like waterfowl, birds of prey, and rodents [6]-[8]. The aim of the present study was to characterize the serotypes, phylogroups, virulence-gene profiles and antimicrobial resistances of a group of *E. coli* strains isolated from asymptomatic griffon vulture, and to establish if griffon vulture could play a role in the spread of resistant or potentially pathogenic *E. coli* strains.

2. Materials and Methods

The samples were collected in 2011 from a *muladar* (a place where farm carrion are discarded by scavenger's species) in Alicante (south-east of Spain). Seventeen healthy griffon vultures (15 immature, 1 sub-adult and 1 adult) were tested for *E. coli* via cloacae swabs with stuart transport medium. Samples were cultured within 24 h of collection. For isolation, swabs were plated directly onto lactose MacConkey agar (L-MC) (Oxoide Ltd.) and incubated at 37˚C for 18 to 24 h. From each L-MC plate, two colonies with typical *E. coli* morphology were selected, identified by the API 20E system (bioMèrieux) and further studied.

Determination of O and H antigens was carried out using the method previously described by Guinée *et al.* [9], with all available O (O1-O181) and H (H1-H56) antisera. Isolates that did not react with O and H antiserum were classified as non-type able (ONT and HNT, respectively).

The phylogenetic group (A, B1, B2 and D) was established by the multiplex PCR-based method of Clermont *et al.* [10].

The presence of 34 virulence genes was analyzed as documented previously [3] [5], using primers specific for genes and operons that encode extraintestinal virulence factors characteristic of ExPEC (*fimH*; *fimAv*$_{MT78}$; *papEF*; *papG* I; *papG* II; *papG* III; *sfa/focDE*; *sfaS*; *focG*; *afa/draBC*; *cnf*1; *cdtB*; *sat*; *hlyA*; *iucD*; *iron*; *kpsM* II, establishing *neuC*-K1, -K2 and -K5 variants; *kpsM* III; *cvaC*; *iss*; *traT*; *ibeA*; *malX*; *usp*; *tsh*), of verotoxigenic *E. coli* (VTEC) (*stx*1; *stx*2; *eae*) and enterotoxigenic *E. coli* (ETEC) (*eltA*; *estA*; *estB*).

Susceptibility to antibiotics was analyzed by disc diffusion. Resistances against ampicillin, amoxicillin/clavulanate acid, cefazoline, gentamicin, trimethoprim/sulfamethoxazole, nalidixic acid and ciprofloxacine were interpreted based on the recommended breakpoints of the CLSI [11]. Suggestive evidence of ESBL production was defined as synergy between amoxicillin/clavulanate and at least one of cefotaxime, ceftazidime, aztreonam or cefepime. Furthermore, PCR was performed using the TEM, SHV and CTX-M specific primers reported previously [5].

3. Results and Discussion

Fourteen different *E. coli* strains were recovered from 10 out of the 17 griffon vultures sampled (4 animals showed *E. coli* strains belonging to 2 different serotypes) (**Table 1**).

Few data are available on *E. coli* serotypes within normal avian microbiota. Serotyping showed that the 14 strains belonged to 8 serogroups, 6 of which (O2, O21, O29, O78, O103 and O141) had already been reported within healthy poultry, and serogroup O60 had also been reported within APEC strains [4] (**Table 1**). However, we have only detected previously four (O2:H18, O21:H19, ONT:H11 and ONT:H19) of the 13 serotypes established in griffon vulture, among 430 serotypes of 1640 APEC strains characterized in a European study and isolated from chickens, turkeys and ducks (data not published).

The 14 *E. coli* strains belonged to phylogroups A (7 strains), B1 (5 strains) and D (2 strains). None isolate

belonged to phylogroup B2 (**Table 1**). This is in consistent with the fact that isolates are obtained via cloacae from healthy birds. Several studies suggest that virulent clonal groups are derived primarily from phylogroup B2, and to a lesser extent from phylogroup D [5] [12].

Eight virulence genes typical of ExPEC (*fimH*, *fimAv*$_{MT78}$, *iroN*, *iucD*, *cvaC iss*, *traT* and *tsh*) were detected of 34 genes screened (**Table 1**). The virulence-gene score (number of virulence genes harbored) ranged from 1 to 7, however none of the 14 strains showed the ExPEC status according to the definition of Johnson *et al*. [13]. A strain satisfied the criteria for being ExPEC if it carried two or more of the following genes: *pap*, *sfa/focDE*, *afa/draBC*, *iucD* and *kpsM* II.

Eleven of the 14 *E. coli* strains showed resistance to any of the 7 antimicrobials tested, being 9 strains ampicillin-resistant and 8 cotrimoxazole-resistant. Furthermore, 2 strains showed intermediate resistance to nalidixic acid and ciprofloxacin (**Table 2**). Although none of the 14 strains tested were ESBL-producing *E. coli*, differently to that reported by some authors in relation with birds of prey different from griffon vulture [6] [7], high resistance-prevalences to ampicillin and cotrimoxazole were detected. In fact, 6 strains showed co-resistance to both antibiotics. These prevalences detected in griffon vultures are similar to those found among healthy poultry strains [14].

Table 1. Serotypes, phylogroups and virulence-gene profile of the 14 *E. coli* strains included in the present study.

Vulture code	Strain code	Serotype	Phylogroup	Virulence-gene profile
1	1 HV	O29:H11	A	*fimH iroN cvaC iss traT tsh*
1	1 HA	ONT:H19	A	*fimH*
2	2 HA	O78:H17	D	*fimH iucD traT*
4	11 HV	ONT:H11	A	*fimH*
4	11 HA	O141:H31	D	*fimH*
6	15 HVA	O60:H20	A	*fimH fimAv*$_{MT78}$ *traT*
9	19 HV	O103:H16	B1	*fimH traT*
9	19 HA	O2:H18	B1	*fimH fimAv*$_{MT78}$
10	20 HV	O21:H19	B1	*fimH iroN iss traT*
10	20 HA	O21:HNT	B1	*fimH fimAv*$_{MT78}$ *iroN iss traT*
13	23 HV	ONT:HNT	B1	*fimH iroN iucD cvaC iss traT tsh*
15	25 HV	O73:H31	A	*fimH*
16	26 HV	O60:H9	A	*fimH iroN iucD iss traT tsh*
17	27 HV	O60:H20	A	*fimH fimAv*$_{MT78}$ *traT*

Table 2. Antibiotic susceptibility of the 14 *E. coli* strains included in the present study. AM = ampicillin; AMC = amoxicillin-clavulanic acid; CZ = cephazolin; GM = gentamicin; SXT = trimethoprim-sulfamethoxazole (cotrimoxazole); NA = nalidixic acid; CIP = ciprofloxacin, R = resistant; S = sensitive; I = intermediate resistance.

Vulture code	Strain code	AM	AMC	CZ	GM	SXT	NA	CIP	Antimicrobial resistances
1	1 HV	**R**	S	S	S	S	S	S	AM
1	1 HA	**R**	S	S	S	**R**	S	S	AM, SXT
2	2 HA	S	S	S	S	S	S	S	
4	11 HV	**R**	S	S	S	**R**	S	S	AM, SXT
4	11 HA	S	S	S	S	S	S	S	
6	15 HVA	**R**	S	**I**	S	**I**	**I**	**I**	AM
9	19 HV	S	S	S	S	**R**	S	S	SXT
9	19 HA	S	S	S	S	**R**	S	S	SXT
10	20 HV	**R**	**I**	**I**	S	**R**	S	S	AM, SXT
10	20 HA	**R**	S	**I**	S	**R**	S	S	AM, SXT
13	23 HV	**R**	S	S	S	S	S	S	AM
15	25 HV	S	S	S	S	S	S	S	
16	26 HV	**R**	S	S	S	**R**	S	S	AM, SXT
17	27 HV	**R**	S	S	S	**R**	**I**	**I**	AM, SXT

Conflict of Interest

The authors affirm that no financial or personal relationship existed that could have inappropriately influenced the content of this manuscript or the opinions expressed.

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