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Pathogenic bacteria and the prevalence of virulence genes in *E. coli* isolated from passerine birds of Iran

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Abstract
Wild birds as source of pathogenic microorganisms infecting livestock and humans are an interesting topic that has received increased attention in recent years. Here we study occurrence of pathogenic bacteria in birds in north-east Iran with focus on to identifying virulence gene in wild and domestic birds. Wild birds were trapped and sampled in different localities in north-east Iran from April to September in 2018 and 2019. From 184 birds representing 32 species of wild passerine birds, potentially pathogenic bacteria were isolated from 171 samples (92.9). *Escherichia coli* was the most frequently isolated bacterium with a prevalence of 70.1%, followed by *Enterobacter* spp. (53.8%) and *Salmonella* spp (3.2%) isolated from 129, 99 and 6 wild birds, respectively. In total 79.6% of the isolates carried virulence factors. A higher frequency of virulence genes was detected in domestic birds (88.6%) than in wild birds (76%). The results showed that birds in north-east Iran can serve as a potential source of bacterial pathogens and virulence factors that may cause disease in both humans and birds. Furthermore, our results introduce new host birds as source of pathogenic microorganisms that can carry and disseminate them in the environment.

Key words: Cloacal swabs, Pathogen, *E.coli*, virulence gene, Wild bird

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INTRODUCTION

Wild birds can serve as a potential reservoir of pathogens that may affect domestic birds, animals, and humans (Jones & James Reynolds, 2008; Vanrompay et al., 2007; Gargiulo et al., 2018). Known for their capacity to move from local movements to long-distance migrations across national and intercontinental borders, birds can act as dispersal agents of pathogenic microorganisms on a spatial and temporal scale (Rappole & Hubalek, 2003; Altizer et al., 2011; Winker et al., 2007; Benskin et al., 2009). Furthermore, many birds, including several common passerine species such as house sparrow *Passer domesticus* and barn swallow *Hirundo rustica*, have adapted to life in the farm environment where their proximity to poultry and other domestic animals may play a role also in human disease (Marzluff, 2001; Capua & Alexander, 2002; Atterby et al., 2016; Tsiodras et al., 2008)

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Most studies on pathogenic bacteria in birds focus on captive and domestic birds, particularly gallinaceous poultry. The knowledge on the distribution of such putative pathogens in wild birds is limited, potentially ignoring the role of these birds that may serve as reservoirs of bacterial pathogens (Broman et al., 2002; Dipineto et al., 2008). In rural areas, the transmission of pathogenic bacteria is facilitated due to close contact between birds and farm animals (Marzluff, 2001; Atterby et al., 2016); for instance, Shiga toxin-producing *E. coli* in wild birds and dairy calves in the farm (Cobbold & Desmarchelier, 2002). In addition to direct transmission of pathogenic bacteria, indirect transmission of pathogenic bacteria from birds to humans can occur via fecal contamination of food and water, resulting in diseases such as salmonellosis, diarrhea, and other illnesses in humans (Tsiodras et al., 2008).
Escherichia coli, Yersinia spp., *Klebsiella* spp., *Salmonella* spp., *Campylobacter* spp., and *Enterobacter* spp. are examples of established and putative pathogenic bacteria that can infect many bird species causing diseases such as gastroenteritis, respiratory problems, septicemia, chronic obstructive pulmonary, and even death in humans (Devriese et al., 1995; Droual et al., 1997; Pasquali et al., 2014). Survey on the role of *Enterobacter* in human and animal disease has received significant attention in recent years. Some species of *Enterobacter* genus such as *E. aerogenes* and *E. cloacae*, have been introduced as multi-resistant and opportunistic pathogens (Davin-Regli, 2015).

Some strains of *E. coli* can cause disease in their host. One of the factors of pathogenicity is the existence of virulence genes that are located on bacterial chromosome and plasmids. Virulence genes, including *fimC, fimH, papC, iss, stx1, stx2* can be shared and spread by different animal species. Among the virulence genes, *fimC* (fimbria type 1 C) plays a major role in epithelial cell adhesion and colonization, and *papC* is responsible for bacterial adhesion (Ievy *et al.*, 2020). Generally, the main factor in determining overall virulence is the number and combination of virulence genes (Wang *et al.*, 2015).

Most studies of putative zoonotic bacteria in birds come from Europe and North America, with much fewer works elsewhere, including important biodiverse areas in the Middle East and Asia.
Iran has a rich breeding bird fauna, but also hosts migratory and wintering bird populations emanating from areas north in Eurasia, thereby bridging the Palearctic, Oriental, and Afrotropical regions, making it an important area for exchanging avifauna (Aliabadian et al., 2005).
The aim of the present study was to identify pathogenic bacteria in wild birds, emphasizing the comparison of virulence genes in isolates isolated from wild and domestic birds.

MATERIAL AND METHODS

COLLECTION OF SAMPLES

Fieldwork was conducted in north-east Iran from June to September in both 2018 and 2019, and cloacal swabs from samples of different avian host sources were collected from nine farms, six residential areas and 21 fields: wild passerine birds, n-120; pigeon, n-28 and poultry, n-23 (Table 1,3). After capture, each bird was housed separately in a cloth bag to reduce stress until sampled. The cloacal samples and,or the swabs of fresh droppings were collected by gently swirling the cloaca with a sterile cotton swab. Swabs were stored in Amies transport medium (Difco co., Italy) and kept cooled in an ice box containing frozen gel packs (for a maximum of 72h) until transferred to the laboratory for microbiological investigations. All captured birds were identified to species level, and type of dispersal movements were classified as resident, summer visitor, winter visitor, and passage migrant from the literature (Porter, 2010).

ISOLATION AND IDENTIFICATION OF BACTERIA

In the laboratory, swabs were transferred to nutrient broth (NB) and incubated at 37° C for 24h. Subsequently, the samples were cultured on MacConkey agar (Difco co., Italy) and Eosin Methylene Blue (EMB) agar and incubated at 37° C for 24h to selectively isolate Gram-negative and enteric bacteria, respectively. For isolation of *Salmonella* and *Shigella*, Gram-negative samples were plated on Hektoen enteric agar (Difco co., Italy) under microaerobic conditions. Furthermore, all pure colonies were identified based on standard microbiologic techniques including Gram staining and cell morphology (Merchant and Packer 1967). Bacterial isolates were further subjected to conventional biochemical tests

using the Microgen™ kit according to manufacturer's instructions. The results were then checked using the 122 Microgen Identification System (MID-60) software that shows the most probable organism among a set in a database.

VIRULENCE GENES

Virulence genes, *hlyA, fimH, AFA, cnf1, aer* and *pape,* were detected using conventional PCR tests. Also, to improve the accuracy of detecting virulence genes positive and negative control strain were used. PCR test was performed in two sets, each for detection of three genes and PCR test was performed in two sets, each for detection of three genes. Briefly, 25-μl reaction mixture was used for PCR assays contained 12.5 μl of 2× Master Mix Red, 1 μl of each primer F&R and 2μl of DNA template. An initial activation step at 95˚C for 5 min was followed by 30 cycles of denaturation at 94˚C for 60 sec, annealing for 30 sec (PCR 1: 62˚C, PCR 2: 60˚C), and extension at 72˚C (PCR 1: 60 sec, PCR 2: 45 sec), followed by one cycle at 72˚C for 10 min. The products of PCR were assayed by electrophoresis through a 1% agarose gel. The sequence primers used showed in table 5.

STATISTICAL ANALYSIS

The data and number of potentially pathogenic bacteria species in different groups of passerine bird were analyzed using the SPSS software. $P < 0.05$ was considered as level of significance.

RESULTS

A total of 184 passerine birds were sampled as part of this survey. These birds belonged to 32 species from 22 bird genera, representing 12 bird families (Table 2). Family Passeridae was the most frequently captured group (34.2%), and *Passer domesticus* was the most frequent captured species (Table 2).

Bacteria were isolated from 171 out of the 184 sampled passerine birds (92.9%). On average 1.7 different bacterial species were isolated from each bird, with a maximum of four bacterial species retrieved from individual *Sturnus vulgaris, Motacilla alba,* and *Turdus merula* (Table 2). *E. coli* was the most abundant isolated bacterium, isolated from 129 birds, followed by the *Enterobacter* genus that was found in 109 sampled birds (Table 4). Of these, 5.4% were determined to be *E. aerogenes* and 19% *E. cloacae*. Interestingly, the overall prevalence of *Salmonella* spp. was 3.2%, with single detections in *Passer montanus, Passer domesticus, Fringilla coelebs, Pica pica* and *Turdus merula*. *Klebsiella* spp and *Serratia* spp, two potential opportunistic pathogens, were isolated from 11 bird species (Table 4).

At the host family level frequency of bacteria was higher in species belonging to Passeridae (100%; n = 63) Sturnidae (100%; n = 7), Turdidae (100%; n = 6), Motacillidae (100%; n =13), Corvidae (100%; n = 3), compared to birds from families Sylviidae (90.5%; n = 53) and Paridae (84.6% n = 13), Fringillidae (83.3% n=12), Muscicapidae (71.4% n=7); families.

Combination of sample size and prevalence precluded detailed analyzes for most of the investigated bacterial species. For *E. coli*, where data is stronger, the bacterium was obtained from all sampled bird families except the Hirundinidae and Emberizidae families (that were only sparsely sampled), suggesting it was widespread in the local avifauna in the study region. In detail prevalence of *E. coli* was higher in passerine birds belong to Corvidae (100%; n=3) and Sylviidae (90.5%; n=53) and Turdidae (83.3%; *n* = 6) families than in birds of the families Fringillidae (41.6%; *n* = 12), Muscicapidae (42.8%; *n* = 7), Alaudidae (33.3%; n=3), Paridae (38.4%; *n* = 13)

Mean number of potentially zoonotic bacterial species for each family of wild passerine birds based on number of bacteria per individual was 1.9 in Passeridae, 1.6 in Sylviidae, 1.7 in Motacillidae, 1.6 in Fringillidae 1.6 in Hirundinidae, 2.2 in Sturnidae, 1.1 in Muscicapidae, 3.0 in Corvidae, 1.3 in Alaudidae, 1.8 in Turdidae. These differences based on ANOVA Tukey's post hoc test analysis were significant ($p<0.05$, $F= 3.659$, $DF=9$). The summary of isolation of bacteria is shown in Table 4.

TABLE 1. Location of 36 point sampling on which family birds and mean number of bacteria.

Type of area	Decimal point	Common wild bird families caught	mean number of bacterial in wild bird
Farm	36.30806°N, 59.52797°E	Passeridae, Fringillidae, Paridae	2
Residential	36.54361°N, 59.2673°E	Passeridae, Muscicapidae	1.3
Residential	36.81028°N, 59.01389°E	Passeridae, Paridae	2
Field	36.07108°N, 60.21983°E	Sylviidae	1.7
Residential	36.06222°N, 60.22944°E	Passeridae,Sylviidae	2
Field	36.21333°N, 59.51306°E	Sylviidae, Passeridae, Motacillidae , Emberizidae	1.7
Field	36.21361°N, 59.51444°E	Passeridae, Sylviidae, Motacillidae	2.1
Field	36.18028°N, 59.60972°E	Fringillidae , Emberizidae	1.3
Residential	36.18023°N, 59.611666°E	Muscicapidae, Turdidae	1.3
Residential	36.2244°N, 59.5742°E	Sylviidae, Passeridae	1.6
Residential	35.25167°N, 58.47472°E	Corvidae, Paridae, Sylviidae	2.2
Field	35.25167°N, 58.44889°E	Alaudidae	1.5
Field	36.65806°N, 59.6575°E	Passeridae, Sturnidae, Sylviidae	2.2
Field	36.15288°N, 59.55062°E	Passeridae, Motacillidae, Sylviidae, Fringillidae	2.0
Field	35.47148°N, 58.4585°E	Passeridae, Fringillidae, Hirundinidae	1.3
Field	36.38956°N, 59.24233°E	Fringillidae ,Motacillidae	1.6
Field	37.60472°N, 57.95917°E	Fringillidae, Motacillidae	1.5
Field	37.45361°N, 57.32017°E	Sylviidae, Passeridae ,Sturnidae	1.6
Field	37.44722°N, 57.66806°E	Sturnidae,Hirundinidae, Passeridae,Turdidae	1.8
Field	36.15842°N, 59.56521°E	Sylviidae, Passeridae, Paridae , Turdidae	2.1
Farm	36.62687°N, 61.12574°E	Muscicapidae, Sturnidae	1.5
Field	36.41809°N, 61.12059°E	Paridae, Sylviida	2.2
Farm	36.25142°N, 58.86659°E	Paridae, Turdidae	1.5
Farm	36.53333°N, 59.28167°E	Fringillidae, Sylviidae	2
Farm	36.79417°N, 59.01722°E	Passeridae, Motacillidae,	1.6
Farm	36.44638°N, 59.2066°E	Fringillidae, Corvidae	3
Field	36.24419°N, 58.86492°E	Sylviidae, Paridae	1
Farm	35.25251°N, 58.44417°E	Passeridae, Paridae	1
Field	35.47278°N, 58.47694°E	Muscicapidae, Sylviidae	1.2
Field	37.52500°N, 59.11583°E	Motacillidae, Paridae Muscicapida	1.2
Farm	36.13470°N, 59.11583°E	Sturnidae	3
Farm	37.59562°N, 58.81309°E	Sylviidae	0.7
Field	36.47619°N, 59.50489°E	Passeridae, Muscicapidae	1.1
Field	35.24558°N, 59.22142°E	Fringillidae, Sylviidae	1.8
Field	36.10889°N, 58.98917°E	Passeridae, Alaudidae	1.1
Field	36.12450°N, 59.115080°E	Passeridae, Fringillidae	1.6

RESULT OF VIRULENCE GENE

Out of the 182 *E. coli* isolates obtained from 184 wild birds and 53 domestic birds host sources, 145 isolates were positive for at least one of the virulence genes studied, with 88.6% in domestic birds and 76% in wild birds showing a higher proportion of virulence genes in domestic bird than wild birds. In our study, none of the isolates were positive for all of the virulence genes simultaneously**.**

The most frequent gene was *fim*H in domestic and wild birds with 60.3% and 22.4%, respectively. All the tested virulence factors were found in domestic birds isolates, whereas in the wild birds' isolates all but *aer* gene were detected. In detail, *hyl*A and *pap*C genes were predominant in wild and domestic birds. The fragment *afa* was found with the same frequency in all studied isolates. Also, several virulence patterns, *aer/pap*C (1.5%-3.7%), *hyl*A*/fim*H 4% and 25%, *hyl*A*/fim*H*/afa* (1.5%-1%), *fim*H/*cnf*-1 (0-3.7%), *aer/fim*H (0-7%) and *pap*C*/fim*H (0%-2%) were found in wild and domestic birds, respectively. Fig 1 shows the occurrence of virulence genes in the studied isolates.

TABLE 2. Wild passerine birds sampled in north-east of Iran April to September 2018 and 2019. Marked species with stars are the new hosts of Enterobacteriaceae.

Family	Species	dispersal Movment	No. of bird captured	Infected
Paridae (7.06)	*Parus major*	Native resident	13(7.06)	11(6.04)
Passeridae (34.23)	*Passer domesticus*	Native resident	27(14.67)	27(15.78)
	Passer hispaniolensis	winter visitors	6(3.26)	6(3.50)
	*Passer montanus**	Native resident	23(12.5)	23(13.45)
	Passer moabiticus	Native resident	1(0.54)	1(0.58)
	Petronia petronia	Summer visitors	3(1.63)	3(1.75)
	Petronia xanthocollis	Summer visitors	3(1.63)	3(1.63)
Sylviidae (28.80)	*Acrocephalus dumetorum*	Passage migrants	3(1.63)	3(1.75)
	Acrocephalus palustris	Passage migrants	2(1.08)	2(1.16)
	Acrocephalus arundinaceus	Passage migrants	1(0.54)	1(0.58)
	Acrocephalus stentoreus	Summer visitors	2(1.08)	2(1.16)
	Sylvia curruca	Passage migrants	16(8.69)	16(9.35)
	Hippolais pallida	Passage migrants	6(3.26)	6(3.50)
	Sylvia nisoria	Passage migrants	9(4.89)	9(5.26)
	Hyppolais languida	Summer visitors	3(1.63)	2(1.16)
	Phylloscopus neglectus	Passage migrants	6(3.26)	4(2.33)
Motacillidae (13.58)	*Motacilla alba**	Native resident	13(7.06)	13(7.60)
Fringillidae (6.52)	*Fringilla coelebs**	Passage migrants	9(4.89)	7(4.09)
	Carduelis carduelis	Native resident	3(1.63)	3(1.75)
Hirundinidae (1.63)	*Hirundo rustica*	Summer visitors	3(1.63)	2(1.16)
Sturnidae (3.80)	*Acridotheres tristis*	Native resident	1(0.54)	1(0.58)
	Sturnus vulgaris	Native resident	6(3.26)	6(3.50)
Muscicapidae (3.80)	*Muscicapa striata*	Passage migrants	2(1.08)	0(0)
	*Ficedula parva**	Passage migrants	5(2.71)	5(2.92)
Corvidae (1.08)	*Pica pica*	Native resident	3(1.63)	3(1.63)
Alaudidae (1.63)	*Galerida cristata*	Native resident	2(1.08)	2(1.16)
	Calandrella rufescens	Passage migrants	1(0.54)	0(0)
Turdidae (3.26)	*Saxicola torquatus*	Summer visitors	2(1.08)	2(1.16)
	Oenanthe alboniger	Native resident	3(1.63)	3(1.75)
	Turdus merula	Winter visitors	1(0.54)	1(0.58)
Emberizidae (1.08)	*Emberiza bruniceps**	Summer visitors	2(1.08)	2(1.16)
Total			**184**	**171**

DISCUSSION

In this study, various potentially zoonotic bacteria were isolated from wild passerine birds in the northeast of Iran. Among the sampled birds, the groups with the most pathogenic species were Corvidae and Sturnidae, respectively. These birds are common in both urban and rural environments, and often come into close contact with domestic animals and refuse from human origin. A high prevalence of putative pathogenic bacteria in these two families may reflect this association.

E.coli with the 70.1% of sampled birds was the most isolated bacterium. This bacterium is widespread in animals, including in birds close to human settlements, and the result is in agreement with previous studies (Benskin et al., 2009; Matias et al., 2016). *E. coli* was more frequent in Corvidae, Sylviidae, and Turdidae families and not isolated in Emberizidae and Hirundinidae families, from which the sample size was small. Although *E. coli* is commonly identified in surveys of bacterial assemblages in wild birds, the reported prevalence rates vary considerably between different birds studied groups. In a study conducted by Steele et al. in the USA; prevalence of *E. coli* was reported 88% in seabirds (Steele et al., 2005), and in another study from Germany, *E. coli* were isolated from 81% of wild Mallards *Anas platyrhynchos* (Ewers et al., 2009). However, *E. coli* prevalence is considered lower in passerine birds than water birds (Gopee et al., 2000), but in our study, prevalence was 70.1% in passerine birds and retrieved from a large number of species. In similar studies, *E.coli* has been found in 50.5 % of passerines from Brazil. Also, among passerine birds prevalence of *E. coli* is higher in omnivorous or carnivorous than in granivorous bird's families, which is in line with our study (Steele et al., 2005).

TABLE 3. The location, number and frequency of *E. coli* isolated from domestic birds.

Location	Number of domestic birds		Number of *E. coli* (%)
	Pigeon (n=28)	Poultry (n=25)	
36.308°N, 59.527°E	5	4	7 (77.7)
36.626°N, 61.125°E	6	4	8 (80)
36.251°N, 58.866°E	4	4	8 (100)
36.533°N, 59.281°E	4	5	9 (100)
36.794°N, 59.017°E	5	4	9 (100)
36.446°N, 59.206°E	4	4	8 (100)

Salmonella was found in 3.2% *Salmonella* of the sampled birds, with isolation from 5 out of 12 bird families, including detections in *Passer montanus, Passer domesticus, Fringilla coelebs, Turdus merula*, and *Pica pica*. Earlier studies have implied that the prevalence of *Salmonella* varies between the group of birds, including high rates in opportunistic bird species such as gulls (Moré et al., 2017; Antilles et al., 2021), and low in passerine birds (Abulreesh et al., 2007; Tizard, 2004; Matias et al., 2016). Their results are consistent with ours, where the prevalence of *Salmonella* was 3.2%. The different prevalence of *Salmonella* among wild birds may be due to their different habitats, feeding habits, and other biological reasons. For instance, the kelp seagull feeds on plates contaminated with waste and sewage treatment; these places are usually contaminated with pathogenic bacteria (Moré et al., 2017). A study conducted by Söderlund et al., 2019 suggested that salmonellosis disease is promoted in passerine birds that are gregarious and feed on the ground (Söderlund et al., 2019).

In our study, the majority of isolates were identified as *Salmonella* ser. Typhimurium, a serovar important for human health, isolated from humans who came into close contact with wild passerine birds in some European countries, New Zealand, and the U.S.A. (Kapperud et al., 1998; Thornley et al., 2003).

Other isolated bacteria included *Klebsiella* spp, retrieved from *Motacillia alba, Ficedula parva* and *Emberiza bruniceps***.** As an opportunistic pathogen, *Klebsiella* spp., can cause disease in birds as well as in humans. (Liao et al., 2019). In our study, *K. pneumoniae* was isolated from bird families such as Passeridae, Motacillidae, and Sylviidae ,while Munoz et al., isolated *K. pneumoniae* from cattle feces (Munoz et al., 2006) . Another study conducted by Rogers implied that eleven bacterial species had the same API 20E code in cattle and passerine birds, which were on the same farm. The results show that there is a potential for transfer of bacteria from cattle to bird and vice versa (Rogers, 2006).

Enterobacter spp. and *Proteus* spp. were also identified in this study. These species can be isolated from both clinical and environmental sources, and in some cases, can cause disease in humans and animals (Wesevich et al., 2020; Schumacher, 2006). The composition of bacterial species varies among wild passerine birds in different areas, and in most cases, it is challenging to discern whether these differences are due to local conditions (habitat, farming, other anthropogenic sources), environmental stressors (humidity, temperature, etc.), or linked to the host. It has been suggested that interaction between specific farm characteristics of microclimate (Jamieson et al., 2002), including soil properties type, the number of livestock present, and the life history of bird species such as diet or migratory tendencies (Hancock et al., 1998), influence pathogen prevalence. To figure out whether bioclimatic factors play a role in bacterial prevalence, further analysis should be done.

Our data is from an under-sampled region regarding bird-borne pathogens, and although in the form of a pilot study, it shows examples of interesting patterns of pathogenic bacteria that warrants further studies, especially to disentangle whether detection in birds on farms translates to risk for domestic animals and humans at the farm and in the food chain. Moreover, we record new host species for *Salmonella* in Iran, including *Fringilla coelebs, Passer montanus*, and for *Klebsiella* spp. including *Motacillidae alba, Ficedula parva* and *Emberiza bruniceps*.

TABLE 4. Enterobacteriacea from cloacal samples of wild passerine birds in the north-east of Iran. The frequency of Enterobacteriaceae isolated from each bird family sampled is shown.

Bacteria isolated	Isolates from each bird family												
	Pa	Fr	Hi	Mo	Mu	Co	Al	Em	Par	St	Sy	TU	Total
	n=63 (34.23)	n=12 (6.52)	n=2 (1.08)	n=13 (7.06)	n=7 (3.80)	n=3 (1.63)	n=3 (1.63)	n=2 (1.08)	n=13 (7.06)	n=7 (3.80)	n=53 (28.80)	n=6 (3.36)	n=184
Escherichia coli	47 (74.6)	5 (41.6)	0 (0)	9 (69.2)	3 (42.8)	3 (100)	1 (33.3)	0 (0)	5 (38.4)	4 (57.1)	48 (90.5)	5 (83.3)	129 (70.1)
Enterobacter spp.	18 (28.5)	1 (8.3)	2 (66.6)	0 (0)	2 (28.5)	1 (50)	1 (33.3)	0 (0)	1 (7.6)	1 (14.2)	11 (20.7)	2 (33.3)	40 (21.7)
Enterobacter aerogenes	3 (4.7)	1 (8.3)	0 (0)	1 (7.6)	0 (0)	0 (0)	0 (0)	0 (0)	1(7.6)	2 (28.5)	2 (3.7)	0 (0)	10 (5.4)
Enterobacter cloacae	17 (26.9)	1 (8.3)	0 (0)	1 (7.6)	1(14.2)	0 (0)	0 (0)	1 (50)	1 (7.6)	2 (28.5)	10 (18.8)	1 (16.6)	35 (19.0)
Enterobacter hormaechei	5 (7.9)	0 (0)	0 (0)	1 (7.6)	0 (0)	0 (0)	0 (0)	1 (50)	2 (15.3)	2 (28.5)	2 (3.7)	1 (16.6)	14 (7.6)
Hafnia alvei	5 (7.9)	3 (25.0)	1 (33.3)	1 (7.6)	0 (0)	2 (100)	1 (33.3)	0 (0)	0 (0)	0 (0)	2 (3.7)	1 (16.6)	16 (8.6)
Serratia spp	11 (17.4)	3 (25.0)	0 (0)	6 (46.1)	0 (0)	0 (0)	0 (0)	0 (0)	3 (23.0)	2 (28.5)	5 (9.4)	0 (0)	30 (16.3)
Serratia marcescens	7 (11.1)	4 (33.3)	2 (66.6)	0 (0)	1 (14.2)	0 (0)	1 (33.3)	1 (50)	0 (0)	1 (14.2)	5 (9.4)	0 (0)	22 (11.9)
Salmonella typhimurium	3 (4.7)	1 (8.3)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.6)	6 (3.2)
Klebsiella spp	4 (6.3)	0 (0)	0 (0)	1 (7.6)	1 (14.2)	1 (50)	0 (0)	1 (50)	0 (0)	1 (14.2)	2 (3.7)	0 (0)	11 (5.9)
Klebsiella . pneumoniae	2(3.1)	0 (0)	0 (0)	1(7.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(1.8)	0 (0)	4(2.1)
Proteus spp	1 (1.5)	1 (8.3)	0 (0)	2 (15.3)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (14.2)	0 (0)	0 (0)	6 (3.2)
Enterobacteriaceae	63 (100)	10 (83.3)	2 (66.6)	13 (100)	5 (71.4)	3 (100)	2 (66.6)	2 (100)	11 (84.6)	7 (100)	48 (90.5)	6 (100)	171 (92.9)

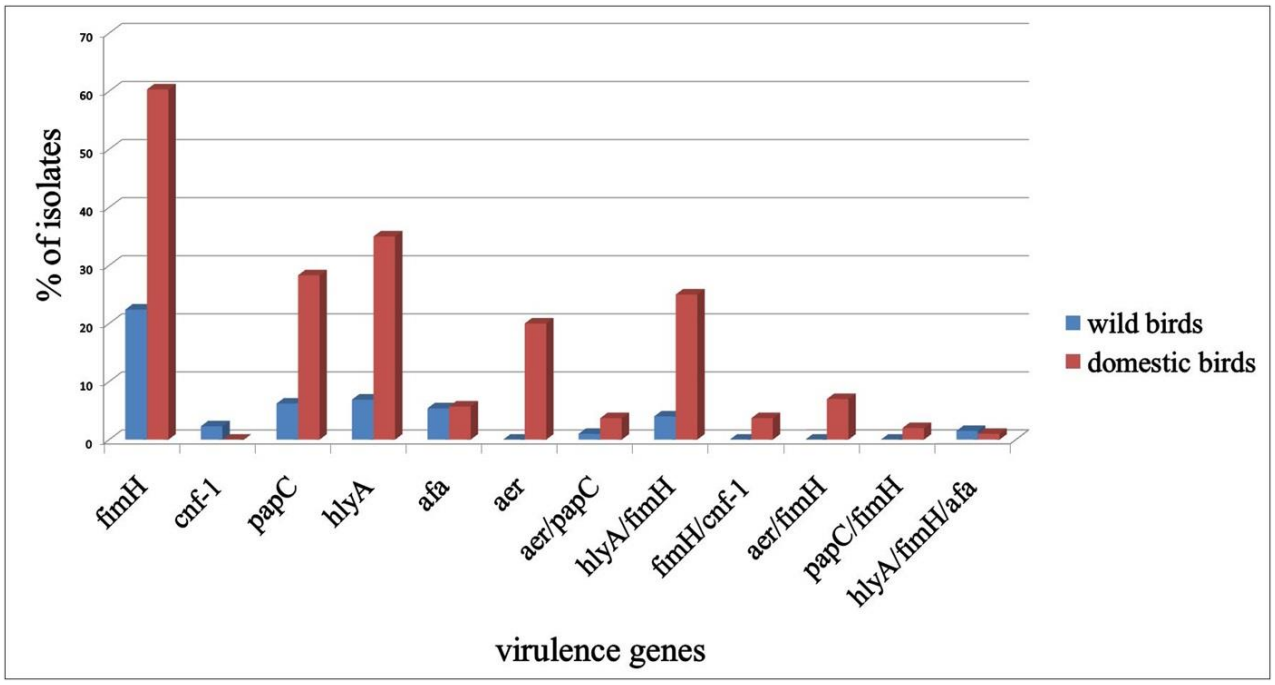

FIGURE 1. Occurrence of virulence genes in the studied isolates.

Virulence genes are important factors that act on synergistically for the growth and survival of microorganisms in the host. Also, they encourage the host to show disease manifestations and increase in pathogenicity (Murugkar et al., 2003). In this study, *E. coli* virulence genes *hly*A, *fim*H, *afa*, *cnf*1, *aer* and *pap*C were detected in wild and domestic birds that are associated with human disease. Among them, *fim*H showed the most prevalent, followed by *hly*A. The *pap*C virulence gene is detected in the *E. coli* strains that cause to urinary infections (Norgren et al., 1987). In our study, all isolates from wild and domestic birds were positive for *pap*C gene, with a medium percentage (34%) in domestic birds and a low percentage (7.2%) in wild birds, which is consistent with the results obtained. by (Capita et al. 2019; Mohamed et al. 2014) that reported a prevalence of 57% of the papC gene in birds' host isolates.

The *cnf*-1 gene, the cytotoxic necrotizing factor, was observed in the strains isolated from domestic birds (3.7%) and in isolates isolated from wild birds with 2.3%. This gene is reported in previous studies in strains of *E. coli* isolated from septicemic poultry (Knobl, 2005) as reported from human and domestic animals with *E. coli* infections (Johnson et al., 2000; Capita et al., 2019). Also, studies were conducted on healthy wild and domesticated pigeons, the cytotoxic necrosis factor 1 was reported in some samples, which showed the potential of spreading the disease by the carriers of these species (Capita et al., 2019; Pedersen et al., 2006**)**.

Our results showed that *pap*C, *hly*A, and *aer* were highly presented among *E. coli* domestic birds compared to wild birds' isolates (figure 1), which is in agreement with the previous study (Ghanbarpour et al., 2011).

Also, out of 182 isolates obtained from wild and domestic birds, 25 isolates showed the occurrence of virulence genes at the same time. As suggested by other authors, these multi-virulent profiles may be related to host adaptation rather than to an increased in virulence (Smoglica et al., 2022). Eventually, as expected and in accordance with the previous studies (Capita et al., 2019), our results showed that there are more virulence genes identified in domesticated birds, which are in close proximity to humans, than in wild birds. However, the occurrence of virulence genes reported in wild birds in previous studies; hence wild birds are considered a threat to human health that could spread microorganisms to the environment (Hughes et al., 2009; La Ragione & Woodward, 2002; Sacristán et al., 2014; Bertelloni et al., 2019).

Considering the limited number of published studies on the occurrence of virulence genes in *E. coli* strains in wild birds, our results show the importance of wild and domestic birds as potential reservoirs and carriers of virulence genes.

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