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Technical Note

Identification of Virulence-Associated Markers in Escherichia Coli Isolated from Captive Red-Browed Amazon Parrot (Amazona Rhodocorytha)

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ABSTRACT

Due to the genetic similarity of pathogenic *Escherichia coli* isolated from birds and pathotypes of human origin, it is suggested that they have a common ancestor and may exchange virulence-associated genes. This study aimed to detect virulence-associated genes in *E. coli* strains isolated from the Red-browed Amazon parrot (*Amazona rhodocorytha*) kept at a conservation institute in Brazil. High genetic variability in virulence was observed, since 12 virulence profiles were found among 14 strains. The number of virulence-associated genes of single strains ranged from 5 to 22 out of 33 genes tested, and only one strain did not present any virulence genes. Regarding adhesion genes, most strains presented from two to five genes, and *crlA* (85.7%) and *fimC* (85.7%) were the most frequent. Frequencies were similar for invasion and iron acquisition genes. Variations among genes were observed for serum resistance and toxin-related genes. Some of the *E. coli* strains isolated from parrots presented virulence genes that are commonly associated with pathotypes of human origin, including newborn meningitis *E. coli*, uropathogenic *E. coli*, and sepsis-associated *E. coli*. It is noteworthy that some of these genes were present in the majority of the analyzed strains. Our results indicate that these strains detected in clinically healthy parrots can be potential reservoirs of several virulence-associated genes. These genes can be transmitted to other *E. coli* strains, including those that affect humans. These *E. coli* strains present a high pathogenic potential of virulence-associated genes in extraintestinal pathogenic *E. coli* strains.

INTRODUCTION

The *Psittacidae* family comprises approximately 350 species, most of which are found in the tropics (British Trust for Ornithology, 2021). Brazil has the greatest diversity of species belonging to this family, ranging from small species such as parakeets to its largest representatives, the macaws (Sigrist, 2006). The Red-browed Amazon (*Amazona rhodocorytha*), also known as Chauá-Parrot, is a species endemic to the Atlantic Forest in eastern Brazil. It is threatened by both habitat loss and trapping for illegal trade (BirdLife International 2021).

Escherichia coli is a part of both the animal and human commensal flora and can be found in several environments. Pathogenic *Escherichia coli* can cause intestinal and extraintestinal infections. Gastrointestinal infections are related to diarrheagenic *E. coli* (DEC), while extraintestinal infections, including sepsis, urinary tract infections and meningitis, are associated with extraintestinal pathogenic *E. coli* (ExPEC) (Cunha *et al.*, 2017; Saka *et al.*, 2019). Despite comprising many lineages, only specific ExPEC isolates are responsible for the majority of infections (Manges *et al.*, 2019). Among the ExPEC isolates, avian pathogenic *E.*

coli (APEC) is responsible for colibacillosis in poultry, an economically important disease that threatens food safety and avian welfare worldwide. (Cunha *et al.*, 2017). Avian colibacillosis is a multifaceted syndrome that can be associated with respiratory disease, septicemia, swollen head syndrome, yolk-sac infection, omphalitis, and cellulitis (Kim *et al.*, 2020; Rocha *et al.*, 2021).

ExPEC strains present a wide range of virulence-associated markers and an important plasticity of the genome. Their virulence traits include adhesins, invasins, iron acquisition and serum resistance factors, toxins, and proteases. These virulence factors are encoded by genes found in pathogenicity islands, plasmids, and other mobile genetic elements (Sarowska *et al.*, 2019). Studies have shown genetic similarity between APEC and pathotypes of human origin, suggesting a common ancestral origin and an ability of potentially pathogenic strains to compromise human health (Ewers *et al.*, 2007).Close similarities in serogroup, virulence factors, phylogenetic group, and sequence type have been shown between APEC and human ExPEC (Cunha *et al.*, 2017), suggesting the important role of APEC strains to be a reservoir of virulence genes for ExPEC strains in humans (Rocha *et al.*, 2017). It is especially important in those species with close contact with humans such as captive birds.

In this context, this study aimed to detect virulence-associated genes in *E. coli* strains isolated from the Red-browed Amazon of a conservation institute in Brazil.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) under protocol number 38772-1. All methods were carried out in accordance with ICMBio relevant guidelines and regulations.

Red-browed Amazon parrot (*Amazona rhodocorytha*)

The birds selected for this study were maintained captive in an enriched vivarium at the National Institute of the Atlantic Forest (Santa Teresa, ES, Brazil). The parrots kept at the Institute come from illegal trade and cannot be returned to their habitat. Husbandry, feed, and water were provided by the employees of the Institute. A total of 14 clinically healthy parrots of different ages and both sexes were randomly selected for this study.

Escherichia coli strains

A total of 14 stool samples were collected using a cloacal swab, individually identified, and stored in a cool box for conservation during transportation to the laboratory. In the laboratory, swabs were individually inoculated into brain heart infusion broth (BHI; Oxoid, Basingstoke, UK) and aerobically incubated at 37 °C. After 24 hr, the content was plated onto eosin methylene blue agar (Oxoid) and aerobically incubated for 24 hr at 37 °C. Colonies morphologically consistent with those of *E. coli* (dark metallic green sheen with a low, convex, and smooth shape) were selected for biochemical tests (Lee *et al.*, 2008). Biochemical tests included Gram stain (Gram negative rods), oxidase (negative), indol (positive), H_2S production (negative), glucose (positive), lactose (positive), lysine (positive), and urease (negative). The confirmed isolates were stored at -20 °C in BHI broth supplemented with 15% glycerin (Synth, Diadema, Brazil) until use. Isolates were reactivated on MacConkey agar (Oxoid) and incubated at 37 °C for 24 hr. A bacterial colony was solubilized in 200 µL ultra-pure water for DNA extraction.

Detection of virulence-associated genes

DNA extraction was carried out by heat treatment as described by Borsoi *et al.* (2009) and stored at -20 °C until use. Isolates were screened for the presence of 33 virulence genes using four multiplex PCRs (Ewers *et al.*, 2007): multiplex-1 (*kpsMTII, hlyA, pic, fimC, hrlA, iha, neuC, afa/draB, malX*, and *sfa/foc*), multiplex-2 (*chuA, ibeA, traT, sitD chr., gimB, iroN*, *ompA*, and *sitD ep.*), multiplex-3 (*EAST-1, iss, irp2, papC, cvi/cva, iucD, tsh,* and *vat*), and multiplex-4 (*crlA, ireA, cnf1/2, tia, sat, fyuA,* and *mat*). Genes functions, primer sequences, and references are listed in Table 1. All PCR amplifications were performed in a mixture (25 µL) consisting of 10 × PCR Buffer [3 µL; 200 mM Tris–HCl (pH 8.4), 500 mM KCl] (2.5 µL), Taq thermostable DNA polymerase (0.3 µL, 5U/µL), $MgCl_2$ (1.25 µL, 25 mM), dNTPs (0.5 µL each, 2.5 mM), extracted template DNA (5 µL) and each of the primers (2 µL each, 20 pmol/L). Sterile Milli-Q water was added in sufficient quantity to achieve a volume of 25 µL. Reaction mixtures were subjected to the following conditions in a thermal cycler (Biocycler Peltier Thermal Cycler MJ96+/MJ96G, Hercules, USA): 3 min at 94 °C, 25 cycles of 30 s at 94 °C, 30 s at 58 °C, and 3 min at 68 °C, with a final cycle of 10 min at 72 °C, followed by a hold at 10 °C (Ewers *et al.*, 2007). For visualization of the PCR products, 10 µL aliquots were subjected to electrophoresis in a 2% agarose gel in Tris–acetylated EDTA buffer. DNA

Table 1 – Genes: functions, primers sequences, and primer references.

Function-related group	Gene	Primer (5′ → 3′)	Amplicon size (bp)	Primer reference
Adhesion	*afa/draB*	F: TAAGGAAGTGAAGGAGCGTG R: CCAGTAACTGTCCGTGACA	810	Ewers *et al.*, 2007
	crlA	F: TTTCGATTGTCTGGCTGTATG R: CTTCAGATTCAGCGTCGTC	250	Maurer *et al.*, 1998
	fimC	F: GGGTAGAAAATGCCGATGGTG R: CGTCATTTTGGGGGTAAGTGC	477	Janssen *et al.*, 2001
	hrlA	F: TCACTTGCAGACCAGCGTTTC R: GTAACTCACACTGCTGTCACCT	537	Ewers *et al.*, 2007
	iha	F: TAGTGCGTTGGGTTATCGCTC R: AAGCCAGAGTGGTTATTCGC	609	Ewers *et al.*, 2007
	papC	F: TGATATCACGCAGTCAGTAGC R: CCGGCCATATTCACATAAC	501	Janssen *et al.*, 2001
	sfa/focD	F: GTCCTGACTCATCTGAAACTGCA R: CGGAGAACTGGGTGCATCTTA	1242	Ewers *et al.*, 2007
	tsh	F: ACTATTCTCTGCAGGAAGTC R: CTTCCGATGTTCTGAACGT	824	Ewers *et al.*, 2007
	mat	F: TATACGCTGGACTGAGTCGTG R: CAGGTAGCGTCGAACTGTA	899	Ewers *et al.*, 2007
Invasion	*gimB*	F: TCCAGATTGAGCATATCCC R: CCTGTAACATGTTGGCTTCA	736	Ewers *et al.*, 2007
	ibeA	F: TGGAACCCGCTCGTAATATAC R: CTGCCTGTTCAAGCATTGCA	342	Ewers *et al.*, 2007
	tia	F: AGCGCTTCCGTCAGGACTT R: ACCAGCATCCAGATAGCGAT	512	Ewers *et al.*, 2007
Iron aquision	*chuA*	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	278	Clermont *et al.*, 2000
	fyuA	F: GCGACGGGAAGCGATGACTTA R: CGCAGTAGGCACGATGTTGTA	774	Schubert *et al.*, 2002
	ireA	F: ATTGCCGTGATGTGTTCTGC R: CACGGATCACTTCAATGCGT	384	Ewers *et al.*, 2007
	iroN1	F: ATCCTCTGGTCGCTAACTG R: CTGCACTGGAAGAACTGTTCT	847	Ewers *et al.*, 2007
	irp2	F: AAGGATTCGCTGTTACCGGAC R: TCGTCGGGCAGCGTTTCTTCT	413	Schubert *et al.*, 2002
	iucD	F: ACAAAAAGTTCTATCGCTTCC R: CCTGATCCAGATGATGCTC	714	Janssen *et al.*, 2001
	sitD chr.	F: ACTCCCATACACAGGATCTG R: CTGTCTGTGTCCGGAATGA	554	Ewers *et al.*, 2007
	sitD ep.	F: TTGAGAACGACAGCGACTTC R: CTATCGAGCAGGTGAGGA	1052	Ewers *et al.*, 2007
Serum resistance	*cvi/cva*	F: TCCAAGCGGACCCCTTATAG R: CGCAGCATAGTTCCATGCT	598	Ewers *et al.*, 2007
	iss	F: ATCACATAGGATTCTGCCG R: CAGCGGAGTATAGATGCCA	309	Ewers *et al.*, 2007
	neuC	F: GGTGGTACATTCCGGGATGTC R: AGGTGAAAAGCCTGGTAGTGTG	676	Ewers *et al.*, 2007
	kpsMT II	F: GCGCATTTGCTGATACTGTTG R: CATCCAGACGATAAGCATGAGCA	280	Genbank accession number X53819
	ompA	F: AGCTATCGCGATTGCAGTG R: GGTGTTGCCAGTAACCGG	919	Ewers *et al.*, 2007
	traT	F: GTGGTGCGATGAGCACAG R: TAGTTCACATCTTCCACCATCG	430	Ewers *et al.*, 2007
Toxins	*cnf1/2*	F: TCGTTATAAAATCAAACAGTG R: CTTTACAATATTGACATGCTG	446	Ewers *et al.*, 2007
	sat	F: TGCTGGCTCTGGAGGAAC R: TTGAACATTCAGAGTACCGGG	667	Ewers *et al.*, 2007
	astA	F:TGCCATCAACACAGTATATCC R: TAGGATCCTCAGGTCGCGAGTGACGGC	116	Yamamoto *et al.*, 1996
	vat	F: TCCTGGGACATAATGGTCAG R: GTGTCAGAACGGAATTGTC	981	Ewers *et al.*, 2007
	hlyA	F: GTCCATTGCCGATAAGTTT R: AAGTAATTTTTGCCGTGTTTT	352	Ewers *et al.*, 2007
Autotransporter serine protease	*pic*	F: ACTGGATCTTAAGGCTCAGG R: TGGAATATCAGGGTGCCACT	409	Ewers *et al.*, 2007
Pathogenicity island associated gene	*malX*	F: GGACATCCTGTTACAGCGCGCA R: TCGCCACCAATCACAGCCGAAC	922	Johnson *et al.*, 2001

Legend: Base pair (bp); Forward (F); Reverse (R).

bands were stained with ethidium bromide for 2 hr at 100 V, viewed under an ultraviolet transilluminator, and photographed. Positive controls included four international *E. coli* reference strains [IMT5155 (APEC), IMT2470 (APEC), IMT7920 (uropathogenic *E. coli* – UPEC), and IMT9267 (neonatal meningitis *E. coli* – NMEC)] and three APEC strains isolated from broiler sources that belong to our stock collection (TK3, CC192, and CC158). Negative control included a mixture of all constituents of the PCR mixed without the addition of extracted DNA.

Statistical Analysis

The obtained data were subjected to statistical analysis using the PASW Statistics software. Descriptive statistics were used to determine the frequency distribution. Chi-square and Fisher's exact tests were used to compare the frequencies of virulence-associated genes. Statistical significance was defined at $p<0.05$ and Bonferroni correction was applied to adjust the confidence intervals for multiple hypothesis testing.

RESULTS AND DISCUSSION

Previous studies have shown that there are differences in the gut microbiota between captive and wild birds. Wild birds usually have a small number of Gram-negative bacteria in the gut microbiota, most of which are transient in healthy birds (Lopes *et al.*, 2016). In contrast, captive birds usually present abundant amounts of *E. coli* in their microbiota (Saidenberg *et al.*, 2017). In these birds, intestinal colonization by *E. coli* is dependent on the animal's health status as well as the environment in which they are kept, because the birds that live in the same enclosure usually use the same feeders and drinkers (Lopes *et al.*, 2016).

The frequency of detection of virulence-associated genes according to their function is shown in Table 2. *E. coli* was present in all parrots. However, the detection of this microorganism does not characterize enteric disturbance or disease in the animal since the birds appeared healthy (Calaça *et al.*, 2020). Of the 33 genes analyzed, only four genes were not detected (*tsh*, *ireA*, *sitD ep.*, *astA*). The number of virulence-associated genes of single strains ranged from 5 to 22, except for one strain (#5) that did not contain any virulence genes. Our results indicate high genetic variability in virulence since we found a total of 12 virulence profiles among 14 strains (Table 3). Profiles I and VII represent two strains each. In Profile XII, no virulence gene was detected. In Profile XI, only five genes were detected, and VI is the profile with the highest number of detected genes (22 of 33 genes). Other profiles presented, from 9 to 19 virulence markers.

Considering the nine genes associated with adhesion (*afa/drab, crlA, fimC, hrlA, iha, papC, sfa/focD, tsh,* and *mat*), only one strain (#5) did not present any gene; other strains presented from two to five genes. The presence of *crlA* (85.7%) and *fimC* (85.7%) genes was significantly higher ($p<0.007$) than *afa/draD* (21.4%), *hrlA* (14.3%), *iha* (14.3%), *papC* (14.3%), and *sfa/focD* (7.1%). Invasion genes (*gimB, ibeA,* and *tia*) presented similar ($p>0.05$) frequencies of detection among strains, and two strains (#5 and #14) did not present any genes. Only two strains (#5 and #11) did not present any iron acquisition genes (*chuA, fyuA, ireA, iroN1, irp2, iucD, sitD chr.,* and *sitD ep.*) and the frequencies of detection were similar ($p>0.05$) among the strains. The presence of serum resistance genes *cvi/cva* (78.6%) and *ompA* (85.7%) was significantly higher ($p<0.008$) than *iss* (21.4%). Two strains (#5 and #14) did not present any toxin-related genes. The *cnf1/2* (85.7%) gene showed significantly higher ($p<0.010$) detection than *sat* (28.6%). The autotransporter serine protease associated gene (*pic*) and the pathogenicity island associated gene (*malX*) were not detected in eight (#1, #2, #4, #5, #9, #10, #12, and #14) and three (#5, #11, and #14) strains, respectively. It is likely that the absence of some specific genes does not reflect a decrease in virulence, since they may be replaced by other functionally related groups (Beceiro *et al.*, 2013).

Of the 33 analyzed genes, 12 genes were frequently foundin *E. coli* isolated from birds (*fimC, papC, crlA, ibeA, iucD, iroN1, sitD ep., sitD chr., traT, iss, cvi/cva, vat*). However, the others are commonly found in *E. coli* of human origin, including newborn meningitis *E. coli* (NMEC), uropathogenic *E. coli* (UPEC), and sepsis-associated *E. coli* (SEPEC) (Sarowska *et al.*, 2019). Some of these genes were present in the majority of the analyzed strains, including *cnf1/2*(85.7%)*, neuC* (71.4%)*, mat* (71.4%)*, kpsMTII* (64.3%)*,* and *chuA* (57.1%). According to Robins-Browne *et al.* (2016), the mobility of most of the genes that encode virulence is common and may often occur. However, our results indicate that these strains detected in clinically healthy parrots may be opportunistic pathogens and may cause disease in immunosuppressed birds (Calaça *et al.*, 2020). It is a concerning result, since poultry may be a vehicle or even a reservoir for human ExPEC strains, and can be considered potential zoonotic agents (Ewers *et al.*, 2007; Sarowska *et al.*, 2019). This is of special concern in captive parrots because of their direct contact with humans.

Table 2 – Relative (%) and absolute frequencies (n) of virulence genes, according to the function-related groups, and individual results.

Function-related group	Gene	Frequencies % (n)[1]	Strain identification													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Adhesion	*afa/draB*	21.4 (3)[b]	1	1	0	0	0	0	0	0	0	1	0	0	0	0
	crlA	85.7 (12)[a]	1	1	1	1	0	1	1	1	1	1	0	1	1	1
	fimC	85.7 (12)[a]	1	1	1	1	0	1	1	1	1	1	0	1	1	1
	hrlA	14.3 (2)[b]	0	0	0	0	0	0	1	0	0	0	1	0	0	0
	iha	14.3 (2)[b]	0	0	0	0	0	0	1	0	0	0	1	0	0	0
	papC	14.3 (2)[b]	0	0	1	0	0	0	0	0	0	1	0	0	0	0
	sfa/focD	7.1 (1)[b]	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	tsh	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	mat	71.4 (10)[a]	1	1	1	1	0	0	1	1	1	1	0	1	0	1
	Total of detected genes		*4*	*4*	*4*	*3*	*0*	*2*	*5*	*3*	*3*	*5*	*3*	*3*	*2*	*3*
Invasion	*gimB*	21.4 (3)[a]	1	1	0	0	0	0	0	0	0	0	1	0	0	0
	ibeA	50.0 (7)[a]	0	0	1	0	0	1	1	1	1	0	0	1	1	0
	tia	57.1 (8)[a]	0	0	1	1	0	1	0	1	1	1	0	1	1	0
	Total of detected genes		1	1	2	1	0	2	1	2	2	1	1	2	2	0
Iron aquision	*chuA*	57.1 (8)[a]	0	0	1	1	0	1	1	1	1	0	0	1	1	0
	fyuA	42.9 (6)[a]	0	0	1	0	0	1	0	1	1	0	0	1	1	0
	ireA	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	iroN1	42.9 (6)[a]	0	0	1	0	0	1	1	1	0	1	0	0	0	1
	irp2	42.9 (6)[a]	0	0	1	0	0	1	0	1	1	0	0	1	1	0
	iucD	57.1 (8)[a]	1	1	1	1	0	0	1	1	1	0	0	1	0	0
	sitD chr.	35.7 (5)[a]	1	1	0	1	0	1	0	0	0	1	0	0	0	0
	sitD ep	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total of detected genes		*2*	*2*	*5*	*3*	*0*	*5*	*3*	*5*	*4*	*2*	*0*	*4*	*3*	*1*
Serum resistance	*cvi/cva*	78.6 (11)[a]	1	1	1	1	0	0	1	1	1	1	1	1	1	0
	iss	21.4 (3)[b]	0	0	0	0	0	1	1	1	0	0	0	0	0	0
	neuC	71.4 (10)[ab]	1	1	1	1	0	1	0	1	1	0	1	1	1	0
	kpsMTII	64.3 (9)[ab]	1	1	1	1	0	0	1	1	1	1	0	1	0	0
	ompA	85.7 (12)[a]	1	1	1	1	0	1	1	1	1	1	0	1	1	1
	traT	42.9 (6)[ab]	0	0	0	0	0	1	1	1	1	0	0	1	1	0
	Total of detected genes		*4*	*4*	*4*	*4*	*0*	*4*	*5*	*6*	*5*	*3*	*2*	*5*	*4*	*1*
Toxins	*cnf1/2*	85.7 (12)[a]	1	1	1	1	0	1	1	1	1	1	1	1	1	0
	sat	28.6 (4)[b]	0	0	0	1	0	1	0	1	0	1	0	0	0	0
	astA	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	vat	50.0 (7)[ab]	0	0	1	0	0	1	1	1	1	0	0	1	1	0
	hlyA	42.9 (6)[ab]	0	0	0	0	0	0	1	1	1	1	1	1	0	0
	Total of detected genes		*1*	*1*	*2*	*2*	*0*	*3*	*3*	*4*	*3*	*3*	*2*	*3*	*2*	*0*
Autotransporter serine protease	*pic*	42.9 (6)	0	0	1	0	0	1	1	1	0	0	1	0	1	0
Pathogenicity island associated gene	*malX*	78.6 (11)	1	1	1	1	0	1	1	1	1	1	0	1	1	0

[1] Different letter indicates significant differences among genes within function-related group (Fisher's exact test; adjusted *p*-value).

Table 3 – Virulence profiles identified among *Escherichia coli* strains (n=14).

Profile identification	Detected genes	Strain identification
I	*afa/draB, crlA, fimC, mat, gimB, iucD, sitD chr., cvi/cva, neuC, kpsMT, ompA, cnf1/2, malX*	1 and 2
II	*crlA, fimC, papC, mat, beA, tia, chuA, fyuA, iroN1, irp2, iucD, cvi/cva, neuC, kpsMT, ompA, malX*	3
III	*crlA, fimC, mat, tia, chuA, iucD, sitD chr., cvi/cva, neuC, kpsMT, ompA, cnf/12, sat, malX*	4
IV	*crlA, fimC, ibeA, tia, chuA, fyuA, iroN1, irp2, sitD chr., iss, neuC, ompA, traT, cnf1/2, sat, vat, pic, malX*	6
V	*crlA, fimC, hrlA, iha, mat, ibeA, chuA, iroN1, iucD, cvi/cva, iss, kpsMT, ompA, traT, cnf1/2, vat, hlyAm pic, malX*	7
VI	*fimC, hrlA, mat, ibeA, tia, chuA, fyuA, iroN1, irp2, iucD, cvi/cva, iss, neuC, kpsMT, ompA, traT, cnf1/2, sat, vat, hlyA, pic, malX*	8
VII	*crlA, fimC, mat, ibeA, tia, chuA, fyuA, irp2, iucD, cvi/cva, neuC, kpsMT, ompA, traT, cnf1/2, vat, hlyA, malX*	9 and 12
VIII	*afa/draB, crlA, fimC, papC, mat, tia, iroN1, sitD chr., cvi/cva, kpsMT, ompA, cnf1/2, sat, hlyA, malX*	10
IX	*hrlA, iha, sfa/focD, gimB, cvi/cva, neuC, cnf1/2, hlyA, pic*	11
X	*crlA, fimC, ibeA, tia, chuA, fyuA, irp2, cvi/cva, neuC, ompA, traT, cnf1/2, vat, pic, malX*	13
XI	*crlA, fimC, mat, iroN1, ompA*	14
XII	No virulence genes detected	5

The strains showed high genetic variability in virulence. Our results indicate that these strains detected in clinically healthy parrots can be potential reservoirs of several virulence-associated genes and can be transmitted to other *E. coli* strains, including those that affect humans (UPEC, NEMEC, and SEPEC). These *E. coli* strains present a high pathogenic potential of virulence-associated genes in ExPEC strains. The use of techniques to group similar bacteria together is helpful to understand the distribution of virulence markers. Further studies can include the whole genome sequencing (WGS) of *E. coli* strains. The combination of WGS techniques and clinical and epidemiological data would allow the determination of which strains within a subtype are more virulent than others.

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REFERENCES

Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clinical Microbiology Reviews 2013;26:185–230.

BirdLife International. Data aone - red-browed Amazon- *Amazona rhodocorytha*. 2021. [cited 2021 Jan 24]. Available from: http://datazone.birdlife.org/species/results?cmn=&cty=0&fam=0&gen=0&kw=parrots&reg=0&spc=&thrlev1=&thrlev2=&so=cn.

Borsoi A, Santin E, Santos LR, Salle CTP, Moraes HLS, Nascimento VP. Inoculation of newly hatched broiler chicks with two Brazilian isolates of Salmonella Heidelberg strains with different virulence gene profile, antimicrobial resistance and pulsed field gel electrophoresis pattern to intestinal changes evaluation. Poultry Science 2009;88:750-8.

British Trust for Ornithology. Bird facts species group: Psittacidae – Parrots. [cited 2021 Jan 14]. Available from: https://www.bto.org/understanding-birds/birdfacts/bird-families/parrots.

Calaça KL, Cervi RC, Reis SA, Nunes IA, Jayme VS, Andrade MA. Occurrence of *Escherichia coli* in captive psittaciformes: antimicrobial susceptibility and virulence genes. Ciência Animal Brasileira 2020;21:1-12.

Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Applied and Environmental Microbiology Journal 2000;66:4555-8.

Cunha MPV,Saidenberg AB, Moreno AM, Ferreira AJP, Vieira MAM, Gomes TAT, *et al.* Pandemic extra-intestinal pathogenic Escherichia coli (ExPEC) clonal group O6-B2-ST73 as a cause of avian colibacillosis in Brazil. PLoS One 2017;12:e0178970.

Ewers C, Li G, Wilking H, Kieβling S, Alt K, Antáo EM, *et al.* Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: how closely related are they? International Journal of Medical Microbiology 2007;297:163-76.

Janssen T, Schwarz C, Preikschat P, Voss M, Philipp HC, Wieler LH. Virulence-associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having died from colibacillosis. International Journal of Medical Microbiology 2001;291:371–8.

Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. The Journal of Infectious Diseases 2000;181:261–72.

Kim YB, Yoon MY, Ha JS, Seo KW, Noh EB, Son SH, *et al.* Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis. Poultry Science 2020;99(2):1088-95.

Lee MD, Nolan LK, Dufour-Zavala L. Colibacillosis. In: Dufour-Zavala L, editor. A laboratory manual for the isolation, identification and characterization of avian pathogens. Georgia: AAAP; 2008. p.10-11.

Lopes ES,Maciel WC, Teixeira RSC, Albuquerque AH, Vasconcelos RH,Machado DN, *et al.* Isolation of *Salmonella* spp. and *Escherichia coli* from psittacine: public health importance. Arquivos do Instituto Biológico 2016;83:1-10.

Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clinical Microbiological Reviews 2019;32:e00135-18.

Maurer JJ, Brown TP, Steffens WL, Thayer SG. The occurrence of ambient temperature-regulated adhesins, curli, and the temperature-sensitive hemagglutinin *tsh* among avian *Escherichia coli*. Avian Disease 1998;42:106–18.

Robins-Browne RM, Holt KE, Ingle DJ, Hocking DM, Yang J, Tauschek M. Are *Escherichia coli* pathotypes still relevant in the era of whole-genome sequencing? Frontiers in Cellular and Infection Microbiology 2016;6:141.

Rocha DT, Salle FO, Borges KA, Furian TQ, Nascimento VP, Moraes HLS, *et al.* Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC): characterization and comparison. The Journal of Infection in Developing Countries 2021;15(7):962-71.

Rocha SLS, Furian TQ, Borges KA, Rocha DT, Moraes HLS, Salle CTP, *et al.*Classification of avian pathogenic *Escherichia coli* (APEC) and human uropathogenic *Escherichia coli* (UPEC) in phylogenetic groups and association with pathogenicity *in vivo*. Acta Scientiae Veterinariae2017;45:1-8.

Saidenberg ABS, Knöbl T, Melville PA, Zuniga E, Salaberry SRS, Benites NB, *et al.* An atypical enteropathogenic *Escherichia coli* isolate with possible human and domestic animal origin from a free-living Lear's Macaw (*Anodorhynchusleari*). Journal of Wildlife Disease 2017;53:396–8.

Saka HK, Dabo NT, Muhammad B, García-Soto S, Ugarte-Ruiz M, Alvarez J. Diarrheagenic Escherichia coli pathotypes from children younger than 5 years in Kano State, Nigeria. Front Public Health 2019;7:348.

Sarowska J, Koloch BF, Kmiecik AJ, Madrzak MF, Ksiazczyk M, Ploskonska GB, *et al.* Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathogens 2019;11:1-16.

Schubert S, Picard B, Gouriou S, Heesemann J, Denamur E.*Yersinia* high-pathogenicity island contributes to virulence in *Escherichia coli* causing extraintestinal infections. Infection and Immunity 2002;70:5335-7.

Sigrist T. Birds of Brazil: an artistic view. 2nd ed. São Paulo: Avis Brasilis; 2006.

Tejkowski TM. Uso de redes neurais artificiais para classificação da patogenicidade de Escherichia coli de origem aviária [dissertation]. Porto Alegre (RS): Universidade Federal do Rio Grande do Sul; 2013.

Yamamoto T, Echeverria P. Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E. coli* strains pathogenic for humans. Infection and Immunity 1996;64:1441-5.