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Original Article

Diverse *Escherichia coli* pathovars of phylogroups B2 and D isolated from animals in Tunisia

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Abstract

Introduction: The virulent *Escherichia coli* strains responsible for extraintestinal infections were mainly belonged to B2 and D phylogroups. However, no past studies have determinate via the presence of virulence genes the frequency of *E. coli* pathovars recovered from animals housed in farms in Tunisia. The aims of this study were to investigate 26 *E. coli* isolated from healthy and diarrheic animals and to determinate via the presence of virulence genes the frequency of pathovars.

Methodology: Twenty-six *E. coli* isolates of phylogroups B2 (n = 14), $B2_2$ (n = 9), $B2_3$ (n = 5), and D_2 (n = 12) were characterized. Genes encoding virulence factors (*fimH*,*eaeA*,*aggC*,*papC*, *papG* allele III, *hlyA*, *east1*, *cnf1, exhA,stx1*, *stx2*, *iutA, fyuA*, *ibeA*,and *ipaH*), and antibiotic resistance as well as class 1 and 2 integrons were searched by polymerase chain reaction (PCR). The genetic relationship of isolates was done by PFGE.

Results: According to the occurrence of specific genes the 26 isolates were classified as:9 EAEC, 2 EHEC**,** 4 UPEC, 3 EPEC/EHEC and 1 NTEC. Therefore, 2 Ex-PEC and 5 APEC were presented amongst our strains. Some isolates (12) were clonal and the remaining was unrelated.

Conclusions: Higher diversity of pathovars which carried diverse combinations of virulence genes in healthy isolates. In addition, it seems that the infections were caused by different mechanisms.

Key words: *Escherichia coli*; virulence genes; genetic diversity; pathovars.

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Introduction

The majority of *Escherichia coli* bacterial populations are harmless commensals of mammals [1]. However, in some conditions, they can cause either intestinal or extraintestinal infections. Manifestation of clinical symptomatology and pathology appears to be closely associated with the possession of certain virulence gene combinations that have a range of functions, including toxin production, attachment/invasion, and immune evasion [2-5].

Diarrheagenic *E. coli* strains are classified into six major groups: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffusely adhering *E. coli* (DAEC) [6-8]. Extraintestinal pathogenic *E. coli* (ExPEC) strains are divided into three major pathotypes: uropathogenic (UPEC) strains that cause urinary tract infections (UTIs), neonatal meningitis (MENEC), and necrotoxigenic strains (NTEC) that cause septicemia [9]. ExPEC strains possess virulence gene combinations distinctive from those found in their counter parts that cause intestinal diseases [10].

In poultry farms, another pathotype of ExPEC avian pathogenic *E. coli* (APEC) strains can cause colibacillosis, which responsible for the mortality of 3%–4% of the animals on a farm, and for a 2%–3% reduction in egg production [11].

Phylogenetic analysis has shown that *E. coli* comprises four main phylogenetic groups: A, B1, B2, and D. Strains belonging to groups A and B1 are found primarily in the commensal flora. However, pathogenic strains associated with severe acute diarrhea or extraintestinal infections mainly belong to B2 and D phylogroups [12].

E. coli isolated from animals with multiple antibiotic-resistant phenotypes have been reported in Tunisia and worldwide [13,14]. This situation has

resulted in a need for more epidemiological information on the prevalence of resistance to various antibiotics and their relevant genes, such as virulence gene combinations in animal isolates.

The aims of this study were to determinate the frequency of the occurrence of potentially pathogenic *E. coli* strainsbelonging to B2 and D2 phylogroups isolated from healthy and diseased animals in Tunisia, and to detect their virulotypes and their genetic relationship.

Methodology

Bacterial isolates collection

A total 116 *E. coli* isolates from healthy and diseased animals (chickens, bovines, and ovines) were recovered from different farms located in nine different governorates in Tunisia between September 2009 and March 2012. Isolates were from poultry feces (n = 61), oral swabs and different organs (n = 13), bovine feces (n = 27), ovine feces (n = 6), and poultry meat (n = 9). Two grams of each fecal sample were homogenized with 2 mL of brain-heart infusion broth, spread onto MacConkey agar plates, and incubated overnight at 37°C.

Table 1. List of 15 virulence genes used in this study to identify the different *E. coli* pathotypes associated with human and animal diseases.

Virulence gene/activity	Primer name	Oligonucleotide sequence (5'→3')	Amplicon size (pb)	Description/function	*E. coli* pathotype
Adhesins					
fimH	fimH-F fimH-R	TGCAGAATGTGCGCCGCGTGG GCAGTCACCTGCCCTCCGGTA	508	D-mannose-specific adhesin, type 1 fimbriae	ExPEC
aggC	aggC-F aggC-R	GCCAAGATCCGAGATTGA TATTAAACCGATGGTAGCG	528	Fimbrialantigen-specificgene	EAEC
eaeA	eaeA-F eaeA-R	GACCCGGCACAAGCATAAGC CCACCTGCAGCAACAAGAGG	384	Intimin	EPEC, EHEC
papC	papC-F papC-R	GTGGCAGTATGAGTTAATGACCGTTA ATATCCTTTCTGCAGGGATGCAATA	200	Pilus assembly, central region of *pap* operon	ExPEC
*papG*allele III	allele III-F allele III-R	GGCCTGCAATGGATTTACCTGG CCACCAAATGACCATGCCAGAC	258	Cystitis-associated (*prs*or *pap-2*) *papG*variant	ExPEC
Toxins					
cnf1	cnf1-F cnf2-R	AAGATGGAGTTTCCTATGCAGGAG CATTCAGAGTCCTGCCCTCATTATT	498	Cytotoxic necrotizing factor 1	ExPEC, NTEC
east1	east 11a east 11b	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	111	EaggEC heat-stable enterotoxin	EAEC
exhA	ehxA-F ehxA-R	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGTTAAGCT	534	Enterohemolysin	EPEC, EHEC
hlyA	hly-F hly-R	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177	α-hemolysin	ExPEC
stx1	stx1-F stx1-R	GGCACTGTCTGAAACTGCTCC TCGCAGTTATCTGACATTCTG	255	Shiga toxin I	EHEC
stx2	stx2-F stx2-R	ATAAATCGCCTATCGTTGACTAC AGAACGCCCACTGAGATCATC	180	Shiga toxin II	EHEC
Siderophores					
fyuA	fyuA-F fyuA-R	TGATTAACCCCGCGACGGGAA CGCAGTAGGCACGATGTTGTA	880	*Yersinia* siderophore receptor (ferric yersiniabactin uptake)	ExPEC
iutA	aerJ-F aerJ-R	GGCTGGACATCATGGGACTGG CGTCGGGAACGGGTAGAATCG	300	Ferric aerobactin receptor (iron uptake/transport)	ExPEC, UPEC
Invasins					
ipaH	ipaHIII ipaHIV	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGATAC	600	Invasion plasmidantigen	EIEC
ibeA	ibe10-F ibe10-R	AGGCAGGTGTGCGCCGCGTAC TGGTGCTCCGGCAAACCATGC	170	Invasion of brain endothelium	ExPEC, APEC

ExPEC: extraintestinalpathogenic *E. coli*; EAEC: enteroaggregative *E. coli*; EPEC: enteropathogenic *E. coli*; EHEC: enterohemorrhagic *E. coli*; NTEC: necrotizing factor-producing *E. coli*; UPEC: uropathogenic *E. coli*; EIEC: enteroinvasive *E. coli*; APEC: avianpathogenic *E. coli.*

Table 2. Phenotypic and genotypic characteristics of B2 (n =14) and D group (n = 12) *E. coli* isolates.

Reference of isolate	Origin	Farm/region	Source	Phylogroups	Integron	Resistance profile	Virulotypes[a]	Score	Pathovar	PFGE
EC1	Healthychickens	Farm in Sousse (central-east region of Tunisia)	Feces	D2	1	S, SXT, TET	*fimH*+ ***iutA***	2	UPEC	P1
EC2			Feces	$B2_2$	_	TET, NA	*fimH* + ***ibeA***+***iutA*** + *fyuA*	4	APEC	P2
EC3			Feces	D2	1	AMX, S, SXT, TET, NA, CIP	*fimH* + *papC* + ***iutA***	3	UPEC	P3
EC4		Tunis (supermarket)	Meat	$B2_3$	1	AMX, TET, NA	*fimH* +***iutA***	2	UPEC	P4
EC5			Meat	D2	_	AMX, TET, NA, SXT, S	*fimH*+ ***east1*** + *iutA*	3	EAEC	P5
EC6	Diarrheal chickens	Farm in wed Ellil region	Feces	D2	1	AMX, AMC, K, TET, SXT, NA, CIP, S	*fimH* + ***east1***	2	EAEC	P6
EC7			Oral swab	D2	1	AMX, AMC, TET, SXT, NA, CIP, S	*fimH* + ***east1***+ *iutA*	3	EAEC	P7
EC8			Oral swab	$B2_2$	1	AMX,AMC, TET, SXT, SSS	*fimH* + ***east1***+ *iutA*	3	EAEC	P8
EC9			Oral swab	D2	1	AMX, AMC, TET, NA, CIP	*fimH* + ***iutA***	2	UPEC	P9
EC10	One chicken with diarrhea	Farm in Ben Arous region	Trachea	$B2_3$	1	TET, NA, NOR	*fimH* + *east1* + ***ibeA*** + ***iutA*** + *fyuA*	5	APEC	P11
EC11			Liver	$B2_3$	1	AMX, SXT, TET, SSS, S	*fimH* + ***ibeA***+ ***iutA*** + *fyuA*	4	APEC	P11
EC12			Intestine	$B2_3$	1	TET, NA, NOR	*fimH* + ***ibeA*** +***iutA*** + *fyuA*	4	APEC	P11
EC13			Heart	$B2_3$	1	TET, NA, NOR	*fimH* + ***ibeA*** + ***iutA***+ *fyuA*	4	APEC	P11
EC14	Healthy turkey	Farm in Ben Arous region	Feces	$B2_2$	1	AMX, TET, S, SSS, SXT, NA	*fimH* + ***stx1*** + *east1*	3	EHEC	P12
EC15			Feces	$B2_2$	1	AMX, TET, NA, SSS, CIP, SXT, S	*fimH* + ***eaeA***	2	EPEC or EHEC	P12
EC16			Feces	D2	_	AMX, CIP, SSS, S, NA	*fimH* + ***east1*** + *iutA*	3	EAEC	P12
EC17			Feces	D2	1	AMX, SXT, TET, SSS, S, CIP	*fimH* +***eaeA*** + *fyuA*	3	EPEC or EHEC	P12
EC18			Feces	D2	1	AMX, NA, TET, CAZ, CTX, SSS, S	*fimH* + *papGIII* + ***east1*** + *iutA* + *fyuA*	5	EAEC	P12
EC19			Feces	D2	_	AMX, TET, S	*fimH* + ***eaeA*** + *iutA*	3	EPEC or EHEC	P12
EC20			Feces	D2	_	SXT, SSS, S	*fimH* + *eaeA* + ***stx1*** ***+ stx 2*** + *east1*	5	EHEC	P12
EC21			Feces	D2	1	AMX, TET, SXT, SSS, S	***east1***	1	EAEC	P12
EC22	Healthy cows	Farm in MenzelBourguiba (north of Tunisia)	Feces	$B2_2$	_	_	*fimH* +***east1*** + *iutA*	3	EAEC	P13
EC23			Feces	$B2_2$	_	S, TET	*fimH* + ***east1***	2	EAEC	P15
EC24			Feces	$B2_2$	_	AMX, TET, S	*fimH* + *papC*+ ***cnf1*** + *astA* + *iutA* + *fyuA*	5	NTEC	P16
EC25			Feces	$B2_2$	1	AMX, TET	***fimH***	1	ExPEC	P17
EC26	Healthy sheep		Feces	$B2_2$	1	_	***fimH***	1	(ExPEC)	

SXT: trimethoprim/sulfametoxazole; S: streptomycin; TE: tetracycline; AMX: amoxicillin; CAZ: ceftazidime; CTX: cefotaxime; SSS: sulfonamides; NA: nalidixic acid; CIP: ciprofloxacin; AN: NOR: norfloxacin; K: Kanamycin; ExPEC: extra intestinal pathogenic *E. coli*; EHEC: enterohemorrhagic *E. coli*; STEC: shigatoxin-producing *E. coli*; EAEC: enteroaggregative *E. coli*; NTEC: necrotizing factor-producing *E. coli*; APEC: avianpathogenic *E. coli*; UPEC: uropathogenic *E. coli*; EPEC: enteropathogenic *E. coli ;* [a] Virulence-associated genes shown in bold face are the genes characteristics of EPEC, UPEC, EAEC, EHEC, APEC, and ExPEC pathovars.

Table 3. Pathovar distribution based on B2 and D2 phylogenetic groups.

Phylogenetic group	ExPEC	UPEC	APEC	EHEC	EAEC	EHEC/ EPEC	NTEC	N (%)
B2	2	1	5	1	3	1	1	14 (53.8)
D2	0	3	0	1	6	2	0	12 (46.15)
Total	**2**	**4**	**5**	**2**	**9**	**3**	**1**	**26 (100)**

ExPEC: extraintestinalpathogenic *E. coli*; UPEC: uropathogenic *E. coli*; APEC: avianpathogenic *E. coli*; EHEC: enterohemorrhagic *E. coli*; EAEC: enteroaggregative *E. coli*; EPEC: enteropathogenic *E. coli*; NTEC: necrotizing factor-producing *E. coli.*

For organs (trachea, liver, intestine, and heart) and poultry meat, 25 grams were homogenized for 2 minutes with 225 mL of buffered peptone water (Bio-Rad, Marnes la Coquette, France), seeded onto MacConkey agar plates, and incubated for 24 hours at 37°C. Isolates with typical *E. coli* morphology were selected (one per sample), and the presumptive identification was confirmed by classical biochemical methods and by the API20E system (BioMerieux, Marcy l'Etoile, France).

Determination of phylogenetic groups

E. coli isolates were allotted to phylogenetic groups A, B1, B2, or D using a triplex polymerase chain reaction (PCR) assay targeting the *chuA* and *yjaA* genes and the DNA fragment TSPE4.C2, which was reported by Clermont *et al.* [15]. Strains were sub-grouped according to Escobar-Paramo *et al.* [12] ; subgroupA_0: *chuA*-, *yjaA*-, andTspE4.C2-; subgroupA_1: *chuA*-, *yjaA*+, and TspE4.C2-; groupB1: *chuA*-, *yjaA*+/-, and TspE4.C2+; subgroup$B2_2$: *chuA*+, *yjaA*+, and TspE4.C2-; subgroup$B2_3$: *chuA*+, *yjaA*+, andTspE4.C2+; subgroupD1: *chuA*+, *yjaA*-, and TspE4.C2-; subgroupD2: *chuA*+, *yjaA*, and TspE4.C2+. Appropriate positive and negative controls were included in the assay. Only isolates belonging to phylogroups B2 and D were further studied.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the standard disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI)'s 2012 guidelines [16]. The following antibiotics were tested: amoxicillin, amoxicillin/clavulanic acid, ceftazidime, cefotaxime, imipenem, colistin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, sulfonamide, nalidixic acid, ciprofloxacin, norfloxacin, and gentamicin (Oxoid, Madrid, Spain).

Detection of virulence genes

The presence of 15 virulence genes encoding toxins (*stx1*, *stx2*, *cnf1, east1*, *ehxA*, *hlyA*), adhesins (*fimH*, *eaeA*, *papC*, *papG* allele III, *aggC*), invasins (*ibeA*, *ipaH*),and the siderophores (*iutA*, *fyuA)* were analyzed by PCR [6]. Details regarding amplicon sizes and oligonucleotide primers, as well as description/functions of virulence genes and their corresponding *E. coli* pathotypes, are illustrated in Table 1. Pathovars were determined according to the occurrence of specific virulence genes: ExPEC (*fimH*, *fyuA*, *hlyA*, *papC*, and *papG* allele III), UPEC (*iutA*), EHEC (*stx1* and/or *stx2*), EAEC (*east1*, *aggC*), EPEC orEHEC (*eaeA*, *exhA*), APEC (*ibeA*), NTEC (*cnf1*), and EIEC (*ipaH*) (Table 1) [6,7,9].

Statistical analysis

A virulence score was determined for each strain and calculated as the sum of virulence genes detected. Statistical testing was done using EpiInfo software version 6.04 (CDC, Atlanta, USA). Comparisons of proportions were determined using the Chi-squared test or Fisher's exact test.

Detection of class 1 and 2 integrons, phylogenetic groups, and pulsed-field gel electrophoresis (PFGE)

The occurrence of class 1 and class 2 integrons was investigated by PCR [17]. PFGE was performed as described previously by Kaufmann [18], and PFGE profiles were interpretable as recommended by Tenover *et al.* [19].

Results

Phylogenetic group classifications of E. coli isolates and characterization of the 26 isolates

Among 116 isolates, different phylogroups were detected: A_0 (n = 30), A_1 (n = 35), B1 (n = 25), $B2_2$ (n = 9), $B2_3$ (n = 5), and D2 (n = 12). The 26 *E. coli* isolates belonging to groups D_2 (n = 12) and B2 (n = 14) were isolated from healthy and diarrheic animals (housed in different farms) in the area of Sousse (in the central-east region of Tunisia), Bizerte (in the north of Tunisia), and Tunis-Ben Arous (southeast of Tunis). They were isolated from poultry meat (n = 2; EC4, EC5); feces of healthy animals (chickens [n = 3; EC1–EC3], turkeys [n = 8; EC14–EC21], cows [n = 4; EC22–EC25], and sheep [n = 1; EC26]); oral swabs (n = 3; EC7–EC9) and feces of diarrheic chickens (n = 1; EC6), and from the

organs (liver, intestine, trachea and heart [n = 4; EC10–EC13]) of one diarrheic chicken (Table 2).

Antimicrobial susceptibilities

Among the 26 isolates of *E. coli* studied, 22 were resistant to tetracycline, 18 to streptomycin, 17 to amoxicillin, 14 to nalidixic acid, 12 to trimethoprim/sulfamethoxazole,7 to ciprofloxacin, and 9 to sulfonamides. The strain resistant to ceftazidime and cefotaxime (EC15) was an extended-spectrum beta-lactamase (ESBL) producer. No resistance to imipenem or gentamicin was observed. Only 2 isolates were susceptible to all antibiotics, and 18 isolates were multidrug resistant (Table 2).

Occurrence of integrons and genetic relatedness

Class 1 integrons were found in 18 isolates (Table 2). However, class 2 integrons were not detected. All *E. coli* isolated from the feces of healthy turkeys showed the same pulsotype (P12). Similarly, the 4 *E. coli* isolates (EC10; EC11, EC12, and EC13 collected from different organs of 1 chicken with diarrhea were clonally related and belonged to the same pulsotype (P11). However, the remaining strains presented unrelated PFGE patterns.

Virulence genes and pathovars classification

Genes encoding the production of toxins detected were *stx1* (2 isolates; EC14, EC20), *stx2* (1 isolate; EC20), *cnf1* (1 isolate; EC24), *east1* (13 isolates; EC5–EC8, EC10, EC14 EC16, EC18, and EC20–EC24). The adhesin-encoding gene *fimH* was detected in all isolates except EC21, and the *eaeA*, *papC*, and *papG* allele III genes were detected in 4 (EC15, EC17, EC19, EC20), 2 (EC3, EC24), and 1 (EC18) isolates, respectively. For the invasins, the *ibeA* gene was detected in 5 isolates (EC2, EC10–EC13), whereas the siderophores were manifested by the presence of 2 genes, *iutA* and *fyuA*, in 17 (EC1–EC5, EC7–EC13, EC16, EC18, EC19, EC22, EC24) and 8 (EC2, EC10–EC13, EC17, EC18, EC24) isolates, respectively (Table 2). In total, 7 types of genes combination were detected: *stx1*+*stx2*+*eaeA* (n = 1); *stx1*+ *east1* (n = 1); *fimH*+ *fyuA* (n = 8); *fyuA*+ *iutA* (n = 7), *fimH*+ *iutA* (n = 10); *fimH*+*ibeA*+*fyuA*+*iutA* (n = 5), and *cnf1*+*papC* (n = 1).

Based on the occurrence of specific genes or combinations, the 26 isolates were classified as9EAEC (34.6%), 2 EHEC (7.6%), 4 UPEC (15.3%),3 EPEC/EHEC (11.5%), and 1 NTEC (3.8%). Therefore, 2 ExPEC (7.6%) and 5 APEC (39.1%) were detected among the isolates. UPEC pathovar harbored the phylogroup D_2, unlike the APEC pathovars, which belonged only to the B2 phylogroup. The EAEC pathovar belonged to the B2 and D_2 phylogroups (Table 3).

Statistical analysis

The median virulence score was 3 and ranged from 1 to 6; the *ibeA* gene was significantly associated with the diarrheic chicken ($p = 0.02$) and with susceptibility to amoxicillin ($p = 0.03$).

Discussion

The multidrug resistance trait of *E. coli* is a cause of concern worldwide. In this study, we found a high level of resistance to tetracycline, streptomycin, amoxicillin, nalidixic acid, trimethoprim-sulfamethoxazole, sulfonamides, and ciprofloxacin. The results also showed low levels of resistance to amoxicillin/clavulanic acid, ceftazidime, and cefotaxime. Similar results have been reported in *E. coli* strains isolated from animal origins, especially avian isolates, in many countries including Tunisia [20,21]. High rates of antimicrobial resistance in *E. coli* have been reported in Tunisian patients [22-24]. This finding might be linked to the excessive use of antibiotics in clinical settings. However, animal-to-human transmission of resistant *E. coli* isolates cannot be excluded. Indeed, identical or closely related isolates from humans and animals have been previously reported in the Netherlands, suggesting a likely transmission of *E. coli* isolates from animals to humans, most probably via the food chain [25].

In our collection, 2 isolates were susceptible to all antibiotics tested, 3 were resistant to 2 families of antibiotics, and 21 isolates were multidrug resistant. It is also interesting to note that all multi-resistant drug isolates were from feces of avian origin, while the other isolates from cows and sheep or meat were resistant just to 2 or 3 families of antibiotics.

Multidrug resistance is mainly linked to integrons. In our study, the presence of class 1 integrons was demonstrated in 18 isolates, while class 2 integrons were detected in only 1 strain. These results are consistent with other studies that showed the dominance of class 1 integrons over class 2 integrons in *E. coli* of human and animal origin [20,21]. The class 1 integrons were functional and capable of integrating multiple genes cassettes in their variable regions, including their expression, and consequently by providing a common promoter [26].

The 26 strains studied were subdivided into phylogroups $B2_2$ (n = 9), $B2_3$ (n = 5), and D_2 (n = 12). Our selected strains were therefore potentially

pathogenic. It is important to note that there is a high risk of pathogenic bacteria spreading to humans via the food chain or through direct contact with farmers and veterinarians, as well as contamination of agricultural soil by animal manure (used as organic fertilizers).

In our study, we looked for 15 different genes encoding virulence factors in *E. coli* using a PCR technique. The *iutA* gene was foundin 4 strains without combination with another group of virulence genes such as *stx1*, *stx2*, *ibeA*, *eaeA*, *east1*, and *cnf1*. Therefore, among the 26 *E. coli* isolates, 4 (15.3%)were UPEC according to the presence of the siderophore-encoding gene *iutA*,which is responsible for urinary tract infections [9], and 9 (34.6%) were EAEC by the presence of the *east1* gene. The UPEC and EAEC pathovars would therefore be most frequently involved in human diarrhea in our environment[27-28]. *E. coli* is known as the first agent of urinary tract infections [24] in which the UPEC and NTEC pathovars are mostly involved. In our collection, only one NTEC isolate was identified, which was isolated from a healthy cow.

The *eaeA* intimin gene responsible for attachment and erasing was often associated with EPEC and EHEC pathovars [29]. EPEC/EHEC was found in 11.5% (n = 3) of isolates. Many studies of EPEC showed that these pathovars were the leading causes of diarrhea in infants and children in Côte d'Ivoire [7], Nigeria [8], India [30], and Bangladesh [31]. Two strains of shiga toxin-producing *E. coli* (STEC) harboring the *stx1*/*stx2* genes, isolated from healthy turkeys, were identified. EHEC (STEC or verotoxin-producing *E. coli* [VTEC]) are important etiological agents of diarrhea associated with *E. coli* in Tunisia and worldwide [8,32,33]. Such pathovar contains the two well-known serotypes *E. coli* O104:H4 and *E. coli* O157:H7 [6,34].

Another group of virulence factors was the adhesins that were considered essential virulence factors in *E. coli*. These adhesins were encoded by several genes; among them, *fimH*, *eaeA*, *papC*, and *papG* allele III were decoded in our isolates. The *fimH* gene was detected in almost of our isolates; this finding is in agreement with the literature, which shows that this gene is the most frequently detected with respect to genes encoding the other adhesins and the rest of the virulence genes [6,29]. In addition, it is usually associated with ExPEC [6].

The *ibeA* gene was detected in five strains isolated from poultry, which were therefore classified as APEC (19.2%). This virulence factor is known to be involved in crossing the blood-brain barrier in *E. coli* strains, responsible for neonatal meningitis in humans. It has been reported that the strains harboring this gene are exclusively of avian origin (APEC) [11]. This was confirmed by statistical testing in our collection; we found that the *ibeA* gene was significantly associated with diarrheic poultry.

PFGE showed that the eight fecal turkey isolates were indistinguishable (PFGE pattern P12). This finding supported intra-transmission of a common clone within this turkey farm, highlighting the well-known phenomenon of rapid and easy transmission of pathogens within an avian herd. APEC strains cause a wide range of localized and systemic infections commonly called avian colibacillosis, which is one of the leading causes of mortality and morbidity associated with economic losses in the industry throughout the world. In our study, the occurrence of four APEC isolates recovered from different organs of one chicken suffering from diarrhea (P11) supports the systemic form of colibacillosis from a respiratory origin that induces colisepticemia, leading to the dissemination of such strains to different organs.

Conclusions

Our results showed multidrug resistance in the majority of our *E. coli* isolates, which is in agreement with many reported results of *E. coli* isolates of animal origins in Tunisia and worldwide. This multidrug resistance trait seems to be linked to the occurrence of class 1 integrons, found in 18 of 26 isolates. Moreover, the occurrence of *ibeA* and *stx1*/*stx2* genes in some strains is worrisome for human health. The great diversity of pathovars supports the necessity of surveying healthy avian, bovine, and ovine *E. coli* isolates that could easily be transferred to humans via the food chain, and of successfully identifying risk factors and the major routes of contamination, which determines the control of infections associated with pathovars.

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