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

Hajer Kilani¹,², Mohamed Salah Abbassi¹,², Sana Ferjani²,³, Rakia Ben Salem¹, Riadh Mansouri⁴, Noureddine Ben Chehida¹, Ilhem Boutiba-Ben Boubaker²,³

¹ Veterinary Research Institute of Tunisia, University of Tunis El Manar, Bab Saadoun, Tunis, Tunisia
² LR99E09 Laboratory of Antibiotic Resistance, Faculty of Medicine, University of Tunis El Manar, Tunis, Tunisia
³ Department of Microbiology, Hospital of Charles Nicolle, Tunis, Tunisia
⁴ Emergency Center for Transboundary Animal Diseases (FAOSNE), Tunis, Tunisia

Abstract

Introduction: The virulent *Escherichia coli* strains responsible for extraintestinal infections were mainly belonged to B2 and D phylogroups. However, no past studies have determine 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 determine via the presence of virulence genes the frequency of pathovars.

Methodology: Twenty-six *E. coli* isolates of phylogroups B2 (n = 14), B2₂ (n = 9), B2₃ (n = 5), and D₂ (n = 12) were characterized. Genes encoding virulence factors (fimH, eaeA, aggC, papC, papG allele III, hlyA, eae₁, cnf₁, exh₄, 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.

J Infect Dev Ctries 2017; 11(7):549-556. doi:10.3855/jidc.8579

(Received 20 April 2016 – Accepted 06 December 2016)

Copyright © 2017 Kilani et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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), enteraggregative *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 determine the frequency of the occurrence of potentially pathogenic *E. coli* strains belonging 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.

#### Bacterial isolates collection

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 (bp) | Description/function | *E. coli* pathotype |
|-------------------------|-------------|---------------------------------|-------------------|----------------------|---------------------|
| **Adhesins**            |             |                                 |                   |                      |                     |
| fimH                    | fimH-F      | TGCAGAATGTGCACCGGCCTGG         | 508               | D-mannose-specific    | ExPEC               |
|                         | fimH-R      | GCAGTCACCGTCGTCCTCCGGTGA       |                   | fimbrin               |                     |
| aggC                    | aggC-F      | GCAAGATCCGAGATTGA              | 528               | Fimbral antigen       | EAE'C               |
|                         | aggC-R      | TATTAACCCGATGGTGAGCC           |                   |                      |                     |
| eaeA                    | eaeA-F      | GACCCCGCACAAGCATAAGC           | 384               | Intimin              | EPEC                |
|                         | eaeA-R      | CCACTCGACGCAACAAGGG            |                   |                      | EHEC                |
| papC                    | papC-F      | GTGCGACTATGAGTTAATGACGGT       | 200               | Pilius assembly, central region of *pap* operon | ExPEC |
|                         | papC-R      | ATATCCTTCTCAGGAGATGCAATA       |                   |                      |                     |
| papG allele III          | allele III-F| GCCCTGCAATGGTTACCTGG           | 258               | Cystitis-associated (*prs* or *pap*-2) operon | ExPEC, papG variant |
|                         | allele III-R| CCACAACATGACCATGCGAC           |                   |                      |                     |
| **Toxins**              |             |                                 |                   |                      |                     |
| cnf1                    | cnf1-F      | AAGATGGAGTTTCTATGCAAGAG        | 498               | Cytotoxic necrotizing factor I | ExPEC, NTEC |
|                         | cnf2-R      | CATTCAAGTCTCTCCCTCATATT        |                   |                      |                     |
| east1                   | east1 1a    | CCATCAACACAGTATATCCGA          | 111               | EaeEC heat-stable enterotoxin | exPEC |
|                         | east1 1b    | GTCGCACGTACGGCTHTGT            |                   |                      |                     |
| exhA                    | exhA-F      | GCATCATCACGCTACGTCC            | 534               | Enterohemolysin       | EPEC                |
|                         | exhA-R      | AATGAGCCACACGCTTAAAGCT         |                   |                      | EHEC                |
| hlyA                    | hly-F       | AAAAAAGTAAACGACTGCTCTGCT       | 1177              | α-hemolysin           | ExPEC               |
|                         | hly-R       | ACCATATAAGCGGTACTCGCTAC        |                   |                      |                     |
| stx1                    | stx1-F      | GGCACGTTCAGAACGCTATCC          | 255               | Shiga toxin I         | ExPEC               |
|                         | stx1-R      | TCACGTATCTGACATTCTG            |                   |                      |                     |
| stx2                    | stx2-F      | ATAAACGCTCATATGCTTGACT         | 180               | Shiga toxin II        | ExPEC               |
|                         | stx2-R      | AGAACCCGACCTGAGATC             |                   |                      |                     |
| **Siderophores**        |             |                                 |                   |                      |                     |
| fyuA                    | fyuA-F      | TGATTACACCGCAGGGAA             | 880               | *Yersinia* siderophore receptor (ferric yersiniabactin uptake) | ExPEC |
|                         | fyuA-R      | CGCAATGGCAGCGCTAGTGA           |                   |                      |                     |
| iutA                    | aerJ-F      | GGCTGGACATCATGGGACTGG          | 300               | Ferric aerobactin receptor (iron uptake/transport) | ExPEC, UPEC |
|                         | aerJ-R      | CGTCGGGAAACGCTAGAATCG          |                   |                      |                     |
| **Invasins**            |             |                                 |                   |                      |                     |
| ipaH                    | ipaHIII     | GTTCTTTCGCCGCTCTCCGATACCCTGC  | 600               | Invasion plasmidantigen | EIEC               |
|                         | ipaHIV      | GCGGTCTGACACCGCCTCCTGAGATAC   |                   |                      |                     |
| ibeA                    | ibe10-F     | AGGCGAGGTGGTCGGCAGCTAC         | 170               | Invasion of brain endothelium | ExPEC, APEC |
|                         | ibe10-R     | TGGTGCTGCCGACACACCGTAC         |                   |                      |                     |

ExPEC: extraintestinal pathogenic *E. coli*; EAE'C: 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*.

Kilaniet al. – *Escherichia coli* pathovars from animals in Tunisia

*J Infect Dev Ctries* 2017; 11(7):549-556.
### 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* | Score | Pathovar | PFGE |
|----------------------|--------|-------------|--------|-------------|----------|-------------------|--------------|-------|----------|------|
| EC1                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces | B2         | 1         | S, SXT, TET       | fimH+ iutA   | 2     | UPEC     | P1   |
| EC2                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces | B2         | 1         | TET, NA           | fimH + ibeA + iutA + fnuA | 4     | APEC     | P2   |
| EC3                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces | D2         | 1         | AMX, S, SXT, TET, NA, CIP | fimH + pepC + iutA | 3     | UPEC     | P3   |
| EC4                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Meat  | B2         | 1         | AMX, TET, NA     | fimH + iutA   | 2     | UPEC     | P4   |
| EC5                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Meat  | D2         | 1         | AMX, TET, NA, SXT, S | fimH + east1 + iutA | 3     | EAEC     | P5   |
| EC6                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Oral swab | D2 | 1         | AMX, AMC, K, TET, SXT, NA, CIP | fimH + east1 | 2     | EAEC     | P6   |
| EC7                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Oral swab | B2         | 1         | AMX, AMC, TET, SXT, NA, CIP | fimH + east1 + iutA | 3     | EAEC     | P7   |
| EC8                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Oral swab | B2         | 1         | AMX, AMC, TET, SXT, SSS | fimH + east1 + iutA | 3     | EAEC     | P8   |
| EC9                  | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Oral swab | D2         | 1         | AMX, AMC, TET, SXT, SSS | fimH + iutA   | 2     | UPEC     | P9   |
| EC10                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Trachea | B2         | 1         | TET, NA, NOR     | fimH + east1 + ibeA + iutA + fnuA | 5     | APEC     | P11  |
| EC11                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Liver | B2         | 1         | AMX, SXT, TET, SSS, S | fimH + iutA   | 3     | EAEC     | P12  |
| EC12                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Intestine | B2         | 1         | TET, TA, NOR     | fimH + east1 + iutA + fnuA | 4     | APEC     | P13  |
| EC13                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Heart | B2         | 1         | TET, TA, NOR     | fimH + east1 + iutA + fnuA | 4     | APEC     | P14  |
| EC14                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | AMX, TET, S, SXT, NA | fimH + stx1 + east1 | 3     | EHEC     | P15  |
| EC15                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | AMX, TET, NA, SXT, NA | fimH + eaeA   | 2     | EPEC or EHEC | P16  |
| EC16                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | D2         | 1         | AMX, CIP, SSS, S, NA | fimH + east1 + iutA | 3     | EAEC     | P17  |
| EC17                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | D2         | 1         | AMX, SXT, TET, SSS, S | fimH + eaeA + fnuA | 3     | EPEC or EHEC | P18  |
| EC18                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | D2         | 1         | AMX, TET, S, SXT, NA | fimH + pepGIII + east1 + iutA + fnuA | 5     | EAEC     | P19  |
| EC19                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | D2         | 1         | AMX, TET, S | fimH + eaeA + iutA | 3     | EPEC or EHEC | P20  |
| EC20                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | D2         | 1         | SXT, SSS, S | fimH + eaeA + stx1 + stx2 + east1 | 5     | EHEC     | P21  |
| EC21                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | D2         | 1         | AMX, TET, SXT, SSS, S | east1        | 1     | EAEC     | P22  |
| EC22                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | S, TET            | fimH + east1 + iutA | 3     | EAEC     | P23  |
| EC23                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | S, TET            | fimH + east1 | 2     | EAEC     | P24  |
| EC24                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | AMX, TET, S | fimH + pepC + cnfl + astA + iutA + fnuA | 5     | NTEC     | P25  |
| EC25                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | AMX, TET            | fimH        | 1     | ExPEC     | P26  |
| EC26                 | Healthy chickens | Farm in Sousse (central-east region of Tunisia) | Feces  | B2         | 1         | AMX, TET            | fimH        | 1     | (ExPEC) |      |

SXT: trimethoprim/sulfamethoxazole; S: streptomycin; TE: tetracycline; AMX: amoxicillin; CIP: cefotaxime; 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: enteraggregative E. coli; NTEC: necrotizing factor-producing E. coli; APEC: avianpathogenic E. coli; UPEC: uropathogenic E. coli; EPEC: enteropathogenic E. coli. * Virulence-associated genes shown in bold face are the genes characteristics of EPEC, UPEC, EAEC, EHEC, APEC, and ExPEC pathovars.
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]; subgroupA0: chuA-, yjaA-, and TspE4.C2-; subgroupA1: chuA-, yjaA+/-, and TspE4.C2-; subgroupB1: chuA-, yjaA+/-, and TspE4.C2+; subgroupB2: chuA+, yjaA+, and TspE4.C2-; subgroupB2+: chuA+, yjaA+, and TspE4.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, trimethoprism-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, eaad1, 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 (eaeA, exha), APEC (ibeA), NTEC (cnfI), 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 EpilInfo 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: A0 (n = 30), A1 (n = 35), B1 (n = 25), B2 (n = 9), B2 (n = 5), and D2 (n = 12). The 26 E. coli isolates belonging to groups D2 (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 Tunisia). They were isolated from poultry meat (n = 2; EC4, EC5); feces of healthy animals (chickens [n = 3; EC1–EC3], turkeys [n = 8; EC1–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

**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: extraintestinal pathogenic E. coli; UPEC: uropathogenic E. coli; APEC: avian pathogenic E. coli; EHEC: enterohemorrhagic E. coli; EAEC: enteroaggregative E. coli; EPEC: enteropathogenic E. coli; NTEC: necrotizing factor-producing E. coli.
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), cnfl (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: stxl+stx2+eaeA (n = 1); stxl+east1 (n = 1); fimH+fyuA (n = 8); fyuA+ubeA (n = 7); fimH+iutA (n = 10); fimH+ubeA+fyuA+iutA (n = 5), and cnfl+papC (n = 1).

Based on the occurrence of specific genes or combinations, the 26 isolates were classified as 9EAEC (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 D2, unlike the APEC pathovars, which belonged only to the B2 phylogroup. The EAEC pathovar belonged to the B2 and D2 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].
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 found in 4 strains without combination with another group of virulence genes such as *stxl, 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 *iutA* gene was detected in five strains isolated from poultry, which were therefore classified as 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 *stxl/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.

**Acknowledgements**

This work was supported by the Tunisian Ministry of Higher Education and Technology.

**References**

1. Bruszczewicz E, Thürmer A, Schuldes J, Leimbach A, Liesegang H, Meyer FD, Boelter J, Petersen H, Gottschalk G, Daniel R (2011) Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: entero-aggregative-haemorrhagic *Escherichiacoli*. Arch Microbiol 193: 883-891.
2. Johnson JR, Stell AL (2000) Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 181: 261-272.
3. Marris CF, Zhang L, Tallman P, Manning SD, Somsel P, Raz P, Colodner R, Jantunen ME, Siitonen A, Saxen H, Foxman B
Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral Escherichia coli. J Med Microbiol 51: 138-142.

Jakobsen L, Spangholm DJ, Pedersen K, Jensen LB, Emborg HD, Agersø Y, Aarestrup FM, Hammerum AM, Frimodt-Møller N (2010) Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in Escherichia coli isolated from community dwelling humans and UTI patients. Inter J Food Microbiol 142: 264-272.

Grauke LJ, Kudva IT, Yoon JW, Hunt CW, Williams CJ, Hovde CL (2002) Gastrointestinal tract location of Escherichia coli O157:H7 in ruminants. Appl Environ Microbiol 68: 2269-2277.

Chapman T, Xi-Yang Wu, Barchia I, Karl A, Bettelheim, Driesen S, Darren T, Wilson M, Chin JJ (2006) Comparison of virulence gene profiles of Escherichia coli strains isolated from healthy and diarrheic Swine. App Env Microbiol 72: 4782-4792.

Dadie A, Kouassi N, Daku E, Dje M, Dosso M (2014) Virulence, serotype and phylogenetic groups of diarrhoeagenic Escherichia coli isolated during digestive infections in Abidjan, Côte d’Ivoire. African J Biol 13: 998-1008.

Ifeanyi CI, Ikeneche NF, Bassey BE, Al-Gallas N, Ben Aissa R, Boudabous A (2015) Diarrheagenic Escherichia coli pathotypes isolated from children with diarrhea in the Federal Capital Territory Abuja, Nigeria. J Infect Dev Ctries 9: 165-174. doi:10.3855/jidc.5528.

Hancock V, Ferrières L, Klemm P (2008) The ferric yersiniabactin uptake receptor fyuA is required for efficient biofilm formation by urinary tract infectious Escherichia coli in human urine. Microbiology 15: 167-175.

Bekal S, Broussseau R, Masson L, Prefontaine G, Fairbrother J, Harel J (2003) Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays. J Clin Microbiol 41: 2113-2115.

Solá-Ginés M, Cameron-Veas K, Badiola I, Dolz R, Majó1 N, Dahbi G, Viso S, Mora, Jorge Blanco A, Piedra-Carrasco N, González-López JJ, Migura-Garcia L (2015) Diversity of multi-drug resistant avian pathogenic Escherichia coli (APEC) causing outbreaks of colibacillosis in Broilers during 2012 in Spain. PLoS One 10: e0143191.

Escobar-Páramo P, Le Mena‘ch A, Le Gall T, Amorin C, Gouriot S, Picard B, Skurnik D, Denamur E (2006) Identification of forces shaping the commensal Escherichiacoli genetic structure by comparing animal and human isolates. Environ Microbiol 8: 1975-1984.

Ben Sällem R, Ben Slama K, Rojo-Bezares B, Porres-Osante N, Jouini A, Klibi N, Boudabous, Torres C (2014) IncI1 plasmids carrying blacTX,M-1 or blacMY-2 genes in Escherichia coli from healthy humans and animals in Tunisia. Microb Drug Resist 20: 495-500.

Debbichi N, Abbassi MS, Sâenz Y, Khamiri M, Majouri D, Ben Rayena C, Ben Salem R, Kilani H, Ben Hassen A, Hammami S (2014) Low antibiotic resistance rates and high genetic heterogeneity of Escherichia coli isolates from urinary tract infections of diabetic patients in Tunisia. J Chemother 28: 89-94.

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Jamar A, van der Zwaluw K, Huijsdens X, Kluytmans JA (2011) The ferric uptake regulator plays a role in pathogenesis of diarrhoeagenic Escherichia coli in chicken meat and meat from pigs. Mol Microbiol 37: 1217-1229.

Hall RM, Brooks DE, Stokes HW (1991) Site-specific insertion of genes into integrons: role of the 59-base element and determination of the recombination crossover point. Mol Microbiol 5: 1941-1959.

Al-Gallas N, Bahri O, Bouratbeen A, Ben Hassen A, Ben Aissa R (2007) Etiology of acute diarrhea in children and adults in Tunisia, with emphasis on diarrheagenic Escherichia coli: Prevalence, phenotyping, and molecular epidemiology. Am Soc Trop Med Hyg 77: 571-582.

Gassama-Sow A, Sow PS, Gueye M, Gueye-N'diaye A, Perret JL, M'boup S, Aidara-Kane A (2004) Characterization of pathogenic Escherichia coli in human immunodeficiency virus-related diarrhoea in Senegal. J Infect Dis 189: 75-78.
Kilani et al. – *Escherichia coli* pathovars from animals in Tunisia

J Infect Dev Ctries 2017; 11(7):549-556.

29. Wu Xi-Yang, Chapman T, Darren J, Bettelheim K, Do TN, Driesen S, Walker MJ, Chin J (2007) Comparative analysis of virulence genes, genetic diversity, and phylogeny of commensal and enterotoxigenic *Escherichia coli* isolates from weaned pigs. Appl Environ Microbiol 73: 83-91.

30. Bhan MK, Raj P, Levine MM, Kaper JB, Bhari N, Srivastava R, Koumar R, Sazawal S (1989) Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J Infect Dis 159: 1061-1064.

31. Albert MJ, Ansaruzzaman M, Faruque SM, Neogi PK, Haider K, Tzipori S (1991) An ELISA for the detection of localized adherent classic enteropathogenic *Escherichia coli* serogroups. J Infect Dis 164: 986-989.

32. Al-Gallas N, Ben Aissa R, Annabi T, Bahri O, Boudabous A (2002) Isolation and characterization of shiga toxin producing *Escherichia coli* from meat and dairy products. Food Microbiol 19: 389-398.

33. Al-Gallas N, Bahri O, Ben Aissa R (2006) Prevalence of shiga toxin-producing *Escherichia coli* in a diarrheagenic Tunisian population, and the report of isolating STEC O157:H7 in Tunis. Curr Microbiol 53: 483-490.

34. Hiko A, Asrat D, Zewde G (2008) Occurrence of *Escherichia coli* O157:H7 in retail raw meat products in Ethiopia. J Infect Dev Ctries 2: 389-393. doi:10.3855/jidc.203.

**Corresponding author**

Mohamed Salah Abbassi
Veterinary Research Institute of Tunisia
20 Street Jebel Lakhdhar, Bab Saadoun, Tunis 1006, Tunisia
Phone: 00216 71 561 070
Fax: 00216 71 569 692
Email: salahtoumi_mohamed@yahoo.com

**Conflict of interests**: No conflict of interests is declared.