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Original Article

Prevalence and Diversity of Integrons and Associated Resistance Genes in *Escherichia coli* Isolates from Poultry Meat in Tunisia

Leila Soufi,[1,2] Mohamed Salah Abbassi,[1] Yolanda Sáenz,[2,3] Laura Vinué,[2] Sergio Somalo,[2] Myriam Zarazaga,[2,3] Asad Abbas,[2] Rafika Dbaya,[1] Latifa Khanfir,[1] Assia Ben Hassen,[4] Salah Hammami,[1] and Carmen Torres[2,3]

Abstract

Fifty-five *Escherichia coli* isolates were acquired from chicken and turkey meat obtained from two slaughterhouses in Tunis. Eighty-nine percent, 80%, 78%, 67%, 45%, 27%, 7%, 4%, and 2% of these isolates showed resistance to tetracycline, trimethoprim/sulfamethoxazole, streptomycin, nalidixic acid, ampicillin, chloramphenicol, ciprofloxacin, colistine, and gentamicin, respectively. No resistance was detected to cefotaxime, ceftazidime, or amikacin. bla_{TEM} gene was found in 22 of 25 ampicillin-resistant isolates, and 1 isolate harbored bla_{OXA-1} gene. Tetracycline resistance was predominately mediated by the *tetA* gene. The *sul1*, *sul2*, and *sul3* genes, alone or combined, were detected in 46 of 48 sulfonamide-resistant isolates, and *sul1* and *sul3* were included in class 1 integrons in some cases. Sixty percent of isolates harbored integrons (class 1, 30 isolates; class 2, 5 isolates). Class 2 integrons contained in all cases the *dfrA1-sat1-aadA1-orfX* gene cassette arrangement. Nine gene cassette arrangements have been detected among class 1 integrons, containing different alleles of *dfrA* (five alleles) and *aadA* (2 alleles) genes, which encode trimethoprim and streptomycin resistance, respectively. An uncommon gene cassette array (*sat-psp-aadA2-cmlA1-aadA1-qacH*-IS*440*-*sul3*) has been identified in three class 1 integron-positive isolates, and one additional isolate had this same structure with the insertion of IS*26* inside the *aadA1* gene (included in GenBank with accession no. FJ160769). The 55 studied isolates belong to the four phylogenic groups of *E. coli*, and phylogroups A and D were the most prevalent ones. At least one virulence-associated gene (*fimA*, *papC*, or *aer*) was detected in 44 of the 55 (80%) studied isolates. *E. coli* isolates of poultry origin could be a reservoir of antimicrobial-resistance genes and of integrons, and its evolution should be tracked in the future.

Introduction

Antimicrobial use in food-animal production is an issue of growing concern, because the selective pressure exerted by these agents might have an impact on commensal bacteria not only to develop resistance but also to be a potential reservoir of resistance genes available for dissemination to pathogenic bacteria. The spread of genetic markers encoding antimicrobial resistance in human pathogenic bacteria via the food chain has gained a great attention during last years (Sunde and Norström, 2006). Retail foods—especially, meat and meat products—may be an important vehicle of community-wide dissemination of antimicrobial-resistant *Escherichia coli* and extraintestinal pathogenic *E. coli* (Johnson *et al.*, 2005). In fact, *E. coli* has been emerging as an important pathogen in clinical sets as well as in the community. High frequencies of resistance to third-generation cephalosporins (due to production of extended spectrum beta-lactamases [ESBL]), aminoglycosides, fluoroquinolones, tetracycline, and trimethoprim-sulfamethoxazole have been reported worldwide (Guerra *et al.*, 2003; Machado *et al.*, 2008; Vinué *et al.*, 2008). The coexistence of antibiotic resistance genes on mobile genetic elements, as plasmids, transposons, and integrons, enhances its rapid dissemination among bacteria (Mazel, 2006; Sunde and Norström, 2006). Integrons are genetic structures able to capture, integrate, and express gene cassettes

[1]Institute of Veterinary Research of Tunisia, Tunis, Tunisia.
[2]Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain.
[3]Centro de Investigación Biomédica de La Rioja, Logroño, Spain.
[4]Laboratory of National Bone Marrow Transplantation Centre, Tunis, Tunisia.

associated with antimicrobial resistance, playing an important role in the dissemination of antimicrobial resistance (Carattoli, 2001; Fluit and Schmitz, 2004; Mazel, 2006). Further, the capture of genes is particularly important when these integrons are mobilized by broad host-range conjugative plasmids or transposons. Integrons include an *intI* gene encoding an integrase (site-specific recombinase), a recombination site (*attI*), and a common promoter (P_c) implicated in the expression of the captured gene cassettes (Hall and Collis, 1995). To date, class 1 and class 2 integrons, characterized by the presence of the *intI1* and *intI2* genes that encode specific integrases, are the most important ones associated with antimicrobial resistance, and a large variety of resistance gene cassettes have been described (Carattoli, 2001; Fluit and Schmitz, 2004; Sunde, 2005; Mazel, 2006). In common class 1 integrons, the *intI1* gene is located in the 5′-conserved segment, whereas the *qacE*Δ*I* and *sul1* genes are located in the 3′-conserved segment (3′-CS). Single or multiple gene cassettes can be inserted between the 5′- and 3′-regions, which lead to multiple drug resistance in bacteria (Mazel, 2006). However, uncommon class 1 integrons, characterized for lacking 3′-CS and for being associated to the sulfonamide resistance *sul3* gene, have been reported (Bischoff *et al.*, 2005; Antunes *et al.*, 2007; Sunde *et al.*, 2008). Complex genetic events have been further elucidated especially in *Salmonella* spp. and *E. coli* strains where insertion sequences IS*26* and IS*440* would have played a major role in the rearrangement of the genetic structure of class 1 integrons (Antunes *et al.*, 2007).

The aims of this study were to investigate the prevalence and diversity of integrons in *E. coli* strains isolated from poultry meat in Tunisia and to analyze the associated resistance and virulence genes as well as their phylogenetic groups.

Materials and Methods

Samples and E. coli *isolates*

A total of 55 food samples (25 from turkey meat and 30 from chicken meat) were collected from two different slaughterhouses in Tunis (Tunisia), corresponding to 17 different flocks of animals. Twenty-five grams of poultry meat was homogenized for 2 minutes with 225 mL of buffered peptone water (Bio-Rad, France), and then were seeded on Tryptone Bile X-glucuronide agar plates (Bio-Rad, Hercules, CA), and incubated for 24 hours at 44°C according to the procedure described in ISO16649-2 (International Organization for Standardization, 2001). Isolates with typical *E. coli* morphology were selected (one per sample), and the presumptive identification was confirmed by classical biochemical methods and by API20E system (BioMérieux, Marcy I'Etoile, France).

Antimicrobial susceptibility testing

The susceptibility to 17 antimicrobial agents (ampicillin [10 μg/disk], cefalothin [30 μg], amoxicillin/clavulanic acid [20/10 μg], ceftazidime [30 μg], cefotaxime [30 μg], gentamicin [10 μg], amikacin [30 μg], kanamycin [30 μg], tobramycin [10 μg], streptomycin [10 μg], tetracycline [30 μg], nalidixic acid [30 μg], ciprofloxacin [5 μg], sulfonamide [200 μg], trimethoprim/sulfamethoxazole [1.25/23.75 μg], chloramphenicol [30 μg], and colistine [50 μg]) (Oxoid, Basingstoke, UK) was determined on Mueller-Hinton agar by the disk diffusion method, according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2007). Zone breakpoints were those recommended by CA-SFM. *E. coli* ATCC 25922 was used as a quality-control strain. The double-disk test with cefotaxime or ceftazidime in the proximity to amoxicillin-clavulanic acid was used for the screening of ESBL.

DNA extraction, characterization of integrons, and detection of antimicrobial resistance genes

Genomic DNA for *E. coli* isolates was extracted by the boiling method, as previously described (Al-Gallas *et al.*, 2002), and used as the target for polymerase chain reaction (PCR) assays. The presence of the *intI1* and *intI2* genes, as well as the *qacE*Δ1 + *sul1* genes, was also analyzed by PCR in all isolates. The characterization of the variable region of class 1 and class 2 integrons was performed by PCR and subsequent DNA sequencing (Sáenz *et al.*, 2004). The primer-walking strategy with further sequencing of all the obtained amplicons was used for the characterization of class 1 integrons lacking the *qacE*Δ1 + *sul1* genes in some of our *E. coli* isolates.

The presence of genes associated to ampicillin resistance (bla_{TEM}, bla_{SHV}, and bla_{OXA-1}), tetracycline resistance [*tet*(A), *tet*(B), and *tet*(C)], chloramphenicol resistance (*cmlA*), streptomycin resistance (*aadA*), and sulfonamides resistance (*sul1*, *sul2*, and *sul3*) was also analyzed by PCR (Sáenz *et al.*, 2004).

Detection of phylogenetic groups and virulence factors

All isolates were assigned to one of the four main *E. coli* phylogenetic groups (A, B1, B2, and D) by triplex PCR amplification as described by Clermont *et al.* (2000). The presence of five virulence genes (*fimA*, *papC*, *papGIII*, *cnf1*, and *aer*) was investigated by PCR using primers and conditions previously described (Ruiz *et al.*, 2002).

Conjugal transfer experiments

Mating experiments were carried out using *E. coli* J53-2 (*pro*, *met*, Rif^R) as the recipient strain. Overnight cultures of donor and recipient strains grown in Mueller-Hinton broth at 37°C were mixed together at 1:10 (v/v) proportion and incubated at 37°C for at least 4 hours without shaking. Then, 0.1 mL of the mixture was spread onto the surface of Mueller-Hinton agar plates containing rifampicin (150 mg/L) plus streptomycin (50 mg/L). Plates were incubated at 37°C and inspected at 24 and 48 hours. Transconjugants growing on the selection medium were recovered and subjected to antibiotic susceptibility testing and PCR to confirm the possible acquisition of integron-related gene cassettes.

Results

A total of 55 *E. coli* isolates were obtained from chicken and turkey meat (30 and 25 isolates, respectively), and the susceptibility to 17 antimicrobial agents for these isolates is shown in Table 1. A high level of resistance was detected to tetracycline (89%), sulfonamide (87%), trimethoprim/sulfamethoxazole (80%), streptomycin (78%), nalidixic acid (67%), ampicillin (45%), kanamycin (40%), chloramphenicol (27%), and cefalothin and ciprofloxacin (7%), and lower levels were detected to amoxicillin-clavulanic acid or tobramycin

Table 1. Number and Percentages of Resistance for the Different Antimicrobial Agents in Relation to the Origin of the *E. coli* Isolates Included in This Study

Antimicrobial agent	*Chicken* (n = *30) No. (%)*	*Turkey* (n = *25) No. (%)*	*Total isolates* (n = *55) No. (%)*
Ampicillin	16 (53)	9 (36)	25 (45)
AMC	0	2 (8)	2 (4)
Cefalothin	2 (7)	2 (8)	4 (7)
Cefotaxime	0	0	0
Ceftazidime	0	0	0
Tetracycline	25 (83)	24 (96)	49 (89)
SXT	23 (77)	21 (84)	44 (80)
Sulfonamide	26 (87)	22 (88)	48 (87)
Chloramphenicol	2 (7)	13 (52)	15 (27)
Gentamicin	1 (3)	0	1 (2)
Tobramycin	2 (7)	1 (4)	3 (5)
Kanamycin	13 (43)	9 (36)	22 (40)
Amikacin	0	0	0
Streptomycin	24 (80)	19 (76)	43 (78)
Nalidixic acid	20 (67)	17 (68)	37 (67)
Ciprofloxacin	2 (7)	2 (8)	4 (7)
Colistin	0	2 (8)	2 (4)

AMC, amoxicillin-clavulanic acid; SXT, trimethoprim/sulfamethoxazole.

(4–5%), colistine (4%), and gentamicin (2%). None of our isolates showed resistance to amikacin, cefotaxime, or ceftazidime, and the screening of ESBLs was negative in all isolates.

Ninety-six percent of the 55 *E. coli* isolates showed a multiresistance phenotype (defined as resistance to three or more different classes of antimicrobial agents), and coresistance to nalidixic acid, tetracycline, trimethoprim/sulfamethoxazole, sulfonamides, and streptomycin antimicrobial agents was the most frequently combination detected.

PCR screening for integrons and characterization of gene cassettes

The presence of integrons was detected in 33 of the 55 studied isolates (60%): the *intI1* gene was identified in 28 of these isolates, the *intI2* gene in 3 isolates, and both *intI1* and *intI2* genes in 2 additional isolates (Table 2). In two of the *intI1*-positive isolates, two different arrangements of resistance gene cassettes were detected in their class 1 variable regions. Thirty-two class 1 integron and five class 2 integron structures were detected in the 33 integron-positive isolates. The characterization of the class 1 variable regions revealed the presence of a wide variety of gene cassette arrangements that are shown in Table 2 and in Fig. 1. Twenty of the 32 class 1 integrons (62.5%) lacked the *qacE*Δ1-*sul1* segment, and the genetic composition of their variable regions was studied in detail by primer-walking PCR strategy.

Nine different gene cassette arrangements of class 1 integrons were detected (Table 2). The *sat-psp-aadA2-cmlA1-aadA1*Δ-IS*26*-Δ*aadA1-qacH*-IS*440-sul3* combination was found in *E. coli* strain Ec21, recovered from a turkey meat sample, and includes an *aadA1* gene disrupted by the insertion of IS*26* (Fig. 1). This novel arrangement has been included in GenBank with the accession number FJ160769. A large variety of

Table 2. Phylogenetic Groups and Arrangements of Resistance Gene Cassettes Detected in the 30 Class 1 Integron-Positive *Escherichia coli* Isolates

Arrangements of resistance gene cassettes	*No. of isolates*	*Phylogenetic group (no. of isolates)*
sat-psp-aadA2-cmlA1-aadA1-qacH-IS*440-sul3*	2[a]	A (1), D (1)
Two class 1 integrons: (1) *sat-psp-aadA2-cmlA1-aadA1-qacH*-IS*440-sul3* (2) *dfrA25-qacE*Δ1-*sul1*	1[b]	B_2
Two class 1 integrons: (1) *sat-psp-aadA2-cmlA1-aadA1*Δ-IS*26*-Δ*aadA1-qacH-IS440-sul3* (2) *dfrA1* + *aadA1*	1[b]	A
*sat-psp-aadA2-qacE*Δ1-*sul1*	1	A
*dfrA12-orf-aadA2-qacE*Δ1-*sul1*	2	D (2)
*dfrA1-aadA1-qacE*Δ1-*sul1*	8	A (2), B_1 (4), B_2 (1), D (1)
dfrA1-aadA1	12	A (5), B_1 (1), B_2 (4), D (2)
dfrA5-ereA	1	B_1 (1)
dfrA5	1	D (1)
dfrA14	1	B_1 (1)

[a]These two strains harbor also a class 2 integron with the *dfrA1-satA1-aadA1* gene cassette array.
[b]It was possible to transfer by conjugation the *sul3*-linked class 1 integron of this strain to *E. coli* strain J53-2.

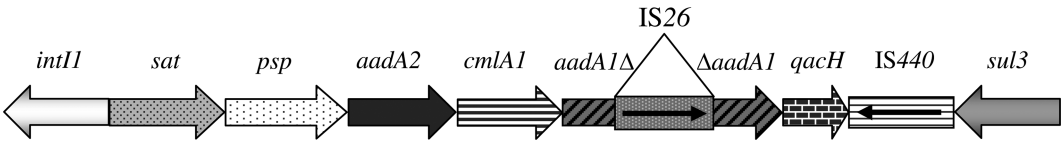

FIG. 1. Class 1 integron lacking *qacE*Δ1-*sul1* segment in *Escherichia coli* isolate Ec21 (sequence included in GenBank with accession number FJ160769).

genes that are involved in resistance to streptomycin (*aadA1*, *aadA2*), trimethoprim (*dfrA1*, *dfrA5*, *dfrA12*, *dfrA14*, *dfrA25)*, sulfonamide (*sul1*, *sul3*), chloramphenicol (*cmlA*), streptothricin (*sat*), erythromycin (*ereA*), and quaternary ammonium compounds (*qac* genes) have been detected in the genetic structures of class 1 integrons.

Mating experiments with the recipient strain *E. coli* J53-2 were performed in the four isolates with class 1 *sul3*-associated integrons, and it was possible to obtain transconjugants from two of the four studied *E. coli* isolates (*E. coli* Ec21 and Ec24) (Table 2), and the *sul3*-linked class 1 integron was transferred to *E. coli* strain J53-2 in both cases (demonstrated by specific PCRs).

The gene cassette arrangements identified in the five *E. coli* isolates that harbored class 2 integrons were in all cases *dfrA1-satA1-aadA1-orfX*, containing resistance genes for trimethoprim, streptothricin, and streptomycin.

Characterization of other antimicrobial resistance genes

The presence of other resistance genes, in addition to the ones included into integrons, was also studied by PCR and sequencing. Twenty-five ampicillin-resistant isolates were detected, and 22 of them harbored a bla_{TEM} gene, and another isolate contained the $bla_{\text{OXA-1}}$ gene. No β-lactamase genes were identified in the remaining two ampicillin-resistant isolates (Table 3). Among 49 tetracycline-resistant isolates, 32 isolates carried only the *tetA* gene, 4 isolates carried only *tetB*, and both the *tetA* and *tetB* genes were concomitantly found in 6 isolates. The remaining seven tetracycline-resistant isolates did not contain any of the tetracycline resistance evaluated genes. Forty-six of the 48 sulfonamide-resistant *E. coli* isolates contained the *sul* genes, and 17 of them harbored simultaneously more that one *sul* gene (Table 3).

Table 3. Resistance Genes Detected in the *Escherichia coli* Isolates in Relation to Its Specific Phenotype of Resistance

Antibiotics	*No. of resistant isolates*	*Genes detected (no. isolates)*
Tetracycline	49	*tetA* (32)
		tetB (4)
		tetA + *tetB* (6)
Sulfonamide	48	*sul1* (3)
		sul2 (10)
		sul3 (16)
		sul1 + *sul2* (8)
		sul1 + *sul3* (3)
		sul2 + *sul3* (3)
		sul1 + *sul2* + *sul3* (3)
Ampicillin	25	bla_{TEM} (22)
		$bla_{\text{OXA}-1}$ (1)
Streptomycin	43	*aadA1* (24)
		aadA2 (2)
		aadA1 + *aadA2* (4)

Phylogenetic groups and virulence factors

The phylogenetic groups A and D were the most prevalent among the 55 *E. coli* isolates (17 isolates in each group, 62%), followed by those of group B1 (15 isolates) and B2 (6 isolates). Regarding the origin of the isolates, the phylogenetic groups A and B1 were the most prevalent (40% and 28%, respectively) in the isolates recovered from turkey meat, and the groups D and B1 (37% and 27%, respectively) in those from chicken meat.

The presence of five virulence-associated genes (*fimA*, *papC*, *papGIII*, *aer*, and *cnf1*) was studied in our 55 *E. coli* isolates, and 44 of them (80%) harbored at least one virulence gene, with *fimA* (encoding type 1 fimbriae) being detected in 78% of isolates. The *aer* (encoding aerobactin) and *papC* (encoding P fimbriae) genes were detected in 40% and 4% of the isolates, respectively, whereas the *papGIII* (class III PapG adhesin) and *cnf1* (cytotoxic necrotizing factor 1) genes were not identified in our bacterial collection. The following associations of virulence genes were detected: *fimA* + *aer* (20 isolates), *fimA* + *papC* (1 isolate), and *fimA* + *aer* + *papC* (1 isolate). The distribution of the 55 *E. coli* isolates in relation to the virulence-associated genes and the phylogenetic groups is presented in Table 4. The prevalence of virulence-associated genes in the isolates belonging to phylogroups B2 + D and to A + B1 was 91% and 72%, respectively.

Table 5 shows the virulence genes detected among the integron-positive and integron-negative isolates. It is worthy to note that the *aer* gene was found more frequently in the integron-positive isolates (54%) than in the integron-negative ones (18%), whereas the prevalence of *fimA* gene was similar

Table 4. Correlation Between Virulence Factors and Phylogenetic Groups in the 55 *Escherichia coli* Isolates of Poultry Origin

Virulence genes	*No. of isolates*	*No. of isolates with the phylogroup*			
		A	*B1*	*B2*	*D*
fim A	21	4	7	0	10
aer	1	0	0	0	1
fim A + *aer*	20	5	6	4	5
fim A + *papC*	1	1	0	0	0
fim A + *aer* + *papC*	1	0	0	1	0
No virulence genes detected	11	7	2	1	1

Table 5. Genes Encoding Virulence Factors Detected in the Integron-Positive and Integron-Negative *Escherichia coli* Isolates

	Integron-positive isolates (n = 33)	*Integron-negative isolates* (n = 22)
Virulence genes	*No. of isolates (%)*	*No. isolates (%)*
fim A	10 (30)	11 (50)
Aer	1 (3)	0
fim A + aer	16 (48)	4 (18)
fim A + papC	0	1 (4)
fim A + aer + papC	1 (3)	0
No virulence genes detected	5 (15)	6 (27)

in both kinds of isolates (81% in the integron-positive isolates and 72% in the negative ones). Table 6 shows the distribution of isolates in relation with the content in virulence genes and also the number of families of antimicrobial agents to which they are resistant. Forty-two percent of the isolates showing a phenotype of multiresistance contained two or three of the five tested virulence genes, and the remaining 58% of the isolates contained less than two virulence genes.

Discussion

Antimicrobial resistance in *E. coli* isolates recovered from food-producing animals might constitute a threat to human health, as these isolates could lead to human infections, especially in children and older people. In Tunisia, little is known about the epidemiology of *E. coli* in food products especially from poultry meat at the slaughterhouse level (Jouini *et al.*, 2007, 2009). In the present study, the highest rates of antimicrobial resistance in *E. coli* isolates from poultry meat were detected for tetracycline, trimethoprim/sulfamethoxazole, sulfonamides, streptomycin, nalidixic acid, and ampicillin (range, 45–89%); however, the resistance level to ciprofloxacin and to gentamicin was 7% and 2%, respectively. Similar phenotypic results have been previously reported in *E. coli* isolates of food products (Guerra *et al.*, 2003; Jouini *et al.*, 2009; Van *et al.*, 2008). It is worthy to note that most of our isolates showed a multiresistance phenotype (96%), and the concomitant resistance to tetracycline, trimethoprim/sulfamethoxazole, sulfonamides, and streptomycin has been observed in our and other studies (Jouini *et al.*, 2009; Guerra *et al.*, 2003). It is known that oxytetracycline, trimethoprim/sulfamethoxazole, and streptomycin are highly used in poultry farms for therapeutic or prophylactic purposes in Tunisia, and this could have contributed to the high rates of resistance detected for those antimicrobial agents.

Among integrons, those of class 1 are the most frequently reported (Carattoli, 2001; Fluit and Schmitz, 2004; Sunde, 2005; Mazel, 2006), and also detected in our poultry isolates (54% of the 55 isolates). Class 1 integrons are able to integrate a wide variety of gene cassettes owing to the synthesis of functional integrases encoded by *intI1*, unlike class 2 integrons, which harbor a nonfunctional *intI2* gene by virtue of possessing a premature in-frame stop codon at amino acid 179 (Márquez *et al.*, 2008). In relation to this fact, the five class 2 integrons identified in our work presented in their variable region the same gene cassette array, *dfrA1-sat1-aadA1-orfX*, which is frequent also in other studies (Sunde, 2005; Jouini *et al.*, 2007; Machado *et al.*, 2008; Vinué *et al.*, 2008). Most of the gene cassettes found within the variable region of class 1 integrons in our *E. coli* isolates correspond to different alleles of the *dfrA* (five alleles) and *aadA* (two alleles) genes, conferring resistance to trimethoprim and streptomycin, respectively. The *dfrA1-aadA1* combination was the most frequently detected, not only in our study (in 21 out of 32 class 1 integrons) but also in *E. coli* isolates recovered from humans, animals, and food in others studies (Sunde, 2005; Machado *et al.*, 2008; Vinué *et al.*, 2008).

Twenty of our 32 class 1 integrons (62.5%) did not contain the common 3′-region (the *qacE*Δ1-*sul1* genes); this phenomenon has been previously reported in *E. coli* recovered from different origins (Sáenz *et al.*, 2004; Bischoff *et al.*, 2005; Antunes *et al.*, 2007; Cocchi *et al.*, 2007; Sunde *et al.*, 2008; Vinué *et al.*, 2008), but at lower frequencies in contrast to our finding. The *qacE*Δ1-*sul1* fragment was replaced by *qacH*-IS*440*-*sul3* sequence in four integrons. All but one of these *sul3*-carrying class 1 integrons presented the following gene cassette organization: *sat-psp-aadA2-cmlA1-aadA1-qacH*-IS*440*-*sul3* (8300 bp), which has been previously described in porcine *E. coli* (Bischoff *et al.*, 2005) and in *Salmonella* strains (Antunes *et al.*, 2007). The remaining structure *sat-psp-aadA2-cmlA1-aadA1*Δ-IS*26*-Δ*aadA1-qacH*-IS*440*-*sul3* (9107 bp), detected and described for the first time in this work, is identical to the above-mentioned one, but includes the element IS*26* into the *aadA1* gene cassette. IS*26* is associated with several antibiotic resistance genes (such as $bla_{\text{SHV-5}}$ and $bla_{\text{CTX-M-group 1}}$) and is implicated in the dissemination of resistance genes via several routes (Miriagou *et al.*, 2005; Jouini

Table 6. Correlation Between Genes Encoding Virulence Factors and Resistance to Different Families of Antimicrobial Agents in the Series of 55 *E. coli* Isolates of the Study

	Number of isolates resistant to the following number of antimicrobial families					*Multidrug resistance phenotype*[a]
Virulence genes	*≤2* (n = 2)	*3* (n = 6)	*4* (n = 6)	*5* (n = 19)	*≥6* (n = 22)	*No. of isolates (%)* (n = 53)
fim A	2	3	4	7	5	19 (36)
aer	–	–	1	–	–	1 (2)
fim A + aer	–	1	1	6	12	20 (38)
fim A + papC	–	–	–	–	1	1 (2)
fim A + aer + papC	–	–	–	1	–	1 (2)
No virulence genes detected	–	2	–	4	5	11 (21)

[a]Multiresistance phenotype has been considered as resistance to three or more different families of antimicrobial agents.

et al., 2007). To our knowledge, this is the second report of insertion of IS*26* into the *aadA1* gene cassette; the first report was in an *Acinetobacter baumannii* strain containing other gene cassette arrays (Han *et al.*, 2008). IS*26* was inserted near the middle of the *aadA1* gene cassette in both cases, and also in the same orientation.

Genes encoding antibiotic resistance were also detected outside integrons. Indeed, 22 out of 25 ampicillin-resistant isolates harbored the bla_{TEM} gene and the bla_{OXA-1} gene in one additional isolate. On the other hand, although the detection of ESBL-containing *E. coli* strains of different origins is frequently reported worldwide, in our study, none of the *E. coli* isolates showed an ESBL phenotype.

Tetracycline resistance was observed in 89% of the isolates, and very high rate of resistance to this antibiotic has been also previously reported from food isolates and to a lesser extent from clinical isolates (Lapierre *et al.*, 2008). The *tetA* and *tetB* genes associated with tetracycline efflux pumps were reported to be predominant in *E. coli* isolates from livestock and food animals (Guerra *et al.*, 2003; Sáenz *et al.*, 2004; Jouini *et al.*, 2007; Van *et al.*, 2008), and similar to our results it seems that *tetA* is the most frequent gene encoding tetracycline resistance. A genetic linkage on large conjugative plasmids of *tetA* and class 1 integrons has been previously reported (Sunde and Norström, 2006), and all our integron-positive strains also contained the *tetA* gene.

Sulfonamide resistance in Gram-negative bacilli generally rises from the presence of the *sul1*, *sul2*, and/or *sul3* genes (Sköld, 2000; Perreten and Boerlin, 2003; Hammerum *et al.*, 2006; Trobos *et al.*, 2008), as in our study, where 46 out of 48 sulfonamide-resistant *E. coli* isolates amplified at least one of the three *sul* genes. The *sul1* gene was part of class 1 integrons in all but four of *sul1*-positive isolates, and similar results have been previously reported (Hammerum *et al.*, 2006; Trobos *et al.*, 2008; Vinué *et al.*, 2008). The *sul3* gene has been previously found in *E. coli* strains from livestock and food although the *sul1* or *sul2* genes were more frequently described (Guerra *et al.*, 2003; Perreten and Boerlin, 2003; Sáenz *et al.*, 2004; Hammerum *et al.*, 2006), which is in contrast with our isolates, where *sul3* was predominant. This would be related to the lack of the *sul1* gene in the 3′-CS, observed in the majority of class 1 integron-positive isolates, or alternatively to the emergence and the rapid spread of successful mobile genetic element including the *sul3* gene. Indeed, *sul3* gene has been reported on conjugative plasmid-borne transposase-like sequences *qacH-IS440-sul3-orf1-IS26* (Perreten and Boerlin, 2003; Bischoff *et al.*, 2005; Antunes *et al.*, 2007), which is an efficient genetic platform that might have contributed to the emerging spread of *sul3* reported worldwide as well as in our *E. coli* isolates. Conjugation experiments carried out with two of four *sul3*-linked class 1 integron-containing *E. coli* isolates were positive, and in both cases it was possible to transfer these integrons to the *E. coli* recipient strain J53-2.

Regarding phylogenetic groups, it has been previously described that *E. coli* isolates belonging to B2 and D phylogroups usually carry more virulence-associated genes and present less antimicrobial resistance rates than the so-called nonpathogenic commensal strains (phylogenetic group A and B1) (Boyd and Hartl, 1998; Cocchi *et al.*, 2007). In our study, a high prevalence (91%) of virulence-associated genes was found in the *E. coli* isolates that belonged to phylogenetic groups B2 and D. Virulence-associated genes are usually encoded on pathogenicity islands providing a mechanism for coordinated horizontal transfer of virulence genes, thus favoring dissemination of pathogenic determinants in every ecosystem.

In conclusion, we found that up to 50% of the *E. coli* strains isolated from poultry meat in Tunisia were integron positive and harbored a diversity of gene cassette arrays. In addition, antibiotic resistance can also occur on nonintegron structures. Our study highlights the emergence and the expansion of the *sul3* gene over the *sul1* gene, a finding that somehow can be related to the lack of the *sul1* gene, the common 3′-CS of class 1 integrons in 60% of strains. The major strains belong to the nonpathogenic groups A, B1, and D, which reduce the possibility to induce human infections. However, the dissemination of genes encoding antibiotic resistance and virulence-associated genes in the commensal groups (A and B1) is a cause for great concern, especially if they are located on mobile elements.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Carmen Torres, Ph.D.
Área de Bioquímica y Biología Molecular
Universidad de La Rioja
Madre de Dios 51
26006 Logroño
Spain

E-mail: carmen.torres@unirioja.es