Comparison of Antimicrobial Susceptibility of Escherichia Coli Isolated from Fecal Poultry and Bovine Housed in Tunisian Farms; Phylogroup Diversity and Detection of Tetracycline and Sulfonamides Resistant Genes With Integron Class1

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Abstract

Little detailed documentation researched the excessive use of antimicrobials such as tetracycline and sulfonamides in veterinary medicine in Tunisia and more studies are needed. A total of 58 of commensal Escherichia coli isolates recovered from fecal samples of healthy poultry (n=31) and bovin (n=27) recovered from farms in Tunisia were examined for 20 antimicrobial as well as, researched the presence of integron, variable regions (VRs), phylogroupes, tetracycline (tetA, tetB et tetC) and sulfonamides(sul1, sul2, sul3) resistance genes. The most frequently resistance in poultry origin were to tetracycline (94.3%), sulfonamide (70.69%), nalidixic acid (61.29), amoxicillin (58%), to trimthetoprim-sulfamethoxazole and streptomycin with the same rate (64.51%), ticarcilline (58%). Whereas, the bovine isolates were most resistant to streptomycin (55.5%), to amoxicillin (18.5), to tetracycline (37%), and have a moderate same rates to kanamycine, to trimthetoprim-sulfamethoxazole 11.11, to nalidixic acid and to sulfonamide 7.4%, For poultry and bovine class 1 integron were detected in 20, 6 isolates, respectively as well as class 2 integron were found in 2 and 1 isolates, respectively. Class 1 integrons were significantly associated with poultry origin (p=0.001). For poultry sul1, sul2, and sul3 genes were detected in 14 (46.2 %), 7 (23.8 %), and 4 (8.9 %) resistant isolates, respectively. Whereas, for bovine 5 isolates were resistant to sulfonamide and sul1 and sul2 genes were detected in 4 and 1 isolates with absence of sul3 genes. and tetracycline genes tetA, tetB genes were observed in 27 (84.37 %) and 8 (25%) resistant isolates, respectively while, TetC was not detected amongst our isolates. Seven arrangement gene cassette were detected; dfrA1-satA1-aadA1 in one identical DNA fragments with approximate size of 2000 bp and six arrangements of resistance gene cassettes of class 1 integron were detected; dfrA1+aadA1 (5isolates); for dfrA17+aadA1, dfrA12+ orfF+aadA2 each one two 2isolates; one isolate for aadA1 and dfrA5, respectively. In poultry, 16 isolates were found to belong to phylogroup A (sub- groupA1: 12, sub- groupA0: 4); 9 to B1, 1 to B2 and 5 to phylogroup D. However, in bovine 9 isolates have the phylogroups A1, 7 isolates B1, 4 isolates B2, and 3 isolates found to phylogroup D. Our results showed that the prevalence of resistance in E. coli isolates from poultry was much higher than that in bovin.

Keywords: Escherichia Coli, Antimicrobial Resistance, Integrons, Poultry, Bovin.

1. Introduction

Escherichia coli can cause a variety of diarrheal and other extra-intestinal infections in humans and animals. The emergence of *E. coli* isolates with multiple antibiotic resistance phenotypes, has been previously reported and is considered as a serious health concern [1,2]. In *Enterobacteriaceae* and *particularly* in *E. coli*, resistance to beta-lactams due to Extended Spectrum Beta-Lactamases (ESBL), quinolones, and aminoglycosides have drawn considerable attention worldwide [3]. ESBL-pro-ducing isolates are usually resistant to other antibiotics such as aminosides, tetracycline, chloramphenicol, trimethoprim, sulfonamides, or quinolones, often due to the presence of different resistance genes on transferable elements such as plasmids, transposons, or integrons [4-8]. In the last decade, it has been observed that ESBL-producers and multi-drug resistant *E. coli* isolates are frequently detected in food-producing animals or food products, and thus health authorities are worried about the potential transmission of these resistant microorganisms to

humans through the food chain. Likewise, in Tunisia many reports have highlighted a high prevalence of antibiotic resistance and ESBL production in *E. coli* isolates from food-producing animals or food products, a situation that requires more investigations and vigilance to monitoring large dissemination of such resistant isolates [9-12].

Mobile genetic elements such as plasmids, transposons, and integrons are able to disseminate genes encoding antibiotic resistance by horizontal transfer, and play important role in the evolution and dissemination of multidrug resistance in Gram-negative bacteria [13,14]. Five classes of integrons related to antibiotic resistance have been described based on the homology of their integrase genes; however, class 1 and 2 were the most prevalent integrons in *Enterobacteriaceae* [15-17]. Class 1 integron comprises two conserved segments, the 5'conserved segment (5'CS) and the 3' conserved segment (3'CS), and an internal variable region. Variable regions are able to contain many gene cassettes and up to 6 cassettes have been reported, which explain, in part, the multiresistance trait of some reported isolates [18].

The aim of this study was to investigate the presence of integrons in commensal *E. coli* isolates from animal origins (poultry and bovine) housed in farms located in four different governorates of Tunisia (Sousse, Tunis, Bizerte and Gbeli), with a focus on antimicrobial resistance of the isolates and phylogroups as well as genetic characterization of genes encoding sulphonamides, tetracycline- resistance (owing to their high rates of resistance in our collection and their excessive use in veterinary medicine in Tunisia).

2. Materials and Methods
2.1 Bacterial Strains and Epidemiological Background
Fifty-eight fecal *E. coli* isolates were collected from poultry (31 isolates, 4 farms (two farms in Sousse in the Central-East region of Tunisia, farm in borej cédri, farm in Tunis) and bovine (27 isolates, 2 farms; farm in Bizerte (in the north of Tunisia) and farm in Gbeli (the southern of Tunisia) during 2012. All animals were healthy and in on each farm, fecal samples were obtained from different flocks that contained from 1000 to 1500 and immediately after collection were cultivated on Mac-Conkey agar and incubated overnight at 37°C. One colony with typical *E. coli* trait from each sample was picked and re-isolated on Mac-Conkey agar, and followed by biochemical identification using Api20E (Bio-Mérieux, France).

2.2 Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing was carried out by the agar disk diffusion method on Mueller–Hinton agar plates according to recommendation of Clinical and Laboratory Standards Institute guidelines (CLSI, 2012). The antibiotics tested were the following: amoxicillin (25 µg), amoxicillin + clavulanic acid (20 µg + 10 µg), ticarcillin (75 µg), ticarcillin + clavulanic acid (75 µg + 10 µg), piperacillin (75 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), imipenem (10 µg), streptomycin (10 UI), gentamicin (10 UI), kanamycin (30 UI), tobramycin (10 µg), tetracycline (30 UI), nalidixic acid (30 µg), ofloxacin (5

µg), pefloxacin (5 µg), ciprofloxacin (5 µg), sulfonamide (200 µg), trimethoprim/sulfamethoxazole (1,25 µg + 23,75 µg) (Bio-Rad). *E. coli* ATCC 25922 was used as a control strain. For the screening of ESBL production The Double-Disc Synergy Test (DDST) with cefotaxime or ceftazidime in the proximity to amoxicillin-clavulanic acid was used [19].

2.3 Detection and Characterization of Integron Class 1 and 2
For integrons of class 1 and 2, the 3'CS of class 1 integrons, and the variable regions of integrons class 1 and 2 were amplified by PCR as previously reported and randomly 16 chosen poultry and bovine isolates of class 1 and 2 integrons were sequenced [20].

2.4 Detection of the Genes Encoding Tetracycline and Sulfonamide Resistance
The presence of the genes encoding tetracycline (*tetA*, *tetB*, and *tetC*) and sulfonamides-resistance (*sul1, sul2, and sul3*) was investigated by PCR for all resistant isolates to these markers as reported by Saénz *et al.*, [20].

2.5 Phylogenetic Analysis of E.coli Isolates
E. coli phylogenetic grouping was determined by a triplex PCR assay as reported by Clermont et al, (2000). The combination of PCR products obtained allowed classification of *E. coli* strains into one of the four major *E. coli* phylogenetic lineages: A, B1, B2 or D. Appropriate positive and negative controls were included in the assay.

2.6 Statistical Analysis
Statistical testing was done using Epi Info (version 6.04) softwares to check if there were a significance relation with; origin-phylogroups, origin-integron and phylogroups-integrons. Comparisons of proportions were determined using the chi-square test or Fisher's exact test.

3. Results
3.1 Susceptibility to Antibiotics
E. coli isolates were collected from poultry (31 isolates), bovine (27 isolates). Among 31 poultry *E. coli* isolates (Table 1), high rates of resistance were observed for the tetracycline (90.32%), sulfonamide (70.51%), trimethoprim/sulfamethoxazole and streptomycin with same rate (64.51%), nalidixic acid (61.29%), amoxicillin and ticarcillin with same rate (58 %). Moderate frequencies of resistance were noted for ciprofloxacin (32.25%) as well as a lowly rate were observed to amoxicillin/clavulanic acid, cefotaxim and ceftazidime with a same rate (3.2 %). In addition, for bovin *E. coli* isolates high rates of resistance were observed for the streptomycin (55.5%), tetracycline (37%), Moderate lowly frequencies of resistance was noted for amoxicillin (18.5%), trimthoprim/sulfamethoxazole (11.11%), and sulfonamide, nalidixic acid with same rate (7.4%). Lowely frequencie of resistance was noted for ciprofloxacin (3.7%). No resistance to imipenem was detected. No ESBL producing *E. coli* isolate was detected. However, it was remarkable that the majority of the resistant isolates were of poultry origin (Table 1).We found that 10 (32.25%) poultry isolates were (resistant to five antibiotic families or more) multiresistants.

Reference of isolate	Source / Farm	Phylogroups	Resistance profile	Int*	VRs** des intégrons (bp) / arrangements of gene Cassettes***	tet	sul	Qac-sul1
1	Poultry / farm Sousse	B1	AMX, TIC, S, SXT, SSS, TE, AN, CIP	1	1000+280/ dfrA12+orfF+aadA2+cmlA1+aadA1+qacH+IS440+-sul3	A	Sul1- 3	-
2		B1	AMX, TIC, S, SXT, SSS , TE, AN	1	1500/ dfrA1+aadA1	A	Sul1-2-3	+
3		A1	AMX, TIC, S, SXT, SSS ,TE, AN	1	1500+700+280/ dfrA1+aadA1	A	Sul 2-3	+
4		A1	AMX, TIC, S, SXT, SSS, TE, AN	1	1500+700+280/ dfrA12+orfF+aadA2	B	Sul1-2	+
5		D2	S, SXT, SSS, TE	1	280/ dfrA1+aadA1	A	Sul1	+
6		A1	AMX, TIC, SSS , TE, AN, CIP,	1	1500/ dfrA1+aadA1	-	Sul1	+
7		B22	TE, AN	-	-	A	-	-
8		A1	AMX, TIC, S, SXT, SSS, TE	1	1500/ dfrA5	-	Sul2	+
9		A0	AMX, TIC, S, SXT, SSS, TE	1	1500/ dfrA17+aadA1	A	Sul2	+
10		B1	S, GM, SSS, TE, AN	1	1000/ aadA1	-	Sul1	+
11		D1	AMX, TIC, S, SXT, SSS, TE	1	1000+280+600	-	-	-
12		A1	AMX, TIC, S, SXT, SSS, TE	-	-	A	-	-
13		B1	SXT, SSS , TE, AN, CIP	1	350+1700	-	Sul1	+
14		D1	AMC, TIC, S, SSS, SXT, TE, AN	1	-	A	-	-
15		B1	AMX, TIC, S, TE, SSS, AN, CIP	-	-	A	-	-

16		B1	AMX, AMC, S, TE, AN, CIP, CAZ, CTX	1	-	B	-	-
17		B1	AMX, TIC, S, SSS, SXT, TE, AN, CIP	1	1500	-	Sul1	+
18		D2	AMX, TIC, S, SXT, TE, AN, CIP	1	300	A	-	-
19		B1	AMX, TIC, S, SXT, , SSS, TE, AN	1	-	A	Sul2	-
20	Poultry / Farm Sousse (2)	A1	TE	-	-	A	-	-
21		A1	TE	-	-	B	-	-
22		A0	SENSIBLE	-	-	-	-	-
23		A1	TE	1	1500	A	-	-
24		A0	SXT, SSS	-	-	-	Sul1	+
25		A0	SENSIBLE	-	-	-	-	-
26	Poultry/ Borej cedria	B1	TIC, S, SXT, SSS, TE,AN, CIP	1	1500 dfrA12+orfF+aadA2	A-B	Sul1-2	+
27		A1	AMX, TIC, SXT, SSS, TE,AN, CIP	1	1000 dfrA1+aadA1	A	Sul1	+
28		A1	S, SXT, SSS, TE,AN, CIP	-	1500/ dfrA17+aadA1	B	Sul1	+
29	Poultry/ tunis	A1	AM, TIC, S, SXT, SSS, TE	2	2000/dfrA1-satA1-aadA1	A-B	Sul1-3	+
30		D1	K, TE,AN	2	2000/ dfrA1-satA1-aadA1	A	-	-
31		A1	AM, TIC, K, S, SXT, SSS, TE,AN	1	1500	B	Sul1	+
1	bovin/ Far-momElhani Bizerte	B1	K, S, SXT, SSS, TE	1	1500/	A	Sul1	+
2		B1	K, TE	-	-	A	-	-
3		A1	SXT, TE	1	-	A	-	-

				Int*	VRs**/Arrangements of Gene Cassettes***			
4		B1	AMX, AMC, TE, AN, CIP	1	-	A	-	-
5		B1	AMX, AMC, K, S, TE, SXT, SSS, AN	1	2000+1500+1200+500+280/ dfrA1+aadA1	A-B	Sul 1	+
6		A0	S	-	-	-	-	-
7		A0	S	-	-	-	-	-
8		D1	S, TE	-	-	A	-	-
9		A0	S	-	-	-	-	-
10		A0	S	-	-	-	-	-
11		B1	S	-	-	-	-	-
12		A0	S	-	-	-	-	-
13		A0	S	-	-	-	-	-
14		B1	S	-	-	-	-	-
15		B22	S	-	-	-	-	-
16		A0	S	-	-	-	-	-
17		A0	SENSIBLE	-	-	-	-	-
18		D1	SENSIBLE	-	-	-	-	-
19		B22	S,TE	-	-	A	-	-
20		B22	AMX, S, TE	-	-	A	-	-
21		B22	AMX, TE	1	-	A	Sul1	+
22	Bovin / Farm Gbeli	A1	AMX, S, TE	2	2000 / dfrA1-satA1-aadA1	A	Sul2	-
23		D1	S	-	-	-	-	-
24		A1	S	1	280	-	Sul1	-
25		A0	S	-	-	-	-	-
26		A1	S	-	-	-	-	-
27		B1	SENSIBLE	-	-	-	-	-

Int* ;integron, VRs** ; variable regions; ***Arrangements of Gene Cassettes detected was obtained in 16 selected isolates with randomly chosen

Table 1: Phenotypic and genotypic characteristics of fecal poultry (N=31) and bovine (N=27) isolates.

3.2 Occurrence of Integrons and Determination of the Variable Region of the Integron Class 1 and 2 by PCR and Occurrence of Arrangements of Resistance Gene Cassettes Detected By Sequencing

For poultry *E.coli* isolates class 1 and 2 integrons were detected in 22 isolates; twenty-six isolates harbored the class 1 integrons and only in three isolates was found class 2 integron,. According to the origin of integron-positive isolates, 22 were poultry isolates and 7 bovine isolates. The 3'CS (*qacEΔ- sul1*) mainly present in the typical class 1 integron were found only in 17 among 22 class 1 integron-positive poultry isolates, whereas, 3 isolates (37 %) among 7 class 1 integron-positive isolates were detected in bovin origin. It is interesting to note that isolates containing integrons were more resistant to antimicrobial agents than integron-negatives isolates (Tables 1). Both of class 1 and 2 integrons were not found in our isolates. Statistical analysis showed that class 1 integrons were significantly associated with poultry origin (p=0.001).

The VRs were detected in twenty four isolates (82.75%) amongst twenty nine class 1 and 2 integron. The size were from 280bp to 1700 in eighteen poultry int1-positive isolates; 1500 (9 isolates), 1000+700+280 (2 isolates) and for six isolates everyone have a alone or combination of VRs likes; 1000pb+280pb+600pb, 1700pb+350pb, 1000pb+280pb, 1000pb, 300pb and 280pb respectively. Whereas, in bovine origin the VRs of class1 integron were detected in 3 isolates with a size of 1500pb and in one isolate with 280pb. While, the combination of VRs; 1200pb+1500pb+ 1200pb+500pb+ 280pb was detected only one in isolate with the arrangements of resistance gene cassettes detected *dfrA12+ orfF+aadA2+ cmlA1+aadA1+qacH+IS440+sul3.* The VRs of class2 integron were detected in poultry and bovin in 2 and 1 isolates, respectively, and showed the arrangement gene cassette; *dfrA1-satA1-aadA1* in one identical DNA fragments with approximate size of 2000 bp: Six arrangements of resistance gene cassettes of class 1 integron were detected; dfrA1+aadA1 (5isolates); for *dfrA17+aadA1, dfrA12+ orfF+aadA2* each one two 2isolates; one isolate for *aadA1 and dfrA*5, respectively.

3.3 Genes Encoding Tetracycline-, Sulphonamide- Resistance and ESBL Production

Thirty eight were resistant to tetracycline. Genes of *tet-type* were carried in 32 devised in 22 (78.75%) poultry isolates and 10 bovin isolates. The genes *tetA, tetB* genes were observed in 27 (84.37 %) and 8 (25%) resistant isolates, respectively. *TetA-tetB* genes were simultaneously found in 3 isolates, while tetC was not detected in our collection. Among thirty one poultry isolates, twenty two (70.96%) isolates were sulfonamide resistant and *sul*-type genes were detected in eighteen isolates while qac-sul1 was detected in 16 isolates.

The genes *sul1, sul2,* and *sul3* were detected in 14 (77.77 %), 7 (38.88%), and 4 (22.22%) isolates, respectively. The combinations of following genes were identified (number of isolates): *sul1+sul2*: (2); *sul1+sul3* (2); *sul2+sul3* (1) and *sul1+sul2+sul3* (1). In addition, sulfonamide resistant encoded by sul1 and sul2 genes in 4 and 1   bovin isolates, respectively with absence of sul3-type. We showed a positive relation between the presence of integrons and the detection of genes encoding resistance to tetracycline and sulfonamides (Table 1).

3.4 Determination of Phylogroups

In poultry, 16 isolates were found to belong to phylogroup A (sub- groupA1: 12, sub- groupA0: 4); 9 to B1, 1 to B2 and 5 to phylogroup D. However, in bovine 9 isolates have the phylogroups A1, 7 isolates B1, 4 isolates B2, and 3 isolates found to phylogroup D.

4. Discussion

We collected fifty eight *E. coli* strains isolated from healthy poultry and bovins recovred from farms which located in four different governorates of Tunisia (Sousse, Tunis, Bizerte and Gbeli). We studied the antibiotic susceptibility and the results showed that among 31 poultry *E. coli* isolates high rates of resistance were observed for the tetracycline (90.32%), nalidixic acid (61.29%), sulfonamide (70.96%), and trimethoprim-sulfamethoxazole (64.51%). Our results were in agreement with other Tunisia studie of 136 fecal poultry *E. coli* isolated from thirty six different farms which showed high rates resistance of tetracycline (94%), nalidixic acid (89.5%) and trimethoprim-sulfamethoxazole (73. 1%) [11]. Moreover, our previous study suggest that poultry isolates were considerate trimethoprim-sulfamethoxazole resistance rate, it is similar to our previous results [21].

However, moderate frequencies of resistance were noted for amoxicillin and ticarcillin with same rate (58%), ciprofloxacin (32.25%), as well as, a lowly rate were observed to amoxicillin/clavulanic acid, cefotaxim and ceftazidime with a same rate (3.2 %). In addition, for bovin E. coli isolates, high rates of resistance were observed for the streptomycin (55.5%), tetracycline (37%). Moderate frequencie of resistance was noted for amoxicillin (18.5%) such as lowly rate of resistance were noted to trimethoprim-sulfamethoxazole (11.11%), nalidixic acid and sulfonamides with same rate (7.4%) and ciprofloxacin (3.7%). Our results were in agreement with other studies (Jiang et al., 2009; Lei et al., 2010) which showed high rates of tetracycline resistance (90.8 %- 95.2 %) and streptomycin (46 %).

Our study presented high rates of the two antimicrobial tetracycline and streptomycin in poultry and bovin *E. coli* isolates, this was having been mainly reported from farmed animals worldwide [2,22,23]. However, the prevalence to streptomycin resistance in E. coli was variable according to geographic area and husbandary conditions [2,22,23]. In addotion, high rate of resistance against trimethoprim-sulfamethoxazole and sulfonamides were observed in poultry origin among our collection, in contrast lowly prevalences of resistance to the same two antimicrobials were found in bovin isolates. Also, this finding is similar to that reported in other countries for E. coli strains isolated from healthy animals and food-products of animal origins [24-28].

No ESBL-producing isolate was identified amongst our collection, while, similary Soufi et al., have not identified any ESBL-producing *E. coli* isolate from a collection of 164 isolates; therefore, in our work the ESBL-producer population might be underestimated owing to the randomly selection of one colony per sample grown on unselective media [21,29]. Recently,

ESBL-producing *E. coli* isolates have been reported in animal worldwide especially from poultry origin [30-33]. In Tunisia, the presence of ESBL producers has been previously reported in *E. coli* from poultry, pets, dromedary, and meat of various animals [9,31,10-12,34]. In those studies, ESBL production was detected by using a selective protocol.

Amongst fifty eight isolates class 1 and class 2 integrons were found in twenty-nine isolates of *E. coli*. Integrons of class 1 were found in twenty six isolates while class 2 only in three isolates, according to the origin of integron-positive isolates, 22 were poultry isolates and 7 bovine isolates, our results were in agreement with other works which showed the dominance of integron class 1 in animal-derived and human *E. coli* isolates [21,29]. However, the presence of integrons were most frequently in poultry origin then bovin origin and this was confirmed by the statistical analysis which found that there is a correlation between poultry origin and class 1 integron (p=0.001).

Class 1 integron is a dynamic genetic system encoding a functional integrase protein enabling integration of several gene cassettes in the variable regions, which were all expressed due to the presence of a common promoter. This genetic trait, explain in part the high dominance of class 1 integrins in *Enterobacteriaceae*, particularly in resistant isolates and in a rich-antibiotic environment. The 3'CS (*qacEΔ- sul1*) found generally in class 1 integron was detected only in 17 poultry among 22 class 1 integron-positive isolates, whereas, 3 isolates (37%) among 7 class 1 integron-positive isolates were detected in bovin origin. This finding was in agreement with other recent studies reporting the absence of this region in class 1 integron [21,29,31,35].

The size of VRs detected by PCR were from 280bp to 1700pb in int1-positive isolates and an approximate size of 2000pb in class 2 integrons which unable to integrate new gene cassettes into the variable region and was not functional [5].The variable region of class2 integron mainly carries *dfrA1* (encoding trimethoprim resistance), *sat1* (encoding streptomricin resistance) and *aadA1* (encoding streptomycin/spectinomycin resistance) [5,21,29]. The fragment of the VR of class 1 integron sized 280pb cannot correspond to any gene cassette. In a genetic point of view it is plausible that currently a phenomenon of crucial genetic rearrangements in class 1 integron is happening. This shifting might argument for the evolution toward more successful structures in capitation and dissemination of resistance. Indeed, this region was replaced in classical class 1 integron by a 'transposon-like' structure, a qacHΔ-IS440-sul3, which could facilitate the dissemination of class 1 integron by a mechanism of transposition [36].

We choose randomly for sequencing of VRs for poultry origin 12, 2 isolates which harbored class 1 integrons and 2, respectively and 2 isolates for bovine each one harbored different class of integron. In 15 class 1-positive isolates, the VR showed the presence of six arrangements of gene cassettes; we notice that the gene cassette array dfrA1-aadA1 was the most frequently detected, found in ten isolates (5/15, 33.33 %). Furthermore, there were one isolates harbouring long arrangement of gene cassettes with un-classical 3' CS: *dfrA12-orf F-aadA2-cmlA1-aadA1-qa-*

cHΔ-IS440-sul3. This structure was also reported by other authors [29,37]. This phenomenon of "substitution» (*qacEΔ-sul1/ qacHΔ-IS440-sul3*) might be for perfection rather than 'change of function'. Indeed, the qacΔH and sul3 genes inserted code for same functions as the genes qacEΔ (resistance the quaternary ammonium) and sul1 (resistance in sulfonamides), respectively. Genetically, this could be explained by the continuous use of the ammonium-quaternary and sulfonamides till the current days in avian industries. The variable regions in class 2 integrons, presented in three isolates, were identical, dfrA1-sat1-aadA1. This structure is mainly common in all class 2 integrons identified worldwide [35].

We showed a positive relation between the presence of integrons and the detection of genes encoding resistance to tetracycline and sulfonamides. Tetracycline resistance was observed in 32 out of 58 isolates (84.2 %), a rate closer to that reported in our previous work [21]. The absence of detection of resistance genes in some of our resistant isolates could indicate that they might contain other non-tested or unknown resistance genes. Genes of tet-type were carried in 32 devised in 22 (78.75%) poultry isolates and 10 bovin isolates. The genes tetA, tetB genes were observed in 27 (84.37 %) and 8 (25%) resistant isolates, respectively. TetA-tetB genes were simultaneously found in 3 isolates, while tetC was not detected in our collection. The genes tetA and tetB were simultaneously found in six isolates. The predominance of tetA gene was also reported by other studies while the tetC gene is scarcely reported [38-40]. A genetic linkage on conjugative plasmids of tetA gene and class 1 integron was reported by Sunde and Norstrom, this is in agreement with our results where the majority of isolates containing tetA also harboured the integron of class 1 [27]. This suggests the possibility of co-localization of the genes of tet-type and integrons; however, more thorough genetic studies were necessary to confirm this hypothesis.

Among thirty one poultry isolates, twenty two (70.96%) isolates were sulfonamide resistant and sul-type genes were detected in eighteen isolates, resistance in the remaining isolates might be due to chromosome mutation in dihydropteroatic synthetase DHPS [41].

The genes sul1, sul2, and sul3 were detected in 14 (77.77 %), 7 (38.88%), and 4 (22.22%) isolates, respectively while qac-sul1 was detected in 16 isolates. The combinations of following genes were identified (number of isolates): sul1+sul2: (2); sul1+sul3 (2); sul2+sul3 (1) and sul1+sul2+sul3 (1). In addition, sulfonamide resistant encoded by sul1 and sul2 genes in 4 and 1 bovin isolates, respectively with absence of sul3-type.

In agreement with our results, the gene sul1 remains by far the most frequently found gene followed by the gene sul2, while sul3 generally less frequent [42,43]. However, in one of the previous studies of our laboratory, the sul3 gene was the most prevalent one in avian E. coli isolates followed by sul1 and sul 2, respectively [29]. It is interesting to note that the majority of integron containing isolates also harboured the sul genes, that might be linked to the structure of class 1 integron which contain sul1 gene in classical 3 'CS and also sul3 in some isolates. For the distribution of isolates according to different phylogroups 29

isolates were found to belong to phylogroup A and 16 to phylogroups B1, in the literature groupsA and B1 in human E.coli strains are considered non- virulent commensal strains contrary to animal E.coli isolates belonged to phylogroup A and B1 such as the ESBL strains, which mainly virulent [11,44,34]. Generally, many studies noted that pathogenic strains producing extra-intestinal infections (ExPEC) belong mainly to the B2 group and D group. Among our collection we detected only 5 isolates belonged to phylogroup B2 and 8 to phylogroup D [45-50].

Taken together, these findings highlight the importance of poultry E. coli as reservoir of antibiotic resistance that certainly linked to the excessive use of antibiotic especially for tetracycline and sulfonamide in avian husbandry in Tunisia more than in bovine farms. This dramatically situation is certainly not specific to Tunisia. Therefore, this is worrisome for global human health, especially with the increasing consumption of poultry meat in Tunisia and in other part of the world owing to its relatively lower cost comparing to red meat.

Conflict of interest statement
None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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