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Research Article

Antimicrobial Resistance of *Escherichia coli* Involved in Algerian Bovine Carriage, ESBL Detection, Integron Characterization and Genetic Lineages

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Abstract

This study aimed to characterize the fecal carriage of antimicrobial-resistant *Escherichia coli* isolates in healthy bovine in Northern Algeria. Fecal samples of 233 cows were collected and cultured on MacConkey agar. *E. coli* isolates were recovered, identified and tested for antibiotic susceptibility by disk diffusion method. Screening of extended-spectrum-betalactamase (ESBL)-production was performed by double-disk synergy test and characterization of ESBL genes by PCR and sequencing. All isolates were typed for phylogenetic groups and multilocus-sequence-typing (MLST) analysis was performed on phylogroup B2 and ESBL-producing isolates. The presence of antimicrobial resistant genes was analyzed in the collection of *E. coli* isolates and integrons in SXT-resistant isolates. Overall, 39.9% of *E. coli* isolates (89/223) were resistant to at least one antimicrobial agent, and 41.5% of them showed multi-drug resistance (MDR). High resistance rates were detected for tetracycline (32.3%), streptomycin (18.4%), sulphamethoxazole/trimethoprim (15.7%) and ampicillin (15.2%). Two ESBL-producing *E. coli* isolates were identified: A/ST617/CTX-M-15 and A/ST48/SHV-12. Sequence types ST95, ST998 and ST145 were detected among the phylogroup B2 isolates. From 35 SXT^R isolates, class-1 and class-2 integrons were detected in 82.9% (29/35) and 12.9% (1/35), respectively. Six gene-cassette-array structures were detected in the variable region of class-1 *(dfrA1-aadA*; *dfrA12-aadA2, aadA1/2; dfrA12-orfF-aadA2-cml-sul3*-linked and *dfrA17-aadA5)* and class-2 integrons *(dfrA1-sat2-aadA1)*. Our study highlights the potential dynamics of animal *E. coli* isolates in farms.

Keywords: Antimicrobial resistance, bovine, *E. coli*, Integrons, MLST, Phylogenetics

Introduction

In veterinary husbandry, antibiotics are used as therapeutic or prophylactic agents, for the treatment and control of infectious diseases, or, in some countries, as growth promoters to improve weight gain [1]. Nowadays, antibiotics are highly used in dairy industry and in animal farming, and in some countries with scarce control; in this sense, a study refer that 56% of farmers in a sub-Saharan country use non-prescribed antibiotics and about 25% of countries at world level use antibiotics as growth promoters of animals [2,3]. These practices associated with insufficient hygiene and biosecurity led to the emergence and spread of antimicrobial resistance globally [4]. Along the food chain, antimicrobial resistance is considered as a major global public health concern, because many food animals are carrying antibiotic-resistant strains, such as extended spectrum-beta-lactamase (ESBL) producing Enterobacteriaceae [5].

The acquisition of new resistance mechanisms leading to antimicrobial resistance, and the declining flow of new antimicrobial agents continue to threaten our ability to treat common infections, particularly infections caused

by multidrug-resistant (MDR) microorganisms [6,7]. Bacterial infections with MDR are of particular concern because it limits treatment options, can be transferred between pathogenic bacteria, and increases superbug morbidity [8]. ESBL-producing *E. coli* is an emerging MDR bacteria resistant to third-generation cephalosporins and monobactams [9]. *E. coli* is a normal inhabitant of the human intestine, which could under some circumstances cause severe sepsis and urinary tract infections, among hospital-level infections [10]. In animals, diarrhea and several infectious diseases caused by *E. coli* are considered the main causes of economic losses associated with poor growth, drug costs and animal death [11]. The intensification of cattle breeding and the intensive use of antibiotics make cattle important reservoirs of resistant bacteria that can be disseminated at the human-animal-environment interface [12]. Although antimicrobial resistant *E. coli* from cattle have been reported in many parts of the world, information on cattle as potential reservoir of *E. coli* resistant to antimicrobials, particularly in Algeria, is more-scarce. Analysis of antimicrobial resistance genes and molecular typing of *E. coli* isolates from cattle will provide useful data for predicting potential risks associated with mammals' *E. coli* in Algeria.

This study aimed at determining the frequency of ESBL-producing *E. coli*, the genetic characteristics and antibiotic-resistant profiles among *E. coli* recovered from cattle feces in northern Algeria

Material and Methods

Ethical Statement

The study protocol was approved by the Veterinary Science Institute Scientific Committee of the university Saad Dahlab of Blida1 (Ref: CSI/N°12/2015).

Sampling and Bacterial Isolation

From January 2017 to September 2019, 30 farms were visited in three department districts of northern Algeria. Most of the farms (21/30, 70%) were located at Tizi-Ouzou while the remaining were distributed between Algiers (4 farms) and Blida (5 farms). After obtaining consent from the farm's owners, accessible animals inside the stable were submitted to fecal sampling. Up to 50 grams of fecal matter were directly taken from the rectum of each animal in a sterile jar and transported immediately to the laboratory under cold storage for processing.

Fecal samples were diluted (1:10) in buffered peptone water (Pasteur Institute of Algiers, Algeria) and incubated at 37°C for 24 h. The enriched culture was inoculated on MacConkey agar plates (Conda, Spain) and incubated at 37°C for 24 h. One presumptive *E. coli* colony per sample was randomly selected and identified by classical biochemical methods (gram-staining, oxidase test, TSI, indol) and API 20E gallery (BioMerieux, France). The identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry method (MALDI-TOF MS, Bruker) in the Laboratory of Biochemistry and Molecular Biology in the University of La Rioja (Logroño, Spain). One *E. coli* isolate per sample was maintained for further studies. *Table 1* shows the isolates recovered from each of the farms tested.

Antimicrobial Susceptibility and ESBL Phenotypic Tests

Antibiotic susceptibility testing for ampicillin (AMP), amoxicillin/clavulanate (AMC), cefotaxime (CTX), ceftazidime (CAZ), cefoxitin (FOX), imipenem (IMP),

Table 1. *Number of E. coli isolates obtained from faecal samples of bovine of 30 different farms located in 3 different departments of Algeria*

Farm Number	Region	No. of the Tested Samples	No. of *E. coli* Isolates
1	Blida	3	3
2	Blida	3	3
3	Algiers	3	3
4	Tizi-Ouzou	6	6
5	Tizi-Ouzou	7	7
6	Tizi-Ouzou	7	7
7	Tizi-Ouzou	15	15
8	Blida	7	7
9	Tizi-Ouzou	7	7
10	Tizi-Ouzou	9	9
11	Tizi-Ouzou	12	12
12	Tizi-Ouzou	11	11
13	Tizi-Ouzou	14	14
14	Tizi-Ouzou	19	19
15	Tizi-Ouzou	6	6
16	Tizi-Ouzou	8	8
17	Tizi-Ouzou	4	4
18	Tizi-Ouzou	3	3
19	Tizi-Ouzou	8	8
20	Tizi-Ouzou	17	17
21	Tizi-Ouzou	4	4
22	Tizi-Ouzou	8	8
23	Algiers	9	9
24	Algiers	4	4
25	Blida	7	7
26	Tizi-Ouzou	7	7
27	Tizi-Ouzou	4	4
28	Tizi-Ouzou	5	5
29	Blida	4	4
30	Algiers	2	2
Total		223	223

ciprofloxacin (CIP), gentamicin (GEN), chloramphenicol (CHL) and sulfamethoxazole/trimethoprim (SXT) was performed by the disk diffusion method as recommended by EUCAST [13]. For streptomycin (STR) and tetracycline (TET), the CLSI recommendation interpretative criteria were followed [14]. The screening for ESBL production was carried out by double-disk test (DDST), using third generation cephalosporins (CTX and CAZ) and a beta-lactamase inhibitor (AMC). Isolates showing resistance to at least three families of antimicrobial agents were considered as multidrug resistant (MDR).

Characterization of Antimicrobial Resistance Genes

Bacterial DNA was extracted by boiling three to five colonies in 1 mL of sterile Milli-Q water for 8 min. The suspension was centrifuged at 12.000 rpm for 2 min; the supernatant was collected and stored at -20°C for later use. *E. coli* isolates resistant to beta-lactams were tested by PCR for beta-lactamase genes: bla_{TEM}, bla_{SHV}, bla_{OXA-1}, bla_{CTX-M}-universal, and $bla_{CTX-M-1}$ group. The PCRs for bla_{CTX-M}-universal and $bla_{CTX-M-1}$ group were performed for the ESBL-producing isolates. Positive amplicons were sequenced to identify the beta-lactamase gene subtype. *E. coli* isolates were screened for the presence of resistance genes such as: *tet*(A)/*tet*(B) for tetracycline, *sul1/sul2/sul3* for sulphonamide, *cmlA*/*floR* for chloramphenicol, *qnrA/qnrB/qnrS/aac(6')*-Ib-cr for ciprofloxacin and *aac*(3)-II for gentamicin resistance [15].

Integron Analysis

SXT resistant (SXT^R) *E. coli* isolates were tested for the integrase of class 1, 2 and 3 integrons *(intI1, intl2,* and *intl3,* respectively). The variable regions of class 1 and class 2 integrons were amplified by PCR in all *intl*1-positive and *intI2*- positive isolates and amplicons were sequenced to obtain the gene cassette arrays [16].

Phylogenetic Groups and Multi Locus Sequence Typing

E. coli isolates were assigned to one the 8 phylogenetic groups (A, B1, B2, C, D, E, F and Clade I) by using the quadruplex PCR strategy as well as the specific PCRs designed for phylogroups C and E [17]. To identify the genetic lineages of selected *E. coli* isolates (ESBL-producing isolates and those affiliated into the phylogenetic group B2), Multilocus sequence typing (MLST) of seven housekeeping genes *(adk, fumC, gyrB, icd, mdh, purA* and *recA)* was performed by PCR and sequencing to determine the sequence type (ST) *(http://mlst.warwick.ac.uk/)* [18].

Data Analysis

Raw data were entered to Microsoft Excel (2016; Microsoft Corp., Redmond, WA, USA) and imported to MedCalc version 2019 (Ostend, Belgium) for statistical analysis. Binary logistic regression was used to determine the association between the phylogenetic groups and the presence of antimicrobial resistance. In this model phylogroup A was as a reference. A *p*-value of 0.05 was used to determine the significance level.

Results

Antimicrobial Resistance Phenotype and Genotype

A total of 223 *E. coli* isolates were obtained of 223 samples of cattle feces (one isolate per sample) *(Table 1)*. Antibiotic susceptibility results showed that 134 (60.1%) of the isolates were susceptible to all antimicrobial drugs tested, while 89 isolates (39.9%) were resistant to at least one antibiotic. Resistance to cefoxitin and imipenem was not found while resistance levels for other antibiotics were as follows (percentage of resistance): tetracycline (32.3%), streptomycin (18.4%), sulfamethoxazole/trimethoprim (15.7%), ampicillin (15.2%), amoxicillin/clavulanic acid (10.8%), gentamicin (6.7%), chloramphenicol (5.4%), ciprofloxacin (3.1%), and cefotaxime and ceftazidime (0.4%). Two of the 223 *E. coli* isolates showed an ESBL phenotype, and the remaining 221 were ESBL-negative.

Resistance Genes Detected Among the ESBL-negative *E. coli* Strains

Table 2 shows the percentage of antibiotic resistance among the 221 non-ESBL-producing *E. coli* isolates of bovine origin analysed in this study. From the 32 ampicillin resistant isolates, 24 (75%) carried the bla_{TEM} gene and 1 (3.1%) carried the bla_{OXA-1} gene. Tetracycline resistance (70 strains) was associated with the presence of *tet*(A) (22.8%), *tet*(B) (22.8%) or *tet*(A)+*tet*(B) genes (4.3%). The *sul*2, *sul*3, *sul1+sul2* and *sul2+sul3* genes were detected in 54.5%, 3%, 24.2% and 15.1 % of SXT resistant isolates, respectively. The *cmlA* gene was found in 58.3% (7/12) of chloramphenicol-resistant isolates. The *qnrS* gene was identified in 22.8% (1/6) of ciprofloxacin resistant isolates. Finally, the *aac*(3)-II gene was revealed in 28.6% (4/14) of gentamicin resistant isolates. *Table 3* shows the phenotypes of resistance shown by all the *E. coli* isolates of the study.

Out of the 89 tested isolates, 50.5% showed resistance to a minimum of two antibiotics. Upon the resistant strains, fourteen patterns of resistance were identified. Two ESBL producing *E. coli* isolates were obtained in two farms from Tizi-Ouzou and Blida *(Table 3)*. Multi-drug resistance (resistance to at least three families of antibiotics) was observed in 41.5% (37/89) of the tested strains *(Table 4)*.

Characteristics of ESBL-producing Strains

Two ESBL-producing *E coli* isolates were identified in this study and the characteristics are shown in *Table 4*. One of them was ascribed to lineage ST617 and phylogroup A, showed a MDR phenotype [AMP-AMC-CTX-CAZ-TET-

***Table 2**. Percentage of antibiotic resistance among the 221 non-ESBL-producing E. coli isolates of bovine origin analysed in this study*

Antibiotic	No. of Isolates Showing Resistance	Rates of Resistance	Resistance Genes (No. of Isolates/%)
Ampicillin	32	14.5	bla_{TEM} (24/75%) bla_{OXA1} (1/3.1%)
Amoxicillin/clavulanic acid	23	10.4	bla_{TEM} (15/65.2%)
Cefotaxime + ceftazidime	0	0.0	-
Ciprofloxacin	6	2.7	*qnrS* (1/22.8%)
Sulphamethoxazole/ Trimethoprim	33	14.9	*sul2* (18 / 54.5%) *sul3* (1/3%) *sul1+sul2* (8/24.2%) *sul2+sul3* (5/15.1%) *dfrA1* (10/30.3%) *dfrA12* (2/6%)
Tetracycline	70	31.7	*tetA* (16/22.8 %) *tetB* (16/22.8%) *tetA+tetB* (3/4.3%)
Gentamicin	14	6.3	*aac3-II* (4/28.6%)
Streptomycin	41	18.5	*aadA1* (11/26.8%) *aadA2* (2/4.9%) *aadA1/2* (5/12.2%)
Chloramphenicol	12	5.4	*cmlA* (7/58.3%)
Imipenem Cefoxitin	0 0	0.0 0.0	-

***Table 3.** Phenotypes of antimicrobial resistance exhibited by the collection of 223 E. coli isolates obtained of bovine fecal samples*

Phenotype of Antibiotic Resistance[a,b]	No. of Isolates	Percentages
Susceptible	134	60.1
TET	28	12.5
AMP-AMC-TET^{17}-SXT^{13}-STR^{14}-GEN^{4}-CHL^{8}-CIP^{3}	19	8.5
AMP-TET^{5}-SXT^{7}-STR^{7}-CHL^{1}-CIP^{1}	8	3.6
AMP-AMC AMP	4 1	1.8 0.4
AMP-AMC-CTX-CAZ-TET-SXT-CIP-GEN-ESBL^{+} AMP-TET-SXT-ESBL^{+}	1 1	0.4 0.4
TET-SXT-STR-GEN^{2}-CHL^{1}	13	5.8
TET-STR TET-GEN-STR^{2}-CIP^{1}	4 3	1.8 1.3
GEN	3	1.3
GEN-CIP	1	0.4
GEN-STR	1	0.4
CHL	2	0.9

[a] *AMP, ampicillin; AMC, amoxicillin/clavulanicacid, CTX, cefotaxime; CAZ, ceftazidime; FOX, cefoxitin; CIP, ciprofloxacin; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline; GEN, gentamicin; STR, streptomycin; CHL, chloramphenicol. ESBL+: ESBL-producer phenotype*

[b] *Those in superscript indicate the number of isolates that showed the specific resistance for the indicated antibiotic, in case that not all of isolates of the group were resistant*

SXT-CIP-GEN] and carried the gene encoding CTX-M15, as well as the beta-lactamase resistance gene bla_{OXA-1}, aminoglycoside resistance gene *aac*(3)-II, tetracycline resistance gene *tet*(B) and the sulphamethoxazole resistance genes *sul1* and *sul2*. The second isolate was typed as ST48/phylogroup A, contained the gene encoding

Table 4. Characteristics of the seven E. coli isolates showing an ESBL-phenotype or being included into phylogenetic group B2

Isolate Code	Farm/Region	ESBL-test	Phenotype of Antimicrobial Resistance [a]	Antimicrobial Resistance Genes	Integron 1 (Gene Cassette Array)	MLST	Phylogenetic Group
X2535	29/Blida	+	AMP-AMC-CTX-CAZ-CIP-SXT-TE-GEN	*bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *tetB*, *sul1,sul2,aac3-II, aac(6')-Ib-cr*	+ (*dfrA17-aadA5*)	ST617	A
X2525	26/Tizi-Ouzou	+	AMP-SXT-TE	*bla*$_{SHV12}$, *tetA*, *sul3*	+	ST48	A
X2325	3/Algiers	-	AMP-AMC-TE	*bla*$_{TEM}$	-	ST998	B2
X2384	11/Tizi-Ouzou	-	AMP	*bla*$_{TEM}$	-	ST14	B2
X2393	11/Tizi-Ouzou	-	Susceptible	-	-	ST95	B2
X2509	24/Algiers	-	CHL	-	-	ST95	B2
X2515	25/Blida	-	Susceptible	-	-	ST95	B2

[a]*AMP, ampicillin; AMC, amoxicillin/clavulanicacid, CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline; GEN, gentamicin; CHL, chloramphenicol*

SHV-12, and carried the genes *tet*(A), *intl1,* and *sul3*; this isolate showed phenotypic resistance to AMP-TET-SXT, but presented a positive screening ESBL test *(Table 4)*.

Characterization of Integrons

Out of 35 SXT^R *E. coli* isolates typed for integrons, 29 (82.8%) carried the *intI1* gene and 1 (2.8%) isolate carried the *intI2* gene, No class 3 integrons were detected. Different gene cassette arrays were found in the class 1 integrons: *aadA1/2* (5 isolates), *dfrA1-aadA1* (10 isolates), *dfrA17-aadA5* (1 isolate), *dfrA12-aadA2* (1 isolate), and *dfrA12-orfF-aadA2-cmlA/aadA1* (1 isolate). In addition, the *dfrA1-sat2-aadA1* array was detected in the variable region of a class 2 integron of one additional *E. coli* isolate *(Fig. 1)*.

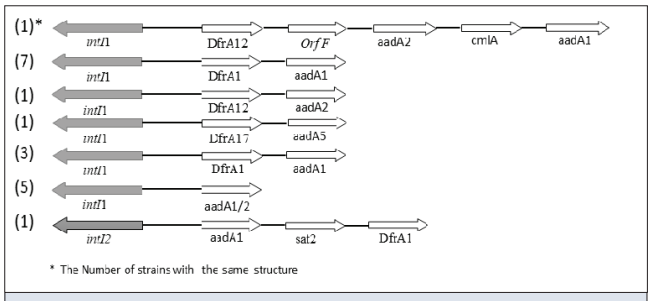

Fig 1. Different structures of class1 and class 2 integrons

Table 5. Distribution of Isolates by phylogenetic groups and correlation with antimicrobial resistance

Phylogroup[a]	No. of Isolates (%)	No. of Isolates (%) Showing Resistance to at Least One Antimicrobial	OR (95% CI)	P Value	No. of isolates Showing Resistance to the Following Number of Antimicrobial Families:						No. and (%) of MDR Isolates	OR (95% CI)	P Value
					1	2	3	4	5	6			
A	70 (31.4)	22 (31.4)	Referent	Referent	9	2	4	3	3	1	11 (15.7)	Referent	Referent
B1	131 (58.7)	59 (45)	1.79 (0.97-1.29)	0.062	27	11	12	4	4	1	21 (16)	1.02 (0.4624-2.27)	0.953
Others	22 (2.2)	8 (22.7)	1.25 (0.46-3.40)	0.667	2	1	1	3	1	-	5 (2.2)	1.19 (0.34-4.20)	0.785
Total	223 (100)	89 (39.9)	NA	NA	38	14	17	10	8	2	37 (16.6)	NA	NA

[a]*Phylogenetic group according to Clermont et al.*[17], [b] *Reference group (Phylogroup A) was chosen arbitrarily, NA = Not applicable for statistical analysis*

Phylogenetic Typing of the *E. coli* Isolates

Seven distinct phylogroups were distinguished among the 223 *E. coli* isolates of this study, with a predominance of the groups B1 and A with 58.7% and 31.4% of isolates, respectively *(Table 5)*. The phylogroups E and B2 represented 4.9% (11/223) and 2.2 % (5/223) respectively, while the phylogroups C, D and F shared three isolates. The 5 isolates of the phylogroup B2 were typed by MLST as ST998, ST14 and ST95. The isolates of phylogroup B2 were recovered from four farms belonging to three different regions of Algeria *(Table 4)*. No statistical correlation was found between phylogenetic groups and the frequency of resistance to at least one antimicrobial agent, or with the rate of resistance to increasing number of antimicrobial families (P>0.05) *(Table 5)*.

Discussion

The unregulated use of antibiotics in bovine farms may enhance the spread of drug-resistant bacteria, particularly ESBL-producing *E. coli*, in the community. These latter

have emerged as a major problem around the world. Primarily, ESBL-producing *E. coli* isolates were only observed in human clinical isolates, but these bacteria have increased drastically in food-producing animals, making them a natural reservoir and contributing to its spread [19]. In the present study, overall, 39.9% of *E. coli* isolates were resistant to at least one antimicrobial agent, whose 41.5% with multi-drug resistant (MDR). The apparent prevalence of resistance to antibiotics recorded in the present survey is lower than those reported in formerly published reports on *E. coli* involved in poultry and pig carriage [20,21]. Only two ESBL-producing *E. coli* isolates were detected in the present study. Unlike to our findings, higher rates of resistance to cefotaxime were observed in *E. coli* isolates recovered from fecal samples of the farms keeping beef cattle (70%) and dairy cattle (85%) in Germany [22]. It is important to remark that no selective media for ESBL-producing *E. coli* recovery was used in our study; so, the prevalence could be higher if antibiotic-supplemented media would be used for ESBL-*E. coli* isolation.

Resistance to tetracycline (31.7%) and streptomycin (18.5%) were the most prevalent phenotypes observed in the tested *E. coli* isolates in our study. Reports from Iran showed higher levels of resistance to streptomycin (98.25%) and tetracycline (98.09%) in *E. coli* isolated from diarrheic calves [23]. The variation between these studies could be due to differences in regulations on antimicrobial use in animals adopted by these countries and therapy traditions followed by veterinarians. Tetracyclines have been used frequently for many decades as efficient and inexpensive antimicrobial agents for animals. The rate of tetracycline resistance detected in our study (31.7%), is in the frame of data obtained in other studies in which higher and lower resistant rates were detected (range4.8-54.5%) [24-27]. Our results concur with the resistance rates to amoxicillin/clavulanic acid (11.62%), sulphamethoxazole/trimethoprim (15.15%) and chloramphenicol (4.04%) reported previously in eastern Algeria [25]. However higher resistance levels were observed in the study of Barour et al.[25] to ampicillin (59.1%) and ciprofloxacin (7.1%).

In the present study, resistance to beta-lactams was mainly associated with the presence of bla_{TEM} gene. This latter was blamed in 24 tested AMP^R isolates while only one AMP^R isolates carried bla_{OXA1}. In a previous report from Tanzania, Madoshi et al.[28] stated that most of beta-lactam resistant *E. coli* isolates recovered from cattle carried bla_{TEM} gene. Sulphonamide resistance genes including *sul2* (51.4%), *sul3* (5.7%), *sul1+sul2* (25.7%), *sul2+sul3* (14.3%) and tetracycline resistance genes *tetA* (23.6%), *tetB* (23.6%) and *tetA+tet B* (4.2%). Accordingly, previous studies reported that sulphonamide resistance genes (*sul1/sul2*) were often found together with tetracycline resistance genes *tet(A) and tet(B)* [29]. The genes *tet(A)* and/or *tet(B),* encoding efflux mechanisms, have been reported to be the most common tetracycline resistance determinant in *E. coli* isolates from humans and animals in many countries [30]. They were associated with 50% (35/70) of the *E. coli* isolates with TET^R phenotypes tested in this study. The number of the isolates harboring exclusively *tet(A)* is similar to those harboring exclusively *tet*(B) genes. Our findings are consistent with the earlier reports showing equal *tet*-gene patterns distribution in *E. coli* isolates recovered from animals, including cattle [30,31]. Other studies reported discordant results with either higher frequencies of *tet(A)* determinant in *E. coli* isolates recovered from cattle [32] or higher frequencies of *tet*(B) genes in *E. coli* isolates [33].

In relation to integron analysis of SXT^R isolates, class 1 integron was detected in 82.8% (29/35) of SXT^R *E. coli* isolates. Five gene-cassette-arrays structures were detected in their variable region: *aadA1/2* (5 isolates), *dfrA1-aadA1*(10 isolates), *dfrA17-aadA5* (1 isolate), *dfrA12-aadA2* (1 isolate), and *dfrA12-OrfF-aadA2-cmlA/aadA1* (1 isolate). One isolate carried the *intI2* with the gene cassette array *dfrA1-sat2-aadA1*. A study conducted in China showed that 66% of *E. coli* strains carried class 1 integron and gene cassette arrays of *aadA1* (most prevalent with 20%), *aadA7, aadA5, aadA17, dfrA1, dfrA5, dfrA1-aadA1, dfrA12-aadA2 and dfrA17-aadA5* [30]. Sequence analysis showed that, the genes *aadA* and *dfrA,* associated to streptomycin and trimethoprim resistance, were dominant in the gene cassette arrays in this study which concurs with previous reports in *E. coli* isolates from cattle [34].

In regards to the phylogenetic groups, B1 (58.7%) and A (31.4%) were the predominant among the *E. coli* isolates. The phylogroups A and B1 are commensals in the intestine and are commonly shed in feces of healthy animals including cattle [35], and blamed in 67.4% of mastitis cases in dairy cattle in China [36]. A study from Brazil showed that most of bovine clinical mastitis associated *E. coli* isolates were assigned to phylogroups A (52%) and B1(38%) [37]. Upon bivariate logistic regression, there was no association between *E. coli* phylogenetic groups and antimicrobial resistance frequencies ($p>0.05$) found during our survey. The major multidrug-resistant *E. coli* isolates belonged to phylogroups A (16%) and B1 (15.7%). In Beijing, 58.6% of antibiotic-resistant *E. coli* strains were affiliated to group B1 and 35.7% were in the group A [38]. Additionally, *E. coli* isolates with MDR were mainly classified in phylogenetic groups A or B1 [39]. The combination of different phylogeny and antimicrobial resistance of *E. coli* may improve the recognition of new subgroups of virulent bacteria.

In cattle, bla_{SHV12} is frequently detected among ESBL producing *E. coli* isolates [19,40]. Molecular analysis of the

two ESBL producing *E. coli* revealed the following patterns [Phylogroup A/ST617/$bla_{CTX-M-15}$] and [Phylogroup A/ST48/bla_{SHV-12}]. Similar findings were reported in Iran [41]. The $bla_{CTX-M-15}$ gene encoding for CTX-M-15 enzyme is often detected in the hospital environment and has been associated with the epidemic lineage ST131/B2 [42]. CTX-M-15 is the most important CTX-M enzyme due to their large diffusion and relation to outbreaks and severe extra-intestinal infections in humans [40]. It has been reported in all continents with reports in all major ecological niches including humans, animals and environment [10,43]. Several studies showed that, the sequence type (ST617) was highly distributed among various livestock species and humans in many African countries [44-47]. The public health threat associated to ESBL-producing CTX-M-15 has to be monitored in different ecological niches and to be considered under the prism of the one health approach. ESBL-producing *E. coli* isolates were multidrug resistant with *blaOXA-1, tetB, sul, sul2, tetA,* and *aac3-II* accessory genes. Similar observation was previously reported by Ibrahim et al.[48] and Lee et al.[49]. These represent a snapshot of resistance genes diversity present in the *E. coli* isolates, including resistance to historically used antibiotics as well as cephalosporins in contemporary use. MLST typing of *E. coli* isolates belonging to the phylogenetic group B2 revealed three ST95 isolates while the remaining belonged to ST14 and ST998. *E. coli* isolates belonging to lineages ST95/B2, ST14/B2, and ST998/B2 are often found in isolates of human origin [50]. Our study highlights an increasing resistance to antibiotics in *E. coli* from cattle carriage. To overcome the problems of multidrug resistant bacteria alternative treatments such as zinc oxide, could be used instead of common antibiotics to treat the *E. coli* and *S. aureus* related diseases [51].

Multi-drug resistance could spread through the food chain if beef meat is contaminated during slaughtering and butchering of cattle as well as through use of livestock feces as manure. Accordingly, hygiene should be adequately enforced at abattoirs to prevent contamination of meat. There is need for formulation and enforcement of policies to regulate use of antimicrobials in the country; antimicrobial surveillance program is also necessary. Public health education about health implications of indiscriminate use of antimicrobials is important.

Declarations

Availabilty of Data and Materials: All data supporting the findings of this study are available from the corresponding author (M. Akkou) upon a reasonable request

Ethical Statement: The study protocol was approved by the Veterinary Science Institute Scientific Committee of the university Saad Dahlab of Blida1 (Ref: CSI/N°12/2015).

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Author Contributions: MS performed the experiments SM-Á, IC, RF-F, and INA contributed significantly to analysis and manuscript preparation. MS and MA performed the data analysis and wrote the manuscript. AH, M-NM and CT helped perform the analysis with constructive discussions.

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