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Diversity of CTX-M Extended-Spectrum β-Lactamases in *Escherichia coli* Isolates from Retail Raw Ground Beef: First Report of CTX-M-24 and CTX-M-32 in Algeria

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The aim of this study was to investigate the prevalence and molecular features of extended-spectrum cephalosporin resistance in *Escherichia coli* isolates contaminating ground beef at retail in Algeria. Of 371 ground beef samples, 27.5% were found to contain cefotaxime-resistant *E. coli* isolates distributed into A (24.5%), B1 (60.8%), and D (14.7%) phylogroups. A rate of 88.2% of isolates had a multidrug-resistance phenotype. All strains were producers of CTX-M type extended-spectrum β-lactamases (ESBLs): CTX-M-1, CTX-M-3, CTX-M-14, CTX-M-15, CTX-M-24, or CTX-M-32. Conjugation assays allowed the transfer of $bla_{\text{CTX-M-1}}$ in association with IncI1 plasmids, $bla_{\text{CTX-M-15}}$ with IncI1 and IncK+B/O plasmids, $bla_{\text{CTX-M-3}}$ with IncK plasmids, and $bla_{\text{CTX-M-14}}$ with IncF1B or IncK plasmids. Sequence analysis of *gyrA* and *parC* genes showed mutations in 98.6% of ciprofloxacin-resistant isolates. The patterns ''GyrA: S83L+D87N, ParC: S80I'' (46.5%) and ''ParC: S80I'' (42.3%) were predominant. *qnrS1*, *qnrB*, and *aac(6′)-Ib-cr* were detected in 18.7% of isolates. The *tet* genes, *tetA*, *tetB*, and *tetA+tetB*, were present in 95.7% of tetracycline-resistant isolates. The *sul* genes (*sul1*, *sul2*, *sul3*, *sul1+sul2*, *sul2+sul3*, and *sul1+sul3*) and the *dfr* gene clusters (*dfrA1*, *dfrA5*, *dfrA7*, *dfrA8*, *dfrA12*, *dfrA5+dfrA12*, *dfrA1+dfrA5*, *dfrA7+dfrA12*, *dfrA5+dfrA7*, and *dfrA1+dfrA5+dfrA7*) were found in 96.4% and 85.5% of sulfamethoxazole/trimethoprim-resistant isolates, respectively. Classes 1 and 2 integrons were detected in 67.6% and 9.8% of isolates, respectively. This study highlighted the significant presence of resistance genes, in particular those of CTXM ESBLs, in the beef meat, with the risk of their transmission to humans through food chain.

Keywords: *Escherichia coli*, ground beef, antibiotic resistance, ESBL

Introduction

Antibiotic resistance in bacteria has reached alarming levels worldwide in both hospitals and the community, resulting in frequent treatment failures. This is inherent in indiscriminate use of antibiotics in human and veterinary medicine and in agriculture, which provides selection pressure and in turn promotes horizontal genetic exchange and recombination events resulting in the emergence and spread of antibiotic resistance mechanisms. Extended-Spectrum β-Lactamase (ESBL) production is the main mechanism for β-lactam resistance in *Enterobacteriaceae*.[1,2] These plasmid-mediated serine enzymes inactivate all penicillins and cephalosporins and aztreonam, but not cephamycins and carbapenems, and are inhibited by clavulanic acid.[3]

Originally, ESBLs were derived by point mutations from TEM-1, TEM-2, and SHV-1 penicillinases; thereafter, subsequent to the introduction of extended-spectrum cephalosporins (ESC) in the 1980s, new ESBLs have emerged, of which CTX-M is now the most prevalent and widely distributed in the world.[4] ESBL CTX-M would have as origin chromosomal β-lactamases of various species of *Kluyvera* and are divided into six phyla (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and CTX-M-45) on the basis of their amino acid sequences.

Dominant variants of CTX-M differ between countries, with the exception of the common worldwide alleles CTX-M-15 and CTX-M-14.[4] The association of $bla_{\text{CTX-M}}$ genes with mobile genetic elements, epidemic plasmids, and successful clones ensured them a rapid and wide dissemination that changed the epidemiology of antibiotic resistance worldwide.[1,2,4] Furthermore, their association with resistance to other classes of antibiotics (fluoroquinolones, aminoglycosides, and co-trimoxazole), due to common genetic

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platforms or resistance mechanisms, significantly restricts therapeutic possibilities.[2,5] Among ESBL-producing *Enterobacteriaceae,* in particular CTX-M, *Escherichia coli* is the most prevalent in the world, followed by *Klebsiella pneumoniae*.[4] Agriculture animals, especially poultry and cattle, are recognized as important vectors of ESBL carriers *E. coli*, in particular CTX-M,[6–8] and contaminated meat is thought to be a possible vehicle, among others, for their transmission to humans through consumption or handling.[7]

Many studies have been conducted worldwide on the occurrence and epidemiology of acquired resistance to ESCs in food-producing animals and food, while very little is known about the situation in North Africa, especially Algeria, where there are no data on this issue. The objective of this study was to investigate prevalence and molecular features of ESC resistance in *E. coli* isolates recovered from ground beef samples collected from butcher shops in different districts of Algiers.

Materials and Methods

Sampling, bacterial isolation, and identification

A total of 371 samples of fresh ground beef were collected during 2013–2014 from 230 butcheries of 20 different districts of Algiers (Algeria), some of which are several kilometers apart (up to 25 km). One sample or two samples (at an interval of several days) were taken, respectively, from 89 and 141 butcheries. The latter were more important in terms of commercial activity; the amount of processed meat exceeds 100 kg per day, up to one animal.

The samples were taken using sterile spatula and plastic bags and transferred to the laboratory in a cooler with ice packs within a maximum of 4 hr. Twenty-five grams of ground meat aseptically weighed was suspended and homogenized in peptone water and incubated at 37°C overnight.[9] Homogenates were plated onto Hektoen agar plates supplemented with cefotaxime at a concentration of 2 mg/L and incubated for 24 hr at 37°C. Isolates with cultural characteristics of *E. coli* were identified using standard microbiological techniques, API20E system (bioMérieux, Marcy l'Étoile, France) and polymerase chain reaction (PCR), for detection of *iudA* gene encoding β-glucuronidase.[10] One *E. coli* isolate per sample was retained for further analysis.

Antimicrobial susceptibility testing and phenotypic detection of ESBLs

Isolates were subjected to susceptibility testing for 16 antibiotics by the disc diffusion method according to the guidelines of the antibiogram committee of the French Society for Microbiology (CASFM) (www.sfm-microbiologie.org). The following disks of antibiotics (Bio-Rad, Marnes la Coquette, France) were used (μg/disk): amoxicillin/clavulanic acid (AMC) (20 μg/10 μg), cefoxitin (FOX) (30 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), cefpirome (CPO) (30 μg), imipenem (IMP) (10 μg), nalidixic acid (NA) (30 μg), ciprofloxacin (CIP) (5 μg), pefloxacin (PEF) (5 μg), ofloxacin (OFX) (5 μg), tetracycline (TE) (30 IU), kanamycin (K) (30 IU), gentamicin (GM) (15 μg), amikacin (AN) (30 μg), sulfamethoxazole/trimethoprim (SXT) (23.75/1.25 μg), and colistin (CS) (50 μg). *E. coli* ATCC 25922 was used as a control strain. An isolate is classified as multidrug resistant (MDR) when it exhibits resistance to antibiotics of at least three different classes.

The screening of isolates for ESBL production was performed by the Double-Disc Synergy Test (DDST).[11]

The minimal inhibitory concentrations (MICs) of cefotaxime, ceftazidime, ciprofloxacin, tetracycline, and SXT were determined by the agar dilution method according to the guidelines of CASFM.

Clonality and phylogrouping of the isolates

Genetic relatedness between isolates was analyzed by pulsed-field gel electrophoresis (PFGE) after total chromosomal DNA digestion with *Xba*I as previously described.[12] Phylogenetic grouping was performed using the PCR-based method.[13]

Molecular detection of antibiotic resistance genes, ISEcp1 sequence, and integrons

The DNA template for PCR was extracted by the boiling method.[14] Simplex and multiplex PCR were used to detect the presence of the following resistance genes as previously described: class A β-lactamases: bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$[15]; plasmid-mediated quinolone resistance (PMQR) determinants: *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, *oqxA*, and *oqxB*[16,17]; aminoglycoside-modifying enzyme: *aac(6′)-Ib*[16]; tetracycline efflux pumps: *tetA* and *tetB*; dihydropteroate synthases: *sul1*, *sul2*, and *sul3*; and dihydrofolate reductase gene clusters: *dfrA1*, *dfrA5*, *dfrA7*, *dfrA8*, and *dfrA12*.[18]

PCR-obtained products were sequenced as previously described[19] and analyzed with the BLAST and FASTA programs of the National Center for Biotechnology Information (www.ncbi.nlm.nhi.gov).

Amplification of quinolone-resistance determining regions (QRDR) of *gyrA* and *parC* genes was performed by PCR[20] on quinolone-resistant isolates, and the nucleotide sequences and the deduced amino acid of amplified fragments were compared with those previously reported for *gyrA* (GenBank accession no. X06373) and *parC* (M58408) using the online ClustalW2 sequence alignment program.

The association of $bla_{CTX\text{-}M}$ genes with insertion sequence IS*Ecp1* was sought by PCR as already described.[15,21]

The presence of class 1 and class 2 integrons was searched by PCR, targeting *intI1* (integrase), *sul1* (sulfonamide resistance), and *qacEΔ1* (quaternary ammonium resistance) genes for the class 1 and *int2* gene for the class 2.[18]

Conjugation experiments and plasmid analysis

Mating experiments were carried out on all isolates using sodium azide–resistant *E. coli* BM21 as a recipient. Briefly, exponential culture of donor isolate (1 volume) and recipient (2 volumes) was inoculated as a spot on Brain Heart Infusion Agar. After overnight incubation at 37°C, transconjugants were selected on Mueller Hinton agar supplemented with cefotaxime (4 mg/L) and sodium azide (200 mg/L).

Plasmid DNA was extracted by alkaline lysis method[22] and analyzed by electrophoresis on 0.7% (wt/vol) agarose gels at 5 volts/cm.

Plasmid incompatibility groups were determined by the PCR replicon-typing method.[23]

Statistical analysis

For comparison of rates, Fisher's exact test was used; a p-value of <0.05 was considered statistically significant.

Results

Of 371 ground beef samples, 102 (27.5%) were found to contain cefotaxime-resistant *E. coli* isolates; phylogenetic analysis showed their distribution into A ($n=25$, 24.5%), B1 ($n=62$, 60.8%), and D ($n=15$, 14.7%) phylogroups. In particular, phylogroup B2 was not present in this collection. Besides resistance to cefotaxime, antibiotic susceptibility testing showed a marked resistance to tetracycline (92.2%), cefpirome (85.3%), nalidixic acid (82.4%), ofloxacin (80.4%), pefloxacin (72.6%), and ciprofloxacin (70.6%), followed by SXT (53.9%), kanamycin (30.4%), amoxicillin/clavulanic acid (27.5%), and ceftazidime (21.6%), while low resistance rates were observed for cefoxitin (3.9%) and gentamicin (2.9%). All isolates were susceptible to imipenem, amikacin, and colistin (Table 1).

Agar dilution MICs ranged from 64 to >512 mg/L for cefotaxime, 16 to 128 mg/L for ceftazidime, 2 to >64 mg/L for ciprofloxacin, ≥1216/64 mg/L for sulfamethoxazole–trimethoprim, 64–256 mg/L for gentamicin, and 256–>512 mg/L for tetracycline. The combinations of resistance phenotypes allowed to distinguish 46 antibiotic resistance patterns, including 3–11 antibiotics, and 88.2% of isolates had a MDR phenotype, they resist to at least three antibiotic classes (Table 2).

All isolates were positive for the DDST presumptive of ESBL production. PCR detection showed $bla_{\text{CTX-M}}$ genes in all isolates, 74 (72.5%) of CTX-M-1 group and 28 (27.5%) of CTX-M-9 group. A rate of 47.3% of CTX-M-1 group–producing isolates belonged to B1 phylogroup, 32.4% to A, and 20.3% to D. For CTX-M-9 group–producing isolates, 96.4% belonged to B1 and 3.6% to A.

Sequencing analysis revealed six different $bla_{\text{CTX-M}}$ alleles as follows: $bla_{\text{CTX-M-1}}$ ($n=47$, 46%), $bla_{\text{CTX-M-15}}$ ($n=24$, 23.5%), $bla_{\text{CTX-M-3}}$ ($n=2$, 2%), and $bla_{\text{CTX-M-32}}$ ($n=1$, 1%) among the 74 CTX-M-1 group genes and $bla_{\text{CTX-M-14}}$ ($n=27$, 26.5%) and $bla_{\text{CTX-M-24}}$ ($n=1$, 1%) among the 28 CTX-M-9 group genes (Tables 2 and 3). The distribution of $bla_{\text{CTX-M}}$ genes according to phylogroups showed a perfect positive association (100%) between $bla_{\text{CTX-M-14}}$ and the phylogroup B1 ($p<0.0001$) and a negative association between $bla_{\text{CTX-M-15}}$ and the phylogroups A ($p=0.002$) and B1 ($p=0.0005$) (Table 3).

The $bla_{\text{TEM-1}}$ gene resulting in production of narrow spectrum β-lactamase was detected in 39 isolates (38.2%) in association with all CTX-M variants (Tables 2 and 3).

The IS*Ecp1* insertion sequence was found upstream of all $bla_{\text{CTX-M}}$ genes with a spacer region of 48 bp (W sequence) for $bla_{\text{CTX-M-15}}$ ($n=24$) and $bla_{\text{CTX-M-1}}$ ($n=26$), 45 bp for $bla_{\text{CTX-M-3}}$ ($n=2$) similar to W sequence with only one nucleotide substitution, and 79 bp to 82 bp (sequences X + W) for $bla_{\text{CTX-M-1}}$ ($n=20$) and $bla_{\text{CTX-M-32}}$ ($n=1$). In two isolates harboring $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-32}}$, IS*Ecp1B* was found disrupted by an IS*Kpn26* insertion sequence (IS5 family).

Conjugation assays performed on all isolates allowed the transfer of $bla_{\text{CTX-M-1}}$ ($n=20$), $bla_{\text{CTX-M-15}}$ ($n=4$), $bla_{\text{CTX-M-3}}$ ($n=2$), and $bla_{\text{CTX-M-14}}$ ($n=1$) genes to *E. coli* BM21. Plasmid replicon analysis showed the association of $bla_{\text{CTX-M-1}}$ with IncI1 plasmids of about 128 kb, $bla_{\text{CTX-M-15}}$ with IncI1 and IncK+B/O plasmids of about 128 kb and 80 kb, $bla_{\text{CTX-M-3}}$ with IncK plasmids of about 95 kb, and $bla_{\text{CTX-M-14}}$ with IncF1B or IncK plasmids of about 150 and 48 kb, respectively (Table 4).

Table 1. Prevalence of Antibiotic Resistance in Total *Escherichia coli* Strains and According to Phylogroups

Antibiotic (disc load)	*Resistance phenotypes in total isolates* (n = *102*) n *(%)*	*Resistance phenotypes according to phylogroups* n *(%)*		
		A (n = *25*)	*B1* (n = *62*)	*D* (n = *15*)
ß-Lactams				
Amoxicillin/Clavulanate (20/10 μg)	28 (27.5)	8 (32)	17 (27.4)	3 (20)
Cefotaxime (30 mg)	102 (100)	25 (100)	62 (100)	15 (100)
Ceftazidime (30 μg)	22 (21.6)	11 (44)	6 (9.7)	5 (33.3)
Cefpirome (30 μg)	87 (85.3)	19 (76)	53 (85.5)	15 (10)
Cefoxitin (30 μg)	4 (3.9)	2 (8.00)	2 (3.2)	0 (0)
Imipenem (10 μg)	0 (0)	0 (0)	0 (0)	0 (0)
Aminoglycosides				
Amikacin (30 μg)	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin (15 μg)	3 (2.9)	3 (12)	0 (0)	0 (0)
Kanamycin (30 IU)	31 (30.4)	12 (48)	15 (24.2)	4 (26.7)
Quinolones-fluoroquinolones				
Nalidixic acid (30 μg)	84 (82.4)	21 (84)	51 (82.3)	12 (80)
Ofloxacin (5 μg)	82 (80.4)	21 (84)	51 (82.3)	10 (66.7)
Ciprofloxacin (5 μg)	72 (70.6)	17 (68)	46 (74.2)	9 (60)
Pefloxacin (5 μg)	74 (72.6)	17 (68)	48 (77.4)	9 (60)
Others				
Tetracycline (30 IU)	94 (92.2)	22 (88)	59 (95.2)	13 (86.7)
Trimethoprim/Sulfamethoxazole (1.25/23.75 μg)	55 (53.9)	16 (64)	30 (48.4)	9 (60)
Colistin (50 μg)	0 (0)	0 (0)	0 (0)	0 (0)

Table 2. Antibiotic Resistance Phenotypes and Genotypes of *E. coli* Isolates ($N=102$)

Resistance phenotypes (no isolates)	Minimal inhibitory concentration mg/L					ESBL gene	Other resistance genes and integrons	QRDR mutations		Phylogroup	PFGE profile
	CTX	CAZ	CIP	TE	SXT			GyrA	ParC		
AMC-CTX-CAZ-CPO-K-NA-OFX-CIP-PEF-TE-SXT ($n=1$)	128	16	>64	>512	64	$bla_{\text{CTX-M-15}}$	bla_{TEM}, *aac(6′)-Ib-cr, tetB, sul2,int1*	S83L D87N	S80I	A	P68
AMC-CTX-CPO-FOX-K-NA-OFX-CIP-PEF-TE-SXT ($n=1$)	256	≤1	64	>512	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul2, sul3, dfrA5, int1*	S83L D87Y	S80I	B1	P62
CTX-CAZ-CPO-GM-K-NA-OFX-CIP-PEF-TE-SXT ($n=2$)	128–256	16	32	>512	>64	$bla_{\text{CTX-M-15}}$	bla_{TEM}, *qnrS1, tet A, tetB, sul1, dfrA5, dfrA12, int1/qacEΔ1*	S83L D87N	S80I	A	P41
AMC-CTX-CAZ-CPO-NA-OFX-CIP-PEF-TE-SXT ($n=1$)	>512	64	8	512	64	$bla_{\text{CTX-M-15}}$	bla_{TEM}, *tetA, sul1, sul2, dfrA7, int1/qacEΔ1*		S80I	D	P12
AMC-CTX-CPO-K-NA-OFX-CIP-PEF-TE-SXT ($n=5$)	256	≤1	8	256	>64	$bla_{\text{CTX-M-1}}$	*tetA, sul2, dfrA5, dfrA7, int1*		S80I	A	P24
	256	≤1	8	>512	>64	$bla_{\text{CTX-M-14}}$	bla_{TEM}, *tetA, sul3, dfrA1, dfrA5, int1, int2*	S83L D87N	S80I	B1	P27
	256	≤1	2	>512	>64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul1, sul2, dfrA7, dfrA12,int1/qacEΔ1*	S83A D87Y	S80I	B1	P51
	256	≤1	8	512	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tet A, sul1, sul2, dfrA12, int1/qacEΔ1*		S80I	D	P56
	512	≤1	32	>512	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *qnrS1, tetA, tetB, sul1, sul2, dfrA12,int1/ qacEΔ1*		S80I	A	P71
CTX-CAZ-CPO-FOX-K-NA-OFX-CIP-PEF-TE ($n=1$)	256	16	>64	>512	≤2	$bla_{\text{CTX-M-15}}$	bla_{TEM}, *aac(6′)-Ib-cr, tet A,int1*	S83L D87N	S80I	A	P49
CTX-CAZ-CPO-FOX-NA-OFX-CIP-PEF-TE-SXT ($n=1$)	>512	64	64	>512	64	$bla_{\text{CTX-M-15}}$	bla_{TEM}, *tetB, sul1, dfrA12, int1/qacEΔ1*	S83L D87N	S80I E84G	A	P64
CTX-CAZ-CPO-K-NA-OFX-CIP-PEF-TE-SXT ($n=4$)	512	16	64	512	64	$bla_{\text{CTX-M-1}}$	*tetA, sul1, sul2, dfrA12, int1/qacEΔ1*		S80I	A	P14
	512	32	64	>512	64	$bla_{\text{CTX-M-1}}$	*tetA, sul1, sul2, dfrA12, int1/qacEΔ1*		S80I	A	P14
	128	16	4	256	>64	$bla_{\text{CTX-M-15}}$	*qnrS1, tetA, dfrA1, dfrA5, int1, int2*			A	P26
	512	128	64	>512	>64	$bla_{\text{CTX-M-15}}$	*tetA, tetB, sul1, sul3, dfrA12, int1/qacEΔ1*	S83L D87N	S80I	D	P60
AMC-CTX-CPO-K-NA-OFX-CIP-PEF-TE ($n=1$)	128	≤1	8	512	≤2	$bla_{\text{CTX-M-14}}$	bla_{TEM}, *tetA, int1*	S83L D87N	S80I	B1	P54

(*continued*)

Table 2. (Continued)

Resistance phenotypes (no isolates)	*Minimal inhibitory concentration mg/L*					*ESBL gene*	*Other resistance genes and integrons*	*QRDR mutations*		*Phylogroup*	*PFGE profile*
	CTX	*CAZ*	*CIP*	*TE*	*SXT*			*GyrA*	*ParC*		
AMC-CTX-CPO-NA-OFX-CIP-PEF-TE-SXT ($n=5$)	64–128	≤1	4–8	512	>64	$bla_{\text{CTX-M-1}}$	*tetA, sul3, dfrA1, int2*		S80I	B1	P3, P32
	256	≤1	64	>512	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetB, sul1, sul2, dfrA7, int1/qacEΔ1*	S83L D87N	S80I E84G	B1	Not typed
	64	≤1	8	512	>64	$bla_{\text{CTX-M-14}}$	*tetA, sul1, sul3, dfrA1, int1/qacEΔ1*	S83L D87N	S80I	B1	P27
	512	≤1	64	512	>64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul2, sul3, dfrA12, int1*	S83L D87N	S80I	B1	P66
AMC-CTX-K-NA-OFX-CIP-PEF-TE-SXT ($n=3$)	512	≤1	8	256	64	$bla_{\text{CTX-M-24}}$	bla_{TEM}, *qnrS1, tet A, sul2,int1*		S80I	A	P13
	128	≤1	8	64	>64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *sul2, dfrA5, dfrA7, int1*	S83I	S80R	A	Not typed
	128	≤1	8	512	>64	$bla_{\text{CTX-M-14}}$	bla_{TEM}, *tetA, sul3, int1*		S80I	B1	P33
AMC-CTX-GM-NA-OFX-CIP-PEF-TE-SXT ($n=1$)	64	≤1	8	256	64	$bla_{\text{CTX-M-32}}$	bla_{TEM}, *dfrA12, sul3, int1*		S80I	A	P22
CTX-CAZ-CPO-NA-OFX-CIP-PEF-TE-SXT ($n=2$)	512	16	32	256–512	>64	$bla_{\text{CTX-M-15}}$	*tetA, sul1, dfrA12, int1/qacEΔ1*		S80I	B1	P16
CTX-CPO-FOX-K-NA-OFX-CIP-PEF-TE ($n=1$)	512	≤1	64	>512	≤2	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tet A, int1*	S83L D87Y	S80I	B1	P61
CTX-CPO-K-NA-OFX-CIP-PEF-TE-SXT ($n=2$)	256	≤1	16	512	>64	$bla_{\text{CTX-M-1}}$	*tetA, sul1, sul2, dfrA7, dfrA12, int1/qacEΔ1*	S83L D87N	S80I	D	P43
	128	≤1	8	256	>64	$bla_{\text{CTX-M-14}}$	*tetA, sul2, int1*	S83L D87N	S80I	B1	P27
AMC-CTX-CPO-NA-OFX-CIP-PEF-TE ($n=3$)	128	≤1	8–16	256–512	≤2	$bla_{\text{CTX-M-14}}$	*tetA, int1*	S83L D87N	S80I	B1	P21, P27, P57
AMC-CTX-NA-OFX-CIP-PEF-TE-SXT(I) ($n=1$)	128	≤1	8	256	64	$bla_{\text{CTX-M-14}}$	*tetA, sul3, int1*		S80I	B1	P30
CTX-CPO-K-NA-OFX-CIP-PEF-TE ($n=4$)	64	≤1	32	>512	≤2	$bla_{\text{CTX-M-3}}$	*tetB*		E84K	D	Not typed
	128	≤1	8	256	≤2	$bla_{\text{CTX-M-14}}$	*tetA, int1*	S83L D87N	S80I	B1	P27
	64	≤1	8	256	≤2	$bla_{\text{CTX-M-14}}$	bla_{TEM}, *tetA, int1*	S83L D87N	S80I	B1	P55
	512	≤1	32	>512	≤2	$bla_{\text{CTX-M-1}}$	*tetA*	S83L D87N	S80I	B1	P59
CTX-CPO-NA-OFX-CIP-PEF-TE-SXT ($n=7$)	128	≤1	8	512	>64	$bla_{\text{CTX-M-1}}$	*tetA, sul3, dfrA1, int2*		S80I	B1	P3
	512	≤1	4–8	256–512	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul1, sul2, dfrA12, int1/qacEΔ1*		S80I	D	P10 ($n=2$)
	128	≤1	4	256	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tet A, sul2, dfrA7, int1*		S80I	B1	P25
	128	≤1	32	512	>64	$bla_{\text{CTX-M-1}}$	*tetA, sul2, dfrA7, int1*	S83L D87N	S80I	B1	P42
	512	≤1	64	256	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul2, sul3, dfrA8*	S83L D87N	S80I	B1	P70
	256	≤1	8	>512	64	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul2*	S83L D87N	S80I	D	Not typed

(*continued*)

TABLE 2. (CONTINUED)

Resistance phenotypes (no isolates)	*Minimal inhibitory concentration mg/L*					*ESBL gene*	*Other resistance genes and integrons*	*QRDR mutations*		*Phylogroup*	*PFGE profile*
	CTX	*CAZ*	*CIP*	*TE*	*SXT*			*GyrA*	*ParC*		
CTX-K-NA-OFX-CIP-PEF-TE-SXT ($n=1$)	128	≤1	8	>512	64	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{TEM}}$, *tet A, tetB, sul1, sul2, dfrA12, int1/qacEΔ1*		S80I	A	P39
AMC-CTX-CAZ-CPO-OFX-TE-SXT ($n=1$)	512	32	≤0.5	512	64	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$, *qnrS1, tetA, sul2, dfrA5, int1*			D	P65
CTX-CAZ-CPO-NA-OFX-CIP-PEF ($n=1$)	>512	16	8	≤4	≤2	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$	S83L D87N	S80I	A	P67
CTX-CAZ-CPO-NA-OFX-TE-SXT ($n=1$)	>512	64	≤0.5	512	64	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$, *qnrS1, tetA, sul2, dfrA5, int1*			A	P58
CTX-CPO-K-NA-OFX-TE-SXT ($n=1$)	128	≤1	≤0.5	>512	64	*bla*$_{\text{CTX-M-3}}$	*bla*$_{\text{TEM}}$, *tetB, sul2, dfrA5, int1*			B1	P38
CTX-CPO-NA-OFX-CIP-PEF-TE ($n=17$)	64–256	≤1	4–16	256–512	≤2	*bla*$_{\text{CTX-M-14}}$	*tetA, int1*	S83L D87N	S80I	B1	P15 ($n=6$), P20, P21, P27, P35, P40, P44, P45, P72
	128	≤1	16	256	≤2	*bla*$_{\text{CTX-M-1}}$	*int2*		S80I	D	P31
	256	≤1	4–8	>512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA*	S83L D87N	S80I	B1	P50, P52
CTX-K-NA-OFX-CIP-PEF-TE ($n=1$)	256	≤1	16	256	≤ 2	*bla*$_{\text{CTX-M-14}}$	*tetA*		S80I	B1	P27
CTX-K-NA-OFX-PEF-TE-SXT ($n=1$)	256	≤1	≤0.5	256	64	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{TEM}}$, *tetA, tetB, sul3, dfrA5, int1*			B1	P47
CTX-NA-OFX-CIP-PEF-TE-SXT ($n=3$)	64–128	≤1	8	256	64	*bla*$_{\text{CTX-M-1}}$	*tetA, sul3, dfrA1, int2*		S80I	B1	P1, P32
	256	≤1	64	256	>64	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{TEM}}$, *tetA, sul2, sul3, dfrA8, int1*	S83L D87N	S80I	B1	P70
CTX-CPO-NA-OFX-PEF-TE ($n=1$)	128	≤1	≤0.5	256	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA*			B1	P34
CTX-CPO-NA-OFX-TE-SXT ($n=1$)	256	≤1	≤0.5	>512	64	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$, *qnrS1, tetA, sul2, int1*			A	P28
CTX-NA-OFX-CIP-PEF-TE ($n=3$)	64	≤1	8	256	≤ 2	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{TEM}}$, *tetA*	S83L D87N	E84K	A	Not typed
	64	≤1	8	512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA*	S83L D87N	S80I	A	P36
	128	≤1	16	256	≤ 2	*bla*$_{\text{CTX-M-14}}$	*tetA, int1*	S83L D87N	S80I	B1	P37
AMC-CTX-CAZ-CPO-TE ($n=1$)	128	16	≤0.5	>512	≤2	*bla*$_{\text{CTX-M-1}}$				B1	P29
AMC-CTX-CPO-TE-SXT ($n=1$)	256	≤1	≤0.5	256	>64	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{TEM}}$, *tetA, sul2, dfrA1, dfrA5, dfrA7,int1*			B1	P48

(*continued*)

TABLE 2. (CONTINUED)

Resistance phenotypes (no isolates)	*Minimal inhibitory concentration mg/L*					*ESBL gene*	*Other resistance genes and integrons*	*QRDR mutations*		*Phylogroup*	*PFGE profile*
	CTX	*CAZ*	*CIP*	*TE*	*SXT*			*GyrA*	*ParC*		
CTX-CAZ-CPO-TE-SXT (*n*=2)	128	16	≤0.5	256	64	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$, *qnrS1, tetA, sul2, dfrA5, int1*			B1	P9
	128	16	≤0.5	256	>64	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$, *qnrS1, tetA, sul2, dfrA5, dfrA12, int1*			B1	P11
CTX-CPO-K-TE-SXT (*n*=1)	256	≤1	≤0.5	>512	64	*bla*$_{\text{CTX-M-1}}$	*tetB, sul2, dfrA7, int1*			B1	P5
CTX-CPO-NA-OFX-TE (*n*=2)	128	≤1	≤0.5	256	≤2	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{TEM}}$, *tetA*			B1	P6
	128	≤1	≤0.5	512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA*			A	P23
CTX-CPO-NA-TE-SXT (*n*=1)	256	≤1	≤0.5	>512	64	*bla*$_{\text{CTX-M-15}}$	*tetA, sul2, dfrA1, int2*			A	P69
AMC-CTX-CPO-TE (*n*=3)	256	≤1	≤0.5	512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA*			A	P18
	256	≤1	≤0.5	512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA*			A	P18
	512	≤1	≤0.5	256	≤2	*bla*$_{\text{CTX-M-1}}$	*qnrB, tetA*			B1	P53
CTX-CAZ-CPO-SXT (*n*=1)	>512	16	≤0.5	≤4	64	*bla*$_{\text{CTX-M-15}}$	*qnrS1*			D	Not typed
CTX-CPO-NA-OFX (*n*=1)	256	≤1	≤0.5	≤4	≤2	*bla*$_{\text{CTX-M-15}}$	*qnrS1*			B1	P8
CTX-CPO-NA-TE (*n*=3)	512	≤1	≤0.5	>512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA, tetB*			D	Not typed
	512	≤1	≤0.5	>512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA, tetB*			D	
	512	≤1	≤0.5	>512	≤2	*bla*$_{\text{CTX-M-1}}$	*tetA, tetB*			D	
CTX-CPO-TE-SXT (*n*=2)	512	≤1	≤0.5	>512	64	*bla*$_{\text{CTX-M-1}}$	*tetB, sul2, dfrA7, int1*			B1	P2
	512	≤1	≤0.5	256	64	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM}}$, *qnrS1, tetA, sul2, dfrA5, int1*			B1	P7
CTX-K-TE-SXT (*n*=1)	512	≤1	≤0.5	>512	64	*bla*$_{\text{CTX-M-1}}$	*tetB, sul2, dfrA7, int1*			B1	P5
CTX-CAZ-CPO (*n*=3)	512	32	≤0.5	≤4	≤2	*bla*$_{\text{CTX-M-15}}$	*qnrS1, int2*			D	P4
	256	16	≤0.5	≤4	≤2	*bla*$_{\text{CTX-M-15}}$				A	P17
	512	16	≤0.5	≤4	≤2	*bla*$_{\text{CTX-M-15}}$	*qnrS1*			B1	P63
CTX-CPO-OFX (*n*=1)	128	≤1	≤0.5	≤4	≤2	*bla*$_{\text{CTX-M-15}}$	*qnrS1*			A	P19
CTX-CPO-SXT (*n*=1)	256	≤1	≤0.5	≤4	64	*bla*$_{\text{CTX-M-1}}$	*sul2, dfrA7, int1*			B1	P46

AMC, amoxicillin–clavulanate; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; CPO, cefpirome; TE, tetracycline; SXT, sulfamethoxazole–trimethoprim; NA, nalidixic acid; PEF, pefloxacin; OFX, ofloxacin; CIP, ciprofloxacin; K, kanamycin; ESBL, extended-spectrum β-lactamase; PFGE, pulsed-field gel electrophoresis; QRDR, quinolone-resistance determining regions.

Table 3. Prevalence of Resistance Genes in Total Isolates and According to Phylogroups

Resistance genes	*Total isolates* (n = *102)* n *(%)*	*Phylogenetic groups* n *(%)*		
		A (n = *25)*	*B1* (n = *62)*	*D* (n = *15)*
$bla_{\text{CTX-M-1}}$	47 (46.1)	11 (44)	27 (43.5)	9 (60)
$bla_{\text{CTX-M-14}}$	27 (26.5)	0 (0) [b,*]	27 (43.5)[a]	0 (0)[c,*]
$bla_{\text{CTX-M-15}}$	24 (23.5)	12 (48)[c,*]	7 (11.3)[b,*]	5 (33.3)
$bla_{\text{CTX-M-3}}$	2 (2)	0 (0)	1 (1.6)	1 (6.7)
$bla_{\text{CTX-M-24}}$	1 (1)	1 (4)	0 (0)	0 (0)
$bla_{\text{CTX-M-32}}$	1 (1)	1 (4)	0 (0)	0 (0)
$bla_{\text{TEM-1}}$	39 (38.2)	14 (56)	19 (30.6)	6 (40)
qnrS1	16 (15.7)	8 (32)[d]	5 (8.1)[d,*]	3 (20)
qnrB	1 (1)	0 (0)	1 (1.6)	0 (0)
aac(6′)-Ib-cr	2 (2)	2 (8)	0 (0)	0 (0)
tet A	82 (80.4)	18 (72)	53 (85.5)	11 (73.3)
tet B	17 (16.7)	6 (24)	6 (9.7)[d,*]	5 (33.3)
sul1	18 (17.6)	7 (28)	5 (8.1)[c,*]	6 (40)[d]
sul2	36 (35.3)	11 (44)	18 (29)	7 (46.7)
sul3	16 (15.7)	1 (4)	14 (22.6)[d]	1 (6.7)
dfrA1	10 (9.8)	2 (8)	8 (12.9)	0 (0)
dfrA5	15 (14.7)	6 (24)	8 (12.9)	1 (6.7)
dfrA7	13 (12.7)	2 (8)	9 (14.5)	2 (13.3)
dfrA8	2 (2)	0 (0)	2 (3.2)	0 (0)
dfrA12	18 (17.6)	8 (32)[d]	5 (8.1)[c,*]	5 (33.3)
int1	69 (67.6)	16 (64)	46 (74.2)	7 (46.7)
qacEΔ1	18 (17.6)	7 (28)	5 (8.1)[c,*]	6 (40)[d]
int2	10 (9.8)	2 (8)	6 (9.7)	2 (13.3)

Letters (a, b, c, d) indicate significant association by the Fisher test.
[a]$p \leq 0.0001$; [b]$0.0001 < p \leq 0.001$; [c]$0.001 < p \leq 0.01$; [d]$0.01 < p < 0.05$.
*Negative association.

The screening for PMQR determinants of all isolates showed the presence of *qnrS1*, *qnrB*, and *aac(6′)-Ib-cr* in 16 (15.7%), 1 (1%), and 2 (2%) isolates, respectively (Tables 2 and 3), while *qnrA*, *qnrC*, *qnrD*, *qepA*, *oqxA*, and *oqxB* were absent. It should be noted the presence of *qnrS1* ($n = 6$) and *qnrB* ($n = 1$) genes in quinolone susceptible isolates. *qnrS1* gene was cotransferred with $bla_{\text{CTX-M-15}}$ from one isolate (Table 4). Sequence analysis of the QRDR of *gyrA* and *parC* genes showed mutations in 71/72 ciprofloxacin-resistant isolates (98.6%), while no mutations were observed in pefloxacin- and/or ofloxacin-resistant isolates. The amino acid substitution pattern ''GyrA: S83L+D87N, ParC: S80I'' (46.5%) and the single substitution ''ParC: S80I'' (42.3%) were predominant, followed by the mutation patterns ''GyrA: S83L+D87Y, ParC: S80I'' (2.8%), ''GyrA: S83L+D87N, ParC: S80I+E84G'' (2.8%), ''GyrA: S83A+D87Y, ParC: S80I'' (1.4%), ''GyrA: S83L+D87N, ParC: E84K'' (1.4%), ''GyrA: S83I, ParC: S80R'' (1.4%), and the single mutation ''ParC: E84K'' (1.4%) (Table 2).

Among the 94 tetracycline-resistant strains, *tet* genes were detected in 90 isolates (95.7%), with a prevalence of 81.1% ($n = 73$) for *tetA*, 8.9% ($n = 8$) for *tetB*, and 10% ($n = 9$) for *tetA+tetB* (Tables 2 and 3). *tetA* gene was cotransferred with $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ from 14 and 2 isolates, respectively (Table 4).

The *sul* genes were present in 53 of 55 SXT-resistant isolates (96.4%), the rates of *sul1*, *sul2*, *sul3*, *sul1+sul2*, *sul2+sul3*, and *sul1+sul3* were of 9.4% ($n = 5$), 39.6% ($n = 21$), 18.9% ($n = 10$), 20.8% ($n = 11$), 7.5% ($n = 4$), and 3.8% ($n = 2$), respectively (Tables 2 and 3). *sul2* gene was cotransferred with $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-1}}$ from six and one isolates, respectively, and *sul3* with $bla_{\text{CTX-M-15}}$ from one isolate (Table 4).

In total, 47 of 55 (85.5%) SXT-resistant isolates harbored *dfr* genes. The gene clusters *dfrA1*, *dfrA5, dfrA7*, *dfrA8*, and *dfrA12* were detected in 14.9% ($n = 7$), 14.9% ($n = 7$), 17% ($n = 8$), 4.3% ($n = 2$), and 27.7% ($n = 13$) of isolates, respectively. The concomitant presence of *dfrA5+dfrA12*, *dfrA1+dfrA5*, *dfrA7+dfrA12*, *dfrA5+dfrA7*, and *dfrA1+ dfrA5+ dfrA7* was observed in 6.4% ($n = 3$), 4.3% ($n = 2$), 4.3% ($n = 2$), 4.3% ($n = 2$), and 2.1% ($n = 1$) of isolates, respectively (Tables 2 and 3). The association *sul+dfr* was found in 83.6% of SXT-resistant isolates. *dfrA7* gene cluster was cotransferred with $bla_{\text{CTX-M-1}}$ from three isolates and *dfrA5* with $bla_{\text{CTX-M-15}}$ or $bla_{\text{CTX-M-1}}$ from one isolate (Table 4).

Class 1 and class 2 integrons were detected in 69 (67.6%) and 10 (9.8%) isolates, respectively, while class three integrons were absent. Fifty-one (73.9%) of class 1 integrons lacked the 3′-conserved sequence (3′-CS) comprising the *qacEΔ1-sul1* genes. Class 1 integrons were cotransferred with $bla_{\text{CTX-M-15}}$ and with $bla_{\text{CTX-M-1}}$ from two and three isolates, respectively (Tables 2 and 3).

The clonal relationship between 94 isolates investigated by PFGE revealed 72 DNA profiles. Similarities between profiles were observed within 33 isolates mostly originating from different districts, they are divided into 10 clusters of 2 isolates and two clusters of 6 isolates (PFGE profile P15) and 7 isolates (PFGE profile P27) (Table 2). The latter harbored CTX-M-14 and belonged to phylogroup B1. As regards resistance profiles, the six-isolate cluster had a same

Table 4. Resistance Genes and Associated Plasmids Transferred in Transconjugants ($N=27$)

Wild-type isolates				*Transconjugants*		
Strain	*CTX-M gene*	*Other resistance genes*	*Plasmid replicon types*	*CTX-M gene*	*Cotransferred genes*	*Plasmid replicon types (size kb)*
EC 1	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA*	I1	+	*tetA*	I1 (128)
EC 15	$bla_{\text{CTX-M-15}}$	bla_{TEM}, *qnrS1, tetA, sul2, dfrA5, dfrA12, int1*	K, B/O	+	*qnrS1, tetA, sul2, dfrA5, int1*	K, B/O (80)
EC 19	$bla_{\text{CTX-M-1}}$	*tetA, sul1, sul2, dfrA12*	I1, FIB	+	*tetA, sul2*	FIB, I1 (141, 128)
EC 28	$bla_{\text{CTX-M-1}}$	*tetA*	I1	+	*tetA*	I1 (128)
EC 39	$bla_{\text{CTX-M-3}}$	*tetB*	FIB, K, H1	+		FIB, K (141, 95)
EC 41	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetB, sul1, sul2, dfrA7, int1*	I1, FIB, K	+	*sul2, dfrA7, int1*	I1 (128)
EC 42	$bla_{\text{CTX-M-1}}$	*tetA*	I1	+	*tetA*	I1 (128)
EC 44	$bla_{\text{CTX-M-1}}$	*tetA, sul2, dfrA5, dfrA7, int1*	I1, FIB	+	*tetA, sul2, dfrA5, dfrA7, int1*	I1 (128)
EC 45	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, sul2, dfrA7*	I1, FIB	+	*tetA, sul2*	I1 (128)
EC 50	$bla_{\text{CTX-M-1}}$		I1, K	+		I1 (128)
EC 58	$bla_{\text{CTX-M-1}}$		I1, FIB	+		I1 (128)
EC 66	$bla_{\text{CTX-M-1}}$	*tetA*	I1, FIB	+	*tetA*	I1 (128)
EC68	$bla_{\text{CTX-M-14}}$	*tetA*	I1, FIB, K	+		FIB, K (150, 48)
EC 73	$bla_{\text{CTX-M-3}}$	bla_{TEM}, *tetB, sul2, dfrA5*	I1, FIB, K	+		K (95)
EC 75	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, tetB, sul1, sul2, dfrA12*	I1, FIB	+	*tetA, sul2*	I1 (128)
EC 91	$bla_{\text{CTX-M 1}}$	*sul2, dfrA7, int1*	I1, FIB	+	*dfrA7, int1*	I1 (128)
EC 92	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *tetA, tetB, sul3, dfrA5*	I1, FIB	+	*tetA*	I1 (128)
EC 100	$bla_{\text{CTX-M-1}}$	*qnrB, tetA*	I1, FIB	+	*tetA*	I1 (128)
EC 105	$bla_{\text{CTX-M 1}}$	bla_{TEM}, *tetA, sul1, sul2, dfrA12*	I1, FIB	+	*sul2*	I1 (128)
EC 109	$bla_{\text{CTX-M-1}}$	*tetA*	I1, FIB	+	*tetA*	I1 (128)
EC 110	$bla_{\text{CTX-M-15}}$	*tetA, tetB, sul1, sul3, dfrA12, int1*	N, I1, FIB, P	+	*tetA, sul3, int1*	I1 (128)
EC 117	$bla_{\text{CTX-M-1}}$	*tetA, tetB*	I1, FIB	+	*tetA*	I1 (128)
EC 118	$bla_{\text{CTX-M-1}}$	*tetA, tetB*	I1, FIB	+	*tetA*	I1 (128)
EC 119	$bla_{\text{CTX-M-1}}$	*tetA, tetB*	I1, FIB	+	*tetA*	I1 (128)
EC 126	$bla_{\text{CTX-M-15}}$	bla_{TEM}	I1	+		I1 (128)
EC 127	$bla_{\text{CTX-M-15}}$	*qnrS1*	I1, FIB	+		I1 (128)
EC130	$bla_{\text{CTX-M-1}}$	bla_{TEM}, *qnrS1, tetA, tetB, sul1, sul2, dfrA12*	I1, FIB	+	*tetA, sul2*	I1 (128)

profile, while the seven-isolate cluster presented differences in their resistance profiles (Table 2).

Discussion

In this study, we investigated the prevalence and molecular features of resistance to ESC in *E. coli* recovered from ground beef samples at retail in Algeria. A total of 102/371 (27.5%) ground beef samples were found to contain ESBL-producing cefotaxime-resistant *E. coli*. The occurrence of ESBL-carrying *E. coli* in beef varies among countries, and comparisons are difficult because of the variety of methods used in studies; rates of 59%, 26%, 7%, 0%, and 0% were reported in studies from Spain, Tunisia, Turkey, Switzerland, and Czech Republic, respectively.[10,24–27] The contamination of meat with ESBL-producing *E. coli* may have resulted from their fecal carriage by animals exposed to antibiotics for prophylaxy and growth promotion.[28,29]

Phylogenetic grouping of isolates showed that B1 was the main phylogroup followed by phylogroup A and to a lesser extent by D. This finding matches with what was observed in CTX-M–producing *E. coli* from livestock (including cattle).[8,30] Thus, even though other hypotheses may include cross-contamination of human origin through the handling of the beef samples before getting at retail, those phylogroups, and particularly the absence of phylogroup B2 which is prevalent in humans, rather argue for an animal origin.

Besides beta-lactam resistance, high prevalence resistance toward fluoroquinolone, SXT, and tetracycline and to a lesser extent to kanamycin was observed, and 88.2% of the isolates were MDR phenotype; this may have resulted from co-selection with ESBL due to associated chromosomally- or plasmid-encoded resistance mechanisms.[5,8,24]

All ESBLs detected in this study were members of CTX-M family, in accordance with the worldwide prevalence of CTX-Ms in *Enterobacteriaceae*, particularly *E. coli*.[4] Among the CTX-M variants identified in our study, CTX-

M-1 (46%), CTX-M-14 (24.5%), and CTX-M-15 (23.5%) were largely predominant; this is consistent with the frequent detection of CTX-M-1 and CTX-M-14 variants in food-producing animals and foods.[6,7,10,24,25,30] Of note, cattle are the animal group, compared to others, where CTX-M-15 has been recognized at a significant prevalence.[8,31,32] Therefore, it may not be surprising to find CTX-M-15 producers in those meat samples as they originate from beef, and this also constitutes an argument that this colonization may rather originate from the animal sector. The most CTX-M variants detected up until now in Algeria were of CTX-M-1 group; these are CTX-M-3 and CTX-M-28 in clinical isolates,[15,33] CTX-M-15 in clinical and chicken isolates,[15,34] and CTX-M-1 in chicken isolates.[35] The second group described was CTX-M-9 through only the CTX-M-14 variant.[36] CTX-M-24 of CTX-M-9 group and CTX-M-32 of CTX-M-1 group found in our isolates are therefore reported for the first time in Algeria. These findings are indicative of the potential role of cattle and beef meat in the dissemination of CTX-M ESBLs, particularly those of CTX-M-9 group, which up until now are rare in clinical setting in Algeria. The association of $bla_{\text{TEM-1}}$ with $bla_{\text{CTX-M}}$ is very common; they often coexist on the same plasmids.[15]

The described associations between CTX-M variants and phylogroups vary according to studies, depending on strains and their origin[8,30,37]; it was reported that the emergence of a type of ESBL results from its interaction with the genetic background of the strain and the surrounding selection pressure.[38] In this study, CTX-M-14 was 100% associated with phylogroup B1; this genotype seems well established in beef; it certainly contributes in the spread of resistance through food chain.

All $bla_{\text{CTX-M}}$ genes were found flanked upstream by an IS*Ecp1*B insertion sequence known to be involved in the expression and mobility of these genes[21]; this association at high rate is well documented.[4] The sequences of spacer regions between IS*Ecp1*B and $bla_{\text{CTX-M}}$ of group 1 previously found in clinical and environmental Algerian strains were W and V + W[15,20]; this is the first time that X + W and the 45-bp sequences were described in Algeria, while they were previously reported in other countries.[10,39,40] This finding suggests a foreign origin of at least some of our strains, possibly carried by imported animals. In two isolates harboring $bla_{\text{CTX-M-1}}$ or $bla_{\text{CTX-M-32}}$, IS*Kpn26*, first described in *Klebsiella pneumoniae* (GenBank: NC_016845), was localized inside IS*Ecp1*B; such a genetic environment was already reported in *E. coli* (GenBank: AB976580).

The conjugation transfer of $bla_{\text{CTX-M}}$ genes was positive for only 26.5% of our isolates, when plasmid horizontal transfer is known as an important contributor in the epidemiology of these genes[1,2,4]; in this regard, transfer rates of 86% and 100% of $bla_{\text{CTX-M}}$ gene were reported.[41,42] Our result could be explained by a chromosomal localization of $bla_{\text{CTX-M}}$ genes[4] or a very low transfer frequency of plasmids.

Plasmid replicon analysis showed that all transferred $bla_{\text{CTX-M-1}}$ were associated with IncI1 plasmid group, which is among the main groups involved in the spread of $bla_{\text{CTX-M}}$.[1,4,6] Many studies reported a close association between CTX-M-1 and IncI1 plasmid types in human, animal, food, and environmental isolates, and the emergence of plasmid IncI1/$bla_{\text{CTX-M-1}}$ was first observed in animals and subsequently in humans.[1,6,7,42,43] Our results support the role of IncI1 plasmids in the dissemination of $bla_{\text{CTX-M-1}}$ and the food chain in their transmission to humans. IncI1 plasmids are considered to be epidemic resistance plasmids; their successful dissemination is probably related, among others, to plasmid-intrinsic factors such as addiction systems (*e.g.*, *pndAC* and *relBE* systems) and colonization factors (*e.g.*, type IV pili).[1,44] $bla_{\text{CTX-M-15}}$ was found associated with IncI1 and IncK+B/O plasmids; the association CTX-M-15-IncI1 was already described, especially in an outbreak of *E. coli* O104:H4 in Northern Europe,[6] whereas linkage of CTX-M-15 with a multireplicon plasmid IncK-B/O is reported for the first time in this study. The multireplicon plasmids have advantage of being stable even in the presence of a second plasmid carrying a homologous replicon. $bla_{\text{CTX-M-14}}$ was found associated either with IncF1B or IncK plasmids; a close association between $bla_{\text{CTX-M-14}}$ and IncK plasmids was previously documented in different studies; indeed, this plasmid type was described as being behind the diffusion of CTX-M-14 among animals and humans from different European countries.[1,6,7,37] $bla_{\text{CTX-M-3}}$, described as dominantly harbored by IncL/M and IncI1, is reported for the first time on an IncK plasmid in this study.

A rate of 80.4% of the isolates was resistant to fluoroquinolones, the association between ESBLs, especially CTX-M, and fluoroquinolone resistance was commonly reported.[2,7] A rate of 18.6% of isolates harbored PMQR determinants with a predominance of *qnr*S1; this is consistent with previous reports.[45] Some strains harboring *qnrB* or *qnrS1* were susceptible to quinolones, this is in accordance with the very low level resistance (below the resistance threshold, according to EUCAST-CASFM) conferred by these genes; however, PMQR determinants can facilitate the emergence of high-level resistant mutants.[46] Furthermore, animal experiments showed that *qnr* genes can reduce bactericidal activity of ciprofloxacin like a *gyrA* mutation.[47]

Almost all ciprofloxacin-resistant isolates (98.6%) had mutations in *gyrA* and/or *parC* genes, all substitutions detected were already described in *E. coli*,[48] and the prevalent mutation profiles were the pattern ''GyrA: S83L+D87N, ParC: S80I'' followed by the single substitution ParC: S80I. ''GyrA: S83L+D87N, ParC: S80I'' was previously reported among the predominant profiles in clinical fluoroquinolone-resistant isolates in Algeria[49] and worldwide.[50,51] The abundance of this genotype may be related to elimination of fitness cost associated with S83L+D87N by additional ParC S80I substitution.[52] High levels of resistance up to 64 mg/L were observed with the single ParC S80I substitution; the same finding was reported.[53] However, previous studies showed that this mutation alone is not associated with any increase in the MIC of ciprofloxacin[52]; thus, the resistance levels observed are probably related to other mechanisms such as decreased expression of outer membrane porins or overexpression of multidrug efflux pumps.

Tetracycline is a broad-spectrum antibiotic used with other first-generation tetracyclines, such as chlortetracycline and oxytetracycline as animal growth promoter. Second-generation tetracyclines, such as minocycline and doxycycline, are commonly used in human and veterinary medicine. Almost all of our tetracycline-resistant isolates harbored *tetA* and/or *tetB* genes with a clear predominance of *tetA* gene, in agreement with the fact that generally efflux mechanisms are most prominent, and the *tetA* gene is commonly encountered in *E. coli* isolated from human,

animals, and animal-derived foods in many countries.[54–59] *tetB* gene was found at low prevalence in our strains; however, it confers a high level of resistance, in particular to minocycline and doxycycline.[58]

Commonly, sulfonamides and trimethoprim resistance in *E. coli* arise from the acquisition of *sul* and *dfr* genes, encoding drug-resistant variants of dihydropteroate synthases and dihydrofolate reductases.[60] Consistent with our results, *sul2* gene was reported as the most prevalent in *E. coli* from human, animals, and animal-derived foods.[29,54,55,57,59] *E. coli* carrying *sul2* gene was described as able to colonize both animals and humans, and animal strains may be implicated in human infections such as UTI and septicemia.[61] *sul3* gene, reported as rare, was detected at a substantial rate in our isolates, almost equivalent to that of *sul1* gene usually reported as most frequent after *sul2*.[54,57,59] Concerning trimethoprim resistance genes, clusters *dfrA1*, *dfrA5*, *dfrA7*, *dfrA8*, *and dfrA12* detected in our study are among the most reported.[10,29,59,62]

Integrons constitute a mechanism for acquisition and expression of resistance genes; they play an important role in the emergence and spread of MDR.[63] Over half of our isolates harbored class 1 integrons, while class 2 integrons were detected in only about 10%; this is in accordance with the predominance of class 1 integrons in *E. coli* from food animals and foods.[9,64,65] The use of antimicrobials, such as tetracycline and sulfonamides, as growth promoters for farm animals as beef cattle, may promote the presence and maintenance of class 1 integrons in *E. coli*.[29] The majority of detected integrons (73.9%) lacked the 3′-conserved sequence comprising the *qacEΔ1-sul1* genes; this truncated structure was already described, it generally contains a *sul3* at the 3′-end and is linked to various insertion sequences probably responsible for this rearrangement.[66]

The PFGE analysis revealed a genetic diversity within the 94 typed isolates; however, clonality was observed among some isolates of which two clusters of six and seven CTX-M-14–producing isolates were unrelated to districts; these findings are indicative of a possible clonal spread of CTX-M-14 producers. The difference in resistance genetic content of some clonal isolates may be related to changes due to selective pressure or genetic exchange.

Conclusions

To the best of our knowledge, this is the first report to study bacterial antibiotic resistance in fresh retail beef meat in Algeria; it highlighted the relatively massive presence of *E. coli* isolate producers of various CTX-M ESBLs. The CTX-M variants, CTX-M-24 and CTX-M-32, are reported for the first time in Algeria. Almost all of the isolates were MDR, with resistance to major antibiotics used in human medicine such as broad spectrum beta-lactams, fluoroquinolones, aminoglycosides, and trimethoprim–sulfamethoxazole. Evidence of both clonal and horizontal dissemination of resistance genes was also demonstrated. These findings are indicative of possible failures in the management of the use of antibiotics in the food sector and/or lack of hygiene during the food process and at retail. From a one health perspective, this is of considerable importance in view of the possibility of antimicrobial resistance transfer between animals and humans.

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Disclosure Statement

No competing financial interests exist.

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