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Research Note

Lineages and Virulence Gene Content among Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Strains of Food Origin in Tunisia

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ABSTRACT

Nineteen extended-spectrum β-lactamase (ESBL)–positive *Escherichia coli* strains recovered from food samples in Tunisia were characterized by multilocus sequence typing and phylogenetic typing, and the virulence gene and plasmid content were also determined. These strains presented unrelated pulsed-field gel electrophoresis patterns and contained genes coding for the following ESBLs (the number of strains is in parentheses): CTX-M-1 (15), CTX-M-14 (2), CTX-M-8 (1), and SHV-5 (1). Twelve different sequence types (STs) were identified among the 19 ESBL-positive strains, which included two new STs (ST2022 in 2 $bla_{\text{CTX-M-14}}$–containing strains and ST1970 in 2 $bla_{\text{CTX-M-1}}$–containing strains). ST155 and ST602 were detected in four and three $bla_{\text{CTX-M-1}}$–containing strains, respectively, and ST405 was detected in one $bla_{\text{CTX-M-8}}$–producing strain. All ESBL-positive strains were ascribed to the phylogenetic groups A and B1. Most of the $bla_{\text{CTX-M-1}}$–containing strains harbored an IncI1 plasmid, except for the four $bla_{\text{CTX-M-1}}$–positive strains of beef origin and ST155, which harbored an IncN plasmid. The two $bla_{\text{CTX-M-14}}$–containing strains contained an IncI1 plasmid. The virulence gene *fimA* was detected in all strains. Most strains also carried the *aer* gene, and six strains carried the *eae* gene. All strains were negative for the virulence genes *sxt, papG*-III, *papC, hly, cnf1,* and *bfp*. We conclude that ESBL-producing *E. coli* strains of food origin in Tunisia show high diversity and that plasmids harboring ESBL genes could be implicated in the dissemination of this resistance phenotype.

In the past few years, there has been increasing concern in the scientific community about the emergence and dissemination of *Escherichia coli* strains producing extended-spectrum β-lactamases (ESBLs), especially of the CTX-M class, which are very frequently associated with community infections *(20)*. Recently, different reports have indicated the dissemination of ESBL-positive *E. coli* strains among the intestinal microbiota of healthy humans *(37),* in food-producing animals, and in food products *(5, 7, 14, 16, 19, 34)*. These resistant bacteria could be transferred to humans through the food chain, which represents a problem for public health. Comparison of human and animal ESBL-producing isolates is important to enhance the knowledge of the potential routes of transfer of these bacteria and resistance genes in different ecosystems.

CTX-M-15 is the most frequent type of ESBL detected among human clinical *E. coli* isolates in Tunisia *(3, 18, 23, 30),* as well as in other countries *(12),* and is mainly associated with the dissemination of the sequence type 131 (ST131) clone of the phylogenetic group B2 *(27)*. However, the CTX-M-15 type of β-lactamase has not been detected in food isolates or in fecal samples from healthy humans in Tunisia, in which the CTX-M-1 variant was frequently identified, although there is no information about the *E. coli* clones that harbor these resistance genes *(2, 4, 19)*. CTX-M-15–producing *E. coli* isolates have been found sporadically in food and animal isolates in other countries *(21, 22, 29, 34)*.

Pulsed-field gel electrophoresis (PFGE) is the most widely used tool for molecular typing of bacterial strains at the local level, but new DNA fingerprinting techniques, including multilocus sequence typing (MLST), are emerging as alternatives, particularly when information regarding evolutionary history is needed *(26)*. MLST is used to characterize *E. coli* isolates and other bacteria on the basis of sequence variations of seven housekeeping loci. The different allele numbers are assigned according to the nucleotide sequence of each locus, and then each strain is defined by the alleles of the seven loci (allelic profile). Closely related organisms can be grouped in clonal complexes. Analyses of *E. coli* isolates by MLST help to determine the genetic relatedness of the isolates, which is important for molecular epidemiological and evolutionary studies *(11)*. MLST is used to study the population biology of microorganisms and provides an understanding of the

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population structure of important microorganisms, such as *E. coli*. Isolates that exhibit similar or identical MLST genotypes are very closely related and are descended from a recent common ancestor *(36)*.

The aim of this study was to characterize ESBL-producing *E. coli* isolates recovered from food samples in previous studies in Tunisia *(4, 19),* with special attention to their plasmid and virulence gene content. Comparison of the results obtained with those reported for ESBL-positive isolates of other origins (sick and healthy humans) provides further insight into the potential of ESBL-containing food isolates as contributors towards the dissemination of ESBL-positive bacteria in humans.

MATERIALS AND METHODS

***E. coli* strains.** Nineteen PFGE-unrelated *E. coli* strains, previously recovered from food samples in different butcheries, supermarkets, and local markets in Tunisia during 2006 and 2007 *(4, 19),* were included in this study. The strains were recovered from the following origins (the number of strains is in parentheses): chickens (11), beef (5), sheep (2), and a turkey (1). They harbored different variants of ESBL genes (number of strains), including $bla_{\text{CTX-M-1}}$ (15), $bla_{\text{CTX-M-14}}$ (2), $bla_{\text{CTX-M-8}}$ (1), and $bla_{\text{SHV-5}}$ (1) (Table 1).

Virulence genotyping of *E. coli*. The presence of nine virulence genes (*fimA, papG* allele III, *hlyA, cnf1, papC, aer, sxt, eae,* and *bfp*) was analyzed by PCR in all ESBL-positive *E. coli* strains *(32)*. They were also screened for the presence of the diffuse adhesion-encoding *afa*/*dra* operon *(6)*.

Genetic typing of strains and plasmid characterization. The *E. coli* strains were characterized by MLST after PCR amplification of the following seven standard housekeeping loci *(35)*: *adk* (adenylate kinase), *fumC* (fumarate hydratase), *gyrB* (DNA gyrase), *icd* (isocitrate/isopropylmalate dehydrogenase), *mdh* (malate dehydrogenase), *purA* (adenylosuccinate dehydrogenase), and *recA* (ATP/GTP binding motif). Amplicons were sequenced and compared with MLST database (http://mlst.ucc.ie/) to determine the specific allele combination and the sequence type.

The strains were assigned to phylogenetic group A, B1, B2, or D using a PCR strategy with specific primers for *chuA, yjaA,* and *TspE4.C2* determinants as previously described *(9)*. The phylogenetic group of some of the strains was already known *(4)*. The plasmids carried by the strains were classified according to their incompatibility group using the PCR-based replicon-typing method *(8)*.

RESULTS AND DISCUSSION

The 19 ESBL-positive *E. coli* strains of food origin included in this study presented unrelated PFGE patterns and contained different variants of CTX-M β-lactamases (CTX-M-1, CTX-M-8, and CTX-M-14) or SHV-5. Table 1 shows a summary of the characteristics and genes of these ESBL-positive strains. It is interesting to note that 12 different sequence types were identified among these strains, indicating high clonal diversity. Four sequence types were detected in more than one strain (ST155 in four strains of beef origin, ST602 in three strains of chicken origin, and ST2022 and ST1970 each in two strains of chicken origin) (Table 1). Two of the 12 sequence types detected among our strains were new and were registered in the MLST database with the names ST2022 and ST1970. Sequence type ST2022 was identified in two CTX-M-14–producing strains, and ST1970 in two CTX-M-1–producing strains (Table 1). The *E. coli* strain carrying the unusual $bla_{\text{CTX-M-8}}$ gene was ascribed to sequence type ST405. All *E. coli* strains of this study were classified into phylogenetic group A ($n = 8$) or B1 ($n = 11$). No strains of phylogroups B2 or D, often associated with extraintestinal infections, were identified in our collection.

Few reports have addressed the presence of ST131 in animal strains or in food samples *(28),* and in accordance, this clonal lineage, often identified among clinical CTX-M-15–producing human *E. coli* strains *(12, 31, 33),* was not detected among our ESBL-positive food strains. ST10 and ST155 were detected in one and four CTX-M-1–producing strains, respectively, in our study. These sequence types have been previously detected in human and food-producing animal *E. coli* strains carrying the CTX-M-1 β-lactamase *(2, 29),* suggesting the potential transfer of these resistant clones within different ecosystems and the potential involvement of the food chain in this process. In Tunisia, these two sequence types (ST10 and ST155) were previously detected in CTX-M-1–producing *E. coli* strains from healthy humans *(2)*.

In our study, three of the four ST155 *E. coli* strains belonged to phylogenetic group B1 and the remaining strain, in addition to the ST10 *E. coli* strain, belonged to phylogenetic group A, in agreement with other studies *(29)*.

The high clonal diversity detected among our $bla_{\text{CTX-M-1}}$–carrying strains suggests that horizontal transmission of ESBL-containing plasmids has occurred among different genetic lineages of *E. coli*; this may be responsible for the recent rapid spread of this variant in this country and requires further studies.

All ESBL-positive strains of food origin examined in this study harbored the *fimA* virulence gene; 16 also carried the *aer* gene, and 6 of them also carried the *eae* gene. None of the ESBL-producing strains harbored the *sxt, papG*-III, *papC, hly, cnf1,* or *bfp* virulence genes.

Different plasmids were detected among the ESBL-positive strains of this study. Eleven of the 15 $bla_{\text{CTX-M-1}}$–containing strains (73.3%) harbored an IncI1 plasmid. It has been previously reported that IncI1 plasmids are carriers of this ESBL gene *(10, 15, 16)*. The remaining four $bla_{\text{CTX-M-1}}$–positive strains of beef origin that belonged to ST155 harbored the IncN plasmid (Table 1), which has also been previously reported to carry $bla_{\text{CTX-M-1}}$ *(13, 24, 25)*. The two $bla_{\text{CTX-M-14}}$–containing strains harbored an IncI1 plasmid, which could also contain this ESBL gene, as found in other studies *(1, 17)*.

In conclusion, the ESBL-positive *E. coli* strains of food origin recovered in Tunisia presented a high clonal diversity, indicating the potential horizontal transfer of ESBL genes in different *E. coli* clones, mostly belonging to phylogenetic groups A and B1. Plasmids of the IncI1 and IncN types might be implicated in the mobilization of $bla_{\text{CTX-M-1}}$ or $bla_{\text{CTX-M-14}}$ genes among food strains. More

TABLE 1. *Genetic lineages and virulence factors of the 19 PFGE-unrelated ESBL-producing* E. coli *strains of food origin recovered in Tunisia*

E. coli strain	Food of origin	β-Lactamase(s)	MLST sequence type/clonal complex	PFGE type	Phylogenetic group	Virulence factor(s)	Plasmid type(s)
C920	Beef	CTX-M-1, TEM-1b	ST155/CC155	P1	B1	*fimA*+*aer*+*eae*	IncN
C923	Beef	CTX-M-1	ST155/CC155	P2	B1	*fimA*+*aer*+*eae*	IncHI1, IncN
C924	Beef	CTX-M-1	ST155/CC155	P3	B1	*fimA*+*aer*+*eae*	IncHI1, IncN
C925	Beef	CTX-M-1	ST155/CC155	P4	A	*fimA*+*aer*	IncN
C1116	Chicken	CTX-M-1	ST602/CC446	P5	B1	*fimA*+*aer*	IncI1, IncFIB, IncFII
C1606	Chicken	CTX-M-1	ST602/CC446	P6	B1	*fimA*+*aer*+*eae*	IncI1, IncFIB, IncFII, IncY
C1616	Chicken	CTX-M-1	ST602/CC446	P7	B1	*fimA*+*aer*	IncI1, IncFIB, IncFII, IncY
C1614	Chicken	CTX-M-1	ST1970[a]/None	P8	A	*fimA*+*aer*	IncI1, IncFIB, IncFII
C1615	Chicken	CTX-M-1	ST1970[a]/None	P9	A	*fimA*	IncI1, IncFIB, IncFII, IncN
C922	Beef	CTX-M-1	ST10/CC10	P10	A	*fimA*+*aer*	IncI1, IncN, IncP
C926	Turkey	CTX-M-1	ST23/CC23	P11	A	*fimA*+*aer*	IncI1, IncN, IncFIB, IncFII
C1103	Chicken	CTX-M-1	ST101/CC101	P12	B1	*fimA*+*aer*	IncI1, IncFIB, IncFII
C1612	Sheep	CTX-M-1	ST1638/None	P13	A	*fimA*	IncI1
C1618	Chicken	CTX-M-1, TEM-1b	ST522/CC522	P14	A	*fimA*	IncI1
C1610	Sheep	CTX-M-1, TEM-20	ST224/None	P15	B1	*fimA*+*aer*+*eae*	IncI1, IncFIB, IncFII
C921	Chicken	CTX-M-8	ST405/CC405	P16	A	*fimA*+*aer*	IncN, IncFIB, IncFII
C929	Chicken	CTX-M-14, TEM-1b	ST2022[b]/None	P17	B1	*fimA*+*aer*	IncI1, IncN, IncY, IncFII, IncFIB
C930	Chicken	CTX-M-14, TEM-1b	ST2022[b]/None	P18	B1	*fimA*+*aer*	IncI1, IncN, IncY, IncFIB, IncFII
C928	Chicken	SHV-5	ST889/None	P19	B1	*fimA*+*aer*+*eae*	IncI1, IncFIB, IncFII

[a] New sequence type (ST1970) because of a new *recA* allele (185).
[b] New sequence type (ST2022) because of a new *fumC* allele (312).

studies should be performed in the future to track the evolution of ESBL types and their frequencies in different ecosystems.

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