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First Characterization of CTX-M-15-Producing *Escherichia coli* Strains Belonging to Sequence Type (ST) 410, ST224, and ST1284 from Commercial Swine in South America

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We report for the first time the isolation of CTX-M-15-producing *Escherichia coli* strains belonging to sequence type (ST) 410, ST224, and ST1284 in commercial swine in Brazil. The $bla_{\text{CTX-M-15}}$ gene was located on F-::A9::B1 and C1::A9::B1 IncF-type plasmids, surrounded by a new genetic context comprising the IS*26* insertion sequence truncated with the IS*Ecp1* element upstream of $bla_{\text{CTX-M-15}}$. These results reveal that commercial swine have become a new reservoir of CTX-M-15-producing bacteria in South America.

The rapid spread of the $bla_{\text{CTX-M-15}}$ gene, which encodes the currently most widely distributed extended-spectrum β-lactamase (ESBL) enzyme in Gram-negative bacteria, is a global challenge to human and veterinary medicine. In this context, food-producing animals have acquired an important role as reservoirs for CTX-M-15-producing bacteria, which can be transmitted to the community (1, 2). In this report, we describe for the first time the isolation of CTX-M-15-producing *Escherichia coli* in commercial swine in Brazil, highlighting a new reservoir of CTX-M-15-producing isolates with zoonotic potential in South America.

In 2012, during a study conducted to assess the occurrence of ESBL-producing bacteria in swine production, eight (3%) ceftiofur-resistant CTX-M-15-producing *E. coli* isolates were recovered from 267 fecal swabs collected from male and female nursery (40 days) and finishing (90 days) pigs from 28 farms in seven Brazilian states. In this regard, ceftiofur-resistant strains were isolated by using MacConkey agar supplemented with 2 μg/ml of ceftiofur. *E. coli* isolates were identified by the MALDI Biotyper (Bruker Daltonics, Germany), and the antibiotic resistance profiles were determined by the Kirby-Bauer method, with MICs determined by the microdilution technique by using Sensititre ESBL-confirmatory MIC plates (Trek Diagnostic Systems, Thermo Fisher) and/or the agar dilution method (3, 4). ESBL production was screened by the double-disk synergy test, and the presence of $bla_{\text{CTX-M}}$-type genes was examined by PCR amplification and sequencing. *E. coli* isolates were further characterized by phylogenetic grouping (5) and multilocus sequence typing (MLST) (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Next, plasmids were extracted and used to transform electrocompetent *E. coli* TOP10 recipient cells (Invitrogen) by electroporation. The transformant *E. coli* TOP10 strains were selected on Mueller-Hinton agar supplemented with 2 μg/ml of cefotaxime and further analyzed by replicon typing (6), plasmid MLST experiments (http://pubmlst.org/plasmid/), and partial plasmid sequencing to elucidate the $bla_{\text{CTX-M-15}}$ genetic environment.

The eight ceftiofur-resistant *E. coli* isolates produced CTX-M-15 and exhibited high MICs of human and veterinary cephalosporins (Table 1). Additionally, *E. coli* strains were resistant to ciprofloxacin, enrofloxacin, norfloxacin, tetracycline, sulfonamide-trimethoprim, and gentamicin (7). Otherwise, all isolates remained susceptible to amikacin, cephamycins, and carbapenems. The isolates belonged to three clonal lineages of sequence type (ST) 224 (5 strains from farm X in Minas Gerais State, southeastern Brazil), ST410 (CC23; 1 strain from farm Y in Minas Gerais State), and ST1284 (2 strains from farm Z in Paraná State, southern Brazil). While none of the eight isolates belonged to phylogroup B2, phylogroups A and B1 were identified among CTX-M-15-producing *E. coli* strains. For *E. coli* ST224 and ST410 strains, the $bla_{\text{CTX-M-15}}$ gene was successfully transferred to TOP10 *E. coli* isolates by transformation, being associated with the presence of IncF incompatibility plasmid groups, with sizes ranging from 40 to 90 kb (Table 1). Additional resistance to gentamicin, sulfonamides, and tetracycline was cotransferred, and the presence of *aacA4*, *aac(3)-IIa*, *aadA1*, *sul1*, and *tetA* genes was confirmed in both donor and receptor *E. coli* strains, whereas PCR analysis of 16S rRNA methylase genes was negative. On the other hand, fluoroquinolone resistance was not cotransferred, and, indeed, no plasmid-mediated quinolone resistance (PMQR) genes were found.

Moreover, representative IncF-positive plasmids from *E. coli* ST224 and ST410 were selected for partial plasmid sequencing and replicon sequence typing, yielding the FAB allele formulas F-:; A9:;B1 and C1:;A9:;B1, respectively (BioProject accession numbers PRJNA279532 and PRJNA291430). In this regard, reports on the FAB formula of ST410 (C1:;A9:;B1) have been rare among CTX-M-15 producers, since most studies have been limited to $bla_{\text{CTX-M}}$-type identification, where plasmid characterization is

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TABLE 1 Epidemiological and microbiological characteristics of CTX-M-15-producing *E. coli* strains isolated from commercial swine, *E. coli* TOP10 (transformed) and *E. coli* TOP10

E. coli strain	Farm/state[b]/yr	ST/phylogroup	MIC (μg/ml)[c] for:															
			AMP	CEF	CTX	CRO	EFT	CPD	CAZ	FEP	FOX	IPM	MER	CIP	ENO	NOR	GEN	AMI	TET	SUL	SXT
180A	X/MG/2012	224/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	**16**	8	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	16	**≥128**	**≥512**	**≥32**
T-180A			**≥32**	**≥32**	**32**	**8**	**16**	**≥64**	4	2	≤4	≤0.5	≤1	0.0035	0.007	0.06	≤4	2	4	≤12	≤2
180B	X/MG/2012	224/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	**16**	16	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	16	**≥128**	**≥512**	**≥32**
181	X/MG/2012	224/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	8	8	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	16	**≥128**	**≥512**	**≥32**
T-181			**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	8	8	≤0.5	≤1	0.0035	0.007	0.12	**≥32**	2	**≥128**	**≥512**	≤2
187[a]	X/MG/2012	224/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	**16**	16	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	16	**≥128**	**≥512**	**≥32**
T-187[a]			**≥32**	**≥32**	**≥128**	**128**	**≥128**	**≥64**	**16**	8	≤4	≤0.5	≤1	0.0035	0.0035	0.06	**≥32**	2	4	**≥512**	≤2
188	X/MG/2012	224/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	**16**	16	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	8	**≥128**	**≥512**	**≥32**
T-188			**≥32**	**≥32**	**≥128**	**≥256**	**128**	**≥64**	**16**	**16**	8	≤0.5	≤1	0.0035	0.007	0.12	**≥32**	2	4	**≥512**	≤2
223B[a]	Y/MG/2012	410/A	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**32**	**16**	16	≤0.5	≤1	**≥64**	**≥64**	**≥128**	≤4	8	**≥128**	**≥512**	**≥32**
T-223B[a]			**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**32**	**16**	≤4	≤0.5	≤1	0.0035	0.0035	0.12	≤4	8	**≥128**	**≥512**	≤2
468	Z/PR/2012	1284/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	**16**	16	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	16	**≥128**	**≥512**	**≥32**
470	Z/PR/2012	1284/B1	**≥32**	**≥32**	**≥128**	**≥256**	**≥128**	**≥64**	**16**	**≥32**	8	≤0.5	≤1	**≥64**	**≥64**	**≥128**	**≥32**	8	**≥128**	**≥512**	**≥32**
TOP10			≤8	≤8	≤0.2	≤1	0.5	1	0.5	≤1	≤4	≤0.5	≤1	0.0035	0.0035	0.06	≤4	2	4	≤12	≤2

[a] Representative IncF-positive plasmids from *E. coli* ST224 and ST410 were selected for replicon sequence typing, obtaining the FAB allele formulas F-::A9::B1 and C1::A9::B1, respectively.

[b] MG, Minas Gerais State; PR, Paraná State.

[c] MICs were determined by microdilution technique using Sensititre ESBL-confirmatory MIC plates (TREK Diagnostic Systems, Thermo Fisher) or agar dilution methods; resistance is indicated in bold by using CLSI criteria (3, 4). AMP, ampicillin; CEF, cephalothin; CTX, cefotaxime; CRO, ceftriaxone; EFT, ceftiofur; CPD, cefpodoxime; CAZ, ceftazidima; FEP, cefepime; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; ENO, enrofloxacin; NOR, norfloxacin; GEN, gentamicin; AMI, amikacin; TET, tetracycline; SUL, sulfonamide; SXT, sulfamethoxazole-trimethoprim.

restricted to Inc type and size determination (8). Therefore, this FAB formula has most likely been underestimated.

Finally, these results revealed that both plasmids carried an IS*Ecp1* truncated by an IS*26* transposase upstream of the $bla_{\text{CTX-M-15}}$ gene and an intact *orf477* gene downstream from $bla_{\text{CTX-M-15}}$ (Fig. 1).

In this study, we report for the first time the emergence of $bla_{\text{CTX-M-15}}$-carrying *E. coli* in commercial healthy pigs in Brazil. In this regard, the identification of CTX-M-15-producing *E. coli* from commercial swine has until now been reported only from Asian and European countries (9, 10). Of particular interest is the description of CTX-M-15-producing *E. coli* belonging to ST410 (CC23), which was previously isolated from broiler and clinical samples, providing evidence for the transmission of CTX-M-15-producing *E. coli* between animals and humans (11). Indeed, CTX-M-15-producing *E. coli* ST410 (CC23), phylogroup A, has been predominant among ESBL-producing *E. coli* isolates from hospitals in Brazil (12). In contrast, *E. coli* strains belonging to ST224 have been identified in inpatients and outpatients and associated with the production of different clinically important β-lactamases, such as NDM-1, KPC-2, and even CTX-M-15 (13–15), and more recently, the production of CTX-M-8 in *E. coli* ST224 isolated from dairy buffalo was documented in Brazil (16). Representative *E. coli* strains belonging to ST410 and ST224 present distinct genetic arrangements upstream of the $bla_{\text{CTX-M-15}}$ gene (Fig. 1). In this context, the ST223B strain presents the IS*Ecp1* flanked by IS*26* upstream the $bla_{\text{CTX-M-15}}$ gene in a similar structure described previously (17) but with a deletion of 727 bp in the IS*Ecp1* sequence. The $bla_{\text{CTX-M-15}}$ gene was previously found together with IS*Ecp1* truncated by an IS*26* element in *E. coli* strains belonging to ST410 from both clinical and food samples from southern Spain (8); however, IS*26* was identified in the opposite orientation (Fig. 1). On the other hand, a new genetic context of $bla_{\text{CTX-M-15}}$ was observed in *E. coli* strains belonging to ST224, which presents a 1,177-bp IS*Ecp1* truncated by an incomplete IS*26* transposase upstream of the $bla_{\text{CTX-M-15}}$ gene, similar to genetic structures found in CTX-M-15-producing *E. coli* isolated from bovine mastitis in the United Kingdom (18).

Finally, the detection of phylogenetic groups A and B1 in this study suggests that selection of silent $bla_{\text{CTX-M-15}}$ carriers among commensal *E. coli* in healthy commercial swine is ongoing, which is a worrisome prospect, since ESBL-producing *E. coli* of commensal origin can play a key role as opportunistic pathogens in humans and other animals that can serve as hosts. Most likely, as previously hypothesized, therapeutic and prophylactic use of ceftiofur in the swine industry may be contributing to the selection and recovery of enteric *E. coli* with resistance to cephalosporin drugs (19), where $bla_{\text{CTX-M}}$-type ESBL genes can rapidly disseminate among healthy pigs. However, given that all isolates were also resistant to fluoroquinolones, the use of enrofloxacin may have exerted a selection pressure. In this regard, in Brazil, both ceftiofur and enrofloxacin are used for the treatment of enteric, urinary, or systemic infections, and in some herds, ceftiofur is used for systematic prophylaxis in 1-day-old piglets.

In summary, surveillance of antimicrobial resistance in bacteria from food-producing animals and derived food products needs to be a priority. Moreover, strategies for the rational use of antimicrobial agents in food animals need to be undertaken urgently, in order to inhibit the release of bacteria harboring clinically important resistance genes. In this regard, the dissemination

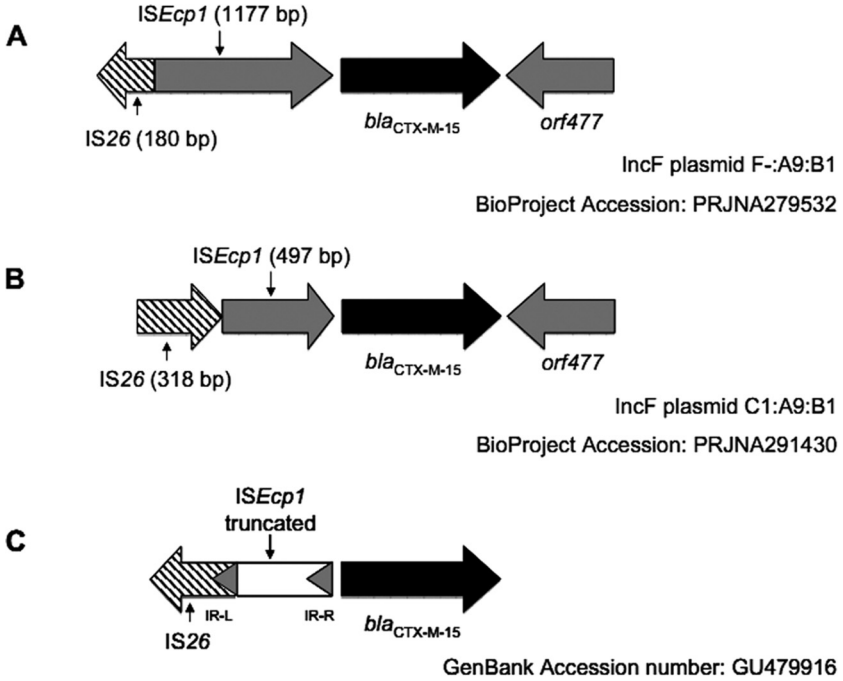

FIG 1 Schematic representations of the genetic environment surrounding the $bla_{CTX-M-15}$ gene in swine *E. coli* isolates. (A) The plasmid $bla_{CTX-M-15}$ gene from *E. coli* 181 ST224 (BioProject accession number PRJNA279532). (B) The plasmid $bla_{CTX-M-15}$ gene from *E. coli* 223B ST410 (BioProject accession number PRJNA291430). (C) Genetic environment surrounding $bla_{CTX-M-15}$ found in an *E. coli* strain belonging to ST410, isolated from clinical and food samples from southern Spain (8) (GenBank accession number GU479916). Sections, open reading frames and genes surrounding the $bla_{CTX-M-15}$ gene; arrows, orientation of each coding sequence. Gene names are shown under the corresponding section.

of cephalosporin-resistant bacteria carrying $bla_{CTX-M-15}$ has the potential to impact both veterinary and human therapeutic treatment options.

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