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First Characterization of CTX-M-15-Producing *Escherichia coli* ST131 and ST405 Clones Causing Community-Onset Infections in South America▽

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CTX-M-15-producing *Escherichia coli* has emerged worldwide as an important pathogen associated with community-onset infections, but in South America reports are scarce. We document the presence of CTX-M-15-producing *E. coli* of the international ST131 and ST405 clones in Colombia and present the first molecular characterization of these isolates in South America.

Since the end of the 1990s, a new family of extended-spectrum β-lactamases (ESBL), the CTX-M type, has spread among continents, becoming the most prevalent in the world (1). Within this family, *Escherichia coli* strains producing CTX-M-15 have emerged as an important causative agent of community-onset infections (COI), predominantly urinary tract infections (UTI) (1). Moreover, the successful dispersion of CTX-M-15 has been associated with specific clones, such as ST131 and ST405, which belong to virulent phylogenetic groups B2 and D, respectively (4, 14). Other genes normally present on the same plasmid carrying $bla_{\text{CTX-M-15}}$, like bla_{TEM} and bla_{OXA}, as well as *aac-(6′)-Ib-cr* and *qnr* determinants, explain the multiresistant phenotype of CTX-M-15-producing bacteria (6, 14).

In South America, CTX-M-2, CTX-M-9, and CTX-M-12 have been reported previously among *Enterobacteriaceae* (15). However, reports of CTX-M-15-producing *Enterobacteriaceae* remain scarce in this region, and only recently a single report from Brazil of the clone ST131 was presented in an abstract lacking clinical data or further molecular characterization (9).

In this study, we present the first molecular characterization of CTX-M-15-producing *Escherichia coli* isolates belonging to the clones ST131 and ST405 associated with COI in Colombia and South America, describing demographic and clinical data of patients from which these isolates were cultured.

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From January 2010 to May 2010, ESBL-positive *E. coli* isolates were sent from three Colombian hospitals to our reference laboratory at CIDEIM in Cali, Colombia. All isolates were obtained from patients who presented to the emergency room with community-onset infections, defined by the patient's having no history of being in a hospital, long-term-care facility, or nursing home, receiving home care, or having an invasive procedure done in the last 3 months. Bacterial identification was confirmed using the Vitek 2 automatic system (bioMérieux, Marcy l'Etoile, France). Detection of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, and β-lactamase genes was performed by PCR, and all isolates in which cluster 1 $bla_{\text{CTX-M}}$ was detected were sequenced.

CTX-M-15-producing *E. coli* isolates were further characterized. Susceptibility testing was performed using the broth microdilution method (Sensititre panels; TREK Diagnostic Systems, Westlake, OH), and the results were interpreted according to the CLSI breakpoints (3). Multiplex PCR for *qnr* determinants (*qnrA*, *qnrB*, *qnrS*) was carried out as previously described (13). PCR for *aac(6′)-Ib* was performed in all isolates, and those that tested positive were further analyzed by digestion with BstF51 to identify strains harboring *aac(6′)-Ib-cr* (8). Detection of virulence-associated genes was performed based on published methods (5); virulence markers screened included *usp* (uropathogenic-specific protein), *ompT* (outer membrane protein), two adhesin-encoding genes (*papC* and *fimH*), two siderophore-related genes (*iutA* and *fyuA*), a serum survival-related gene (*traT*), and a pathogenicity-associated island (PAI) marker. Determination of the *E. coli* phylogenetic groups (A, B1, B2, and D) was carried out by a modified multiplex PCR (2, 12). These isolates underwent typing using the DiversiLab system (bioMérieux), following the manufacturer's instructions, and multilocus sequence typing (MLST) (16), according to the recommended procedure at the *E. coli* MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

From 33 ESBL-positive isolates received at CIDEIM, 18 harbored cluster 1 $bla_{\text{CTX-M}}$, and DNA sequence analysis revealed the presence of $bla_{\text{CTX-M-15}}$ in 14 isolates. Within the remaining four isolates, one harbored $bla_{\text{CTX-M-12}}$ and the other three harbored $bla_{\text{CTX-M-12a}}$. Table 1 summarizes

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TABLE 1. Clinical data of patients and microbiological and molecular data of CTX-M-15-producing isolates

Patient	Sex[a]	Age (yr)	Diagnosis	Underlying disease(s)[b]	ATB last 3 mo[c]	MIC (μg/ml)[f]								Virulence factor genes	Other *bla* genes detected	Presence of[e]:		Phylogenetic group	MLST[d]	
						CTX	CAZ	FEP	TZP	ETP	MEM	CIP	AMK			*qnr*	*aac(6′)-Ib-cr*		CC	ST
1	F	85	Cystitis	R-UTI, UI	NIT, CIP	>32	8	8	≤8/4	≤0.5	≤0.12	>8	≤8	PAI, *iutA*, *traT*	None	−	+	A	10	617
2	F	80	Cystitis	R-UTI	No	>32	32	16	≤8/4	≤0.5	≤0.12	>8	≤8	PAI, *iutA*, *traT*	None	−	+	A	10	617
3	M	30	Peritonitis	Ulcerative colitis	No	>32	8	8	≤8/4	≤0.5	≤0.12	>8	≤8	*fimH*, *iutA*, *traT*	None	−	+	A	10	44
4	F	80	Cystitis	UI, HTN	CIP	16	≤2	2	≤8/4	≤0.5	≤0.12	8	16	*fimH*, *iutA*, *traT*	None	−	+	A	10	44
5	F	69	Cystitis	R-UTI	NIT	>32	16	32	16/4	≤0.5	≤0.12	>8	≤8	*fimH*, *fyuA*	bla_{TEM-1}	−	+	D	405	405
6	F	75	Cystitis	R-UTI, UI	NIT	>32	32	16	≤8/4	≤0.5	≤0.12	>8	≤8	*fimH*, *fyuA*	bla_{TEM-1}	−	+	D	405	405
7	F	88	Pyelonephritis	CRF, CHF, DM2	No	16	8	8	≤8/4	≤0.5	≤0.12	>8	≤8	PAI, *fimH*, *fyuA*, *iutA*, *traT*	None	−	+	D	405	405
8	F	18	Cystitis	None	No	>32	16	16	≤8/4	≤0.5	≤0.12	>8	≤8	*ompT*, PAI, *fyuA*, *traT*	bla_{TEM-1}	−	−	D	None	2043
9	F	81	Pyelonephritis	Alzheimer's disease	No	>32	32	32	16/4	≤0.5	≤0.12	>8	16	*ompT*, PAI, *fyuA*	bla_{TEM-1}	−	+	D	648	648
10	M	80	Cystitis	R-UTI, COPD	No	>32	32	32	32/4	2	0.25	>8	≤8	PAI, *ompT*, *fimH*, *fyuA*, *traT*	None	−	+	D	648	648
11	M	2	Cystitis	None	No	>32	>32	64	32/4	≤0.5	≤0.12	8	32	PAI, *fimH*, *iutA*, *traT*	None	−	+	D	None	1722
12	F	2	Cystitis	None	No	>32	8	16	≥128/4	≤0.5	≤0.12	>8	≤8	*usp*, *ompT*, PAI, *fimH*, *fyuA*, *iutA*, *traT*	bla_{TEM-1}	−	+	B2	None	131
13	F	69	Cystitis	R-UTI, UI, HTN	CIP	>32	>32	32	32/4	≤0.5	≤0.12	>8	≤8	*usp*, *ompT*, PAI, *fimH*, *fyuA*, *iutA*, *traT*	bla_{TEM-1}	−	+	B2	None	131
14	F	64	Cystitis	UI, DM2, HTN	No	32	32	2	≤8/4	≤0.5	≤0.12	>8	≤8	*usp*, *ompT*, PAI, *papC*, *fimH*, *fyuA*, *iutA*	bla_{TEM-1}	−	+	B2	None	2042

[a] F, female; M, male.
[b] R-UTI, repetitive urinary tract infection; UI, urinary incontinence; HTN, arterial hypertension; CRF, chronic renal failure, CHF, congestive heart failure; DM2, diabetes mellitus 2; COPD, chronic obstructive pulmonary disease.
[c] ATB, antibiotic; NIT, nitrofurantoin; CIP, ciprofloxacine.
[d] ST, sequence type; CC, clonal complex.
[e] +, positive; −, negative.
[f] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin-tazobactam; ETP, ertapenem; MEM, meropenem; AMK, amikacin.

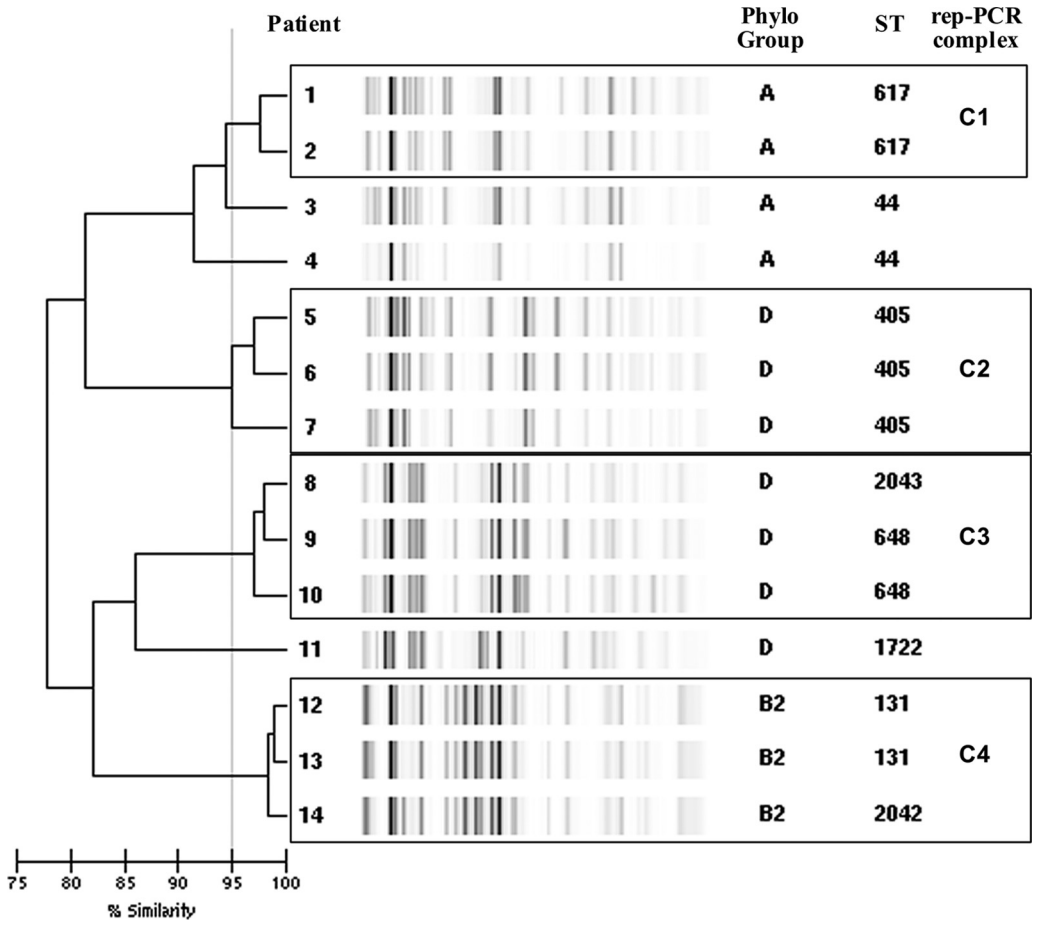

FIG. 1. CTX-M-15-producing *E. coli* isolates typed by DiversiLab Rep-PCR using 95% as a cutoff value.

the clinical, microbiological, and molecular data of patients and CTX-M-15 isolates.

DiversiLab Rep-PCR, using 95% as a cutoff value, allowed the identification of four different repetitive PCR (rep-PCR) complexes of related isolates, while three strains yielded single rep-PCR types, as shown in Fig. 1. Within complex 4, two *E. coli* isolates (12 and 13) belong to ST131. The DiversiLab system was able to identify clone ST131, as previously reported by Pitout et al. (10); in addition, isolate 14 is a new single-locus variant (SLV) of ST131, designated ST2042, which might demonstrate a local variation of the ST131 clone. These three *E. coli* isolates had coresistance to quinolones (ciprofloxacin MIC, >8 μg/ml) harboring *aac(6′)-Ib-cr* without *qnr* determinants, as well as $bla_{\text{TEM-1}}$. Like ST131 isolates recovered in other geographic areas, they were also found to carry the virulence factor genes *fimH*, *fyuA*, and *usp* (6, 14).

Isolates belonging to the virulent phylogenetic group D were grouped in two different rep-PCR complexes: complex 2 correspond to isolates belonging to the ST405, and complex 3 is formed by three isolates, two that belong to the ST648 and the other to the newly designated ST2043 (double-locus variant [DLV] of ST648). Isolate 11 (ST1722) represents a unique rep-PCR profile within phylogenetic group D. The remaining four isolates (isolates 1 to 4) belong to the phylogenetic group A and to the sequence type ST10 complex; two were ST617 and grouped within rep-PCR complex 1, and the other two were ST44. The CC10 has been also associated with other ESBLs such as CTX-M-14 or SHV-12 in isolates producing UTI in community patients (7). All previous isolates harbor several virulence factors and have coresistance to ciprofloxacin; all but one harbor *aac(6′)-Ib-cr*, and none harbored *qnr* determinants (Table 1).

Patients' ages ranged between 2 and 88 years, with most of them (10 of 14) over 60 years old; all but one presented with community-onset UTI. Recurrent UTI (6/11) and urinary incontinence (5/11) were the most common underlying conditions detected among the patients. Two patients had a history of previous intake of ciprofloxacin, two of nitrofurantoin, and one of both antibiotics during the last 3 months (Table 1).

We report for the first time in Colombia CTX-M-15-producing *E. coli* in several sequence types (STs), including the presence of ST131 and ST405, which are multidrug-resistant virulent clones involved in the intercontinental dissemination of $bla_{\text{CTX-M-15}}$. As previously reported, ST131 has been associated with UTI (4, 11). The presence of CTX-M-15-producing *E. coli* causing COI and its potential spread in the community is an important public health concern, as Colombia may become part of the so-called "CTX-M pandemic" (1). There is a serious need to monitor the spread of these multidrug-resistant clones since, for instance, ST131 has been implicated in severe

COI, including bacteremias (11). It is therefore mandatory to study the prevalence, clinical impact, and risk factors for the acquisition of CTX-M-producing *E. coli* in the community in Colombia and South America.

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