

CTX-M-32 beta-lactamase-producing uropathogenic *Escherichia coli* isolated in Latin America

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Background

Uropathogenic *Escherichia coli* (UPEC) is the most important pathogen involved in urinary tract infections (UTIs). Beta-lactam antibiotics have been widely used to treat UPEC infections; however, extended-spectrum beta-lactamases (ESBLs) create significant therapeutic problems by inactivating almost all beta-lactams except cephamycins and carbapenems [1,2]. The CTX-M-type beta-lactamases are members of a lineage of ESBLs currently recognized as the most widespread worldwide, both in clinical and community settings. Based on their amino-acid sequence diversity, more than 135 of the CTX-M identified variants have been classified into six major clusters: CTX-M-1, -2, -8, -9, -25 and -45. (www.lahey.org/studies/webt.stm) [3-5]. These beta-lactamases predominantly hydrolyze cefotaxime (or ceftriaxone) but are weakly active against ceftazidime. However, to date the CTX-M-1 cluster is the most diversified group and includes more efficient CTX-M variants with a strong hydrolytic activity against ceftazidime, as is the case for CTX-M-32 [6]. In 2004, *bla*_{CTX-M-32} was first reported in an *E. coli* isolate from Spain [7]. Since then, CTX-M-32 continues to spread mainly in Mediterranean countries [3]. In Latin America, the prevalence of CTX-M beta-lactamases is represented by the CTX-M-2, but other variants such as CTX-M-1, CTX-M-8, CTX-M-9, CTX-M-12, CTX-M-14, CTX-M-15, CTX-M-16, CTX-M-24 and CTX-M-56 have been identified in this continent [4,8]. In this report, we describe the first detection of CTX-M-32 in uropathogenic *Escherichia coli* from

Venezuela, which is also the first report of this enzyme in Latin America.

The study

In September 2011, a 46-year-old male diabetic patient was hospitalized in the internal medicine long-stay unit service of University of The Andes Hospital (Mérida, Venezuela) with a history of recurrent urinary tract infection (UTI). This patient had more than three microbiologically documented episodes of symptomatic UTI during the last 6 months. Initially, the patient was managed with empirical first-line antibiotic therapy (amoxicillin or cephalexin) and later with wide-spectrum cephalosporins (ceftriaxone or cefotaxime). One cephalosporin-resistant strain of *E. coli* LMM23/11 was isolated from a urine sample at the Public Health Laboratory.

The strain was identified using conventional biochemical tests and confirmed using the API 20E system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was performed using the agar dilution method according to the recommendations of the CLSI guidelines [9]. This isolate showed a multidrug resistance phenotype including third-generation cephalosporins, gentamicin, tobramycin and ciprofloxacin, but was susceptible to carbapenems, tigecycline and colistin. ESBLs activity was confirmed by restoring susceptibility to cefotaxime and ceftazidime in the presence of clavulanic acid and also by the double-disk synergy test (Table 1). Few data are available on the role of diabetes mellitus itself as a risk factor for the development of antimicrobial resistance of

Table 1. Antibiotic susceptibility of ESBL-producing *E. coli* LMM23/11 and transconjugants LMM23/11Tc

Antibiotics tested	MIC (mg/liter) for			
	<i>E. coli</i> LMM23/11	<i>E. coli</i> LMM23/11Tc	<i>E. coli</i> MKD135	<i>E. coli</i> ATCC 25922
Cefoxitin	8	8	4	2
Cefotaxime	> 256	128	0.064	0.125
Cefotaxime/clavulanic acid ^a	0.25	0.25	0.125	0.125
Ceftazidime	>256	64	0.025	0.25
Ceftazidime/clavulanic acid ^a	0.25	0.25	0.25	0.125
Aztreonam	4	4	0.25	0.25
Imipenem	0.064	0.064	0.064	0.064
Ertapenem	0.064	0.064	0.032	0.032
Meropenem	0.064	0.064	0.064	0.032
Gentamicin	> 64	32	0.5	0.5
Amikacin	8	4	0.5	1
Tobramycin	16	4	0.125	0.5
Ciprofloxacin	8	0.125	0.25	0.0125
Colistin ^b	1	0.5	0.25	0.25
Tigecycline ^b	0.25	0.125	0.125	0.032
Other test				
DDST ^c	+	+	-	-

^aClavulanate was used at fixed concentration of 4 mg/liter

^bMIC interpretation according to European Committee on Antimicrobial Susceptibility Testing (EUCAST). Version 3.0, (2013)

^cDDST: double disk synergy test

uropathogens. However, recurrent infections are of particular concern because of the repeated morbidity and necessity for frequent administration of antibiotics, with the possible adverse selection consequences of antimicrobial-resistant strains [2]. The patient was treated according to the susceptibility test with i.v. meropenem.

E. coli LMM23/11 was submitted to the Molecular Microbiology Laboratory of The Andes University for confirmation of unusual resistance to wide-spectrum cephalosporins, determination of resistance mechanisms, characterization, and additional testing.

Detection of antimicrobial resistance *bla*_{CTX-M} and *bla*_{CTX-M-1} genes was performed by PCR amplification using conditions previously described [10] and the following sets of primers: CTX-M-GF 5'-ATGTGCAGYACCAGTAARGTKATGGC-3' and CTX-M-GR 5'-CCGCTGCCGGTYTTATCVCCBAC-3' for *bla*_{CTX-M}; CTX-M-1F 5'-GGTAAAAAATCACTGCGTC-3' and CTX-M-1R 5'-TTGGTGACGATTTTAGCCGC-3' for *bla*_{CTX-M-1}. The presence of other beta-lactamase genes was determined by previously described primers [11] with negative results. Amplification products were purified (PCR-Accuprep kit Bioneer). Nucleotide

and amino acid sequence alignments, analyzed using the Basic Local Alignment Search Tool (BLAST) suite of programs (<http://www.ncbi.nlm.nih.gov>), revealed the presence of the *bla*_{CTX-M-32} gene, with a 98% degree of identity corresponding to the nucleotide sequence reference GenBank accession N° AJ557142.

Conjugation studies were performed to determine if ESBLs could be transferred from *E. coli* LMM23/11 to *E. coli* MKD135 (Rifampicin). Transconjugants obtained were selected on Mueller Hinton Agar II plates (Becton Dickinson and Co., Sparks, MD, USA) containing rifampicin (400 mg/liter) and cefotaxime (2 mg/liter) as required. Transconjugants (LMM23/11Tc) showed the ESBL phenotype, as determined by MICs and positive PCR results for *bla*_{CTX-M}.

E. coli LMM23/11 was classified as belonging to the B2 phylogenetic group by a multiplex PCR assay using a combination of three DNA markers (*chuA*, *yjaA* and the DNA fragment TspE4.C2) as described elsewhere [12]. It is generally accepted that the capacity of UPEC to cause disease among humans varies according to the presence and type of virulence factors within each lineage. However, other studies report that bacterial pathogens that acquire resistance determinants and express a multiresistant phenotype

also show a reduced virulence [13]. Four of the six virulence genes (*usp*, *fyuA*, *KpsMTII* and *fimH*), detected by PCR using specific primers as described previously [14], underline the possible emergence of a highly virulent multiresistant strain in Venezuela (Table 2).

Conclusion

We report the presence of the *bla*_{CTX-M-32} gene in uropathogenic *Escherichia coli* from a diabetic patient in Venezuela and, to our knowledge, this provides the first description of a CTX-M-32 beta-lactamase in Latin America. A few CTX-M-32-producing strains have been isolated from clinical samples in Spain, Italy, Greece and Portugal, suggesting the existence of community reservoirs for CTX-M enzymes and an ongoing widespread dissemination in the Mediterranean area [3,5,12].

In terms of evolution, CTX-M-3 was likely the enzyme at the origin of diversification in the CTX-M-1 cluster, which was coincident with positive selection acting on several amino acid positions [6,15]. Analysis of sequencing, showing that CTX-M-32 differs from CTXM-1 by a single Asp240-Gly substitution, is a key factor in the evolution of CTX-M beta-lactamases for its increased level of resistance to ceftazidime. As a matter of fact, our *bla*_{CTX-M-32}-containing *E. coli* LMM23/11 strain showed a ceftazidime high MIC value (>256 mg/liter).

The results obtained suggest that the *E. coli* LMM23/11 expressing CTX-M-32 is a potent uropathogenic strain belonging to the phylogenetic group B2. This strain can efficiently colonize the intestine because of the presence of virulence factors that subsequently might infect the urinary tract, regardless of its selection as a result of antibiotic

pressure. We hope that these findings will contribute to the knowledge of the population genetic structure of UPEC and their propensity to spread, or emerge, with multidrug resistant phenotypes in developing countries.

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Table 2. Genetic characteristics of uropathogenic *E. coli* LMM23/11

Characteristic	<i>E. coli</i> LMM23/11
beta-lactamase	
<i>bla</i> _{TEM}	-
<i>bla</i> _{SHV}	-
<i>bla</i> _{CTX-M}	+ (CTX-M-32)
Virulence factors	
<i>fimH</i>	+
<i>KpsMT II</i>	+
<i>fyuA</i>	+
<i>usp</i>	+
<i>papAH</i>	-
PAI	-
Phylogenetic group	B2

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