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Changing Epidemiology of Extended-Spectrum β-Lactamases in Argentina: Emergence of CTX-M-15

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A multicenter survey, carried out in 2010 in Argentina, showed an increased prevalence of extended-spectrum β-lactamase (ESBL)-producing enterobacteria, with some changes in the molecular epidemiology of circulating ESBLs. While enzymes of the CTX-M-2 group remain endemic, the emergence of CTX-M-15 and of enzymes of the CTX-M-8 and CTX-M-9 groups was observed. The CTX-M-15-positive isolates represented 40% of CTX-M producers and included representatives of *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST11.

Extended-spectrum cephalosporin resistance in enterobacteria is mostly mediated by extended-spectrum β-lactamases (ESBLs). Among them, the CTX-M-type ESBLs (initially reported in the second half of the 1980s) are the most prevalent enzymes worldwide (5, 6). To date, the CTX-M family of enzymes comprises at least 124 allotypes, subclassified by amino acid similarities into six sublineages, namely, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and CTX-M-45 (http://www.lahey.org/Studies/) (23).

Since its first detection, CTX-M-2 has become the most prevalent ESBL in Argentina, and enzymes of the CTX-M-2 group have been the only CTX-Ms reported in this country (21, 22).

In this work, we report the results of a recent multicenter survey conducted to analyze the prevalence and nature of ESBLs in Argentina, which showed a notable evolution in the molecular epidemiology of circulating enzymes.

A total of 1,586 consecutive and nonrepetitive enterobacterial clinical isolates were recovered during October 2010 from patients at 15 community hospitals distributed in three different regions of Argentina: (i) Ciudad Autónoma de Buenos Aires (CABA) ($n = 5$) and Buenos Aires ($n = 2$), (ii) Santa Fe ($n = 4$), and (iii) Chubut ($n = 4$). Isolates were identified by both conventional and automated methods (Vitek; bioMérieux). Antimicrobial susceptibility tests were performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) (9). ESBL confirmatory tests were performed by synergy tests using cefotaxime (CTX) and ceftazidime (CAZ) compared to CTX-clavulanic acid and CAZ-clavulanic acid-containing disks (10) for all noninducible AmpC-producing enterobacteria. In inducible AmpC producers, ESBL detection was performed using cefepime (FEP) compared to FEP-clavulanic acid-containing disks (M. Quinteros, M. Radice, P. Power, M. Matteo, M. Mollerach, J. Di Conza, N. Costa, and G. Gutkind, presented at the International Congress on Beta-Lactamases, L'Aquila, Italy, 1999). Screening for AmpC β-lactamases was assayed using a 300-μg phenyl boronic acid-containing disk placed 2 cm from the CAZ-containing disks (25).

Two hundred seven isolates exhibiting inhibition zones for CTX of ≤27 mm and/or CAZ of ≤22 mm were collected during the study period (13.1% of all screened enterobacterial isolates) (Table 1). Reduced susceptibility to expanded-spectrum cephalosporins was higher than the 9% observed in a surveillance study performed in Buenos Aires in 2003 ($P < 0.05$) (21), even if in that study only microorganisms recovered from inpatients were considered, while in the present study, samples recovered from both inpatients and outpatients were included.

TABLE 1 Number of isolates of each species recovered within the study period, extended-spectrum cephalosporin resistance, and number of resistant isolates that were further studied

Species	No. of isolates	No. (%) of ESC[a]-resistant isolates	ESC-resistant isolates recovered within 1 wk	
			No. of isolates	No. of ESBL producers/AmpC producers
Escherichia coli	1,120	64 (5.7)	16	14/2
Klebsiella pneumoniae	193	87 (45.1)	22	22/0
Proteus mirabilis	115	14 (12.2)	6	5/1
Enterobacter cloacae	37	11 (29.7)	3	1/2
Morganella morganii	29	11 (37.9)		
Klebsiella oxytoca	20	6 (30)	4	4/0
Citrobacter freundii	18	5 (27.8)		
Serratia spp.	18	5 (27.8)	3	3/0
Providencia spp.	13	2 (15.4)	1	1/0
Citrobacter spp.	8	–		
Proteus vulgaris	7	2 (28.6)		
Enterobacter aerogenes	3			
Salmonella sp.	2			
Shigella spp.	2			
Proteus penneri	1			
Total	1,586	207 (13.1)	55	50/5

[a] ESC, extended-spectrum cephalosporin.

Confirmatory tests for ESBL production were performed with all of the isolates exhibiting reduced susceptibility to expanded-

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TABLE 2 Primers used in this study

Name	Sequence (5′→3′)	Reference
CTX-M-group-1F	GTTACAATGTGTGAGAAGCAG	17
CTX-M-group-1R	AACGGAATGAGTTTCCCCATT	17
CTX-M-group-2F	ACCAGGCTCAATTGTGGA	This study
CTX-M-group-2R	AGATGAGGGTTCGTTGCAA	This study
CTX-M-group-8F	CACGGATTCAATTTTCAGGAG	3
CTX-M-group-8R	GAGCGCTCCACATTTTTTAG	3
CTX-M-group-9F	GTTACAATGTGTGAGAAGCAG	17
CTX-M-group-9R	CAGCCAGAAAGTTATGGAG	This study
CTX-M-group-25F	GGATGATGAGAAAAAGCGTAAGGC	This study
CTX-M-group-25R	GGACTAATAACCGTCGGTGAC	This study

spectrum cephalosporins collected during the first week of the study ($n = 55$). This sample was considered to be representative of the whole study period, since the relative frequencies of the most prevalent species were similar during the whole month of study (Table 1). The molecular epidemiology of ESBL determinants was investigated in all confirmed ESBL-producing isolates ($n = 50$). The remaining 5 isolates were high-level AmpC producers (Table 1). Molecular detection of ESBL genes was conducted by PCR amplification using alkaline lysis-extracted total genomic DNA as the template and the primers listed in Table 2. Amplicons were sequenced in both strands using an ABI Prism 3700 DNA sequencer.

Of the 50 ESBL producers, 47 were found to carry CTX-M-type determinants (94%) and the simultaneous presence of two different $bla_{\text{CTX-M}}$ determinants have been observed in 2 of them. Among the CTX-M producers, CTX-M-2 group determinants were found in 26 isolates (55%; 25 CTX-M-2 and 1 CTX-M-56), CTX-M-1 group determinants in 19 isolates (40%; all CTX-M-15), CTX-M-9 group determinants in 3 isolates (6%; all CTX-M-14), and CTX-M-8 group determinants in 1 isolate (2%; CTX-M-8) (Table 3).

Although CTX-M enzymes remain the most prevalent ESBL determinants, the dominance of CTX-M-2 reported previously (21) was diluted by the emergence and remarkable spread of CTX-M-15 and, to a lesser extent, by the emergence of other CTX-M groups. The emergence of CTX-M-15 was observed in both *Escherichia coli* and *Klebsiella* spp.

TABLE 3 CTX-M-producing enterobacteria collected during a 1-week study[a] in 15 hospitals distributed in different regions of Argentina

Species (no. of isolates)	ESBL determinant(s) (no. of isolates)
Klebsiella pneumoniae (21)	$bla_{\text{CTX-M-15}}$ (10) $bla_{\text{CTX-M-2}}$ (9) $bla_{\text{CTX-M-2}}$ + $bla_{\text{CTX-M-15}}$ (1) $bla_{\text{CTX-M-8}}$ (1)
Escherichia coli (13)	$bla_{\text{CTX-M-15}}$ (7) $bla_{\text{CTX-M-14}}$ (3) $bla_{\text{CTX-M-2}}$ (3)
Proteus mirabilis (5)	$bla_{\text{CTX-M-2}}$ (4) $bla_{\text{CTX-M-56}}$ (1)
Klebsiella oxytoca (4)	$bla_{\text{CTX-M-2}}$ (3) $bla_{\text{CTX-M-2}}$ + $bla_{\text{CTX-M-15}}$ (1)
Serratia spp. (3)	$bla_{\text{CTX-M-2}}$ (3)
Providencia spp. (1)	$bla_{\text{CTX-M-2}}$ (1)

[a] October 2010.

TABLE 4 Genotypic characterization of CTX-M-15-producing *E. coli* and *K. pneumoniae* isolates

Species and isolate	City	Hospital[a]	Phylogenetic group	Clone	Genetic context of $bla_{\text{CTX-M-15}}$[b]
E. coli					
CM2	Buenos Aires	H6	B2	Ec1	II
L4	Buenos Aires	H3	B2	Ec2	I
M1	Buenos Aires	H7	A	Ec3	II
SM4	Buenos Aires	H8	A	Ec4	II
SM5	Buenos Aires	H8	A	Ec5	I
T1	Chubut	H13	B2	Ec2	I
T3	Chubut	H13	B2	Ec6	I
K. pneumoniae					
B4	Buenos Aires	H4	ND[c]	Kp2	I
CL1	Buenos Aires	H1	ND	Kp3	I
CL4	Buenos Aires	H1	ND	Kp1	I
CL6	Buenos Aires	H1	ND	Kp7	II
CL9	Buenos Aires	H1	ND	Kp4	I
CM4	Buenos Aires	H6	ND	Kp1	I
CV1	Buenos Aires	H7	ND	Kp5	II
I3	Santa Fe	H5	ND	Kp1	I
I4	Santa Fe	H5	ND	Kp1	I
L5	Buenos Aires	H3	ND	Kp6	I
T8	Chubut	H13	ND	Kp8	I

[a] H1, Hospital de Clínicas, Universidad de Buenos Aires; H3, Hospital Alemán, Ciudad Autónoma de Buenos Aires (CABA); H4, Hospital Británico, CABA; H5, Hospital Iturraspe, Santa Fe; H6, CEMIC, CABA; H7, Sanatorio Mater Dei, CABA; H8, Hospital Eva Perón, Buenos Aires; H13, Hospital de Trelew, Chubut.
[b] I, international $bla_{\text{CTX-M-15}}$ genetic environment (GenBank accession no. NC013121.1; II, truncated IS*Ecp1*-$bla_{\text{CTX-M-15}}$ genetic environment (GenBank accession no. HQ157353) (11).
[c] ND, not determined.

The genetic environments surrounding the most prevalent CTX-M determinants, $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-15}}$, were investigated by PCR mapping and sequencing. The $bla_{\text{CTX-M-2}}$ gene was always located downstream of an IS*CR1* element, as previously described (1, 13). Different genetic environments were found surrounding $bla_{\text{CTX-M-15}}$: in 13 isolates, it was associated with a complete IS*Ecp1* located 48 bp upstream of $bla_{\text{CTX-M-15}}$, in agreement with the worldwide genetic context named "the international $bla_{\text{CTX-M-15}}$ genetic environment" (GenBank accession no. NC013121.1); in 5 isolates, $bla_{\text{CTX-M-15}}$ was associated with a truncated IS*Ecp1* (still conserving a complete promoter), as recently described in the United Kingdom (GenBank accession no. HQ157353) (11) (Table 4).

To investigate the dissemination of CTX-M-15, we performed a genotype analysis of the isolates producing this CTX-M variant (7 *E. coli* and 11 *K. pneumoniae* isolates). Genotyping was performed by determination of the four main *E. coli* phylogenetic groups (7) and by PCR-based fingerprinting using random amplification of polymorphic DNA (RAPD) with the 1290 decamer (19) and repetitive extragenic palindromic PCR (REP-PCR) (16). (Isolates were assigned to a same clone when identical band profiles were obtained with the two PCR-based fingerprinting methods.) Clonal heterogeneity was observed among both *E. coli* and *K. pneumoniae* isolates (Table 4). All of the CTX-M-15-producing *E. coli* isolates belonging to phylogenetic group B2 ($n = 4$) were identified as ST131 by the PCR-based method proposed by Clermont et al. (8) and confirmed by multilocus sequence typing (MLST) [http://mlst.ucc.ie/mlst/dbs/Ecoli/documents/primers

Coli_html] with two *E. coli* isolates (L4 and CM2). Moreover, MLST analysis of the CTX-M-15-producing *K. pneumoniae* isolates (12) assigned the most prevalent clone (Kp1, including 4 isolates circulating in both Buenos Aires and Santa Fe) to sequence type 11 (ST11) (Table 4).

Nowadays, it is worth noting that although some of the CTX-M enzymes have been associated with specific countries, such as CTX-M-9 and CTX-M-14 in Spain (14, 18), CTX-M-1 in Italy (4), and CTX-M-2 in Israel, Japan, and most South American countries (6, 21), others, such as CTX-M-15, have been detected worldwide (2, 4, 15, 20). The present data indicate that the cosmopolitan CTX-M-15 ESBL is becoming widespread also in Argentina and is often associated with clones distributed worldwide, such as *E. coli* ST131 and *K. pneumoniae* ST11 (24), further underscoring the dissemination potential of this enzyme. The new epidemiological scenario may have followed an allodemic rather than an epidemic pattern, reflecting the dissemination of both multiple clones and/or several mobile genetic elements.

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