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Dissemination of CTX-M-15 β-Lactamase Genes Carried on Inc FI and FII Plasmids among Clinical Isolates of *Escherichia coli* in a University Hospital in Istanbul, Turkey▽

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The CTX-M-1 group was found in 86.8% of the *Escherichia coli* isolates from Istanbul. A subset study revealed all isolates carrying $bla_{CTX-M-15}$ genes flanked by the insertion element IS*Ecp1*. Plasmid typing of transconjugates carrying $bla_{CTX-M-15}$ showed that most isolates belonged to the Inc/rep FII group but that one isolate also belonged to the FI group.

CTX-M-type enzymes were reported in Germany and Argentina in 1989, and so far, more than 67 CTX-M-type β-lactamases have been identified, mostly in *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica* serovar Typhimurium isolates (http://www.lahey.org). The global spread is now a major concern in certain areas such as Latin America, Asia, Europe, Africa, and North America (5).

Data on the prevalence and distribution of CTX-M-type enzymes are very limited in Turkey. Two studies revealed that CTX-M enzymes were present and disseminated among *Enterobacteriaceae* family isolates in Turkey (1, 10). The aim of this study was to determine the current prevalence of the CTX-M-1 group and the molecular characteristics in *E. coli* clinical isolates in a university hospital in Istanbul, Turkey.

(This work was presented in part as a poster at the "Microbes in a Changing World" Congress of the International Union of Microbiological Societies (IUMS), San Francisco, CA, 23 to 28 July 2005.)

A total of 1,010 consecutive nonrepetitive isolates of *E. coli* obtained from inpatients and outpatients at the hospital of Istanbul Faculty of Medicine over a 2-year period (2002 to 2004) were screened for extended-spectrum β-lactamase (ESBL) production, using the double-disc synergy test. A total of 61 *E. coli* isolates (27 from inpatients and 34 from outpatients) with ESBL phenotypes were included in this study, the majority of which were from the urine and respiratory specimens of patients treated in six different medical and surgical wards.

MICs were determined by the agar dilution method. MICs for cefotaxime, ceftriaxone, and ceftazidime used alone and in combination were determined, with 4 μg/ml clavulanic acid for phenotypic detection of ESBLs.

Conjugation experiments for the transfer of cefotaxime resistance were carried out with all CTX-M-1-producing *E. coli* isolates (8). Crude extracts of β-lactamases were subjected to isoelectric focusing (IEF) (3).

All isolates were screened for the CTX-M-1 group (13) and bla_{TEM} (16) and bla_{SHV} (9). Strains which had pI bands at 7.3 were subjected to bla_{OXA-1}-like PCR using the primers OXA-1-A (5′-GAATCGCATTATCACTTATGG-3′) and OXA-1-B (5′-GATACATGTTCTCTATGG-3′; this study). PCR amplicons for linking IS*Ecp1* with bla_{CTX-M} were obtained by anchoring one primer at the 3′ end of bla_{CTX-M} (bla_{CTX-M} reverse, 5′CACTTTGTCGTCTAAGGCG3′) and the other to the 5′ end of IS*Ecp1* (5′AATACTACCTTGGCTTTCTGA3′).

Sequencing was carried out with both strands by using the dideoxy chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer. Sequence analysis was performed using a Lasergene DNASTAR software package. Sequence alignments were done using Clustal W software and a PAM 250 matrix.

After isoelectric focusing was carried out, a bioassay was assigned to visualize and confirm the hydrolytic activity of the enzymes detected by the isoelectric focusing (4).

Randomly amplified polymorphic DNA (RAPD) analyses were performed using an ERIC2 primer (7).

A subset of *E. coli* isolates had their plasmids fully characterized. Plasmids were isolated by the alkaline lysis method (12) and used to transform *E. coli* TOPO cells (Invitrogen, Paisley, United Kingdom) via electroporation. Transformants were checked by PCR for carriage of the CTX-M type 1 gene. Plasmids were restricted using EcoR1, their sizes were assessed (2), and they were typed according to the method described by Carattoli et al. (6). Initially, multiplex PCR was used and then refined, with single PCR used to obtain clear amplicons for sequencing.

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TABLE 1. MICs, isoelectric points, PCR results, and sequence and plasmid analyses for ESBL-positive *E. coli* isolates

Isolate	MIC (μg/ml) (drug sensitivity)[a]		pI value			PCR or sequencing result[b]				RAPD PCR result[c]	Result of conjugation of CTX-M-15[d]	$bla_{CTX\text{-}M\text{-}15}$ Inc type[e]
	CTX	CAZ				bla_{TEM}	bla_{SHV}	$bla_{CTX\text{-}M\text{-}15}$	OXA-1-like			
1	128 (R)	16 (I)			8.6	−	−	+		a	+	FI
2	16 (I)	4 (S)	5.6		8.4	+	−	+		b	−	
3	128 (R)	64 (R)	5.6		8.5	+	−	−		b		
4	128 (R)	64 (R)	5.6		8.4	+	−	+		c	+	FII
5	64 (R)	8 (S)			8.4	−	−	+		a	+	ND
6	32 (I)	8 (S)	5.6		8.4	+	−	+		d	+	FII
7	128 (R)	64 (R)	5.4		8.4	+	−	+			−	
8	32 (I)	4 (S)		7.4	8.4	−	−	+		e	+	ND
9	128 (R)	64 (R)	5.4	7.3	8.4	+	−	+	+	c	+	FII
10	256 (R)	64 (R)	5.4	7.3	8.4	+	−	+	+	f	+	FII
11	128 (R)	64 (R)	5.4	7.3	8.6	+	−	+	+	b	+	FII
12	128 (R)	16 (I)	5.4	7.2	8	+	−	+		a	−	
13	128 (R)	64 (R)	5.4	7.3	8.4	+	−	+	+	g	+	FII
14	128 (R)	64 (R)	5.4	7.3	8.4	+	−	+	+	g	+	FII
15	32 (I)	64 (R)	5.4	7.3	8.6	+	−	+	+	b	+	FII
16	256 (R)	64 (R)		7.3	8.6	−	−	+	+	a	−	
17	512 (R)	256 (R)	6.2	7.3	8.4	−	−	+	+	h	−	
18	128 (R)	128 (R)	5.4		8.6	+	−	+		i	+	FII
19	256 (R)	64 (R)	5.4		8.6	+	−	+		i	+	FII
20	32 (I)	8 (S)	5.4		8.4	+	−	+		b	−	
21	64 (R)	128 (R)		7.3	8.4	−	+	+	+	j	−	
22	32 (I)	4 (S)	5.4		8.4	+	−	+		k	+	ND
23	256 (R)	64 (R)	5.4	7.3	8.6	+	−	+	+	g	+	FII
24	128 (R)	32 (R)	5.4	7.3	8.6	+	−	+	+	g	+	FII
25	128 (R)	32 (R)	5.6	7.3	8.6	+	−	+	+	g	−	
26	256 (R)	128 (R)	5.6	7.3	8.6	+	−	+	+	g	+	FII
27	256 (R)	64 (R)	5.6		8.4	+	−	+		b	+	ND
28	256 (R)	64 (R)			8.6	−	−	+		a	−	
29	512 (R)	128 (R)	5.6	7.3	8.4	+	−	+	+	e	+	ND
30	128 (R)	32 (R)	5.6	7.4	8.4	+	−	+		l	+	ND
31	256 (R)	32 (R)		7.3	8.6	−	−	+	+	l	+	FII
32	256 (R)	32 (R)	5.4	7.3	8.6	+	−	+	+	m	+	FII
33	8 (S)	2 (S)	5.4	8.4	8.9	+	−	+		e	−	
34	512 (R)	128 (R)	5.4	7.3	8.9	+	−	+	+	b	+	ND
35	128 (R)	8 (S)	5.4	7.2	8.9	+	+	+		a	−	
36	64 (R)	64 (R)	5.4		8.4	+	−	+		l	−	
37	256 (R)	8 (S)	5.4		8.4	+	−	+		b	−	
38	128 (R)	64 (R)			8.9	−	−	+		k	+	ND
39	64 (R)	256 (R)	5.4		8.4	+	−	+		l	+	FII
40	>512 (R)	256 (R)		7.3	8.9	−	−	+	+	a	−	
41	32 (I)	8 (S)			8.4	−	−	+		a	−	
42	128 (R)	64 (R)		7.3	7.6	−	−	+	+	l	−	
43	128 (R)	256 (R)	5.2			+	−	−		g		
44	16 (I)	4 (S)	5.4		8.4	+	−	+		l	−	
45	256 (R)	128 (R)	5.2	7.3		+	−	−	+	l		
46	64 (R)	8 (S)		7.3	8	−	−	−	+	b		
47	128 (R)	128 (R)		7.3	7.6	−	+	+	+	l	+	ND
48	2 (S)	16 (I)		7		−	+	−		b		
49	256 (R)	64 (R)	5.2			+	−	−		l		
50	128 (R)	256 (R)	5.4		8.6	+	−	+		l	+	FII
51	128 (R)	32 (R)	5.4	7.3	7.6	+	+	+	+	l		
52	32 (I)	16 (I)	5.4		8.4	+	−	+		b	+	ND
53	32 (I)	64 (R)	6.4	6.8	7	−	+	−		b		
54	256 (R)	256 (R)	IB[f]	IB	IB	+	−	+		l	−	
55	32 (I)	16 (I)	IB	IB	IB	+	−	+		k	−	
56	>512 (R)	512 (R)			8	+	−	+		l	+	ND
57	256 (R)	>512 (R)	5.4	7.2	8.4	+	−	−		b		
58	256 (R)	128 (R)	5.4	7.3	8.9	+	+	+	−		−	
59	256 (R)	128 (R)	5.4		8.9	+	−	+		b	−	
60	512 (R)	128 (R)		7.3	8.9	−	+	+	+	a	−	
61	256 (R)	64 (R)	5.4	7.3	8.9	+	+	+	+	l	−	

[a] CTX, cefotaxime; CAZ, ceftazidime; I, insensitivity; R, resistance; S, sensitivity.
[b] Sequencing was carried out with $bla_{CTX\text{-}M}$ amplicons only to confirm the identification as $bla_{CTX\text{-}M\text{-}15}$.
[c] Lowercase letters represent the different clones of *E. coli* isolates.
[d] Where there is no plus or minus given, the $bla_{CTX\text{-}M\text{-}15}$ gene has not been detected.
[e] Plasmid typing was carried out with transconjugates from selected isolates based on RAPD typing and phenotypic characteristics. ND, indicates where $bla_{CTX\text{-}M\text{-}15}$ genes were detected from transconjugates but the plasmids remained uncharacterized.
[f] IB, indefinite bands.

Sixty-one (6%) out of the 1,010 *E. coli* isolates were found to be positive for the presence of ESBLs, in our study. There was a high rate of resistance against ciprofloxacin (80.4%). The most active antibiotics for ESBL-producing *E. coli* were amikacin (91.9%) and imipenem (100%).

The pI values of the β-lactamases and the PCR results for ESBL-encoding genes are shown in Table 1. All of the CTX-M-1-group-positive *E. coli* isolates were confirmed to possess cefotaxime-hydrolyzing activity in the subsequent bioassay experiments, corroborating the pI values with cefotaximase activity. Positive amplicons were sequenced, and all were shown to be $bla_{\text{CTX-M-15}}$. As $bla_{\text{CTX-M-15}}$ is ubiquitously associated with IS*Ecp1* (13, 14), the genetic context was sought by using the PCR strategy described previously. In all CTX-M-15-positive isolates, $bla_{\text{CTX-M-15}}$ was found directly adjacent to the IS*Ecp1*.

The results of RAPD analyses indicated that there was no evidence for the spread of a single bacterial clone responsible for the high prevalence of CTX-M-15 isolates. Conjugation experiments were performed with all 53 CTX-M-1 group ESBL-producing *E. coli* strains, to examine the rate of transfer. Transconjugants were obtained for 29 isolates (Table 1). The high conjugation efficiency of about 10^{-3} to 10^{-4} recombinants per donor cell (*E. coli* K-12 W3110 Rif^{r} Lac) was observed, and all transconjugants expressed the ESBL-related resistance phenotypes.

Plasmid analysis indicated that in all cases, the strains possessed more than one plasmid, with some strains possessing up to five. The presence of the $bla_{\text{CTX-M-15}}$ genes in cefotaxime-resistant transconjugates were confirmed by PCR and sequencing. Once transconjugates were confirmed, they were randomly selected, and plasmids were isolated as described previously. Multiplex PCR was undertaken to determine the plasmid Inc/rep type, followed by simplex PCR as previously described (6, 11). In one isolate (Table 1, isolate 1), the $bla_{\text{CTX-M-15}}$ gene was carried on a plasmid of approximately 65 kb and possessed an F1 Inc/rep type. The other plasmids ($n = 17$) that were typed possessed a large plasmid of approximately 140 kb, which possessed an Inc/rep type of FII (Table 1).

Data show that the isolation of the CTX-M-1-group-positive *E. coli* accounted for 86.8% of the ESBL-positive *E. coli* isolates. The abundance of different phenotypes and the presence of multiple different enzymes in each strain indicate that the epidemiology of ESBLs in our hospital is complex. The high clinical incidence of $bla_{\text{CTX-M-15}}$ since 2004 suggests that the gene pool was well established prior to this date. Interestingly, the transconjugates demonstrating $bla_{\text{CTX-M-15}}$ carriage were predominantly Inc/rep type FII but also type FI, and while the FII and $bla_{\text{CTX-M-15}}$ relationship is well established, this is not the case with FI (11, 15).

The high prevalence of the CTX-M-15 group underlines the need for further surveillance and further investigation for better understanding of the epidemiology and the genetic background of this specific resistance trend, but the present results demonstrate that CTX-M-15 has emerged and spread in a very short period in our hospital.

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