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First Extended-Spectrum-β-Lactamase (CTX-M-15)-Producing *Salmonella enterica* Serotype Typhimurium Isolate Identified in Lebanon

In *Salmonella*, most extended-spectrum β-lactamases (ESBLs) are derivatives of TEM and SHV β-lactamase families, although other groups, including PER and CTX-M, have been described recently (1, 2). CTX-M-type ESBLs display levels of resistance to cefotaxime and ceftriaxone significantly higher than those to ceftazidime (2). Since 1995, CTX-M-type ESBLs have disseminated dramatically in several parts of the world (1).

In January 2004, a *Salmonella enterica* serotype Typhimurium isolate, CAM18, was recovered from a stool specimen collected from a 6-year-old child upon admission to a hospital in Northern Lebanon. The child had no history of travel. The isolate had a high resistance level to cefotaxime and ceftazidime and was also resistant to several aminoglycosides, sulfamethoxazole-trimethoprim, and tetracycline. It was susceptible to cefoxitin, imipenem, quinolones, and chloramphenicol. ESBL production was detected by the double-disk synergy test (6).

The ESBL phenotype was transferred to *Escherichia coli* K-12 resistant to nalidixic acid with an efficiency of 10^{-2} per donors. Certain transconjugants also acquired resistance to tetracycline and kanamycin (TC-pCAM18-1), while others acquired resistance to tetracycline alone (TC-pCAM18-2). The addition of clavulanic acid reduced the MICs of all β-lactams tested (Table 1).

Results of isoelectric focusing (8) and of amplifications using TEM, SHV, CTX-M, and OXA-1 primers (3, 10, 11) are shown in Table 1. The β-lactamase with a pI of 5.4 was most probably TEM-1. Sequencing of $bla_{\text{OXA-1}}$ amplicons revealed the presence of OXA-30, a β-lactamase with a pI of 7.3 differing from OXA-1 by one amino acid and recently reported in *E. coli* (4) and *S. enterica* serotype Typhimurium (5). Sequencing of $bla_{\text{CTX-M}}$ amplicons revealed that coding regions were 100% identical to the coding region of the $bla_{\text{CTX-M-15}}$ gene (GenBank accession number AY044436). Amplification and sequencing of the entire coding sequence of $bla_{\text{CTX-M-15}}$ (14) confirmed that the β-lactamase with a pI of 8.6 was CTX-M-15, an enzyme that has been previously identified by several researchers (for a recent review, see reference 1). Unlike most CTX-M enzymes, CTX-M-15, an Asp-240-Gly variant of CTX-M-3, increased the catalytic efficiency against ceftazidime (12), as observed with MIC results (Table 1).

The presence of IS*Ecp1*, a mobile sequence located upstream of several CTX-M genes, was investigated by a PCR assay as previously described (13). Sequencing of PCR products obtained with isolate CAM18 and transconjugants revealed that the IS*Ecp1* element was located in the same position as that found in Indian and Turkish isolates (7, 9). Moreover, the 160-bp sequence upstream of $bla_{\text{CTX-M-15}}$ was 100% identical to the corresponding sequences of Indian (GenBank accession number AY044436) and Canadian (accession number AY458016) isolates.

Kanamycin resistance was associated only with strain TC-pCAM18-1, while tetracycline resistance was exhibited by both transconjugants. This result in addition to plasmid purification revealed that bla_{TEM} and kanamycin resistance genes reside on a plasmid of 20 kb and $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-30}}$, and tetracycline resistance genes reside on a larger plasmid of 60 kb.

We report for the first time the isolation of a CTX-M-type ESBL in Lebanon. In addition, this is also the first report of an ESBL-producing *Salmonella* in Lebanon. A study done in 2003 at St. George hospital (Beirut, Lebanon) on 49 *Salmonella* strains showed 100% susceptibility to ceftazidime and cefotaxime (Z. Daoud, personal communication). The appearance of the CTX-M-15-producing *S. enterica* serotype Typhimurium in the community in 2004 is a serious threat to public health.

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TABLE 1. Characteristics of *S. enterica* serotype Typhimurium CAM18, *E. coli* K-12, and two transconjugants

Strain	MIC (μg/ml)[a]				β-Lactamase		Plasmid size (kb)	Resistance[b]	
	CT/CTL	TZ/TZL	PM/PML	TX	pI value[c]	*bla* gene		Tc	K
Salmonella serotype Typhimurium CAM18	>256/0.19	128/0.75	>256/0.064	>256	5.4	bla_{TEM}	~20	R	R
					7.3	$bla_{\text{OXA-30}}$	~60		
					8.6	$bla_{\text{CTX-M-15}}$			
E. coli									
TC-pCAM18-1[d]	>256/0.064	32/0.75	128/<0.064	>256	5.4	bla_{TEM}	~20	R	R
					7.3	$bla_{\text{OXA-30}}$	~60		
					8.6	$bla_{\text{CTX-M-15}}$			
TC-pCAM18-2	>256/0.064	32/0.75	128/<0.064	>256	**7.3**	$bla_{\text{OXA-30}}$	~60	R	S
					8.6	$bla_{\text{CTX-M-15}}$			
K-12	0.19/0.19	0.19/0.19	0.047/<0.064	0.047				S	S

[a] Abbreviations: CT, cefotaxime; CTL, cefotaxime-clavulanate; TZ, ceftazidime; TZL, ceftazidime-clavulanate; PM, cefepime; PML, cefepime-clavulanate; TX, ceftriaxone.
[b] Abbreviations: Tc, tetracycline; K, kanamycin; R, resistant; S, susceptible.
[c] pI values in bold type indicate the β-lactamases that were positive in the bioassay with cefotaxime as a substrate.
[d] Transconjugants indicated by TC prefix in the strain designation.

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