

Dissemination of the CTX-M-25 family β -lactamases among *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* and identification of the novel enzyme CTX-M-41 in *Proteus mirabilis* in Israel

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Objectives: The CTX-M-25 family of β -lactamases is a closely related family of enzymes found rarely in the world. We aimed to describe the occurrence and to understand the dissemination of this extended-spectrum β -lactamase family among Enterobacteriaceae strains in our hospital.

Methods: Fifty-four CTX-M-producing Enterobacteriaceae strains collected from 2000 to 2005 were screened for *bla*_{CTX-M-25} genes by PCR and sequencing. Genetic relatedness was analysed by PFGE. Antibiotic susceptibilities were determined by VITEK-2. Plasmids encoding *bla*_{CTX-M-25}-type genes were isolated, transformed and analysed by Southern blot using a *bla*_{CTX-M-25} probe. Chromosomal location of *bla*_{CTX-M-25}-type was studied by *I-CeuI* restriction analysis. The *bla*_{CTX-M-25} genetic environment was characterized by PCR mapping and partial sequencing.

Results: Ten out of 54 CTX-M-producing isolates (18.5%) carried *bla*_{CTX-M-25} genes, including *Klebsiella pneumoniae* ($n = 4$), *Escherichia coli* ($n = 3$), *Enterobacter cloacae* ($n = 1$) and *Proteus mirabilis* ($n = 2$). Isolates were genetically unrelated. Four β -lactamases were found: CTX-M-25, CTX-M-26, CTX-M-39 and CTX-M-41, a new member of the family (accession no. DQ023162) that differed from CTX-M-25 in three amino acids, Ala80Val, Val106Ile and Ile126Ser. *bla*_{CTX-M-25}-type genes were plasmid-mediated in all genera but *P. mirabilis*, organized in a class I integron and located downstream of an *ISEcp1* element. The genes were encoded on different plasmids with varying degree of similarities. Several antibiotic-resistant determinants conferring resistance to trimethoprim and aminoglycosides existed on the same integron.

Conclusions: *bla*_{CTX-M-25} exists in Israel in different enteric species. Spread of these enzymes within and between species is due to transfer of plasmids with common regions and by dissemination of determinants encoding these genes. CTX-M-41, a novel member of this family, was identified in the chromosome of *P. mirabilis*.

Keywords: plasmids, spread, integrons

Introduction

CTX-M-type extended-spectrum β -lactamases (ESBLs) are currently the most widespread ESBL family worldwide.^{1–3} These β -lactamases have been classified into five phylogenetic families on the basis of their amino acid identities: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25.³ The CTX-M-25 family includes CTX-M-25, which was reported in a single *Escherichia coli* strain from Canada in 2000, and CTX-M-26, which was

described in a *Klebsiella pneumoniae* outbreak strain from the UK in 2002.⁴ These two enzymes share 99% amino acid identity and differ in only three amino acids. The genes encoding these ESBLs were identified on large plasmids and were located downstream of *ISEcp1*, the most common insertion sequence of the CTX-M-type enzymes.^{1,2}

In a previous study performed in our institution, a 1200-bed tertiary-care teaching hospital, comprising 45 wards, with almost 100 000 admissions annually, we found 80 ESBL-producing

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isolates of 400 unique-patient isolates screened.⁵ Fifty-four of these isolates carried *bla*_{CTX-M}-type genes (67.5%): 44 isolates carried *bla*_{CTX-M-2}-type genes and 10 isolates carried *bla*_{CTX-M-25}-type genes. Sequencing of these *bla*_{CTX-M-25}-type genes revealed a new CTX-M-25-related enzyme, CTX-M-39, in *Enterobacter* and in *E. coli*.^{6,7} CTX-M-39 revealed 99% homology with CTX-M-26, with a substitution of arginine for glutamine at position 225. The goals of this study were to elucidate the elevated occurrence of these rarely occurring CTX-M-25 family enzymes in various clinical isolates of Enterobacteriaceae, including *K. pneumoniae*, *E. coli*, *Enterobacter cloacae* and *Proteus mirabilis*, and to understand the mechanism of dissemination of this ESBL family among these bacteria. The possibilities of clonal spread and horizontal transfer of plasmid carrying the *bla*_{CTX-M-25}-related gene or transfer of the intact gene were examined.

Materials and methods

Bacterial strains

Of 54 isolates belonging to the Enterobacteriaceae group collected at random in our institution in 2000–05, which were found to carry a *bla*_{CTX-M-25}-related gene, 10 were included in this study.⁵ The isolates belonged to the following species: *K. pneumoniae* (*n* = 4), *E. coli* (*n* = 3), *Enterobacter* spp. (*n* = 1) and *P. mirabilis* (*n* = 2). Bacterial identification was performed by the VITEK-2 (bioMérieux, Hazelwood, MO, USA). Susceptibility testing was performed using the VITEK-2 AST GN09 card (bioMérieux) and using Etest (AB Biodisk, Solna, Sweden). An ESBL-producing phenotype was determined based on the ESBL confirmatory disc diffusion assay recommended in the CLSI guidelines,⁸ using the clavulanic acid combination disc method (Oxoid, Hampshire, England, UK) with both cefotaxime and ceftazidime.⁹

Pulsed-field gel electrophoresis

Clonal relatedness within each species was determined by PFGE. DNA preparation and cleavage was performed using 20 U of *SpeI* endonuclease (New England Biolabs, Beverly, MA, USA) for *E. coli*, *K. pneumoniae* and *E. cloacae* isolates, and using *SmaI* (New England Biolabs) for *P. mirabilis* strains. Electrophoresis was performed in a 1% agarose gel (BMA Products, Rockland, ME, USA) prepared and run in 0.5× Tris/borate/EDTA buffer on a CHEF-DR III apparatus (Bio-Rad Laboratories, Ltd, Rishon LeZion, Israel). For *SpeI* digests, the initial switch time was 3 s, the final switch time was 20 s and the run time was 23 h at 6 V/cm at 14°C. For *SmaI* digests, the initial switch time was 5 s, the final switch time was 20 s and the run time was 24 h. Gels were stained in ethidium bromide, de-stained in distilled water and photographed using a Bio-Rad GelDoc 2000 camera (Bio-Rad Laboratories). PFGE DNA patterns were compared between isolates belonging to the same genera.

Detection of *bla*_{CTX-M}, *bla*_{CTX-M-25}-family and other β-lactamase genes by PCR, cloning and sequencing

The presence of *bla*_{CTX-M} and, specifically, of *bla*_{CTX-M-25}-family ESBLs in all the studied isolates was determined by PCR and sequencing. The specific primers that were used for different *bla*_{CTX-M}-type gene detection are summarized in Table 1.

Table 1. Sequences of the primers used to detect *bla*_{CTX-M} genes and their genetic environment

Primer name	Sequence	Reference
<i>bla</i> _{CTX-M-2}	F: ATGATGACTCAGAGCATTTCG R: TTATTGCATCAGAAACCGTG	10
<i>bla</i> _{CTX-M-9}	F: GTGACAAAGAGAGTGCAACGG R: ATGATTCTCGCCGCTGAAGCC	11
<i>bla</i> _{CTX-M-10}	F: GCAGACCAGTAAAGTGATGG R: GCGATATCGTTGGTGGTACC	12,13
<i>bla</i> _{CTX-M-8}	F: ATGATGAGACATCGCGTTAAG R: CGGTGACGATTTTCGCGGCAG	6
<i>bla</i> _{CTX-M-25}	F: CACACGAATTGAATGTTTCAG R: TCACTCCACATGGTGAGT	6
<i>ISEcpI</i>	F: AAAAATGATTGAAAGGTGGT	14
<i>sulI</i> R	R: GCTCAAGAAAAATCCCATCCCC	14
<i>IntI</i> D	F: GCCAACTTTCAGCACATG	15
<i>IntI</i> U	R: GTTCGGTCAAGGTTCTG	15
<i>qacEΔIF</i>	F: ATCGCAATAGTTGGCGAAGT	15
5'CS	F: GGCATCCAAGCAGCAAG	16
3'CS	R: AAGCAGACTTGACCTGA	16

F, forward; R, reverse.

The presence of other β-lactamase genes was determined using previously described primers.^{6,16} Bacterial cell lysates were used as DNA templates, and PCR conditions were as described previously.⁶ PCR products were cloned into pGEM-T Easy Vector System and transformed into *E. coli* JM109 (Promega), after which inserts were bi-directionally sequenced, analysed and compared via the National Center for Biotechnology Information (NCBI) web site. Isolates that carried *bla*_{CTX-M-25}-type ESBLs were chosen for further characterization.

Conjugation experiments

Transconjugation experiments were performed by filter mating with five strains representing each of the CTX-M-producing genera (*E. cloacae* 1018, *K. pneumoniae* 1016, *E. coli* 1430 and two *P. mirabilis* strains 1270 and 1336). *E. coli* HB101 was used as a recipient strain. Transconjugants were selected on Luria-Bertani (LB) agar plates containing streptomycin (500 mg/L) and ceftriaxone (16 mg/L).

CTX-M-25-type plasmid analysis, transformation and Southern blot hybridization

Plasmid DNA from all *bla*_{CTX-M-25}-type carrying isolates was isolated using a NucleoBond PC 100 kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The DNA preparations were electrophoresed on 1% agarose gels. BAC-Tracker supercoiled DNA ladder (Epicentre, Madison, WI, USA) was used as a size marker for *bla*_{CTX-M-25}-type carrying plasmids. Transformation experiments were carried out by electroporation of plasmid DNA to *E. coli* strain GeneHogs (Invitrogen, UK) using an Electroporator 2510 (Eppendorf, Hamburg, Germany). Transformant colonies were selected on LB agar plates containing ampicillin (100 mg/L). For Southern blot analysis, plasmid DNA from donor strains and transformants were digested with *Apal* (does not recognize CTX-M-type genes) and *EcoRV* (recognizes CTX-M-type genes at nucleotide

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744) endonucleases (New England Biolabs), electrophoresed, transferred to a Hybond N⁺ membrane (Amersham Biosciences) and cross-linked with UV light. A *bla*_{CTX-M-25} gene radioactively labelled with random primer DNA-labelling mixture (Biological Industries, Beit Haemek, Israel) was used as a probe.

Location of *bla*_{CTX-M-25}-type genes

Chromosomal location of *bla*_{CTX-M-25}-type genes was confirmed by I-CeuI digestion (New England Biolabs) followed by PFGE electrophoresis and hybridization with radioactive-labelled probes of 16S and *bla*_{CTX-M-25} genes. *P. mirabilis* ATCC strain 25933 lacking *bla*_{CTX-M-25} was used as a control strain. Separation of DNA fragments was performed as described previously.¹⁷ *Saccharomyces cerevisiae* chromosomal DNA (Bio-Rad) was used as a size marker.

Genetic environment of *bla*_{CTX-M} genes

The genetic organization of the *bla*_{CTX-M-25} gene environment was examined in all the *bla*_{CTX-M-25}-carrying isolates by PCRs using specific primers (Table 1) and partial sequencing. PCR mapping analysis was performed on plasmid preparations of transformed strains, except for *E. coli* 1466, whose mapping was performed on plasmid DNA isolated from the clinical strain, and for *P. mirabilis* strains, whose mapping was performed on chromosomal DNA. The insertion sequence forward primer ISEcpl with the *bla*_{CTX-M-25} reverse primer was used to amplify the CTX-M-25 upstream region. Specific primers Int1D and Int1U were used to detect the presence of integrase 1 gene. Primers qacE Δ 1F and sul1R were used for identification of *bla*_{CTX-M-25} genes downstream region. Primers 5'CS and 3'CS were used for identifying the presence of additional resistant determinants in the integron.

Nucleotide sequence accession number

The nucleotide sequence data for CTX-M-41 have been submitted to the GenBank nucleotide sequence database under accession no. DQ023162.

Results

Detection of CTX-M-25 β -lactamase family among CTX-M-producing Enterobacteriaceae

DNA derived from 10 isolates of the 54 CTX-M-producing Enterobacteriaceae isolates was amplified by CTX-M-25 primers (Table 1) and comprised the subject of this study (Table 2). Sequence analysis revealed four different enzymes: CTX-M-25, CTX-M-26, CTX-M-39 reported previously in *E. cloacae* from Israel⁶ and a new member of this family identified in this study in *P. mirabilis* designated CTX-M-41, according to the Lahey clinic nomenclature (<http://www.lahey.org/Studies/>). Sequencing of the novel *bla*_{CTX-M-41} gene revealed three point mutations when compared with the *bla*_{CTX-M-25} gene: C239T, G316A and T377G, resulting in the amino acid substitutions Ala80Val, Val106Ile and Ile126Ser. Analysis of a deduced amino acid sequence of CTX-M-41 using ClustalW through the EMBL-EBI site (<http://www.ebi.ac.uk/>) showed an identity of 99% with both CTX-M-25 (accession no. AF518567)¹⁸ and CTX-M-26 (accession no. AY455830).⁴ Of the four members of the CTX-M-25 family, CTX-M-39 was the most prevalent enzyme (6 of 10 strains).

Table 2. β -Lactamase genes and plasmids identified in the study isolates

Species	Isolate	<i>bla</i> _{CTX-M-25} family	Other <i>bla</i> genes
<i>K. pneumoniae</i>	1032	CTX-M-39	SHV-1 ^a
	1016	CTX-M-39	SHV-1 ^a , TEM-1
	1268	CTX-M-25	SHV-14 ^a
	1320	CTX-M-26	TEM-1, SHV-27 ^a
<i>E. coli</i>	1466	CTX-M-39	TEM-1, CTX-M-2
	1430	CTX-M-39	TEM-1
	1393	CTX-M-39	TEM-1
<i>E. cloacae</i>	1018	CTX-M-39	TEM-1
<i>P. mirabilis</i>	1270	CTX-M-25	OXA-2
	1336	CTX-M-41	ND

ND, not detected.

^a*bla* genes that were not transferred with *bla*_{CTX-M-25}-type genes.

Among all species, *K. pneumoniae* isolates possessed three different variants of this ESBL family (Table 2). In addition to the CTX-M-25 family, two *Klebsiella* isolates (1268 and 1320) and one *E. coli* isolate (1466) also carried other ESBLs such as SHV-14, SHV-27 and CTX-M-2, respectively (Table 2).

Apparent susceptibilities to various cephalosporins and to aztreonam varied between isolates, even among those carrying the same CTX-M enzyme. Most (8 of 10) strains were resistant to trimethoprim/sulfamethoxazole. Co-resistance to quinolones and gentamicin also varied. All isolates were susceptible to imipenem and amikacin, except for *E. coli* 1430, which was amikacin intermediate (Table 3).

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Genotyping. The 10 bacterial strains that carried *bla*_{CTX-M-25} were isolated from 10 patients who were hospitalized in six different wards in the hospital and did not exhibit any apparent epidemiological relation. Seven of the 10 bacterial strains were isolated from wounds, 2 from peritoneal fluid and 1 from blood. The clonal relatedness of isolates belonging to each genus, assessed by PFGE, showed a different DNA pattern, indicating non-clonal transmission of the CTX-M-25-type-producing strains.

Location of the CTX-M-25-family genes, CTX-M-25-family-encoding plasmid analysis and transfer experiments

The location of *bla*_{CTX-M-25} was determined in order to understand the occurrence of this gene family in various clones and in four different species (*Klebsiella*, *E. coli*, *E. cloacae* and *P. mirabilis*).

*bla*_{CTX-M-25}-encoding plasmids were isolated from all isolates, except for the two *P. mirabilis* isolates, suggesting a chromosomal origin of *bla*_{CTX-M-25} and *bla*_{CTX-M-41} in this genus. An attempt to transfer *bla*_{CTX-M-25} by transconjugation of five of the *bla*_{CTX-M-25}-producing strains was unsuccessful. In the case of *Proteus*, I-CeuI endonuclease digestion was used with two *bla*_{CTX-M-25}-type genes carrying *P. mirabilis* isolates and a control strain. The analysis generated fragments ranging from 2.2 Mb to a fragment smaller than 2.25 kb. The rRNA

Table 3. Antibiotic susceptibilities of clinical isolates carrying the *bla*_{CTX-M-25} genes and their transformants (T)

Species	Isolate #	MIC (mg/L)											
		AMP	PIP	TZP	ATM	CRO	CAZ ^a	FEP	IPM	CIP	AMK	GEN ^a	SXT
<i>K. pneumoniae</i>	1032	>32	>128	<4	<2	32	0.125	<8	<1	<0.25	<2	<1	>2/38
	T-1032	>32	>128	<4	2	16	0.125	2	<1	<0.25	<2	<1	>2/38
	1016	>32	>128	<4	>16	8	96	<8	<1	>2	16	>16	>2/38
	T-1016	>32	>128	<4	16	8	64	<1	<1	<0.25	16	>16	>2/38
	1268	>32	>128	<4	4	>32	4	32	<1	<0.25	<2	4	<2/38
	T-1268	>32	>128	<4	16	>64	16	32	<1	<0.25	<2	4	<2/38
	1320	>32	>128	<4	>16	8	256	<8	<1	<0.25	8	1.5	>2/38
	T-1320	>32	>128	<4	16	8	256	<1	<1	<0.25	<2	1.5	>2/38
<i>E. coli</i>	1466 ^c	>32	>128	>64	<2	32	2	<8	<1	<1	16	>16	>2/38
	1430	>32	>128	<4	<1	<4	0.38	<8	<1	<0.25	16	>16	>2/38
	T-1430	>32	>128	<4	<1	4	1.5	<1	<1	<0.25	16	>16	>2/38
	1393	>32	>128	<4	<8	32	1.5	>16	<1	<0.25	<2	0.125	>2/38
	T-1393	>32	>128	<4	2	8	1.5	<1	<1	<0.25	<2	2	>2/38
<i>E. cloacae</i>	1018	32	>128	<4	32	>32	256	>16	<1	>4	<2	128	>2/38
	T-1018	>32	>128	<4	32	16	256	4	<1	<0.25	<2	0.75	>2/38
<i>P. mirabilis</i>	1270 ^b	>32	>128	<4	>16	>32	0.75	>16	<1	>2	12	16	<2/38
	1336 ^b	>32	>128	<4	<2	>32	1.5	>16	<1	>2	8	8	>2/38
Recipient <i>E. coli</i>	GeneHogs [®]	4	<4	<4	<1	<1	<1	<1	<1	<0.25	<2	<1	<2/38

AMK, amikacin; AMP, ampicillin; ATM, aztreonam; CRO, ceftriaxone; CAZ, ceftazidime; CIP, ciprofloxacin; FEP, cefepime; GEN, gentamicin; IPM, imipenem; PIP, piperacillin; TZP, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole.

^aMICs of ceftazidime and gentamicin were tested by Etest.

^bChromosomal location.

^cTransformation failed.

probe hybridized with all fragments of all three strains except fragments 2.2 and 1.6 Mb, whereas the *bla*_{CTX-M-25} probe hybridized only with the 2.2 Mb fragment of *P. mirabilis* isolate 1270 and with 785 kb fragment of isolate 1336 further supporting the chromosomal location of these genes in *P. mirabilis* (data not shown).

Transformation of plasmid DNA carrying *bla*_{CTX-M-25}-type-family genes (verified by PCR and subsequent sequencing) into a susceptible *E. coli* GeneHogs recipient strain was successful in seven isolates (four *K. pneumoniae*, two *E. coli* and one *E. cloacae*). Plasmid DNA containing *bla*_{CTX-M-39} of *E. coli* 1466 was isolated but was non-transformable on repeated transformation attempts and therefore not studied further.

Transformants showed antibiotic susceptibility patterns similar to those of their donors upon acquisition of the plasmids. The MICs of ceftriaxone and ceftazidime were similar among donors and transformants, with the exception of *E. coli* 1393 transformant, in which acquisition of the *bla*_{CTX-M-39} plasmid conferred resistance to ampicillin and piperacillin, and increased the MIC of ceftriaxone from <1 to 8 mg/L and of gentamicin from <1 to 2 mg/L (Table 3).

The size of all *bla*_{CTX-M-25}-type-carrying plasmids was estimated to be ~165 kb (data not shown). Restriction analysis of plasmid DNA from all transformed strains using *Apa*I and *Eco*RV endonucleases showed different restriction profiles with varying degree of similarities (see Figure 1a for *Eco*RV restriction). Southern analysis revealed an identical hybridization profile in two pairs of transformed isolates originating from *K. pneumoniae* strains 1032 and 1320, and from *E. coli* 1430

and *K. pneumoniae* 1016. The other two transformants from *K. pneumoniae* isolates (1268 and 1016) showed a different hybridization pattern. The transformant of *E. cloacae* 1018 showed a unique pattern (Figure 1b).

PCR analysis of transformed plasmids showed that TEM-1 co-transferred with the CTX-25-family gene in all of the isolates, whereas SHV-1, SHV-14 and SHV-27 did not (Table 2).

Genetic environment of the *bla*_{CTX-M-25} family

Since CTX-M-type enzymes are associated with *ISEcp1*-like insertion sequences and class I integrons, the genetic organization of the *bla*_{CTX-M-25} gene's environment was studied in all 10 isolates, including the two *P. mirabilis* strains in which this gene was chromosomally encoded and the *E. coli* isolate 1466 that failed to transform. *bla*_{CTX-M-25} genes in all isolates were located on a class I integron. The integron included *int1*, the gene encoding for integrase 1, *qacEΔ1*, the truncated form of *qacE* gene that confers resistance to quaternary ammonium compounds,¹⁹ *sulI*, which confers resistance to sulphonamides²⁰ and one of the CTX-M-25-family genes. *ISEcp1*, the most common insertion sequence of genes encoding CTX-M enzymes,² was located 126–128 bp upstream of the start codon of the CTX-M-25-type gene. The resistance elements located between 5'CS and 3'CS conserved segments varied among the different isolates (Figure 2). PCR mapping and partial sequencing of this region in seven selected isolates (three *K. pneumoniae* strains, two *E. coli* strains, one *E. cloacae* strain and one *P. mirabilis* strain) revealed the presence of different resistance

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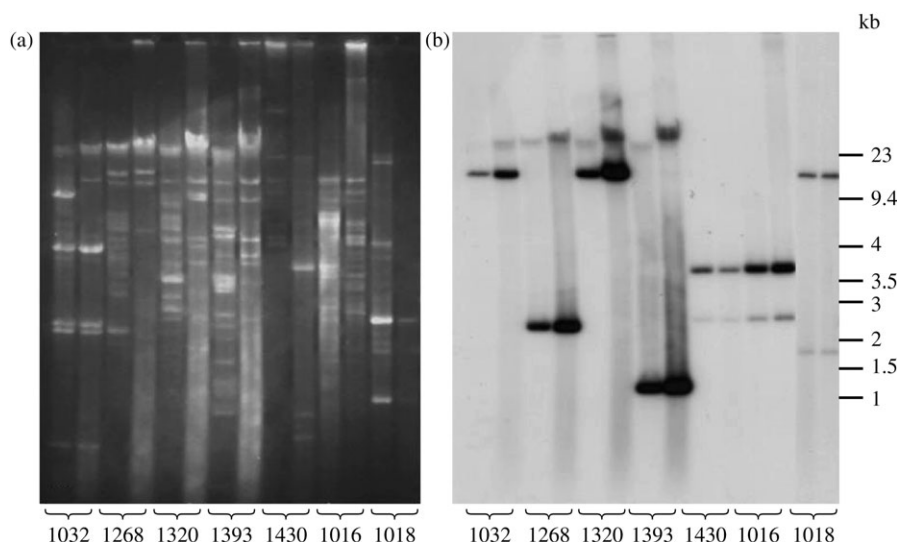


Figure 1. Restriction analysis of plasmid DNA of donors and transformants (marked together in the same brackets), carrying *bla*_{CTX-M-25}-family genes using *EcoRV* endonuclease (a) and the respective Southern blot analysis hybridized with the CTX-M-25 probe (b).

determinants. Dihydrofolate reductase (*dhfr* type VII), which confers resistance to trimethoprim,^{21,22} was the most common resistant determinant present in four strains (*K. pneumoniae* 1032 and 1320, *E. coli* 1393 and *E. cloacae* 1018). Aminoglycoside adenylyltransferase (*aadA1*), which confers resistance to streptomycin and spectinomycin,²³ was found in *K. pneumoniae* 1016 and *E. coli* 1430. The *aac(6')-Ib* gene encoding an aminoglycoside 6'-*N*-acetyltransferase that confers resistance to amikacin, kanamycin and tobramycin²⁴ and the *bla*_{OXA-2} gene were found in the *P. mirabilis* isolate 1270 (Figure 2).

Discussion

The CTX-M group has become the most widespread ESBL family but the CTX-M-25 family, the most recently reported CTX-M subgroup, is still rare in the world. We describe the high occurrence of this β -lactamase family and examine their mode of dissemination between *K. pneumoniae*, *E. coli*, *E. cloacae* and *P. mirabilis*. We describe a new member of the CTX-M-25 family, CTX-M-41, in *P. mirabilis* and we report for the first time the occurrence of this ESBL family in *E. cloacae*. Other CTX-M-type β -lactamases such as CTX-M-1, CTX-M-2 or CTX-M-9 were described previously in these two genera.^{25–29} Phylogenetically, CTX-M-41, found in this study in *P. mirabilis*, is the closest enzyme to CTX-M-25, reported initially in *E. coli*

from Canada. CTX-M-39, found in this study in *K. pneumoniae*, *E. coli* and *E. cloacae*, is closer to CTX-M-26, reported initially in *K. pneumoniae* from the UK.

Of the CTX-M-25 family, CTX-M-39 was the most common β -lactamase, identified in 6 of 10 isolates and in 3 different genera (Table 2). The presence of four different members of the CTX-M-25 family β -lactamases in four different species in our hospital is intriguing and we attempted to reveal their mode of dissemination.

There were no epidemiological relationships between the isolates, and PFGE confirmed that the isolates belonged to different clones within each genus ruling out the possibility of clonal spread. *bla*_{CTX-M-25} genes were found to be encoded on different large plasmids with varied degrees of similarities except in *P. mirabilis* where *bla*_{CTX-M-41} and *bla*_{CTX-M-25} were found to be chromosomally encoded.

The presence of *bla*_{CTX-M-25}-family genes among different species encoded in different plasmids and located even in the chromosome suggests an integron-based dissemination. Indeed, *bla*_{CTX-M-25}-family genes were found to be located in a class I integron associated with the *ISEcp1* insertion sequence adjacent to the gene which presumably acts as a key factor in the dispersion of these genes, as was reported previously.³⁰ This mode of spread may explain the existence of the same gene in several plasmids, such as in the case of *bla*_{CTX-M-39} carried on five different plasmids of which some were highly similar, for

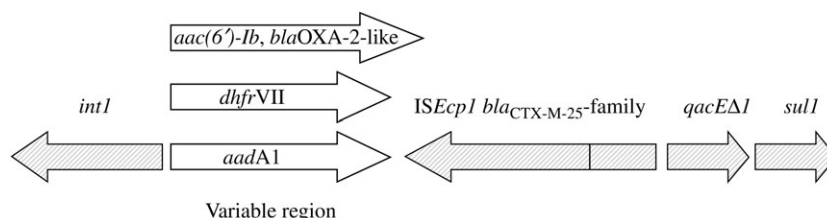


Figure 2. Structural organization of *ISEcp1-bla*_{CTX-M-25}-containing DNA region. Striped arrows indicate resistance determinants that were common in all of the studied isolates including *P. mirabilis* strains whose integron was chromosomally located. White arrows indicate variable DNA regions containing the indicated resistant determinants. The resistant determinants carried in each of the studied isolates are described in detail in the Results section. Arrows indicate the direction of transcription.

example, plasmids of *E. coli* 1430 and *K. pneumoniae* 1016, and others that were highly different, such as plasmids of *K. pneumoniae* 1032 and *E. cloacae* 1018 (Figure 1).

In spite of the presence of different plasmids each encoding a different enzyme as in the case of *K. pneumoniae* 1032 (encoding CTX-M-39) and 1320 (encoding CTX-M-26), a similar hybridization pattern was obtained with the *bla*_{CTX-M-25}-labelled probe (Figure 1b) suggesting the presence of similar fragments.

The apparent differences between plasmids carrying the same ESB� gene but exhibiting variability in the genetic environment of these genes (Figure 2) have been documented before, in the case of *bla*_{CTX-M-9} in genetically unrelated *E. coli* strains³¹ and in the structure of other *bla*_{CTX-M} genes.^{2,32} *bla*_{TEM} was found to be present on the same plasmids carrying the *bla*_{CTX-M-25}-family genes, as was also reported in other *bla*_{CTX-M} genes.³¹

The origin of the CTX-M-25 family is yet undetermined. Other CTX-M-type enzymes seem to have descended from chromosomal β -lactamases of *Kluyvera* spp.² The enzyme that was found to be most closely related to the CTX-M-25 family is KLUG-1 from *Kluyvera georgiana*, the progenitor of CTX-M-8,³³ which is the closest enzyme to the CTX-M-25 subgroup³⁴ but is considered as a different subgroup.³ CTX-M-25 and CTX-M-26, respectively, show 89.4% and 89.7% identities with KLUG-1, which similar to CTX-M-39 and CTX-M-41, respectively, show 90% and 89% identities to KLUG-1. These high similarities indicate that these four enzymes probably originate from the same but a different progenitor gene, yet to be identified.

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Transparency declarations

None to declare.

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