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Novel Chromosome-Encoded CTX-M-78 β-Lactamase from a *Kluyvera georgiana* Clinical Isolate as a Putative Origin of CTX-M-25 Subgroup▽

While TEM- and SHV-derived extended-spectrum β-lactamases (ESBLs) arose by point mutations in genes encoding broad-spectrum variants that were extensively disseminated into mobile elements, CTX-M β-lactamases are derived from natural (chromosomal) counterparts that already have oxyimino-cephalosporinase activity (11). To date, the CTX-M/KLU β-lactamases account for nearly 110 representatives clustered in five main groups (http://www.lahey.org/studies/webt.asp): CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. A hypothetical chromosomal counterpart for the CTX-M-25 subfamily is still missing.

Kluyvera georgiana 14751 was isolated from a bloodstream infection in a 62-year-old male patient through the SENTRY Antimicrobial Surveillance Program in Louisville, KY, on 2 December 2002. Biochemical classification, ambiguous between *Kluyvera georgiana* and *Kluyvera ascorbata*, was resolved by 16S rRNA gene sequencing (GenBank accession no. AM933755), displaying 99.7% identity with the sole available sequence from *K. georgiana* ATCC 51603 (GenBank accession no. AF047186). Identity to all deposited *K. ascorbata* sequences ranged from 97.4 to 98.5% (6).

The strain was resistant to ampicillin, gentamicin, nalidixic acid (Table 1) and trimethoprim-sulfamethoxazole (TMP-SMX) (by disc diffusion). Conjugation to *Escherichia coli* CAG12177 resulted in *E. coli* CK10 harboring an IncFII group conjugative plasmid of ca. 30 kb (pTC10; partial sequence deposited in EMBL under accession no. FN568351), responsible for TMP-SMX and aminoglycoside resistance (Table 1) by a class 1 integron harboring *dfrA17* and *aadA5* gene cassettes (previously reported individually in different *Kluyvera intermedia* isolates; EMBL accession no. EU523051 and EU523052). Upstream of the integron we found a $bla_{\text{TEM-1b}}$ gene followed by a region, including the Tn*3-tnpR*, the first 91 bp of Tn*3-tnpA*, and an IS*26* which is also found in plasmids carrying some $bla_{\text{CTX-M}}$ genes. However, no CTX-M could be recovered from the transferred plasmid.

Partially EcoRI-digested chromosomal DNA from *K. georgiana* 14751 was cloned in a pK19 vector (Kan^r) and transformed into *E. coli* Top10F′ (*E. coli* TKE14751-1KA2), yielding pTKE-1KA2 with an 8-kb insert (Fig. 1) harboring a *bla* gene (876 bp) which encodes a novel CTX-M-78 (EMBL accession no. AM982522) closely related to CTX-M-39 (96.2% amino acid identity) (1) and clustered in the CTX-M-25 subgroup. As seen in Table 1, the MIC increases for cefotaxime, while that of ceftazidime is almost unaffected, correlating with the expected (and experimental [data not shown]) kinetics for most CTX-M enzymes.

As in other chromosome-encoded $bla_{\text{CTX-M/KLU}}$, a 1,227-bp *att*-like gene encoding a putative aspartate aminotransferase is located upstream, with at least 96% identity with other entries from *Kluyvera*. Downstream, an *orf3*-like gene similar to a putative *ttrR* response regulator from *Kluyvera georgiana* (5) and similar to the *orf3* version from the complex class 1 integrons associated with $bla_{\text{CTX-M-2}}$ (found as a fusion gene *orf3*::*qacEΔ1*) (7), and an *orf4*-like gene encoding a putative autotransporter in *Kluyvera ascorbata* (2), were also found. In contrast to other kluyveras, in which sequences similar to IRr-IS*Ecp1* were found downstream of $bla_{\text{CTX-M/KLU}}$ (8), we did not observe any sequence that could serve as putative right inverted repeats (IRrs) there (Fig. 1). A 35-bp sequence seems to represent the boundary between the chromosome-derived information and the plasmid-acquired information. In $bla_{\text{CTX-M-25}}$ and $bla_{\text{CTX-M-26}}$ (GenBank accession no. AF518567 and AY455830, respectively) (4), the immediate upstream region is occupied by IS*Ecp1*, also associated with other $bla_{\text{CTX-M}}$ genes (3). A putative 5-bp target site for IS*Ecp1B* (AATAC) was found immediately upstream of the 35-bp sequence in the chromosomal DNA from *K. georgiana* 14751.

CTX-M-78 possesses high similarity with members of the CTX-M-25 subgroup, making this enzyme the closest representative to be considered one of the probable progenitors of the subfamily.

TABLE 1. MICs of antibiotics for *Kluyvera georgiana* 14751, the recipient strain, and the derived recombinant clone

Antibiotic(s)	MIC (μg/ml)[a]		
	K. georgiana 14751	*E. coli* TKE14751-1KA2	*E. coli* Top10F′
Ampicillin	256	256	2
Ampicillin-clavulanate	4/2	2/1	2/1
Cephalothin	8	256	2
Cefoxitin	1	2	2
Cefotaxime	0.032	1	≤0.016
Ceftazidime	0.063	0.25	0.125
Cefepime	0.032	0.5	0.016
Aztreonam	≤0.016	0.5	0.032
Kanamycin	16	256	1
Gentamicin	64	≤0.5	ND
Tetracycline	≤0.5	256	ND
Nalidixic acid	128	2	ND

[a] For ampicillin-clavulanate, the MIC values for ampicillin and clavulanate are shown before and after the slash, respectively. ND, not determined.

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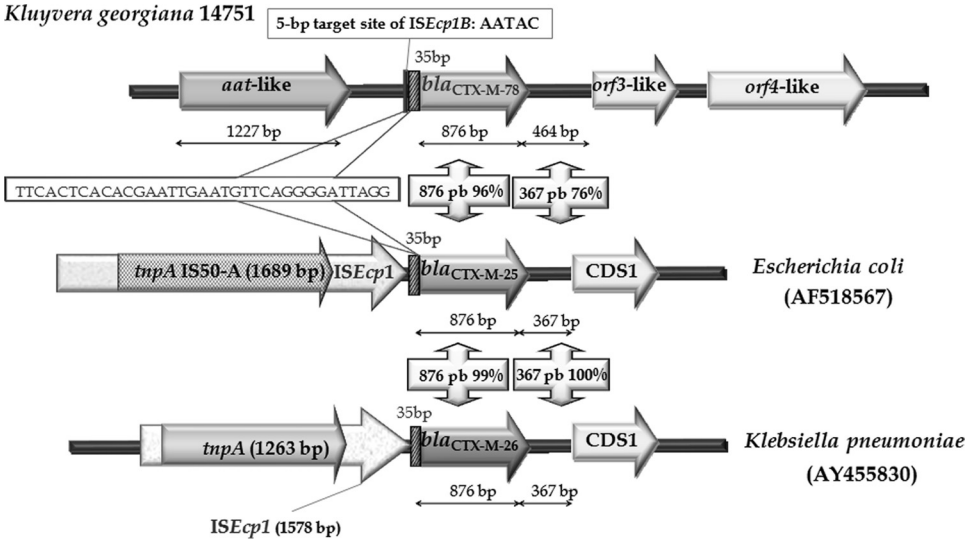

FIG. 1. Schematic representation of the insert of pTKE-1KA2 from *Kluyvera georgiana* 14751 (top) and comparison with available sequences harboring $bla_{CTX\text{-}M\text{-}25}$ (middle) and $bla_{CTX\text{-}M\text{-}26}$ (bottom).

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