ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2005, p. 441–443 Vol. 49, No. 1
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.1.441–443.2005

Emergence of CTX-M-15-Producing Enterobacteria in Cameroon and Characterization of a $bla_{CTX\text{-}M\text{-}15}$-Carrying Element

J. Gangoue-Pieboji,[1,2] V. Miriagou,[1]* S. Vourli,[1] E. Tzelepi,[1] P. Ngassam,[2] and L. S. Tzouvelekis[1,3]

Laboratory of Bacteriology, Hellenic Pasteur Institute,[1] and Department of Microbiology, Medical School, University of Athens,[3] Athens, Greece, and Laboratory of General Biology, Faculty of Sciences, University of Yaounde I, Yaounde, Cameroon[2]

Received 23 April 2004/Returned for modification 17 July 2004/Accepted 18 September 2004

CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* emerged recently in Cameroon. CTX-M-15 was encoded by two different multiresistance plasmids, of which one carried an IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$ element flanked by a 5-bp target site duplication and inserted within a Tn*2*-derived sequence. A truncated form of this element in the second plasmid was identified.

Extended-spectrum β-lactamase (ESBL)-positive enterobacteria are frequently isolated in hospitals in Cameroon. Up to 1999, SHV-12 and SHV-2a were the dominant ESBLs (J. Gangoue-Pieboji, B. Bedenic, S. Koula-Shiro, et al., Program Abstr. 9th Int. Congr. Infect. Dis., abstr. 15419, 2000). In a PCR-based screening for *bla* types applied to enterobacteria collected during July and August 2002 in Yaounde Central Hospital, it was found that 14 out of 17 ESBL-positive isolates produced SHV ESBLs, confirming previous findings. The remaining isolates (one *Klebsiella pneumoniae* isolate and two *Escherichia coli* isolates), however, were $bla_{CTX\text{-}M}$ positive. CTX-M is a rapidly growing family of ESBLs that preferentially hydrolyze cefotaxime. The $bla_{CTX\text{-}M}$ genes are commonly found in plasmids carried by enterobacteria. CTX-M ESBLs have been reported worldwide, the highest prevalence being observed in Latin America, Eastern Europe, and the Far East (3, 16). We report here on the emergence of CTX-M producers also in Cameroon.

The three clinical isolates studied (*K. pneumoniae* YC-17 and *E. coli* YC-5b and YC-14) had been derived from patients with urinary tract infection acquired during hospitalization. The isolates were resistant to amoxicillin, amoxicillin-clavulanate, piperacillin, cefotaxime, ceftazidime, cefepime, and aztreonam, as determined by the agar dilution method. Activity of cefotaxime and ceftazidime was restored by clavulanic acid. MICs of piperacillin-tazobactam, cefoxitin, and imipenem were within the susceptibility range. Isolates were also resistant to various non-β-lactam antibiotics by a disk diffusion assay (Table 1).

β-Lactamases were extracted by ultrasonic treatment and characterized by isoelectric focusing. Isolates produced β-lactamases with apparent isoelectric points (pIs) equal to 7.3 and 8.8. *E. coli* YC-5b produced an additional β-lactamase focusing at 5.4. Isolates were positive in a PCR specific for $bla_{CTX\text{-}M\text{-}3}$-related genes (6). Sequencing the PCR products showed 100% homology with $bla_{CTX\text{-}M\text{-}15}$ (accession no. AY044436) (6). CTX-M-15 corresponded to the β-lactamase with a pI of 8.8. Also, by PCR with bla_{TEM}- and bla_{OXA}-specific primers (1, 15) and the sequencing of the amplicons the β-lactamases with pIs of 7.3 and 5.4 were identified as OXA-30 and TEM-1. Therefore, oxyimino-β-lactam resistance was mainly due to CTX-M-15.

In conjugation experiments performed in liquid media *E. coli* YC-14 and *K. pneumoniae* YC-17 transferred resistance to oxyimino-β-lactams and aminoglycosides to an *E. coli* K-12 host (Table 1). Plasmid analysis indicated transfer of 90-kb plasmids that produced similar PstI restriction patterns. Additionally, in both preparations, PstI fragments equal in size (5.3 kb) hybridized with a digoxigenin-labeled $bla_{CTX\text{-}M\text{-}15}$ probe, suggesting spread of a single plasmid (pYC-14). *E. coli* YC-5b harbored a 50-kb plasmid (pYC-5b) that was used to transform *E. coli* DH5α. Transformants exhibited the resistance phenotype of *E. coli* YC-5b (Table 1). The PstI-generated restriction pattern of pYC-5b was different from that of pYC-14. Hybridization of the $bla_{CTX\text{-}M\text{-}15}$ probe occurred on a 3.4-kb PstI fragment of pYC-5b. Isoelectric focusing and PCR experiments showed that pYC-5b and pYC-14 coded also for the penicillinases produced by the respective clinical isolates.

Plasmids pYC-5b and pYC-14 were partially digested with Sau3A, and the fragments were ligated into pBCSK(+) (Stratagene). Recombinant plasmids were used to transform *E. coli* DH5a. Selection was performed in media containing either cefotaxime or ampicillin. Colony hybridization with a $bla_{CTX\text{-}M}$ probe was also applied to facilitate selection. Nucleotide sequences of overlapping fragments were determined with an ABI 377 sequencer (Applied Biosystems).

In pYC-5b, an IS*EcpI* insertion sequence, comprising an intact *tnpA* gene and two 30-bp imperfect inverted repeats (IRL and IRR) characteristic of this element (accession no. AJ242809) (9), was located 48 bp upstream of $bla_{CTX\text{-}M\text{-}15}$. The promoter driving $bla_{CTX\text{-}M}$ transcription was identified within the 3′ noncoding sequence of IS*EcpI* (13). An 18-bp sequence corresponding to the external part of IRR of IS*EcpI* (putative IRR) was found 373 bp downstream of $bla_{CTX\text{-}M\text{-}15}$. The intervening 373-bp sequence had 55% homology with the respective chromosomal region of *Kluyvera cryocrescens* (from nucleotide [nt] 3304 to 3677 in the sequence with accession no. AY026417) (4). The IS*EcpI*-$bla_{CTX\text{-}M\text{-}15}$-containing sequence was flanked by 5-bp direct repeats and inserted within *tnpA* (*tnpAΔ1*, 214 nt from the 5′ end; *tnpAΔ2*, 2,246 nt) of a Tn*2*-

* Corresponding author. Mailing address: Laboratory of Bacteriology, Hellenic Pasteur Institute, Vas. Sofias 127, Athens 11521, Greece. Phone: 30 210 6478810. Fax: 30 210 6423498. E-mail: miriagou@mail.pasteur.gr.

TABLE 1. Antibiotic susceptibility of CTX-M-15-producing strains

Strain	MICs (μg/ml) of[a]:											Other resistance markers[b]
	AMX	AMC	PIP	TZP	FOX	CTX	CTX+	CAZ	CAZ+	ATM	FEP	IMI	
E. coli YC-14	≥256	32	≥256	32	8	≥256	0.5	32	1	64	64	0.25	Gm, Tb, Sul, Tmp, Cm
E. coli K-12(pYC-14)	≥256	32	≥256	16	8	≥256	0.25	32	0.5	32	32	0.12	Gm, Tb
K. pneumoniae YC-17	≥256	64	≥256	32	16	≥256	1	128	2	128	128	0.5	Gm, Tb, Sul, Tmp, Cm
E. coli K-12(pYC-17)	≥256	32	≥256	16	8	≥256	0.25	32	0.5	32	32	0.12	Gm, Tb
E. coli YC-5b	≥256	32	≥256	32	16	≥256	0.5	64	1	64	64	0.12	Gm, Tb, Sul, Tmp
E. coli DH5α(pYC-5b)	≥256	32	≥256	8	4	≥256	0.12	32	0.5	32	16	≤0.06	Gm, Tb, Sul, Tmp
E. coli K-12	4	2	1	1	4	≤0.06	—[c]	0.25	—	≤0.06	≤0.06	≤0.06	
E. coli DH5α	2	2	1	1	4	≤0.06	—	0.12	—	≤0.06	≤0.06	≤0.06	

[a] AMX, amoxicillin; AMC, amoxicillin-clavulanic acid (2:1); PIP, piperacillin; TZP, piperacillin plus tazobactam (4 μg/ml); Fox, cefoxitin; Ctx, cefotaxime; CTX+, cefotaxime plus clavulanic acid (4 μg/ml); CAZ, ceftazidime; CAZ+, ceftazidime plus clavulanic acid (4 μg/ml); ATM, aztreonam; IMI, imipenem.
[b] Gm, gentamicin; Tb, tobramycin; Sul, sulfonamides; Tmp, trimethoprim; Cm, chloramphenicol.
[c] —, not done.

derived sequence. The latter also contained part of the respective *tnpR* (*tnpR*Δ; 173 nt from the 5′ end) and was flanked by directly repeated IS*26* elements (Fig. 1A). The truncated forms of transposase and resolvase of Tn*2* were, most likely, not functional. A homologous segment, extending from the 3′ end of the *tnpA* gene of IS*Ecp1* up to the IS*26* of the right end, was carried by the self-transferable plasmid pYC-14. This sequence was preceded by IS*26* (Fig. 1B).

Since its first description in 2001, CTX-M-15 has been identified in multiple locations in Asia and Europe (2, 5–8, 10–12, 17). This study documents for the first time the emergence of CTX-M-15-producing enterobacteria in an African country. CTX-M-15 differs from CTX-M-3 by an Asp-240→Gly substitution that increases activity against ceftazidime (14). The enhanced substrate spectrum of CTX-M-15 is probably a factor contributing to its spread.

IS*Ecp1*-like sequences have been associated with various $bla_{\text{CTX-M}}$ genes of the three major evolutionary groups (3). The presence of a 5-bp duplication at the boundaries of the IS*Ecp1*-$bla_{\text{CTX-M-15}}$ element and the resemblance of its right end to the IRR of IS*Ecp* are indicative of transposition. Similar sequence characteristics in the recently described IS*Ecp1B*-$bla_{\text{CTX-M-19}}$ element led to the hypothesis that IS*Ecp1* mediates a regular transposition process (13). However, the putative IRRs of these elements had less than 60% homology with the corresponding region of IRR and also differ from each other by 9 nt (50% homology). Therefore, the possibility for a one-ended transposition mechanism cannot be definitely excluded (P. D. Stapleton, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1457, 1999).

Geographical and temporal clusters of identical $bla_{\text{CTX-M}}$ genes carried by apparently different plasmids have also been reported in previous studies (reviewed in reference 3). Notably, the sequence homology of the CTX-M-encoding loci in pYC-5b and pYC-14 extends beyond IS*EcpI*-$bla_{\text{CTX-M-15}}$, including parts of the Tn*2* flanking segments. Recently, Lartigue et al. described plasmids carrying IS*Ecp1*-$bla_{\text{CTX-M-15}}$ elements inserted within *tnpA* of a Tn*2*-like transposon harbored by *E. coli* isolates from France and India (8). Furthermore, a GenBank search revealed a plasmid from *E. coli* isolated in Canada (pC15-1a) that also contained a Tn*2*-inserted IS*Ecp1*-$bla_{\text{CTX-M-15}}$ (from nt 17077 to 23482 in the sequence with accession no.

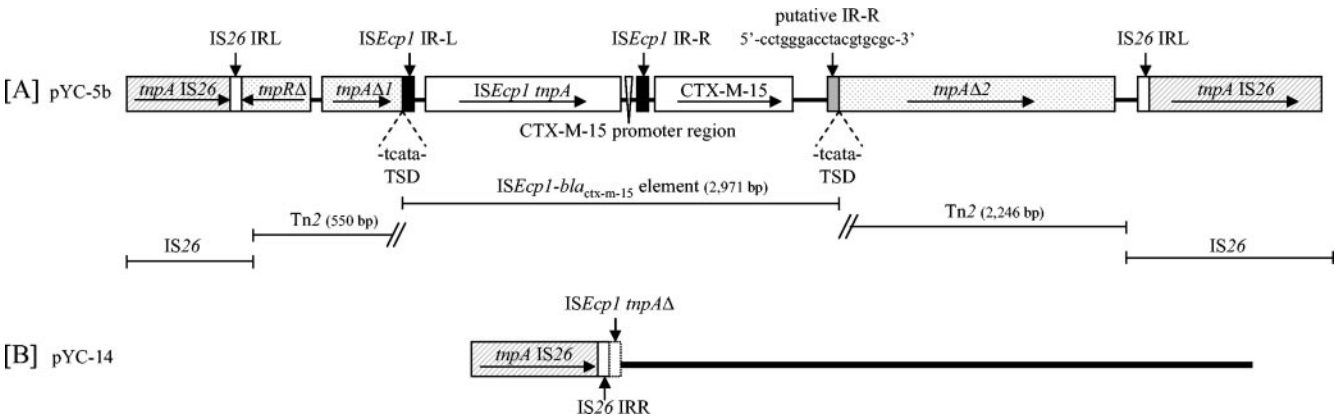

FIG. 1. Schematic representation of the IS*Ecp1*-$bla_{\text{CTX-M-15}}$-containing sequences in plasmids pYC-5b (A) and pYC-14 (B). Inverted repeat sequences (IR) and target site duplications (TSD) are shown. Arrows indicate direction of transcription. The thick line (B) denotes homology with the sequence in panel A.

AY458016 [M. R. Mulvey et al., unpublished data]). This sequence was homologous to that found in pYC-5b except that it lacked the left-hand IS*26*. Also, in silico restriction analysis of pC15-1a indicated patterns different from that of pYC-5b. Since IS*Ecp1* does not exhibit marked target site selectivity, it can be hypothesized either that the CTX-M-15-encoding plasmids discussed here diverged from an ancestral IS*Ecp1-bla*$_{\text{CTX-M}}$-carrying plasmid or that the IS*Ecp1-bla*$_{\text{CTX-M-15}}$ sequence was independently acquired as part of a larger mobile element.

Nucleotide sequence accession numbers. The described sequences have been assigned GenBank accession numbers AY604721 and AY604722.

REFERENCES

1. **Arlet, G., G. Brami, D. Decre, A. Flippo, O. Gaillot, P. H. Lagrange, and A. Philippon.** 1995. Molecular characterisation by PCR-restriction fragment length polymorphism of TEM β-lactamases. FEMS Microbiol. Lett. **134:** 203–208.
2. **Baraniak, A., J. Fiett, W. Hryniewicz, P. Nordmann, and M. Gniadkowski.** 2002. Ceftazidime-hydrolysing CTX-M-15 extended-spectrum-β-lactamase (ESBL) in Poland. J. Antimicrob. Chemother. **50:**393–396.
3. **Bonnet, R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
4. **Decousser, J. W., L. Poirel, and P. Nordmann.** 2001. Characterization of a chromosomally encoded extended-spectrum class A β-lactamase from *Kluyvera cryocrescens*. Antimicrob. Agents Chemother. **45:**3595–3598.
5. **Edelstein, M., M. Pimkin, I. Palagin, I. Edelstein, and L. Stratchounski.** 2003. Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrob. Agents Chemother. **47:**3724–3732.
6. **Karim, A., L. Poirel, S. Nagarajan, and P. Nordmann.** 2001. Plasmid-mediated extended-spectrum-β-lactamase (CTX-M-3 like) from India and gene association with insertion sequence IS*Ecp1*. FEMS Microbiol. Lett. **201:**237–241.
7. **Lartigue, M. F., L. Poirel, C. Heritier, V. Tolun, and P. Nordmann.** 2003. First description of CTX-M-15-producing *Klebsiella pneumoniae* in Turkey. J. Antimicrob. Chemother. **52:**315–316.
8. **Lartigue, M. F., L. Poirel, and P. Nordmann.** 2004. Diversity of genetic environment of *bla*$_{\text{CTX-M}}$ genes. FEMS Microbiol. Lett. **234:**201–207.
9. **Mahillon, J., and M. Chandler.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
10. **Mushtaq, S., N. Woodford, N. Potz, and D. M. Livermore.** 2003. Detection of CTX-M-15 extended-spectrum-β-lactamase in the United Kingdom. J. Antimicrob. Chemother. **52:**528–529.
11. **Neuwirth, C., E. Siebor, M. Pruneaux, M. Zarnayova, C. Simonin, J. P. Kisterman, and R. Labia.** 2003. First isolation of CTX-M15-producing *Escherichia coli* from two French patients. J. Antimicrob. Chemother. **51:**471–473.
12. **Pagani, L., E. Dell'Amico, R. Migliavacca, M. M. D'Andrea, E. Giacobone, G. Amicosante, E. Romero, and G. M. Rossolini.** 2003. Multiple CTX-M-type extended-spectrum-β-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in northern Italy. J. Clin. Microbiol. **41:**4264–4269.
13. **Poirel, L., J.-W. Decousser, and P. Nordmann.** 2003. Insertion sequence IS*Ecp1B* is involved in expression and mobilization of a *bla*$_{\text{CTX-M}}$ β-lactamase gene. Antimicrob. Agents Chemother. **47:**2938–2945.
14. **Poirel, L., M. Gniadkowski, and P. Nordmann.** 2002. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum-β-lactamase CTX-M-15 and of its structurally related β-lactamase CTX-M-3. J. Antimicrob. Chemother. **50:**1031–1034.
15. **Siu, L. K., J. Y. C. Lo, K. Y. Yuen, P. Y. Chau, M. H. Ng, and P. L. Ho.** 2000. β-Lactamases in *Shigella flexneri* isolates from Hong Kong and Shanghai and a novel OXA-1-like β-lactamase, OXA-30. Antimicrob. Agents Chemother. **44:**2034–2038.
16. **Tzouvelekis, L. S., E. Tzelepi, P. T. Tassios, and N. J. Legakis.** 2000. CTXM-type beta-lactamases: an emerging group of extended-spectrum enzymes. Int. J. Antimicrob. Agents **14:**137–142.
17. **Yu, W.-L., K.-C. Cheng, L.-T. Wu, M. A. Pfaller, P. L. Winokur, and R. N. Jones.** 2004. Emergence of two *Klebsiella pneumoniae* isolates harboring plasmid-mediated CTX-M-15 β-lactamase in Taiwan. Antimicrob. Agents Chemother. **48:**362–363.