ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2004, p. 3758–3764 Vol. 48, No. 10
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.10.3758–3764.2004

Complete Nucleotide Sequence of a 92-Kilobase Plasmid Harboring the CTX-M-15 Extended-Spectrum Beta-Lactamase Involved in an Outbreak in Long-Term-Care Facilities in Toronto, Canada

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Received 6 January 2004/Returned for modification 21 April 2004/Accepted 26 June 2004

A major outbreak involving an *Escherichia coli* strain that was resistant to expanded-spectrum cephalosporins occurred in Toronto and surrounding regions in 2000 to 2002. We report the complete sequence of a plasmid, pC15-1a, that was found associated with the outbreak strain. Plasmid pC15-1a is a circular molecule of 92,353 bp consisting of two distinct regions. The first is a 64-kb region that is essentially homologous to the non-R-determinant region of plasmid R100 except for several point mutations, a few small insertions and deletions, and the absence of Tn*10*. The second is a 28.4-kb multidrug resistance region (MDR) that has replaced the R-determinant region of the R100 progenitor and consists mostly of transposons or partial transposons and five copies of the insertion element IS*26*. All drug resistance genes found in pC15-1a, including the beta-lactamase genes $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, and $bla_{\text{TEM-1}}$, the tetracycline resistance gene *tetA*, and aminoglycoside resistance genes *aac*(*6′*)*-Ib* and *aac*(*3*)*-II*, are located in the MDR. The $bla_{\text{CTX-M-15}}$ gene was found downstream of IS*Ecp1* as part of a transposition unit, as determined from the surrounding sequence. Examination of the plasmids from CTX-M-15-harboring strains isolated from hospitals across Canada showed that pC15-1a was found in several strains isolated from a site in western Canada. Comparison of pC15-1a and pCTX15, found in an *E. coli* strain isolated in India in 1999, revealed that the plasmids had several features in common, including an R100 backbone and several of the resistance genes, including $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, *tetA*, and *aac*(*6′*)*-Ib*.

Plasmid-mediated extended-spectrum beta-lactamase (ESBL) enzymes are most commonly of the TEM, SHV, or CTX-M type (8). To date more than 120 TEM enzymes, more than 50 SHV enzymes, and more than 30 CTX-M enzymes have been reported (www.lahey.org/studies/). Members of these groups are class A enzymes and, for the most part, are inhibited by clavulanic acid.

The CTX-M-type beta-lactamases are increasingly found in enterobacterial species throughout the world; more than half have been reported within the last 4 years (7, 28). They are generally most active against cefotaxime and show little activity against ceftazidime. Phylogenetically, they are grouped into five clusters based on their amino acid identities: the CTX-M-1 cluster (CTX-M-1, -3, -10, -11, -12, -15, etc.), the CTX-M-2 cluster (CTX-M-2, -4, -5, -6, -7, and -20, TOHO-1, etc.), the CTX-M-8 cluster (CTX-M-8), the CTX-M-25 cluster (CTX-M-25 and -26), and the CTX-M-9 cluster (CTX-M-9, -13, -14, -16, -17, and -19, TOHO-2, etc.). The probable progenitors of two of the clusters have been identified as the chromosomal beta-lactamases of *Kluyvera ascorbata* (CTX-M-2 cluster) (10) and *Kluyvera georgiana* (CTX-M-8 cluster) (20).

CTX-M-15 was first described on large plasmids isolated from *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* strains from patients hospitalized in New Delhi, India, in 1999 (11). However, it should be noted that the first report of the $bla_{\text{CTX-M-15}}$ sequence in the public domain was made in 2001; the sequence, designated $bla_{\text{UOE-1}}$, was found on a plasmid from an *E. coli* isolate from Japan (GenBank accession no. AY013478). CTX-M-15 differs from CTX-M-3 by a single amino acid change, Asp240Gly (Ambler numbering) (3). Both beta-lactamases are inhibited by clavulanic acid and tazobactam, but unlike CTX-M-3, CTX-M-15 displays a catalytic activity toward ceftazidime (19). This activity is thought to be due to the Asp240Gly substitution, as has been shown for CTX-M-16 and CTX-M-27 (5, 6). CTX-M-15 has also been identified in Poland (4), Bulgaria (I. Schneider, E. Kueleyom, R. Makovska, and A. Bauernfeind, Abstr. 12th Cong. Clin. Microbiol. Infect. Dis., abstr. P430, 2002), and Canada (12, 13).

From August 2000 to April 2002, a major outbreak involving a multidrug-resistant *E. coli* strain associated with long-term care facilities occurred in Toronto and surrounding regions

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(12). A representative isolate that conferred resistance to expanded-spectrum cephalosporins was shown to harbor a plasmid containing $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, and $bla_{TEM\text{-}1}$ (M. P. Muller, D. Boyd, A. Ashi-Sulaiman, C. Larocque, M. Mulvey, A. McGeer, and B. Willey, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-2233, 2001). This report describes the complete nucleotide sequence of the plasmid as well as its isolation from a single hospital in western Canada. The plasmid is genetically very similar to the one isolated from strains from patients hospitalized in New Delhi, India, in 1999 (11).

MATERIALS AND METHODS

Bacterial strains. All isolates were identified at participating sites by routine procedures performed at each laboratory. Some isolates were obtained through an ESBL surveillance initiative conducted by the Canadian Nosocomial Infection Surveillance Program (13). Upon receipt at the National Microbiology Laboratory, strains were subcultured to Columbia blood agar to ensure viability and purity. Stock cultures were stored at −70°C in Microbank vials (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). The identification of all submitted isolates was confirmed by using Vitek Gram-Negative Identification cards (bioMérieux). Control strains used in this study included *K. pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 25922.

Antimicrobial susceptibility testing. Potential ESBL isolates were confirmed by the disk diffusion method as described by NCCLS (16) by using disks containing ceftazidime (CAZ; 30 mg/liter), ceftazidime-clavulanic acid (CAZ-CLA; 30 and 10 mg/liter, respectively), cefotaxime (CTX; 30 mg/liter), and cefotaxime-clavulanic acid (CTX-CLA; 30 and 10 mg/liter, respectively) supplied by Mast Diagnostics (Bootle, Merseyside, United Kingdom). Susceptibilities to other classes of antimicrobial agents were determined by using the Vitek Gram-Negative Susceptibility-121 cards (bioMérieux).

Molecular characterization of study strains. Strains were subtyped by using pulsed-field gel electrophoresis (PFGE) following DNA extraction and digestion with XbaI using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention (26). PFGE-generated DNA profiles were digitized and entered into the BioNumerics software program (version 2.5; Applied Maths, Kortrijk, Belgium) for analysis. DNA fragments on each gel were normalized by using the *Salmonella enterica* serovar Braenderup "Universal Marker" (kindly provided by B. Swaminathan, Centers for Disease Control and Prevention) as a molecular weight standard. Isolates were considered to be genetically related if their macrorestriction DNA patterns differed by fewer than seven bands (27).

The numbers and isoelectric points (pI's) of the beta-lactamases present in the strains in this study were determined by isoelectric focusing with pH 3 to 10 precast gels in a Mini-Protean II apparatus (Bio-Rad). Crude cell extracts were prepared by sonication, and beta-lactamase activity was visualized by a colorimetric assay using nitrocefin (1 mg/ml) in an agar overlay.

Total DNA was isolated as described previously (22) and was used in PCRs with universal primer sets to detect bla_{SHV} (17), bla_{TEM}, and $bla_{OXA\text{-}1}$ (24). The bla_{CTX} genes were identified by using in-house designed universal primers CTX-M-U1 (5′-ATGTGCAGYACCAGTAARGTKATGGC) and CTX-M-U2 (5′-TGGGTRAARTARGTSACCAGAAYCAGCGG) (where R stands for purine, Y stands for pyrimidine, and S stands for G or C) in PCRs with conditions similar to those described for bla_{TEM}, except for the alteration of the annealing temperature to 58°C. Amplicons were purified by using commercially available methods (Amicon), and sequence identification was conducted on an ABI 3100 sequencer at the DNA Core Facility (National Microbiology Laboratory). DNA and amino acid sequence comparisons were conducted by using the BLAST suite of programs (2).

Plasmid DNA from clones and from clinical strains (large plasmids) were isolated by using commercial isolation kits (QIAGEN). Plasmid DNA was used to transform electrocompetent *E. coli* DH10B (Invitrogen) by using a Gene Pulser apparatus (Bio-Rad). Transformants were selected on LB agar containing CTX (5 mg/liter). Plasmid DNA digested with restriction enzymes was separated on a 0.7% agarose gel using 0.5× Tris-borate-EDTA for 16 h at 2.8 V/cm with circulating buffer. Digitized images of gels were analyzed by using the BioNumerics software program (version 2.5; Applied Maths) with the One Kilobase Extension Ladder (Invitrogen) as a standard.

DNA cloning and sequence analysis. DNA for plasmid pC15-1a was randomly sheared and cloned into pCR4Blunt-TOPO by using the TOPO Shotgun subcloning kit (Invitrogen). Plasmid DNA from the resulting subclones was purified prior to sequencing by using the Wizard SV96 kit (Promega), and end sequencing was performed by using BigDye (version 3.0; Perkin-Elmer Applied Biosystems Inc.) and running the reactions on an ABI377 or ABI3100 sequencer (Perkin-Elmer Applied Biosystems Inc.). Custom primers were also employed in PCRs to extend sequences for specific plasmids or to fill gaps and ensure that double-strand data were obtained for the complete sequence of pC15-1a. All primers used were synthesized on an Oligo1000M (Beckman) by using standard phosphoramidite chemistry. The resulting sequence data were assembled by using the Staden package (25), and annotation and analysis of the sequence were performed by using Artemis (21) and the tools available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

Nucleotide sequence accession number. The complete sequence of pC15-1a has been submitted to the GenBank nucleotide sequence database under accession number AY458016.

RESULTS AND DISCUSSION

Initial plasmid characterization. During an outbreak investigation involving six long-term care facilities and two acute care facilities in the Toronto region, PFGE analysis revealed that more than 200 patients were colonized with an indistinguishable strain of multidrug-resistant *E. coli* (12). Isoelectric focusing on nine strains revealed that all produced three beta-lactamases with pI's of 8.6, 7.4, and 5.4, respectively (data not shown). Plasmid analysis revealed that all strains harbored a large plasmid of ~92 kb that could easily be transferred by conjugation to *E. coli* J53-2; the transconjugants produced the three beta-lactamases (data not shown). PCR and sequence analysis identified the beta-lactamases as TEM-1 (pI 5.4), OXA-1 (pI 7.4), and CTX-M-15 (pI 8.6) (Muller et al., 41st ICAAC). For further study, the plasmid from a single *E. coli* clinical isolate, N00-0666, was electrotransformed into *E. coli* DH10B. The plasmid, designated pC15-1a, was restricted with HpaI, and analysis of the fragments confirmed the size of the plasmid to be ~92 kb (Fig. 1, lane A).

Distribution of pC15-1a in Canada. In 1999, a national surveillance project involving 12 hospitals from across the country was initiated to determine the molecular epidemiology and rates of ESBLs in Canada (13). In this study 11 out of 116 strains harboring ESBLs were found to contain $bla_{CTX\text{-}M\text{-}15}$. All of these strains had a positive result with TEM PCR, and nine gave a positive result with OXA-1 PCR (Table 1). Comparison of HpaI plasmid profiles to the pC15-1a HpaI profile revealed that three strains from Vancouver General Hospital, Vancouver, British Columbia, harbored a plasmid indistinguishable from pC15-1a. Two other strains from this hospital were indistinguishable by PFGE analysis and were assumed to harbor pC15-1a. One strain from this hospital harbored a pC15-1a variant (four band differences), and another harbored plasmid pC15-2a, which was unrelated to pC15-1a. One strain from a Toronto hospital harbored pC15-1a, and another strain from this hospital harbored plasmid pC15-2b, which was related to pC15-2a. As with the original Toronto long-term-care facility outbreak strain, the other clinical strains harboring pC15-1a displayed a multidrug resistance phenotype, and all but one were resistant to ciprofloxacin (Table 1). It was determined that *E. coli* ESBL35, which was resistant to all beta-lactams and beta-lactam–inhibitor combinations, overproduced its chromosomal AmpC beta-lactamase (data not shown). *E. coli* ESBL475 and *E. coli* ESBL373 (presumed to harbor pC15-1a) were also both resistant to amoxicillin-clavulanic acid. Not

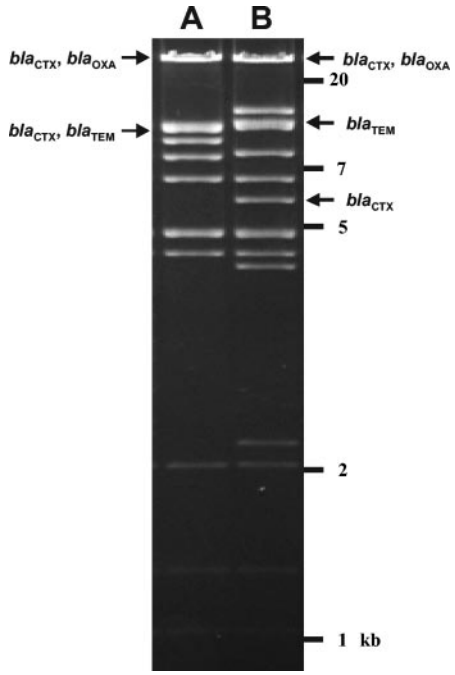

FIG. 1. HpaI digests of pC15-1a (lane A) and pCTX15 (lane B). Fragments hybridizing with $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1}$, and $bla_{OXA\text{-}1}$ probes are indicated. The molecular weight marker was the One Kilobase Extension Ladder (Invitrogen).

shown in Table 1 is the fact that the other strains harboring pC15-1a were intermediate to amoxicillin-clavulanic acid. Differences in growth characteristics of the strains and/or expression of the beta-lactamases are the most likely reason for this discrepancy.

Complete nucleotide sequence of pC15-1a. The presence of the plasmid-mediated ESBL CTX-M-15, associated with a large outbreak in Toronto, in addition to the identification of pC15-1a in genetically unrelated strains of *E. coli* found in a geographically distinct area of Canada, prompted us to examine the genetic make-up of this plasmid. The complete nucleotide sequence of plasmid pC15-1a was determined by sequencing randomly sheared fragments of pC15-1a cloned into pCR4Blunt-TOPO. Plasmid pC15-1a was found to be a circular molecule of 92,353 bp with a 52.6% G+C content. The plasmid was found to be composed of two distinct regions: the first is a 64-kb fragment (bases 28439 to 92353; 51.7% G+C) that is homologous to most of the region corresponding to bases 21910 to 92048 of plasmid R100 (accession no. AP000342), and the second is a unique multidrug resistance region (bases 1 to 28438; 54.6% G+C) that has replaced bases 92048 to 21909 of R100 (Fig. 2A). Plasmid R100 (also called NR1) was originally isolated from *Shigella flexneri* in Japan in the late 1950s (15). R100 is a 94-kb self-conjugative, multiple antibiotic resistance plasmid from the FII incompatibility group that has been found worldwide among the *Enterobacteriaciae*, including *E. coli* (9).

A nucleotide identity of 98% was found between the pC15-1a R100-derived region and the corresponding region in R100. The majority of the changes either were found to be synonymous mutations, occurred in noncoding regions, or resulted in conserved amino acid substitutions. Significant changes between these two sequences are summarized in Table 2. Several genes of unknown function have disrupted reading frames, but it is not known how this affects plasmid physiology. Two mobile elements were found: (i) an IS*682* variant inserted near the end of *yehA* and (ii) IS*1a* inserted between *yigA* and *yigB*. More interestingly, Tn*10* is absent, though the inverted repeats defining the transposon ends, and direct repeats defining the insertion site, were identified at the location in pC15-1a where Tn*10* resides in the R100 sequence.

The 28.4-kb unique region, which contained all the drug resistance genes of pC15-1a, was found to be composed primarily (73%) of elements associated with transposition (Fig. 2). Interestingly, this region replaces a 24.1-kb region in R100 that consists mostly of the R determinant harboring all the drug resistance genes except for *tetB* of Tn*10* (Fig. 2A). There were no direct and/or inverted repeats at the junctions to suggest that the complete unique region of pC15-1a replaced the R100 R determinant by a transposition process. However,

TABLE 1. Characteristics of the *E. coli* strains harboring pC15-1a used in this study[a]

Strain	Date of isolation	Location[b]	PFGE profile[c]	Plasmid	Resistance phenotype[d]
N00-0666	2000	LTC outbreak	A1	pC15-1a	Amp Cfz Cro Cip Gen Tob
ESBL35	1 Dec. 1999	VGH	B1	pC15-1a variant	Amc Amp Cfz Caz Cro Cip Gen Tzp Tob Sxt
ESBL123	3 April 2000	VGH	C1	pC15-2a	Amp Cfz Cro Cip Sxt
ESBL304	20 May 2000	VGH	D1	pC15-1a	Amp Cfz Cro Cip Gen Tob Sxt
ESBL361	30 Aug. 2000	VGH	E1	pC15-1a	Amp Cfz Cro Gen Tob Sxt
ESBL370	14 Sept. 2000	VGH	F1	pC15-1a	Amp Cfz Cro Cip Gen Tob Sxt
ESBL373	17 Sept. 2000	VGH	F1	ND[e]	Amc Amp Cfz Cro Cip Gen Tob Sxt
ESBL374	18 Sept. 2000	VGH	F1	ND	Amp Cfz Cro Cip Gen Tob Sxt
ESBL475	7 May 2000	UHN	G1	pC15-1a	Amc Amp Cfz Cro Cip Gen Tob Sxt
ESBL480	27 Sept. 2000	UHN	H1	pC15-2b	Amp Cfz Cro Cip Gen Tob

[a] All strains were positive by PCR analysis for CTX-M-, TEM-, and OXA-1-type genes and contained beta-lactamases with pls of 5.4, 7.4, and 8.6 as determined by isoelectric focusing.
[b] LTC outbreak; long-term-care outbreak, Toronto region. VGH, Vancouver General Hospital; UHN, University Health Network, Toronto, Ontario, Canada.
[c] The same letter-number combination means that fingerprints were indistinguishable, A different letter-number combination means that fingerprints had >7 band differences.
[d] Abbreviations indicate drug resistance as follows: Amc, amoxicillin-clavulanic acid; Amp, ampicillin; Cfz, cefazolin; Caz, ceftazidime; Cro, ceftriaxone; Cip, ciprofloxacin; Gen, gentamicin; Tzp, piperacillin-tazobactam; Tob, tobramycin; Sxt, trimethoprim-sulfamethoxazole.
[e] ND, not done.

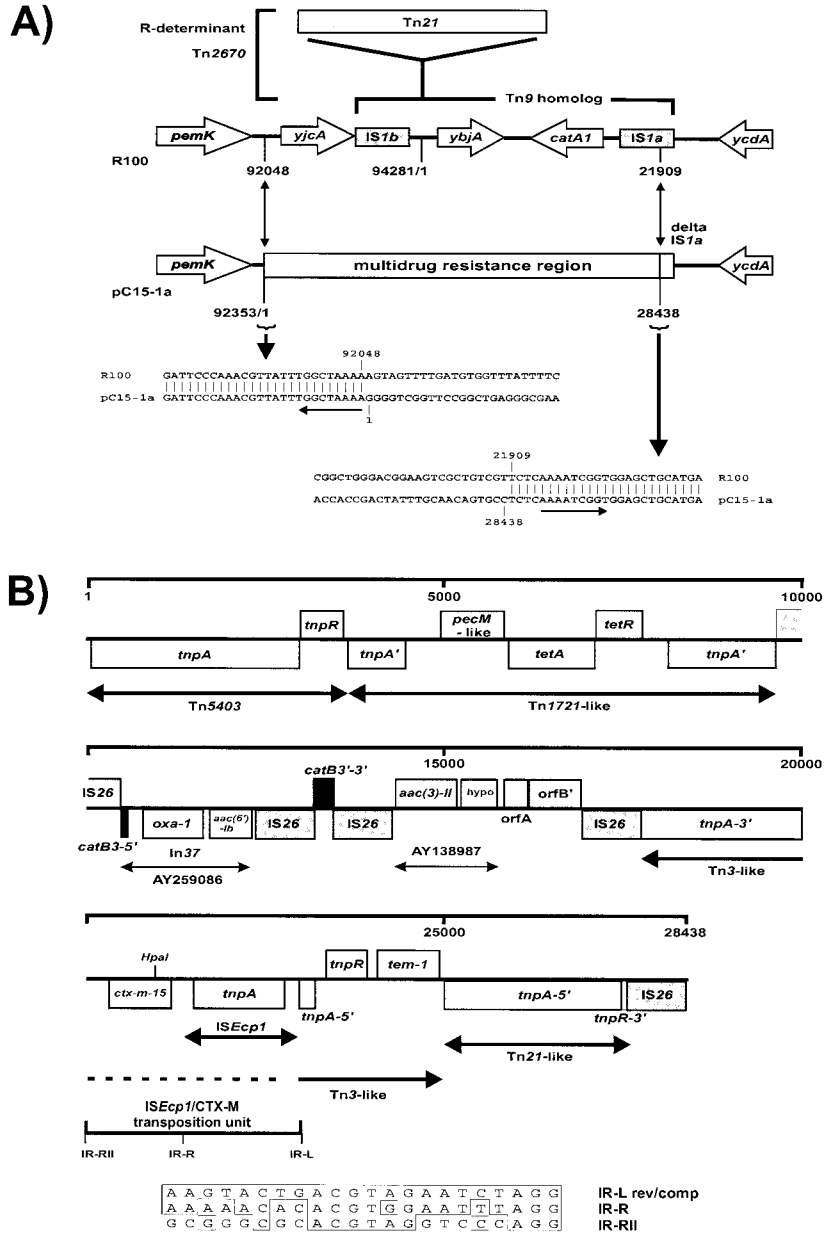

FIG. 2. (A) Schematic diagram comparing the region between *pemK* and *ycdA* from R100 with that from pC15-1a. Tn*21*, which is inserted between IS*1b* and *ybjA* in R100, is not drawn to scale. Junction regions are shown at the bottom; arrows below these sequences indicate the 9-bp region that is repeated in reverse order. (B) Schematic diagram showing the open reading frames of the multidrug resistance region of pC15-1a. Rectangles above the line, open reading frames transcribed from left to right; rectangles below the line, open reading frames transcribed from right to left. The putative origin of the sequence, when known, is shown below the open reading frames. A designation with a prime, e.g., *tnpA'*, indicates a partial open reading frame. An alignment of the IS*Ecp1* left inverted repeat (IR-L; reverse complement of the sequence), the IS*Ecp1* right inverted repeat (IR-R), and the right inverted repeat of the transposition unit (IR-RII) is shown at the bottom.

a 9-bp sequence at the left junction is repeated in reverse order 4 bp away from the right junction (Fig. 2A). The functional significance of this finding is not known. The genetic arrangement of this region is diagrammed in Fig. 2B, and its features are listed in Table 3. Tn*5403* and partial sequences of a Tn*1721*-like element, a Tn*3*-like element, and a Tn*21*-like element account for 58.6% of this region. Five copies of IS*26* account for an additional 14.8%. Two drug resistance genes, *tetA* of Tn*1721* and bla_{TEM-1} of Tn*3*, are found on partially deleted transposons. Two other genes appear to have originated from the drug resistance cassette of a class 1 integron. The aminoglycoside resistance gene *aac*(*6'*)*-1b* and bla_{OXA-1} are found on a 1,863-bp region 100% identical to a region of In*37* (29). This region also includes the sequence coding for the first 39 amino acids of *catB3*. Interestingly, the sequence coding for amino acids 38 to 147 of *catB3* is located 2.7-kb downstream (bases 13145 to 13475) in the opposite orientation between two copies of IS*26*. The aminoglycoside resistance

TABLE 2. Significant sequence divergences of pC15-1a from R100

Variation[a]	R100 coordinates[b]	Effect in pC15-1a[c]
Substitution	29694	G-to-T substitution introduces stop codon and truncates *ycjB*
Deletion	29885–29902	After the early stop introduced by substitution above in pC15-1a, a 6-aa deletion (LLLLLL) in the R100 *ycjB* region
Deletion	30210	C deleted; causes frameshift in *ydaA* approximately midway in ORF; resulting protein diverges at this point
Deletion	33242–33247	2-aa deletion (SS; AGTAGC) in repetitive region of *yddA*
Deletion	36157–45253	Deletion of Tn*10*; inverted repeats from IS*10-L* and IS*10-R* are still present
Insertion	47797/47798	IS*682* variant (not 100% identical); disrupts *yehA*; last 8 aa originate from IS*682* sequence
Insertion	48227/48228	1-bp insertion in noncoding region downstream of *yehA*
Deletion	58087–58489	AAC deleted; causes fusion of *yfhA* and *yfiA*; last 28 aa of ORF are from *yfiA*
Substitution	65886	T-to-G substitution eliminates stop between *traN* and *traZ*; also noted in R100-1 (AF005044)
Deletion	78171–78187	6-aa deletion (PQQPQQ; CCACAACAGCCACAACAG) in repetitive region of *traD*
Insertion	86627–86628	1-bp insertion in noncoding region downstream of *yigA*
Insertion	86648–86649	IS*1a* inserted here; found in R100 but located in region not contained in pC15-1a
Insertion	86640–86648	8-bp insertion site of IS*1a* (GGTGCTATT); duplicated in pC15-1a as direct repeats flanking IS*1a*

[a] Nucleotide substitutions were included only if they affected the length or arrangement of the R100 coding regions. Locations of deletions in repetitive regions are arbitrary.
[b] Based on GenBank accession number AP000342.
[c] Effect of the change at the R100 coordinates on the equivalent region in the pC15-1a R100-derived region. aa, amino acids; ORF, open reading frame.

gene *aac*(*3*)*-II* is found on a 1,466-bp region 100% identical to a region from *Acinetobacter baumannii* (23). Interestingly, in *A. baumannii* the *aac*(*3*)*-II* gene is found downstream from an *intI1* gene that has been disrupted by IS*26*. In pC15-1a, the promoter sequences found in the 5′ conserved region of class 1 integrons that drive expression of inserted cassettes are not present in either region, raising the question of whether the genes are expressed. Each region was found adjacent to a copy of IS*26*, and analysis of the sequence revealed putative hybrid promoter-like elements in which a putative −35 box (TTGCAA) is found in a inverted repeat of IS*26* and a putative −10 box is found 17 bp away in the integron-specific sequence, thus providing a mechanism for expression of the downstream resistance genes (Fig. 3). A similar hybrid promoter involving IS*26* inverted-repeat sequences was previously shown to be involved in expression of *aac*(*3*)*-III* genes (1) and in expression of $bla_{\text{SHV-2a}}$ in a *P. aeruginosa* isolate (14). Isoelectric focusing results indicated that $bla_{\text{OXA-1}}$ was expressed, providing evidence that these promoters are active in pC15-1a. However, expression analysis using primer extension would provide more

TABLE 3. Features and/or open reading frames of the multidrug resistance region of pC15-1a

Location	Feature(s) and/or open reading frame(s)	Gene(s)	% Homology[a]	GenBank accession no.
1–3664	Tn*5403*; transposase, resolvase	*tnpA*, *tnpR*	99.8	X75779
3663–9641	Central core of Tn*1721* (bp 4091–10096); partial transposases, PecM-like transcriptional regulator, tetracycline resistance protein, tetracycline resistance repressor	*tnpA* partial, *pecM*-like, *tetA*, *tetR*	99.4	X61367
9642–10461	IS*26*	*tnpA*	100	AF550679
10462–10499	Partial chloramphenicol *O*-acetyltransferase	*catB3*	100	AJ310778
10718–11548	Beta-lactamase	$bla_{\text{OXA-1}}$	100	AF326777
11679–12278	Aminoglycoside N6′-acetyltransferase	*aac*(*6′*)*-Ib*	100	AY259086
12325–13144	IS*26*	*tpnA*	100	AF550679
13145–13475	Partial chloramphenicol *O*-acetyltransferase	*catB3*	100	AJ310778
13476–14295	IS*26*	*tnpA*	100	AF550679
14339–15199	Aminoglycoside N3′-acetyltransferase	*aac*(*3*)*-II*	100	AY138987
15212–15754	Hypothetical; identical to hypothetical protein AAN34369 in *Acinetobacter baumannii*	hypo	100	AY138987
15825–16157	IS*3* family transposase; *Agrobacterium tumefaciens* C58 OrfA	orfA	95.1 (aa)	NP356057
16154–16894	Partial; putative transposase protein of *Sinorhizobium meliloti*	orfB	89.5 (aa)	NP387207
16896–17715	IS*26*	*tnpA*	99.6	AF550679
17716–19960	Tn*3*-like partial transposase; disrupted by IS*Ecp1*/CTX-M transposition element	*tnpA*	100	AB103092
20353–21228	Beta-lactamase	$bla_{\text{CTX-M-15}}$	100	AY044436
21277–22932	IS*Ecp1*	*tnpA*	100	AY044436
22933–23146	Tn*3*-like partial transposase; disrupted by IS*Ecp1*/CTX-M transposition element	*tnpA*	100	AB103092
23145–25057	Tn*3*-like resolvase, beta-lactamase	*tnpR*, $bla_{\text{TEM-1}}$	99.7	AB103092
25057–27528	Partial transposase, identical to Tn*21 tnpA* from R100	*tnpA*	100	NC_002134
27531–27628	Partial resolvase, identical to Tn*21 tnpR* from R100	*tnpR*	100	NC_002134
27619–28438	IS*26*	*tnpA*	100	AF550679

[a] Unless otherwise indicated, percent homologies are bases on nucleic acid comparisons. Amino acid (aa) homologies were used if they proved to be more informative of gene function. In many cases, numerous BLAST hits were obtained, all with similar degrees of homology. In such cases, only one was selected for reference purposes.

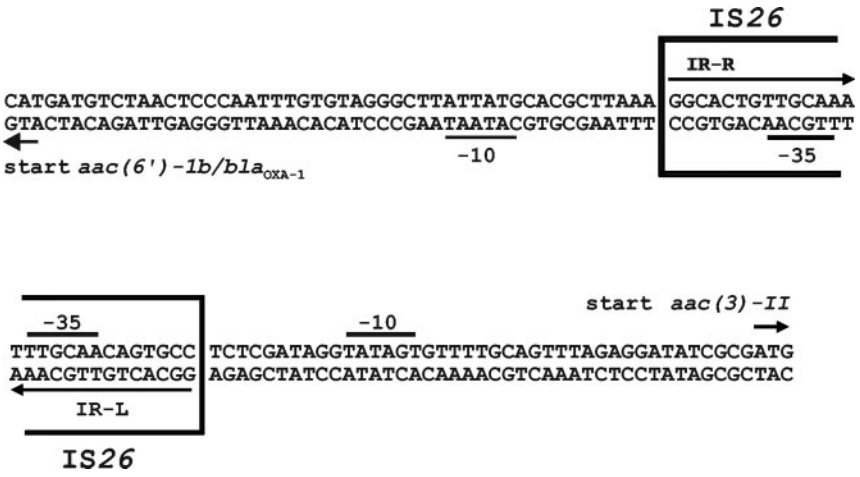

FIG. 3. Putative hybrid promoter regions of *aac*(*6′*)-*Ib*/*bla*$_{OXA-1}$ (A) and *aac*(*3*)-*II* (B) as found in pC15-1a.

direct proof of the involvement of the hybrid promoter elements in resistance gene expression.

The beta-lactamase gene, *bla*$_{CTX-M-15}$, was located downstream of an IS*Ecp1* element, as was found for *bla*$_{CTX-M-15}$ in enterobacterial isolates from Poland and India (4, 11). All 11 isolates harboring *bla*$_{CTX-M-15}$ identified by the Canadian ESBL surveillance project (13) also had this genetic arrangement (data not shown). IS*Ecp1* elements have been identified upstream of other CTX-M-type genes, including *bla*$_{CTX-M-19}$, found in a *K. pneumoniae* isolate from Vietnam (18). Extensive analysis showed that IS*Ecp1* was responsible for mobilization of a transposition unit including itself and *bla*$_{CTX-M-19}$ (18). The mechanism was shown to involve IS*Ecp1* inverted repeat IR-L and a second sequence (IR-RII), downstream of *bla*$_{CTX-M-19}$, that resembled inverted repeat IR-R. In addition, transposition was shown to create a 5-bp duplication of the target sequence. Examination of the sequence downstream of *bla*$_{CTX-M-15}$ in pC15-1a revealed a 22-bp region (IR-RII) with homology to IR-R of IS*Ecp1* (Fig. 2B). Furthermore, IR-L and IR-RII were flanked by 5-bp direct repeats (TATGA), suggesting that probably the same mechanism described for *bla*$_{CTX-M-19}$ was responsible for the location of *bla*$_{CTX-M-15}$ in pC15-1a. These findings provide further evidence to support a mechanism of mobilization of CTX-M genes which are located downstream of the IS*Ecp1* element.

Comparison to the *bla*$_{CTX-M-15}$ plasmid from India. The *bla*$_{CTX-M-15}$ gene was first characterized on a series of large (145- to 155-kb) plasmids from several *Enterobacteriaceae* isolated from patients hospitalized in New Delhi, India (11). Analysis of pC15-1a revealed that *bla*$_{CTX-M-15}$ is located downstream of IS*Ecp1* at the exact location found in an Indian plasmid and that the sequences in these region are 100% identical (data not shown). In addition, each plasmid from India also harbored *bla*$_{TEM-1}$ and *bla*$_{OXA-1}$. Comparison of the pC15-1a HpaI profile to that of pCTX15, isolated from an *E. coli* strain from the Indian outbreak, showed that they shared at least 10 bands. Use of the coordinates of the R100 HpaI sites in the analysis revealed that several bands are very close in size to bands from R100, indicating that pCTX15 may also contain an R100-like backbone as well (Fig. 1). PCR analysis showed that, in addition to the beta-lactamase genes mentioned, pCTX15 also contains *aac*(*6′*)-*Ib* (located upstream of *bla*$_{OXA-1}$), *tetA*, IS*26*, and *tetB*, but *aac*(*3*)-*II* is absent. The presence of *tetB* indicates that pCTX15 likely harbors Tn*10*, thus accounting for some of the band differences, since Tn*10* contains four HpaI sites. Southern hybridization analysis showed that whereas the upstream regions of *bla*$_{CTX-M-15}$ and *bla*$_{TEM-1}$ are found on the same fragment in pC15-1a, they are located on different fragments in pCTX15 (Fig. 1). This result, and the absence of *aac*(*3*)-*II* in pCTX15, likely indicates differences in the corresponding resistance regions of the two plasmids, though sequencing of pCTX15 would be required to precisely define its organization and its relationship to R100.

Conclusions. This is the first report of the complete nucleotide sequence of a plasmid harboring the CTX-M-15 gene. This plasmid has been associated with multiple isolates in Canada at geographically distinct locations. In addition, molecular studies have revealed a possible relationship of the Canadian plasmid with a plasmid associated with outbreaks in India. Although the sequence information did not reveal a mechanism that may explain the broad geographical distribution of this plasmid, the study has highlighted the evolution of a relatively well studied plasmid (R100) into a new variant containing novel antimicrobial resistance elements. In addition, the study has provided support for the transposition theory of CTX-M genes linked to IS*Ecp1*. Further studies are required to determine why this particular ESBL-containing plasmid has been identified in both acute and long-term care settings in Canada.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Jennifer Campbell, Romeo Hizon, Rebekah Murphy, and Darrell Johnstone.

Contributing members of the Canadian Nosocomial Infection Surveillance Program are E. Bryce, Vancouver General Hospital, Vancouver, British Columbia; J. Conly, University of Calgary, Calgary, Alberta; J. Embil and J. Embree, Health Sciences Centre, Winnipeg, Manitoba; M. Gardam, University Health Network, Toronto, Ontario; M. Gourdeau, Hôpital de l'Enfant-Jésus, Quebec City, Quebec; K. Green, Community and Hospital Infection Control Association—Canada, Toronto, Ontario; M. John, St. Joseph's Health Centre, London,

Ontario; B. A. Henderson, Peter Lougheed Centre, Calgary, Alberta; J. Hutchinson, Health Sciences Centre, St. John's, Newfoundland; M. Ishak, Centre Hospitalier Angrignon, Verdun, Quebec; V. Roth, The Ottawa Hospital, Ottawa, Ontario; L. Johnston, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia; J. Langley, I. W. K. Grace Health Science Centre, Halifax, Nova Scotia.; M. Loeb, Hamilton Health Sciences Corp., Hamilton, Ontario; A. Matlow, Hospital for Sick Children, Toronto, Ontario; A. McGeer, Mount Sinai Hospital, Toronto, Ontario; M. Miller, Jewish General Hospital, Montreal, Quebec; D. Moore, Montreal Children's Hospital, Montreal, Quebec; M. Mulvey, Canadian Science Centre for Human and Animal Health, Health Canada, Winnipeg, Manitoba; M. Ofner-Agostini and S. Paton, Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa, Ontario; A. Simor and M. Vearncombe, Sunnybrook and Women's College Health Sciences Centre, Toronto, Ontario; G. Taylor, University of Alberta, Edmonton, Alberta; A. Khan, The Moncton Hospital, Moncton, New Brunswick; K. Weiss, Hôpital Maisonneuve-Rosemont, Montreal, Quebec; A. Wong, Royal University Hospital, Saskatoon, Saskatchewan; and D. Zoutman, Kingston General Hospital, Kingston, Ontario.

REFERENCES

1. **Allmansberger, R., B. Brau, and W. Piepersberg.** 1985. Genes for gentamicin-(3)-*N*-acetyl-transferase III and IV. II. Nucleotide sequences of three AAC(3)-III genes and evolutionary aspects. Mol. Gen. Genet. **198:**514–520.
2. **Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
3. **Ambler, R. P., A. F. Coulson, J. M. Frere, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A beta-lactamases. Biochem. J. **276:**269–270.
4. **Baraniak, A., J. Fiett, W. Hryniewicz, P. Nordmann, and M. Gniadkowski.** 2002. Ceftazidime-hydrolysing CTX-M-15 extended-spectrum beta-lactamase (ESBL) in Poland. J. Antimicrob. Chemother. **50:**393–396.
5. **Bonnet, R., C. Dutour, J. L. Sampaio, C. Chanal, D. Sirot, R. Labia, C. De Champs, and J. Sirot.** 2001. Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-240→Gly. Antimicrob. Agents Chemother. **45:**2269–2275.
6. **Bonnet, R., C. Recule, R. Baradue, C. Chanal, D. Sirot, C. De Champs, and J. Sirot.** 2003. Effect of D240G substitution in a novel ESBL CTX-M-27. J. Antimicrob. Chemother. **52:**29–35.
7. **Bonnet, R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
8. **Bradford, P.** 2001. Extended-spectrum β-lactamases. Clin. Microbiol. Rev. **14:**933–951.
9. **Davies, J. E., and R. Rownd.** 1972. Transmissible multiple drug resistance in *Enterobacteriaceae*. Science **176:**758–768.
10. **Humeniuk, C., G. Arlet, V. Gautier, P. Grimont, R. Labia, and A. Philippon.** 2002. β-Lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. Antimicrob. Agents Chemother. **46:**3045–3049.
11. **Karim, A., L. Poirel, S. Nagarajan, and P. Nordmann.** 2001. Plasmid-mediated extended-spectrum beta-lactamase (CTX-M-3 like) from India and gene association with insertion sequence IS*Ecp1*. FEMS Microbiol. Lett. **201:**237–241.
12. **Muller, M., A. McGeer, B. M. Willey, D. Reynolds, R. Malanczyj, M. Silverman, M. A. Green, and M. Culf.** 2002. Outbreaks of multi-drug resistant *Escherichia coli* in long-term care facilities in the Durham, York, and Toronto regions of Ontario, 2000–2002. Can. Commun. Dis. Rep. **28**:113–118.
13. **Mulvey, M. R., E. Bryce, D. Boyd, M. Ofner-Agostini, S. Christianson, A. E. Simor, S. Paton, The Canadian Hospital Epidemiology Committee, and The Canadian Nosocomial Infection Surveillance Program, Health Canada.** 2004. Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* spp. in Canadian hospitals. Antimicrob. Agents Chemother. **48:**1204–1214.
14. **Naas, T., L. Philippon, L. Poirel, E. Ronco, and P. Nordmann.** 1999. A SHV-derived extended-spectrum beta-lactamase in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **43:**1281–1284.
15. **Nakaya, R., A. Nakamura, and Y. Murata.** 1960. Resistance transfer agents in *Shigella*. Biochem. Biophys. Res. Commun. **3:**654–659.
16. **NCCLS.** 2000. Methods for disk susceptibility tests for bacteria that grow aerobically. NCCLS approved standard. NCCLS M7-A5. NCCLS, Wayne, Pa.
17. **Nuesch-Inderbinen, M. T., H. Hachler, and F. H. Kayser.** 1996. Detection of genes coding for extended-spectrum SHV beta-lactamases in clinical isolates by a molecular genetic method, and comparison with E test. Eur. J. Clin. Microbiol. Infect. Dis. **15:**398–402.
18. **Poirel, L., J.-W. Decousser, and P. Nordmann.** 2003. Insertion sequence IS*Ecp1B* is involved in expression and mobilization of a bla_{CTX-M} β-lactamase gene. Antimicrob. Agents Chemother. **47:**2938–2945.
19. **Poirel, L., M. Gniadkowski, and P. Nordmann.** 2002. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum beta-lactamase CTX-M-15 and of its structurally related beta-lactamase CTX-M-3. J. Antimicrob. Chemother. **50:**1031–1034.
20. **Poirel, L., P. Kampfer, and P. Nordmann.** 2002. Chromosome-encoded Ambler class A β-lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **46:**4038–4040.
21. **Rutherford, K., J. Parkhill, J. Crook, T. Horsnell, P. Rice, M.-A. Rajandream, and B. Barrell.** 2000. Artemis: sequence visualisation and annotation. Bioinformatics **16:**944–945.
22. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Segal, H., R. Thomas, and B. G. Elisha.** 2003. Characterization of class 1 integron resistance gene cassettes and the identification of a novel IS-like element in *Acinetobacter baumannii*. Plasmid **49:**169–178.
24. **Speldooren, V., B. Heym, R. Labia, and M. H. Nicolas-Chanoine.** 1998. Discriminatory detection of inhibitory-resistant β-lactamases in *Escherichia coli* by single-strand conformation polymorphism-PCR. Antimicrob. Agents Chemother. **42:**879–884.
25. **Staden, R., K. F. Beal, and J. K. Bonfield.** 2000. The Staden package, 1998. Methods Mol. Biol. **132:**115–130.
26. **Swaminathan, B., T. Barrett, S. B. Hunter, and R. Tauxe.** 2001. PulseNet; the molecular subtyping network for foodborne disease surveillance in the United States. Emerg. Infect. Dis. **7:**382–389.
27. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
28. **Tzouvelekis, L. S., E. Tzelepi, P. T. Tassios, and N. J. Lagakis.** 2000. CTX-M-type β-lactamases: an emerging group of extended-spectrum enzymes. Int. J. Antimicrob. Agents **14:**137–142.
29. **Wang, M., J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, and D. C. Hooper.** 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. **47:**2242–2248.