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Complete Nucleotide Sequence of Plasmid pTN48, Encoding the CTX-M-14 Extended-Spectrum β-Lactamase from an *Escherichia coli* O102-ST405 Strain▽

Typhaine Billard-Pomares,[1,2,3] Olivier Tenaillon,[2,3] Hervé Le Nagard,[4,5] Zoé Rouy,[6] Stéphane Cruveiller,[6] Claudine Médigue,[6] Guillaume Arlet,[7,8] Erick Denamur,[2,3] and Catherine Branger[1,2,3]*

AP-HP, Hôpital Louis Mourier, Service de Microbiologie-Hygiène, Colombes, France[1]; INSERM, UMR-S 722, Paris F-75018, France[2]; Université Paris Diderot, UMR-S 722, Faculté de Médecine, Site Xavier Bichat, Paris F-75018, France[3]; INSERM, UMR-S 738, Paris F-75018, France[4]; Université Paris Diderot, UMR-S 738, Paris F-75018, France[5]; Laboratoire de Génomique Comparative, CNRS UMR 8030, Institut de Génomique, CEA Genoscope, Evry, France[6]; Université Pierre et Marie Curie-Paris 6, Faculté de Médecine, Site Saint-Antoine, Laboratoire de Bactériologie, ER8, Paris, France[7]; and AP-HP, Hôpital Tenon, Service de Bactériologie-Hygiène, Paris, France[8]

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The sequence of pTN48, a plasmid of the FII-FIB replicon type that encodes a CTX-M-14 enzyme in an *Escherichia coli* strain of the phylogenetic group D_2 O102-ST405 clone, was determined. pTN48 is, for the most part, a mosaic of virulence, antibiotic resistance, and addiction system modules found in various other plasmids. The presence of multiple addiction systems indicates that the plasmid should be stably maintained in the *E. coli* clone, favoring dissemination of the CTX-M-14 enzyme.

The epidemiology of extended-spectrum beta-lactamases (ESBLs) has drastically changed in recent years. An explosive spread of CTX-M-type enzymes, with *Escherichia coli* as the main host, has occurred in both hospital and community settings worldwide (4, 23). Two phenomena may explain such an epidemic profile: the spread of plasmids bearing antibiotic resistance genes between bacterial strains and the spread of bacterial clones bearing resistance-encoding plasmids. Recently, the application of multilocus sequence typing revealed that a few *E. coli* clones with the ability to capture a large panel of ESBLs have disseminated internationally (5, 7, 14, 16, 18, 19). Furthermore, it has been shown that two of these clones, the O25b sequence type 131 (ST131) clone of the B2 phylogenetic group and the O102-ST405 clone of the D_2 phylogenetic group, were highly virulent in a mouse model of septicemia (6, 16). The dissemination of such resistant and virulent clones constitutes a major public health concern and prompted us to examine ESBL-encoding plasmids associated with these clones. We therefore sequenced pTN48, a nonconjugative plasmid of the FII-FIB replicon type carrying a CTX-M-14 ESBL gene and originating from a strain of the *E. coli* D_2 phylogenetic subgroup I O102-ST405 clone (8). Strain TN48 was isolated from an adult patient with a urinary tract infection in 2004 in Paris and was resistant to ciprofloxacin, streptomycin, kanamycin, gentamicin, tobramycin, netilmicin, tetracycline, nalidixic acid, chloramphenicol, trimethoprim, and sulfonamides; the DH10B electroporant containing pTN48 (DH10B/pTN48) displayed the same resistance phenotype, except that it remained susceptible to ciprofloxacin.

The plasmid DNA was extracted from the electroporant (8) by using the Qiagen Large Construct kit (Qiagen, Courtaboeuf, France), and Solexa technology was used for sequencing. The reads generated were assembled *de novo* into 38 contigs with VELVET software (28). Combinatorial PCRs were used to assemble the contigs and to fill in gaps. MaGe (Magnifying Genomes) software was used for gene annotation and comparative analysis as described elsewhere (26). Manual validation of the automatic annotation was performed using the MaGe interface.

Plasmid pTN48 is a circular molecule of 165,657 bp (overall G+C content of 50.21%) harboring 194 predicted open reading frames (ORFs); 134 were assigned known functions. Twelve unique ORFs corresponding to about 18,000 bp had no homolog in public databases and thus could be considered specific to this plasmid. Thirty-six coding sequences as part of 25 insertion sequences, in particular, IS*1* and IS*26*, were found throughout the plasmid. pTN48 can be divided into three functional modules that are involved in antimicrobial resistance, virulence, and plasmid transfer and maintenance (Fig. 1).

Antibiotic resistance is encoded within a continuous region of 42,794 bp (Fig. 2) divided into six subregions sharing strong homology with different plasmids by five copies of IS*26* (the third one being truncated). The first subregion of 1,600 bp encompassed $bla_{TEM\text{-}1b}$ associated with a remnant of transposon Tn*2*. The second subregion, a 12,000-bp region composed of a class 1 integron (*dfrA17*, *aadA5*, *qacEΔ1*, *sul1*), *chrA*, *padR*, IS*6100*, *mphA*, *mrx*, and *mphR* showed >99% similarity to a CTX-M-15-encoding plasmid, pEK499 (27). The third subregion, a 9,800-bp sequence, showed >99% similarity to a *Klebsiella pneumoniae* CTX-M-19-encoding plasmid, pILT-3 (24). It included *orf1* of Tn*1721*, into which were inserted

* Corresponding author. Mailing address: Service de Microbiologie et Hygiene, Hôpital Louis Mourier, 178 rue des Renouillers, 92701 Colombes Cedex, France. Phone: 33 147 606 010. Fax: 33 147 606 048. E-mail: catherine.branger@lmr.aphp.fr.

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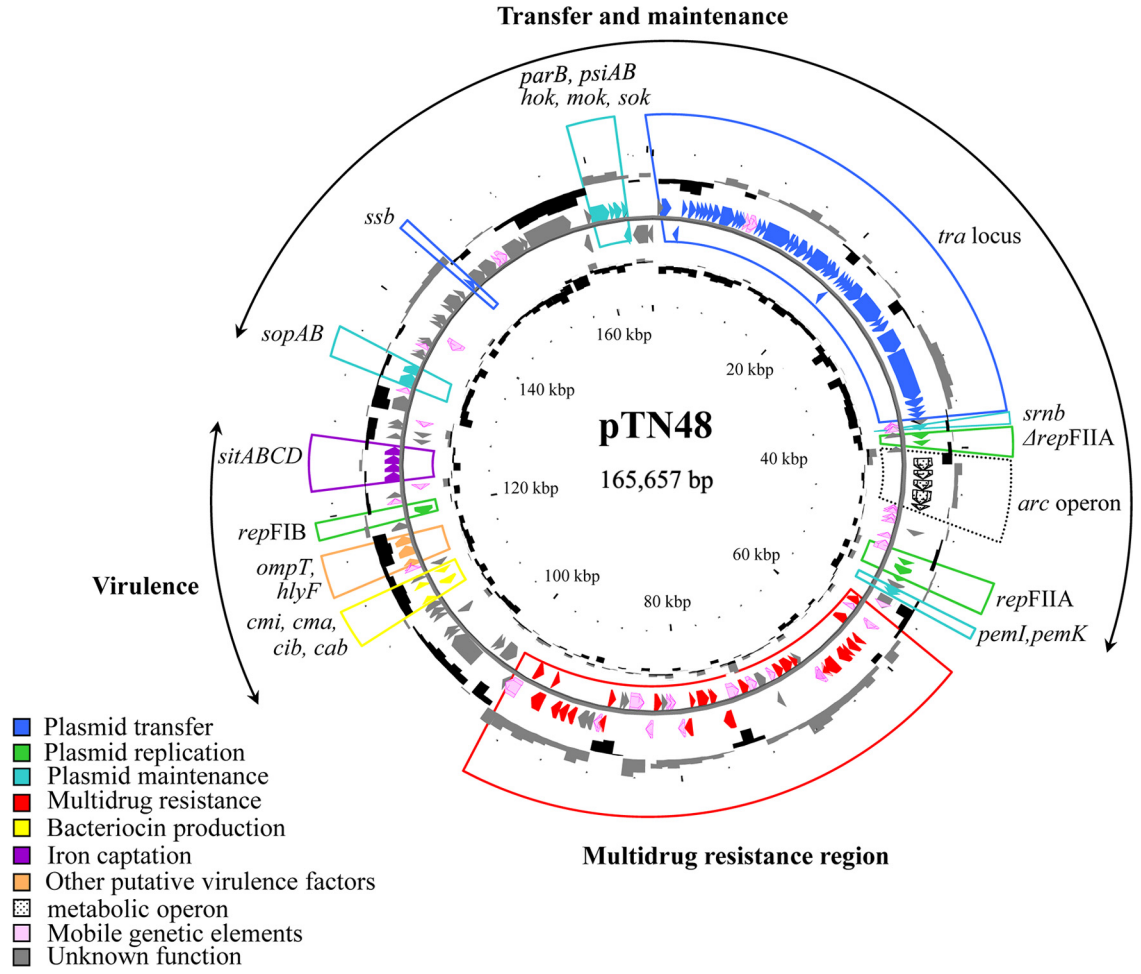


FIG. 1. Circular representation of plasmid pTN48. The circles display (from the outside) (i) GC percent deviation (GC window − mean GC) in a 1,000-bp window, (ii) predicted coding sequences transcribed in a clockwise direction, (iii) predicted coding sequences transcribed in a counterclockwise direction, (iv) GC skew ([G + C]/[G − C]) in a 1,000-bp window, and (v) coordinates in kilobase pairs from the origin of replication. The color code for the various gene functions is shown below the map.

$bla_{CTX-M-14}$ and its environment (IS*903* and IS*Ecp1B*), and a second class 1 integron, in inverted orientation with respect to the previous one, containing *aacA4* and *cmlA*. The next subregion, a 3,200-bp sequence containing *aac*(*3*)-*II*, was found to be identical to a CTX-M-15-encoding plasmid, pC15-1a (2). The fifth subregion was a 5,000-bp sequence containing an IS*CR14*-like element associated with truncated *groEL* and *ermBC* (3, 25). Finally, the last subregion, an 11,000-bp sequence, can be divided into two parts, a first part containing *repA* and *repC* of the *repABC* operon, an antisense RNA reg-

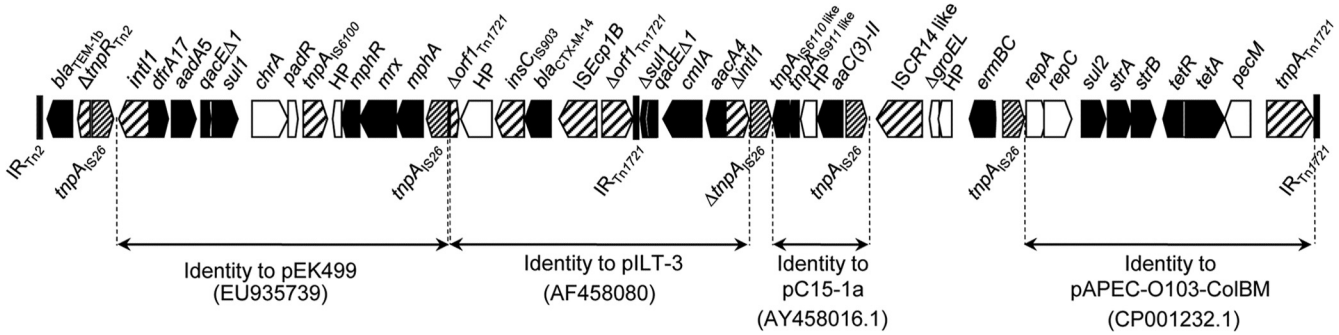
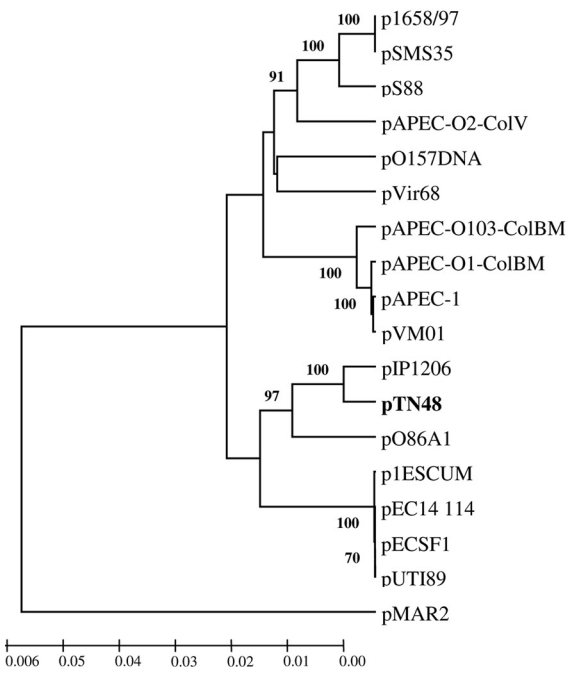


FIG. 2. Schematic representation of the antibiotic resistance region of plasmid pTN48. Black arrows represent resistance genes, hatched black arrows represent mobile genetic elements, and white arrows represent the other genes or hypothetical-protein-encoding genes. Truncated genes are indicated by a Δ symbol. Vertical bars indicate the inverted 38-bp repeats of Tn*2* and Tn*1721*.



	Virulence genes				
	hlyF	*ompT*	*mig14*	*sitABCD*	*colBM*
p1658/97	+	+	+	-	-
pSMS35	+	+	+	+	+
pS88	+	+	+	+	-
pAPEC-O2-ColV	+	+	+	+	-
pO157DNA	-	+	-	-	-
pVir68	-	+	-	-	-
pAPEC-O103-ColBM	+	-	+	+	+
pAPEC-O1-ColBM	+	+	+	+	+
pAPEC-1	+	+	+	+	-
pVM01	+	+	+	+	+
pIP1206	-	-	-	-	-
pTN48	+	+	+	+	+
pO86A1	-	-	-	-	-
p1ESCUM	-	-	-	-	-
pEC14 114	-	-	-	-	-
pECSF1	-	-	-	-	-
pUTI89	-	-	-	-	-
pMAR2	-	-	-	-	-

FIG. 3. Phylogenetic tree of 18 FIIA-FIB *E. coli* backbone plasmids reconstructed from the concatenated DNA sequences of five genes (*repA1*, *repA*, *traM*, *traX*, and *finO*) by the UPGMA. Bootstrap values exceeding 70% are indicated at the nodes. The DNA sequences of plasmids p1658/97 (accession no. AF550679.1), pAPEC-O2-ColV (AY545598.5), pVM01 (EU330199.1), pSMS35_130 (CP000971.1), pECOS88 (CU928146.1), pAPEC-O103-ColBM (CP001232.1), pAPEC-1 (CP000836.1), pUTI89 (CP000244.1), pMAR2 (FM180569.1), pVir68 (CP001162.1), pO157 DNA (AB011549.2), pEC14_114 (GQ398086.1), pO86A1 DNA (AB255435.1), pECSF1 DNA (AP009379.1), p1ESCUM (CU928148.1), pIP1206 (AM886293.1), and pAPEC-O1-ColBM (DQ381420.1) were extracted from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/), but that of plasmid pTN48 (in bold) was not. The presence of virulence genes is indicated at the right by plus signs.

ulating system, *sul2*, and *strAB*, and a second part containing *tetR*, *tetA*, *pecM*, and *tnpA* of Tn*1721*. Both parts are similar (>99% identity) to a region of plasmid pAPEC-O103-ColBM, but the second part is in the reverse orientation (12). The resistance phenotypes of the TN48 and DH10B/pTN48 strains were consistent with the antibiotic resistance genes identified. Two kinds of macrolide resistance-mediating genes were also identified, *mphA*, a gene that is commonly present in *Enterobacteriaceae*, and the *ermBC* genes, which are common in streptococci but rarely isolated in *E. coli* (22).

The virulence region of approximately 17,000 bp contained a subset of virulence factor coding genes found on ColBM plasmids (12, 13). This region had >99% identity and conserved synteny with the multidrug resistance plasmid pSMS35_130 (9). These included, in order, the *colBM* gene cluster, *ompT*, *hlyF*, *mig-14*, and *sitABCD*. To assess the role of these plasmid-borne virulence genes, we tested the extraintestinal virulence of TN48 and DH10B/pTN48 in a mouse model of septicemia as described in reference 10. The TN48 strain was highly virulent, as it killed all 10 of the inoculated mice, as did highly virulent control strain CFT073 (10), whereas strain DH10B/pTN48 was not virulent, as it did not kill any of the 10 mice inoculated, as did the two commensal derived K-12 strains MG1655 (10) and DH10B. This indicates that the plasmid by itself is not able to transform, in our model, an avirulent strain into a virulent strain.

The replication-and-maintenance region contained a complete transfer locus of 33,264 bp composed of 24 *tra* genes, 9 *trb* genes, *artA*, and *finO*. This region was similar in a conserved synteny (98 to 99% identity) to pAPEC-O1-ColBM (11), pSMS35_130 (9), and pIP1206 (21). However, the *traV* gene, implicated in pore construction, was truncated at the 5′ end by the insertion of IS*629*. This truncation could putatively impair conjugation efficiency, which is consistent with the observation that pTN48 was not transferable by conjugation *in vitro* (8). In addition, pTN48 carried several plasmid maintenance and partitioning modules (*srnB*, *pemI pemK*, *hok mok sok*, *parB*, and *sopAB*), ensuring stable plasmid inheritance. Actually, the *E. coli* TN48 and DH10B/pTN48 strains were not cured of the plasmid by sodium dodecyl sulfate (20) or novobiocin (15) treatment or after 200 generations in batch cultures without antibiotic pressure.

Plasmid pTN48 has several replicons. A first 17,253-bp region contains two copies of *rep*FII (a and b) separated by an *arcABCD* gene cluster encoding proteins involved in the arginine deiminase pathway (1); this region was 99% identical to the FII region of pIP1206 (21). In addition, *rep*FIB was located downstream of the *ompT hlyF mig-14* virulence region.

To assess the phylogenetic history of the pTN48 backbone, a tree was built as described in reference 12, by using the concatenated gene sequences of conserved regions, *traM*, *traX*, *finO*, *repA1* (FII), and *repA* (FIB), from 18 *E. coli* plasmids of the FIIA-FIB replicon type. The phylogenetic tree generated by the unweighted-pair group method using average linkages (UPGMA) showed that pTN48 did not cluster with the plasmids that share its virulence region, pAPEC and related plasmids (12), which include pS88, a plasmid of *E. coli* neonatal meningitis (20), and pSMS35 (9), but clustered with plasmids lacking this region, such as pIP1206 (21) (Fig. 3). This suggests that acquisition of the virulence region can occur on FIIA-FIB plasmids with distinct phylogenetic backgrounds.

In conclusion, pTN48 is a mosaic of antibiotic resistance, virulence, and addiction system modules which appeared to have evolved through stepwise events of integration and/or recombination of DNA modules from various virulence or resistance plasmids. This suggests a high rate of gene flow between bacteria harboring these public-health-threatening plasmids. Yet, similar to the CTX-M-15-encoding F plasmids in O25b-ST131 strains described by Woodford et al. (27), the presence of numerous addiction systems in pTN48 should contribute to plasmid maintenance and therefore multidrug resistance and CTX-M enzyme dissemination (17).

Nucleotide sequence accession number. The nucleotide sequence of pTN48 has been submitted to the EMBL/GenBank database under accession number FQ482074.

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