Complete nucleotide sequence of plasmid pTN48 encoding the CTX-M-14 extended spectrum \(\beta\)-lactamase from an *Escherichia coli* O102-ST405 strain

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Running title: Sequence of plasmid pTN48 encoding \(\text{bla}_{\text{CTX-M-14}}\)

Key words: CTX-M-14, plasmid, *Escherichia coli*, O102-ST405

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Abstract

The sequence of pTN48, a plasmid of FII-FIB replicon type that encodes CTX-M-14 enzyme in a *Escherichia coli* strain of the phylogenetic group D, 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 the dissemination of CTX-M-14 enzyme.
The epidemiology of extended-spectrum beta-lactamases (ESBLs) has drastically changed in the recent years. An explosive spread of CTX-M type enzymes with *Escherichia coli* as the main host has occurred both in hospital and community settings worldwide (4, 23). Two phenomena may explain such an epidemic profile: the spread of plasmids bearing the antibiotic resistance genes between bacterial strains and the spread of bacterial clones bearing the resistance-encoding plasmids. Recently the application of multilocus sequence typing (MLST) revealed that a few *E. coli* clones with 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 (ST) 131 clone of B2 phylogenetic group, and the O102-ST405 clone of D2 phylogenetic group, were highly virulent in a mouse model of septicemia (6, 16). The dissemination of such resistant and virulent clones constitutes a major concern for public health and prompted us to examine ESBL-coding plasmids associated to these clones. We therefore sequenced pTN48, a non-conjugative plasmid of FII-FIB replicon type carrying a CTX-M-14 enzyme and originating from a strain of the *E. coli* D2 phylogenetic subgroup I O102-ST405 clone (8). Strain TN48 was isolated from a urinary tract infection in an adult patient in 2004 in Paris and was resistant to ciprofloxacin, streptomycin, kanamycin, gentamicin, tobramycin, netilmicin, tetracycline, nalidixic acid, chloramphenicol, trimethoprim and sulfonamide; 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 the 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 165,692 bp (overall G + C content of 50.21%), harbouring 194 predicted open reading frames (ORFs); 134 were assigned to known functions. Twelve unique ORFs corresponding to about 18,000 bp had no homolog in public databases and thus could be considered specific of this plasmid. Thirty six coding sequences as part of 25 ISs, in particular IS1 and IS26 were found throughout the plasmid.

pTN48 can be divided in 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) that 5 copies of IS26 (the third one being truncated) partitioned in six subregions sharing strong homologies with different plasmids. The first subregion of 1,600bp encompassed blaTEM-1b associated with a remnant of Tn2 transposon. The second subregion, a 12,000 bp region composed of a class 1 integron (dfrA17, aadA5, qacEA1, sul1), chrA, padR, IS6100 and mphA, mrx, mphR, showed >99% similarity with a CTX-M-15-encoding plasmid, pEK499 (27). The third subregion, a 9,800 bp sequence, showed >99% similarity with a Klebsiella pneumoniae CTX-M-19-encoding plasmid, pILT-3 (24). It included orf1 of Tn1721 in which was inserted blaCTX-M-14 and its environment (IS903 and ISEcp1B) and a second class 1 integron, in inverted orientation with the previous one, containing aacA4 and cmlA. The next 3,200 bp subregion, containing aac(3)-II was found identical with a CTX-M-15-encoding plasmid, pC15-1a (2). The fifth subregion was a 5,000 bp sequence containing a ISCR14 like element associated with a truncated groEL and ermBC (3, 25).

Finally, the last 11,000 bp subregion can be divided in two parts, a first part containing repA and repC of the repABC operon, an antisense RNA regulating system, sul2, strAB and a second part containing tetR, tetA, pecM and tnpA of Tn1721. Both parts are similar (> 99% identity) to a region of plasmid pAPEC-O103-ColBM, but the second part being in
reverse orientation (12). The resistance phenotypes of the TN48 and DH10B-pTN48 strains were consistent with the antibiotic resistant genes identified. Two kinds of macrolide resistance mediating genes were also identified, *mphA* gene which is commonly present in *Enterobacteriaceae* and *ermBC* genes which are common in *Streptococci* but scarcely 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 a respected synteny with the multidrug resistant plasmid pSMS35_130 (9). These included in order the *colBM* gene cluster, *ompT*, *hlyF*, *mig-I4* and *sitABC*. 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 in (10). The TN48 strain was highly virulent as it killed all ten of the inoculated mice as did the highly virulent control strain CFT073 (10), whereas DH10B-pTN48 strain was not virulent as it did not kill any mouse over the 10 inoculated as 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 (98-99% identity) to pAPEC-O1-ColBM (11), pSMS35_130 (9) and pIP1206 (21). However, the *traV* gene, implicated in the pore construction, was truncated at the 5’ extremity by the insertion of an IS629. This truncation putatively could impair the 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*, *sopAB*) ensuring stable plasmid inheritance. Actually, we could not cure the plasmid from the *E. coli* TN48 and DH10B-pTN48 strains, using sodium dodecyl sulphate (20) or novobiocin (15) treatments or after 200 generations in batch cultures without antibiotic pressure.
Plasmid pTN48 has several replicons. A first 17,253 bp region contains two repFII (a and b) separated by an arcABC gene cluster encoding proteins involved in the arginine deiminase pathway (1), this region had 99% identity with the FII region of pIP1206 (21). In addition, repFIB was located downstream the ‘ompT-hlyF-mig-14’ virulence region.

To assess the phylogenetic history of pTN48 backbone, a tree was built as in (12) using the concatenated gene sequences of conserved regions, traM, traX, finO, repA1 (FII) and repA (FIB) from 18 E. coli plasmids of FIIA-FIB replicon type. The phylogenetic tree generated by the UPGMA method showed that pTN48 was not clustered with the plasmids that share its virulence region, the pAPEC and their 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 the 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 virulent or resistant plasmids. This suggests a high gene flow between bacteria harboring these public health threatening plasmids. Yet, similarly to the CTX-M-15-encoding F plasmids in O25b-ST131 strains described by Woodford (27), the presence of numerous addiction systems in pTN48 should contribute to the plasmid maintenance and therefore the multiresistance and the CTX-M enzyme dissemination (17).

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


**Figure legends**

**Fig. 1. Circular representation of plasmid pTN48.** Circles display (from the outside): (1) GC percent deviation (GC window - mean GC) in a 1,000-bp window, (2) predicted coding sequences (CDSs) transcribed in the clockwise direction, (3) predicted CDSs transcribed in the counter-clockwise direction, (4) GC skew (G+C/G-C) in a 1,000-bp window, (5) coordinates in kilobase pairs (kbp) from the origin of the replication. The colour code for the various gene functions is indicated below the map.

**Fig. 2. Schematic representation of the antibiotic resistant 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 (HP) genes. Truncated genes are indicated by a Δ symbol. Vertical bars indicate the inverted 38 bp repeats of Tn2 and Tn1721.

**Fig. 3. Phylogenetic tree of 18 FIIA-FIB E. coli backbone plasmids reconstructed from the concatenated DNA sequences of 6 genes (repA1, repA, traM, traX and finO) using the UPGMA method.** Bootstrap values exceeding 70% are indicated at the nodes. DNA sequences were extracted from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) except for plasmid pTN48 (in bold): p1658/97 (AF550679.1), pAPEC-O2-ColV (AY545598.5), pVM01 (EU330199.1), plasmid 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), pAPEC-O1-ColBM (DQ381420.1). The presence of the virulence genes is indicated in the right part of the figure by a + sign.
Fig. 3

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