**Klebsiella pneumoniae** Multiresistance Plasmid pMET1: Similarity with the *Yersinia pestis* Plasmid pCRY and Integrative Conjugative Elements

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**Abstract**

**Background:** Dissemination of antimicrobial resistance genes has become an important public health and biodefense threat. Plasmids are important contributors to the rapid acquisition of antibiotic resistance by pathogenic bacteria.

**Principal Findings:** The nucleotide sequence of the *Klebsiella pneumoniae* multiresistance plasmid pMET1 comprises 41,723 bp and includes Tn1331.2, a transposon that carries the blaTEM1 gene and a perfect duplication of a 3-kbp region including the aac(6')-Ib, aadA1, and blaOXA-9 genes. The replication region of pMET1 has been identified. Replication is independent of DNA polymerase I, and the replication region is highly related to that of the cryptic *Yersinia pestis* 91001 plasmid pCRY. The potential partition region has the general organization known as the parFG locus. The self-transmissible pMET1 plasmid includes a type IV secretion system consisting of proteins that make up the mating pair formation complex (Mpf) and the DNA transfer (Dtr) system. The Mpf is highly related to those in the plasmid pCRY, the mobilizable high-pathogenicity island from *E. coli* ECOR31 (HPICOR31), which has been proposed to be an integrative conjugative element (ICE) progenitor of high-pathogenicity islands in other *Enterobacteriaceae* including *Yersinia* species, and ICEKp1, an ICE found in a *K. pneumoniae* strain causing primary liver abscesses. The Dtr MobB and MobC proteins are highly related to those of pCRY, but the endonuclease is related to that of plasmid pK245 and has no significant homology with the protein of similar function in pCRY. The region upstream of mobB includes the putative oriT and shares 90% identity with the same region in the HPICOR31.

**Conclusions:** The comparative analyses of pMET1 with pCRY, HPICOR31, and ICEKp1 show a very active rate of genetic exchanges between *Enterobacteriaceae* including *Yersinia* species, which represents a high public health and biodefense threat due to transfer of multiple resistance genes to pathogenic *Yersinia* strains.

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**Introduction**

Plasmids are important contributors to the rapid acquisition of antibiotic resistance by pathogenic bacteria through their ability to acquire resistance genetic determinants and to transfer them among bacteria belonging to the same or different genera and species [1,2]. These resistance genes are usually located inside transposable elements, integrons, or ISCRs (insertion sequence common regions), which facilitate their mobility at the molecular level and at least in the case of transposons could help expanding plasmid’s host range [1,3,4,5]. In the past few years, considerable efforts have been made to completely sequence resistance plasmids and understand their biological properties [1].

*K. pneumoniae*, an important opportunistic pathogen, is the causative agent of community-acquired infections and more frequently of infections in the urinary tract and soft tissue, pneumonia, septicemia, and meningitis in hospitalized patients [6]. Hospital outbreaks caused by multiresistant *K. pneumoniae* strains have been described throughout the world [7,8]. It has been proposed that Crohn’s disease as well as ankylosing spondylitis are linked to *Klebsiella* infection possibly through the mechanism of molecular mimicry [9]. *K. pneumoniae* has also been identified as the causative agent of liver abscesses [10]. However, *K. pneumoniae* infections are especially dangerous in neonatal wards [6]. With the rise in the number of strains harboring resistance genes, usually plasmid-borne, the mortality of *K. pneumoniae* infections has increased and treatment has become more complicated [11,12,13]. A multiresistant *K. pneumoniae* isolate that caused high mortality in a neonatal ward harbors the self-transmissible plasmid pMET1, which carries the genetic determi-
| ORF | Position | G+C Content (%) | Protein (aa) | Best Blast-X Hit | $E$ value | Identity/ similarity | Accession No. |
|-----|----------|----------------|-------------|------------------|-----------|---------------------|--------------|
| 1   | 1,387–674| 45             | 238 Putative RepA protein [pCRY, Yersinia pestis biovar Microtus str. 91001] | 2e-119 | 89/95 | NP_995415 |
| 2   | 2,198–1,839| 42.6          | 118 Putative transcriptional regulator [pCRY, Yersinia pestis biovar Microtus str. 91001] | 2e-42 | 72/84 | NP_995417 |
| 3   | 2,451–2,906| 48.7          | 151 Transcription antiterminator [pCRY, Yersinia pestis biovar Microtus str. 91001] | 2e-83 | 99/100 | NP_995418 |
| 4   | 3,534–4,244| 57.3          | 236 Type IV secretory pathway VirB1 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 2e-132 | 99/99 | NP_995421 |
| 5   | 4,241–4,546| 56.2          | 101 Type IV secretory pathway VirB2 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 9e-31 | 98/99 | NP_995422 |
| 6   | 4,559–7,297| 52.8          | 912 PI3x-4/VirB3-4-like protein [Escherichia coli] | 0.0 | 99/100 | AAP70302 |
| 7   | 7,316–8,026| 49.2          | 236 Type IV secretion system VirB5 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 2e-98 | 84/90 | NP_995424 |
| 8   | 8,275–9,351| 46.4          | 358 Type IV secretory pathway VirB6 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 1e-155 | 87/95 | NP_995426 |
| 9   | 9,443–9,980| 57.4          | 45 Hypothetical protein [pCRY, Yersinia pestis biovar Microtus str. 91001] | 9e-13 | 91/91 | AE017044.1 |
| 10  | 9,620–10,255| 55.4          | 211 Type IV secretion system VirB8 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 4e-105 | 99/100 | NP_995427 |
| 11  | 10,252–11,160| 56.9          | 302 Type IV secretion pathway VirB9 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 1e-170 | 99/100 | NP_995428 |
| 12  | 11,201–12,475| 57.4          | 424 Type IV secretion pathway VirB10 component [pCRY, Yersinia pestis biovarMicrotus str. 91001] | 0.0 | 96/97 | NP_995429 |
| 13  | 12,465–13,490| 47.6          | 341 Type IV secretion pathway VirB11 component [pCRY, Yersinia pestis biovar Microtus str. 91001] | 0.0 | 99/100 | NP_995430 |
| 14  | 13,487–13,885| 46.5          | 132 Hypothetical protein YF_pCRY17 [pCRY, Yersinia pestis biovar Microtus str. 91001] | 8e-73 | 98/100 | NP_995431 |
| 15  | 13,921–14,226| 42.4          | 101 Hypothetical protein YF_pCRY18 [pCRY, Yersinia pestis biovar Microtus str. 91001] | 3e-52 | 99/99 | NP_995432 |
| 16  | 14,251–14,565| 40            | 104 Putative dpa decarboxylase protein remnant [Yersinia pestis biovar Microtus str. 91001] | 8e-41 | 96/97 | NP_995433 |
| 17  | 15,331–17,082| 39.8          | 583 Putative mobilization MobB protein [pCRY, Yersinia pestis biovar Microtus str. 91001] | 0.0 | 99/99 | NP_995436 |
| 18  | 17,079–17,846| 48.7          | 255 Putative mobilization protein MobC [pCRY, Yersinia pestis biovar Microtus str. 91001] | 9e-144 | 99/100 | NP_995437 |
| 19  | 17,949–18,749| 54.2          | 168 Putative endonuclease [pK245, Klebsiella pneumoniae] | 2e-54 | 62/76 | ABG56794 |
| 20  | 19,631–18,663| 53.3          | 320 Antirestriction protein ArdC [p29930, Yersinia enterocolitica] | 3e-152 | 86/92 | CAD58578 |
| 21  | 20,016–20,351| 33.7          | 112 Hypothetical protein AmetDRAFT_2972 [Alkaliphilus metallirediges genes QYMF] | 2e-14 | 39/86 | ZP_00799598 |
| 22  | 20,348–20,680| 31.1          | 111 Hypothetical protein AmetDRAFT_2972 [Alkaliphilus metallirediges genes QYMF] | 2e-05 | 43/60 | ZP_00799598 |
| 23  | 21,353–21,060| 45.8          | 99 Transcriptional regulator [Escherichia coli] | 5e-34 | 72/86 | CAH19146 |
| 24  | 21,706–21,356| 47.8          | 117 Hypothetical protein [Escherichia coli] | 1e-43 | 71/84 | CAH19145 |
| 25  | 22,100–22,717| 45.8          | 205 Plasmid partitioning protein ParF [p29930, Yersinia enterocolitica] | 3e-84 | 77/90 | CAD58556 |
| 26  | 22,772–23,011| 47.3          | 79 Partitioning protein ParG[p29930, Yersinia enterocolitica] | 9e-16 | 53/70 | CAD58557 |
| 27  | 24,886–23,630| 46.8          | 417 Hypothetical protein V12B01_09786 [Vibrio splendidus 12B01] | 6e-134 | 59/74 | ZP_00991171 |
| 28  | 26,905–24,890| 46.6          | 675 Hypothetical protein V12B01_09791 [Vibrio splendidus 12B01] | 0.0 | 73/84 | ZP_00991172 |
| 29  | 24,886–23,630| 46.8          | 892 Hypothetical protein V12B01_09796 [Vibrio splendidus 12B01] | 0.0 | 67/80 | ZP_00991173 |
| 30  | 30,731–29,871| 50.3          | 286 Beta-lactamase precursor (TEM-1)* [pJHCMW1, Tn1331, Klebsiella pneumoniae] | 2e-152 | 100/100 | NP_608310 |
| 31  | 32,270–31,431| 50.3          | 279 Beta-lactamase precursor * (Penicillinsae) (Oxacillinase) (OXA-9) | 1e-157 | 100/100 | NP_608309 |
| 32  | 33,103–32,315| 53.6          | 262 Adenylyltransferase [pJHCMW1, Tn1331, Klebsiella pneumoniae] | 6e-145 | 99/100 | NP_608308 |
| 33  | 33,778–33,173| 54.1          | 201 Aminoglycoside 6’-N-acetyltransferase type Ib [AAC(6’)-Ib] * [pJHCMW1, Tn1331, Klebsiella pneumoniae] | 1e-112 | 100/100 | NP_608307 |
| 34  | 35,317–34,478| 50.3          | 279 Beta-lactamase precursor * (Oxacillinase, OXA-9) [pJHCMW1, Tn1331, Klebsiella pneumoniae] | 1e-157 | 100/100 | NP_608309 |
nents for resistance to several aminoglycosides and β-lactams [12]. In this paper we report the complete sequence and analysis of pMET1, and a comparative study with the Yersinia pestis cryptic plasmid pCRY, the mobilizable high-pathogenicity island found in Escherichia coli strain ECOR31 [HPIECOR31], an integrative conjugative element (ICE) that is a possible progenitor of high-resistance genes to pathogenic agents of high public health and biodefense threat due to transfer of multiple resistance genes to pathogenic Yersinia strains.

Results and Discussion

The 41,723 bp multiresistance plasmid pMET1 (GenBank accession number EU383016) includes the replication region, a type 4 secretion system (T4SS), a partition system, and a transposable element that includes two genes coding for aminoglycoside modifying enzymes and two β-lactamases. Table 1 and Fig. 1 summarize the characteristics of pMET1 including the predicted functions of the ORFs on the basis of amino acid sequence comparison.

The transposable element Tn1331.2

The plasmid pMET1 includes an 11,042-bp transposon, named Tn1331.2 [12] that is highly related to Tn1331 (see Fig. 2) [14,15]. Tn1331 can be considered an evolutionary product of Tn9 by the addition of a 3,047-bp DNA region, which has the structure of the variable portion of the integrons, flanked by 520-bp direct repeats (Fig. 2). This region includes three resistance genes with a structure consisting of aac(6')-Ib-attC and adaA1-bla9_NA-aac(6')-Ib-attC, potentially defining two gene cassettes including aac(6')-Ib-attC and adaA1-bla9_NA-aac(6')-Ib-attC [14,16,17]. As a result of the presence of these three genes and blaTEM cells harboring Tn1331 are resistant to several aminoglycosides and β-lactams. Possible mechanisms for the generation of Tn1331 have been discussed previously [16,18]. Tn1331.2 has a perfect duplication of the 3,047-bp DNA region and as a consequence a 520-bp including a C-terminal fragment of tspR is found as three direct repeats flanking the potential gene cassettes (Fig. 2). As it is the case for Tn1331 [14], the Tn1331.2 tspR differs from the Tn9 version of the gene in the 9-nucleotide repeats that in Tn9 code for the amino acid sequence GHFGFH. Tn1331.2 includes only one copy of this 9-bp fragment coding for the amino acid sequence GHF.

The duplication of the 3,047-bp DNA fragment including the resistance genes could be a strategy to increase the gene dosage when the transposon is present in a low copy number plasmid. Mechanisms of gene duplication when they are flanked by direct repeats have been described before [19,20,21]. Although we have observed a transition from Tn1331.2 to Tn1331 by the loss of one of the 3,047-bp repeats in the laboratory, we do not know if this is a process that happens in nature (dashed arrow, Fig. 2). While for many years no evolutionary derivatives of Tn1331 other than the duplication to render Tn1331.2 had been isolated, there was a recent report of such a phenomenon. The transposon Tn1332, isolated from Pseudomonas putida, can be considered as Tn1331 with the insertion of three DNA fragments that include the metallo-β-lactamase gene blaVIM-2, and the insertion sequences ISrpm17 and ISrpm18, respectively (Fig. 2) [22].

Replication and partition

 Blast analysis revealed a pMET1 region of high homology to the Y. pestis 91001 plasmid pCRY putative replication region [23] (schematically shown in Fig. 1A). The characteristics of this region include two AT-rich DNA segments flanking ORF1, named RepA, whose amino acid shown sequence showed 89% identity and 95% similarity to the pCry RepA putative protein (Fig. 3A and B). Lower but still significant similarities were found to other replication proteins from plasmids isolated from Buchnera aphidicola [24,25] and Y. enterocolitica plasmid pYVe227 [26]. To functionally identify the replication region of pMET1, three fragments 12, 34, and 45 (coordinates 41298-1655, 522-2039, and 1-2039 respectively) were ligated to the kanamycin resistance fragment and used to transform E. coli TOP10. Kanamycin resistant colonies were obtained only when the recombinant clones included the fragments 12 or 45 (Fig. 3A). These results were confirmed by cloning all three fragments using pCR2.1 as vector, and attempting to transform the DNA polymerase I deficient E. coli.

Table 1. cont.

| ORF | Position | G+C Content (%) | Protein | Best Blast-X Hit | $E$ value | Identity/ similarity | Accession No. |
|-----|----------|-----------------|---------|------------------|-----------|---------------------|--------------|
| 35  | 36,150-35,362 | 53.6 | 262 | Adenyltransferase [pHCMW1, Tn1331, Klebsiella pneumoniae]* | 6e-145 | 99/100 | NP_608308 |
| 36  | 36,825-36,220 | 54.1 | 201 | Aminoglycoside 6'-N-acetyltransferase type Ib [AAC(6')-Ib] * [pHCMW1, Tn1331, Klebsiella pneumoniae] | 1e-112 | 100/100 | NP_608307 |
| 37  | 37,565-37,208 | 54.3 | 185 | Resolvase [pHCMW1, Tn1331, Klebsiella pneumoniae] * | 2e-95 | 99/100 | NP_608306 |
| 38  | 37,694-40,732 | 51.8 | 1015 | Transposase [pHCMW1, Tn1331, Klebsiella pneumoniae] * | 0.0 | 100/100 | NP_608305 |
| 39  | 41,223-40,942 | 52.7 | 93 | Predicted transcriptional regulator [pCRY, Yersinia pestis biovar Microtus str. 91001] | 7e-37 | 100/100 | NP_995443 |
| 40  | 41,157-41,516 | 52.1 | 120 | Hypothetical protein YP_pCRY30 [pCRY, Yersinia pestis biovar Microtus str. 91001] | 1e-50 | 97/98 | NP_995444 |
| 41  | 41,545-41,204 | 53 | 107 | Hypothetical protein pLU0442 [Photorhabdus luminescens subsp.laumondii TT01] | 2e-27 | 60/73 | NP_927795 |

*Best hit selected according to genetic coherence rather than a minimal difference in score.

**Done with tblastn

**Including stop codon

Accession No.: NP_608308
coli C2110. While all three recombinant plasmids replicated in a wild-type *E. coli* strain, only recombinant clones including fragment 12 or 45 could be stably maintained in *E. coli* C2110. The recombinant clone harboring the fragment 34 was unable to replicate in the absence of DNA polymerase I (Fig. 3A). Therefore the results of both experiments defined a replication region encompassing nucleotides 1 to 1655, which includes two essential features: one complete ORF, named *repA*, and the AT-rich sequence between nucleotides 1 and 522 (Fig. 3A). This latter region likely includes the *ori* locus. These two features are conserved in the pCRY replication region: the AT rich sequence is conserved as the G+C content profile is almost identical to pMET1, and *repA* shows 95% similarity and 89% identity to *pMET1 RepA* when translated (Fig. 3B). On the other hand, the overall identity at the nucleotide level between pMET1 and pCRY for this locus is 89%. This high relatedness between the pMET1 and pCRY replication regions strongly suggests that pMET1 can be replicated in *Yersinia* species.

Plasmid pMET1 includes a putative partition region that has the general organization of that of plasmid pTP228, also known as the *parFG* locus [27]. The pMET1 partition region encompasses nucleotides 22,100 to 23,011 and includes two ORFs (ORF25 and ORF26, Table 1) encoding two putative proteins with strong homologies to ParF and ParG. The ParF homolog is a 205-amino acids protein that has shown significant homology with numerous proteins that belong to the type Ib subgroup of the ParA superfamily that is related to the MinD subgroup of cell division proteins [28,29,30]. These proteins are Walker-type ATPases that include a deviant motif A, which contains a conserved ‘signature’ lysine (XKGGXXKT) [29,31,32]. This motif as well as motif A'...
Figure 2. Genetic organization of transposons Tn3, Tn 1331, Tn 1332, and Tn 1331.2. The horizontal arrows indicate the location and direction of transcription of the genes. The genes are named only once for the sake of clarity. The light gray blocks show the 520-bp region direct repeats. Note that in Tn1332 one of the direct repeats is interrupted by an insertion of ISPpu17. The upper vertices of the dark gray triangles show points of insertion of DNA fragments that generate a new transposable element. The length of the base of each triangle defines the sizes of such DNA insertions. The curved dashed arrow to the right indicates that the transition Tn1331.2 to Tn1331 by deletion of one of the 3,047-bp occurred in the laboratory but it has not been confirmed to occur in nature. Stippled bar below Tn1331 indicates potential gene cassettes (GC).

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[29] are shown in Fig. 4A. The maximum pMET1 ParF identities and similarities were found with those encoded by the cryptic Y. enterocolitica plasmid p29930 [33] (90% similarity and 77% identity), the multidrug resistance plasmid pTP228 from Salmonella enterica subsp. Enterica serovar Newport [27] (87% similarity and 72% identity), and the Erwinia amylovora Ea88 plasmid pEA29 [34] (85% similarity and 73% identity).

Unlike with ParF, the pMET1 ParG has significant homologues only in p29930 (70% similarity and 53% identity), pTP228 (71% similarity and 58% identity), and pEA29 (68% similarity and 51% identity), generally known as ParB-like proteins. Studies on the pTP228 ParG protein showed that it acts as a dimer, may interact in a sequence-specific manner with a direct repeats-containing DNA region located upstream of parF known as parH, stimulates ATP hydrolysis by ParF, and modulates polymerization of ParF [35]. In addition, the ParG dimer binds an operator and acts as an autorepressor of the parFG operon [31]. Structures of parB-like proteins alone or in complex with DNA have been determined [36,37,38]. Other plasmids that have ParF homologues include proteins that are functional analogs but heterologs to ParG. The nucleoprotein complex has been recently called “segrosome” and is essential to ensure proper partition of the plasmid molecules [31]. Analysis of the pMET1 region upstream of the parF and parG genes showed an organization similar to that found in pTP228, which includes the parH locus and a putative operator (Fig. 4A) [31,39]. Interestingly, although the ParG proteins from pTP228 and pMET1 share homology, the repeats found upstream of parF in pMET1 are unrelated to those in pTP228. This region includes eight imperfect direct repeats that stretch over the promoter region. A graphical representation (sequence logo) of the multiple alignment of these repeats is shown in Fig. 4B. A region that includes some or all of these direct repeats could play the role of parH in pMET1 partition. Fig. 4A also shows the putative promoter, the −35 sequence is located within two partially overlapping direct repeats and upstream of this structure the repeat number 6 is part of an inverted repeat. One or more of these structures could function as operators, binding of ParG to one or both of these two structures may result in inhibition of transcription of parF and parG. Within the putative parH region there is also a perfect inverted repeat (Fig. 4A) unrelated to the putative ParG binding sites. The function of this structure is unknown.

Conjugation

Type 4 secretion systems (T4SSs) encoded by plasmids are involved in bacterial conjugation and transport of effectors to eukaryotic cells during infection [40]. Bacterial conjugation plays an important role in dissemination of antibiotic resistance genes at the cellular level [41,42]. Previous studies indicated that pMET1 is self-transmissible [12], analysis of its nucleotide sequence shows that the transfer region of pMET1 lies within a ca. 16-kbp DNA segment, the ATG of the first gene, virB1, occurs at coordinate 3534 and the ATG of the last gene, ardC (which is transcribed opposite to all the rest) at coordinate 19,631. A block of predicted coding products (named VirB1–VirB11) are highly homologous to proteins that make up the mating pair formation complex (Mpf) in a large number of plasmids and other DNA elements with the highest identity with those in the Y. pestis 91001 plasmid pCRY [23], the high pathogenicity island HPIECOR31 [43], and ICEKp1, an ICE found in the primary liver abscess causing K. pneumoniae NTUH-K2044 [44] (see Fig 1B, Table 1, and Table 2). The putative functions of these proteins are discussed in several reviews [40,42,45]. In all four cases the virB3 and virB4 genes are fused. An homolog of published VirB7 proteins is absent in all four structures. However, a 45-amino acids hypothetical protein (ORF9) is present between virB6 and virB8 in all four elements. This high conservation and the synergy suggest that this ORF might play a role equivalent to that of VirB7. The pMET1 vir-like genes are followed by ORFs of unknown function and the predicted DNA transfer (Dtr) group of genes that potentially code for MobB, MobC, and an endonuclease. While the pMET1 MobB and MobC showed 99% identity to those in the Y. pestis plasmid pCRY, the endonuclease showed highest identity with that of the K. pneumoniae plasmid pK245, which carries genes coding for resistance to quinolones and extended-spectrum β-lactamases [46], and the cryptic Y. enterocolitica plasmid p29930 [33]. Interestingly, no significant homology was found with the nuclease encoded by pCRY. Further analysis of the pMET1 mobilization region showed a structure oriT-mobB-mobC, similar to those in

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other elements such as the mobilizable HPI\textsubscript{ECOR31} [43] or the plasmid CloDF13 [47]. The pMET1 region upstream of \textit{mobB} has 90\% identity with that of the HPI\textsubscript{ECOR31}. As it is the case in this element, there are two \textit{nic} (or \textit{oriT}) sites in pMET1 due to their location inside inverted repeat 2 (IR2). It is then possible that either strand of pMET1 can be transferred to the recipient cell, an unusual phenomenon [48]. Furthermore, studies on the plasmid R1162, which also possesses two \textit{oriT}s, suggested that transfer can be initiated at one of them and terminated at the other with the inverted repeat playing a role in termination [49].

Downstream of the endonuclease gene, there is an ORF that is transcribed in the opposite direction to all the genes described above with homology to the p29930 \textit{ardC} [33], which codes for a putative antirestriction protein. The pSA ArdC protein has been studied in some detail. It has been shown to bind ssDNA and to protect single-stranded but not double-stranded plasmid DNA \textit{in vitro} against the activity of \textit{HhaI}, an enzyme that cleaves both single- and double-stranded DNA. It was proposed that ArdC is transported to the recipient cell with the transferred single-stranded DNA and protects the incoming single-stranded DNA from the host endonucleases [50].

**Comparative analysis of pMET1 and the \textit{Y. pestis} pCRY plasmid**

pCRY is a 21,742-bp cryptic plasmid recently isolated from \textit{Y. pestis} strain 91001 [23]. Comparative analysis of pMET1 and pCRY DNA sequences revealed highly similar backbones (see Fig. 1A). These analyses showed that the ca. 18-kb fragment including the replication region and the T4SS involved in conjugation of pMET1 shares high sequence homology as well as gene synteny with that of pCRY. These results suggest that they have a common ancestor and it would not be surprising if other plasmids with the same backbone but carrying other mobile genetic elements including resistance genes were found in other \textit{Enterobacteriaceae} isolates. In the particular case of \textit{Y. pestis}, our findings, taken together with the recent description of other
multiresistance plasmids of *Yersinia* that share their backbone with plasmids of *Salmonella* [51], and the finding of a common ancestry between the *Y. pestis* pMT1/pFra plasmid and a cryptic plasmid from a clinical isolate of *S. enterica* serovar Typhi [52] indicate that there must be an active transfer of plasmids between *Yersinia* and other *Enterobacteriaceae* by direct contact or through other intermediate bacteria resulting in a high risk of dissemination of antibiotic resistance genes to pathogenic *Y. pestis* strains.

Comparative analysis of pMET1 and the integrative and conjugative element of *E. coli* ECOR31

The pMET1 T4SS is related to those found in the ICEKp1 and HPIECOR31 (Fig. 1B). A study on HPIECOR31 showed that it shares common structures, such as the genes for synthesis of the siderophore yersiniabactin, with other high-pathogenicity islands found in *Enterobacteriaceae* including *Yersinia* species; but differs in the presence of a DNA region within an end of the element, known as RB-HPIECOR31 (right border-high pathogenicity island ECOR31) (see Fig. 1B, ICEs from *E. coli* ECOR31 and *Y. pestis* KIM) [43]. This region includes a pMET1-related T4SS, which made HPIECOR31 the first pathogenicity island to be found harboring a complete set of conjugative plasmid genes [43]. Furthermore, the complete HPIECOR31 can be excised from the chromosome, circularized, and transferred indicating that it is an integrative conjugative

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**Table 2.** Comparison of amino acid sequences of VirB/PliX-like proteins

|                | pCRY AE017044.1 | ICEECOR31 AV233333.1 | ICEKp1 AB298504.1 |
|----------------|-----------------|----------------------|--------------------|
| VirB1          | 99/99           | 63/77                | 63/77              |
| VirB2          | 98/99           | 76/85                | 76/85              |
| VirB3-4-like   | 97/98           | 99/100               | 98/99              |
| VirB5          | 84/90           | 83/98                | 84/89              |
| VirB6          | 87/95           | 87/92                | 95/96              |
| ORF9           | 97/97           | 100/100              | 97/100             |
| VirB8          | 99/100          | 98/100               | 99/99              |
| VirB9          | 98/100          | 99/99                | 98/99              |
| VirB10         | 96/97           | 95/96                | 95/95              |
| VirB11         | 99/100          | 99/100               | 97/99              |

Values indicate identity/similarity obtained by blastx comparison of each pMET1 ORF and the corresponding hypothetical protein from pCRY, ICEECOR31 and ICEKp1.

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Materials and Methods

Bacterial strains and plasmids

Plasmid pMET1 was originally isolated from *K. pneumoniae* FC1, a strain isolated in a pediatric unit in Mendoza, Argentina (Hospital Luis C. Lugomaggiore) [12]. Plasmid pMET1 DNA was introduced into *Escherichia coli* XL1-Blue (recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F' proAB lacI217AM15 Tn10]) [Stratagene] by transformation for purification and further manipulations. Recombinant clones including fragments of pMET1 were hosted in *E. coli* XL1-Blue or *E. coli* TOP10 F*− metD AckA redK12 mutM-supE44 recA1 araD139 (endA1 leu2-3,112) 7697 araL-amh lacZΔM15 galU galK xylS xylR (Stbl3) endA1 napG (Invitrogen). *E. coli* C2110 polA1 gcvR RifR [54] was used as host to isolate the pMET1 replication region. Recombinant clones were generated using pUC18 [55], pCR2.1 (Invitrogen), or pSMART (Lucigen) as cloning vectors. Plasmid pUC4K [56] was the source of the kanamycin resistance fragment (*aph* gene).

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