

1 **CMB-13-0249 revised**

2 **Molecular characterization of an atypical IncX3 plasmid pKPC-NY79 carrying *bla*<sub>KPC-2</sub> in a *Klebsiella***  
3 ***pneumoniae***

4

5 Pak-Leung Ho<sup>a,\*</sup>, Yuk-Yam Cheung<sup>a</sup>, Wai-U Lo<sup>a</sup>, Zhen Li<sup>a</sup>, Kin-Hung Chow<sup>a</sup>, Chi-Ho Lin<sup>b</sup>,  
6 Jasper Fuk-Woo Chan<sup>a</sup>, Vincent Chi-Chung Cheng<sup>a</sup>

7

8 <sup>a</sup>*Department of Microbiology, Queen Mary hospital and Carol Yu Centre for Infection, The University of*  
9 *Hong Kong, Hong Kong, China*

10 <sup>b</sup>*Centre for Genomic Sciences, The University of Hong Kong, Hong Kong, China*

11

12 **Keywords:** carbapenem resistance; carbapenemases; beta-lactamases; plasmid replicon typing; epidemiology

13

14 \*Corresponding author.

15 Department of Microbiology, The University of Hong Kong, Queen Mary hospital, Pokfulam Road, Pokfulam,  
16 Hong Kong, China. Tel: +852-2855 4897; Fax: +852-2855 1241; E-mail: [plho@hkucc.hku.hk](mailto:plho@hkucc.hku.hk)

17

18

19 **Abstract**

20 The IncX family of plasmids has recently been expanded to include at least four subtypes, IncX1-IncX4. The  
21 revised classification provides an opportunity for improving our understanding of the sequence diversity of the  
22 IncX plasmids and the resistance genes they carried. We described the complete nucleotide sequence of a novel  
23 IncX3 plasmid, pKPC-NY79 (42,447 bp) from a sequence type 258 *Klebsiella pneumoniae* strain that was  
24 isolated from a patient who was hospitalized in New York, United States. In pKPC-NY79, the plasmid scaffold  
25 and genetic load region were highly similar to homologous regions in pIncX-SHV (IncX3, JN247852) and the  
26 *bla*<sub>KPC</sub> carrying pKpQIL (IncFII<sub>k</sub>, GU595196), respectively, indicating that it has possibly arisen through  
27 recombination of plasmids. The *bla*<sub>KPC-2</sub> gene, as part of a transposon Tn4401a, was found within the genetic  
28 load region. The backbone of pKPC-NY79 differs from pIncX-SHV by a deletion involving the gene tandem  
29 *hns-topB* (encoding H-NS protein and topoisomerase III, respectively) and a putative ATPase gene.  
30 Unexpectedly, the impact of the *hns-topB* deletion on host fitness and plasmid stability was found to be small. In  
31 conclusion, the findings contribute to a better understanding of the plasmid platforms carrying *bla*<sub>KPC</sub> and of  
32 variations in the backbone of the IncX3 plasmids.

33

34 (Word count 199)

35

36

## 37 **Introduction**

38           In *Enterobacteriaceae*, the most frequent class A carbapenemases are the KPC enzymes [26]. The first  
39 KPC-producing isolate was identified in a *Klebsiella pneumoniae* strain from North Carolina, USA in 1996.  
40 Since then, carbapenem-resistant, KPC-producing isolates have increased in frequency, species distribution and  
41 geographical distribution [3,19]. The spread of KPC involves clones, plasmids and transposons. Globally,  
42 KPC-producing *K. pneumoniae* isolates were often found to be sequence type (ST) 258 or its variant ST11  
43 [22,26]. These KPC-producing isolates carry plasmids which encode the *bla*<sub>KPC</sub> genes as part of a transposon  
44 Tn4401 or its variants [18,22]. On the basis of sequence deletions upstream of *bla*<sub>KPC</sub>, five isoforms of Tn4401a  
45 to Tn4401e have been identified which were associated with different levels of *bla*<sub>KPC</sub> gene expression [18]. In  
46 China, a variant of Tn4401, harboring *ISKpn8*, was reported to carry *bla*<sub>KPC</sub> genes in most isolates [22]. While at  
47 least 13 different alleles have been reported, KPC-2 and KPC-3 occur most widely [8,26]. The *bla*<sub>KPC</sub> genes  
48 have been reported on plasmids with narrow (IncFII<sub>k</sub>, ColE) and broad (IncN, IncL/M and IncA/C) host range or  
49 on untypable plasmids. The first *bla*<sub>KPC</sub>-carrying plasmids to be completely sequenced were p9 (IncN,  
50 FJ223607), p12 (IncN, FJ223605) and p15S (ColE, FJ223606) [9]. The other plasmids that have been  
51 completely sequenced were pKP048 (IncFII<sub>k</sub>, FJ628167), pSLMT (IncFII<sub>k</sub>, HQ589350), pKpQIL (IncFII<sub>k</sub>,  
52 GU595196), pKpQIL-IT (IncFII<sub>k</sub>, JN233705), pKPHS2 (IncFII<sub>k</sub>, CP003224) and pKPN101-IT (IncFII<sub>k</sub>,  
53 JX283456).

54           The IncX plasmids are narrow host range plasmids of *Enterobacteriaceae*. Such plasmids have most  
55 often been reported in *E. coli*, *Salmonella* spp. and *Klebsiella* spp [15,20]. They are known to encode type IV  
56 secretion system (T4SS), enabling their own conjugative transfer, and to carry genes encoding biofilm formation  
57 and antimicrobial resistance. PCR-based replicon typing (PBRT) procedures demonstrated that the IncX  
58 plasmids occur infrequently in 1% or less of the *Escherichia coli* populations [2,15]. Recently, comparative  
59 analysis of completely sequenced IncX plasmids demonstrated that the IncX plasmids could be subdivided into  
60 four subgroups, IncX1 to IncX4, and the prototype plasmid R6K belonged to the IncX2 subgroup [14]. The  
61 basic core structures of the IncX plasmids include sequences encoding replication, partitioning, T4SS,  
62 transcriptional activator and putative DNA transfer protein [14,20]. Based upon the *taxC* gene, a revised typing  
63 scheme was proposed and these plasmids were shown to be more prevalent than previously acknowledged [14].  
64 In this study, we report the complete sequence of a novel IncX3 plasmid, designated pKPC-NY79 carrying  
65 *bla*<sub>KPC</sub> originating from a patient with epidemiological link to the United States.

66

## 67 **Material and methods**

68

### 69 Bacterial strain

70 In 2011, we identified a *K. pneumoniae* strain CRE79 carrying *bla*<sub>KPC-2</sub> from a urine sample of a 71 year-old  
71 man who was repatriated from New York, United States, where he had been hospitalized for intracranial  
72 hemorrhage. The organism was identified by the Vitek II (bioMérieux SA, Marcy l'Etoile, France). The disc  
73 diffusion method and Etest (AB Biodisk, Solna, Sweden) were used to determine the susceptibility to antibiotics  
74 [4]. Combined disc test was used to determine the carbapenemase phenotype using EDTA or boronic acid as  
75 inhibitors [10].

76

### 77 Molecular studies

78 The major carbapenemase genes (*bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub> and *bla*<sub>OXA-48</sub>) were detected by PCR using  
79 previously described primers (Supplementary file, Table S1) [10,13]. The multilocus sequence type (MLST) of  
80 the strain was determined using the Pasteur Institute scheme [6]. In brief, internal portions of seven  
81 housekeeping genes were amplified and sequenced: *rpoB* (beta-subunit of RNA polymerase), *gapA*  
82 (glyceraldehyde 3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase),  
83 *phoE* (phosphorine E), *infB* (translation initiation factor) and *tonB* (periplasmic energy transducer). A different  
84 allele number was given to each distinct sequence within a locus, and a distinct sequence type (ST) number was  
85 attributed to each distinct allelic profile. Allele sequences and STs are available on the web site at  
86 <http://www.pasteur.fr/mlst>. The transferability of *bla*<sub>KPC</sub> was tested by filter mating using *Escherichia coli* J53  
87 (azide-resistant) as the recipient [13]. Transconjugants were selected on MacConkey medium containing sodium  
88 azide (100 µg/ml) and meropenem (0.5 µg/ml).

89 Plasmid DNA was extracted with QIAGEN Large Construct kit (Qiagen, Hilden, Germany).  
90 Subsequently, the plasmids were converted to the linear forms by incubation with *Aspergillus oryzae* S1  
91 nuclease (Sigma Chemical Cp., St Louis, MO, USA) and were sized by pulsed-field gel electrophoresis [12].  
92 The complete sequence of the plasmid pKPC-NY79 carrying *bla*<sub>KPC</sub> in a J53 transconjugant (originating from *K.*  
93 *pneumoniae* strain CRE79) was obtained by using the 454 GS FLX system (Roche, USA) according to the  
94 manufacturer's instruction. The library yielded a total of 64,798 reads with average read length of 500 bp. The  
95 reads were assembled by the GS de novo Assembler (version 2.6) into two contigs. The gaps were closed by

96 PCR and Sanger sequencing. The plasmid was annotated by RAST Server and each predicted open reading  
97 frames (ORFs) was further blast against the NCBI non-redundant protein database using BLASTP [1,13].  
98 Additional bioinformatics analyses were conducted as previously described [12,13].

99

100 Plasmid stability and fitness cost

101 Stability tests were conducted as described previously [23]. Three ul of an overnight growth of the bacteria in  
102 Luria-Beranti (LB) broth were inoculated into 3 ml of a fresh LB broth and incubated for 12 hours at 37 °C  
103 (time zero). The above process was repeated every 12-hourly (equivalent to 10 generations each). At time zero  
104 and after passage in the absence of antibiotic for 50, 100, 150 and 200 generations, a sample of the culture was  
105 diluted and spread onto LB plate. One hundred colonies were picked and replica plated onto a pair of plain and  
106 antibiotic-containing (0.5 µg/ml meropenem) LB plates. Plasmid stability was determined through the  
107 percentage of colonial growth on the antibiotic-containing plates. Testing was conducted on two separate  
108 occasions.

109 Two growth parameters including the lag phase and the doubling time in the exponential phase were  
110 used to assess the fitness cost associated with the introduction of pKPC-NY79. The test strains were *E. coli* J53  
111 and a J53 transconjugant with the plasmid (J53/pKPC-NY79). Bacteria were cultured in LB broth at 37 °C with  
112 shaking. Growth was monitored every 15 minutes by the optical density until the readings reach a plateau. A  
113 growth curve of optical density versus time was plotted and the doubling time calculated as previously described  
114 [5]. The lag phase was obtained by extrapolating the tangent at the exponential part of the growth curve back to  
115 the inoculum level [24]. This experiment was carried out on three separate occasions.

116

## 117 **Results and discussion**

118

119 Phenotypic and genotypic characteristics of the bacterial strain

120 The *K. pneumoniae* strain CRE79 was resistant to all β-lactams, including imipenem (>32 µg/ml), ertapenem  
121 (>32 µg/ml), meropenem (>32 µg/ml), and multiple non-β-lactam antibiotics (amikacin, ciprofloxacin,  
122 chloramphenicol, cotrimoxazole, nitrofurantoin). It was susceptible only to gentamicin, colistin and fosfomycin  
123 (8 µg/ml). The extensively drug-resistant phenotype was consistent with those exhibited by KPC epidemic  
124 clones circulating in different countries [8,17,19]. Combined disc testing showed that carbapenem-resistance  
125 could be reversed by boronic acid but not EDTA. MLST identified CRE79 as ST 258, which is a major lineage

126 associated with dissemination of *bla*<sub>KPC</sub> in many countries [3,16]. PCR assays showed that it was positive for  
127 *bla*<sub>KPC</sub> but negative for the other major carbapenemase genes (*bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub> and *bla*<sub>OXA-48</sub>). The *bla*<sub>KPC</sub>  
128 carrying plasmid was conjugatively transferred to *Escherichia coli* J53 at frequency of 10<sup>-6</sup> per donor cells. In  
129 the transconjugant, resistance to β-lactam antibiotics including carbapenems was the only resistance trait  
130 transferred. Pulsed-field gel electrophoresis and S1 nuclease digestion gave a single plasmid band of ~40 kb.  
131 The complete sequence of the plasmid is presented here.

132

133 Nucleotide sequence analysis of pKPC-NY79

134 The plasmid (designated as pKPC-NY79, GenBank accession JX104759) is a 42,447 bp circular  
135 plasmid with an average GC content of 48.5% and 38 putative open reading frames (ORFs) ( Table 1). The  
136 plasmid backbone shares high sequence homology to IncX3 plasmids [8,14], exemplified by pIncX-SHV  
137 (JN247852) and pEC14\_35 (JN935899) which were used as references for annotating pKPC-NY79. When this  
138 plasmid was tested by the initially described PBRT [2], the result was negative. Testing with the revised IncX  
139 typing scheme based upon the *taxC* genes [14] detected the plasmid as belonging to the IncX3 subgroup. Figure  
140 1 shows the backbone regions shared by pIncX-SHV and pKPC-NY79 comprise sequences encoding replication  
141 (replication initiation protein, *pir*; replication accessory protein, *bis*), partitioning (*parA*), conjugation/type IV  
142 secretion system (T4SS, with 11 genes, *pilX1* to *pilX11*), transcriptional activator (*actX*) and putative DNA  
143 transfer proteins (*taxA* and *taxC*). However, pKPC-NY79 was modified by a deletion involving the putative  
144 plasmid stability tandem genes *hns-topB* (encoding a putative DNA-binding protein and a putative type III  
145 topoisomerase, respectively) and a gene encoding ATPase (*atpase*), as comparing with pIncX-SHV. In  
146 pKPC-NY79, the genetic load region between the resolvase, *res* gene and *parB* is 16.4 kb in length, including  
147 the *bla*<sub>KPC-2</sub> carrying Tn4401a transposon and an upstream 8.2 kb region with three transposases (IS26-*tmpA*,  
148 Tn3-*tmpA* and ISAs12-*tmpA*), one resolvase (Tn3-*tmpR*) and a truncated *umuD* which putatively encodes for an  
149 ultraviolet repair protein. The *tmpR* gene in the right extremity of Tn4401a was truncated (Figure 1). Of note, the  
150 genetic load region from position 6441 to 21387 (JX104759) is almost identical (query coverage 100%, identity  
151 99.9%) to the same gene array in the epidemic *bla*<sub>KPC-3</sub> carrying plasmid pKpQIL (GU595196, IncFII<sub>k</sub>) which  
152 was associated with the spread of *bla*<sub>KPC</sub> in the United States, Israel and Italy [8,17,19]. The finding suggested  
153 that pKPC-NY79 might have evolved from recombination events involving IncX3/pIncX-SHV-like and  
154 IncFII<sub>k</sub>/pKpQIL-like plasmid ancestors. In this regard, it is interesting that two such plasmids  
155 (IncX3/pIncX-SHV and IncFII<sub>k</sub>-FIB<sub>k</sub>/pKpQIL-IT) were found in the *K. pneumoniae* strain 55873 [8]. The two

156 *bla*<sub>KPC</sub> alleles that were found in the two highly homologous regions in pKPC-NY79 (*bla*<sub>KPC-2</sub>, His271, codon  
157 CAC) and pKpQIL (*bla*<sub>KPC-3</sub> Tyr271, codon TAC) could possibly arise through nucleotide substitution [27].  
158 Similar phenomena have been reported for *bla*<sub>CTX-M</sub> alleles carried on highly similar plasmid IncFII  
159 (*bla*<sub>CTX-M-14/24</sub>), IncN (*bla*<sub>CTX-M-1/32</sub>) and IncL/M (*bla*<sub>CTX-M-3/15</sub>) platforms [12,21].

160

161 Plasmid stability of pKPC-NY79 and its effect on bacterial host fitness

162 Previous studies have demonstrated that plasmid-encoded H-NS proteins such as sfh have a “stealth” function  
163 that allow the plasmids to be transferred to the new bacterial hosts with minimal effects on their fitness [7].  
164 Given the deletion of the tandem genes *hns-topB* in pKPC-NY79, we assessed the impact of introducing the  
165 plasmid into strain J53 and the stability of the plasmid in absence of antibiotics. The findings showed that  
166 introduction of the plasmid significantly increased the lag phase and doubling time of the host strain but the  
167 magnitude of the effects were small (Table 2). Stability of pKPC-NY79 with the *hns-topB* deletion could be  
168 maintained at 100% over 50 generations of multiplication in the absence of antibiotics. However, a drastic loss  
169 of pKPC-NY79 was observed after 100 generations of multiplication, with 75% and 28.5% retaining the  
170 plasmid at 150 and 200 generations respectively. As reviewed recently, plasmid-encoded *hns*-like genes occur in  
171 a wide range of different plasmids [25]. Unlike previous studies which demonstrated a major adverse effect on  
172 fitness [7], our results showed that this IncX3 plasmid variant with *hns* deletion only has a modest effect on the  
173 host and plasmid stability was only affected after prolonged propagation in the absence of antibiotics. The  
174 discordant observations could possibly be related to host species (*Salmonella* spp. vs. *E. coli*), plasmid size (42  
175 kb vs. >100 kb) and host adaptive mutations [23,25].

176

177 In conclusion, we hereby described the complete sequence of a *bla*<sub>KPC-2</sub>-carrying IncX3 plasmid with some  
178 unique features in the backbone sequences. The findings contribute to a better understanding of the replicon  
179 types of plasmids involved in the dissemination of the *bla*<sub>KPC</sub> genes. The natural distribution of the IncX3 group  
180 of plasmids and the roles that they play in the dissemination of emerging resistance genes remains to be  
181 elucidated [11].

182

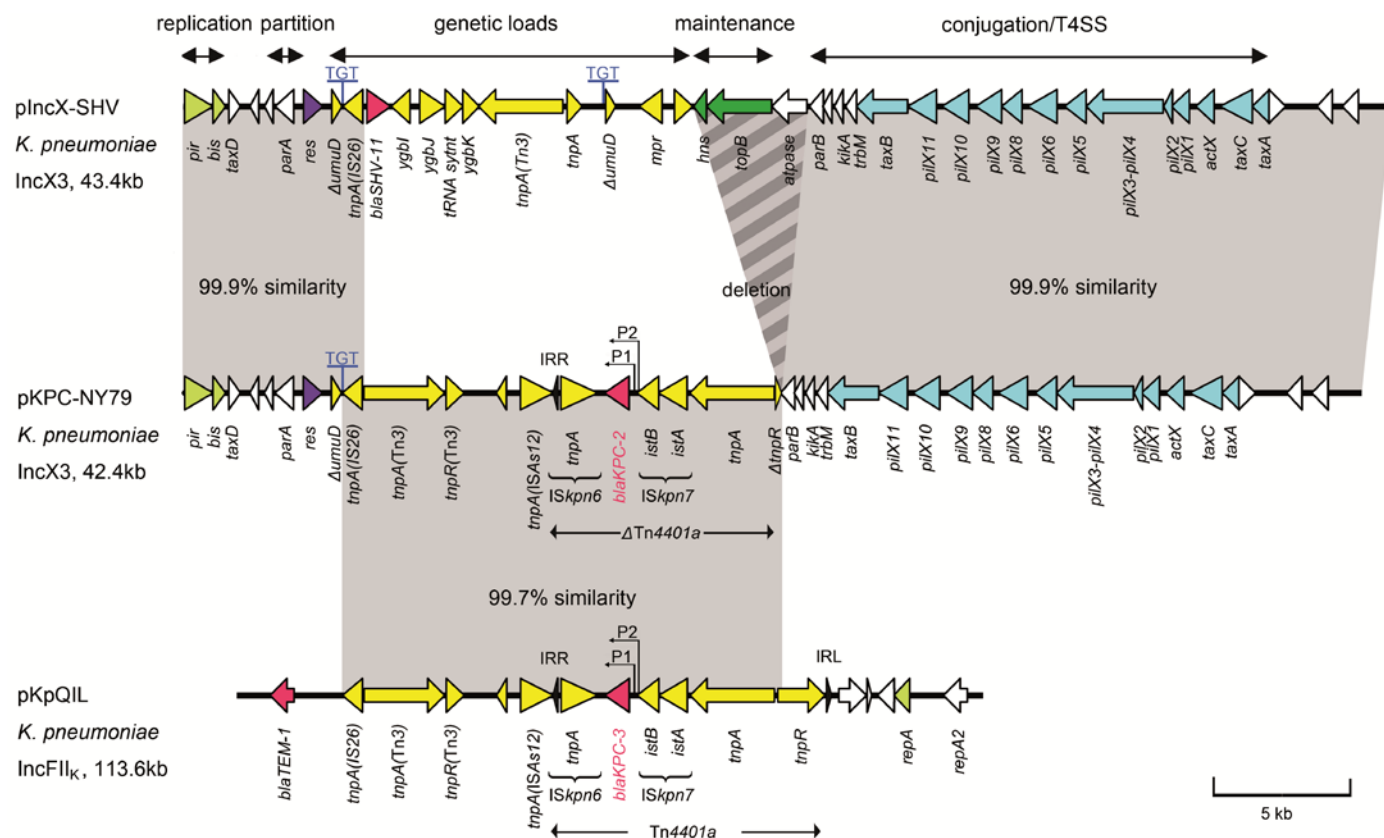
183 **Acknowledgement**

184 This work was supported by grants from the Research Fund for the Control of Infectious Diseases (RFCID) of  
185 the Health and Food Bureau of the Government of the HKSAR. We thank Pak-Chung Sham, Levina Lam, Ben  
186 Ho and Wilson Chan of the Centre for Genomic Science, University of Hong Kong for technical assistance.



187 **Fig. 1** Comparative analysis of linear plasmid maps for two plasmids, pIncX-SHV and pKPC-NY79 and a partial sequence in pKpQIL. The function blocks of the plasmids  
 188 are indicated above the linear maps. The lengths of the ORFs are drawn in proportion to the size of the ORFs. Homologous ORFs in the plasmid maps are represented in the  
 189 same colour. Mobile elements are labelled in yellow. The promoters, P1 and P2 of *bla*<sub>KPC</sub> gene are identified and annotated as described previously [18]. The accession  
 190 numbers were: pIncX-SHV (JN247852); pKPC-NY79 (JX104759) and pKpQIL (GU595196).

191



192

193 **Table 1 Open reading frames identified in pKPC-NY79**

ORF number	Gene name (alternative name)	Position in JX104759	Strand	Size (bp)	Function
1.	<i>pir (repB)</i>	1-1014	+	1014	replication initiator protein
2.	<i>bis</i>	1023-1466	+	444	conserved hypothetical protein
3.	<i>taxD</i>	1585-2007	+	423	DNA distortion polypeptide
4.	<i>hyp</i>	2659-2348	-	312	hypothetical protein
5.	<i>hyp</i>	3176-2865	-	312	hypothetical protein
6.	<i>parA</i>	3911-3249	-	663	plasmid partition protein
7.	<i>res</i>	4274-4954	+	681	resolvase
8.	<i>ΔumuD</i>	5283-5639	+	357	truncated DNA polymerase
9.	<i>tnpA (IS26)</i>	6389-5673	-	717	transposase of IS26
10.	<i>tnpA (Tn3)</i>	6450-9347	+	2898	transposase of Tn3
11.	<i>tnpR (Tn3)</i>	9415-10056	+	642	resolvase of Tn3
12.	<i>hyp</i>	11581-11222	-	360	hypothetical protein
13.	<i>tnpA (ISAs12)</i>	12085-13269	+	1185	transposase of ISAs12
14.	<i>tnpA (ISkpn6)</i>	13546-14865	+	1320	transposase of ISkpn6
15.	<i>blaKPC-2</i>	15996-15115	-	882	Carbapenemase
16.	<i>istB (ISkpn7)</i>	17063-16284	-	780	transposase of ISkpn7
17.	<i>istA (ISkpn7)</i>	18085-17060	-	1026	transposase of ISkpn7
18.	<i>tnpA (Tn4401a)</i>	21221-18192	-	3030	transposase of Tn4401a
19.	<i>ΔtnpR (Tn4401a)</i>	21330-21388	+	59	truncated resolvase of Tn4401a
20.	<i>parB</i>	21966-21454	-	513	plasmid partition protein
21.	<i>hyp</i>	22271-21945	-	327	hypothetical protein
22.	<i>kika</i>	22671-22357	-	315	killer family protein
23.	<i>trbM</i>	23151-22759	-	393	VirB/Tra/Trw family

					protein
24.	<i>taxB</i>	24983-23148	-	1836	conjugal transfer protein
25.	<i>pilX11</i>	26020-24986	-	1035	conjugal transfer protein
26.	<i>pilX10</i>	27181-26216	-	966	conjugal transfer protein
27.	<i>pilX9</i>	28356-27427	-	930	conjugal transfer protein
28.	<i>pilX8</i>	29090-28362	-	729	conjugal transfer protein
29.	<i>pilX6</i>	30340-29285	-	1056	conjugal transfer protein
30.	<i>pilX5</i>	31389-30619	-	771	conjugal transfer protein
31.	<i>pilX3-pilX4</i>	34152-31399	-	2754	conjugal transfer protein
32.	<i>pilX2</i>	34485-34177	-	309	conjugal transfer protein
33.	<i>pilX1</i>	35095-34451	-	645	conjugal transfer protein
34.	<i>actX</i>	35978-35328	-	651	Transcription anti-terminator
35.	<i>taxC</i>	37350-36190	-	1161	DNA transfer relaxase
36.	<i>taxA</i>	37967-37353	-	615	DNA transfer auxiliary protein
37.	<i>hyp</i>	37949-38554	+	606	hypothetical protein
38.	<i>hyp</i>	40225-39674	-	552	hypothetical protein
39.	<i>hyp</i>	41161-40580	-	582	hypothetical protein

---

194

195

196

**Table 2. Effect of pKPC-NY79 on bacterial growth parameters**

	Mean time (minutes) $\pm$ standard deviation		<i>P</i> value
	J53	J53, pKPC-NY79	
Lag phase	134.6 $\pm$ 0.2	144.1 $\pm$ 0.6	<0.001
Doubling time	25.4 $\pm$ 0.1	26.4 $\pm$ 0.4	0.02

## References

1. Aziz R.K., Bartels D., Best A.A., DeJongh M., Disz T., Edwards R.A., Formsma K., Gerdes S., Glass E.M., Kubal M. et al. (2008). The RAST Server: rapid annotations using subsystems technology. *BMC. Genomics* 9: 75.
2. Carattoli A., Bertini A., Villa L., Falbo V., Hopkins K.L., and Threlfall E.J. (2005). Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* 63: 219-228.
3. Castanheira M., Costello A.J., Deshpande L.M., and Jones R.N. (2012). Expansion of clonal complex 258 KPC-2-producing *Klebsiella pneumoniae* in Latin American hospitals: report of the SENTRY Antimicrobial Surveillance Program. *Antimicrob. Agents Chemother.* 56: 1668-1669.
4. Clinical and Laboratory Standards Institute. (2012). *Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement M100-S22*. CLSI, Wayne, PA, USA, 2012).
5. Cottell J.L., Webber M.A., and Piddock L.J. (2012). Persistence of Transferable Extended-Spectrum-beta-Lactamase Resistance in the Absence of Antibiotic Pressure. *Antimicrob. Agents Chemother.* 56: 4703-4706.
6. Diancourt L., Passet V., Verhoef J., Grimont P.A., and Brisse S. (2005). Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J. Clin. Microbiol.* 43: 4178-4182.
7. Doyle M., Fookes M., Ivens A., Mangan M.W., Wain J., and Dorman C.J. (2007). An H-NS-like stealth protein aids horizontal DNA transmission in bacteria. *Science* 315: 251-252.
8. Garcia-Fernandez A., Villa L., Carta C., Venditti C., Giordano A., Venditti M., Mancini C., and Carattoli A. (2012). *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob. Agents Chemother.* 56: 2143-2145.
9. Gootz T.D., Lescoe M.K., Dib-Hajj F., Dougherty B.A., He W., Della-Latta P., and Huard R.C. (2009). Genetic organization of transposase regions surrounding blaKPC carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. *Antimicrob. Agents Chemother.* 53: 1998-2004.
10. Ho P.L., Li Z., Lai E.L., Chiu S.S., and Cheng V.C. (2012). Emergence of NDM-1-producing *Enterobacteriaceae* in China. *J Antimicrob. Chemother.* 67: 1553-1555.
11. Ho P.L., Li Z., Lo W.U., Cheung Y.Y., Lin C.H., Sham P.C., Cheng V.C., Ng T.K., and Chow K.H. (2012). Identification and characterization of a novel incompatibility group X3 plasmid carrying bla<sub>NDM-1</sub> in *Enterobacteriaceae* isolates with epidemiological links to multiple geographical areas in China. *Emerg Micro Infect* 1: e39.

12. Ho P.L., Lo W.U., Wong R.C., Yeung M.K., Chow K.H., Que T.L., Tong A.H., Bao J.Y., Lok S., and Wong S.S. (2011). Complete sequencing of the FII plasmid pHK01, encoding CTX-M-14, and molecular analysis of its variants among *Escherichia coli* from Hong Kong. *J. Antimicrob. Chemother.* **66**: 752-756.
13. Ho P.L., Lo W.U., Yeung M.K., Lin C.H., Chow K.H., Ang I., Tong A.H., Bao J.Y., Lok S., and Lo J.Y. (2011). Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. *PLoS. One.* **6**: e17989.
14. Johnson T.J., Bielak E.M., Fortini D., Hansen L.H., Hasman H., Debroy C., Nolan L.K., and Carattoli A. (2012). Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. *Plasmid* **68**: 43-50.
15. Johnson T.J., Wannemuehler Y.M., Johnson S.J., Logue C.M., White D.G., Doetkott C., and Nolan L.K. (2007). Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. *Appl. Environ. Microbiol.* **73**: 1976-1983.
16. Kitchel B., Rasheed J.K., Patel J.B., Srinivasan A., Navon-Venezia S., Carmeli Y., Brolund A., and Giske C.G. (2009). Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob. Agents Chemother.* **53**: 3365-3370.
17. Leavitt A., Chmelnitsky I., Carmeli Y., and Navon-Venezia S. (2010). Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. *Antimicrob. Agents Chemother.* **54**: 4493-4496.
18. Naas T., Cuzon G., Truong H.V., and Nordmann P. (2012). Role of ISKpn7 and Deletions in blaKPC Gene Expression. *Antimicrob. Agents Chemother.* **56**: 4753-4759.
19. Navon-Venezia S., Leavitt A., Schwaber M.J., Rasheed J.K., Srinivasan A., Patel J.B., and Carmeli Y. (2009). First report on a hyperepidemic clone of KPC-3-producing *Klebsiella pneumoniae* in Israel genetically related to a strain causing outbreaks in the United States. *Antimicrob. Agents Chemother.* **53**: 818-820.
20. Norman A., Hansen L.H., She Q., and Sorensen S.J. (2008). Nucleotide sequence of pOLA52: a conjugative IncX1 plasmid from *Escherichia coli* which enables biofilm formation and multidrug efflux. *Plasmid* **60**: 59-74.
21. Novais A., Canton R., Moreira R., Peixe L., Baquero F., and Coque T.M. (2007). Emergence and dissemination of Enterobacteriaceae isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1, -3, and -32) plasmids. *Antimicrob. Agents Chemother.* **51**: 796-799.
22. Shen P., Wei Z., Jiang Y., Du X., Ji S., Yu Y., and Li L. (2009). Novel genetic environment of the carbapenem-hydrolyzing beta-lactamase KPC-2 among *Enterobacteriaceae* in China. *Antimicrob. Agents Chemother.* **53**: 4333-4338.

23. Sota M., Yano H., Hughes J.M., Daughdrill G.W., Abdo Z., Forney L.J., and Top E.M. (2010). Shifts in the host range of a promiscuous plasmid through parallel evolution of its replication initiation protein. *ISME J.* **4**: 1568-1580.
24. Swinnen I.A., Bernaerts K., Dens E.J., Geeraerd A.H., and Van Impe J.F. (2004). Predictive modelling of the microbial lag phase: a review. *Int. J. Food Microbiol.* **94**: 137-159.
25. Takeda T., Yun C.S., Shintani M., Yamane H., and Nojiri H. (2011). Distribution of genes encoding nucleoid-associated protein homologs in plasmids. *Int. J. Evol. Biol.* **2011**: 685015.
26. Tzouvelekis L.S., Markogiannakis A., Psychogiou M., Tassios P.T., and Daikos G.L. (2012). Carbapenemases in *Klebsiella pneumoniae* and Other *Enterobacteriaceae*: an Evolving Crisis of Global Dimensions. *Clin. Microbiol. Rev.* **25**: 682-707.
27. Wolter D.J., Kurpiel P.M., Woodford N., Palepou M.F., Goering R.V., and Hanson N.D. (2009). Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. *Antimicrob. Agents Chemother.* **53**: 557-562.