Partition locus-based classification of selected plasmids in Klebsiella pneumoniae, Escherichia coli and Salmonella enterica spp.: An additional tool

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1. Introduction

Plasmids are extrachromosomal DNA molecules capable of autonomous replication and are the main vectors of resistance and virulence genes, especially in Enterobacteriaceae. Tracing plasmids conferring drug resistance is important for analysis of evolution, epidemiology and spread of antibacterial resistance. The epidemic plasmids belong to the most frequently occurring plasmid families, however antibiotic resistance gene are not always associated with one particular replicon, transfer or partition system. Therefore is difficult to construct phylogenies of plasmids or to track the spread of particular markers.

Incompatibility (Inc) group identification has been frequently used to classify plasmids. Identification methods include the initial technique based on conjugation (Novick, 1987), hybridization with cloned replication regions (Couturier et al., 1988), PCR-based replicon typing (PBRT) (Carattoli et al., 2005) and relaxase typing (Compain et al., 2014). Moreover MLST schemes for plasmids were developed to assign plasmids to STs, in analogy to the typing developed for bacterial genomes (García-Fernández et al., 2008).

Mitotic segregation of plasmids, termed partition in bacteria, is a fundamental step of the cell cycle that ensures the transmission of the whole genome to daughter cells. It is governed by specific genetic loci named *par*, first identified in low-copy-number plasmids and later found to be present as homologues in most bacterial chromosomes. *par* loci are organized into operons encoding two proteins, an ATPase and a DNA-binding protein, and including a centromeric site. These components interact with each other to direct the subcellular localization that ensures stability of their replicons. Three types of partitioning ATPases are known (Gerdes et al., 2010): the Walker-type ATPases encoded by the *par/sop* gene family (type I partitioning loci which are the most common of the *par* systems), the actin-like ATPase encoded by the *par* locus of plasmid R1 (type II partitioning locus) and the tubulin-like GTPases encoded by plasmids from *Bacillus* sp. (type III partitioning loci). Despite their similarities in genetic organization, these three *par* types use entirely different molecular mechanisms (Guynet and de la Cruz, 2011).

While the acquisition of plasmids often enables bacteria to survive in the presence of antibiotics, it is possible that plasmids also confer vulnerabilities that may be exploited in tailored antibacterial therapy (Williams and Hergenrother, 2008). In order to control the spread of multiresistance plasmids, we need to determine many more variables that affect their replication, maintenance and movement.

Recently we developed a multiplex PCR method called "plasmid relaxase gene typing" (PRaseT). This classification scheme is based on the relaxase, a key protein which is part of the mobilization region of transmissible plasmids. The aim of the present study was to identify different partition systems located on multiresistance plasmids and to design a multiplex PCR method here called "plasmid partition gene typing" (PAR-T). This method could further the classification of plasmids in *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella enterica spp.* and will constitute another option for characterising plasmids.

2. Materials and methods

2.1. Database search, primer design and *in silico* primer assay

An *in silico* analysis was carried out using GenBank BLAST (http://blast.ncbi.nlm.nih.gov/) on plasmids >40 kb from *K. pneumoniae* and *E. coli* conferring multidrug resistance. Due to a low number of plasmids IncHI, IncI1 from *K. pneumoniae*, the *in silico* analysis was extended to some plasmids from *Enterobacter cloacae* and *Citrobacter freundii*. For the eight Inc groups studied (IncF, IncA/C, IncL/M, IncN, IncHI, IncR, IncI1, IncX), the *par* operon was used as template; the presence of partition-specific multidomains was searched for using CD-Search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/). Multiple alignments were performed with ClustalW2 software (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Primer pairs covering most sequences in each family were designed using FastPCR (http://primerdigital.com/fastpcr.html) and Primerblast (http://www.ncbi.nlm.nih.gov/tools/primer-blast) software, while minimizing codon degeneracy (Table 1). Oligonucleotide primers were tested *in silico* for hybridization with plasmids from Enterobacteriaceae referenced in GenBank.

2.2. Bacterial strains and plasmids

For validation of the PCR assays, experiments were conducted with 136 *Escherichia coli* transconjugants (Tc) or transformants (Tf) of Enterobacteriaceae (Table 2). All strains carried plasmids belonging to diverse Inc groups that encoded β-lactamases conferring resistance to third-generation cephalosporins and/or carbapenems (ESBLs, acquired cephalosporinases, carbapenemases). They were part of four collections of, respectively, (i) *E. coli* strains isolated between 1997 and 2002 in various French university hospitals (Marcadé et al., 2009) (Branger et al., 2005)), (ii) *K. pneumoniae* strains from various geographical regions collected since the 1980s (D. Decré and G.Arlet, personal collection), (iii) *Salmonella enterica* subsp. *enterica* strains representing various serovars (collection of the French National Reference Center for *E. coli*, *Shigella sp.*, and *Salmonella* sp., Institut Pasteur), (iv) *E. coli*, *S. enterica*, *K. pneumoniae* strains isolated during 2013 in various Argentina University Hospitals (M. Jure, personal collection). All Tc, Tf and clinical strains used in this study were analyzed in parallel with the PAR-T and PRaseT methods. The IncR plasmids which do not encode relaxases were analyzed only by using PBRT (Carattoli et al., 2005). The Tc used as positive controls in PAR-T reactions are given in Table 2. After optimization using Tc or Tf carrying replicons of various types according to PRaseT, we applied the PAR-T method to a panel of 30 clinical strains (17 *E. coli*, 11 *K. pneumoniae* and 2 *S. enterica*) carrying replicons of one to four different types (Table 3).

2.3. DNA extraction and PCR conditions

Lysis by boiling was used for total DNA extraction as previously described (Dallenne et al., 2010). Multiplex PCR was carried out using the Qiagen Multiplex PCR kit (Qiagen, Courtaboeuf, France). The master mix contained pre-optimized concentrations of HotStarTaq DNA polymerase and $MgCl_2$, deoxynucleotide triphosphate and buffer. To all multiplex PCRs, solution Q (Qiagen) that facilitates the reaction with difficult-to-amplify templates by modifying DNA melting was added. Total DNA in 2 µL of bacterial lysate was subjected to multiplex PCR in a 50 µL volume. The conditions for multiplex PCR were optimized to ensure that all targets were sufficiently amplified for amplicons to be easily visible on 1.5% agarose gels. The optimal primer concentrations are reported in Table 1. PCR conditions consisted of an initial activation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 57°C for 90 s and 72°C for 90 s with a final extension at 68°C for 10 min; for Multiplex I and Multiplex II annealing temperature was elevated to 60°C for 90 s. Simplex PCRs were performed in a 50 µL mix with 2U of Taq DNA polymerase (Roche Diagnostics), 10×PCR buffer/$MgCl_2$ (Roche Diagnostics, Meylan, France), 200 µM of each deoxynucleotide triphosphate (dNTP Mix, Eurobio, Courtaboeuf, France), 0.2 pmol/µL of each primer, 40 µL of sterile water and 2 µL of total DNA extract. PCR conditions consisted in 30 cycles [94°C for 1 min, 55°C for 40 s, 72°C for 1 min], preceded by 1 cycle at 94°C for 5 min and followed by 1 cycle at 72°C for 5 min. PCR products were separated at 100 V for 90 min (180 min for Multiplex IV) on 1.5% agarose gel electrophoresis containing ethidium bromide and visualized using GelDoc (Biorad, Marnes-La-Coquette, France). PCR products were purified using the Exosap purification kit (Illustra Exostar-1 Step, Dutscher, Brumath, France) and subjected to bidirectional DNA sequencing using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an Applied Biosystems 3730 XL capillary sequencer. Sequence analysis was carried out using Sequence scanner (Applied Biosystems), GeneDoc (www.psc.edu/biomed/genedoc) and GenBank BLAST software.

3. Results and discussion

3.1. *In silico* analysis

We mainly focused on plasmid families previously found to be involved in the spread of resistance genes in Enterobacteriaceae (Carattoli, 2013). The majority of plasmids contain a single *par* locus, with the exception of some plasmids such as pR55 plasmid which include two *par* loci (type I *parAB* and type II *parMR*). The type I partitioning loci are the most common of the *par* systems (74%). The partition loci are often close to *rep* gene. In contrast, some of them are located at some distance, e.g. the *parAB* locus of pCTX-M-360 which is 30000 bp by away from RepA. Different designations are used in the annotated sequences for *par* loci: *par, sop, stb*. *In silico* analysis showed that some Inc groups are relatively homogeneous with respect their partition systems, such as IncL/M, N, A/C, I1 or R. The high sequence similarity within these groups (99-100%) allowed designation of a pair of primers for each group. The study of the genetic environment (500 nucleotides on either side of the *par* genes or their equivalent) of the same groups showed high similarity (75 to 100%). In contrast, other groups such as IncF showed more divergent *par* sequences. In addition, IncX plasmids from *K. pneumoniae* (e.g. pIncX-SHV, accession number JN247852 or pKPC-NY79, accession number JX104759) carried a *parA* gene encoding a Walker-type ATPase near the *rep* region and an annotated *parB* gene 25 kb apart from *parA* without a partition-specific multidomain. *In silico* analysis of IncX plasmids in *E. coli* showed a different organization from that found in *K. pneumoniae*, with *parA* and *parB* organized in an operon. *In silico* analysis of IncHI plasmids did not show any correlation between *par* loci and IncHI1 and HI2. Furthermore, the phylogram of *K. pneumoniae* in Fig. 1 reveals partition proteins form distinct subgroups and are connected to Inc group with the exceptions of plasmids IncF.

In silico analysis led to the design of 18 pairs of primers targeting the *par* operons of plasmids from *K. pneumoniae*, *E. coli* and *Salmonella enterica spp.* belonging to Inc groups A/C, F, HI, I1, L/M, N, R. For plasmids belonging to the IncX group we decided to target only the *parA* gene (Table 1). The primer names for IncF (F1 to F9) and IncHI (HIa and HIb) groups were arbitrarily chosen.

3.2. Primer evaluation using transconjugants and transformants

In order to assess the sensitivity and specificity of each PCR, primers were tested using a collection of 136 recipient cells, with PRaseT as the reference method (Table 2). Each primer pair was validated using all recipient cells, first in a simplex and then a multiplex PCR and target DNA of either single cells or cell mixtures was used. PCR conditions were optimized and all amplicons were sequenced. *E. coli* strain J53 was used as negative control in PCR experiments to test for possible cross-hybridization with chromosomal DNA. An example of the results is shown in Fig S1. No non-specific amplification was observed. PAR-T results were largely consistent with the PRaseT results (98.6%) except for two strains which carried an IncX replicon (*E. coli* strains 64 and 110). These results were surprising. If the study of IncX plasmids indicates a diversity in their backbones, no data allow an explanation as to why the *par* loci are organized differently in *K. pneumoniae* and *E. coli* and why there are differences in the *parA* genes between these two species. A complete analysis of all IncX replicon present in the databases will be required (all Enterobacteriaceae).

3.3. Evaluation of PAR-T using clinical strains

Thirty clinical strains, each carrying replicons of one to four different types, were tested by PAR-T to confirm the specificity of the designed primers (Table 3). An example of the results is shown in Fig S1. For 24 strains (80%) there was a perfect correlation between the results obtained with PAR-T and PRaseT. Six strains were positive with PRaseT but negative with PAR-T. Among these, five carried IncX replicons (four *E. coli* strains, i.e. 17, 19, 28 and 34, and one *K. pneumoniae* strain, KpS20) and one (*E. coli* strain 105) carried an IncI1 replicon. As shown by *in silico* analysis, *par* loci in IncX plasmids are organized differently between *K. pneumonia*e and *E. coli*. It is likely that our analysis targeting plasmids >40 kb of *K. pneumoniae* and some *E. coli* did not allow the study of all the *par* loci of IncX plasmids. A complete analysis of all IncX replicon present in the databases will be required (all the Enterobacteriaceae). The strain (*E. coli* 105) that carried an IncI1 plasmid was negative with PAR-T. We considered three possibilities: (i) a divergent IncI1 *par* locus that could not hybridize with our primers was present, (ii) the *par* locus was truncated or (iii) the gene locus was absent. *In silico* analysis has shown that the *par* sequences of IncI1 replicons are well conserved and form a homogen group, thus making the first possibility (i) unlikely.

Overall the specificity were very high (>90%) for all Inc groups studied except IncX.

Finally, four strains (*E. coli* 19 and 34, and *K. pneumoniae* KpS5 and KpS26) which were negative with PRaseT were found to contain IncF and IncR plasmids, respectively, when PAR-T was used. For the *K. pneumoniae* strains, the results were not unexpected as IncR plasmids do not encode relaxases. The two *E. coli* were positive with PBRT with IncFIA, IncFIB and IncFII replicons found in both. Moreover, PCR targeting the genes of the type IV secretion system was negative and the plasmids from neither strain could be transferred to a recipient cell by conjugation (Compain et al., 2014). We considered the possibility of the loss of genes for conjugative transfer.

4. Conclusions

Considering the complexity of constant plasmid evolution and the unavailability of full-length plasmid sequencing in most laboratories, the combined use of several complementary classification methods should be a practical value. Our set of seven multiplex PCRs allowed classification of the most frequently encountered transmissible plasmids in *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella enterica spp*. by targeting their *par* loci, with the exception of IncX replicons. For this group, *in silico* analysis of all plasmids present in the databases must be carried out to design new primers and to improve the value of the method described here.

<u>Conflicting interest</u>

The authors declare that they have no conflicting interests.

References

Branger, C., Zamfir, O., Geoffroy, S., Laurans, G., Arlet, G., Thien, H.V., Gouriou, S., Picard, B., Denamur, E., 2005. Genetic background of Escherichia coli and extended-spectrum beta-lactamase type. Emerg. Infect. Dis. 11, 54–61.

Carattoli, A., 2013. Plasmids and the spread of resistance. Int. J. Med. Microbiol. IJMM 303, 298–304.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., Threlfall, E.J., 2005a. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods 63, 219–228.

Compain, F., Poisson, A., Le Hello, S., Branger, C., Weill, F.-X., Arlet, G., Decré, D., 2014. Targeting relaxase genes for classification of the predominant plasmids in Enterobacteriaceae. Int. J. Med. Microbiol. IJMM 304, 236–242.

Couturier, M., Bex, F., Bergquist, P.L., Maas, W.K., 1988. Identification and classification of bacterial plasmids. Microbiol. Rev. 52, 375–395.

Dallenne, C., Da Costa, A., Decré, D., Favier, C., Arlet, G., 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. 65, 490–495.

García-Fernández, A., Chiaretto, G., Bertini, A., Villa, L., Fortini, D., Ricci, A., Carattoli, A., 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in Escherichia coli and Salmonella of human and animal origin. J. Antimicrob. Chemother. 61, 1229–1233.

Gerdes, K., Howard, M., Szardenings, F., 2010. Pushing and pulling in prokaryotic DNA segregation. Cell 141, 927–942.

Guynet, C., de la Cruz, F., 2011. Plasmid segregation without partition. Mob. Genet. Elem. 1, 236–241.

Marcadé, G., Deschamps, C., Boyd, A., Gautier, V., Picard, B., Branger, C., Denamur, E., Arlet, G., 2009. Replicon typing of plasmids in Escherichia coli producing extended-spectrum beta-lactamases. J. Antimicrob. Chemother. 63, 67–71.

Novick, R.P., 1987. Plasmid incompatibility. Microbiol. Rev. 51, 381–395.

Williams, J.J., Hergenrother, P.J., 2008. Exposing plasmids as the Achilles' heel of drug-resistant bacteria. Curr. Opin. Chem. Biol. 12, 389–399.

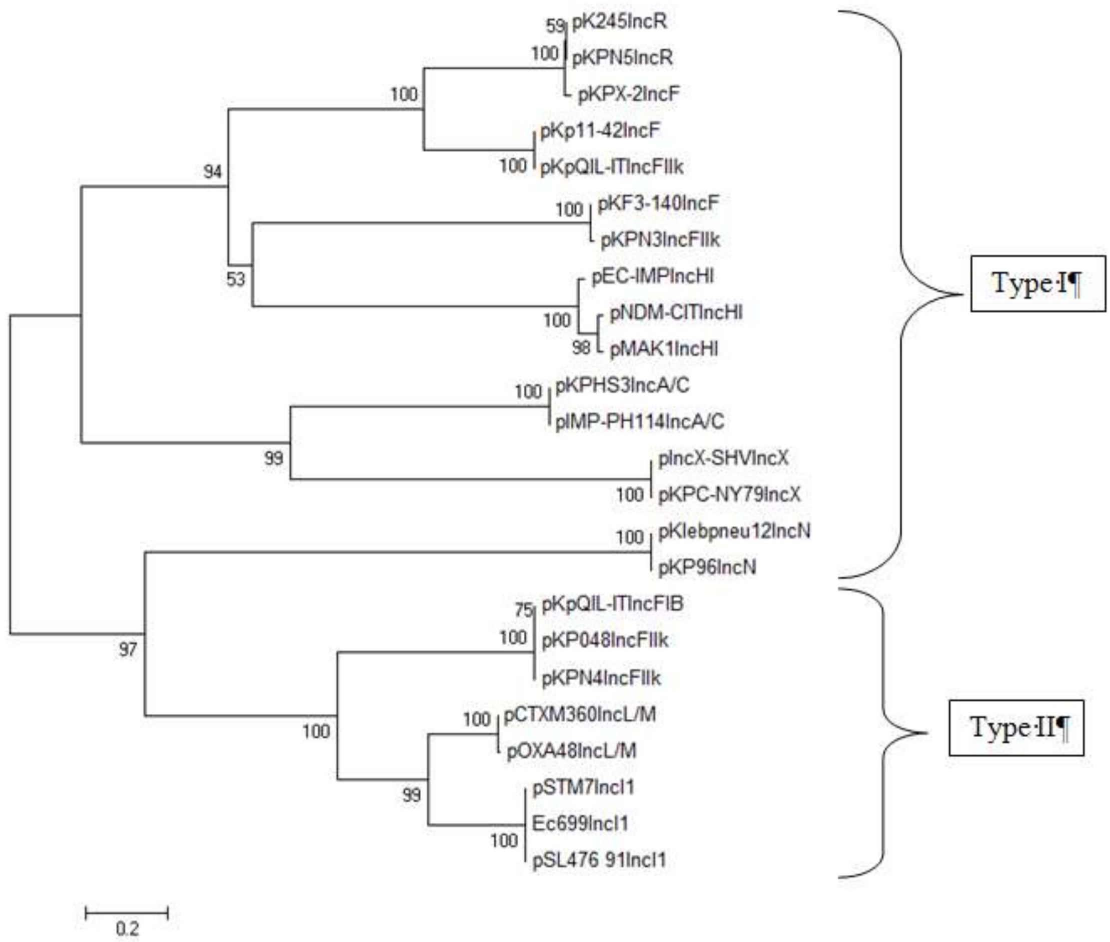

Figure 1. Phylogram of the partition proteins. Unrooted evolutionary tree showing 24 ParA and ParB proteins (or equivalent) from plasmid of *K. pneumoniae, E. coli, Salmonella spp., C. freundii, E. cloacae*. pKpQIL-IT include two *par* loci. Types I and II refer to the two types of plasmid-encoded partitioning loci shown in Fig. 1 and described in the text. The phylogram was constructed using Mega5 (Neighbor-Joining algorithm, bootstrap: 1000 replication).

Table 1. Primers used in this study.

PCR name and plasmid type detected	Primer name	Sequence (5' - 3')	Length (bp)	Plasmid prototype	EMBL accession number	Target site	Amplicon size (bp)	Primer concentration (μM)
Multiplex I, II, III: IncFIA, IncFIB, IncFIC, IncFII, IncFIIk	SopAB-F1-for	CAACCAGTGCATAAGTGCTGGTC	23	pKPN3	CP000648.1	*sopA sopB*	1370	0,4
	SopAB-F1-rev	ACCATACGCGTGAGGCGCTCTCAA	24					
	SopAB-F2-for	ACCAGTGCATAAACGCTGGTC	21	pKF3-140	FJ876827	*sopA sopB*	1130	0,4
	SopAB-F2-rev	GCGTGGTTTAATCAGACGATCGAA	24					
	SopAB-F3-for	GATGACGAAGGCTATTGCCATTGC	24	pKP187	CP000965.1	*sopA sopB*	720	0,4
Multiplex I : *sop* systems *E. coli*, *K. pneumoniae*, *S. enterica*	SopAB-F3-rev	GCGCATACGACATTGATCGTGCCA	24					
	SopAB-F4-for	TGACGAAGGCTATCGCCATTGC	22	pKPN-CZ	JX424424.1	*sopA sopB*	928	0,4
	SopAB-F4-rev	ATGCTTCCCCAGGCATCCCGAA	22					
	SopAB-F5-for	ACCAGTGCATAAACGCTGGTC	21	pH2332-166	KJ484626.1	*sopA sopB*	1533	0,4
	SopAB-	CTGTAAGTGCAGCAGCTTTA	24					

	F5_rev	CGAC						
Multiplex II : *stb* systems *K. pneumoniae*	KP-StbAB-F2-for	TTTGAAGGCGATGAGCTTCAGACC	24	pKP048	FJ628167	*stbA stbB*	1211	0,2
	KP-StbAB-F2-rev	GCCCCACCATTTTCGGGCTCCATCC	24					
	KP-StbAB-F4-for	GTCACGGTATTTGTTGTACACTCC	24	pKpQIL-IT	JN233705	*stbA stbB*	453	0,2
	KP-StbAB-F4-rev	GTTGATACGGTTTTGGATACGCCA	24					
Multiplex III : *stb* systems *E. coli*	EC-StbAB-F1-for	GAACGTATACTGCGATGATGG	21	pEC14_114	GQ398086	stbA stbB	905	0,2
	EC-StbAB-F1-rev	CTTTTTGCCCAAGATGGTGCCA	22					
	EC-StbAB-F2-for	CCGGAATGGTCTATGACGCTGCA	23	pKPC-LKEc	KC788405	*stbA stbB*	1061	0,2
	EC-StbAB-F2-rev	ATCAGGAACGGCAATCGTTCATCC	24					
	EC-StbAB-F3-for	GCGGTCGCAAAATTGCCGAAGCTG	24	pO145-13516	CP006263.1	*stbA stbB*	1194	0,2
	EC-StbAB-F3-rev	GAATTTTGCTTGTTTCCCAGAC	22					
Multiplex IV : IncA/C, L/M, N	ParAB-AC-for	AGGCCTTTTATCTGGCGTTA	20	pKPHS3	CP003225	*parA parB*	1817	0,4
	ParAB-AC-rev	GACAGTAGACGGAACCAGAG	20					
	ParAB-LM-for	CCACCAACATCAAACTGGC	19	pOXA-48	JN626286	*parA parB*	1378	0,1
	ParAB-LM-rev	CAGATGCTGGACGTTCTTAC	20					
	StbABC-N-	TCCCGGCATTATTGATAAA	24	pKP96	EU195449	*stbA stbB*	1093	0,1

	for	GAGTT				*stbC*		
	StbABC-N-rev	ACGGGTT-AAACGTCTCAGC	18					
Multiplex V : IncHI	ParAB-HIa-for	AAGATCGCCCTCGTTGGTCAG	21	pEC-IMP	EU855788	*parA* *parB*	2020	0,4
	ParAB-HIa-rev	CAACTTTTTGAGCAACCTGGAG	22					
	ParAB-HIb-for	AAGATCGCCCTTGTCGGTCAACG	23	pMAK1	AB366440	*parA* *parB*	1765	0,4
	ParAB-HIb-rev	TGGTAACAAATCCATGCTCTTCCA	24					
Multiplex VI : IncR, IncI1	ParAB-R-for	TC-ACGA-CCAGCAAAAAGAGGAA	20	pKP91	CP000966	*parA* *parB*	2032	0,4
	ParAB-R-rev	GCTAAACTCATAAGTCAGCGT	20					
	ParAB-I1-for	GACGGCGAGAAGTTTTCATT	20	Ec699	Replicolscope	*parA* *parB*	1227	0,4
	ParAB-I1-rev	TTCAGCGTTTCTTCTGGTCT	20					
Simplex VII : IncX	ParA-X-for	GAGCTTCAACAGCAGAACAG	21	pIncX-SHV	JN247852	*parA*	633	0,2
	ParA-X-rev	ATTGCATCATGTCTGGCTTG	20					

Table 2. Recipient cells used in validation experiments. Tc : transconjugants, Tf : transformant, PRaseT : plasmid relaxase gene typing, PBRT : PCR-based replicon typing, PAR-T : plasmid partitioning genes typing. † : Tc used as a positive control in partitioning genes typing.

N°	Parental strain	Tc/Tf	β-Lactamase	Plasmid classification	
				PRaseT (and/or PBRT)	PAR-T
48	*E. coli*	Tc	TEM-52	X1	X
51	*E. coli*	Tc	TEM-3	L/M	X
52	*E. coli*	Tf	TEM-52	X1	X
57	*E. coli*	Tc	SHV-12	F	F
62	*E. coli*	Tc	SHV-2	FIB	F
64	*E. coli*	Tc	SHV-5	X4	-
73	*E. coli*	Tc	SHV-4	FIIK	FIIK
85	*E. coli*	Tf	CTX-M-1	FIA	F
91	*E. coli*	Tc	CTX-M-3	N	N
94†	*E. coli*	Tc	CTX-M-9	FII	F
98	*E. coli*	Tc	CTX-M-1	X1	X
100	*E. coli*	Tc	CTX-M-1	FIA, FII	F
102	*E. coli*	Tc	CTX-M-1	HI2	HIa
104	*E. coli*	Tf	CTX-M-1	FIA, FIB, FII	F
105	*E. coli*	Tc	CTX-M-1	L/M	L/M
108	*E. coli*	Tf	CTX-M-1	FIA	F
110	*E. coli*	Tc	CTX-M-1	X4	-
111†	*E. coli*	Tc	CTX-M-1	FIA	F
114	*E. coli*	Tc	CTX-M-1	L/M	L/M
115	*E. coli*	Tc	CTX-M-9	FII	F
118	*E. coli*	Tc	CTX-M-1	FIA, FIB	F
120	*E. coli*	Tc	CTX-M-3	FIB	F
122	*E. coli*	Tc	CTX-M-9	FII	F
125	*E. coli*	Tc	CTX-M-1	FII	F
126	*E. coli*	Tc	CTX-M-1	N	N
127	*E. coli*	Tc	CTX-M-3	FIB	F
Levy	*E. coli*	Tc	OXA-48	L/M	L/M
AD-48	*E. coli*	Tc	OXA-48	L/M	L/M
AD-50	*E. coli*	Tc	OXA-48	L/M	L/M
AD-17	*E. coli*	Tc	OXA-48	L/M	L/M
AD-44-2	*E. coli*	Tc	NDM-1	N	N
AD-2	*E. coli*	Tc	OXA-48	L/M	L/M
Goe-137	*E. coli*	Tc	VIM	HI2	HIa
Goe-132	*E. coli*	Tc	VIM	A/C	A/C
KATS	*E. coli*	Tc	NDM-1, CTX-M-1	F	F
M-2	*E. coli*	Tc	KPC-2	L/M	L/M
M-3	*E. coli*	Tc	KPC-2	L/M	L/M
M-5	*E. coli*	Tc	KPC-2	L/M	L/M
Ec50	*E. coli*	Tc	TEM-52	I1	I1
S1	*K. pneumoniae*	Tc	CMY-4	A/C	A/C

S3	*K. pneumoniae*	Tc	FOX-3	A/C	A/C
S4	*K. pneumoniae*	Tc	DHA-1	A/C	A/C
S6	*K. pneumoniae*	Tc	SHV-5	A/C	A/C
S8	*K. pneumoniae*	Tc	CMY-2	A/C	A/C
S9	*K. pneumoniae*†	Tc	SHV-4	FIIk	F
S10	*K. pneumoniae*	Tc	ACC-1	A/C	A/C
S11	*K. pneumoniae*	Tc	CMY-4	A/C	A/C
S12	*K. pneumoniae*	Tc	SHV-2, DHA-1	FIIk, R	F, R
S13	*K. pneumoniae*	Tc	SHV-1	A/C	A/C
S14†	*K. pneumoniae*	Tc	CTX-M-15, CMY-4, VIM-4	A/C	A/C
S16	*K. pneumoniae*	Tc	CTX-M-3	A/C	A/C
S18	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	F	F
S19†	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	N	N
S20	*K. pneumoniae*	Tc	OXA-1, SHV-2a, CTX-M-15	F	F
S21	*K. pneumoniae*	Tc	TEM-3	A/C	A/C
S23	*K. pneumoniae*	Tc	CTX-M- 3	N, A/C	N, A/C
S24	*K. pneumoniae*	Tc	TEM-3	A/C	A/C
S26	*K. pneumoniae*	Tc	SHV-12	N	N
S28	*K. pneumoniae*	Tc	DHA-1	L/M	L/M
S30†	*K. pneumoniae*	Tf	OXA-1, DHA-1	R	R
S33	*K. pneumoniae*	Tf	DHA-1	R	R
S34†	*K. pneumoniae*	Tc	DHA-1	L/M	L/M
S36	*K. pneumoniae*	Tc	OXA-1, CTX-M-3	FII	F
S43	*K. pneumoniae*	Tc	CTX-M-3	N	N
S45	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	L/M	L/M
S46	*K. pneumoniae*	Tc	CTX-M-15	L/M	L/M
S47	*K. pneumoniae*	Tc	CTX-M-3	L/M	L/M
S48	*K. pneumoniae*	Tc	CTX-M-15	L/M	L/M
S49	*K. pneumoniae*	Tc	CTX-M-15	L/M	L/M
S51	*K. pneumoniae*	Tc	SHV-12	FIIk	F
S53	*K. pneumoniae*	Tc	SHV-12	R	R
S55	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	FIIk	F
S56	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	FIIk R	F, R
S61	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	F	F
S68	*K. pneumoniae*	Tc	TEM-129	A/C	A/C
S72	*K. pneumoniae*	Tc	SHV2a	F	F
S73	*K. pneumoniae*	Tc	SHV-4	A/C	A/C
S75†	*K. pneumoniae*	Tc	OXA-1, CTX-M-15	FIIk	F
S76	*K. pneumoniae*	Tc	TEM-12	F	F
S77	*K. pneumoniae*	Tf	SHV-12	R	R
S78	*K. pneumoniae*	Tc	CTX-M-15	FII	F

S79	*K. pneumoniae*	Tc	CTX-M-15	F	F
S82	*K. pneumoniae*	Tc	CTX-M-15	FII	F
S83	*K. pneumoniae*	Tc	TEM-3	A/C	A/C
S86	*K. pneumoniae*	Tc	CMY-4	A/C	A/C
S88	*K. pneumoniae*	Tc	SHV-2a	FIIk	F
S89	*K. pneumoniae*	Tc	TEM-21	A/C	A/C
S90†	*K. pneumoniae*	Tc	KPC	X3	X
FM1	*K. pneumoniae*	Tc	OXA-48, CTX-M-15	L/M	L/M
FM2	*K. pneumoniae*	Tc	OXA-48, CTX-M-15	L/M	L/M
FUR-STA	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
CLE-TN	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
PET-TN	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
MUR-STA	*K. pneumoniae*	Tc	NDM-1	FIIk	F
2966	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
LD-1131	*K. pneumoniae*	Tc	VIM	R	R
LD-3856	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
Z-19760	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
Z-45518	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
Z-16300	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
Z-47994	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
Z-4359	*K. pneumoniae*	Tc	OXA-48	L/M	L/M
MIKH	*K. pneumoniae*	Tc	OXA-48, CTX-M9	HI1	HIb
BHR	*K. pneumoniae*	Tc	VIM-4, CTX-M-1	HI1	HIb
M-4	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-6	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-7	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-14	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-20	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-32	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-40	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-41	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-50	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-52	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
M-53	*K. pneumoniae*	Tc	KPC-2	L/M	L/M
TNDHA-5	*K. pneumoniae*	Tc	DHA-1, SHV-12	HI2	HIb
TNDHA-6	*K. pneumoniae*	Tc	DHA-1, SHV-12	HI2	HIb
TNDHA-7	*K. pneumoniae*	Tc	DHA-1, SHV-12	HI2	HIb
TNDHA-8	*K. pneumoniae*	Tc	DHA-1, SHV-12	HI2	HIb
S00056	*S. enterica* Typhimurium	Tc	CTX-M-2	HI2	HIa
S00319†	*S. enterica* Havana	Tc	CTX-M-15	HI2	HIa

S01331	*S. enterica* Tel el kebir	Tc	CTX-M-15	HI2	HIa
S01477†	*S. enterica* Typhimurium	Tc	CTX-M-1/CMY-2	HI1, I1	I1, HIb
S01650	*S. enterica* Brandeburg	Tc	CTX-M-14	FrepB	F
S03207	*S. enterica* Typhimurium	Tf	CTX-M-15	FIA, FIB	F
S03663	*S. enterica* Grumpensis	Tc	CTX-M-15	HI2	HIa
S03664	*S. enterica* Typhimurium	Tc	CTX-M-15	N	N
S04662	*S. enterica* Virchow	Tc	CTX-M-32	N	N
S05343	*S. enterica* Concord	Tc	CTX-M-15	HI2	HIa
S07364	*S. enterica* Miami	Tc	SHV-2	N	N
S09118	*S. enterica* Keurmassar	Tc	SHV-12	HI2, FI	F, HIa
S27078	*S. enterica* Carmel	Tc	CTX-M-15	FrepB	F
S7917	*S. enterica* Derby	Tc	ND	FIA	F
S7981	*S. enterica* Saintpaul	Tc	OXA-48	L/M	L/M
M-1	S. *enterica* Enteritidis	Tc	KPC-2	L/M	L/M
S1106†	*S. enterica* Virchow	Tc	SHV-12	I1	I1

Table 3. Plasmid partition gene typing of 30 clinical strains of Enterobacteriaceae.

N°	Parental species	β-Lactamase	PRase T	PAR-T				
				Multiplex I and II	Multiplex III	Multiplex IV	Multiplex V	Simplex VI
3	*E. coli*	TEM-24	A/C		A/C			
15	*E. coli*	TEM-24	A/C, I1		A/C		I1	
17	*E. coli*	TEM-24	A/C, F, HI2, X4	F	A/C	HI		-
19	*E. coli*	TEM-21	A/C, N, X1	F	A/C, N			-
23	*E. coli*	TEM-24	A/C, F, I1	F	A/C		I1	
26	*E. coli*	TEM-24	A/C, F, HI1	F	A/C	HIa		
28	*E. coli*	TEM-21	A/C, X1		A/C			-
33	*E. coli*	TEM-24	A/C		A/C			
34	*E. coli*	TEM-24	A/C, N, X1	F	A/C, N			-
40	*E. coli*	TEM-24	A/C, F	F	A/C			
50	*E. coli*	TEM-52	I1				I1	
84	*E. coli*	CTX-M-1	F HI1, N	F	N	HIb		
88	*E. coli*	CTX-M-1	F, HI1, N	F	N	HIa		
101	*E. coli*	CTX-M-2	HI2			Hia		
105	*E. coli*	CTX-M-1	L/M, I1		L/M		-	
106	*E. coli*	CTX-M-1	F, HI1, N	F	N	HIa		
107	*E. coli*	CTX-M-2	I1, F	F			I1	
KpS 5	*K. pneumoniae*	DHA-1	FIIK, L/M	F	L/M		R	
KpS 19	*K. pneumoniae*	CTX-M-15, DHA-1	FIIK, F, L/M, N	F	L/M, N			
KpS 20	*K. pneumoniae*	CTX-M-15,SH	F, X4	F				-

		V-2a						
KpS 26	*K. pneumoniae*	SHV-12	FIIK, N	F	N		R	
KpS 47	*K. pneumoniae*	CTX-M-3	HI2, L/M		L/M	HIa		
KpS 63	*K. pneumoniae*	DHA-1	FIIK, L/M	F	L/M			
KpS 83	*K. pneumoniae*	TEM-3	A/C, F, N		A/C, N			
KpS 88	*K. pneumoniae*	SHV-2a	FIIK	F				
KpS 92	*K. pneumoniae*	CTX-M-14, VIM-1	F, FIIK, I1	F			I1	
FM-2	*K. pneumoniae*	OXA-48	FIIK, L/M, N	F	L/M, N			
FM-10	*K. pneumoniae*	OXA-48	A/C, L/M		A/C, L/M			
S10-1477	*S. enterica* Typhimurium	CTX-M-1, CMY-2	HI1, I1			HIb	I1	
S10-1526	*S. enterica* Typhimurium	CTX-M-1, CMY-2	HI1, I1			HIb	I1	

Highlights

-Low-copy-number plasmids utilize partition systems for plasmid maintenance.

-*par* loci are organized into operons encoding two proteins, an ATPase and a DNA-binding protein, and including a centromeric site.

-The method called "plasmid partition gene typing" showed high specificity for the classification of resistance plasmids (IncA/C, FIA, FIB, FIC, FIIk, FII, HI1, HI2, I1, L/M, N) except for IncX replicons.