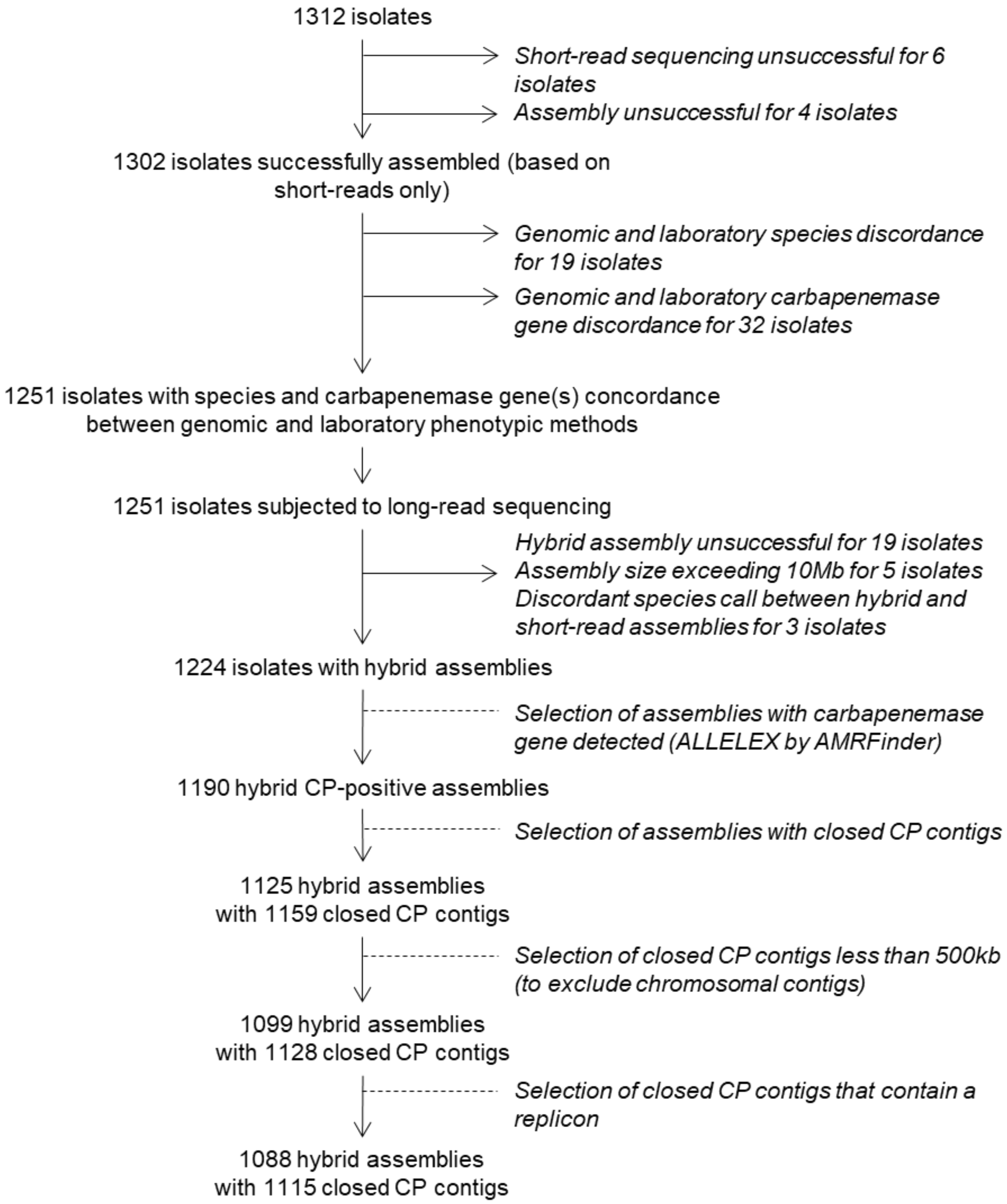
Supplementary Information

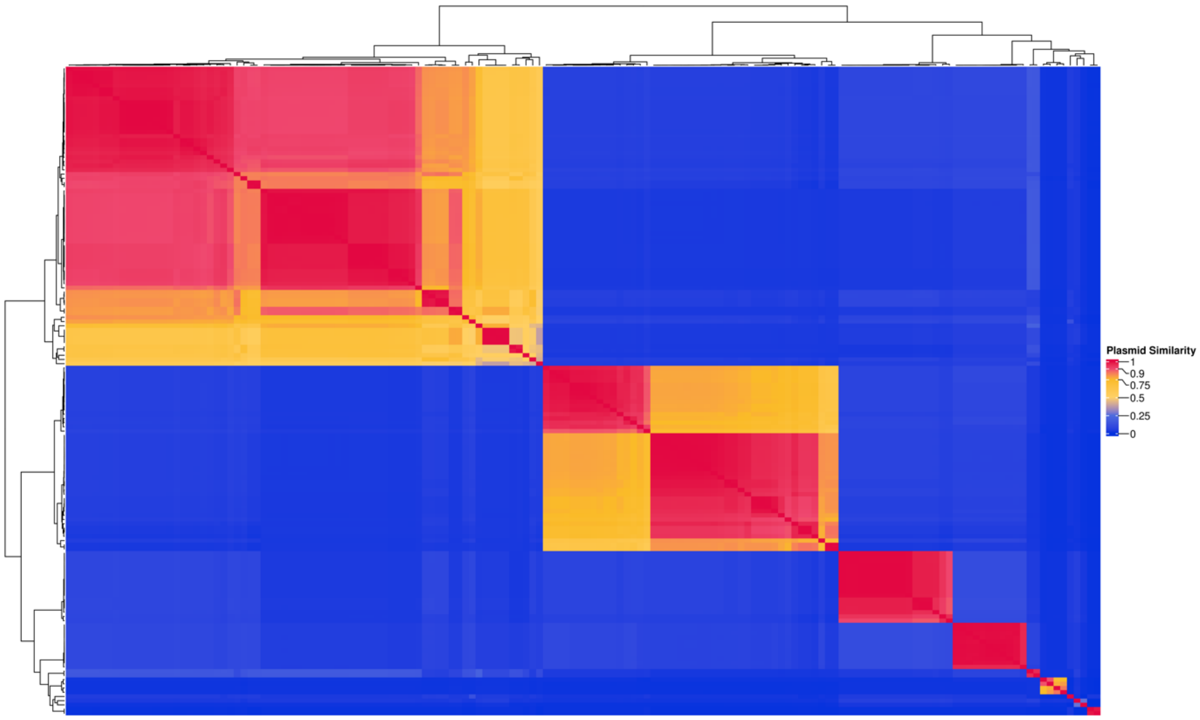
Original Research

Title: Plasmid dynamics driving carbapenemase gene dissemination in healthcare environments: a nationwide analysis of closed Enterobacterales genomes

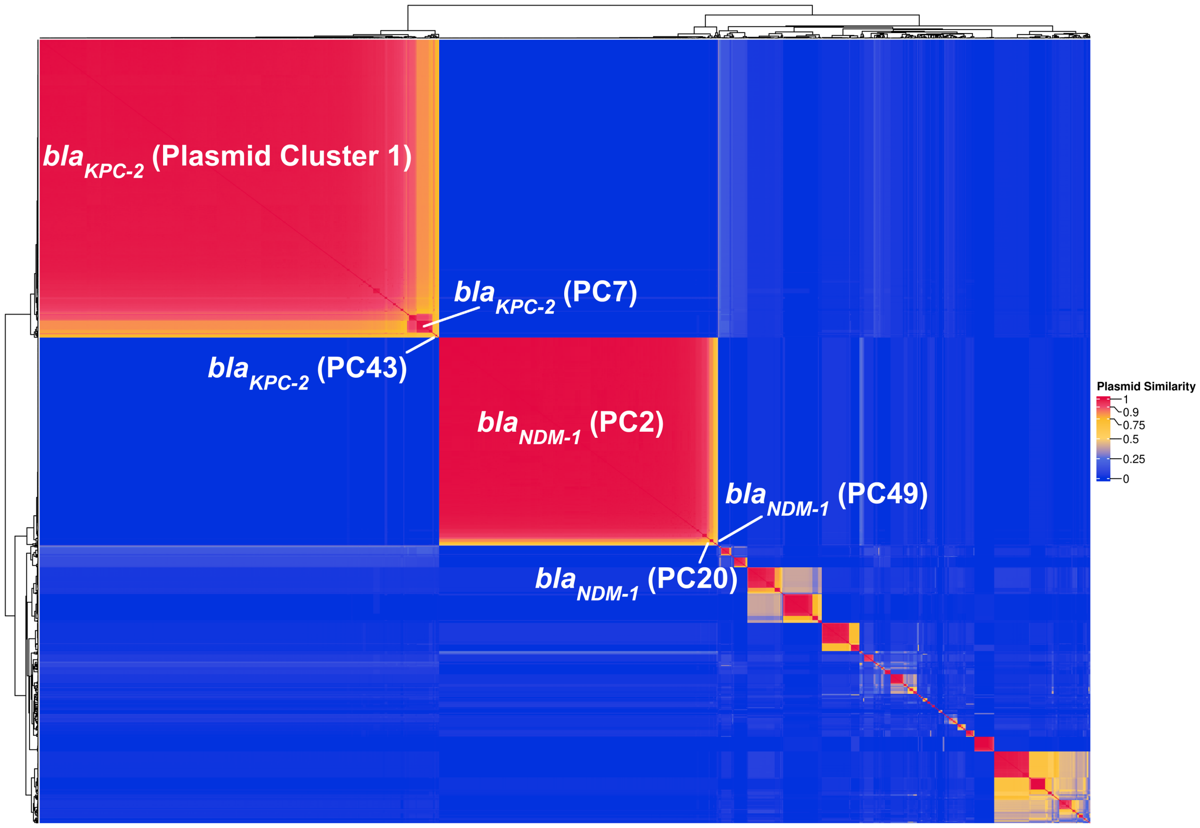
Supplementary Figures



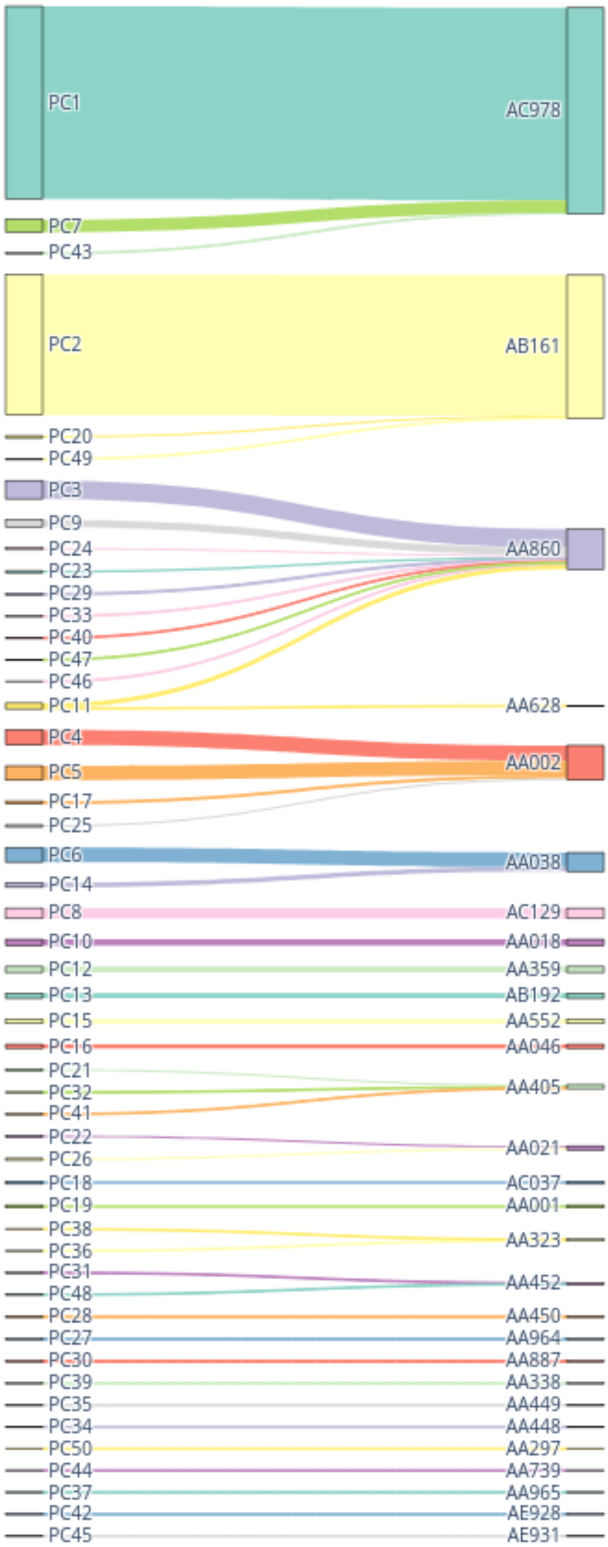
Supplementary Figure 1. Disposition of isolates analysed in the study. From September 6, 2010 to April 28, 2015, 1,251 carbapenemase-producing Enterobacterales isolates (from 791 patients) confirmed by both genomic and phenotypic methods were collected across participating study sites. Hybrid assemblies were constructed for 1,088 isolates, for which 1,115 closed carbapenemase-producing plasmids were identified.



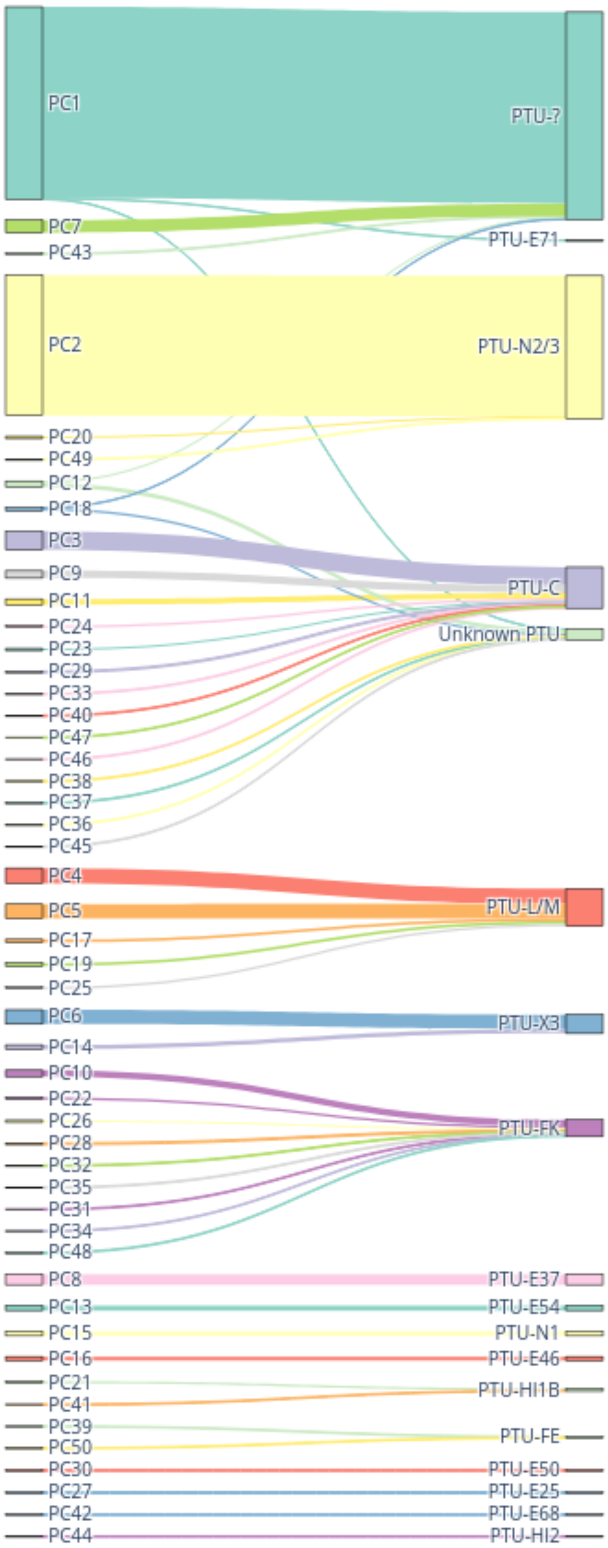
Supplementary Figure 2. The same plasmid clustering method was applied on 154 circularized *bla*$_{\text{IMP-4}}$ plasmids from another an institutional collection of mostly clinical carbapenem-resistant isolates systematically collected from 2002 to 2020[7]. This grouped 140 plasmids into 12 plasmid clusters, leaving 14 unclustered.



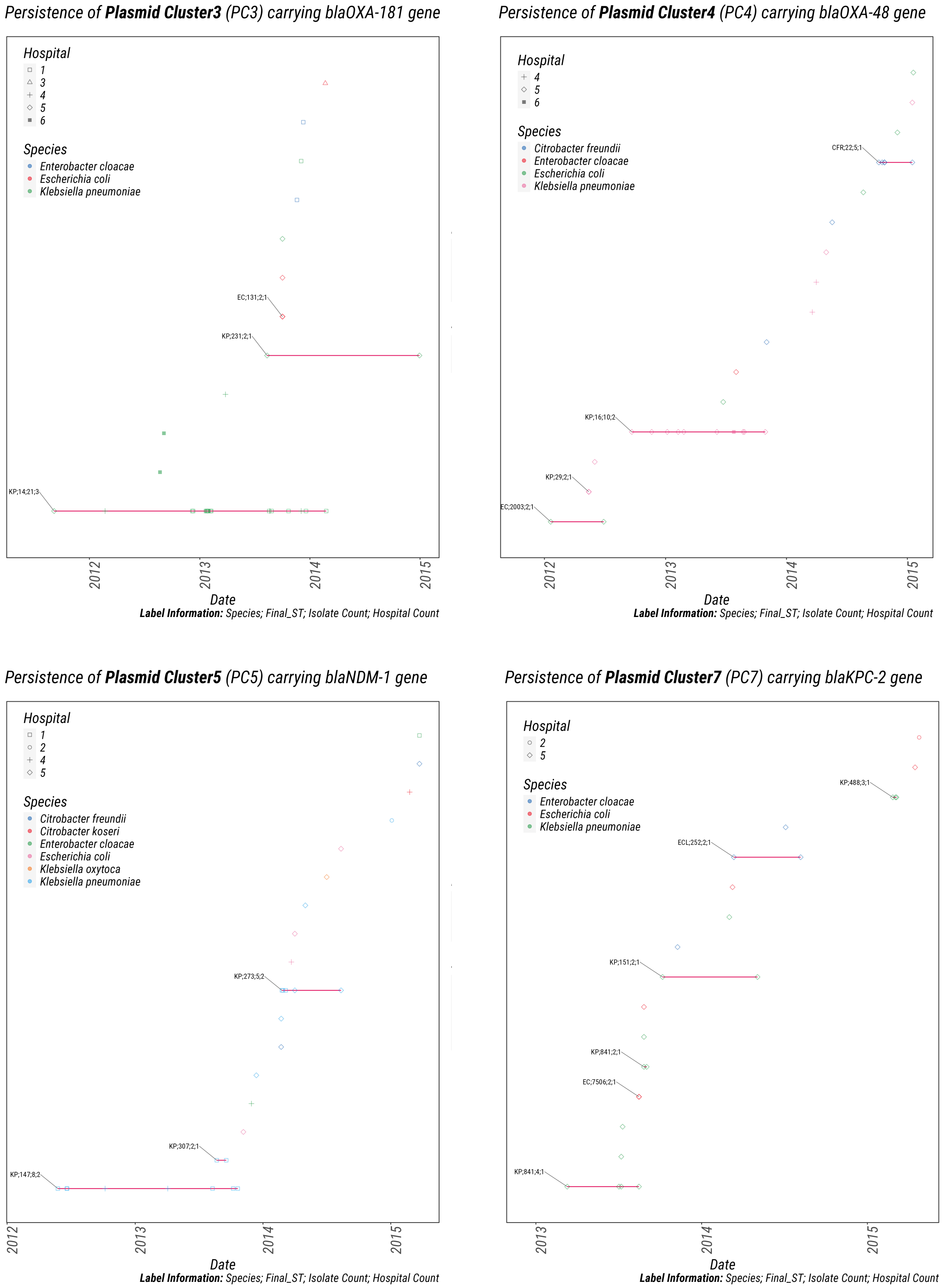
Supplementary Figure 3. Heatmap showing the plasmids grouped into 50 plasmid clusters based on pairwise k-mer (21bp) similarity. The two largest plasmid clusters, PC1 and PC2, as well as the clusters comprising plasmids carrying the same carbapenemase genes and closest in similarity to PC1 (PC7 and PC43) and PC2 (PC20 and PC49) are annotated. Intra-cluster pairwise k-mer similarity was 81.8 to 99.8%, 82.0% to 99.5% and 98.9% for PC1, PC7 and PC43 respectively. Intra-cluster pairwise k-mer similarity was 83.3% to 100%, 97.0% to 99.8% and 94.7% for PC2, PC20 and PC49 respectively.



Supplementary Figure 4. Sankey diagram mapping plasmid clusters from our method to MOBsuite cluster codes



Supplementary Figure 5. Sankey diagram mapping plasmid clusters from our method to COPLA-defined plasmid taxonomic units

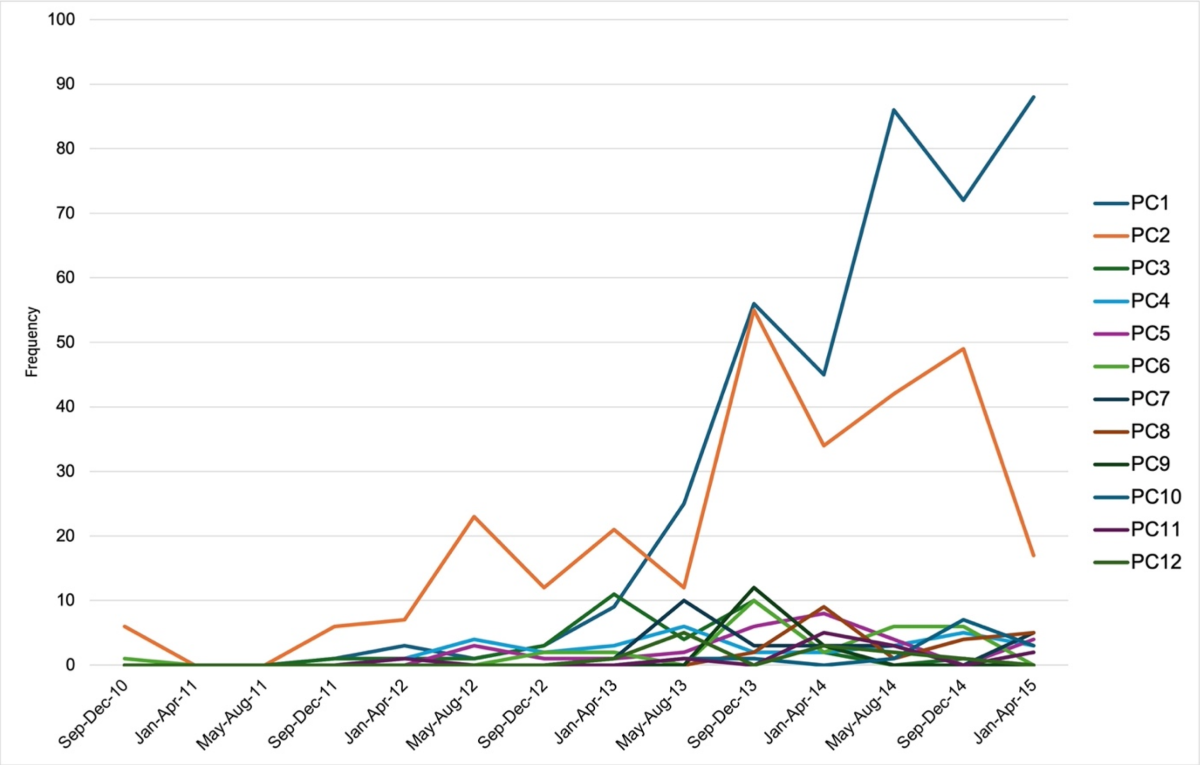

Persistence of **Plasmid Cluster3** (PC3) carrying blaOXA-181 gene

Persistence of **Plasmid Cluster4** (PC4) carrying blaOXA-48 gene

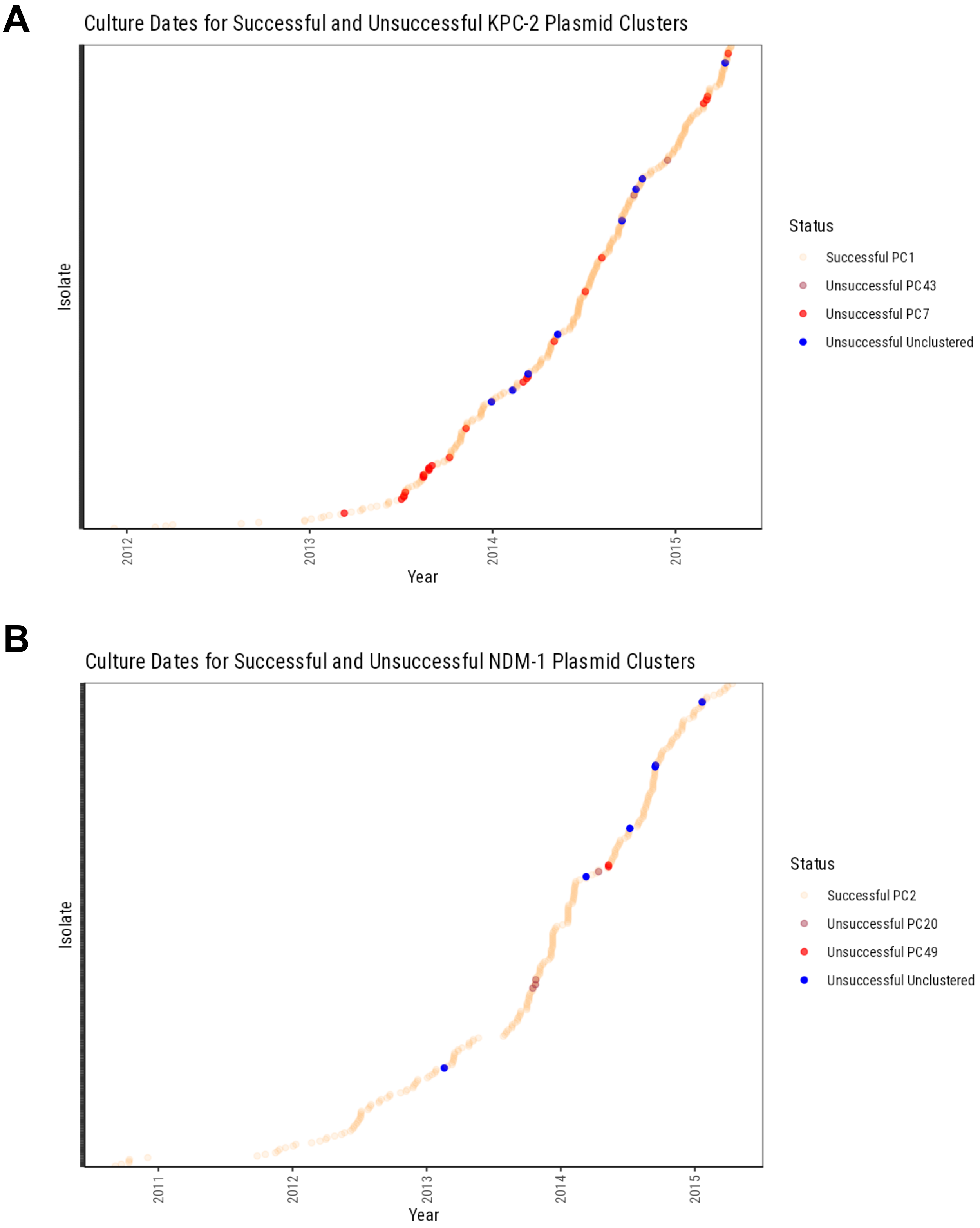
Persistence of **Plasmid Cluster5** (PC5) carrying blaNDM-1 gene

Persistence of **Plasmid Cluster7** (PC7) carrying blaKPC-2 gene

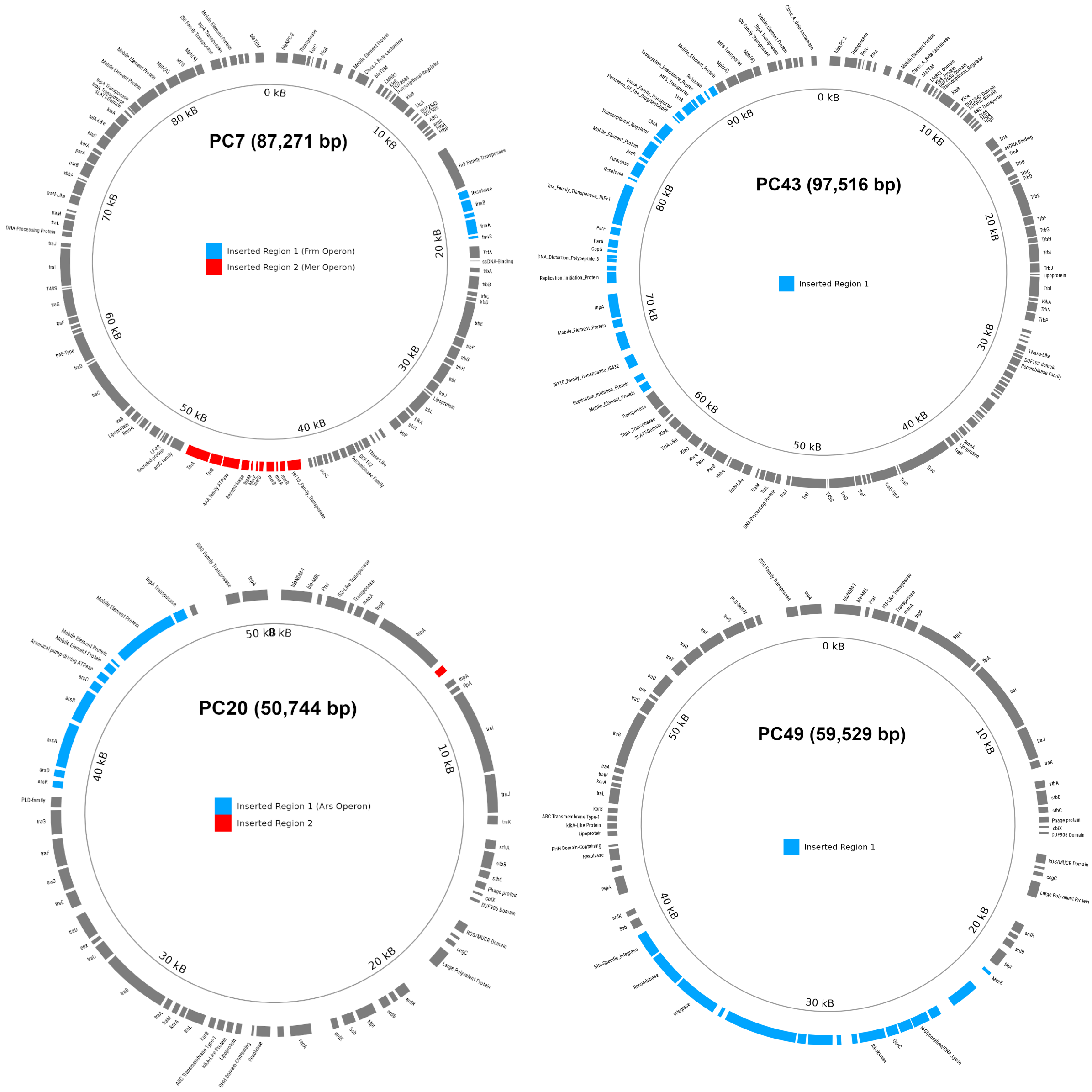
Supplementary Figure 6. Transmission dynamics of selected carbapenemase-encoding plasmid clusters over the five-year study period. Each point represents the incidence of an isolate carrying the plasmid of interest. The points are ordered along the x-axis based on the date of detection, with displacement along the y-axis for clarity. Plasmids that occurred in isolates that met pairwise clonal linkage criteria are visually connected by pink horizontal lines. For each clonal lineage, the following information is annotated in this format: "abbreviated species; ST; number of isolates; number of hospitals where the clone was detected"



Supplementary Figure 7. Temporal trends of plasmid clusters that accounted for >1% of all plasmids (n=12).



Supplementary Figure 8. Date of culture for (A) PC1, PC7, PC43 and other unclustered *bla*$_{\text{KPC-2}}$-positive plasmids. (B) PC2, PC20, PC49 and other unclustered *bla*$_{\text{NDM-1}}$-positive plasmids



Supplementary Figure 9. Representative closed PC7 and PC20 plasmids

Supplementary Tables

Supplementary Table 1. Distribution of n=1,088 CPE isolates by species and hospital.

Species	**Hospital (%, by hospital)**					
	1 (n=72)	**2 (n=33)**	**3 (n=25)**	**4 (n=76)**	**5 (n=753)**	**6 (n=129)**
K. pneumoniae (n=486)	48 (66.7)	16 (48.5)	10 (40.0)	46 (60.5)	323 (42.9)	43 (33.3)
E. coli (n=339)	17 (23.6)	10 (30.3)	11 (44.0)	20 (26.3)	232 (30.8)	49 (38.0)
E. cloacae (n=145)	6 (8.3)	4 (12.1)	2 (8.0)	2 (2.6)	106 (14.1)	25 (19.4)
C. freundii (n=62)	1 (1.4)	2 (6.1)	2 (8.0)	5 (6.6)	47 (6.2)	5 (3.9)
Other *Citrobacter spp.*[a] (n=21)	0	1 (3.0)	0	1 (1.3)	18 (2.4)	1 (0.8)
E. aerogenes (n=15)	0	0	0	1 (1.3)	13 (1.7)	1 (0.8)
K. oxytoca (n=15)	0	0	0	0	13 (1.7)	2 (1.6)
M. morganii (n=4)	0	0	0	1 (1.3)	1 (0.1)	2 (1.6)
S. marcescens (n=1)	0	0	0	0	0	1 (0.8)

[a] Refers to *C. amalonaticus* (n=10), *C. koseri* (n=7), *C. farmeri* (n=2) and *C. rodentium* (n=2).

Supplementary Table 2. Distribution of the most common sequence-types among the three predominant CPE species.

Species	**ST**	**No. of isolates (%)**
K. pneumoniae (n=486)	14	50 (10.3)
	147	30 (6.2)
	16	28 (5.8)
	11	28 (5.8)
	231	25 (5.1)
	273	21 (4.3)
	20	18 (3.7)
	841	16 (3.3)
	34	14 (2.9)
	307	13 (2.7)
	15	12 (2.5)
	17	12 (2.5)
	Others	219
E. *coli* (n=339)	131	64 (18.9)
	410	17 (5.0)
	101	15 (4.4)
	1193	12 (3.5)
	167	11 (3.2)
	38	10 (2.9)
	68	9 (2.7)
	162	8 (2.4)
	224	8 (2.4)
	10	7 (2.1)
	Others	178
E. cloacae (n=145)	93	32 (22.1)
	592	8 (5.5)
	171	8 (5.5)
	418	8 (5.5)
	54	8 (5.5)
	66	6 (4.1)
	1595	5 (3.4)
	270	4 (2.8)
	125	4 (2.8)
	127	4 (2.8)
	1	4 (2.8)
	17	4 (2.8)
	Others	50

Supplementary Table 3. Distribution of n=1,115 carbapenemase genes by hospital.

Hospital	**Carbapenemase gene (%, by hospital)**														
	***bla*$_{\text{KPC-2}}$**	***bla*$_{\text{NDM-1}}$**	***bla*$_{\text{OXA-181}}$**	***bla*$_{\text{OXA-48}}$**	***bla*$_{\text{IMP-1}}$**	***bla*$_{\text{OXA-232}}$**	***bla*$_{\text{NDM-7}}$**	***bla*$_{\text{NDM-5}}$**	***bla*$_{\text{NDM-4}}$**	***bla*$_{\text{NDM-9}}$**	***bla*$_{\text{OXA-23}}$**	***bla*$_{\text{IMP-4}}$**	***bla*$_{\text{IMP-26}}$**	***bla*$_{\text{VIM-4}}$**	***bla*$_{\text{KPC-6}}$**
1 (n=72)	5 (6.9)	42 (58.3)	22 (30.6)	0	0	0	0	1 (1.4)	0	2 (2.8)	0	0	0	0	0
2 (n=33)	8 (24.2)	13 (39.4)	1 (3.0)	6 (18.2)	2 (6.1)	1 (3.0)	2 (6.1)	0	0	0	0	0	0	0	0
3 (n=25)	3 (12.0)	15 (60.0)	1 (4.0)	0	0	1 (4.0)	2 (8.0)	2 (8.0)	1 (4.0)	0	0	0	0	0	0
4 (n=83)	5 (6.0)	43 (51.8)	14 (16.9)	2 (2.4)	1 (1.2)	2 (2.4)	1 (1.2)	6 (7.2)	2 (2.4)	1 (1.2)	2 (2.4)	0	2 (2.4)	1 (1.2)	1 (1.2)
5 (n=770)	441 (57.3)	228 (29.6)	19 (2.5)	28 (3.6)	11 (1.4)	15 (1.9)	14 (1.8)	9 (1.2)	3 (0.4)	1 (0.1)	1 (0.1)	0	0	0	0
6 (n=132)	15 (11.4)	93 (70.5)	3 (2.3)	5 (3.8)	8 (6.1)	2 (1.5)	1 (0.8)	0	0	0	1 (0.8)	4 (3.0)	0	0	0

Supplementary Table 4. Distribution of carbapenemase genes across of n=1,115 plasmids classified by replicon type.

Inc group	**Carbapenemase gene**														
	bla$_{\text{KPC-2}}$	***bla***$_{\text{NDM-1}}$	***bla***$_{\text{OXA-181}}$	***bla***$_{\text{OXA-48}}$	***bla***$_{\text{IMP-1}}$	***bla***$_{\text{OXA-232}}$	***bla***$_{\text{NDM-7}}$	***bla***$_{\text{NDM-5}}$	***bla***$_{\text{NDM-4}}$	***bla***$_{\text{NDM-9}}$	***bla***$_{\text{OXA-23}}$	***bla***$_{\text{IMP-4}}$	***bla***$_{\text{IMP-26}}$	***bla***$_{\text{VIM-4}}$	***bla***$_{\text{KPC-6}}$
IncU (n=432)	432	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IncU with other replicon types (n=43)	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IncN (n=296)	1	294	0	0	0	0	0	0	0	1	0	0	0	0	0
IncN with other replicon types (n=8)	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0
IncC (n=45)	0	23	3	0	13	0	0	0	0	0	0	4	1	1	0
IncC with other replicon types (n=55)	0	7	42	0	5	0	0	0	0	0	0	0	1	0	0
IncL/M (n=48)	0	6	0	41	1	0	0	0	0	0	0	0	0	0	0
IncL/M with other replicon types (n=31)	0	30	0	0	1	0	0	0	0	0	0	0	0	0	0
IncF (n=60)	1	40	0	0	0	0	0	8	4	3	4	0	0	0	0
IncF with other replicon types (n=13)	0	11	2	0	0	0	0	0	0	0	0	0	0	0	0
IncX3 (n=32)	0	2	0	0	0	0	19	10	1	0	0	0	0	0	0
IncX3,rep_cluster_1195 (n=10)	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
IncR (n=10)	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
IncR with other replicon types (n=1)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
IncH with other replicon types (n=6)	0	4	0	0	0	0	1	0	1	0	0	0	0	0	0
rep_cluster_1195 (n=22)	0	0	1	0	0	21	0	0	0	0	0	0	0	0	0
rep_cluster_1254 (n=1)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
rep_cluster_573 (n=1)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
rep_cluster_2335 (n=1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Supplementary Table 5. Distribution of carbapenemase genes across of n=1,115 plasmids classified by MOB type.

MOB type	**Carbapenemase gene**														
	$bla_{\text{KPC-2}}$	$bla_{\text{NDM-1}}$	$bla_{\text{OXA-181}}$	$bla_{\text{OXA-48}}$	$bla_{\text{IMP-1}}$	$bla_{\text{OXA-232}}$	$bla_{\text{NDM-7}}$	$bla_{\text{NDM-5}}$	$bla_{\text{NDM-4}}$	$bla_{\text{NDM-9}}$	$bla_{\text{OXA-23}}$	$bla_{\text{IMP-4}}$	$bla_{\text{IMP-26}}$	$bla_{\text{VIM-4}}$	$bla_{\text{KPC-6}}$
MOBP (n=503)	363	43	10	35	2	21	19	9	1	0	0	0	0	0	0
MOBF (n=381)	30	334	0	0	0	0	0	7	4	4	2	0	0	0	0
MOBH (n=104)	1	34	43	0	18	0	1	0	1	0	0	4	1	1	0
MOBC (n=4)	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Multiple MOB (n=13)	3	6	3	0	0	0	0	0	0	0	0	0	1	0	0
Not typed[a] (n=110)	79	14	4	6	2	0	0	2	0	0	2	0	0	0	1

[a] Relaxase not detected

Supplementary Table 6. Top 10 most common STs for the two most common host species for PC1 and PC2.

Plasmid cluster	Host species	ST	No. of plasmids
PC1	*Klebsiella pneumoniae*	231	11
		17	10
		323	10
		526	10
		5606	10
		65	9
		70	7
		841	7
		20	5
		307	5
	Escherichia coli	131	14
		68	8
		1193	7
		162	7
		167	4
		224	4
		38	4
		10	3
		216	3
		2797	3
PC2	*Escherichia coli*	131	45
		101	6
		162	6
		4532	6
		410	4
		1193	3
		448	3
		6272	3
		69	3
		354	2
	Klebsiella pneumoniae	34	12
		11	7
		14	6
		307	6
		15	4
		16	4
		231	4
		496	4
		2028	3
		273	3

Supplementary Table 7. Representative best hit for n=35 plasmid clusters with at least one match (weighted average identity >99%) in the PLSDB database outside of this dataset.

Plasmid Cluster	Hit Accession	Hit Long Name	Hit Length (bp)	Hit Coverage (%)	Weighted Average Identity	Location
PC2	NC_024954.1	Escherichia coli plasmid pNDM-ECS01, complete sequence	41190	100	99.981	Thailand
PC3	NZ_CP099753.1	Escherichia coli strain 806883-11-2019 plasmid pJEF1-OXA-181, complete sequence	177859	99.3	99.974	Switzerland
PC4	NZ_CP040036.1	Klebsiella pneumoniae strain KPC160117 plasmid pOXA48-L117, complete sequence	65499	100	99.991	Taiwan
PC5	NZ_CP032193.1	Salmonella enterica subsp. enterica serovar Senftenberg strain AR_0127 plasmid unnamed2, complete sequence	87450	100	99.974	NA
PC6	NZ_AP023210.1	Escherichia coli strain TUM18781 plasmid pMTY18781-5, complete sequence	46161	100	99.998	NA
PC8	LC521850.1	Klebsiella pneumoniae KP161 plasmid pKP161_OXA232 DNA, complete sequence	6141	100	100	NA
PC9	NZ_CP015835.1	Escherichia coli strain MS6198 plasmid pMS6198A, complete sequence	137565	92.1	99.969	United Kingdom
PC10	NZ_CP104680.1	Klebsiella pneumoniae strain 2021CK-00608 plasmid unnamed2, complete sequence	112409	100	99.987	USA
PC11	NZ_CP046773.1	Vibrio alginolyticus strain 2014V-1011 plasmid unnamed2, complete sequence	131705	97.85	99.954	USA
PC12	NZ_OQ821025.1	Klebsiella pneumoniae strain 03A20CPO005 plasmid p03A20005_B_KPC, complete sequence	7580	100	99.96	NA
PC14	NZ_CP024806.1	Escherichia coli strain AMA1167 plasmid pAMA1167-OXA-181, complete sequence	51479	100	99.994	Denmark
PC16	NZ_CP126852.1	Enterobacter hormaechei strain Ehh_7 plasmid pEh7_1, complete sequence	110786	100	99.975	Switzerland
PC18	NZ_CP023725.1	Klebsiella pneumoniae strain TVGHCRE225 plasmid unnamed3, complete sequence	103454	100	99.964	Taiwan
PC19	NZ_CP068295.1	Klebsiella pneumoniae strain RIVM_C016775 plasmid	47537	100	99.969	Netherlands

		pRIVM_C016775_6, complete sequence				
PC20	CP067706.1	Klebsiella pneumoniae strain ARLG-4851 plasmid pC349_3, complete sequence	41190	100	99.978	USA
PC21	NZ_CP071280.1	Klebsiella pneumoniae subsp. pneumoniae strain SA-KpST14 plasmid pSA-KpST14-NDM-1, complete sequence	269326	98.3	99.945	Saudi Arabia
PC22	NZ_CP150006.1	Klebsiella pneumoniae strain 2024CK-00033 plasmid unnamed2, complete sequence	146946	100	99.964	USA
PC24	NZ_CP021952.1	Klebsiella pneumoniae strain AR_0148 plasmid tig00000169_pilon, complete sequence	176349	98.61	99.975	NA
PC26	NZ_CP033947.1	Klebsiella pneumoniae subsp. pneumoniae strain ARLG-3135 plasmid p1, complete sequence	142764	98.8	99.974	NA
PC27	NZ_KJ958926.1	Klebsiella pneumoniae strain Kpn-3002cz plasmid pB-3002cz, complete sequence	97649	91.92	99.941	NA
PC28	NZ_CP098376.1	Klebsiella pneumoniae strain KP14ND1 plasmid pKP14ND1-1, complete sequence	116699	100	99.964	Vietnam
PC29	NZ_CP040171.1	Vibrio cholerae strain VC1374 plasmid pVC1374, complete sequence	140291	97.97	99.959	China
PC31	CP132211.1	Enterobacter hormaechei strain 2022CK-00285 plasmid unnamed2, complete sequence	140050	97.41	99.903	USA
PC32	NZ_CP079140.1	Klebsiella pneumoniae subsp. pneumoniae strain WRC05_CMC387PC plasmid pCMC387P_P2, complete sequence	211813	99.68	99.935	India
PC34	NZ_CP125089.1	Klebsiella pneumoniae strain BAK085 plasmid pBAK085a, complete sequence	113626	100	99.967	Singapore
PC35	NZ_KP008371.1	Klebsiella pneumoniae strain 565 plasmid PKPCAPSS, complete sequence	127970	100	99.95	NA
PC36	NZ_MT621569.1	Escherichia coli plasmid pSDCDK-IncFNDM5, complete sequence	80348	100	99.864	NA
PC37	NZ_CP028178.1	Klebsiella pneumoniae strain CFSAN054111 plasmid pGMI16-006_2, complete sequence	100222	97.48	99.95	Germany

PC38	NZ_CP095664.1	Escherichia coli strain dm900 plasmid p_dm900_NDM5, complete sequence	80574	90.62	99.852	Bangladesh
PC39	LC744497.1	Escherichia coli MyNCGM557 plasmid pMyNCGM557 DNA, complete sequence	94588	100	99.913	NA
PC41	NZ_CP008933.1	Klebsiella pneumoniae strain PMK1 plasmid pPMK1-NDM, complete sequence	304526	97.61	99.864	Nepal
PC44	NZ_CP053693.1	Enterobacter hormaechei strain Eh27 plasmid pEh27_2, complete sequence	274120	98.86	99.976	China
PC46	NZ_KJ802405.1	Providencia stuartii isolate GN576 plasmid pNDM-PstGN576, complete sequence	147886	99.04	99.972	NA
PC49	CP067706.1	Klebsiella pneumoniae strain ARLG-4851 plasmid pC349_3, complete sequence	41190	100	99.978	USA
PC50	CP077295.1	Escherichia coli strain FDAARGOS 1386 plasmid unnamed1, complete sequence	133266	95.12	99.731	USA

Supplementary Table 8. List of n=60 clonal lineages comprising isolates carrying *bla*$_{KPC-2}$-positive PC1 plasmids, ordered according to date of culture of the earliest-detected isolate.

Clonal lineage	Species	ST	No. of isolates[a]	Date of earliest isolate[b] (DD/MM/YYYY)	Date of latest isolate (DD/MM/YYYY)	No. of hospitals
1	*Klebsiella pneumoniae*	11	2	07/12/2011	19/10/2013	1
2	*Klebsiella pneumoniae*	841	4	27/02/2012	14/12/2013	1
3	*Enterobacter cloacae*	93	30	17/08/2012	07/04/2015	1
4	*Enterobacter aerogenes*	116	2	22/12/2012	24/01/2013	1
5	*Klebsiella pneumoniae*	231	10	29/01/2013	29/10/2014	2
6	*Klebsiella pneumoniae*	23	1	07/06/2013	19/12/2014	1
7	*Klebsiella pneumoniae*	327	1	02/08/2013	08/01/2014	2
8	*Klebsiella pneumoniae*	65	8	22/08/2013	28/03/2014	1
9	*Klebsiella pneumoniae*	1626	1	01/09/2013	10/11/2013	1
10	*Escherichia coli*	131	2	04/10/2013	13/07/2014	1
11	*Klebsiella pneumoniae*	5605	3	10/10/2013	25/02/2015	1
12	*Escherichia coli*	8890	1	10/10/2013	10/10/2013	1
13	*Klebsiella pneumoniae*	17	4	28/10/2013	11/11/2013	1
14	*Escherichia coli*	2797	1	29/10/2013	11/11/2013	1
15	*Klebsiella pneumoniae*	307	2	31/10/2013	13/11/2014	1
16	*Escherichia coli*	-	1	10/11/2013	13/12/2013	1
17	*Escherichia coli*	1193	2	04/12/2013	03/04/2015	1
18	*Klebsiella pneumoniae*	5606	6	09/12/2013	22/04/2015	1
19	*Klebsiella pneumoniae*	742	1	31/12/2013	02/01/2014	1
20	*Klebsiella pneumoniae*	70	4	05/02/2014	10/09/2014	1
21	*Enterobacter cloacae*	78	1	18/02/2014	18/06/2014	1
22	*Enterobacter cloacae*	54	3	19/02/2014	29/08/2014	1
23	*Klebsiella pneumoniae*	1191	1	15/03/2014	29/07/2014	1
24	*Escherichia coli*	131	4	31/03/2014	10/04/2014	1
25	*Klebsiella pneumoniae*	12	1	21/04/2014	04/08/2014	1
26	*Klebsiella pneumoniae*	323	8	23/04/2014	10/03/2015	1
27	*Klebsiella pneumoniae*	526	1	24/04/2014	22/06/2014	1
28	*Klebsiella pneumoniae*	526	1	24/04/2014	27/04/2014	1
29	*Enterobacter cloacae*	133	1	27/04/2014	22/06/2014	1

30	*Escherichia coli*	83	1	08/05/2014	22/09/2014	2
31	*Klebsiella pneumoniae*	86	1	24/05/2014	16/04/2015	1
32	*Klebsiella pneumoniae*	5606	2	04/06/2014	28/07/2014	1
33	*Citrobacter freundii*	-	1	24/06/2014	02/07/2014	1
34	*Klebsiella pneumoniae*	1308	1	24/06/2014	25/06/2014	1
35	*Klebsiella pneumoniae*	661	1	11/07/2014	29/07/2014	1
36	*Escherichia coli*	191	1	12/07/2014	14/10/2014	1
37	*Klebsiella pneumoniae*	307	1	16/07/2014	27/07/2014	1
38	*Klebsiella pneumoniae*	792	1	25/07/2014	07/09/2014	1
39	*Escherichia coli*	162	4	28/07/2014	27/11/2014	2
40	*Klebsiella pneumoniae*	978	1	10/08/2014	16/09/2014	2
41	*Klebsiella pneumoniae*	15	1	14/08/2014	25/08/2014	1
42	*Klebsiella pneumoniae*	20	2	22/08/2014	08/04/2015	1
43	*Escherichia coli*	38	1	23/08/2014	25/08/2014	1
44	*Klebsiella pneumoniae*	526	1	29/08/2014	30/08/2014	1
45	*Escherichia coli*	-	1	08/09/2014	09/09/2014	1
46	*Enterobacter cloacae*	66	1	09/09/2014	10/09/2014	1
47	*Klebsiella pneumoniae*	736	1	09/09/2014	09/09/2014	1
48	*Enterobacter cloacae*	599	1	14/09/2014	14/04/2015	1
49	*Escherichia coli*	131	1	16/09/2014	16/09/2014	1
50	*Klebsiella pneumoniae*	17	2	07/11/2014	15/04/2015	1
51	*Enterobacter cloacae*	614	1	30/12/2014	06/01/2015	1
52	*Klebsiella pneumoniae*	3520	1	08/01/2015	13/02/2015	1
53	*Escherichia coli*	7090	2	11/01/2015	30/01/2015	1
54	*Klebsiella oxytoca*	135	1	12/01/2015	28/01/2015	1
55	*Escherichia coli*	9602	1	16/02/2015	25/02/2015	1
56	*Escherichia coli*	68	7	26/02/2015	10/03/2015	1
57	*Klebsiella pneumoniae*	5609	1	01/04/2015	08/04/2015	1
58	*Escherichia coli*	167	3	03/04/2015	04/04/2015	1
59	*Klebsiella pneumoniae*	37	1	04/04/2015	10/04/2015	1
60	*Escherichia coli*	218	1	09/04/2015	09/04/2015	1

[a] This count excludes the earliest-detected isolate in each clonal lineage, as that isolate is considered to have acquired the plasmid by horizontal transmission

[b] Refers to the earliest-detected isolate in each clonal lineage

Supplementary Table 9. List of n=38 clonal lineages comprising isolates carrying *bla*$_{\text{NDM-1}}$-positive PC2 plasmids, ordered according to date of earliest-detected isolate.

Clonal lineage	Species	ST	No. of isolates[a]	Date of earliest isolate[b] (DD/MM/YYYY)	Date of latest isolate (DD/MM/YYYY)	No. of hospitals
1	*Klebsiella pneumoniae*	48	1	13/10/2010	14/10/2010	1
2	*Enterobacter cloacae*	592	4	16/11/2011	08/12/2012	1
3	*Klebsiella pneumoniae*	147	1	10/01/2012	12/06/2012	1
4	*Klebsiella pneumoniae*	16	2	01/04/2012	26/06/2012	1
5	*Klebsiella pneumoniae*	273	2	07/06/2012	02/08/2012	1
6	*Escherichia coli*	131	30	16/06/2012	28/11/2014	3
7	*Klebsiella pneumoniae*	11	5	22/06/2012	04/02/2014	2
8	*Escherichia coli*	131	1	30/08/2012	26/11/2012	1
9	*Escherichia coli*	131	1	08/01/2013	16/01/2013	1
10	*Enterobacter cloacae*	-	1	26/02/2013	15/03/2013	1
11	*Klebsiella oxytoca*	108	3	07/04/2013	17/12/2014	2
12	*Klebsiella pneumoniae*	231	3	26/04/2013	20/01/2015	2
13	*Citrobacter freundii*	18	3	07/05/2013	09/09/2014	1
14	*Klebsiella pneumoniae*	307	4	21/08/2013	31/03/2015	1
15	*Citrobacter amalonaticus*	-	2	29/08/2013	15/10/2013	1
16	*Escherichia coli*	569	1	13/09/2013	13/09/2013	1
17	*Klebsiella oxytoca*	108	5	01/10/2013	01/07/2014	1
18	*Klebsiella pneumoniae*	15	2	08/10/2013	17/12/2013	1
19	*Klebsiella pneumoniae*	34	11	10/10/2013	07/02/2014	1
20	*Citrobacter freundii*	22	1	04/11/2013	05/11/2013	1
21	*Klebsiella pneumoniae*	14	5	09/12/2013	10/12/2013	1
22	*Escherichia coli*	101	3	10/12/2013	10/12/2013	1
23	*Enterobacter cloacae*	418	1	17/12/2013	26/09/2014	1
24	*Escherichia coli*	4532	5	20/01/2014	22/01/2014	1
25	*Citrobacter koseri*	933	1	20/01/2014	21/01/2014	1
26	*Klebsiella pneumoniae*	2028	1	21/01/2014	21/01/2014	1
27	*Citrobacter freundii*	22	1	25/05/2014	23/03/2015	2
28	*Escherichia coli*	448	2	29/05/2014	29/05/2014	1
29	*Citrobacter freundii*	22	1	12/06/2014	24/06/2014	1
30	*Enterobacter cloacae*	127	1	04/07/2014	04/07/2014	1

31	*Klebsiella pneumoniae*	496	3	02/08/2014	15/09/2014	2
32	*Escherichia coli*	131	1	11/08/2014	09/09/2014	2
33	*Enterobacter cloacae*	270	2	15/08/2014	30/08/2014	1
34	*Enterobacter cloacae*	719	1	20/08/2014	23/10/2014	1
35	*Klebsiella pneumoniae*	86	1	11/09/2014	14/09/2014	1
36	*Escherichia coli*	6272	1	11/09/2014	16/11/2014	1
37	*Enterobacter cloacae*	66	1	14/09/2014	16/11/2014	1
38	*Escherichia coli*	131	1	30/09/2014	15/11/2014	1

[a] This count excludes the earliest-detected isolate in each clonal lineage, as that isolate is considered to have acquired the plasmid by horizontal transmission

[b] Refers to the earliest-detected isolate in each clonal lineage

Supplementary Table 10. List of genes n=17 genes present in the representative PC7 plasmid that are not present in the representative PC1 plasmid.

Region (coordinates)	Gene name	Accession number[a]	Function
Inserted Region 1 (19758-20033)	FrmR	UniProtKB:P0AAP3	Formaldehyde Detoxification
Inserted Region 1 (18618-19727)	FrmA	UniProtKB:P25437	Formaldehyde Detoxification
Inserted Region 1 (18178-18576)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (17276-18127)	FrmB	UniProtKB:P51025	Formaldehyde Detoxification
Inserted Region 1 (16613-17248)	Resolvase	BAN82974.1	Integration/Excision
Inserted Region 2 (41365-42369)	IS110 Family Transposase IS4321	AUK19617.1	Integration/Excision
Inserted Region 2 (42448-42882)	MerR	AYD68532.1	Mercury Detoxification
Inserted Region 2 (42932-43144)	Pyridine nucleotide-disulphide oxidoreductase, dimerization domain containing protein (MerA)	InterPro:IPR004099	Unknown
Inserted Region 2 (43180-43818)	MerB	UniProtKB:P77072	Mercury Detoxification
Inserted Region 2 (43930-44295)	MerD	AYD68537.1	Mercury Detoxification
Inserted Region 2 (44292-44528)	MerE	AYD68538.1	Mercury Detoxification
Inserted Region 2 (44661-44864)	TnpM	SPD96334.1	Integration/Excision
Inserted Region 2 (44903-45517)	Recombinase Family Protein	QGX86091.1	Integration/Excision
Inserted Region 2 (45578-46795)	Hypothetical	ab initio prediction	Integration/Excision

Inserted Region 2 (46792-47700)	TniB NTP-binding protein	AYD68541.1	Integration/Excision
Inserted Region 2 (47703-49385)	TniA Putative Transposase	AYD68540.1	Integration/Excision

[a] All accession numbers are from Genbank unless otherwise stated

Supplementary Table 11. List of genes n=29 genes present in the representative PC43 plasmid that are not present in the representative PC1 plasmid.

Region (coordinates)	Gene name	Accession number[a]	Function
Inserted Region 1 (63883-64587)	Mobile element protein	QEQ71200.1	Integration/Excision
Inserted Region 1 (64710-65297)	replication initiation protein	QJK41970.1	Replication/Recombination/Repair
Inserted Region 1 (65893-66897)	IS110 family transposase IS4321	AUK19617.1	Integration/Excision
Inserted Region 1 (67315-68751)	Glutathione synthase	UniRef50:A8H4X8	Oxidative stress response
Inserted Region 1 (69018-69722)	Mobile element protein	QEQ71200.1	Integration/Excision
Inserted Region 1 (69770-71635)	TnpA	AHX39443.1	Integration/Excision
Inserted Region 1 (72338-73255)	Replication initiation protein	UCT21151.1	Integration/Excision
Inserted Region 1 (73259-73714)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (73887-74240)	DNA distortion polypeptide 3	UCT21149.1	Unknown
Inserted Region 1 (74193-74498)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (74616-74900)	CopG family transcriptional regulator	UCT21146.1	Copper Resistance
Inserted Region 1 (74980-75642)	ParA	UCT21145.1	Replication/Recombination/Repair
Inserted Region 1 (75917-76555)	ParF	UCT21144.1	Integration/Excision
Inserted Region 1 (76693-76932)	Hypothetical	ab initio prediction	Unknown

Inserted Region 1 (76833-79820)	Tn3 family transposase TnEc1	UniRef50:P08504	Integration/Excision
Inserted Region 1 (80156-80392)	Resolvase	BAN82974.1	Integration/Excision
Inserted Region 1 (80520-81653)	Permease	RefSeq:WP_063120614.1	Small molecule transport
Inserted Region 1 (81759-82082)	ArsR family transcriptional regulator	RefSeq:WP_001175593.1	Arsenic Resistance
Inserted Region 1 (82159-82863)	Mobile element protein	QEQ71171.1	Integration/Excision
Inserted Region 1 (82754-83443)	Hypothetical	ab initio prediction	Uknown
Inserted Region 1 (83591-83896)	Transcriptional regulator	UniProtKB:I6X7F9	Gene expression regulation
Inserted Region 1 (83907-85112)	ChrA putative chromate transport protein	UniProtKB:Q55027	Chromate resistance
Inserted Region 1 (85654-85866)	Permease of the drug/metabolite transporter	QBP89643.1	Small molecule transport
Inserted Region 1 (85889-86401)	EamA family transporter	AUK19672.1	Small molecule transport
Inserted Region 1 (86567-87454)	Tetracycline efflux protein TetA	QBP89642.1	AMR
Inserted Region 1 (87421-87765)	MFS transporter	AOZ40923.1	Small molecule transport
Inserted Region 1 (87844-88395)	Tetracycline resistance repressor	QBP89641.1	AMR
Inserted Region 1 (88551-88793)	Relaxase	QJK42003.1	Transfer
Inserted Region 1 (88941-89486)	Hypothetical	ab initio prediction	Unknown

[a] All accession numbers are from Genbank unless otherwise stated

Supplementary Table 12. List of genes n=9 genes present in the representative PC20 plasmid that are not present in the representative PC2 plasmid.

Region (coordinates)	**Gene name**	**Accession number**[a]	**Function**
Inserted Region 1 (38823-39176)	ArsR	Refseq: WP_001114073.1	Arsenic Resistance
Inserted Region 1 (39224-39586)	ArsD	UniProtKB:P46003	Arsenic Resistance
Inserted Region 1 (39604-41355)	ArsA	UniProtKB:P08690	Arsenic Resistance
Inserted Region 1 (41404-42693)	ArsB	UniProtKB:P0AB93	Arsenic Resistance
Inserted Region 1 (42706-43131)	ArsC	UniProtKB:P08692	Arsenic Resistance
Inserted Region 1 (43162-43536)	Arsenical pump-driving ATPase	UniProtKB:P52145	Arsenic Resistance
Inserted Region 1 (43575-43961)	Mobile element protein	QIM11227.1	Integration/Excision
Inserted Region 1 (43958-44146)	Mobile element protein	QBP89794.1	Integration/Excision
Inserted Region 2 (6620-6988)	Hypothetical	ab initio prediction	Unknown

[a] All accession numbers are from Genbank unless otherwise stated

Supplementary Table 13. List of genes n=16 genes present in the representative PC49 plasmid that are not present in the representative PC2 plasmid.

Region (coordinates)	**Gene name**	**Accession number[a]**	**Function**
Inserted Region 1 (21645-21890)	Antitoxin MazE	UniProtKB:P0AE72	Stability/Transfer/Defense
Inserted Region 1 (22547-23911)	dNTP triphosphohydrolase	QHU48414.1	Nucleotide Catabolism
Inserted Region 1 (24438-25004)	Hypothetical	ab initio prediction	Stability/Transfer/Defense
Inserted Region 1 (25001-25846)	N-glycosylase/DNA lyase	HAMAP:MF_00241	Base-excision Repair
Inserted Region 1 (25790-26473)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (26442-27047)	QueC	HAMAP:MF_01633	Queuosine Biosynthesis
Inserted Region 1 (27044-28285)	Ribokinase	HAMAP:MF_01987	Ribose Metabolism
Inserted Region 1 (28282-28614)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (28987-29286)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (29375-30547)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (30544-31002)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (30992-34300)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (34396-34656)	Hypothetical	ab initio prediction	Unknown
Inserted Region 1 (34808-36841)	Integrase	QHU48412.1	Unknown
Inserted Region 1 (36834-38393)	Recombinase	QHU48411.1	Integration/Excision
Inserted Region 1 (38383-39573)	Site-specific Integrase	QHU48410.1	Integration/Excision

[a] All accession numbers are from Genbank unless otherwise stated