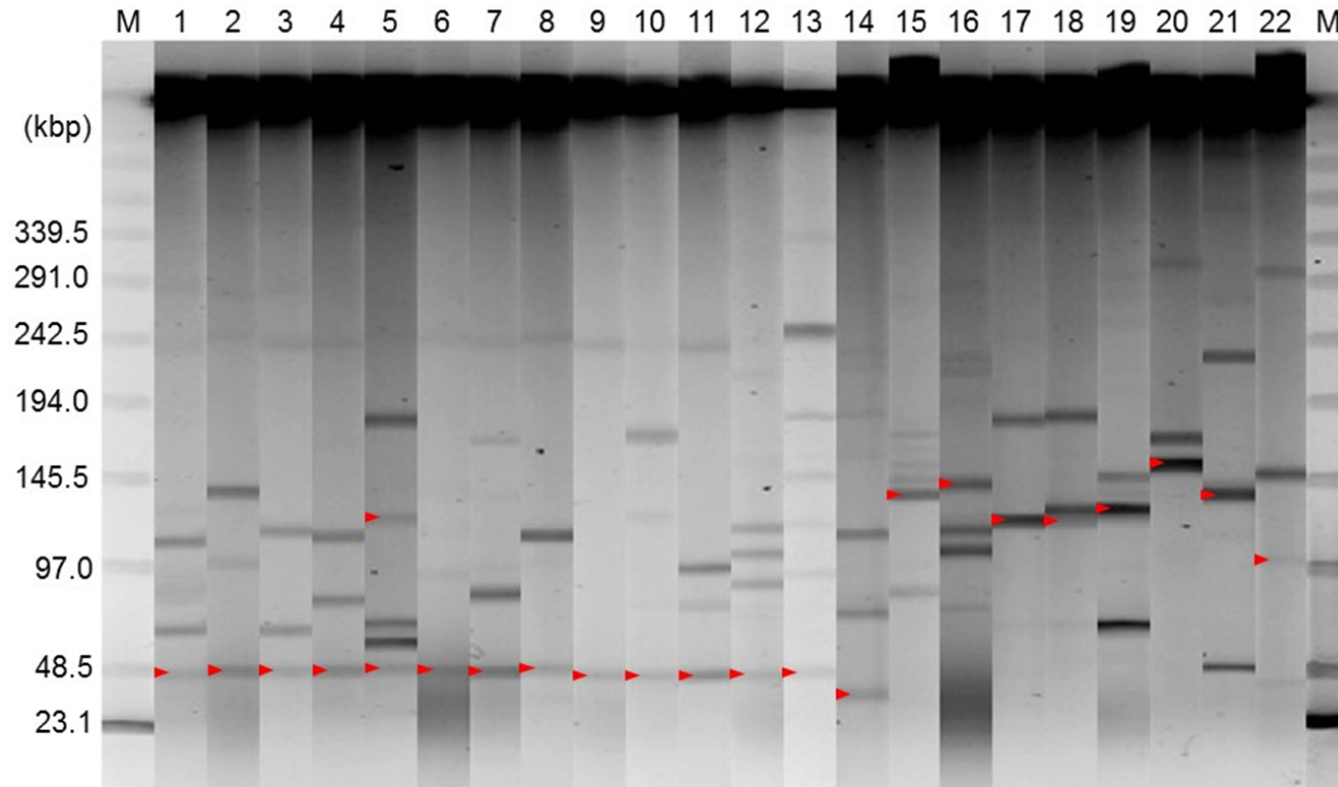


Title:

A prolonged multispecies outbreak of IMP-6 carbapenemase-producing Enterobacterales due to horizontal transmission of the IncN plasmid

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Figure S1. Pulsed-field gel electrophoresis profiles of S1 nuclease-treated DNA of the 22 isolates



The lanes were normalized and rearranged by GelComper II software, version 6.6 (Applied Maths, St-Martens-Latem, Belgium). Bands of plasmid carrying *bla*_{IMP-6} are indicated by red arrowheads. Lane M: CHEF DNA size standards lambda ladder (Bio-Rad, Hercules, CA, USA) and $\square$ -Hind III digest (TaKaRa Bio, Shiga, Japan). Lanes 1-22: isolates from cases 1 to 22 in Figure 1, respectively.

A)

Plasmid type Plasmid name

Legend: replication gene (blue diamond), pseudogene (black diamond), transposase gene (yellow diamond), AMR gene (red diamond), conjugation-related gene (cyan diamond), other (grey diamond)

Genomic map showing the distribution of genes across plasmids. The x-axis represents genomic distance from 1 bp to 50 kb. The y-axis lists plasmids and their types. The legend defines gene categories: replication gene (blue diamond), pseudogene (black diamond), transposase gene (yellow diamond), AMR gene (red diamond), conjugation-related gene (cyan diamond), and other (grey diamond). The map shows the presence of these genes in various plasmids, with some plasmids containing multiple copies of certain genes. The plasmids are grouped by origin: Place (A1, A2, B, C, D, E1, E2), Japan (F), Taiwan (N/A), and China (N/A).

Plasmids and their types:

- A1: pMRY14-225ECO_3
- A2: pMRY14-168KPN_2
- B: pMRY14-210ECL_3
- C: pMRY14-235ECO_1
- D: pMRY14-209ECL_1
- E1: pMRY14-165KPN_2
- E2: pMRY14-187KOX_2
- F: pMRY14-208_1
- N/A: pKPI-6
- N/A: pLK78
- N/A: pIMP-GZ1517

Genomic distance (kb): 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50

Place, Japan, Taiwan, China

										AMR gene															
Plasmid type	Species	Plasmid replicon type	IS26 in intI1	Place	Collection date	Plasmid name	Accession number	aac(6)-lb ^b	aac(6)	tetA	blaTEM-1	aadA16-cr	ant-S	blaOXA-10	bla	dfrA27	fosA5	gmrA1	qnrS1	blaNDP-2	aac(3)-IIa	blaCTX-M-15	blaOXA-1		
A1	E. coli	N	+	Japan	Oct 2013	pMRYY14-225ECO_3	AP018745	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
	E. cloacae	N	+	Japan	Nov 2013	pMRYY14-211ECL_2	AP018744	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	E. coli	N	+	Japan	Dec 2013	pMRYY14-229ECO_3	AP018746	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
	E. coli	N	+	Japan	Dec 2013	pMRYY14-230ECO_3	AP019252-253	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	K. oxytoca	N	+	Japan	Dec 2013	pMRYY14-192KOX_5	AP019230-234	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
	E. coli	N	+	Japan	Feb 2014	pMRYY14-457ECO_1	AP019291-294	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	E. coli	N	+	Japan	Dec 2013	pMRYY14-231ECO_2	AP019254-257	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
	E. coli	N	+	Japan	Nov 2013	pMRYY14-228ECO_2	AP019249-251	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
A2	K. aerogenes	N	-	Japan	Mar 2014	pMRYY14-243KA_E_1	AP019272-273	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
	K. pneumoniae	N	-	Japan	Dec 2013	pMRYY14-168KP_N_2	AP018742	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	E. coli	N	-	Japan	Oct 2013	pMRYY14-226ECO_3	AP019247-248	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-				
	E. cloacae	N	-	Japan	Feb 2014	pMRYY14-233ECL_4	AP019258-260	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	K. pneumoniae	N	-	Japan	Nov 2013	pMRY15-537KP_N_2	AP019295	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
N/A	K. pneumoniae	N	-	Japan	2008	pKIPI-6	AB616660	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
B	E. cloacae	N	-	Japan	Oct 2013	pMRYY14-210ECL_3	AP018743	++	+++	++++	p	-	-	-	-	-	-	-	-	-	-	-			
C	E. coli	N, FIA, FII	+	Taiwan	Sep 2012	pLK75	KJ440076	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+			
D	E. cloacae	untypc	+	Taiwan	Sep 2012	pLK78	KJ440075	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+			
	K. pneumoniae	N	-	China	2002	pKP96	EU195449	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+			
	E. coli	N, FIA, FII	+	Japan	Jan 2014	pMRYY14-235ECO_1	AP019261-271	++	+++	++++	+	+	+	+	+	+	+	+	+	+	+	+			
	E. cloacae	N	+	Japan	Sep 2013	pMRYY14-209ECL_1	AP019241-246	p	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
	K. pneumoniae	N	+	China	Jan 2012	pIMP_SZ1501	KU051708	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	E. coli	N	+	China	Sep 2013	pIMP_GZ1517	KT982618	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	E. cloacae	N	+	China	Aug 2012	pIMP_SH1506	KT989598	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
N/A	C. freundii	N	+	China	Nov 2011	pIMP-HK1500	KT989599	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	K. oxytoca	N, R	+	Japan	Jan 2014	pMRYY14-193KOX_2	AP019235-238	++	+++	++++	-	-	-	-	-	-	-	-	-	-	-	-			
	K. oxytoca	N, R	+	Japan	Nov 2013	pMRYY14-1																			

A) Comparative analysis of IncN plasmid region using BLAST atlas. The coding regions of pMRY14-225ECO_3 were aligned against those of 10 other plasmids using TBLASTX, followed by visualisation using GView server. The coloured bars show the complete identity of translated amino acid sequences between ORF regions in the reference plasmid and the other plasmid sequences. Key: N/A, not available. B) Comparison of AMR gene patterns among 31 plasmids. The AMR genes of plasmids were analysed by ResFinder program. Key: N/A, not available; +, positive; -, negative; p, pseudogene.

Supplementary Table. Plasmid sequence information in this study

Organism	Strain name	Plasmid name	PBRT Inc type	Sequence status	Number of contigs	Total contig size (bp)	S1-PFGE band size (kb)	Ratio (contig size/band size)	Accession number	ID
<i>E. cloacae</i>	MRY14-211	pMRY14-211ECL_2	N	Complete	1	51,983	40	1.30	AP018744	2
<i>E. cloacae</i>	MRY14-210	pMRY14-210ECL_3	N	Complete	1	40,793	30	1.36	AP018743	14
<i>E. cloacae</i>	MRY14-233	pMRY14-233ECL_4	N	Draft	3	56,528	44	1.28	AP019258-AP019260	12
<i>E. cloacae</i>	MRY14-209	pMRY14-209ECL_1	Untyped	Draft	6	144,027	150	0.96	AP019241-AP019246	16
<i>E. cloacae</i>	MRY14-208	pMRY14-208ECL_1	FIB	Draft	2	104,686	100	1.05	AP019239-AP019240	22
<i>E. coli</i>	MRY14-229	pMRY14-229ECO_3	N	Complete	1	51,998	45	1.16	AP018746	3
<i>E. coli</i>	MRY14-225	pMRY14-225ECO_3	N	Complete	1	51,983	50	1.04	AP018745	1
<i>E. coli</i>	MRY14-228	pMRY14-228ECO_2	N	Draft	3	57,776	45	1.28	AP019249-AP019251	8
<i>E. coli</i>	MRY14-231	pMRY14-231ECO_2	N	Draft	4	55,725	45	1.24	AP019254-AP019257	7
<i>E. coli</i>	MRY14-230	pMRY14-230ECO_3	N	Draft	2	51,761	45	1.15	AP019252-AP019253	4
<i>E. coli</i>	MRY14-457	pMRY14-457ECO_1	N	Draft	4	50,875	40	1.27	AP019291-AP019294	6
<i>E. coli</i>	MRY14-226	pMRY14-226ECO_3	N	Draft	2	50,590	45	1.12	AP019247-AP019248	11
<i>E. coli</i>	MRY14-235	pMRY14-235ECO_1	N, FIA, FII	Draft	11	139,357	140	1.00	AP019261-AP019271	15
<i>K. aerogenes</i>	MRY14-243	pMRY14-243KAE_1	N	Draft	2	51,158	50	1.02	AP019272-AP019273	9
<i>K. oxytoca</i>	MRY14-192	pMRY14-192KOX_5	N	Draft	5	53,712	40	1.34	AP019230-AP019234	5.1
<i>K. oxytoca</i>	MRY14-192	pMRY14-192KOX_2	N, R	Draft	14	129,584	130	1.00	AP019216-AP019229	5.2
<i>K. oxytoca</i>	MRY14-187	pMRY14-187KOX_2	N, R	Draft	11	138,178	160	0.86	AP019199-AP019209	20
<i>K. oxytoca</i>	MRY14-247	pMRY14-247KOX_2	N, R	Draft	17	119,351	140	0.85	AP019274-AP019290	21
<i>K. oxytoca</i>	MRY14-193	pMRY14-193KOX_2	N, R	Draft	4	103,391	130	0.80	AP019235-AP019238	17
<i>K. oxytoca</i>	MRY14-191	pMRY14-191KOX_2	N, R	Draft	6	100,909	100	1.01	AP019210-AP019215	18
<i>K. pneumoniae</i>	MRY14-168	pMRY14-168KPN_2	N	Complete	1	50,951	40	1.27	AP018742	10
<i>K. pneumoniae</i>	MRY15-537	pMRY15-537KPN_2	N	Draft	1	52,178	46	1.13	AP019295	13
<i>K. pneumoniae</i>	MRY14-165	pMRY14-165KPN_2	N, R	Complete	1	130,480	130	1.00	AP018741	19

PBRT: PCR-based replicon typing; PFGE: pulsed field gel electrophoresis.