Supplementary file

Table S1. Culture medium and antibiotics used for selection of transconjugants

Recipient strain and feature	Agar medium	Agent or antibiotic added to agar medium in conjugation experiment[a]	
		NDM-carrying IncX3 plasmids	CTX-M-14-carrying pHK01 plasmid
Escherichia coli J53 (azide-R, laboratory strain)	MacConkey	Azide and meropenem	Azide and cefotaxime
E. coli ST405 (Azi-R, clinical isolate)	MacConkey	Azide and meropenem	Azide and cefotaxime
E. coli ST131 (Azi-R, clinical isolate)	MacConkey	Azide and meropenem	Azide and cefotaxime
Shigella flexneri (clinical isolate)	XLD	Meropenem	Cefotaxime
Salmonella enteritidis (clinical isolate)	XLD	Meropenem	Cefotaxime
Klebsiella pneumoniae (clinical isolate)	UriSelect4	Meropenem	Cefotaxime
Pseudomonas aeruginosa (Azi-R, PT224, ATCC 27853, NCTC 10662)	MacConkey	Azide and meropenem	Azide and cefotaxime
Acinetobacter baumannii (Azi-R, ATCC 17959, ATCC 19606)	MacConkey	Azide and meropenem	Azide and cefotaxime

Abbreviaitons: Azi-R, resistant to sodium azide; XLD, xylose Lysine Deoxycholate.

[a] At the following concentration: sodium azide (100 µg ml^{-1}), meropenem (0.25 µg ml^{-1}) and/or cefotaxime (1 µg ml^{-1}).

Table S2. Summary of 49 IncX3 plasmids.

No.	Plasmid Name[a]	NDM allele	Geographical Origin[b]	Species[c]	Accession	Plasmid size, bp (query coverage/identity)[d]	Host source	Year collected	Genetic load region, bp[e]
1	pIncX-SHV	None	Italy	KP	JN247852	43,380 (94.0%/99.9%)	Human	2010	13,287
2	pNDM-HN380	NDM-1	China (HU)	KP	JX104760	54,035 (100.0%/99.9%)	Human	2011	23,944
3	pCRE3694-NDM1*	NDM-1	Hong Kong	EC	MH234505	53,097 (98.7%/99.9%)	Human	2016	23,006
4	pCREC-544_2	NDM-1	Korea (IC)	EC	CP024828	51,455 (100.0%/99.9)	Human	2015	21,365
5	pCRENT-193_2	NDM-1	Korea (SL)	ENTSP	CP024814	44,962 (97.4%/99.9%)	Human	2013	14,871
6	pJEG027	NDM-4	Myanmar	KP	KM400601	46,253 (100.0%/99.8%)	Human	na	16,070
7	pM213_X3	NDM-4	Myanmar	EC	AP018142	43,534 (94.0%/99.9%)	Human	2015	13,443
8	pM216_X3	NDM-4	Myanmar	EC	AP018146	46,161 (100.0%/99.9%)	Human	2015	16,070
9	pCREC-532_3	NDM-1	Korea (BS)	EC	CP024833	46,137 (99.9%/99.9%)	Human	2015	16,070
10	pM110_X3	NDM-7	Myanmar	EC	AP018141	45,122 (97.7%/99.9%)	Human	2015	15,031
11	pEC50-NDM7	NDM-7	China	EC	KX470735	46,161 (100.0%/99.9%)	Human	na	16,070
12	pCREC-629_3	NDM-7	Korea (JN)	EC	CP024818	46,161 (100.0%/99.9%)	Human	2015	16,070
13	pKpN01-NDM7	NDM-7	Canada	KP	CP012990	46,161 (100.0%/99.9%)	Human	2013	16,070
14	pOM26-1	NDM-7	Oman	EC	KP776609	45,122 (97.7%/99.9%)	Human	na	15,031
15	pKW53TNDM	NDM-7	Kuwait	EC	KX214669	46,161 (100.0%/99.9%)	Human	2012	16,070
16	pSCE516-2	NDM-1	China	EC	KX023261	46,254 (100.0%/99.9%)	Chicken	2015	16,071
17	pCRE3855-NDM5*	NDM-5	Hong Kong	EC	MH234498	46,161 (100.0%/100.0%)	Human	2016	16,070
18	pCRE3712-NDM5*	NDM-5	Hong Kong	EC	MH234503	46,161 (100.0%/100.0%)	Human	2016	16,070
19	pP785-NDM5	NDM-5	China (GD)	EC	MF547509	46,161 (100.0%/99.9%)	Pig	2016	16,070
20	pP788A-NDM5	NDM-5	China (GD)	EC	MF547510	46,161 (100.0%/100.0%)	Pig	2016	16,070
21	pP768-NDM5	NDM-5	China (HU)	EC	MF547508	46,161 (100.0%/100.0%)	Pig	2016	16,070
22	pCRE3836-NDM5*	NDM-5	Hong Kong	EC	MH234499	46,161 (100.0%/99.9%)	Human	2016	16,070
23	pCRE3774-NDM5*	NDM-5	Hong Kong	EC	MH234502	45,547 (98.7%/100.0%)	Human	2016	15,456
24	pCRE3871-NDM5*	NDM-5	Hong Kong	EC	MH234497	46,161 (100.0%/100.0%)	Human	2016	16,070
25	pCRE3819-NDM5*	NDM-5	Hong Kong	KP	MH234500	46,161 (100.0%/100.0%)	Human	2016	16,070
26	pCRE3218-NDM5*	NDM-5	Hong Kong	EC	MH234507	46,161 (100.0%/100.0%)	Human	2016	16,070
27	pCRE2967-NDM5*	NDM-5	Hong Kong	EC	MH234509	47,474 (100.0%/99.9%)	Human	2016	17,383
28	pCRE2998-NDM5*	NDM-5	Hong Kong	KP	MH234508	46,161 (100.0%/100.0%)	Human	2016	16,070
29	pCREC-A6-NDM	NDM-1	China	EC	KX960109	46,037 (100.0%/99.7%)	Human	na	16,002
30	pZHDC40	NDM-5	China	EC	KY041843	45,346 (98.2%/100.0%)	Human	2015	15,255

31	pECNDM101	NDM-5	China	EC	KX507346	46,165 (100.0%/99.9%)	Pig	na	16,073
32	pCREC-591_4	NDM-5	Korea (IC)	EC	CP024825	46,161 (100.0%/99.9%)	Human	2015	16,070
33	pEc1929	NDM-5	China	EC	KT824791	46,164 (100.0%/99.9%)	Human	2014	16,071
34	pP855-NDM5	NDM-5	China (GD)	EC	MF547511	46,161 (100.0%/99.9%)	Pig	2017	16,070
35	pP744T-NDM5	NDM-5	China (HN)	EC	MF547507	45,547 (98.7%/100.0%)	Pig	2015	15,456
36	pNDM5_WCHEC0215	NDM-5	China	EC	KY435936	47,337 (100.0%/97.4%)	Human	na	17,268
37	pK518_NDM5	NDM-5	China (ZJ)	KM	CP023188	46,146 (100.0%/99.9%)	Human	2017	16,070
38	pK516_NDM5	NDM-5	China (ZJ)	KM	CP022351	46,149 (100.0%/99.9%)	Human	2017	16,070
39	pNDM-QD28	NDM-5	China (QD)	EC	KU167608	46,161 (100.0%/99.9%)	Human	2013	16,070
40	pNDM-QD29	NDM-5	China (QD)	EC	KU167609	46,161 (100.0%/99.9%)	Human	2013	16,070
41	pNDM5_IncX3	NDM-5	China	KP	KU761328	46,161 (100.0%/99.9%)	Human	2015	16,070
42	p1493-2	NDM-5	China (SZ)	EC	CP019073	46,145 (100.0%/99.9%)	Human	2013	16,068
43	pCRE3706-NDM5*	NDM-5	Hong Kong	KP	MH234504	46,161 (100.0%/99.9%)	Human	2016	16,070
44	pCRE3816-NDM5*	NDM-5	Hong Kong	KP	MH234501	46,161 (100.0%/99.9%)	Human	2016	16,070
45	NUHL24835	NDM-5	China (JX)	KP	CP014006	46,161 (100.0%/99.9%)	Human	2014	16,070
46	pCRCB-101_1	NDM-7	Korea (IC)	CF	CP024820	46,161 (100.0%/99.9%)	Human	2015	16,070
47	pAD-19R	NDM-17	China (SD)	EC	KX833071	46,161 (100.0%/99.9%)	Chicken	2015	16,070
48	pCRE3473-NDM5*	NDM-5	Hong Kong	ECLO	MH234506	46,161 (100.0%/100.0%)	Human	2016	16,070
49	pNDM-MGR194	NDM-5	India	KP	KF220657	46,253 (100.0%/99.9%)	Human	2011-13	16,070

[a] Plasmids sequenced in the present study are marked with an asterisk

[b] BS, Busan; IC, Incheon ; SL, Seoul; GD, Guangdong; BJ, Beijing; HN, Henan; HU, Hunan; JN, Jeonnam; JS, Jiangsu; JX, Jiangxi; QD, Qingdao; SD, Shandong; ZJ, Zhejiang.

[c] CF, *Citrobacter freundii*; EC, *Escherichia coli*; ECLO, *Enterobacter cloacae*; ENTSP, *Enterobacter species*; KP, *Klebsiella pneumoniae*; KM, *Klebsiella michiganensis*

[d] Percentages coverage and identity by using pNDM-HK2998 as a reference

[e] The genetic load region is between the resolvase gene and the *hns* gene. In all the plasmids, IS*L3* was inserted at the same positions in the same orientations.

Figure S1. Sequence alignment of new *fumC*, *icd*, *mdh* and *recA* alleles in two *E. coli* strains (CRE 3725 and CRE 3775) from this study with other closely related alleles.

fumC

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fumC251      ------------------------------------------------------------   60
fumC71       ------------------------------------------------------------   60
fumC709      ------------------------------------------------------------   60
fumC385      -------------G----------------------------------------------   60
CRE3725      CGAGCGCCATTCGTCAGGCGGCGGATGAAGTACTGGCAGGACAGCATGACGACGAATTCC   60
                          14
fumC251      ----------------------------------------T-------------------   120
fumC71       ------------------------------------------------------------   120
fumC709      ------------------------------------------------------------   120
fumC385      ------------------------------------------------------------   120
CRE3725      CGCTGGCTATCTGGCAGACCGGCTCCGGCACGCAAAGTAACATGAACATGAACGAAGTGC   120
                                                    101

fumC251      -------T-----------------T----------------------------------   180
fumC71       -------------------------------T----------------------------   180
fumC709      -------------------------------T----------------------------   180
fumC385      ------------------------------------------------------------   180
CRE3725      TGGCTAACCGGGCCAGTGAATTACTCGGCGGCGTGCGCGGGATGGAACGTAAAGTTCACC   180
                   128               146   152

fumC251      -------C----------------------------------------------------   240
fumC71       -------C--------------------C-------------------------------   240
fumC709      ---------------------------------------------------------G--   240
fumC385      ------------------------------------------------------------   240
CRE3725      CTAACGATGACGTGAACAAAAGCCAAAGTTCCAACGATGTCTTTCCGACGGCGATGCACG   240
                   188                  209                          238

fumC251      ------------------------------------------------------------   300
fumC71       ------------------------------------------------------------   300
fumC709      ------------------------------------------------------------   300
fumC385      ------------------------------------------------------------   300
CRE3725      TTGCGGCGCTGCTGGCGCTGCGCAAGCAACTCATTCCGCAGCTTAAAACCCTGACACAGA   300

fumC251      -G------------------A---------------------------------------   360
fumC71       ------------------------------------------------------------   360
fumC709      ------------------------------------------------------------   360
fumC385      ------------------------------------------------------------   360
CRE3725      CACTGAATGAGAAATCCCGTGCATTTGCCGATATCGTCAAAATTGGTCGTACTCACTTGC   360
             302                321

fumC251      -------------------G-----------------------------------G----   420
fumC71       -------------------G----------------------------------------   420
fumC709      -------------------G-----------------------------------T----   420
fumC385      -------------------A----------------------------------------   420
CRE3725      AGGATGCCACGCCGTTAACTCTGGGGCAGGAGATTTCCGGCTGGGTAGCGATGCTCGAGC   420
                               380                                 416

fumC251      ----------------T--------------------------------       469
fumC71       -------------------------------------------------       469
fumC709      -------------------------------------------------       469
fumC385      -------------------------------------------------       469
CRE3725      ATAATCTCAAACATATCGAATACAGCCTGCCTCACGTAGCGGAACTGGC       469
                            437
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icd

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icd161        ------------------------------------------------------------   60
icd479        ------------------------------------------------------------   60
icd242        ------------------------------------------------------------   60
icd168        ------------------------------------------------------------   60
CRE3725       CGACGCTGCAGTCGAGAAAGCCTATAAAGGCGAGCGTAAAATCTCCTGGATGGAAATTTA   60

icd161        ------------------------------------------------------------   120
icd479        ------------------------------------------------------------   120
icd242        ------------------------------------------------------------   120
icd168        ------------------------------------------------------------   120
CRE3725       CACCGGTGAAAAATCCACACAGGTTTATGGTCAGGATGTCTGGCTGCCTGCTGAAACCCT   120

icd161        ------------------------------------------------------------   180
icd479        ------------------------------------------------------------   180
icd242        ------------------------------------------------------------   180
icd168        ------------------------------------------------------------   180
CRE3725       TGATCTGATTCGTGAATATCGCGTTGCTATTAAAGGCCCGCTGACCACTCCGGTTGGTGG   180


icd161        ---------------G--------------------------------------------   240
icd479        ---------------C--------------------------------------------   240
icd242        ---------------G--------------------------------------------   240
icd168        ------------------------------------------------------------   240
CRE3725       CGGTATTCGTTCTCTTAACGTTGCCCTGCGCCAGGAACTGGATCTCTACATCTGCCTGCG   240
                            196

icd161        ------------------------------------------------------------   300
icd479        ------------------------------------------------------------   300
icd242        ------------------------------------------------------------   300
icd168        ------------------------------------------------------------   300
CRE3725       TCCGGTACGTTACTATCAGGGCACCCCAAGCCCGGTTAAACACCCTGAACTGACCGATAT   300

icd161        ------------------------------------------------------------   360
icd479        ------------------------------------------------------------   360
icd242        ------------------------------------G-----------------------   360
icd168        ------------------------------------------------------------   360
CRE3725       GGTTATCTTCCGTGAAAACTCGGAAGACATTTATGCAGGTATCGAATGGAAAGCTGACTC   360
                                                 336

icd161        ------------------------------------------------------------   420
icd479        ------------------------------------------------------------   420
icd242        ---------C--------------------------------------------------   420
icd168        ------------------------------------------------------------   420
CRE3725       TGCCGACGCTGAGAAAGTGATTAAATTCCTGCGTGAAGAGATGGGCGTGAAGAAAATTCG   420
                      370

icd161        ---------------C-----------C--------------------------------   480
icd479        ---------C-----------------------------A-----------T--------   480
icd242        ---------------------------------------G--------------------   480
icd168        ---------------------------------------G--------------------   480
CRE3725       CTTCCCGGAACATTGTGGTATCGGTATTAAGCCGTGTTCTGAAGAAGGCACCAAACGTCT   480
                      430   436         448         460         472

icd161        ---C-----C----------------------------   518
icd479        --------------------------------------   518
icd242        --------------------------------------   518
icd168        --------------------------------------   518
CRE3725       GGTTCGTGCAGCGATCGAATACGCAATTGCTAACGATC   518
                484   490
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mdh

```
mdh590       ------------------------------------------------------------   60
mdh441       --T---------------------------------------------------------   60
mdh249       ------------------------------------------------------------   60
mdh6         -----------------G------------------------------------------   60
CRE3725      GGCGTAGCGCGTAAACCAGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATC   60
               3              18

mdh590       -----------------------------------------------------------T   120
mdh441       ------------------------------------------------------------   120
mdh249       ------------------------------------------------------------   120
mdh6         ------------------------------------------------------------   120
CRE3725      GTGAAAAACCTGGTACAGCAAGTTGCGAAAACCTGCCCGAAAGCGTGCATTGGTATTATC   120
                                                                        120

mdh590       --------------------------G---------------------------------   180
mdh441       ------------------------------------------------------------   180
mdh249       --------------------T---------------------------------------   180
mdh6         ------------------------------------------------------------   180
CRE3725      ACTAACCCGGTTAACACCACAGTTGCAATTGCTGCTGAAGTGCTGAAAAAAGCCGGTGTT   180
                                141   147
mdh590       ------------------------------------------------------------   240
mdh441       ------------------------------------------------------------   240
mdh249       ------------------------------------------------------------   240
mdh6         ------------------------------------------------------------   240
CRE3725      TATGACAAAAACAAACTGTTCGGCGTTACCACGCTGGATATCATTCGTTCCAACACCTTT   240

mdh590       ------------------------------------------------------------   300
mdh441       ------------------------------------------------------------   300
mdh249       ------------------------------------------------------------   300
mdh6         ------------------------------------------------------------   300
CRE3725      GTTGCGGAACTGAAAGGCAAACAGCCAGGCGAAGTTGAAGTGCCGGTTATTGGCGGTCAC   300

mdh590       ------------------------------------------------------------   360
mdh441       ------------------------------------------------------------   360
mdh249       ------------------------------------------------------------   360
mdh6         ------------------------------------------------------------   360
CRE3725      TCTGGTGTTACCATTCTGCCGCTGCTGTCACAGGTTCCTGGCGTTAGTTTTACCGAGCAG   360

mdh590       ------------------------------------------------------------   420
mdh441       ------------------------------------------------------------   420
mdh249       ------------------------------------------------------------   420
mdh6         ------------------------------------------------------------   420
CRE3725      GAAGTGGCTGATCTGACCAAACGTATCCAGAACGCGGGTACTGAGGTGGTTGAAGCGAAA   420

mdh590       -------------------------------- 452
mdh441       -------------------------------- 452
mdh249       -------------------------------- 452
mdh6         -------------------------------- 452
CRE3725      GCCGGTGGCGGGTCTGCAACCCTGTCTATGGG 452
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recA

```
recA380      ---------------------------T--------------------------------    60
recA221      T-----------------------------------------------------------    60
recA2        ------------------------------------------------------------    60
recA490      ------------------------------------------------------------    60
CRE3775      CGCACGTAAACTGGGCGTCGATATCGACAACCTGCTGTGCTCCCAGCCGGACACCGGCGA    60
             1                          28

recA380      ------------------------------------------------------------    120
recA221      ------------------------------------------------------------    120
recA2        ------------------------------------------------------------    120
recA490      ------------------------------------------------------------    120
CRE3775      GCAGGCACTGGAAATCTGTGACGCCCTGGCGCGTTCTGGCGCAGTAGACGTTATCGTCGT    120

recA380      ------------------------------------------------------------    180
recA221      ---------------------------------------------------C--------    180
recA2        ---------------------------------------------------C--------    180
recA490      ---------------------------------------------------A--------    180
CRE3775      TGACTCCGTGGCGGCACTGACGCCGAAAGCGGAAATCGAAGGCGAAATCGGTGACTCTCA    180
                                                               172
recA380      ------------------------------------------------------------    240
recA221      ------------------------------------------------------------    240
recA2        ------------------------------------------------------------    240
recA490      ------------------------------------------------------------    240
CRE3775      CATGGGCCTTGCGGCACGTATGATGAGCCAGGCGATGCGTAAGCTGGCGGGTAACCTGAA    240

recA380      ------------------------------------------------------------    300
recA221      ------------------------------------------------------------    300
recA2        ------------------------------------------------------------    300
recA490      ------------------------------------------------------------    300
CRE3775      GCAGTCCAACACGCTGCTGATCTTCATCAACCAGATCCGTATGAAAATTGGTGTGATGTT    300

recA380      ------------------------------------------------------------    360
recA221      ------------------------------------------------------------    360
recA2        ------------------------------------------------------------    360
recA490      ------------------------------------------------------------    360
CRE3775      CGGTAACCCGGAAACCACTACCGGTGGTAACGCGCTGAAATTCTACGCCTCTGTTCGTCT    360

recA380      ------------------------------------------------------------    420
recA221      ------------------------------------------------------------    420
recA2        ------------------------------------------------------------    420
recA490      ------------------------------------------------------------    420
CRE3775      CGACATCCGTCGTATCGGCGCGGTGAAAGAGGGCGAAAACGTGGTGGGTAGCGAAACCCG    420

recA380      ------------------------------------------------------------    480
recA221      ------------------------------------------------------------    480
recA2        ------------------------------------------------------------    480
recA490      ------------------------------------------------------------    480
CRE3775      CGTGAAAGTGGTGAAGAACAAAATCGCTGCGCCGTTTAAACAGGCTGAATTCCAGATCCT    480

recA380      ------------------------------   510
recA221      ------------------------------   510
recA2        ------------------------------   510
recA490      ------------------------------   510
CRE3775      CTACGGCGAAGGTATCAACTTCTACGGCGA   510
```