

Table S1. Identification of the 37 *aac(3)-IIg*-positive *E. cloacae* complex isolates based on *hsp60* and *rpoB* sequences

Isolates	<i>hsp60</i> genotyping	<i>rpoB</i> genotyping
Y3	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y4	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y7	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y8	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y10	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>
Y24	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y40	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y43	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y59	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y67	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y75	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y81	<i>E. kobe</i> ^a	<i>E. cloacae</i> IV ^a
Y88	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y108	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>
Y118	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y129	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y130	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y131	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y137	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>
Y150	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y165	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>
Y176	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y178	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y184	<i>E. asburiae</i>	<i>E. asburiae</i>
Y233	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y243	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y249	<i>E. cloacae</i> subsp. <i>dissolvens</i> ^a	<i>E. asburiae</i> ^a
Y261	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y274	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y295	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y308	<i>E. asburiae</i>	<i>E. asburiae</i>
Y315	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i>
Y320	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>
Y323	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>
Y324	<i>E. kobe</i> ^a	<i>E. cloacae</i> IV ^a
Y327	<i>E. kobe</i> ^a	<i>E. cloacae</i> IV ^a
Y2152	<i>E. hormaechei</i> subsp. <i>Oharae</i>	<i>E. hormaechei</i>

^a Identification with sequences of *hsp60* and *rpoB* are conflicting with each other.

Table S2. Primers used in this study

Primers	Sequences (5' to 3')	Use
aac(3)-Ilg-F aac(3)-Ilg-R	AACAATTGCGGCGGACCTTTCACG CGCTGCGGATCGGCATTTCTAGC	Screening for <i>aac(3)-Ilg</i>
strA-F strA-R	GTGGCTTGCCCCGAGGTGATCA CCAAGTCAGAGGGTCCAATC	Screening for <i>strA</i>
strB-F strB-R	ATCGTCAAGGGATTGAAACC GGATCGTAGAACATATTGGC	Screening for <i>strB</i>
aadA5-F aadA5-R	GATCGAGCGCCATCTGGCTG ACGTTCCGCTCGTCACCCTT	Screening for <i>aadA5</i>
aac(6')-Ilc-F aac(6')-Ilc-R	CATGCTTGGCTGAACCGCC CCGGCCTTCTCGTAGCA	Screening for <i>aac(6')-Ilc</i>
aadA1-F aadA1-R	CAACGATGTTACGCAGCAGG ATGTCATTGCGCTGCCATTC	Screening for <i>aadA1</i>
aphA-1-F aphA-1-R	ATGGGCTCGCGATAATGTC CTCACCGAGGCAGTTCCAT	Screening for <i>aphA-1</i>
aac(3)-Ild-F aac(3)-Ild-R	GGCAATAACGGAGGCGCTTCAAAA TTCCAAGCATCGGCATCTCATACG	Screening for <i>aac(3)-Ild</i>
aadA2-F aadA2-R	CATTGAGCGCCATCTGGAAT ACATTTGCTCATCGCCGGC	Screening for <i>aadA2</i>
armA-F armA-R	AGGTTGTTTCCATTTCTGAG TCTCTTCCATTCCCTTCTCC	Screening for <i>armA</i>
aadB-F aadB-R	GTGTAACACGCAAGCACGATGATATTGATCTG CGAGCCTGTAGGACTCTATGTGCTTTGTAGG	Screening for <i>aadB</i>
rmtB-F rmtB-R	GCTTTCTGCGGGCGATGTAA ATGCAATGCCGCGCTCGTAT	Screening for <i>rmtB</i>
aac(6')-Ile-aph(2'')-Ia-F aac(6')-Ile-aph(2'')-Ia-R	TTGAAATAATCGGTAGTGGTTATGATAGTGTG GCA CCCTCAAAAACCTGTTGTTGCATTTAGTCTTTCC	Screening for <i>aac(6')-Ile-aph(2'')-Ia</i>
Pro-aac(3)-Ilg-F Pro-aac(3)-Ilg-R	CAGATCCAGCAGTCTGC ATCAGGGCGAGCCAAAGTGC	Cloning of <i>aac(3)-Ilg</i>

Table S2, continued

Primers	Sequences (5' to 3')	Use
Pro-strA-F Pro-strA-R	CAGATCCAGCAGTCTGC ATCAGGGCGAGCCAAAGTGC	Cloning of <i>strA</i>
Pro-strB-F Pro-strB-R	CGAACGAGAGCTACCGGT CGCTTGATATCTAGTATGACGTCTG	Cloning of <i>strB</i>
Pro-aadA5-F Pro-aadA5-R	TTGAAGTCGAAGGTGATATC GCTCAACGCAAGATTCT	Cloning of <i>aadA5</i>
Pro-aac(6')-IIC -F Pro-aac(6')-IIC -R	ACAGTCTATGCCTCGGGCA GCCATTCATGACCACTTCCCC	Cloning of <i>aac(6')-IIC</i>
Pro-aadA1-F Pro-aadA1-R	ACTTCGCTGCTGCCCAA TTATTTGCCGACTACCTTGGTG	Cloning of <i>aadA1</i>
Pro- <i>aphA-1</i> -F Pro- <i>aphA-1</i> -R	GCCTGAAAACACAACCCGCTAC CTCTGCCAGTGTTACAACC	Cloning of <i>aphA-1</i>
Pro-aac(3)-IId-F Pro-aac(3)-IId-R	GGATGATAAGTTTATCACCACCG CTAACCTGAAGGCTCGCAAG	Cloning of <i>aac(3)-IId</i>
Pro-aadA2-F Pro-aadA2-R	AAGCAGCTATCGTTTTTGCAGTG CGGCTTGAACGAATTGTTAGACATC	Cloning of <i>aadA2</i>
Pro- <i>armA</i> -F Pro- <i>armA</i> -R	CTGGCTGGCGTGAAGGTGATGAACAA TTATTTCTGAAATCCACTAG	Cloning of <i>armA</i>
Pro-aadB-F Pro-aadB-R	AAATGCCTCGACTTCGCTGC GGACGAATTGTTAGGCCGCATATC	Cloning of <i>aadB</i>
Pro- <i>rmtB</i> -F Pro- <i>rmtB</i> -R	GATTAAGCATTGGTAACTGTCAGACC CCTTCTGATTGGCTTATCCATTC	Cloning of <i>rmtB</i>
Pro-aac(6')-Ie- <i>aph(2'')</i> -Ia-F Pro-aac(6')-Ie- <i>aph(2'')</i> -Ia-R	GACCTGATGGAAAAACAATG CCATCTTTCCACTTCCTTT	Cloning of <i>aac(6')-Ie-aph(2'')</i> -Ia
<i>aac(3)</i> -Ilg-probe-F <i>aac(3)</i> -Ilg-probe-R	ATGAACACAAGGGAAACAATTGCGG TCAGGGCGAGCCAAAGTGCCGTTGAA	Generating <i>aac(3)</i> -Ilg probe
<i>hsp60</i> -F <i>hsp60</i> -R	GGTAGAAGAAGGCGTGGTTGC ATGCATTCGGTGGTGATCATCAG	Sequence analysis of the <i>hsp60</i> gene
<i>rpoB</i> -F <i>rpoB</i> -R	AACCAGTTCCGCGTTGGCCTGG CCTGAACAACACGCTCGGA	Sequence analysis of the <i>rpoB</i> gene

Table S3. Antibiotic susceptibilities of the 37 *aac(3)-IIg*-positive *E. cloacae* complex isolates, transconjugants and the recipient control *E. coli* C600

Strains*	MIC ($\mu\text{g/mL}$)										
	GEN	KAN	STR	SPE	AMK	TOB	NEO	SIS	MCR	NET	RIB
C600	≤ 0.25	≤ 1	2	4	≤ 1	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	0.5	≤ 2
Y4	64	512	128	16	≤ 1	8	32	128	512	0.5	1024
JY4	64	512	256	8	≤ 1	4	64	128	64	0.5	>1024
Y8	128	512	256	8	16	64	32	512	512	128	1024
JY8	64	512	256	4	4	16	32	128	256	32	>1024
Y10	128	128	128	16	8	64	4	256	1024	128	512
JY10	64	32	128	2	≤ 1	16	1	128	128	16	64
Y24	128	512	128	16	8	64	32	512	512	64	1024
JY24	64	512	256	4	2	32	32	64	128	16	>1024
Y40	128	512	128	16	8	64	32	512	1024	128	>1024
JY40	64	512	256	4	4	32	32	128	256	32	>1024
Y43	128	512	128	16	8	64	32	256	512	64	1024
JY43	32	1024	256	4	8	32	32	128	256	32	>1024
Y59	128	512	128	16	8	64	32	256	512	64	1024
JY59	64	512	256	16	8	64	64	128	256	32	>1024
Y67	1024	256	128	8	16	128	16	>1024	>1024	64	1024
JY67	128	512	128	4	4	32	32	256	256	16	>1024
Y75	128	512	256	16	8	64	64	512	512	128	>1024
JY75	64	512	256	4	8	16	64	128	256	32	>1024
Y81	128	512	128	16	16	64	64	512	512	256	>1024
JY81	64	512	256	4	4	16	32	128	256	32	>1024
Y88	128	64	128	16	8	64	2	128	512	64	256
JY88	64	128	256	4	8	32	8	128	256	32	256
Y108	128	64	128	16	8	64	2	256	512	128	512
JY108	64	32	256	4	2	32	1	128	256	32	128
Y118	128	128	128	16	16	64	16	256	512	128	512
JY118	64	32	128	≤ 1	≤ 1	16	1	64	256	16	64
Y129	128	512	128	16	8	64	32	512	512	64	1024
JY129	64	512	256	4	2	32	32	64	256	16	>1024
Y130	128	512	128	16	8	64	64	512	512	64	1024
JY130	64	512	256	4	2	32	32	128	256	32	>1024
Y131	128	512	128	16	8	32	32	512	512	64	1024
JY131	64	512	256	4	2	32	32	128	128	16	>1024
Y165	128	512	128	2	16	64	32	256	1024	64	>1024
JY165	32	512	256	4	4	16	32	128	256	32	>1024
Y233	>1024	>1024	128	128	>1024	>1024	512	>1024	>1024	>1024	>1024
JY233	>1024	>1024	128	128	>1024	256	2	128	256	128	4
Y243	128	512	128	8	16	256	32	>1024	>1024	1024	>1024
JY243	64	512	256	4	4	16	32	128	256	32	>1024

Table S3, continued

Strains*	MIC ($\mu\text{g/mL}$)										
	GEN	KAN	STR	SPE	AMK	TOB	NEO	SIS	MCR	NET	RIB
Y249	128	128	128	8	16	128	4	1024	>1024	256	512
JY249	32	32	256	4	16	32	1	128	256	32	128
Y261	64	256	128	16	8	64	16	256	512	64	1024
JY261	64	512	256	4	4	32	32	128	256	32	>1024
Y315	256	256	128	16	8	128	16	1024	1024	64	1024
JY315	64	512	256	4	4	32	32	64	128	32	>1024
Y324	128	128	128	16	16	64	2	256	512	64	512
JY324	64	64	256	4	4	32	1	128	128	32	128
Y327	128	64	128	16	8	64	2	256	512	64	512
JY327	64	64	256	4	2	16	1	128	128	16	64
Y3	64	512	128	16	8	64	16	256	512	64	1024
Y7	128	512	512	8	16	64	32	256	512	128	1024
Y137	128	256	128	16	8	64	32	256	512	64	1024
Y150	128	512	128	8	8	64	64	256	512	64	>1024
Y176	128	256	64	4	8	128	64	256	1024	64	>1024
Y178	256	512	128	16	8	64	64	512	1024	128	>1024
Y184	64	256	64	≤ 1	8	64	16	256	512	64	1024
Y274	>1024	>1024	128	64	>1024	>1024	1024	>1024	>1024	>1024	>1024
Y295	128	512	128	16	8	128	32	512	512	128	>1024
Y308	128	64	128	8	16	64	2	256	512	64	512
Y320	64	256	128	16	≤ 1	8	16	128	512	0.5	512
Y323	128	256	128	512	8	64	16	256	512	64	1024
Y2152	64	128	128	4	≤ 1	4	16	128	256	1	512

* The name of the strains starting with J represents transconjugants.

Abbreviations: GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SPE, spectinomycin; AMK, amikacin; TOB, tobramycin; NEO, neomycin; SIS, sisomicin; MCR, micromonicin; NET, netilmicin; RIB, ribostamycin.

Table S4. Key genomic features of the three whole-genome sequenced *E. cloacae* complex isolates

Characteristic	Y233 (Accession no. CP049046.1)	Y323 (Accession no. CP049188.1)	Y2152 (Accession no. CP049192.1)
Species or subspecies	<i>E. hormaechei</i> subsp. <i>steigerwaltii</i>	<i>E. hormaechei</i> subsp. <i>oharae</i>	<i>E. hormaechei</i> subsp. <i>oharae</i>
MLST	ST461	ST303	ST303
Genome size (bp)	4,719,755	4,782,705	4783459
GC content (%)	55.56	55.27	55.27
No. of coding sequences	4347	4366	4415
No. of plasmids	3	3	3
No. of tRNA genes	86	84	84
No. of rRNA genes	25	25	25
Resistance gene(s)			
Chromosome	<i>bla</i> _{ACT-17} , <i>fosA7</i>	<i>bla</i> _{ACT-25} , <i>fosA7</i> , <i>mcr-9</i>	<i>bla</i> _{ACT-25} , <i>fosA7</i> , <i>mcr-9</i>
Plasmids	pIH12-233 (322.325 kb): <i>aphA-1</i> , <i>catII</i> , <i>tet(D)</i> , <i>mcr-9</i> , <i>dfrA19</i> , <i>strA</i> , <i>strB</i> , <i>aac(6')-IIc</i> , <i>ereA2</i> , <i>sul1</i> , <i>qnrB4</i> , <i>bla</i> _{DHA-1} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-12} , <i>aac(6')-Ib</i> , <i>aac(3)-IIg</i> p233-142 (142.557 kb): <i>bla</i> _{TEM-1} , <i>aadA1</i> , <i>sul1</i> , <i>armA</i> , <i>msr(E)</i> , <i>mph(E)</i> p233-2 (2.507 kb): -	pIH12-323 (394.232 kb): <i>aphA-1</i> , <i>catII</i> , <i>tet(D)</i> , <i>mph(A)</i> , <i>mcr-9</i> , <i>dfrA19</i> , <i>strA</i> , <i>strB</i> , <i>aac(6')-IIc</i> , <i>ereA2</i> , <i>sul1</i> , <i>qnrB4</i> , <i>bla</i> _{DHA-1} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-12} , <i>bla</i> _{SFO-1} , <i>aac(6')-Ib</i> , <i>aac(3)-IIId</i> , <i>aadA2</i> , <i>aac(3)-IIg</i> pY323-2 (5.976 kb): - pY323-3 (2.495 kb): -	pIH12-2152 (303.394 kb): <i>aphA-1</i> , <i>catII</i> , <i>tet(D)</i> , <i>mcr-9</i> , <i>dfrA19</i> , <i>strA</i> , <i>strB</i> , <i>aac(6')-IIc</i> , <i>ereA2</i> , <i>sul1</i> , <i>qnrB4</i> , <i>bla</i> _{DHA-1} , <i>aac(3)-IIg</i> pDC2152-6 (5.976 kb): - pDC2152-2 (2.495 kb): -

Table S5. The *aac(3)-IIg*-containing sequences in the NCBI nucleotide database were from 34 species

Organisms	Number
<i>Enterobacterales</i>	33
<i>Enterobacteriaceae</i>	31
<i>Enterobacter cloacae</i> complex	12
<i>Enterobacter hormaechei</i>	4
<i>Enterobacter cloacae</i>	5
<i>Enterobacter hormaechei</i> subsp <i>hoffmannii</i>	1
<i>Enterobacter asburiae</i>	1
<i>Enterobacter hormaechei</i> subsp <i>steigerwaltii</i>	1
<i>Klebsiella aerogenes</i>	1
<i>Salmonella enterica</i> subsp <i>enterica</i>	1
<i>Klebsiella quasipneumoniae</i> subsp <i>quasipneumoniae</i>	1
<i>Citrobacter freundii</i>	1
<i>Escherichia coli</i>	1
<i>Salmonella enterica</i> subsp <i>enterica</i> serovar	1
<i>Salmonella enterica</i> subsp <i>enterica</i> serovar <i>Braenderup</i>	1
<i>Cronobacter sakazakii</i>	2
<i>Salmonella enterica</i>	2
<i>Klebsiella pneumoniae</i>	1
<i>Salmonella enterica</i> subsp <i>enterica</i> serovar <i>Concord</i>	1
<i>Citrobacter farmeri</i>	1
<i>Salmonella enterica</i> subsp <i>enterica</i> serovar <i>Newport</i>	1
<i>Salmonella enterica</i> subsp <i>enterica</i> serovar <i>Heidelberg</i>	1
<i>Klebsiella pneumoniae</i> subsp <i>pneumoniae</i>	1
<i>Citrobacter braakii</i>	1
<i>Klebsiella oxytoca</i>	1
<i>Providencia stuartii</i>	1
<i>Phytobacter ursingii</i>	1
<i>Aeromonas veronii</i>	1

Table S6. Cluster analysis of the 34 *aac(3)-IIg*-containing sequences

Clusters	GenBank accession no.	Identity (%) ^a
Cluster 1	CP049193.1 (pIH12-2152)	*
Cluster 1	CP049189.1 (pIH12-323)	99.88
Cluster 1	CP049047.1 (pIH12-233)	82.74
Cluster 1	CP014775.1	88.79
Cluster 1	KY978628.1	97.03
Cluster 1	CP028537.1	99.93
Cluster 1	LT994835.1	99.68
Cluster 1	MH399264.1	88.6
Cluster 1	CP028975.1	99.94
Cluster 1	CP040696.1	88.91
Cluster 1	CP043927.1	83.44
Cluster 1	CP044075.1	88.87
Cluster 2	EU855787.1	*
Cluster 2	EU855788.1	88.42
Cluster 2	CP011601.1	88.62
Cluster 2	CP012170.1	89.14
Cluster 2	KY863418.1	99.99
Cluster 2	CP027144.1	88.79
Cluster 2	CP028197.1	100
Cluster 2	CP031568.1	88.4
Cluster 2	CP032842.1	85.91
Cluster 2	MK191835.1	88.56
Cluster 2	MK191844.1	88.4
Cluster 2	CP040382.1	85.49
Cluster 2	CP042552.1	86.98
Cluster 3	CP013215.1	*
Cluster 3	CP022695.1	86.57
Cluster 4	CP016526.1	*
Cluster 4	CP012599.1	85.31
Cluster 5	MF344580.1	*
Cluster 6	CP028197.1	*
Cluster 7	CP030186.1	*
Cluster 8	CP044177.1	*
Cluster 8	CP044215.1	80.42

^a Percent identity when compared with the reference sequence in the same cluster.

* Reference sequence for the cluster analysis.