

Supplementary material

Table S1. Results of in bi- and triparental mating assays of identified IncQ-3 plasmids.

Plasmid donor(s)	Selection (µg/mL)	Plasmid recipients			
		<i>E. coli</i> DH5α (Rif ^r)/ <i>E. coli</i> JCB816 (Tet ^r) (biparental)	<i>E. coli</i> DH5α/ <i>E. coli</i> JCB816 (Tet ^r) + <i>E. coli</i> DH5a with pRK2013 (triparental)	<i>P. aeruginosa</i> PA01161 (Rif ^r) (biparental)	<i>P. aeruginosa</i> PA01161 (Rif ^r) + <i>E. coli</i> DH5a with pRK2013 (triparental)
<i>Aeromonas</i> sp. 5.4 ¹	CAZ16/RIF50	+	+	+	+
<i>Aeromonas</i> sp. 115	CAZ16/RIF50	+	+	+	+
<i>Raoultella</i> sp. 210C ²	CAZ16/RIF50 TRI50/RIF50	+	+	+	+
<i>Aeromonas</i> sp. 426	CIP1/TET50	-	-	ND ³	ND
<i>Aeromonas</i> sp. 458	CIP1/RIF50	-	-	-	-
<i>Kluyvera</i> sp. 435	CAZ16/RIF50	-	-	-	-

Footnotes: ¹The same results also for *Aeromonas* sp. 6.45; ²The same results also for *Raoultella* sp. 274B , *Raoultella* sp. 213C , *Raoultella* sp. 382A , *Raoultella* sp. 293, *Raoultella* sp. 385A , *Raoultella* sp. 299A , *Raoultella* sp. 376, *Raoultella* sp. 286, *Raoultella* sp. 328; ³ND – not done

Table S2. Genes located within analysed IncQ-3 plasmids (antibiotic resistance gens are **in bold**).

Gene	Plasmid	Location	Gene length (bp)	Protein length (aa)	Predicted function
5.4_c400005	p5.4_c4	1..432	432	143	plasmid mobilization relaxosome protein MobC
5.4_c400010	p5.4_c4	416..2857	2442	813	relaxase/mobilization nuclease topoisomerase MobA/primase RepB fusion protein
5.4_c400015	p5.4_c4	2928..3791	864	287	replication protein RepA
5.4_c400020	p5.4_c4	3778..4629	852	283	replication protein RepC
5.4_c400025c	p5.4_c4	complement(4940..5803)	864	287	class A extended-spectrum beta-lactamase GES-7
5.4_c400030c	p5.4_c4	complement(5950..6186)	237	78	trimethoprim-resistant dihydrofolate reductase DfrB3
5.4_c400035	p5.4_c4	6370..7410	1041	346	class 3 integron integrase IntI3
5.4_c400040c	p5.4_c4	complement(7824..7838)	468	155	hypothetical protein
115_p200005	p115_p2	1..432	432	143	plasmid mobilization relaxosome protein MobC

115_p200010	p115_p2	416..2857	2442	813	relaxase/mobilization nuclease topoisomerase MobA/primase RepB fusion protein
115_p200015	p115_p2	2928..3791	864	287	replication protein RepA
115_p200020	p115_p2	3778..4629	852	283	replication protein RepC
115_p200025c	p115_p2	complement(4887..5441)	555	184	AAC(6')-Ib family aminoglycoside 6'-N- acetyltransferase
115_p200030c	p115_p2	complement(5575..6438)	864	287	class A beta-lactamase GES-7
115_p200035c	p115_p2	complement(6585..6821)	237	78	trimethoprim-resistant dihydrofolate reductase DfrB3
115_p200040	p115_p2	7005..8045	1041	346	class 3 integron integrase IntI3
115_p200045c	p115_p2	complement(8526..8993)	468	115	hypothetical protein
426_p300005	p426_p3	1..432	432	143	plasmid mobilization relaxosome protein MobC
426_p300010	p426_p3	416..2857	2442	813	relaxase/mobilization nuclease topoisomerase MobA/primase RepB fusion protein
426_p300015	p426_p3	2928..3791	864	287	replication protein RepA
426_p300020	p426_p3	3778..4659	882	293	replication protein RepC
426_p300025c	p426_p3	complement(5203..5859)	657	218	quinolone resistance pentapeptide repeat protein QnrS2
426_p300030c	p426_p3	complement(6697..7164)	468	155	hypothetical protein
435_c400005	p435_c4	1..387	387	128	plasmid mobilization relaxosome protein MobC
435_c400010	p435_c4	371..2797	2427	808	relaxase/mobilization nuclease topoisomerase MobA/primase RepB fusion protein
435_c400015	p435_c4	2868..3749	882	293	replication protein RepA
435_c400020	p435_c4	4048..4482	435	144	ABC transporter ATPase (frameshifted; incomplete; partial in the middle of a contig; missing start and stop)
435_c400025c	p435_c4	complement(4724..5083)	360	119	ABC transporter ATPase (frameshifted; incomplete; partial in the middle of a contig; missing start and stop)
435_c400030c	p435_c4	complement(5408..6556)	1149	382	cephalosporin-hydrolyzing class C beta-lactamase FOX-15
435_c400035	p435_c4	6671..7975	1305	434	IS4 family transposase
435_c400040	p435_c4	complement(8483..8950)	468	155	hypothetical protein
458_p300005	p458_p3	1..432	432	143	plasmid mobilization relaxosome protein MobC
458_p300010	p458_p3	416..2857	2442	813	relaxase/mobilization nuclease topoisomerase MobA/primase RepB fusion protein
458_p300015	p458_p3	2928..3791	864	287	replication protein RepA
458_p300020	p458_p3	3778..4659	882	293	replication protein RepC

458_p300025c	p458_p3	complement(5203..5859)	657	218	quinolone resistance pentapeptide repeat protein QnrS2
458_p300025c	p458_p3	complement(6586..8244)	1659	552	Aerotaxis sensor receptor protein
458_p300035c	p458_p3	complement(8715..9182)	468	155	hypothetical protein

Table S3. Bacterial strains used in this study.

Bacterial strain	Relevant characteristics	References
<i>E. coli</i> DH5 α	Rif ^R	[1]
<i>E. coli</i> JCB816	Tet ^R	[2]
<i>Pseudomonas aeruginosa</i> PAO1161	Rif ^R	[1]
<i>E. coli</i> strain DH5a pRK2013	Km ^R , with pRK2013 mobilization helper plasmid	[3]
<i>Aeromonas</i> sp. 5.4	Caz ^R , Tri ^R	[4]
<i>Aeromonas</i> sp. 6.45	Caz ^R , Tri ^R	[4]
<i>Aeromonas</i> sp. 115	Caz ^R , Tri ^R	[4]
<i>Kluyvera</i> sp. 435	Caz ^R	[5]
<i>Aeromonas</i> sp. 458	Cip ^R	[4]
<i>Aeromonas</i> sp. 426	Cip ^R	[4]
<i>Raoultella</i> sp. 210C	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 274B	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 213C	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 382A	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 293	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 385A	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 299A	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 376	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 286	Caz ^R , Tri ^R , Mer ^R	[5]
<i>Raoultella</i> sp. 328	Caz ^R , Tri ^R , Mer ^R	[5]

Footnotes: Rif – rifampicin, Tet – tetracycline, Cip – ciprofloxacin, Km – kanamycin, Caz – ceftazidime, Tri – Trimethoprim; Mer – Meropenem

Table S4. Results on Minimum Inhibitory Concentration (MICs) for donor and recipient strains for bi- and triparental mating assays.

Bacterial strain	MIC (μ g/ml)				
	Ceftazidime	Trimethoprim	Ciprofloxacin	Tetracycline	Rifampicin
<i>Aeromonas</i> sp. 5.4	128	>512	ND ³	ND	1
<i>Aeromonas</i> sp. 6.45	>128	>512	ND	ND	2
<i>Aeromonas</i> sp. 115	>128	>512	ND	ND	4
<i>Raoultella</i> sp. 210C ¹	>128	>512	ND	ND	16
<i>Raoultella</i> sp. 382A ²	>128	>512	ND	ND	32

<i>Aeromonas</i> sp. 426	ND	ND	4	1	128
<i>Aeromonas</i> sp. 458	ND	ND	2	1	1
<i>Kluyvera</i> sp. 435	128	ND	ND	ND	2
<i>E. coli</i> DH5 α (Rif ⁺)	0,125	0,5	<1	1	>256
<i>E. coli</i> JCB816 (Tet ⁺)	4	<0,5	<1	128	8
<i>Pseudomonas aeruginosa</i> PAO1161 (Rif ⁺)	2	128	<1	8	>256

Footnotes: ¹The same MIC results also for *Raoultella* sp. 274B , *Raoultella* sp. 213C , *Raoultella* sp. 385A , *Raoultella* sp. 299A , *Raoultella* sp. 376, *Raoultella* sp. 286, *Raoultella* sp. 328; ²The same MIC results also for *Raoultella* sp. 293; ³NP – not done

Table S5. Primers used in this study.

Primer name	Nucleotide sequence	References
MultiGES_for	AGTCGGCTAGACCGGAAAG	[6]
MultiGES_rev	TTTGTCCTGCTCAGGAT	[6]
FOXMF	AACATGGGGTATCAGGGAGATG	[7]
FOXMR	CAAAGCGCGTAACCGGATTGG	[7]
qnrS_for	GCAAGTTCATTGAACAGGGT	[8]
qnrS_rev	TCTAAACCGTGAGTTCGGCG	[8]

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