Table S1. Strains and plasmids used in this work and their relevant features

Strain or plasmid	Genotype/characteristics	Reference[a] or source
Klebsiella pneumoniae		
MGH 78578	*K. pneumoniae* subsp. *pneumoniae* ATCC 700721 type strain (ST38, K52 serotype), harboring *bla*SHV-11, *bla*SHV-12, *bla*TEM-1, and *bla*OXA-9 β-lactamases	American Type Culture Collection (ATCC)
MGH 78578ΔAG	*ampG::aac(3)IV*; MGH 78578 strain-derived knockout mutant with *ampG* (KPN_00395 gene) interrupted by an apramycin resistance gene (*aac(3)IV*) flanked by FRT sites ($Apra^R$)	This work
Kp52.145	Also known as B5055; highly virulent reference strain (ST66, K2 serotype), devoid of β-lactamases	99
52K0	CPS expression-defective mutant derived from Kp52.145	100
Kp52.145R	Rif^r spontaneous mutant of Kp52145 strain. Obtained after plating different dilutions of overnight LB cultures in Müller-Hinton agar plates supplemented with rifampin 100 mg/l	This work
Kp52.145RΔAG	*ampG* (BN49_1369) knockout mutant derived from Kp52145R	This work
Pseudomonas aeruginosa		
PA14	Highly virulent and cytotoxic reference strain	101
Strains used for conjugation of AmpC β-lactamases		
Klebsiella oxytoca 20/065	Clinical strain from a Spanish multicenter study harboring a *bla*DHA-1 β-lactamase codified in a conjugative plasmid	20
Escherichia coli 40/026	Clinical strain from a Spanish multicenter study harboring a *bla*CMY-2 β-lactamase codified in a conjugative plasmid	20
Escherichia coli HB101	Str^r, Kan^r (codified in the plasmid PRK2013). Used as an intermediary strain for conjugation when indicated	Laboratory collection
Strains and plasmids used for construction of knockout mutants		
pMDIAI (Addgene #51655)	pMD18-T simple backbone containing an apramycin resistance gene (*aac(3)IV*) flanked by FRT sites; template for amplification of this resistance cassette	Addgene; 102
pACBSR-Hyg (Addgene #87830)	A p15A replicon plasmid containing an arabinose-inducible λ-Red recombinase and a hygromycin resistance marker	Addgene; 87

pFLP-Hyg (Addgene #87831)	Plasmid bearing a heat-shock inducible FLP recombinase and a hygromycin resistance marker	Addgene; 102
pUCP24	Gm^r; pUC18-based multicopy shuttle vector	103
pUCP-DHA-1	Gm^r; pUCP24 containing the *bla*DHA-1 gene cloned	This work
pUCP-CMY-2	Gm^r; pUCP24 containing the *bla*CMY-2 gene cloned	This work

Abbreviations: $Apra^R$: apramycin resistance cassette; CPS: capsule polysaccharide; FRT: flippase recognition target; FLP: flippase recombinase; Gm^r: gentamicin resistant; Kan^r: kanamycin resistant; Rif^r: rifampin resistant; ST: sequence type; Str^r: streptomycin resistant; kb: kilobase. [a]References numeration corresponds to the Reference section of the manuscript.

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Table S2. Primers designed in this work for the analysis of gene expression by real time RT-PCR

Primer	**Sequence (5′-3′)**	**Target and amplicon size (bp)**
Kleb_coli_rpoD_F	GGAGCAAAACCCGCAGTCACAGC	*K. pneumoniae* Kp52.145 strain *rpoD* housekeeping gene (66 bp). Also used in *K.*
Kleb_coli_rpoD_R	GTCAGATAGCCTTGCTCCTTACC	

		oxytoca and *E. coli rpoD* since hybridization sites are conserved in the three species.
RT_CMY2_F	CGCAATGGACTCCGGGCGCTAAG	*bla*CMY-2 gene (63 bp)
RT_CMY2_R	GCGCGCCAAACAGACCAATGCTG	
RT_DHA1_F	TCTGCCGCAGTGGAAGGGGATCA	*bla*DHA-1 gene (65 bp)
RT_DHA1_R	ACGGCAGTCCGCCTGCGGTATAG	

Table S3. Primers designed in this work for cloning, sequencing, and construction of knockout mutants

Primer	**Sequence (5′-3′)**[a,b]	**PCR size (bp)**	**Application**
MGH_AG_KO_F	TGCCGTCAACAGCGTCCTGAC CGATACCATCGCCGATATGGC GCAGGACACCAGCATCCACGA TTTCATCAAGCAAAACGATTCC GGGGATCCGTCGACC	1532	Amplification of apramycin resistance gene with ca. 80 nts tails corresponding to upstream and downstream sequences of *K. pneumoniae* MGH 78578 strain *ampG* (gene KPN_00395 from GenBank accession number CP000647.1), added to enable homologous recombination
MGH_AG_KO_R	AGACATTGTGAAAACGAAAAA TAAAATCCCCGGAGCTGAATC GGTTAAATTATTTAAAGAATC GAACGAAAACTGTCAATATGT AGGCTGGAGCTGCTTC		
52145_AG_KO_F	AAAACGCGCGTTAATGCTATC GCGAACCCGGCCTCCGCCGGG TTCGTGCTTTATGCTATGATTC CGGGGATCCGTCGACC	1491	Amplification of apramycin resistance gene with added 60 nts tails corresponding to upstream and downstream sequences of Kp52.145 strain *ampG* (gene BN49_1369 from GenBank accession number FO834906.1), added to enable homologous recombination
52145_AG_KO_R	AATAAGCCGTTATAATTACGTT TCGTAATTACAACGGCTAATA ATGATGCTGGATCCTTATGTA GGCTGGAGCTGCTTC		
CMY2-EcoRI-F	AAAA**GAATTC**GCCCGGACACCTT TTTGCTT	1186	Cloning of *bla*CMY-2 gene into pUCP24 vector
CMY2-HindIII-R	TTT**AAGCTT**AATTATTGCAGCTTT		

	TCAAGAATGC		
CMY2-F	GTCAACACGGTGCAAATC	1257	PCR and sequencing of *bla*CMY-2 gene
CMY2-R	AGGCCCAATATCCTGGGC		
CMY2-int-F	AAAAGATTATGCCTGGGGCT	-	Internal primers for *bla*CMY-2 gene sequencing
CMY2-int-R	AGCCCCAGGCATAATCTTTT		
DHA1-EcoRI-F	AAAA**GAATTC**TGAATCTGACGATACTTGCC	1191	Cloning of *bla*DHA-1 gene into pUCP24 vector
DHA1-HindIII-R	TTT**AAGCTT**AATTATTCCAGTGCACTCAAAATA		
DHA1-F	GCTCTTGTTATAAATAACCG	1310	PCR and sequencing of *bla*DHA-1 gene
DHA1-R	ACAGCCCATAAAGCAAATTA		
DHA1-int-F	GGTTATCTCACACCTTTATTA	-	Internal primers for *bla*DHA-1 gene sequencing
DHA1-int-R	TAATAAAGGTGTGAGATAACC		
AR_DHA-1-F	TTACGCCGCCGCCGTATTCA	1307	PCR and sequencing of *ampR* and intergenic region of *ampR-bla*DHA-1 divergon (based on GenBank accession number: HM193083.1)
AR_DHA-1-R	CGGATCATTCAGCGCCATCT		
AR_DHA-1–int-F	CGACTTTCACCCGCTCACG	-	Internal primers for sequencing *ampR* and intergenic region of *ampR-bla*DHA-1 divergon (based on GenBank accession number: HM193083.1)
AR_DHA-1–int-R	CGTGAGCGGGTGAAAGTCG		
KP mpl-F	GACGAATGCGCATTCATATT	1378	PCR and sequencing of *mpl* in *K. pneumoniae* strains. Sequence obtained from Kp52.145 strain chromosome (GenBank accession number FO834906.1, BN49_4810 gene)
KP mpl-R	TTATTCAGCCGCGGCCGC		
KP mpl-int-F	CGAGCAGGAGCTGGTGGG	-	Internal primers for *mpl* sequencing. Sequence obtained from Kp52.145 strain chromosome (GenBank accession number FO834906.1, BN49_4810 gene)
KP mpl-int-R	CCCACCAGCTCCTGCTCG		
KP AD-F	ACGACGTTTCAGTACCATAC	720	PCR and sequencing of *ampD* in *K. pneumoniae* strains. Sequence obtained from Kp52.145
KP AD-R	ATGATCAAGCTGCCAGTGTT		

			strain chromosome (GenBank accession number FO834906.1, BN49_4228 gene)
KP-dacB-F	CCCAGGTCAGCAGCAATTT	1668	PCR and sequencing of *dacB* in *K. pneumoniae* strains. Sequence obtained from Kp52.145 strain chromosome (GenBank accession number FO834906.1, BN49_0527 gene)
KP-dacB-R	ATGACGACTGGGATGAGGA		
KP_dacB_int-F	CTCATCGGAAGCGCAGTATT	-	Internal primers for *dacB* sequencing. Sequence obtained from Kp52.145 strain chromosome (GenBank accession number FO834906.1, BN49_0527 gene)
KP_dacB_int-R	AATACTGCGCTTCCGATGAG		

[a]Restriction sites in primers used for cloning are shown in bold. [b] In the primers used for gene inactivation, the nucleotides corresponding to the FRT sites flanking the apramycin resistance cassette are underlined. Meanwhile, the rest of the sequences of this type of primers correspond to ca. 60-80 nucleotides fragments upstream/downstream (F/R primers respectively) of each gene to be inactivated.