Supplementary

Supplementary Table 1. List of all strains used in this work, all are *E. coli* (unless indicated Sty, meaning *Salmonella enterica* Serovar. Typhimurium).

Strain	Genotype	Origin and description
DA5438	*E. coli* MG1655	Lab collection, Wild type
DA14704		Amd^R UTI isolate, provided by G. Kahlmeter
DA14709		Amd^R UTI isolate, provided by G. Kahlmeter
DA14710		Amd^R UTI isolate, provided by G. Kahlmeter
DA14712		Amd^R UTI isolate, provided by G. Kahlmeter
DA14713		Amd^R UTI isolate, provided by G. Kahlmeter
DA14715		Amd^R UTI isolate, provided by G. Kahlmeter
DA14718		Amd^R UTI isolate, provided by G. Kahlmeter
DA14719		Amd^R UTI isolate, provided by G. Kahlmeter
DA18464	*aspS*(prom)	This work, original Amd^R isolate
DA18465	*thrS*(R609H)	This work, original Amd^R isolate
DA18467	*ubiE*(del bp 346-349 → FS)	This work, original Amd^R isolate
DA18468	*thrS*(S517A), *eutG*(M65V)	This work, original Amd^R isolate
DA18469	*aspS*(prom, -10)	This work, original Amd^R isolate
DA18471	*aspS*(P170L)	This work, original Amd^R isolate
DA18474	*thrS*(K609E)	This work, original Amd^R isolate
DA18476	*aspS*(prom, -35), *tpx*(C95Y)	This work, original Amd^R isolate
DA18477	*rplL*(prom and dupl)	This work, original AmdR isolate
DA18480	*aspS*(P191S)	This work, original Amd^R isolate
DA18481	*ppa*(D11A)	This work, original Amd^R isolate
DA18482	*rpoB*(H447L), *araB*(I450L)	This work, original Amd^R isolate
DA18483	*ubiX*(del bp 246 → FS)	This work, original Amd^R isolate
DA18484	*aspS*(E4G)	This work, original Amd^R isolate
DA18485	*ppa*(del after gene)	This work, original Amd^R isolate
DA18487	*gltX*(S294F)	This work, original Amd^R isolate
DA18488	*thrS*(E519G)	This work, original Amd^R isolate
DA18489	*mrdA*(D389G), *trxB*(G37D)	This work, original Amd^R isolate
DA18490	*mrdA*(D389G)	This work, original Amd^R isolate
DA18491	*ispA*::IS1	This work, original Amd^R isolate
DA18493	*spoT*(S305P)	This work, original Amd^R isolate
AD18494	*aspS*(E4G)	This work, original Amd^R isolate
DA18496	*ppa*(D11A)	This work, original Amd^R isolate
DA18497	*gltX*(D180G)	This work, original Amd^R isolate
DA19261	MG1655 *ompR::cat* from pKD3	Used for amplification of *cat* cassette
DA24033	Amd^R gene unknown	This work, original Amd^R isolate
DA24034	Amd^R gene unknown	This work, original Amd^R isolate
DA24035	*cysB*(del bp 148 → FS)	This work, original Amd^R isolate
DA24036	Amd^R gene unknown	This work, original Amd^R isolate
DA24037	Amd^R gene unknown	This work, original Amd^R isolate
DA24038	Amd^R gene unknown	This work, original Amd^R isolate
DA24039	Amd^R gene unknown	This work, original Amd^R isolate
DA24040	Amd^R gene unknown	This work, original Amd^R isolate
DA24041	Amd^R gene unknown	This work, original Amd^R isolate
DA24042	Amd^R gene unknown	This work, original Amd^R isolate
DA24043	Amd^R gene unknown	This work, original Amd^R isolate

DA24044	Amd^R gene unknown	This work, original Amd^R isolate
DA24045	Amd^R gene unknown	This work, original Amd^R isolate
DA24046	Amd^R gene unknown	This work, original Amd^R isolate
DA24047	Amd^R gene unknown	This work, original Amd^R isolate
DA24048	Amd^R gene unknown	This work, original Amd^R isolate
DA24049	Amd^R gene unknown	This work, original Amd^R isolate
DA24050	Amd^R gene unknown	This work, original Amd^R isolate
DA24051	Amd^R gene unknown	This work, original Amd^R isolate
DA24052	Amd^R gene unknown	This work, original Amd^R isolate
DA24053	Amd^R gene unknown	This work, original Amd^R isolate
DA24054	Amd^R gene unknown	This work, original Amd^R isolate
DA24055	Amd^R gene unknown	This work, original Amd^R isolate
DA24056	Amd^R gene unknown	This work, original Amd^R isolate
DA24057	Amd^R gene unknown	This work, original Amd^R isolate
DA24058	Amd^R gene unknown	This work, original Amd^R isolate
DA24059	Amd^R gene unknown	This work, original Amd^R isolate
DA24060	Amd^R gene unknown	This work, original Amd^R isolate
DA24100	MG1655 /pSIM5-Tet	lambda red system Tet^R plasmid
DA24678	Amd^R clinical isolate	Re-isolation of clinical isolate. Smaller colonies of isolate DA14707. (Provided by G. Kahlmeter)
DA24686	Amd^R clinical isolate	Re-isolation of clinical isolate. Smaller colonies of isolate DA14717. (Provided by G. Kahlmeter)
DA24690	Amd^R clinical isolate	Re-isolation of clinical isolate. Smaller colonies of isolate DA14721. (Provided by G. Kahlmeter)
DA24692	Amd^R clinical isolate	Re-isolation of clinical isolate. Smaller colonies of isolate DA14723. (Provided by G. Kahlmeter)
DA24978	*ubiE*(del bp 346-349 → FS) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18467)
DA24979	*thrS*(K609E) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18474)
DA24980	*rplL*(prom and dupl) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18477)
DA24981	*aspS*(P191S) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18480)
DA24982	*rpoB*(H447L), *araB*(I450L) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18482)
DA24983	*ubiX*(del bp 246 → FS) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18483)
DA24984	*gltX*(S294F) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18487)
DA24985	*mrdA*(D389G) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18490)
DA24986	*ispA*::IS1 */ pSIM5-Tet*	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18491)
DA24987	*spoT*(S305P) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18493)
DA25197	*E. coli* DH5-alpha / pEL3C15-mRFP1	Plasmid based on the BioBrick vector pSB3T5, containing *cat* (CamR), mRFP1 and a minimal p15 ori.
DA25772	*ppa*(D11A) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18481)
DA25773	*mrdA*(D389G), *trxB*(G37D) /pSIM5-Tet	This work, Lambda red plasmid transformant (Donor: 24100, Recipient: DA18489)
DA26124	*thrS*(K609E), 1803045::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26125	*aspS*(P191S), 1942263::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26126	*mrdA*(D389G), *ybeD*::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted

DA26127	*ppa*(D11A), 4455190::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26128	*ubiX*(del bp 246 → FS), 2428872::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26129	*spoT*(S305P), 3816783::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26130	*rplL*(prom and dupl), 4188613::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26131	*rpoB*(H447L), 4188613::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA26132	*cysB*::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA27146	Sty del(CRISPR1-*cas*1)::*spc* del(CRISPR2)::*cat-sacB*-T0 / pSIM5-tet	Used for amplification of *cat-sacB* cassette
DA27708	*ubiE*(del bp 346-349 → FS), 4025329::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA27709	*trxB*(G37D), *dmsA*::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA28348	*thrS*(K609E), 1803045::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26124 , recipient: DA5438)
DA28349	1803045::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26124, recipient: DA5438)
DA28350	*aspS*(P191S), 1942263::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26125, recipient: DA5438)
DA28351	1942263::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26125, recipient: DA5438)
DA28352	*mrdA*(D389G), *ybeD*::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26126, recipient: DA5438)
DA28353	*ybeD*::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26126, recipient: DA5438)
DA28354	*ppa*(D11A), 4455190::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26127, recipient: DA5438)
DA28355	4455190::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26127, recipient: DA5438)
DA28356	*ubiX*(del bp 246 → FS), 2428872::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26128, recipient: DA5438)
DA28357	2428872::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26128, recipient: DA5438)
DA28358	*spoT*(S305P), 3816783::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26129, recipient: DA5438)
DA28359	3816783::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26129, recipient: DA5438)
DA28360	*rplL*(prom and dupl), 4188613::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26130, recipient: DA5438)
DA28361	4188613::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26130, recipient: DA5438)
DA28362	*rpoB*(H447L), 4188613::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA26131, recipient: DA5438)
DA28363	4188613::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA26131, recipient: DA5438)
DA28364	*cysB*::FRT-Cam-FRT	This work, knock out, transductant (donor: DA26132, recipient: DA5438)
DA28366	*ubiE*(del bp 346-349 → FS), 4025329::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA27708, recipient: DA5438)
DA28367	4025329::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA27708, recipient: DA5438)
DA28368	*trxB*(G37D), *dmsA*::FRT-Cam-FRT	This work, Amd^R transductant (donor: DA27709, recipient: DA5438)

DA28369	*dmsA*::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA27709, recipient: DA5438)
DA28372	*cysB::cat-sacB*	This work, Linear transformant, cat-sacB cassette inserted (donor: DA27146 , recipient: DA24100)
DA28432	*ppa*(D11A), 4455190::FRT-scar	This work, reconstructed mutant
DA28433	4455190(scar)	This work, reconstructed mutant
DA28434	*aspS*(P191S), 1942263::FRT-scar	This work, reconstructed mutant
DA28435	1942263(scar)	This work, reconstructed control
DA28436	*ubiE*(del bp 346-349 → FS), 4025329::FRT-scar	This work, reconstructed mutant
DA28437	4025329(scar)	This work, reconstructed control
DA28438	*spoT*(S305P), 3816783::FRT-scar	This work, reconstructed mutant
DA28439	*cysB*(scar)	This work, knock out
DA29703	*thrS*(K609E), 1803045::FRT-scar	This work, reconstructed mutant
DA29704	1803045::FRT scar	This work, reconstructed control
DA29705	*mrdA*(D389G), *ybeD*::FRT-scar	This work, reconstructed mutant
DA29706	*ybeD*::FRT-scar	This work, reconstructed control
DA29707	*ubiX*(del bp 246 → FS), 2428872::FRT-scar	This work, reconstructed mutant
DA29708	2428872::FRT-scar	This work, reconstructed control
DA29709	3816783::FRT-scar	This work, reconstructed control
DA29710	*rplL*(-10 prom), 4188613::FRT-scar	This work, reconstructed mutant
DA29711	4188613::FRT-scar	This work, reconstructed control
DA29712	*rpoB*(H447L), 4188613::FRT-scar	This work, reconstructed mutant
DA29713	4188613::FRT-scar	This work, reconstructed control
DA29715	*trxB*(G37D), *dmsA*::FRT-scar	This work, reconstructed mutant
DA29716	*dmsA*::FRT-scar	This work, reconstructed control
DA29717	*mrdA*(D389G), *ybeD*::FRT-scar, *trxB*(G37D), *dmsA*::FRT-scar	This work, reconstructed mutant
DA30113	*gltX*(S294F), 2536530::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA30114	*ispA*::IS1, 430247::FRT-Cam-FRT	This work, Linear transformant, Cam cassette inserted
DA30116	*gltX*(S294F), 2536530::FRT-Cam-FRT	This work, Amd^R transductant (donor:DA30113 , recipient: DA5438)
DA30117	2536530::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA30113 , recipient: DA5438)
DA30118	*ispA*::IS1, 430247::FRT-Cam-FRT	This work, Amd^R transductant (donor:DA30114 , recipient: DA5438)
DA30119	430247::FRT-Cam-FRT	This work, Amd^S transductant (donor: DA30114 , recipient: DA5438)
DA30120	*gltX*(S294F), 2536530::FRT-scar	This work, reconstructed mutant
DA30121	2536530::FRT-scar	This work, reconstructed control
DA30122	*ispA*::IS1, 430247::FRT-scar	This work, reconstructed mutant
DA30123	430247::FRT-scar	This work, reconstructed control
DA32973	Amd^R clinical isolate	ECO SENS II nr 1403, from Växjö (Greece)
DA32977	Amd^R clinical isolate	ECO SENS nr 2604, from Växjö (Finland)

DA32978	Amd^R clinical isolate	ECO SENS II nr 4005, from Växjö (UK)
DA32979	Amd^R clinical isolate	ECO SENS II nr 4289, from Växjö (UK)
DA34895	*cysB*(G241E)	This work, Linear transformation: *cysB* from clinical strain DA14710 transformed into wildtype over *cat-sacB*.
DA34896	*cysB*(Q103stop)	This work, Linear transformation: *cysB* from clinical strain DA14713 transformed into wildtype over *cat-sacB*.
DA34897	*cysB*(1 bp ins. nt 485→FS)	This work, linear transformation: *cysB* from clinical strain DA14718 transformed into wildtype over *cat-sacB*.
DA34898	*cysB*(K76stop)	This work, linear transformation: *cysB* from clinical strain DA24686 transformed into wildtype over *cat-sacB*.
DA35001	Amd^R clinical isolate	From Gunnar Kahlmeter (DE 118, Germany)
DA37697	MG1655 /pEL3c15-*cysB*	This work, transformant. pEL3c15 cleaved with XbaI and PstI and ligated with insert containg the *cysB* gene including promoter from DA5438. Clone E.
DA37700	*cysB*(G241E) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14710, clone A.
DA37701	*cysB*(G241E) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14710, clone D.
DA37702	*cysB*(Q103stop) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14713, clone A.
DA37703	*cysB*(Q103stop) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14713, clone B.
DA37704	*cysB*(Q103stop) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14713, clone C.
DA37705	*cysB*(1 bp ins. nt 485→FS) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14718, clone B.
DA37706	*cysB*(1 bp ins. nt 485→FS) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14718, clone B.
DA37707	*cysB*(1 bp del. nt 262→FS) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14719, clone A.
DA37708	*cysB*(1 bp del. nt 262→FS) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14719, clone B.
DA37709	*cysB*(1 bp del. nt 262→FS) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14719, clone C.
DA37710	*cysB*(1 bp del. nt 262→FS) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA14719, clone D.
DA37711	*cysB*(K76stop) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA24686, clone A.
DA37712	*cysB*(K76stop) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA24686, clone B.

DA37713	*cysB*(K76stop) /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into clinical strain DA24686, clone D.
DA37715	*cysB*::scar, /pEL3c15-*cysB*	This work, transformant. pEL3c15-*cysB* from DA37697 transformed into *cysB* KO strain DA28439 , clone C

FS, frame shift; del, deletion; dupl, duplication; fus, fusion; ins, insertion

4

5 Supplementary Table 2. All PCR primers used in this work.

Primer name	**Sequence**	**Used for**
aspS_FWD	CAGCACCCGTTTCGTATC	amplification of the *aspS* gene
aspS_REV	AATTTCCAGTATAATAGCCGCC	amplification of the *aspS* gene
aspS_LiTr_FWD	CTTATCCGACCTACGGTTCGGTA TCTCTGGTAGTCCTGGTTGTAGG CTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *znuB* and *ruvB* genes (linked to *aspS*)
aspS_LiTr_REV	ATCCGGCACTGGCAATATGCCTG ATGCGACGCTGTTCGCGCATATG AATATCCTCCTTAG	amplification of the Cam cassette inserted between the *znuB* and *ruvB* genes (linked to *aspS*)
znuB_ruvB_scr_FWD	TGTTTTGGTGGGGATGGTG	screening of insert between *znuB* and *ruvB*
znuB_ruvB_scr_REV	AGAACGTGAAACCATTGAGGA	screening of insert between *znuB* and *ruvB*
cysB_FWD	GAAAAAGACGGAAAGGCGA	amplification of the *cysB* gene and screening of the *cysB* KO
cysB_REV	GCGAGGCGGGTAATTAGA	amplification of the *cysB* gene and screening of the *cysB* KO
cysB_KO_FWD	ATGAAATTACAACAACTTCGCT ATATTGTTGAGGTGGTCATGTA GGCTGGAGCTGCTTC	amplification of the *cat-sacB* cassette used for KO of the *cysB* gene
cysB_KO_REV	TTATTTTTCCGGCAGTTTTATAT CTTTAAACATGACCTCACATATG AATATCCTCCTTAGTTCC	amplification of the *cat-sacB* cassette used for KO of the *cysB* gene
cysB_plasmid_FWD	TATATCTAGAAAAGACGGAAAG GCGACTGG	amplification of the *cysB* gene for insertion on the pEL3c15 plasmid (primer containing restriction enzyme cleavage sites *Xba*I)
cysB_plasmid_REV	TATACTGCAGGATCGCGGCGGGG TTCCTGA	amplification of the *cysB* gene for insertion on the pEL3c15 plasmid (primer containing restriction enzyme cleavage sites *Pst*I)
gltX_FWD	CAGGAAAAAGTCGCGCAA	amplification of the *gltX* gene
gltX_REV	GATCGCAAAACAGACCGT	amplification of the *gltX* gene
gltX_LiTr_FWD	ACTGTTAAAAAAGTGCGCTTTG TTTATGCCGGATGCGGCGTGTAG GCTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *yfeS* and *cysM* genes (linked to *gltX*)
gltX_LiTr_REV	ATTGAATTAGCACGATTTTGTA GGCCGGATAAGGCGTTTACATA TGAATATCCTCCTTAG	amplification of the Cam cassette inserted between the *yfeS* and *cysM* genes (linked to *gltX*)
yfeS_cysM_FWD	TTACGGCGTTTCCTCACGA	screening of insert between *yfeS* and *cysM*
yfeS_cysM_REV	GGGCGGGGATTTAAGGAT	screening of insert between *yfeS* and *cysM*
ispA_FWD	TCGTCGCAGAGTTTCGGT	amplification of the *ispA* gene
ispA_REV	ATTTGCCCTGAGTCCGTT	amplification of the *ispA* gene

ispA_LiTr_FWD	CGTGCTTTTGTTGGCCGGACAAA GCGTTTACGCCGCATCCTGTAGG CTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *yajD* and *tsx* genes (linked to *ispA*)
ispA_LiTr_REV	TTCCCGGCATTTCTGATTGTTAA CAACGTGTGCTTTGTTCCATATG AATATCCTCCTTAG	amplification of the Cam cassette inserted between the *yajD* and *tsx* genes (linked to *ispA*)
yajD_tsx_scr_FWD	GGGGAAGATGCGCAGAAA	screening of insert between *yajD* and *tsx*
yajD_tsx_scr_REV	AACTACGATCACTGGCACT	screening of insert between *yajD* and *tsx*
mrdA_FWD	ATTTTACGCTCCATCATGCC	amplification of the *mrdA* gene
mrdA_REV	TGTCGCCTGCCTGTAAAG	amplification of the *mrdA* gene
mrdA_LiTr_FWD	AGTTGCCTTTGCTGCTTGGTTTT ACCGTTGGGGTGTAGTCTGTAG GCTGGAGCTGCTTC	amplification of the Cam cassette used for KO of the *ybeD* gene (linked to *mrdA*)
mrdA_LiTr_REV	TGATCAGGTGGTTGAAGTGGTA CAGCGCCATGCGCCAGGTCATAT GAATATCCTCCTTAG	amplification of the Cam cassette used for KO of the *ybeD* gene (linked to *mrdA*)
ybeD_scr_FWD	AGCTGGCGGACAAGAATTTTA	screening of the *ybeD* gene KO
ybeD_scr_REV	CGCTGGTTGTGTTGCAAGA	screening of the *ybeD* gene KO
ppa_FWD	GGATGAGGTGGTGGAAGAGG	amplification of the *ppa* gene
ppa_REV	ATGAAAAATGCGGCTACGG	amplification of the *ppa* gene
ppa_LiTr_FWD	TAGCCAGATATAGTATGCGTAG GCCGGATAAGGCGTTCACTGTAG GCTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *mpl* and *ygjD* genes (linked to *ppa*)
ppa_LiTr_REV	ACGCATCAAGCGTCGCATCAGGC ACTATGCTCAAATGCCGCATATG AATATCCTCCTTAG	amplification of the Cam cassette inserted between the *mpl* and *ygjD* genes (linked to *ppa*)
mpl_ygjA_FWD	TGGAGTGGCGATGTGGAT	screening of insert between *mpl* and *ygjA*
mpl_ygjA_REV	ACGCGAAGAAAGAGAAAGAAG	screening of insert between *mpl* and *ygjA*
rplL_FWD	CGACCTACGAAGAAGCAA	amplification of the *rplL* gene
rplL_REV	AGGCACAGCAGTAGTCAAA	amplification of the *rplL* gene
rpoBmut_FWD	AGCGAACATGGAGCTGAG	amplification of the 500 bp around the *rpoB*(H447L) mutation in DA18482
rpoBmut_REV	TCGGACCTTCAGGGGTTT	amplification of the 500 bp around the *rpoB*(H447L) mutation in DA18482
rplL_rpoB_LiTr_FWD	CAGGGCGAAACGAGGAAGAAGC TTTTCGCCCTGGACAACATGTAG GCTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *sroH* and *thiH* genes (linked to *rplL* and *rpoB*)
rplL_rpoB_LiTr_REV	TTGAAAGCGTTGTGTTTTTTTA TGGTAGTGCCGTCAGCAGCATAT GAATATCCTCCTTAG	amplification of the Cam cassette inserted between the *sroH* and *thiH* genes (linked to *rplL* and *rpoB*)
sroH_thiH_scr_FWD	TGCCTTGACCTTGCGATAAT	screening of insert between *sroH* and *thiH*
sroH_thiH_scr_REV	AACAGTTCTCACCGCACGA	screening of insert between *sroH* and *thiH*
spoTmut_FWD	GAAGGGCGTTTGCAGGAAG	amplification of the 500 bp around the *spoT*(S305P) mutation in DA18493
spoTmut_REV	ATAAGCGAAGTCGACGGG	amplification of the 500 bp around the *spoT*(S305P) mutation in DA18493

spoT_LiTr_FWD	AATAGCGATTTCACATAACTAC AAGTTATCAATTTCCCCTTGTAG GCTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *dinD* and *yicG* genes (linked to *spoT*)
spoT_LiTr_REV	GGTAGCGATTTGTGCGCATTGTC ACGATTGAGATTTTTTACATAT GAATATCCTCCTTAG	amplification of the Cam cassette inserted between the *dinD* and *yicG* genes (linked to *spoT*)
dinD_yicG_scr_FWD	ACCCGATGCGTAAGCCAA	screening of insert between *dinD* and *yicG*
dinD_yicG_scr_REV	AATATCGCGCACTGACCC	screening of insert between *dinD* and *yicG*
thrS_FWD	CTCCGGCTTCTTCTGCTT	amplification of the *thrS* gene
thrS_REV	TAACATCGCTCAACCGGG	amplification of the *thrS* gene
thrS_LiTr_FWD	ATAATTTTATTTTTTTCATAAA ACGCAGAAACAGTGACCATGTA GGCTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *arpB* and *yniD* genes (linked to *thrS*)
thrS_LiTr_REV	AAGGGATTTTTTGTTATTACAG CCGTCGGGTGGCGTTGTGCATAT GAATATCCTCCTTAG	amplification of the Cam cassette inserted between the *arpB* and *yniD* genes (linked to *thrS*)
arpB_yniD_scr_FWD	TGGCCGCTAAAAATCATGAC	screening of insert between *arpB* and *yniD*
arpB_yniD_scr_REV	TCTGGCTGTCTTCGTTTGT	screening of insert between *arpB* and *yniD*
trxB_FWD	AAGCCCAGCAGACGAGAA	amplification of the *trxB* gene
trxB_REV	AGAGGTGATAATGACGGGAGAA	amplification of the *trxB* gene
trxB_LiTr_FWD	ATGAAAACGAAAATCCCTGATG CGGTATTGGCTGCTGAGGTGTA GGCTGGAGCTGCTTC	amplification of the Cam cassette used for KO of the *dmsA* gene (linked to *trxB*)
trxB_LiTr_REV	TACACCTTTTCAACCTGAACAAG GTTTGTATGTGACGGATCATAT GAATATCCTCCTTAG	amplification of the Cam cassette used for KO of the *dmsA* gene (linked to *trxB*)
dmsA_scr_FWD	CGCTTTTTTTGTCTCCCTTTGA	screening of the *dmsA* gene KO
dmsA_scr_REV	CCGTTATCTTCCTGCCAGT	screening of the *dmsA* gene KO
ubiE_FWD	CGGCAGGCAATGAAAAAAC	amplification of the *ubiE* gene
ubiE_REV	TACCTCCACGCGCAATAC	amplification of the *ubiE* gene
ubiE_LiTr_FWD	GTATCTGGCAAACCATGCCCGAT GCGACGCTGTCGCGTCTTGTAGG CTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *fre* and *fadA* genes (linked to *ubiE*)
ubiE_LiTr_REV	TAAACGCCTTATCCGGCCTACGG TTCGGACTATTTGTAGGCATAT GAATATCCTCCTTAG	amplification of the Cam cassette inserted between the *fre* and *fadA* genes (linked to *ubiE*)
fre_fadA_scr_FWD	TTGCCCGCGATCTGTTTT	screening of insert between *fre* and *fadA*
fre_fadA_scr_REV	ATGTGTATCGGTCTGGGT	screening of insert between *fre* and *fadA*
ubiX_FWD	TGTGTTGTGTGTTTGCAGG	amplification of the *ubiX* gene
ubiX_REV	AAAGCAGTGCAACGTCAG	amplification of the *ubiX* gene
ubiX_LiTr_FWD	CGCGCATCAATAAAAATGGCGCT GAAAAAATATTCAACGCTGTAG GCTGGAGCTGCTTC	amplification of the Cam cassette inserted between the *cvpA* and *dedD* genes (linked to *ubiX*)
ubiX_LiTr_REV	CATCCGACACGCATTGCCCGATG CCGCAAAGGCATAAAAACATAT GAATATCCTCCTTAG	amplification of the Cam cassette inserted between the *cvpA* and *dedD* genes (linked to *ubiX*)
cvpA_dedD_scr_FWD	AAAAAGCAATCACCGCGA	screening of insert between *cvpA* and *dedD*
cvpA_dedD_scr_REV	GGCTTAAGTGGCGTGGTA	screening of insert between *cvpA* and *dedD*

Supplementary Table 3. Population analysis (comparing the same genes from several strains of the same species to *E. coli* MG1655) accession numbers for the clinical amindinocillin resistant strains are KP670466-KP670849 in GenBank.

Sequence name (gene and strain)	Accession number
alaS_DA14704	KP670466
alaS_DA14709	KP670467
alaS_DA14710	KP670468
alaS_DA14712	KP670469
alaS_DA14713	KP670470
alaS_DA14715	KP670471
alaS_DA14718	KP670472
alaS_DA14719	KP670473
alaS_DA24678	KP670474
alaS_DA24686	KP670475
alaS_DA24690	KP670476
alaS_DA24692	KP670477
argS_DA14704	KP670478
argS_DA14709	KP670479
argS_DA14710	KP670480
argS_DA14712	KP670481
argS_DA14713	KP670482
argS_DA14715	KP670483
argS_DA14718	KP670484
argS_DA14719	KP670485
argS_DA24678	KP670486
argS_DA24686	KP670487
argS_DA24690	KP670488
argS_DA24692	KP670489
aroK_DA14704	KP670490
aroK_DA14709	KP670491
aroK_DA14710	KP670492
aroK_DA14712	KP670493
aroK_DA14713	KP670494
aroK_DA14715	KP670495
aroK_DA14718	KP670496
aroK_DA14719	KP670497
aroK_DA24678	KP670498
aroK_DA24686	KP670499
aroK_DA24690	KP670500
aroK_DA24692	KP670501
aspS_DA14704	KP670502
aspS_DA14709	KP670503
aspS_DA14710	KP670504
aspS_DA14712	KP670505
aspS_DA14713	KP670506
aspS_DA14715	KP670507
aspS_DA14718	KP670508
aspS_DA14719	KP670509
aspS_DA24678	KP670510
aspS_DA24686	KP670511

aspS_DA24690	KP670512
aspS_DA24692	KP670513
crp_DA14704	KP670514
crp_DA14709	KP670515
crp_DA14710	KP670516
crp_DA14712	KP670517
crp_DA14713	KP670518
crp_DA14715	KP670519
crp_DA14718	KP670520
crp_DA14719	KP670521
crp_DA24678	KP670522
crp_DA24686	KP670523
crp_DA24690	KP670524
crp_DA24692	KP670525
cyaA_DA14704	KP670526
cyaA_DA14709	KP670527
cyaA_DA14710	KP670528
cyaA_DA14712	KP670529
cyaA_DA14713	KP670530
cyaA_DA14715	KP670531
cyaA_DA14718	KP670532
cyaA_DA14719	KP670533
cyaA_DA24678	KP670534
cyaA_DA24686	KP670535
cyaA_DA24690	KP670536
cyaA_DA24692	KP670537
cysB_DA14704	KP670538
cysB_DA14709	KP670539
cysB_DA14710	KP670540
cysB_DA14712	KP670541
cysB_DA14713	KP670542
cysB_DA14715	KP670543
cysB_DA14718	KP670544
cysB_DA14719	KP670545
cysB_DA24678	KP670546
cysB_DA24686	KP670547
cysB_DA24690	KP670548
cysB_DA24692	KP670549
rplL_DA14704	KP670550
rplL_DA14709	KP670551
rplL_DA14710	KP670552
rplL_DA14712	KP670553
rplL_DA14713	KP670554
rplL_DA14715	KP670555
rplL_DA14718	KP670556
rplL_DA14719	KP670557
rplL_DA24678	KP670558
rplL_DA24686	KP670559
rplL_DA24690	KP670560
rplL_DA24692	KP670561
cysE_DA14704	KP670562
cysE_DA14709	KP670563
cysE_DA14710	KP670564

cysE_DA14712	KP670565
cysE_DA14713	KP670566
cysE_DA14715	KP670567
cysE_DA14718	KP670568
cysE_DA14719	KP670569
cysE_DA24678	KP670570
cysE_DA24686	KP670571
cysE_DA24690	KP670572
cysE_DA24692	KP670573
ftsA_DA14704	KP670574
ftsA_DA14709	KP670575
ftsA_DA14710	KP670576
ftsA_DA14712	KP670577
ftsA_DA14713	KP670578
ftsA_DA14715	KP670579
ftsA_DA14718	KP670580
ftsA_DA14719	KP670581
ftsA_DA24678	KP670582
ftsA_DA24686	KP670583
ftsA_DA24690	KP670584
ftsA_DA24692	KP670585
ftsQ_DA14704	KP670586
ftsQ_DA14709	KP670587
ftsQ_DA14710	KP670588
ftsQ_DA14712	KP670589
ftsQ_DA14713	KP670590
ftsQ_DA14715	KP670591
ftsQ_DA14718	KP670592
ftsQ_DA14719	KP670593
ftsQ_DA24678	KP670594
ftsQ_DA24686	KP670595
ftsQ_DA24690	KP670596
ftsQ_DA24692	KP670597
ftsZ_DA14704	KP670598
ftsZ_DA14709	KP670599
ftsZ_DA14710	KP670600
ftsZ_DA14712	KP670601
ftsZ_DA14713	KP670602
ftsZ_DA14715	KP670603
ftsZ_DA14718	KP670604
ftsZ_DA14719	KP670605
ftsZ_DA24678	KP670606
ftsZ_DA24686	KP670607
ftsZ_DA24690	KP670608
ftsZ_DA24692	KP670609
galE_DA14704	KP670610
galE_DA14709	KP670611
galE_DA14710	KP670612
galE_DA14712	KP670613
galE_DA14713	KP670614
galE_DA14715	KP670615
galE_DA14718	KP670616
galE_DA14719	KP670617

galE_DA24678	KP670618
galE_DA24686	KP670619
galE_DA24690	KP670620
galE_DA24692	KP670621
gltX_DA14704	KP670622
gltX_DA14709	KP670623
gltX_DA14710	KP670624
gltX_DA14712	KP670625
gltX_DA14713	KP670626
gltX_DA14715	KP670627
gltX_DA14718	KP670628
gltX_DA14719	KP670629
gltX_DA24678	KP670630
gltX_DA24686	KP670631
gltX_DA24690	KP670632
gltX_DA24692	KP670633
ispA_DA14704	KP670634
ispA_DA14709	KP670635
ispA_DA14710	KP670636
ispA_DA14712	KP670637
ispA_DA14713	KP670638
ispA_DA14715	KP670639
ispA_DA14718	KP670640
ispA_DA14719	KP670641
ispA_DA24678	KP670642
ispA_DA24686	KP670643
ispA_DA24690	KP670644
ispA_DA24692	KP670645
lon_DA14704	KP670646
lon_DA14709	KP670647
lon_DA14710	KP670648
lon_DA14712	KP670649
lon_DA14713	KP670650
lon_DA14715	KP670651
lon_DA14718	KP670652
lon_DA14719	KP670653
lon_DA24678	KP670654
lon_DA24686	KP670655
lon_DA24690	KP670656
lon_DA24692	KP670657
mrdA_DA14704	KP670658
mrdA_DA14709	KP670659
mrdA_DA14710	KP670660
mrdA_DA14712	KP670661
mrdA_DA14713	KP670662
mrdA_DA14715	KP670663
mrdA_DA14718	KP670664
mrdA_DA14719	KP670665
mrdA_DA24678	KP670666
mrdA_DA24686	KP670667
mrdA_DA24690	KP670668
mrdA_DA24692	KP670669
mrdB_DA14704	KP670670

mrdB_DA14709	KP670671
mrdB_DA14710	KP670672
mrdB_DA14712	KP670673
mrdB_DA14713	KP670674
mrdB_DA14715	KP670675
mrdB_DA14718	KP670676
mrdB_DA14719	KP670677
mrdB_DA24678	KP670678
mrdB_DA24686	KP670679
mrdB_DA24690	KP670680
mrdB_DA24692	KP670681
mreB_DA14704	KP670682
mreB_DA14709	KP670683
mreB_DA14710	KP670684
mreB_DA14712	KP670685
mreB_DA14713	KP670686
mreB_DA14715	KP670687
mreB_DA14718	KP670688
mreB_DA14719	KP670689
mreB_DA24678	KP670690
mreB_DA24686	KP670691
mreB_DA24690	KP670692
mreB_DA24692	KP670693
mreC_DA14704	KP670694
mreC_DA14709	KP670695
mreC_DA14710	KP670696
mreC_DA14712	KP670697
mreC_DA14713	KP670698
mreC_DA14715	KP670699
mreC_DA14718	KP670700
mreC_DA14719	KP670701
mreC_DA24678	KP670702
mreC_DA24686	KP670703
mreC_DA24690	KP670704
mreC_DA24692	KP670705
mreD_DA14704	KP670706
mreD_DA14709	KP670707
mreD_DA14710	KP670708
mreD_DA14712	KP670709
mreD_DA14713	KP670710
mreD_DA14715	KP670711
mreD_DA14718	KP670712
mreD_DA14719	KP670713
mreD_DA24678	KP670714
mreD_DA24686	KP670715
mreD_DA24690	KP670716
mreD_DA24692	KP670717
ppa_DA14704	KP670718
ppa_DA14709	KP670719
ppa_DA14710	KP670720
ppa_DA14712	KP670721
ppa_DA14713	KP670722
ppa_DA14715	KP670723

ppa_DA14718	KP670724
ppa_DA14719	KP670725
ppa_DA24678	KP670726
ppa_DA24686	KP670727
ppa_DA24690	KP670728
ppa_DA24692	KP670729
rcsB_DA14704	KP670730
rcsB_DA14709	KP670731
rcsB_DA14710	KP670732
rcsB_DA14712	KP670733
rscB_DA14713	KP670734
rcsB_DA14715	KP670735
rcsB_DA14718	KP670736
rcsB_DA14719	KP670737
rcsB_DA24678	KP670738
rcsB_DA24686	KP670739
rcsB_DA24690	KP670740
rcsB_DA24692	KP670741
rcsC_DA14704	KP670742
rcsC_DA14709	KP670743
rcsC_DA14710	KP670744
rcsC_DA14712	KP670745
rcsC_DA14713	KP670746
rcsC_DA14715	KP670747
rcsC_DA14718	KP670748
rcsC_DA14719	KP670749
rcsC_DA24678	KP670750
rcsC_DA24686	KP670751
rcsC_DA24690	KP670752
rcsC_DA24692	KP670753
rcsD_DA14704	KP670754
rcsD_DA14709	KP670755
rcsD_DA14710	KP670756
rcsD_DA14712	KP670757
rcsD_DA14713	KP670758
rcsD_DA14715	KP670759
rcsD_DA14718	KP670760
rcsD_DA14719	KP670761
rcsD_DA24678	KP670762
rcsD_DA24686	KP670763
rcsD_DA24690	KP670764
rcsD_DA24692	KP670765
rcsF_DA14704	KP670766
rcsF_DA14709	KP670767
rcsF_DA14710	KP670768
rcsF_DA14712	KP670769
rcsF_DA14713	KP670770
rcsF_DA14715	KP670771
rcsF_DA14718	KP670772
rcsF_DA14719	KP670773
rcsF_DA24678	KP670774
rcsF_DA24686	KP670775
rcsF_DA24690	KP670776

rcsF_DA24692	KP670777
rpoB_DA14704	KP670778
rpoB_DA14709	KP670779
rpoB_DA14710	KP670780
rpoB_DA14712	KP670781
rpoB_DA14713	KP670782
rpoB_DA14715	KP670783
rpoB_DA14718	KP670784
rpoB_DA14719	KP670785
rpoB_DA24678	KP670786
rpoB_DA24686	KP670787
rpoB_DA24690	KP670788
rpoB_DA24692	KP670789
slt_DA14704	KP670790
slt_DA14709	KP670791
slt_DA14710	KP670792
slt_DA14712	KP670793
slt_DA14713	KP670794
slt_DA14715	KP670795
slt_DA14718	KP670796
slt_DA14719	KP670797
slt_DA24678	KP670798
slt_DA24686	KP670799
slt_DA24690	KP670800
slt_DA24692	KP670801
spoT_DA14704	KP670802
spoT_DA14709	KP670803
spoT_DA14710	KP670804
spoT_DA14712	KP670805
spoT_DA14713	KP670806
spoT_DA14715	KP670807
spoT_DA14718	KP670808
spoT_DA14719	KP670809
spoT_DA24678	KP670810
spoT_DA24686	KP670811
spoT_DA24690	KP670812
spoT_DA24692	KP670813
thrS_DA14704	KP670814
thrS_DA14709	KP670815
thrS_DA14710	KP670816
thrS_DA14712	KP670817
thrS_DA14713	KP670818
thrS_DA14715	KP670819
thrS_DA14718	KP670820
thrS_DA14719	KP670821
thrS_DA24678	KP670822
thrS_DA24686	KP670823
thrS_DA24690	KP670824
thrS_DA24692	KP670825
ubiE_DA14704	KP670826
ubiE_DA14709	KP670827
ubiE_DA14710	KP670828
ubiE_DA14712	KP670829

ubiE_DA14713	KP670830
ubiE_DA14715	KP670831
ubiE_DA14718	KP670832
ubiE_DA14719	KP670833
ubiE_DA24678	KP670834
ubiE_DA24686	KP670835
ubiE_DA24690	KP670836
ubiE_DA24692	KP670837
ubiX_DA14704	KP670838
ubiX_DA14709	KP670839
ubiX_DA14710	KP670840
ubiX_DA14712	KP670841
ubiX_DA14713	KP670842
ubiX_DA14715	KP670843
ubiX_DA14718	KP670844
ubiX_DA14719	KP670845
ubiX_DA24678	KP670846
ubiX_DA24686	KP670847
ubiX_DA24690	KP670848
ubiX_DA24692	KP670849

11

12