

Supplementary information

4.5 years within-patient evolution of a colistin resistant KPC-producing

Klebsiella pneumoniae ST258

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40-word summary:

KPC-producing *Klebsiella pneumoniae* (KPC-Kp) have emerged as a major nosocomial pathogen. This study emphasizes long-term *in-vivo* genome evolution along with the selection of pathoadaptive mutations in a KPC-Kp strain that might have contributed to its long-term carriage, virulence and dissemination

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Supplementary Methods

Bioinformatic analysis

PacBio subreads were assembled with both Canu [1] and the RS_HGAP_Assembly.3 protocol from the SMRT analysis toolkit v2.3. Consensus accuracy was further polished using Quiver [2] and manually corrected with Illumina reads. Assembly of Illumina reads by Velvet [3] allowed to get the sequence of the 13.8 pBIC-1c plasmid missing in the PacBio assembly. Annotation of Kp BIC-1 was performed using Prokka [4]. The assembled genome was uploaded to the ResFinder v2.1 server (<http://cge.cbs.dtu.dk/services/ResFinder/>) to identify resistance genes. Illumina reads were assembled by using SPAdes [5].

Cloning and Bacterial transformation

pBIC-1c plasmids from isolate S5 (susceptible to gentamicin, highly resistant to amikacin) and isolate S11 (resistant to both amikacin and gentamicin) were introduced by electroporation into *E. coli* TOP10 by using a Gene Pulser II (Bio-Rad) [6]. *E. coli* TOP10 carrying pBIC-1c-GentaR (from S5) or pBIC-1c-GentaS (from S11) were and selected on agar plates supplemented with amikacin 15 µg/ml or with gentamicin 15 µg/ml respectively. Wild type sequence (from Kp BIC-1) of *gmlC*, *arnC* and *phoP* were cloned into a modified pUC19, made resistant to apramycin due to an acetyltransferase AAC(3)-IV. Cloning were performed with the NEBuilder® Hifi DNA Assembly cloning kit according to the manufacturer instructions. pUC19-*gmlC*(WT) and pUC-19-*phoP*(WT) were transformed by electroporation in S15 whereas pUC-19-*arnC*(WT) was introduced in S17. Empty pUC19 was also electroporated in S15 and S17 as control. The transformants were selected on agar plates supplemented with apramycin 30 µg/ml.

Gene expression quantification by qRT-PCR

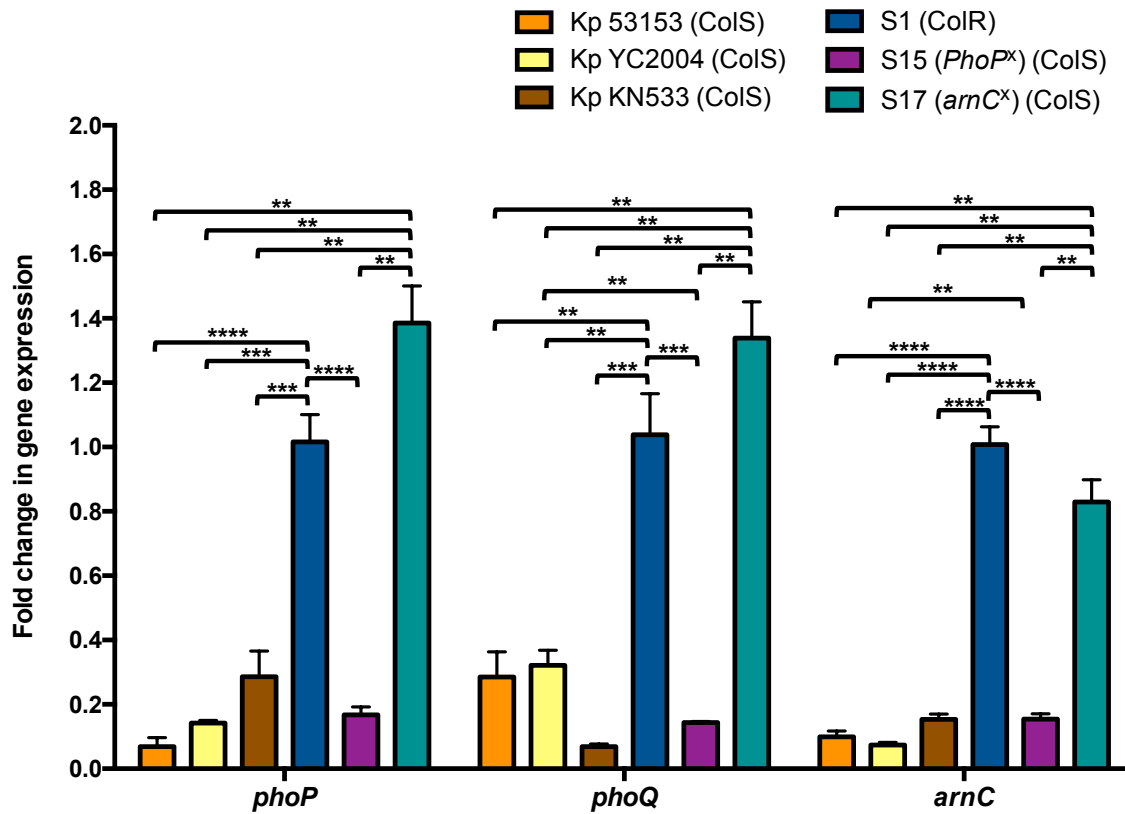
arnC, *phoP* and *phoQ* expression was analysed by qRT-PCR. Total RNAs were extracted from exponential-phase cultures using the RNeasy kit (Qiagen), treated with DNase, and 25 ng of RNA was

used for each reaction. Primer and probe concentrations were adjusted to achieve efficiencies of 90–110% and all experiments were performed in triplicate. The primers used in these experiments are summarized in Supplementary Table 4. All qRT-PCRs included an initial denaturation step of 30 s at 95°C, 35 cycles of denaturation (95°C/1s), annealing (52°C/5s), and extension (72°C/7s). Expression of each gene was normalized using a housekeeping gene coding for a ribosomal protein (*rpsL*). The relative expression of each gene was then calibrated against its expression in isolate S1.



Supplementary Figure 1. Maximum likelihood phylogenetic tree of KPC-Kp ST258.

The core genome phylogeny of Kp BIC-1 and the 17 clinical Kp isolates (highlighted in red) was performed together with published sequences retrieved from the NCBI web server belonging to the ST258 clade a/I (32192, AR_0098, KPNIH30, UHKPC07, UHKPC33, CAV1453, FBRG01.1), ST258 clade b/II (KPNIH32, MNCRE53, MNCRE69, ST258_FL, MPZG03.1, NJST258_2, LXMF01.1). The ST11 strain HS11286 was used as an outgroup and all the other genomes sequences belonging to ST258 were retrieved from the NCBI database. Multiple sequence alignment and phylogeny were performed with Parsnp [7].



Supplementary Figure 2. Effect of PhoP and ArnC mutation on *phoP*, *phoQ* and *arnC* gene expression.

Expression analysis was performed by qRT-PCR on 6 strains either wild type (Kp53153, KpKN533, KpYC2004), mutated in *mgrB* (S1), double mutant *mgrB phoP* (S15) or double mutant *mgrB arnC* (S17). Average fold change of *phoP*, *phoQ* and *arnC* expression are represented (n=3, means \pm SEM). Statistical analysis were performed with Student t-test (only significant results are indicated: **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$). Compared to isolate S1, the expression of *phoP*, *phoQ* and *arnC* is significantly different in isolate S15 but not in isolate S17. Expression of the three genes in isolate S1 is significantly higher than in the three strains with a non-disrupted *mgrB*. * Indicates the presence of a non-synonymous SNP in the gene. ColS = susceptible to colistin; ColR = resistance to colistin

Supplementary Table 1. Acquired resistance genes and mutations contributing to antimicrobials, heavy-metals and arsenic resistance in Kp

Gene	Protein	Genetic support	Function	Resistance
Resistance genes				
<i>bla</i> _{KPC-2}	KPC-2	pBIC-1a	Ambler class A β-lactamase	All β-lactams including carbapenems
<i>bla</i> _{TEM-1}	TEM-1	pBIC-1a	Ambler class A β-lactamase	Narrow-spectrum β-lactams
<i>bla</i> _{SHV-11}	SHV-11	Chromosome	Ambler class A β-lactamase	Narrow-spectrum β-lactams
<i>bla</i> _{SHV-12}	SHV-12	pBIC-1b	Ambler class A β-lactamase	Extended-spectrum cephalosporins
<i>bla</i> _{OXA-9}	OXA-9	pBIC-1a	Ambler class D β-lactamase	Narrow-spectrum β-lactams
<i>aac</i> (6′)- <i>Ib</i>	AAC(6′)-Ib	pBIC-1c	Aminoglycoside acetyltransferase	Kanamycin, Tobramycin, Netilmicin, fluoroquinolones
<i>aph</i> (3′)- <i>Ia</i>	APH(3′)-Ia	pBIC-1a	Aminoglycoside phosphotransferase	Kanamycin
<i>ars operon</i>		pBIC-1a	Efflux pump	Arsenate and arsenite
<i>crp operon</i>		pBIC-1a	Efflux pump	Copper-derivative compound
<i>sil operon</i>		pBIC-1a	Efflux pump	Silver-derivative compound
<i>fosA-like</i>		Chromosome	Fosfomycin glutathione transferase	Fosfomycin
<i>oqxA/oqxB</i>	OqxA/OqxB	Chromosome	Efflux pump	Quinolones, biocides
<i>acrAB-tolC</i>	AcrAB-TolC	Chromosome	Efflux pump	Broad-range of substrates including acriflavine
<i>emrD</i>	EmrD	Chromosome	Efflux pump	Etidium bromide, biocides, quinolone, phenicols
Mutation				
² <i>gyrA</i>	GyrA	Chromosome	Mutated gyrase A subunit	Quinolones and Fluoroquinolones
³ <i>parC</i>	ParC	Chromosome	Mutated topoisomerase IV	Quinolones and Fluoroquinolones
⁴ <i>ompK36</i>	OmpK36	Chromosome	Mutated porin	β-lactams
⁵ <i>ompK35</i>	OmpK35	Chromosome	Truncated porin (frameshift)	β-lactams
⁶ <i>mgrB</i>	MgrB	Chromosome	Activation of the colistin resistance pathway via PhoP/PhoQ activation (<i>mgrB</i> is disrupted by IS <i>Kpn35</i>)	Polymyxins

¹ These genes and mutations were also identified in the 17 isolates from the patient. ² Ser to Leu substitution at position 83; ³ Ser to Ile substitution at position 80; ⁴ L (G insertion at position 1118), ⁶ 8 kb IS*Kpn25* insertion between nucleotides 141 and 142 of *mgrB*.

Supplementary Table S2: mutations identified in the 17 isolates derived from the Kp BIC-1 strain

¹ Position	Mutation	² Mutation annotation	³ Gene(s)	⁴ Annotation	Classification	S1	S2	S3	S4	S5	S6	S7	S8
15372	T→A	IG (-75/-187)	<i>PROKKA_00015</i> ← / → <i>marR_1</i>	inner membrane protein/Multiple antibiotic resistance protein MarR	Conserved protein / Antibiotic resistance								
70826	T→A	N335I (AAT→ATT)	<i>nagC_1</i>	N-acetylglucosamine repressor	Carbon metabolism and transport								
87501	G→T	R132R (CGG→CGT)	<i>fabD_1</i>	Malonyl CoA-acyl carrier protein transacylase	Lipids metabolism						X		
112796	Δ1 bp	coding (205/909 nt)	<i>cynR_1</i>	HTH-type transcriptional regulator CynR	Carbon metabolism and transport								
112800	A→T	S67R (AGT→AGA)	<i>cynR_1</i>	HTH-type transcriptional regulator CynR	Carbon metabolism and transport								
118736	A→T	IG (+169/-26)	<i>ogt_1</i> → / → <i>fnr</i>	Methylated-DNA--protein-cysteine methyltransferase/Fumarate and nitrate reduction regulatory protein	Protein modificaton / Regulation								
212223	G→A	S118S (TCC→TCT)	<i>zntB_2</i>	Zinc transport protein ZntB	Transporter								
252616	G→C	R304G (CGT→GGT)	<i>PROKKA_00239</i>	Phage-related baseplate assembly protein	Phage and mobile genetic element	X		X	X				
257261	T→A	D460V (GAC→GTC)	<i>oprF_1</i>	Outer membrane porin F precursor	Cell wall and cell envelope								
305919	T→C	IG (+174/+25)	<i>yraA</i> → / ← <i>PROKKA_00307</i>	Putative cysteine protease YraA/HD domain protein	Nitrogen and amino acids metabolism / conserved protein								
389853	Δ23 bp	coding (424-446/861 nt)	<i>PROKKA_00392</i>	Bacteriophage replication protein O	Phage and mobile genetic element								
450562	C→A	L19I (CTC→ATC)	<i>pfeA</i>	Ferric enterobactin receptor precursor, outer membrane protein fepA	Cell wall and cell envelope								
452099	G→A	G531D (GGT→GAT)	<i>pfeA</i>	Ferric enterobactin receptor precursor	Cell wall and cell envelope								
460813	27 bp x 2	duplication	<i>PROKKA_00463</i>	hypothetical protein	Conserved protein								
477196	T→C	S83P (TCG→CCG)	<i>ydjZ_1</i>	TVP38/TMEM64 family inner membrane protein YdjZ	Conserved protein					X			
495424	G→A	A358A (GCG→GCA)	<i>sppA</i>	Protease 4, signal peptide peptidase	Protein secretion								
565864	T→A	I201N (ATT→AAT)	<i>phoP_1</i>	Virulence transcriptional regulatory protein PhoP	Regulation								
689934	G→T	E148* (GAG→TAG)	<i>yahA</i>	Cyclic di-GMP phosphodiesterase YahA	Regulation								
729417	G→A	H95H (CAC→CAT)	<i>yxeP_2</i>	putative hydrolase YxeP	Nitrogen and amino acids metabolism								
827364	A→T	L32Q (CTG→CAG)	<i>potF_1</i>	Putrescine-binding periplasmic protein precursor	Nitrogen and amino acids metabolism								
829698	G→A	V8V (GTC→GTT)	<i>nfsA</i>	Oxygen-insensitive NADPH nitroreductase	Energetic metabolism								
1012404	T→C	T26A (ACT→GCT)	<i>sucC</i>	Succinyl-CoA ligase [ADP-forming] β subunit	Energetic metabolism								
1072036	Δ2681 bp	Coding	<i>shlB</i> → / → <i>shlA</i>	Hemolysin transporter protein / Hemolysin precursor	Virulence								
1220202	T→G	IG (-94/+3)	<i>nifA</i> ← / ← <i>PROKKA_01187</i>	Nif-specific regulatory protein/hypothetical protein	Regulation / conserved protein								
1234385	T→A	F93Y (TTT→TAT)	<i>PROKKA_01198</i>	hypothetical protein	Conserved protein								
1350288	G→T	A176E (GCA→GAA)	<i>dnaB_1</i>	Replicative DNA helicase	Replication, DNA repear, recombination								

1424306	C→T	P323S (CCT→TCT)	<i>yjcC_1</i>	putative membrane protein YjcC	Conserved protein					
1574001	A→T	Q339L (CAG→CTG)	<i>proA</i>	Gamma-glutamyl phosphate reductase	Nitrogen and amino acids metabolism					
1601224	G→C	V212L (GTC→CTC)	<i>gstB_2</i>	Glutathione S-transferase GstB	Energetic metabolism (aerobic and anaerobic)					
1686958	G→A	T177M (ACG→ATG)	<i>degP</i>	Periplasmic serine endoprotease DegP precursor	Nitrogen and amino acids metabolism					
1712430	A→G	R196R (CGA→CGG)	<i>epsE_1</i>	Type II secretion system protein E	Protein secretion					
1738791	T→A	N240Y (AAT→TAT)	<i>cueO</i>	Blue copper oxidase CueO precursor	Energetic metabolism				X	
1798536	T→G	D137A (GAT→GCT)	<i>ilvI_1</i>	Acetolactate synthase isozyme 3 large subunit	Carbon metabolism and transport					
2002150	G→C	S205R (AGC→AGG)	<i>rbsC_5</i>	Ribose transport system permease protein RbsC	Carbon metabolism and transport					
2040739	C→T	G193S (GGT→AGT)	<i>xylA_2</i>	Xylose isomerase	Carbon metabolism and transport					
2082086	Δ233 bp	IG (+1047/+112)	<i>PROKKA_02008</i> → / ← <i>iolB</i>	hypothetical protein/5-deoxy-glucuronate isomerase	Conserved protein / arbon metabolism and transport					
2082554	G→A	A229V (GCA→GTA)	<i>iolB</i>	5-deoxy-glucuronate isomerase	Carbon metabolism and transport					
2117070	Δ59 bp	coding (105-163/1308 nt)	<i>licH_2</i>	putative 6-phospho-β-glucosidase	Carbon metabolism and transport					
2130748	C→T	G13S (GGT→AGT)	<i>PROKKA_02051</i>	hypothetical protein	Conserved protein					
2446301	A→G	Q24R (CAG→CGG)	<i>rfaH_1</i>	Transcription antitermination protein RfaH	Regulator					X
2734777	C→A	A28S (GCG→TCG)	<i>chbA_3</i>	N,N'-diacetylchitobiose-specific phosphotransferase enzyme IIA component	Carbon metabolism and transport					
2823602	C→T	W166* (TGG→TAG)	<i>epsI_1</i>	putative glycosyltransferase EpsI	Cell wall and cell envelope					
2847452	C→A	V162L (GTG→TTG)	<i>lldR_1</i>	Putative L-lactate dehydrogenase operon regulatory protein	Regulator					
2887499	C→A	L277L (CTG→CTT)	<i>ghrB</i> ←	Glyoxylate/hydroxypyruvate reductase B	Carbon metabolism and transport					
2901797	C→A	F223L (TTC→TTA)	<i>PROKKA_02778</i>	Ankyrin repeats (3 copies)	Conserved protein					
2939971	T→A	L238Q (CTG→CAG)	<i>dctA_1</i>	Aerobic C4-dicarboxylate transport protein	Carbon metabolism and transport					
2977537	G→A	C161Y (TGC→TAC)	<i>arnC</i>	Undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	Regulator					
3011187	C→T	P199S (CCG→TCG)	<i>ugpA_2</i>	sn-glycerol-3-phosphate transport system permease protein UgpA	Carbon metabolism and transport					
3023465	T→A	IG (+382/-81)	<i>gntU</i> → / → <i>asd_1</i>	Low-affinity gluconate transporter/Aspartate-semialdehyde dehydrogenase	Carbon metabolism and transport / Carbon metabolism and transport					
3124795	A→T	N23I (AAT→ATT)	<i>PROKKA_02988</i>	hypothetical protein	Conserved protein					
3504575	T→A	I325F (ATC→TTC)	<i>yjiL_3</i>	L-galactonate transporter	Carbon metabolism and transport					
3582674	G→T	P180T (CCC→ACC)	<i>fimH</i>	S-fimbrial protein subunit FimH	Cell wall and cell envelope					X
3583070	T→G	T48P (ACC→CCC)	<i>fimH</i>	S-fimbrial protein subunit FimH	Cell wall and cell envelope					X
3589019	+A	IG (-139/+340)	<i>fim</i> ← / ← <i>fimE</i>	Fimbrial subunit type 1 precursor/recombinase FimE	Cell wall and cell envelope / Replication, DNA repear, recombination					X

3589010	319 bp inversion	Intergnic (319 nt)	<i>fim</i> ← / ← <i>fimE</i>	Fimbrial subunit type 1 precursor/recombinase FimE	Cell wall and cell envelope / Replication, DNA repear, recombination					X
3633554	C→T	I110I (ATC→ATT)	<i>lldR_3</i>	Putative L-lactate dehydrogenase operon regulatory protein	Regulator					
3645629	T→C	F603S (TTT→TCT)	<i>ptsI_1</i>	Phosphoenolpyruvate-protein phosphotransferase	Carbon metabolism and transport					
3684369	A→T	E266D (GAA→GAT)	<i>pduF</i>	Propanediol diffusion facilitator	Carbon metabolism and transport					
3684591	T→A	S3T (TCT→ACT)	<i>araC_3</i>	Arabinose operon regulatory protein	Regulator					
3835485	G→A	A86A (GCC→GCT)	<i>norW</i>	Nitric oxide reductase FIRd-NAD(+) reductase	Energetic metabolism					
3914911	T→A	K26* (AAA→TAA)	<i>PROKKA_03778</i> ←	Flavin reductase like domain protein	Energetic metabolism					
4041591	C→A	G146* (GGA→TGA)	<i>recE</i>	Exodeoxyribonuclease 8	Replication, DNA repear, recombination					
4095605	Δ42 bp	coding (881-922/128 7 nt)	<i>uraA</i>	Uracil permease	Nucleotide metabolism					
4139891	C→T	A29A (GCG→GCA)	<i>hemF</i>	Coproporphyrinogen-III oxidase, aerobic	Energetic metabolism					
4189550	A→G	V81A (GTT→GCT)	<i>fadL</i>	Long-chain fatty acid transport protein precursor	Lipids metabolism					
4238854	C→T	P227S (CCA→TCA)	<i>nuoC</i>	NADH-quinone oxidoreductase subunit C/D	Energetic metabolism					
4242163	C→A	P104T (CCG→ACG)	<i>nuoG</i>	NADH-quinone oxidoreductase subunit G	Energetic metabolism					
4246904	G→A	L77L (CTG→CTA)	<i>nuoK</i>	NADH-quinone oxidoreductase subunit K	Energetic metabolism					
4284912	A→T	S425C (AGC→TGC)	<i>rscC</i>	Sensor histidine kinase RcsC	Regulator			X		
4293011	A→G	D20G (GAT→GGT)	<i>ada_2</i>	Bifunctional transcriptional activator/DNA repair enzyme Ada	Regulator					
4382511	C→T	G18D (GGC→GAC)	<i>yafP_2</i>	putative N-acetyltransferase YafP	Replication, DNA repear, recombination					
4390267	C→T	T214M (ACG→ATG)	<i>thiD</i> →	Hydroxymethylpyrimidine/phosphomethylp yrimidine kinase	Vitamins					
4417588	C→T	G558R (GGG→AGG)	<i>mdtC</i>	Multidrug resistance protein MdtC	Antibiotic resistance					
4431396	G→T	T157T (ACG→ACT)	<i>alkA</i>	DNA-3-methyladenine glycosylase 2	Replication, DNA repear, recombination, transcription					
4439693	C→T	IG (+115/-845)	<i>PROKKA_04264</i> → / → <i>PROKKA_04265</i>	PAP2 superfamily protein/hypothetical protein	Conserved protein / Conserved protein			X		
4454944	A→C	S91S (TCA→TCC)	<i>epsI_2</i> →	putative glycosyltransferase EpsI	Cell wall and cell envelope					
4457264	A→G	M346V (ATG→GTG)	<i>wcaJ</i> →	UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase	Cell wall and cell envelope					
4473640	A→T	C157* (TGT→TGA)	<i>PROKKA_04290 (gmIC)</i> ←	Hypothetical glucosyl transferase GtrII family protein	Cell wall and cell envelope					
4498032	T→C	IG (+81/-234)	<i>PROKKA_04313</i> → / → <i>cobS</i>	hypothetical protein/Cobalamin synthase	Conserved protein / Vitamiins					
4561988	C→T	A5V (GCA→GTA)	<i>yedR</i> →	Inner membrane protein YedR	Conserved protein					
4596831	C→T	T234M (ACG→ATG)	<i>otsA</i> →	Alpha,alpha-trehalose-phosphate synthase [UDP-forming]	Carbon metabolism and transport					
4598395	T→A	L12Q (CTG→CAG)	<i>uidB_3</i> →	Glucuronide carrier protein	Carbon metabolism and transport					

4686972	G→A	M1M (ATG→ATA) ⁺	<i>fadR_1</i>	Fatty acid metabolism regulator protein	Regulator					
4746701	G→A	G52R (GGG→AGG)	<i>pkn1_2</i>	Serine/threonine-protein kinase pkn1	Regulator				X	
4746705	A→T	D53V (GAT→GTT)	<i>pkn1_2</i>	Serine/threonine-protein kinase pkn1	Regulator				X	
4795138	G→C	T70R (ACG→AGG)	<i>narL</i>	Nitrate/nitrite response regulator protein NarL	Regulator					
4803773	Δ2 bp	coding (793–794)	<i>narH</i>	Respiratory nitrate reductase 1 beta chain	Energetic metabolism					
5000545	G→A	P148L (CCT→CTT)	<i>gsiD_2</i>	Glutathione transport system permease protein GsiD	Energetic metabolism					
5158597	T→C	IG (+387/+353)	<i>fdnI</i> → / ← <i>PROKKA_04950</i>	Formate dehydrogenase, nitrate-inducible, cytochrome b556(Fdn) subunit/Polyphosphate kinase 2 (PPK2)	Energetic metabolism /Phosphate metabolism					
5211440	A→G	E65G (GAG→GGG)	<i>envZ_2</i>	Similar to osmolarity sensor protein EnvZ	Regulator					
5304269	T→C	F357S (TTT→TCT)	<i>pgtB</i>	Phosphoglycerate transport system sensor protein PgtB	Carbon metabolism and transport					
5318561	T→A	K300N (AAA→AAT)	<i>PROKKA_05103</i>	Alpha/beta hydrolase family protein	Cell wall and cell envelope					
27572 (pBIC-1a)	C→T	V35I (GTA→ATA)	<i>PROKKA_05204</i>	TraX protein	Phage and mobile genetic element					
78017 (pBIC-1a)	+G	intergenic (-202/+57 9)	<i>PROKKA_05260</i> ← / ← <i>PROKKA_05261</i>	hypothetical protein/hypothetical protein	Conserved protein / Conserved protein					X
152672 (pBIC-1a)	Δ1 bp	coding (327/2796 nt)	<i>clpC</i>	ATP-dependent Clp protease ATP-binding subunit ClpC	Phage and mobile genetic element					
4165 (pBIC-1b)	Δ1 bp	coding (315/2754 nt)	<i>virB4</i>	Type IV secretion system protein virB4	Phage and mobile genetic element					
15544 (pBIC-1b)	C→T	IG (+689/-493)	<i>traG</i> → / → <i>yhcR</i>	Conjugal transfer protein TraG/Endonuclease YhcR precursor	Phage and mobile genetic element / Replication, DNA repair, recombination				X	
9622 (pBIC-1c)	A→G	L119S (TTA→TCA)	<i>aac(6)'-Ib</i>	Aminoglycoside N(6')–acetyltransferase type 1	Antibiotic resistance					X

¹Position of the mutation, for deletion, position of the first missing nucleotide; ²IG, Intergenic; ³gene name according to Prokka annotation; ⁴Prokka annotation, ⁵x indicates that the mutation is present

Supplementary Table 3. Effect of the SNP in *aac(6')*-Ib on aminoglycosides susceptibility

Strain	Gentamicin	Susceptibility testing ¹		
		Amikacin	Tobramycin	Netilmicin
S5 (AAC(6')-Ib 119L)	1.5	128	24	>256
S10 (AAC(6')-Ib 119S)	8	32	48	>256
<i>E. coli</i> TOP10 (pBIC-1c-GentaS)	1.5	64	16	64
<i>E. coli</i> TOP10 (pBIC-1c-GentaR)	12	12	12	32
<i>E. coli</i> TOP10	0.25	1.5	0.25	0.125

¹Determined by E-test. Resistant MIC values are highlighted in grey

Supplementary Table 4. Primers used in the study

PCR and primers	Sequence	Reference or source
Conventional PCR		
aac(6')pBIC3 F	CAAATATGTATCCGCTCATG	This work
aac (6')pBIC3 R	CATGAGGGAAGCGGTGATCG	This work
RT-PCR		
rpsL13_F	GCCGTACTTGGAGCGAGCCTG	[2]
rpsL14_R	CCGTGGCGGTCTGTGTTAAAGA	[2]
phoP_F	ATTGAAGAGGTTGCCGCCCCG	[2]
phoP_R	GCTTGATCGGCTGGTCATTCACC	[2]
phoQ_F	ATATGCTGGCGAGATGGGAAAACGG	[2]
phoQ_R	CCAGCCAGGGAACATCACGCT	[2]
arnC F	GAAGGTCTCGGTGGTTATCC	This work
arnC R	CGAGTGCTGGCCGTAGTTAC	This work
fimH_F	ACGGCACCTCTTACCCGTTCC	This work
fimH_R	CCAGATGAACTGGAAGGAGTCGCT	This work
Cloning		
arn-cloning for	GCTTGCATGCCTGCACAACTATTGCGT TCCTGTTCTC	This work
arn-cloning rev	CCTCTAGAGTCGACCATATCATACCCG GCGTCCAGC	This work
phoP-cloning rev	CCTCTAGAGTCGACCAATGACAGCGG GAAGATATGC	This work
phoP-cloning for	GCTTGCATGCCTGCAAATGAAGGAGT TCCATGCAG	This work
gml locus cloning for	GCTTGCATGCCTGCA CGTATTGCGCAGAAAGTGGG	This work
gml locus cloning rev	CCTCTAGAGTCGACC CGCTGATATGTCTGCTGAAG	This work
pUC19-Apra rev	TGCAGGCATGCAAGCTTGCC	This work
pUC19 Apra for	GGTCGACTCTAGAGGATCCC	This work

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