Supplementary Tables

Supplementary Table 1. Strains and plasmids used in this study

	Description	Source
Strains		
R6	Wild type	ATCC BAA 255
R6M2B	R6 clone selected from CIP resistance	This work
T5-R6M2B	R6 clone transform with gDNA from R6M2B selected for CIP resistance	This work
T1-60827	R6 clone transformed with gDNA from 60827 selected for CIP resistance	This work
14599	clinical isolate	Dr. GG Zhanel
14635	clinical isolate	Dr. GG Zhanel
14636	clinical isolate	Dr. GG Zhanel
64933	clinical isolate	Dr. GG Zhanel
45089	clinical isolate	Dr. GG Zhanel
51597	clinical isolate	Dr. GG Zhanel
59774	clinical isolate	Dr. GG Zhanel
60827	clinical isolate	Dr. GG Zhanel
60911	clinical isolate	Dr. GG Zhanel
45693	clinical isolate	Dr. GG Zhanel
60827	clinical isolate	Dr. GG Zhanel
R6 pFF6*0043*	R6 transformed with plasmid pFF6*004*3, KAN^{r}	This work
R6M2B pFF6*patA*	R6 transformed with plasmid pFF6*patA*, KAN^{r}	This work
R6M2B pFF6*patB*	R6 transformed with plasmid pFF6*patB*, KAN^{r}	This work
R6M2B pFF6*0043*	R6M2B transformed with plasmid pFF6*004*3, KAN^{r}	This work
R6M2B pFF6*patA*	R6M2B transformed with plasmid pFF6*patA*, KAN^{r}	This work
R6M2B pFF6*patB*	R6M2B transformed with plasmid pFF6*patB*, KAN^{r}	This work
T5-R6M2B pFF6*0043*	T5-R6M2B transformed with plasmid pFF6*0043*, KAN^{r}	This work
T5-R6M2B pFF6*patA*	T5-R6M2B transformed with plasmid pFF6*patA*, KAN^{r}	This work
T5-R6M2B pFF6*patB*	T5-R6M2B transformed with plasmid pFF6*patB*, KAN^{r}	This work
T1-60827 pFF3*0043*	T1-60827 transformed with plasmid pFF3*0043*,CM^{r}	This work
T1-60827 pFF3*patA*	T1-60827 transformed with plasmid pFF3*patA*, CM^{r}	This work
T1-60827 pFF3*patB*	T1-60827 transformed with plasmid pFF3*patA*, CM^{r}	This work

60827 pFF3*0043*	60827 transformed with plasmid pFF3*0043,* CM^r	This work
60827 pFF3*patA*	60827 transformed with plasmid pFF3*patA*, CM^r	This work
60827 pFF3*patB*	60827 transformed with plasmid pFF3*patB,* CM^r	This work
R6$^{\text{parC-R6M2B}}$	R6 transformed with *parC* PCR fragment from R6M2B	This work
R6$^{\text{spr1902-T5R6M2B,smR}}$	R6 transformed with spr1902 PCR fragment from T5-R6M2B and *rpsL*$^+$ streptomycin resistance marker, SM^r	This work
R6$^{\text{parC, gyrA-R6M2B}}$	R6$^{\text{parC}}$ transformed with *gyrA* PCR fragments from R6M2B	This work
R6$^{\text{parC, gyrA, spr1902-T5R6M2B,smR}}$	R6$^{\text{parC,gyrA-R6M2B}}$ co-transformed with spr1902 PCR fragment from T5-R6M2B and *rpsL*$^+$, SM^r	This work
R6$^{\text{parC, gyrA, spr1544-T5R6M2B,smR}}$	R6$^{\text{parC,gyrA-R6M2B}}$ co-transformed with spr1544 PCR fragment from T5-R6M2B and *rpsL*$^+$, SM^r	This work
R6$^{\text{patB,T1-60827,smR}}$	R6 co-transformed with patB fragment from T1-60827 and *rpsL*$^+$ (contain mutations **T142G;**T357C; T618C;C1260T), SM^r	This work
R6$^{\text{patA,T1-60827, smR}}$	R6 co-transformed with patB fragment from T1-60827 and *rpsL*$^+$ (contain mutations **G335T;** T537C; A540C; C660T) SM^r	This work
T5-R6M2B $^{\text{spr0335R6, smR}}$	T5-R6M2B co-transformed with spr0335 PCR fragment from R6 and *rpsL*$^+$, SM^r	This work
R6M2B $^{\text{spr0129R6, smR}}$	R6M2B co-transformed with spr0129 PCR fragment from R6 and *rpsL*$^+$, SM^r	This work
Plasmids		
pFF3	*S. pneumoniae* non-replicative vector that contains a CM^r resistance marker	Unpublished data
pFF6	pFF3 in which the CM^r resistance marker was replaced with a KM^r resistance marker.	Unpublished data
pFF6*0043*	plasmid pFF6(KAN^r) containing a fragment of spr0043 amplified from R6	This work
pFF6*patA*	pFF6(KAN^r) containing a fragment of *patA* (spr1887) amplified from R6	This work
pFF6*patB*	pFF6(KAN^r) containing a fragment of *patB* (spr1885) amplified from R6	This work
pFF3*0043*	pFF3(CM^r) containing a fragment of *patA* (spr1887) amplified from 60827	This work
pFF3*patA*	plasmid pFF3(CM^r) containing a fragment of *patA* (spr1887) amplified from 60827	This work
pFF3*patB*	Plasmid pFF3(CM^r) containing a fragment of *patB* (spr1885) amplified from 60827	This work

Abbreviations: CIP: Ciprofloxacin; KAN: Kanamycin; CM: Chloramphenicol; SM: streptomycin; CRI; Centre de Recherche en Infectiologie

Supplementary Table 2. Primers used in this study

Primers	**Primer sequences**
Genes inactivations	
*patB*F-KO	CCAACCTCCAGCAGAAAGAG
*patB*R-KO	CCTGACTAGCATCTGGCACA
*patA*F-KO	GGGGTACCGTTGGTTCCATCGCTTCTTT
*patA*R-KO	CGGGATCCATCCTTTGTTTTTGTCCACC
spr0043F-KO	GGGAGATATGACCTTCAAGC
spr0043R-KO	AAACTGCTAGTCGCCTCATC
Mutants reconstruction	
parC-F	TGGGTTGAAGCCGGTTCA
parC-R	CAAGACCGTTGGTTCTTTC
gyrA-F	TTCTCTACGGAATGAATG
gyrA-R	GATATCACGAAGCATTTCCAG
spr0129(L)-F	TGTTGCTTGCGTTTATGGAG
spr0129(L)-R	TGATAGAGAAATTTTTATGA
spr1902-F	ATGGAAAAACAAACCGTCGCCGT
spr1902-R	TTAAGACCACTCATTTTCTGC
spr1902(L)-F	CCATATTGAAGGCCAAGTCCT
spr1902(L)-R	GTTGCAGCTGCCTTTGATATGG
spr0335(L)-F	TGACCGTTTTGGCGCTTGTATC
spr0335(L)-R	ATTTTGTAGCTGACCATTGA
spr1544(L)-F	TTCAGTTCTGTTTTTCAATATCG
spr1544(L)-R	TGGTACGACCTGTAAATTGGT
up*patA*-F	AAACCAAGACTCACTAGTTA
patB-R	TTATTCGAAAACAAATTGATTGTG
qRT-PCR	
RT16sRNA-F	CCTATTGTTAGTTGCCATCATTCAG
RT16srRNA-R	GACTCGTTGTACCAGCCATTGT
RTpatA -R	GGTGGCAAGGATATTCGAGA
RT*patA*-R	AGAATGGCACGTTGGAGAAC
RT*patB*-F	GCCTTTACTGGAGCTGAACG
RT*patB*-R	AGGTTGGAGCCTTTTCAGGT
RTspr0081-F	CCTACTACGAAGCAGCGACAG
RTspr0081-R	TACAAGCGGAGTCAACTGAGG
RTspr1884-F	CGGTGTCCAACTTGTCAATG
RTspr1884-R	GAGTGAGATGTCCCGAAAGG

Abbreviation: F: Forward; R: Reverse;(L): Long fragment; up: upstream; qRT-PCR: quantitative real-time PCR.

Supplementary Table 3. Reversion of the efflux phenotype by reserpine.

Strains	**CIP MIC μg/mL**				
μg/mL reserpine	**0**	**2.5**	**5**	**10**	**20**
R6	0.5	0.5	0.5	0.5	0.5
T1-60827	2	0.5	0.5	0.5	0.5
60827	2	1	1	0.5	0.5
R6M2B	128	64	64	32	32
T5-R6M2B	128	64	64	32	32

Supplementary Table 4: QRDRs mutations, *patA* expression and CIP MIC in *S. pneumoniae* clinical isolates.

	QRDRs mutations		***patA* expression**[a]	**MIC(ug/mL)**[b]	
Strains	***gyrA***	***parC***		**CIP**	**CIP+R**
14599			1.1±0.25	0.5	0.5
14635			1.4±0.3	0.5	0.5
14636			0.734±0.169	0.5	0.5
64933			1.13±0.04	1	1
45089		*K137N*	1.14±0.234	1	1
51597		*S79F*	8.87±1.356	8	2
59774			11.13±2.347	4	0.5
60911			11.09±2.67	4	0.5
45693	*S81F*	*S79F*	6.98±0.03	128	32

[a] As measured by qRT-PCR

[b] CIP(ciprofloxacin); R(reserpine 20ug/mL). MICs have been measured at least in triplicates.

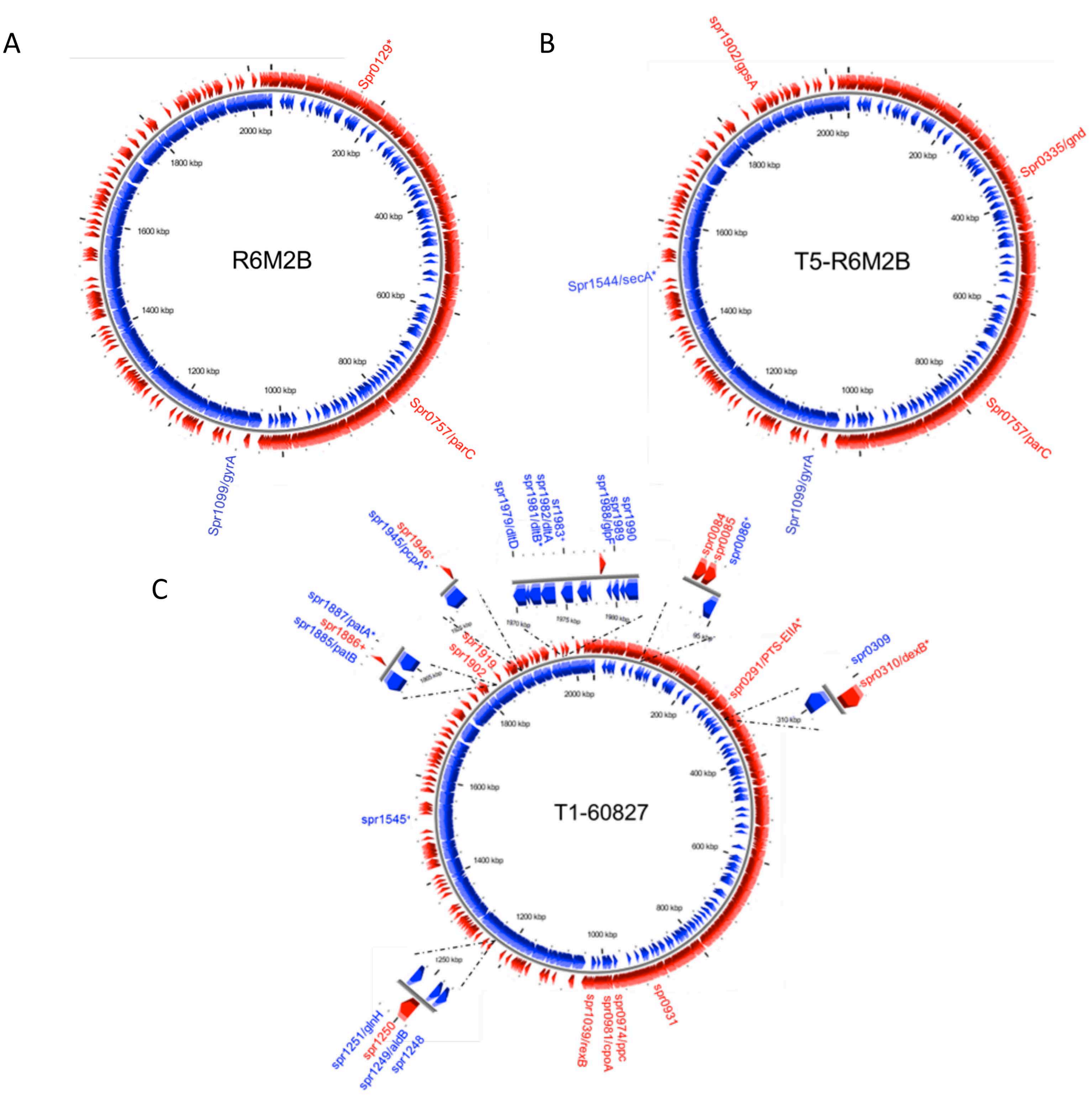
A
Spr0129*
2000 kbp
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
R6M2B
Spr0757/parC
Spr1099/gyrA
B
spr1902/gpsA
Spr0335/gnd
2000 kbp
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
T5-R6M2B
Spr1544/secA*
Spr0757/parC
Spr1099/gyrA
C
spr1946*
spr1945/pcpA*
spr1979/dltD
spr1982/dltA
spr1981/dltB*
sr1983*
spr1988/glpF
spr1989
spr1990
1970 kbp
1975 kbp
1980 kbp
spr0084
spr0085
spr0086*
95 kbp
spr1887/patA*
spr1886+
spr1885/patB
1905 kbp
spr1919
spr1902
spr0291/PTS-EIIA*
spr0309
spr0310/dexB*
310 kbp
2000 kbp
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
T1-60827
spr1545*
1250 kbp
spr1251/glnH
spr1250
spr1249/aldB
spr1248
spr1039/rexB
spr0981/cpoA
spr0974/ppc
spr0931

Figure S1. Schematic representation (in CG view) of the genomic loci mutated in *S. pneumoniae* R6M2B (A), T5-R6M2B (B) and T1-60827 (C). Genes indicated in red are encoded on the forward DNA strand and genes in blue are encoded on the reverse strand. Genes with an asterisk (*) have mutation(s) in the region upstream of the ATG and genes with a plus symbol ($^+$) have mutation(s) downstream of their coding region. Abbreviations: *parC*: DNA topoisomerase IV subunit A, *gyrA*: DNA gyrase subunit A, *gnd*: 6-phosphogluconate dehydrogenase, *secA*: preprotein translocase subunit SecA, *gpsA*: NAD(P)H-dependent glycerol-3-phosphate dehydrogenase, *PTS-EIIA*: PTS system IIA component, *dexB*: glucan 1,6-alpha-glucosidase, *ppc*: phosphoenolpyruvate carboxylase, *cpoA*: glycosyl transferase CpoA, *rexB*: second subunit of major exonuclease, *aldB*: alpha-acetolactate decarboxylase, *glnH*: amino acid ABC transporter amino acid-binding protein, *patB*: ABC transporter ATP-binding protein/permease, *patA*: ABC transporter ATP-binding protein/permease, *pcpA*: choline binding protein PcpA, *dltD*: D-alanine transfer from undecaprenol-phosphate to the poly(glycerophosphate) chain, *dltC*: D-alanine--poly(phosphoribitol) ligase subunit 2, *dltB*: D-alanine transfer from Dcp to undecaprenol-phosphate, *dltA:* D-alanine--poly(phosphoribitol) ligase subunit 1, *glpF*: glycerol uptake facilitator protein.