

Table S1. Clinical Isolates used in this study

Isolate ID	Organism source	Date of Isolation
KP1	Blood Culture	12/04/2016
KP2	Blood Culture	08/03/2016
KP3	Tissue	10/01/2016
KP4	Tissue	07/12/2015
KP5	Blood Culture	05/10/2015
KP6	Blood Culture	25/09/2015
KP7	Blood Culture	01/09/2015
KP8	Ascitic Fluid	23/02/2015
KP9	Tissue/Kidney Stone	05/12/2014
KP10	Blood Culture	27/10/2014
KP11	Screening Swab	10/10/2014
KP12	Blood Culture	01/10/2014
KP13	Groin swab	02/07/2014
KP14	Wound swab	11/06/2014
KP15	Blood Culture	20/05/2014
KP16	Blood Culture	20/04/2014
KP17	Sputum	19/03/2014
KP18	Catheter urine	18/02/2014
KP19	Catheter urine	17/10/2013
KP20	Sputum	01/08/2013
KP21	Sputum	30/07/2013
KP22	Blood Culture	27/07/2013
KP23	Wound swab	23/07/2013
KP24	Mid-stream urine	23/07/2013
KP25	Blood Culture	22/07/2013
KP26	Mid-stream urine	03/07/2013
KP27	Central Line Tip	17/04/2013
KP28	Bronchio-alveolar Lavage (BAL)	11/03/2013
KP30	Blood Culture	07/08/2012
KP31	Blood Culture	06/07/2016
KP32	Tissue	05/07/2016
KP33	Pus	05/07/2016
KP34	Tissue	28/06/2016
KP38	Blood Culture	21/06/2016
KP40	Blood Culture	09/06/2016

KP46	Tissue	22/04/2016
KP47	Blood Culture	22/04/2016
KP48	Peritoneal Fluid	01/04/2016
KP50	Pus	23/03/2016
KP59	Blood Culture	25/02/2016

Table S2. PCR Primers used in this study

Primer	Sequence (5' to 3')	Reference
gyrA - F	AATATGCGCCATCAGCCC	(S1)
gyrA - R	TGCGAGAGAAATTACACC	(S1)
parC - F	ATGAGCGATATGGCAGAGC	This work
parC - R	CCACTTCCCGCAGGTTGT	This work
oqxR - F	TTCCTGACGCCGGTGTTTTA	(S2)
oqxR - R	CTGCGGTGCCAAAAAGAACA	(S2)
ramR - F	CTGCAGTGCCCGGTGAACCCTGGCGT	(S3)
ramR - R	CTGCAGATTTGCTGATTCAGCAGCGAC	(S3)
Qnr synthesis	From: GAGCGCTTGTGTCGCTACAT To: GGTTGCTTCATGGGCAA	As Accession number AY070235.1
AAC-6'-Ib-cr synthesis	From: GAGCAAACGATCAATGCG To: AAGCCTAGCCAATTTGCTAGG	As Accession number JF775514.1

Table S3. Mutations identified in the *oqxR*

Mutant/Isolate	Amino acid position and changes
<i>oqxR1</i>	Tyr109STOP
<i>oqxR2</i>	Leu89FS
<i>oqxR3</i>	Ala37Glu
<i>oqxR4</i>	Ala133Val
<i>oqxR5</i>	Arg93Cys
<i>oqxR6</i>	Glu85STOP
<i>oqxR7</i>	Gln21STOP
<i>oqxR8</i>	Glu116STOP
<i>oqxR9</i>	Asp3Glu
<i>oqxR10</i>	Ala133Pro
<i>oqxR11</i>	Ala37Glu
<i>oqxR12</i>	Gly63 ins SIHLGRNG
KP9	Glu24STOP
KP12	Asp3Tyr
KP20	Ala19Val
KP28	Leu76FS
KP30	Val130Ala

Effects on OqxR sequence of mutations identified in *oqxR* in Ecl8 mutants (alleles *oqxR1* to 12) or in the *K. pneumoniae* clinical isolates (KP9, 12 etc). All these mutations have been shown to result in loss of function, and so OqxAB hyper-production.

Table S4: Disc susceptibility testing according to CLSI protocol (26) for *K. pneumoniae* Ecl8 and mutants determining the effect of acquisition events on Fluoroquinolone susceptibility.

Parental strain ^a , mutation (transformed plasmid)	Susceptibility/Non-Susceptibility to				Mutation/Transformation
	CIP	LEV	NOR	OFL	
Ecl8	S	S	S	S	Wildtype
Ecl8 (pEX-K4)	S	S	S	S	Vector only
Single acquisition events					
<i>gyrA1</i>	S	S	S	S	GyrA:Ser83Phe
<i>gyrA2</i>	S	S	S	S	GyrA Ser83Tyr
<i>gyrA3</i>	S	S	S	S	GyrA Asp87Tyr
<i>oqxR1</i>	S	S	S	S	OqxR:Tyr109STOP
<i>oqxR2</i>	S	S	S	S	OqxR:Leu89FS
<i>oqxR3</i>	S	S	S	S	OqxR:Ala37Glu
<i>oqxR4</i>	S	S	S	S	OqxR:Ala133Val
$\Delta ramR$	S	S	S	S	deleted RamR
<i>ramR</i>	S	S	S	S	RamR:Thr124Pro
(<i>qnrA1</i>)	S	S	S	S	QnrA1
(<i>aac(6')-Ib-cr</i>)	S	S	S	S	Aac(6')-Ib-cr
Double acquisition events					
<i>oqxR1</i> + <i>gyrA2</i>	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr
$\Delta ramR$ + <i>gyrA1</i>	NS	NS	S	NS	deleted RamR + GyrA:Ser83Phe
$\Delta ramR$ + <i>oqxR5</i>	S	S	NS	S	deleted RamR + OqxR:Arg93Cys
(<i>qnrA1</i> + <i>aac(6')-Ib-cr</i>)	S	S	S	S	QnrA1+ Aac(6')-Ib-cr
<i>gyrA1</i> (pEX-K4)	S	S	S	S	GyrA:Ser83Phe Vector only
<i>gyrA1</i> (<i>qnrA1</i>)	S	S	S	S	GyrA:Ser83Phe + QnrA1
<i>gyrA1</i> (<i>aac(6')-Ib-cr</i>)	S	S	S	S	GyrA:Ser83Phe + Aac(6')-Ib-cr

<i>oqxR1</i> (pEX-K4)	S	S	S	S	OqxR:Tyr109STOP Vector only
<i>oqxR1</i> (<i>qnrA1</i>)	NS	NS	NS	NS	OqxR:Tyr109STOP + QnrA1
<i>oqxR1</i> (<i>aac(6')-Ib-cr</i>)	S	S	S	S	OqxR:Tyr109STOP + Aac(6')-Ib-cr
Δ <i>ramR</i> (pEX-K4)	S	S	S	S	deleted RamR Vector only
Δ <i>ramR</i> (<i>qnrA1</i>)	NS	NS	NS	NS	deleted RamR + QnrA1
Δ <i>ramR</i> (<i>aac(6')-Ib-cr</i>)	S	S	S	S	deleted RamR + Aac(6')-Ib-cr

Triple acquisition events

Δ <i>ramR</i> + <i>oqxR5</i> + <i>gyrA1</i>	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe
<i>gyrA1</i> (<i>qnrA1</i> + <i>aac(6')-Ib-cr</i>)	NS	S	NS	S	GyrA:Ser83Phe + QnrA1 + Aac(6')-Ib-cr
<i>oqxR1</i> (<i>qnrA1</i> + <i>aac(6')-Ib-cr</i>)	NS	S	NS	NS	OqxR:Tyr109STOP + QnrA1 + Aac(6')-Ib-cr
Δ <i>ramR</i> (<i>qnrA1</i> + <i>aac(6')-Ib-cr</i>)	NS	S	S	S	deleted RamR + QnrA1+ Aac(6')-Ib-cr
<i>oqxR1</i> + <i>gyrA2</i> (pEX-K4)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr Vector only
<i>oqxR1</i> + <i>gyrA2</i> (<i>qnrA1</i>)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr + QnrA1
<i>oqxR1</i> + <i>gyrA2</i> (<i>aac(6')-Ib-cr</i>)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr + Aac(6')-Ib-cr
Δ <i>ramR</i> + <i>gyrA1</i> (pEX-K4)	NS	NS	S	NS	deleted RamR + GyrA:Ser83Phe Vector only
Δ <i>ramR</i> + <i>gyrA1</i> (<i>qnrA1</i>)	NS	NS	NS	NS	deleted RamR + GyrA:Ser83Phe + QnrA1
Δ <i>ramR</i> + <i>gyrA1</i> (<i>aac(6')-Ib-cr</i>)	NS	S	NS	S	deleted RamR + GyrA:Ser83Phe + Aac(6')-Ib-cr
Δ <i>ramR</i> + <i>oqxR5</i> (pEX-K4)	S	S	S	S	deleted RamR + OqxR:Arg93Cys Vector only
Δ <i>ramR</i> + <i>oqxR5</i> (<i>qnrA1</i>)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + QnrA1
Δ <i>ramR</i> + <i>oqxR5</i> (<i>aac(6')-Ib-cr</i>)	S	S	S	S	deleted RamR + OqxR:Arg93Cys + Aac(6')-Ib-cr

Quadruple acquisition events

<i>oqxR1 + gyrA2</i> (<i>qnrA1 + aac(6')-Ib-cr</i>)	NS	NS	NS	NS	OqxR:Tyr109STOP + GyrA:Ser83Tyr + QnrA1 + Aac(6')-Ib-cr
Δ<i>ramR + gyrA1</i> (<i>qnrA1 + aac(6')-Ib-cr</i>)	NS	NS	NS	NS	deleted RamR + GyrA:Ser83Phe + QnrA1 + Aac(6')-Ib-cr
Δ<i>ramR + oqxR5 +</i> (<i>qnrA1 + aac(6')-Ib-cr</i>)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + QnrA1 + Aac(6')-Ib-cr
Δ<i>ramR + oqxR5 + gyrA1</i> (pEXK4)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe Vector only
Δ<i>ramR + oqxR5 + gyrA1</i> (<i>qnrA1</i>)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe + QnrA1
Δ<i>ramR + oqxR5 + gyrA1</i> (<i>aac(6')-Ib-cr</i>)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe + Aac(6')-Ib-cr

Quintuple acquisition event

Δ<i>ramR + oqxR5 + gyrA1</i> (<i>qnrA1 + aac(6')-Ib-cr</i>)	NS	NS	NS	NS	deleted RamR + OqxR:Arg93Cys + GyrA:Ser83Phe + QnrA1 + Aac(6')-Ib-cr
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^a *K. pneumoniae* Ecl8 is the parental strain in all cases.

^b CIP, ciprofloxacin; LEV, levofloxacin; NOR, norfloxacin; OFL, ofloxacin

Zone diameter values for definition of susceptible (S) /non-susceptible (NS) designation were the means of three repetitions rounded to the nearest integer. Definitions were made by reference to susceptibility breakpoints set by the CLSI (29).

Table S5: Initial predictive rules and “reduced permeability correction” for ciprofloxacin susceptibility in *K. pneumoniae*

Rule number	Mutations identified in proteins			Presence of PMQR		Phenotype	“RP” Phenotype
	GyrA QRDR	OqxR	RamR	Qnr	Aac(6')-Ib-cr	(MIC [mg.mL ⁻¹])	
1	+					S (0.25)	S (1)
2		+				S (0.25)	S (1)
3			+			S (0.125)	S (0.5)
4				+		S (0.063)	S (0.25)
5					+	S (0.016)	S (0.063)
6	+	+				NS	NS
7	+		+			NS	NS
8	+			+		S (1)	NS (4)
9	+				+	S (0.5)	NS (2)
10		+	+			S (0.25)	S (1)
11		+		+		NS	NS
12		+			+	S (0.5)	NS (2)
13			+	+		NS	NS
14			+		+	S (0.063)	S (0.25)
15				+	+	S (0.5)	NS (2)
16	+	+	+			NS	NS
17	+	+		+		NS	NS
18	+	+			+	NS	NS

19	+		+	+		NS	NS
20	+		+		+	NS	NS
21	+			+	+	NS	NS
22		+	+	+		NS	Ns
23		+	+		+	S (1)	NS (4)
24		+		+	+	NS	NS
25			+	+	+	NS	NS
26	+	+	+	+		NS	NS
27	+	+	+		+	NS	NS
28	+	+		+	+	NS	NS
29	+		+	+	+	NS	NS
30		+	+	+	+	NS	NS
31	+	+	+	+	+	NS	NS

+, mutation identified or PMQR present, blank; no mutation or PMQR absent, NS (Non-susceptible) and shaded blue. S; susceptible and not shaded as defined in Table S4

“RP” (Reduced Permeability) rules to be applied when the OmpF:OmpC ratio is <0.5.

MICs were determined when isolates were designated susceptible, RP correction is 4-fold, as described in the text. Susceptibility breakpoints applied for these isolates are MIC breakpoints defined by CLSI (29).

Table S6: Final predictive rules for ciprofloxacin susceptibility in *K. pneumoniae* clinical isolates.

Rule number	Mutations identified in proteins				Presence of PMQR		Phenotype (MIC [mg.mL ⁻¹])
	1st GyrA QRDR	2 nd QRDR (ParC or GyrA)	OqxR	RamR	Qnr	Aac(6')-Ib-cr	
1RP	+						S (1)
2RP			+				S (1)
3RP				+			S (0.5)
4RP					+		S (0.25)
5RP						+	S (0.063)
6RP	+		+				NS
7RP	+			+			NS
8RP	+				+		NS (4)
9RP	+					+	NS (2)
10RP			+	+			S (1)
11RP			+		+		NS
12RP			+			+	NS (2)
13RP				+	+		NS
14RP				+		+	S (0.25)
15RP					+	+	NS (2)
16RP	+		+	+			NS
17RP	+		+		+		NS

18RP	+		+			+	NS
19RP	+			+	+		NS
20RP	+			+		+	NS
21RP	+				+	+	NS
22RP			+	+	+		Ns
23RP			+	+		+	NS (4)
24RP			+		+	+	NS
25RP				+	+	+	NS
26RP	+		+	+	+		NS
27RP	+		+	+		+	NS
28RP	+		+		+	+	NS
29RP	+			+	+	+	NS
30RP			+	+	+	+	NS
31RP	+		+	+	+	+	NS
32RP	+	+					NS (4)
33RP	+	+	+				NS
34RP	+	+		+			NS
35RP	+	+			+		NS
36RP	+	+				+	NS
37RP	+	+	+	+			NS
38RP	+	+	+		+		NS
39RP	+	+	+			+	NS
40RP	+	+		+	+		NS

41RP	+	+		+		+	NS
42RP	+	+			+	+	NS
43RP	+	+	+	+	+		NS
44RP	+	+	+	+		+	NS
45RP	+	+	+		+	+	NS
46RP	+	+		+	+	+	NS
47RP	+	+	+	+	+	+	NS

+, mutation identified or PMQR present, blank; no mutation or PMQR absent,

NS (Non-susceptible) and shaded blue. S; susceptible and not shaded

Supplementary References

- S1. Fu Y, Zhang W, Wang H, Zhao S, Chen Y, Meng F, Zhang Y, Xu H, Chen X, Zhang F. 2013. Specific patterns of gyr A mutations determine the resistance difference to ciprofloxacin and levofloxacin in *Klebsiella pneumoniae* and *Escherichia coli*. *BMC Infect Dis* 13:8.
- S2. Bialek-Davenet S, Lavigne J-P, Guyot K, Mayer N, Tournebize R, Brisse S, Leflon-Guibout V, Nicolas-Chanoine M-H. 2015. Differential contribution of AcrAB and OqxAB efflux pumps to multidrug resistance and virulence in *Klebsiella pneumoniae*. *J Antimicrob Chemother* 70:81–8.
- S3. Hentschke M, Wolters M, Sobottka I, Rohde H, Aepfelbacher M. 2010. ramR mutations in clinical isolates of *Klebsiella pneumoniae* with reduced susceptibility to tigecycline. *Antimicrob Agents Chemother* 54:2720–3.