Table S1. List of primers used in this study

Primer use and target region	Primer	Sequence (5’-3’)
Sequencing		
ramR	ramR-F	CTGCAGTGCCCGGTGAACCCTGGCGT
	ramR-R	CTGCAGATTTGCTGATTCAGCAGCGAC
acrR	acrR-F	ACGTAACCTCTGTAAAGTCAT
	acrR-R	TTAAGCTGACAAGCTCTCCG
oqxR	oqxR-F	TTCCTGACGCCGGTGTTTTA
	oqxR-R	CTGCGGTGCCAAAAAGAACA
rpsJ	S10-FW	TTAACCCAGGCTGATCTGCACG
	S10-RV	ATGCAGAACCAAAGAATCCGTAT
qRT-PCR		
acrA	acrA-F	ATGTGACGATAAACCGGCTC
	acrA-R	CTGGCAGTTCGGTGGTTATT
oqxA	oqxAB-F	AAGGTGCTGGTGAAGTCGAT
	oqxAB-R	GGAGACGAGGTTGGTATGGA
ramA	ramA-F	GCATCAACCGCTGCGTATT
	ramA-R	CGTTGCAGATGCCATTTCG
rarA	rarA-F	TGGATCGACAACCATCTTGA
	rarA-R	AAGGACTGCTGGGAGTCAAA
kpgA	kpgA-F	CGACCTCTTTCTCAAGGCGA
	kpgA-R	GCCTGTTTGATCATCGTGGC
rrsE	rrsE-F	TTGACGTTACCCGCAGAAGAA
	rrsE-R	GCTTGCACCCTCCGTATTACC

Table S2. Expression of *acrA*, *ramA*, *oqxA*, *rarA* and *kpgA*, and mutations in *ramR* and *oqxR* in 43 tigecycline non-susceptible *K. pneumoniae* isolates.

Isolate	MIC[b]	Relative expression[a]					Mutation	
	(mg/L)	*acrA*	*ramA*	*oqxA*	*rarA*	*kpgA*	*ramR*	*oqxR*
Clinical isolates with higher *acrA* expression but not of *rarA* and *oqxA*								
TNSKP1	3	2.9 ± 0.9	1.1 ± 0.2	2.2 ± 1.2	1.1 ± 0.4	75 ± 10	Silent mutation Missense mutation	Silent mutation
TNSKP2	3	2.2 ± 1.0	1.8 ± 0.5	3.2 ± 0.5	1.2 ± 0.3	72 ± 2.2	Missense mutation	Silent mutation
TNSKP3	3	2.2 ± 0.3	1.2 ± 0.2	1.6 ± 0.6	1.4 ± 0.5	1.3 ± 0.1	Silent mutation Missense mutation	Silent mutation Missense mutation
TNSKP4	4	4.7 ± 0.1	0.9 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	>800	Missense mutation	Silent mutation
TNSKP5	3	2.3 ± 1.0	7.7 ± 0.8	3.6 ± 0.7	1.2 ± 0.3	1.1 ± 0.1	Missense mutation	Silent mutation
TNSKP6	3	2.7 ± 0.7	3.7 ± 1.0	2.2 ± 0.9	1.3 ± 0.0	>800	Missense mutation	Silent mutation
TNSKP7	3	4.5 ± 1.3	5.3 ± 1.8	1.3 ± 0.1	0.7 ± 0.1	>800	Nonsense mutation	Silent mutation
TNSKP8	3	6.4 ± 1.6	2.4 ± 0.4	1.0 ± 0.5	0.4 ± 0.1	205 ± 33	Missense mutation	Silent mutation
TNSKP9	3	3.9 ± 0.0	6.2 ± 0.2	2.3 ± 0.1	1.1 ± 0.0	48 ± 7.9	Silent mutation Missense mutation	Silent mutation
TNSKP10	3	3.3 ± 0.1	5.4 ± 0.2	2.6 ± 0.5	1.7 ± 0.2	56 ± 6.6	Missense mutation	Silent mutation
TNSKP11	3	3.4 ± 0.3	4.1 ± 0.1	1.8 ± 0.3	1.2 ± 0.2	25 ± 3.7	Missense mutation	Silent mutation
TNSKP12	3	2.3 ± 0.4	2.6 ± 1.0	2.3 ± 0.3	1.5 ± 0.3	32 ± 12	Missense mutation	Silent mutation
TNSKP13	3	2.5 ± 0.3	4.1 ± 0.2	1.9 ± 0.8	1.0 ± 0.4	1.5 ± 0.7	Missense mutation	Silent mutation
TNSKP14	3	2.3 ± 0.7	4.3 ± 1.0	1.3 ± 0.3	0.8 ± 0.2	>800	Missense mutation	Silent mutation

TNSKP15	4	3.0 ± 1.1	3.5 ± 1.4	2.9 ± 1.5	1.1 ± 0.2	95 ± 21	Missense mutation	Silent mutation
TNSKP16	4	6.4 ± 2.5	5.3 ± 0.3	3.5 ± 1.0	1.8 ± 0.6	>800	Nonsense mutation	Silent mutation
TNSKP17	4	3.4 ± 1.1	4.0 ± 0.4	2.5 ± 0.9	1.8 ± 0.0	>800	Nonsense mutation	Silent mutation
TNSKP18	4	4.2 ± 0.4	8.5 ± 0.5	2.8 ± 0.3	1.4 ± 0.2	1.0 ± 0.1	Frameshift mutation	Silent mutation
TNSKP19	4	2.0 ± 0.1	3.8 ± 0.2	1.4 ± 0.4	0.8 ± 0.2	1.9 ± 0.6	Missense mutation	Silent mutation
TNSKP20	4	2.2 ± 0.3	3.7 ± 0.9	1.8 ± 0.4	1.2 ± 0.3	660 ± 153	Missense mutation	Silent mutation
TNSKP21	4	5.5 ± 0.2	38.4 ± 1.2	0.5 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	Nonsense mutation	Silent mutation
TNSKP22	6	3.7 ± 0.4	6.1 ± 2.3	1.8± 0.6	1.4 ± 0.3	753 ± 111	Nonsense mutation	Silent mutation
TNSKP23	8	4.7 ± 0.1	5.9 ± 0.0	2.2 ± 0.1	0.4 ± 0.0	649 ± 60	Frameshift mutation Missense mutation	Silent mutation
Clinical isolates with higher *oqxA* expression but not of *ramA* and *acrA*								
TNSKP24	3	1.7 ± 0.2	1.0 ± 0.0	36 ± 10.4	33 ± 11.3	>800	Silent mutation	Silent mutation Missense mutation
TNSKP25	3	1.8 ± 0.2	0.9 ± 0.2	99 ± 8.4	19.4 ± 1.3	1.3 ± 0.1	Silent mutation Missense mutation	Silent mutation Missense mutation
TNSKP26	3	1.9 ± 0.2	0.5 ± 0.0	32.5 ± 4.2	26.4 ± 2.8	3.0 ± 0.0	Silent mutation	Missense mutation
TNSKP27	3	1.7 ± 0.1	0.9 ± 0.0	102 ± 5.4	417 ± 19	1.2 ± 0.2	Silent mutation	Missense mutation
TNSKP28	4	1.6 ± 0.5	0.0 ± 0.0	33.4 ± 8.3	16.3 ± 5.8	0.7 ± 0.3	Silent mutation	Missense mutation Nonsense mutation
Clinical isolates with higher *acrA* and *oqxA* expression								
TNSKP29	3	6.8 ± 0.5	7.6 ± 0.4	5.6 ± 1.7	2.6 ± 0.4	88 ± 23	Missense mutation	Silent mutation
TNSKP30	3	9.6 ± 0.1	11.8 ± 0.3	173 ± 10	148 ± 0.8	1.2 ± 0.7	Missense mutation	Silent mutation

								Missense mutation
TNSKP31	3	4.2 ± 0.2	7.6 ± 0.2	8.1 ± 0.9	4.3 ± 0.8	1.0 ± 0.4	Missense mutation	Silent mutation Missense mutation
TNSKP32	3	2.1 ± 0.1	1.1 ± 0.1	27 ± 3.0	8.4 ± 0.8	0.7 ± 0.2	Silent mutation Missense mutation	Silent mutation Missense mutation
TNSKP33	4	2.3 ± 0.6	1.4 ± 0.3	138.0 ± 18.1	520.0 ± 118.9	0.2 ± 0.0	Silent mutation	Silent mutation Missense mutation
TNSKP34	4	4.4 ± 1.4	12.3 ± 4.4	10.6 ± 1.5	4.6 ± 1.3	497 ± 153	Nonsense mutation	Silent mutation
TNSKP35	4	6.2 ± 1.2	27.2 ± 2.3	4.5 ± 0.7	0.6 ± 0.1	53.4 ± 7.8	Nonsense mutation	Silent mutation
TNSKP36	6	3.3 ± 1.7	3.3 ± 1.5	4.4 ± 0.1	1.7 ± 0.4	106 ± 44	Missense mutation	Silent mutation
TNSKP37	6	5.0 ± 1.9	8.6 ± 0.7	9.5 ± 0.4	3.1 ± 0.5	574 ± 107	Silent mutation Missense mutation	Silent mutation
TNSKP38	6	5.5 ± 0.3	8.6 ± 0.0	5.1 ± 1.5	2.4 ± 0.5	15 ± 0.7	Missense mutation	Silent mutation
TNSKP39	8	4.2 ± 1.5	9.8 ± 1.1	4.1 ± 0.1	3.0 ± 0.4	>800	Frameshift mutation	Silent mutation
TNSKP40	8	2.7 ± 1.2	15 ± 2.6	6.7 ± 1.6	3.1 ±1.5	0.5 ± 0.1	Frameshift mutation	Missense mutation
TNSKP41	8	2.8 ± 0.1	1.2 ± 0.1	14 ± 1.1	7.6 ± 0.5	622 ± 56	Nonsense mutation	Silent mutation Missense mutation
Clinical isolates with higher *kpgA* expression with baseline expression of *acrA*, *ramA*, *rarA*, and *oqxA*								
TNSKP42	4	1.0 ± 0.3	1.1 ± 0.0	2.0 ± 0.4	1.2 ± 0.2	>800	Silent mutation Missense mutation	Silent mutation Missense mutation
Clinical isolates with baseline expression of *acrA*, *ramA*, *rarA*, *oqxA*, and *kpgA*								
TNSKP43	3	0.9 ± 0.2	0.8 ± 0.2	1.2 ± 1.1	0.4 ± 0.1	1.2 ± 0.1	Nonsense mutation	Silent mutation

6 [a] Relative expression compared with KP478 (expression = 1). Results are means of 3 runs ± standard deviation.

[b] MIC of tigecycline.