Supplementary table 1. Primer pairs used for PCR amplification for confirmation of mutations in the gene of the S10 ribosomal protein, in *ramR* and for the identification of variants of the 16S rDNA gene in each of the 8 copies of rDNA clusters

Target	Primer pair	Nucleotide primer sequence	Primer position in CP003200 sequence	Amplicon size (bp)
1-16S	16S KPN RV	5'-TTGACGTCATCCCCACCTTC-3'	15687-17374	-
	1-PTR FW	5'-GCTGTGCGCTTAAAGACAT-3'		1688
2-16S	2-GltRac FW	5'-TATGGCTTCTCGACGCTGG-3'	120212-121819	1626
3-16S	3-PhotOX FW	5'-CACAAATGGCGCGCAAATAG-3'	213669-212107	1601
4-16S	4-BPur FW	5'-TTGTTGCATGGTATTAAATCCCC-3'	256988-258817	1830
5-16S	5-Hyp FW	5'-GGGCCAGTTAATGTTGAGTTG-3'	626645-628458	1811
6-16S	6-DD17 FW	5'-GAAAAAGCGGCGGATTGGG-3'	1001674-1003306	1633
7-16S	7-PDCh FW	5'-ATCGAGCTGGTAGTAAAAGACG-3'	4034886-4033224	1703
8-16S	8-Exap FW	5'-GGTGAAAATTGAGCAAATCGTG-3'	4846754-4845201	1594
rpsJ	S10 RV	5'-ATGCAGAACCAAAGAATCCGTAT-3'	4870582-4870893	312
	S10 FW	5'-TTAACCCAGGCTGATCTGCACG-3'		
ramR	RamR RV	5'-GGTAGGTCAGGGCGATAC-3'	1437547-1438219	659/673/1978[a]
	RamR FW	5'-AGATCGGCGGTTTGTTTAAA-3'		

[a]: amplicon size changes according to deletions of 13 nucleotides or insertion of ISKpn18 element in the *ramR* gene

PCR conditions were as follows: PCR for *rpsJ* and *ramR* genes were performed with an initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C 45 sec, annealing at 59°C 15 sec, and extension at 72°C 30 sec (*rpsJ*) or 120 sec (*ramR*) and a final extension of 5 min at 75°C, using the Bioline MyFi Taq Polymerase (Bioline USA Inc). 16S PCRs amplifications were performed using the same 16S KPN RV primer in combination with the different FW primers, with an initial denaturation at 95°C for 1 min followed by 30 cycles of denaturation at 98°C 10 sec, annealing-elongation at 57°C for 120 sec, using the Ranger DNA polymerase (Bioline USA Inc).

Supplementary table 2. Differences identified in predicted coding sequences of genomes of the tigecycline intermediate and resistant *Klebsiella pneumoniae* strains, by comparison with the genome of the susceptible strain

predicted CDS function	**Contig_KP3-S (nt position)**	**KP3-S**	**KP1-I**	**KP2-R**	**KP4-R**	**KP5-R**
putative fimbrial biogenesis, outer membrane usher protein	Contig 12 (81247-78731)	conserved	conserved	conserved	conserved	Thr787→Pro787
putative protoheme IX biogenesis protein	Contig 23 (49594-50820)	conserved	conserved	conserved	conserved	Lys292→Asn292
FimH fimbrial protein	Contig 1 (100850-101794)	conserved	conserved	conserved	conserved	Thr97→Pro97
putative fumarate lyase	Contig 13 (139934-141295)	conserved	conserved	conserved	Pro205→Ser205	conserved
3-(2,3-dihydroxyphenyl) propionate dioxygenase	Contig 5 (150362-149679)	Frameshift at Phe58	conserved	conserved	conserved	conserved
translation elongation factor Tu	Contig 29 (35967-34747)	conserved	Depletion by IS	Depletion by IS	conserved	conserved
hypothetical prophage protein	Contig 7 (61919-63469)	conserved	conserved	conserved	conserved	Depletion by stop codon at Ser281
hypothetical protein	Contig 21 (35237-34776)	conserved	conserved	conserved	Pro88→Gln88	conserved
hypothetical protein	Contig 2 (308119-307970)	conserved	conserved	conserved	Asn5→Ser5	conserved
hypothetical protein	Contig 52 (5065-5979)	conserved	conserved	conserved	conserved	Ile59→Lys59

Supplementary Figure 1

Tigecycline linked to 16S rRNA (pink) via a coordinated Mg2+ ion (yellow ball). The Lys-55 residue in *Thermus thermophilus* S10 is the equivalent of the Val-57 in the *K. pneumoniae* S10, which was mutated in the KP4-R strain. This residue maps in the vertex of a loop that is at 8 Å from the tigecycline binding site

(mmdb_4G5T; 5)