Table S1. Primers used in this study.

Primers for *acrAB* gene deletion (5'-3')	
acrAB-kan-F	TTAACTTTTGACCATTGACCAATTTGAAATCGGACACTCGAGGTT TACATGTGTAGGCTGGAGCTGCTTC
acrAB-kan-R	GTTATGCATAAAAAAGGCCGCTTGCGCGGCCTTAGTGATTACACGT TGTACATATGAATATCCTCCTTAG
Primers for *mlaA* gene deletion and complementation (5'-3')	
mlaA-kan-F	CTTAATAAGAAAAAAGGTGAGTTTTGCGACTCACCTTTTTTGTTT GTTTCGTGTAGGCTGGAGCTGCTTC
mlaA-kan-R	CCGTCATGGATGGCAAACTGCATAAGCCATAAAAAAACAGGGAGA CATTTCATATGAATATCCTCCTTAG
mlaA-com-F	GCGGGAGTTACTCTGAAAATGTAG
mlaA-com-R	ATCGTTTGTGGTTAAGTGCTGG

Table S2. MICs of tigecycline-resistant mutants and parental strains to different antibiotics.

Strains	MIC (μg/mL) [a]												
	CTX[C]	FEP[C]	ATM[C]	IPM[C]	MEM[C]	CIP[c]	LVX[c]	CST[c]	AMK[c]	CHL[c]	TET[c]	MIN[c]	TGC[b]
ATCC 25922	0.19	0.125	0.125	0.5	0.094	0.012	0.032	0.25	3	3	1.5	0.75	0.125
25922-TGC8	0.25	0.19	0.125	1	0.125	0.016	0.047	0.25	3	6	8	8	8
25922Δ*acrAB*	0.047	0.064	0.125	0.38	0.094	0.012	0.008	0.125	2	1	1	0.38	0.0625
25922Δ*acrAB*-TGC8	0.5	0.5	0.38	0.75	0.19	0.016	0.016	0.38	3	12	12	12	8

[a]Abbreviations: CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; CST, colistin; AMK, amikacin; CHL, chloramphenicol; TET, tetracycline; MIN, minocycline; TGC, tigecycline;

[b]Tested by standard broth microdilution tests;

[c]Tested by Etest method.

Table S3. Strains used in this study.

Strains	Description	References
25922	Wild-type	ATCC 25922
25922Δ*mlaA*	ATCC 25922 deletion of *mlaA*	This study
25922Δ*mlaA*/pCR®2.1	ATCC 25922 deletion of *mlaA*, complemented with empty pCR®2.1 vector	This study
25922Δ*mlaA*/pCR®2.1-*mlaA*	ATCC 25922 deletion of *mlaA*, complemented with wild-type *mlaA*	This study
25922Δ*mlaA*/pCR®2.1-*mlaA*+	ATCC 25922 deletion of *mlaA*, complemented with mutational-type *mlaA*	This study
25922Δ*acrAB*	ATCC 25922 deletion of *acrAB*	This study
25922Δ*acrAB*Δ*mlaA*	ATCC 25922 deletion of *acrAB* and *mlaA*	This study
25922Δ*acrAB*Δ*mlaA*/pCR®2.1	ATCC 25922 deletion of *acrAB* and *mlaA*, complemented with empty pCR®2.1 vector	This study
25922Δ*acrAB*Δ*mlaA*/pCR®2.1-*mlaA*	ATCC 25922 deletion of *acrAB* and *mlaA*, complemented with wild-type *mlaA*	This study
25922Δ*acrAB*Δ*mlaA*/pCR®2.1-*mlaA*+	ATCC 25922 deletion of *acrAB* and *mlaA*, complemented with mutational-type *mlaA*	This study

Table S4. Series of tigecycline MICs change during the gene deletion and complementation experiment.

Strain	Tigecycline MIC (μg/mL)
25922	0.125
25922Δ*mlaA*	0.125
25922Δ*mlaA*/pCR®2.1	0.125
25922Δ*mlaA*/pCR®2.1-*mlaA*	0.125
25922Δ*mlaA*/pCR®2.1-*mlaA*+	1
25922Δ*acrAB*	0.0625
25922Δ*acrAB*Δ*mlaA*	0.0625
25922Δ*acrAB*Δ*mlaA*/pCR®2.1	0.0625
25922Δ*acrAB*Δ*mlaA*/pCR®2.1-*mlaA*	0.0625
25922Δ*acrAB*Δ*mlaA*/pCR®2.1-*mlaA*+	0.5

Table S5. The existence of three mutated loci (*mlaA*, *marR* and *rpsJ*) in the series isolates that stored in each step of the induction experiment of 25922-TGC8 (tigecycline MIC from 0.25 μg/mL to 8 μg/mL).

Reference position	Gene mutation	Occurrence of mutations[a]			
		0.25 μg/mL	1 μg/mL	4 μg/mL	8 μg/mL
286573	*mlaA* (Deletion43-44NF)	NA	+	+	+
3541486	*marR* (G104D)	NA	NA	+	+
1300420	*rpsJ* (G57L)	NA	NA	NA	+

[a] This column lists the concentration of tigecycline at which the mutations occurred. NA, mutation not found; +, mutation has occurred.