

Supplementary material

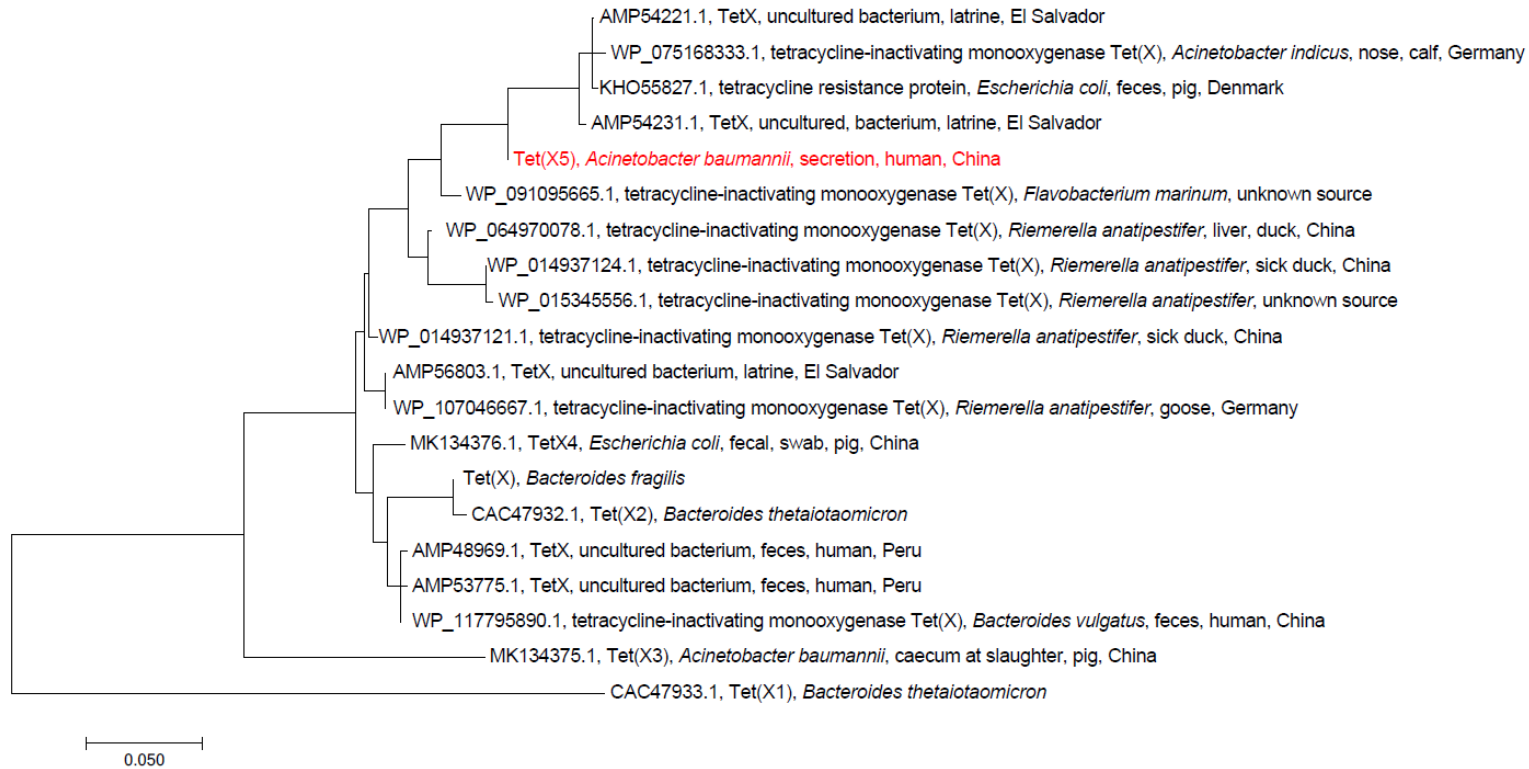


FIG S1 Phylogenetic analysis of the Tet(X5) protein sequence. Phylogenetic tree of the deduced amino acid sequences of putative phosphoethanolamine transferases from different bacterial species. The tree was generated using MEGA 7 by Maximum likelihood method.

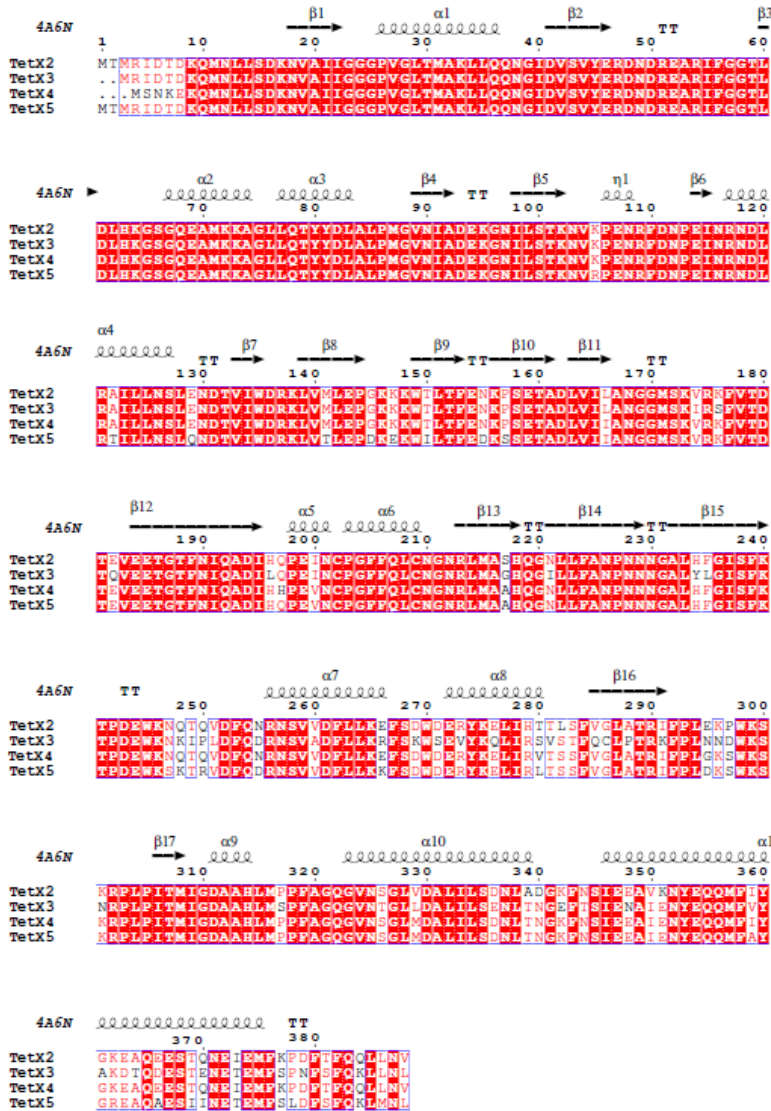


FIG S2 BLAST alignments of amino acid sequences of four *tet(X)* variants using Clustal 2.1 and ESPrict 3.0 (<https://www.genome.jp/tools-bin/clustalw> and <http://esprict.ibcp.fr/ESPrict/cgi-bin/ESPrict.cgi>). Original Tet(X2)-tigecycline complex (PDB ID: 4A6N) served as the reference for secondary structure depiction.

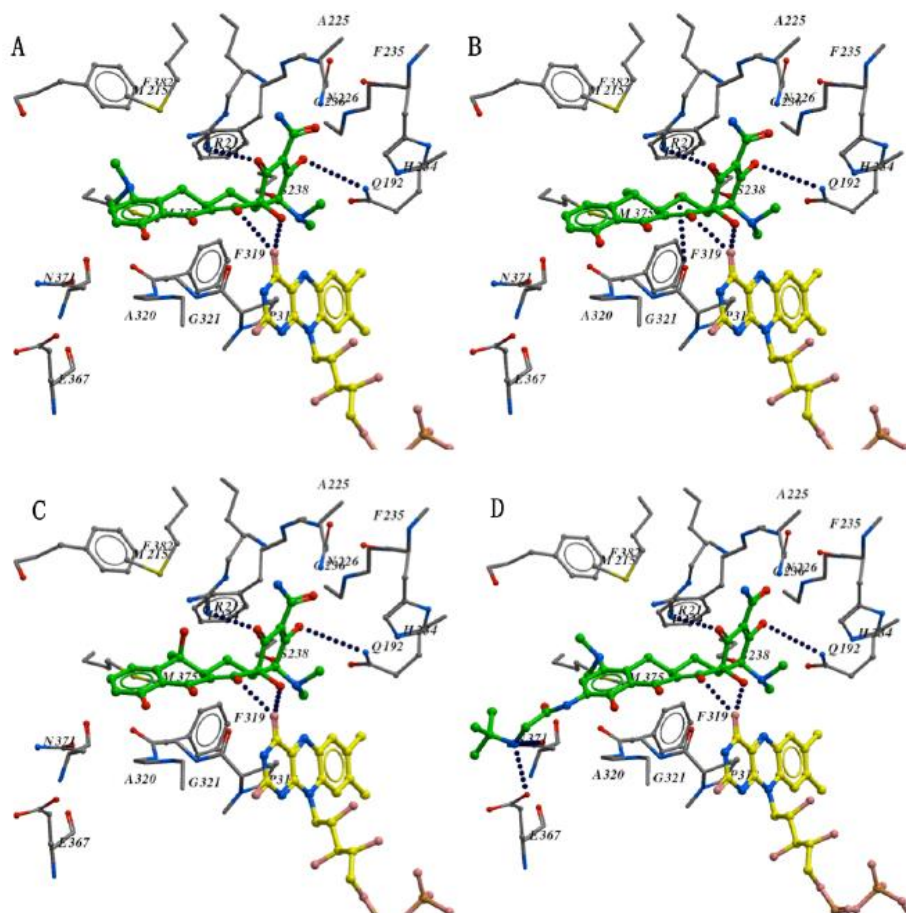


FIG S3 Predicted binding conformations of minocycline (A), doxycycline (B), tetracycline (C) and tigecycline (D) in the substrate-binding site of the modelled Tet(X5) structure. The binding conformations suggest that the tetracycline core has an essentially identical interaction with Tet(X5). Hydrogen bonds are depicted by blue dotted lines.

TABLE S1 Minimum inhibitory concentration (MIC) values of various antimicrobial agents against *Acinetobacter baumannii* AB17H194

	MIC (mg/L) of various antimicrobials
	AB17H194
Tetracycline	256
Doxycycline	32
Minocycline	8
Tigecycline	32
Eravacycline	32
Omadacycline	32
Piperacillin	64
Ceftazidime	16
Cefepime	32
Aztreonam	0.5
Imipenem	0.5
Meropenem	1
Gentamycin	>256
Amikacin	>256
Ciprofloxacin	64
Levofloxacin	32
Colistin	0.6

TABLE S2 Predicted binding affinity of tetracyclines to TetX5.

	Doxycycline	Minocycline	Tetracycline	Tigecycline
Tet(X5)	-6.9	-7.3	-6.5	-8.7