**Supplemental Data**

TITLE: Joint Effects of Antibiotics and Quorum Sensing Inhibitors On Resistance Development in Bacteria

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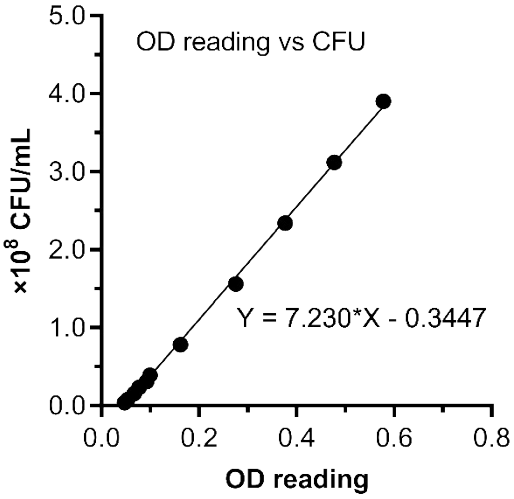



Fig. S1 Linear relationship between the optical density (OD) readings and CFU of *E. coli* in the present study. OD reading s were recorded a Multiskan$^{TM}$ FC Microplate Photometer (ThermoFisher Scientific, USA), while CFU was determined by serially diluting the bacterial suspensions in LB medium and plating them on LB agar plates.


Fig. S2 Comparisons between DNA sequence of reference (1057 bp of β subunit in RpoB) and samples. The red letters and figures mean the base changes and mutation sites, respectively. REF: reference, TET: tetracycline, CHL: chloramphenicol, CIN: cinnamaldehyde, NPO: 4-Nitropyridine-N-oxide.

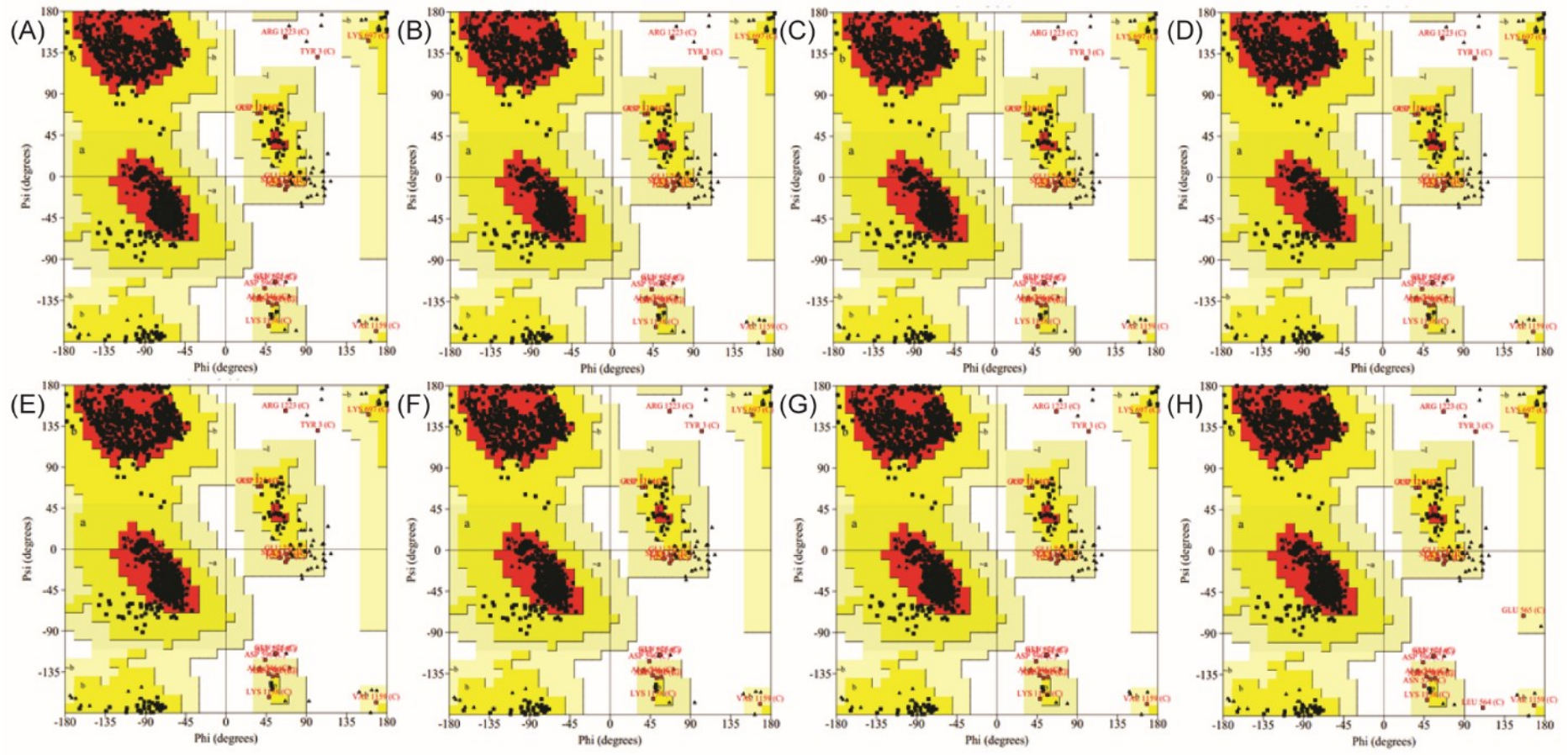



Fig. S3. Ramachandran plots of predicted RpoB mutants. (A) Wild type, (B) Q513E, (C) Q513L & D516G, (D) R529G, (E) S531F, (F) S531Y, (G) T563P (H) P564L

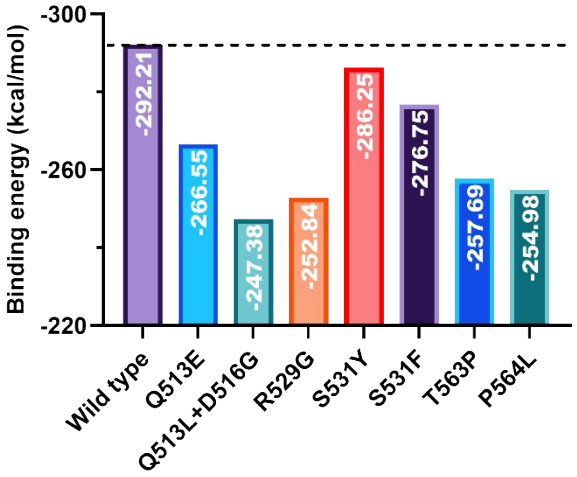



Fig. S4. Binding energies between the rifampicin molecule and the wild type and mutated RpoB.

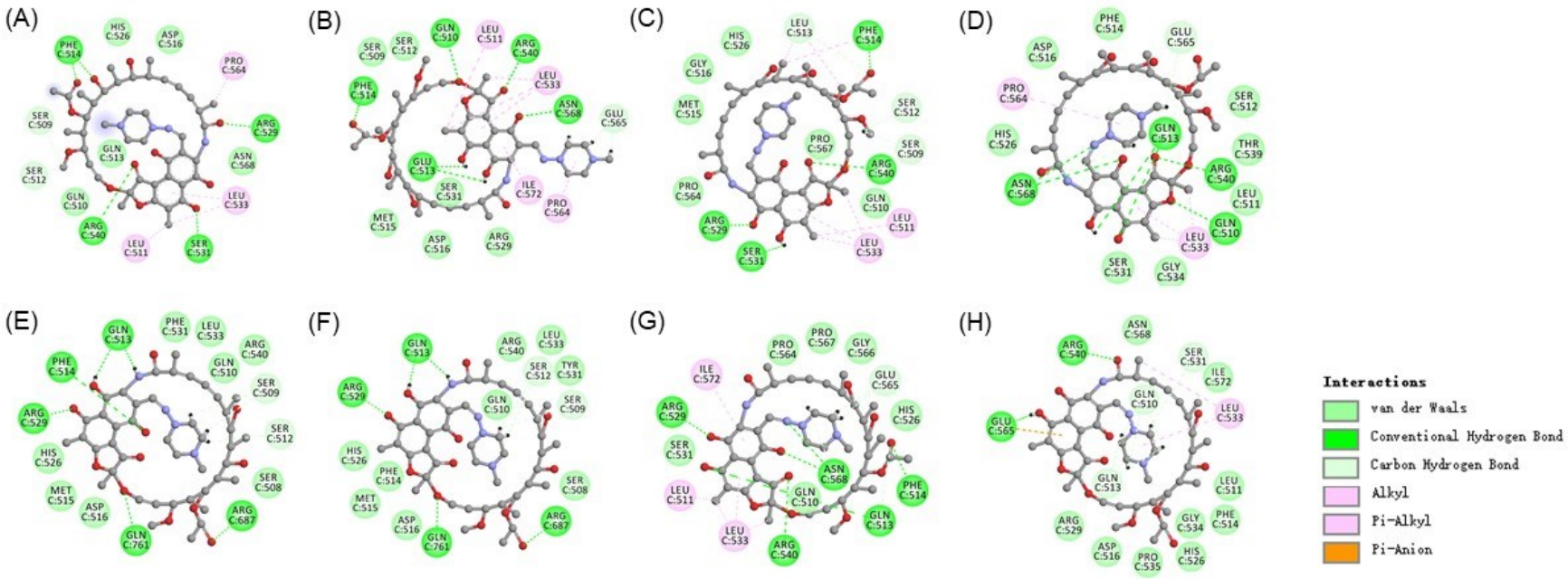



Fig. S5 Diagrams of docking interactions of rifampicin with RpoB protein in wild type and predicted mutants. (A) Wild type, (B) Q513E, (C) Q513L & D516G, (D) R529G, (E) S531F, (F) S531Y, (G) T563P, (H) P564L

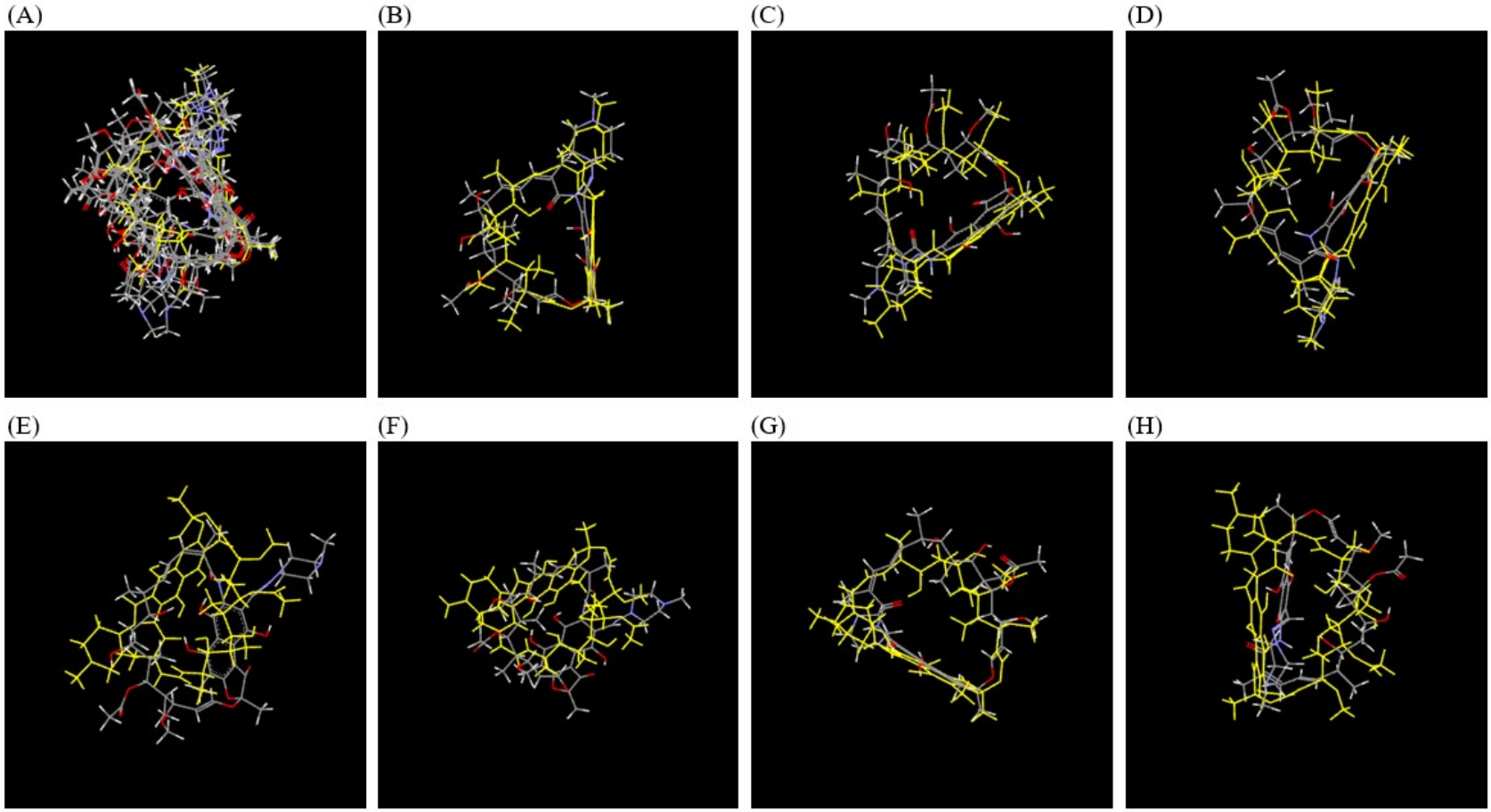



Fig. S6 The differences of docking poses of ligand between wild type and mutant RpoB, the ligand in yellow in all graphs represent the ligand pose after docking with wild type. (A) All poses docking with mutants and wild type, (B) Q513E, (C) Q513L & D516G, (D) R529G, (E) S531F, (F) S531Y, (G) T563P, (H) P564L.

Table S1. Information of the test chemicals, including their CAS number, structures and abbreviations in this work.

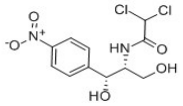
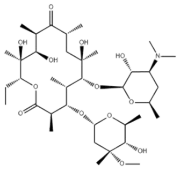
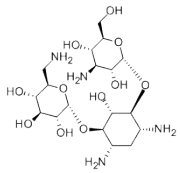
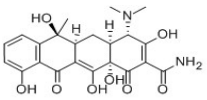
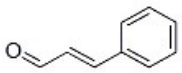
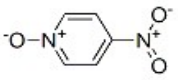
| Class | CAS/Name | Abbreviation | Structure |
|---|---|---|---|
| Antibiotics | 56-75-7 Chloramphenicol | CHL |  |
| | 114-07-8 Erythromycin | ERY |  |
| | 59-01-8 Kanamycin | KAN |  |
| | 60-54-8 Tetracycline | TET |  |
| QSIs | 104-55-2 Cinnamaldehyde | CIN |  |
| | 1124-33-0 4-Nitropyridine-N-oxide | NPO |  |

Table S2. Experimental setup for bacterial resistance mutation screening. Conc. is the abbreviation for concentration. The unit for conc. is mg/L. CHL: chloramphenicol, ERY: erythromycin, KAN: kanamycin, TET: tetracycline; CIN: cinnamaldehyde, NPO: 4-Nitropyridine-N-oxide.

| | Single exposure | | Mixture exposure (QSIs in the mixtures were added at a fixed concentration. | |
|---|---|---|---|---|
| | Group | Antibiotic conc. | CIN conc. | NPO conc. |
| CHL | CHL1 | 0.006 | 42 | 1.0 |
| | CHL2 | 0.06 | 42 | 1.0 |
| | CHL3 | 0.3 | 42 | 1.0 |
| | CHL4 | 0.6 | 42 | 1.0 |
| ERY | ERY1 | 0.16 | 42 | 1.0 |
| | ERY2 | 1.6 | 42 | 1.0 |
| | ERY3 | 8 | 42 | 1.0 |
| | ERY4 | 16 | 42 | 1.0 |
| KAN | KAN1 | 0.01 | 42 | 1.0 |
| | KAN2 | 0.1 | 42 | 1.0 |
| | KAN3 | 0.5 | 42 | 1.0 |
| | KAN4 | 1 | 42 | 1.0 |
| TET | TET1 | 0.001 | 42 | 1.0 |
| | TET2 | 0.01 | 42 | 1.0 |
| | TET3 | 0.05 | 42 | 1.0 |
| | TET4 | 0.1 | 42 | 1.0 |
| CIN | CIN1 | 0.42 | | |
| | CIN2 | 4.2 | | |
| | CIN3 | 21 | | |
| | CIN4 | 42 | | |
| NPO | NPO1 | 0.01 | | |
| | NPO2 | 0.1 | | |
| | NPO3 | 0.5 | | |
| | NPO4 | 1.0 | | |

Table S3. Detailed information of primers of tested genes in *E. coli*.

| Genes | Primer sequence（5’-3’） | | Length（bp） |
|---|---|---|---|
| 16S | Forward | GGACGCACGATAGAGAGTGG | 141 |
| | Reverse | TTCGGTCCGCGATCCTAATG | |
| *uvrD* | Forward | CTACGGTAATCCGGTGGAGC | 143 |
| | Reverse | GTTGCAGGATATGCGGCTTG | |
| *rpoS* | Forward | TGTCCAGCAACGCTTTTTCG | 189 |
| | Reverse | AGCTGAACGTTTACCTGCGA | |
| *recA* | Forward | CTGCTACGCCTTCGCTATCA | 231 |
| | Reverse | TCTACGGCGAACTGGTTGAC | |
| *mutS* | Forward | ACGGGCCTATACCCTGAACT | 124 |
| | Reverse | CAGCGGGTTGGCGATAAATG | |
| *lexA* | Forward | TGACTTGCTGGCAGTGCATA | 175 |
| | Reverse | GAAGCTCTGCTGACGAAGGT | |
| *dinB* | Forward | CGTTGTCACTGGATGAGGCT | 278 |
| | Reverse | TTTTGCCGCTGAGACTTTGC | |

Table S4. The best models of predicted mutants using three different evaluation methods.

| Mutants | 3D-profile Score | DOPE Score | PROCHECK: Most Favored and Allowed Residues Percentage |
|---|---|---|---|
| S531F | 559.63 | -141,849 | 99.6% |
| P564L | 558.21 | -141,613 | 99.6% |
| T563P | 559.58 | -141,563 | 99.6% |
| Q513E | 558.56 | -141,563 | 99.6% |
| S531Y | 559.28 | -141,809 | 99.6% |
| Q513L & D516G | 558.27 | -141,718 | 99.7% |
| R529G | 557.94 | -141,445 | 99.6% |

Table S5. Protein stability prediction of mutants by calculating unfolding Gibbs free energy changes (ΔΔG, kcal/mol) using PremPS. The positive sign of ΔΔG value corresponds to destabilizing mutations.

| Mutant | ΔΔG (kcal/mol) | Prediction |
|---|---|---|
| Q513E | 0.86 | Destability |
| Q513L & D516G | 0.51 | Destability |
| R529G | 1.55 | Destability |
| S531F | 0.52 | Destability |
| S531Y | 0.53 | Destability |
| T563P | 0.74 | Destability |
| P564L | 0.66 | Destability |