**Table S2. Bacterial strains and plasmids used in this study**

| Strain/plasmid | Description | Source |
|---|---|---|
| *P. aeruginosa* | | |
| PAO1 | Reference strain | S. Lory |
| PAO1*carB*::Tn | PW8995; Tn5 inactivation of *carB* | (1) |
| PAO1 (V) | PAO1 pMMB67EH vector control | This study |
| PAO1 (p*carB*) | PAO1 with plasmid-encoded, inducible *carB* | This study |
| PAO1*carB*::Tn (V) | PAO1*carB*::Tn pMMB67EH vector control | This study |
| PAO1*carB::*Tn (p*carB*) | PAO1*carB*::Tn with plasmid-encoded, inducible *carB* | This study |
| *E. coli* | | |
| MG1655 | Reference strain | (2) |
| JW0031 | Keio collection, Δ*carB*::Km | (3) |
| MG1655Δ*carB* | MG1655Δ*carB*::Km transduced from JW0031 | This study |
| MG1655 (p*gfp*) | MG1655 with plasmid-encoded, inducible *gfp* | (4) |
| MG1655Δ*carB* (p*gfp*) | MG1655Δ*carB* with plasmid-encoded, inducible *gfp* | This study |
| DH5α | Cloning strain | Lab stock |
| SM10λpir | Mobilizing strain for biparental mating | (5) |
| *S. aureus* | | |
| HG003 | Reference strain | (6) |

| | | |
|---|---|---|
| NE1454 | JE2 Tn inactivated *carB* | (7) |
| HG003*carB*::Tn | HG003 Tn inactivated *carB* | This study |
| *Plasmids* | | |
| pIT2 | Tn5, $Tet^R$ | (8) |
| pMMB67EH | $P_{tac}$, $Cb^R$ | (9) |
| p*carB* | pMMB67EH with $P_{tac}$::*carB,* $Cb^R$ | This study |
| p*gfp* | pUA66 with $P_{lacZ}$::*gfp,* $Km^R$ | (10) |

Definitions: $Cb^R$, carbenicillin resistance; $Km^R$, kanamycin resistance; $tet^R$, tetracycline resistance

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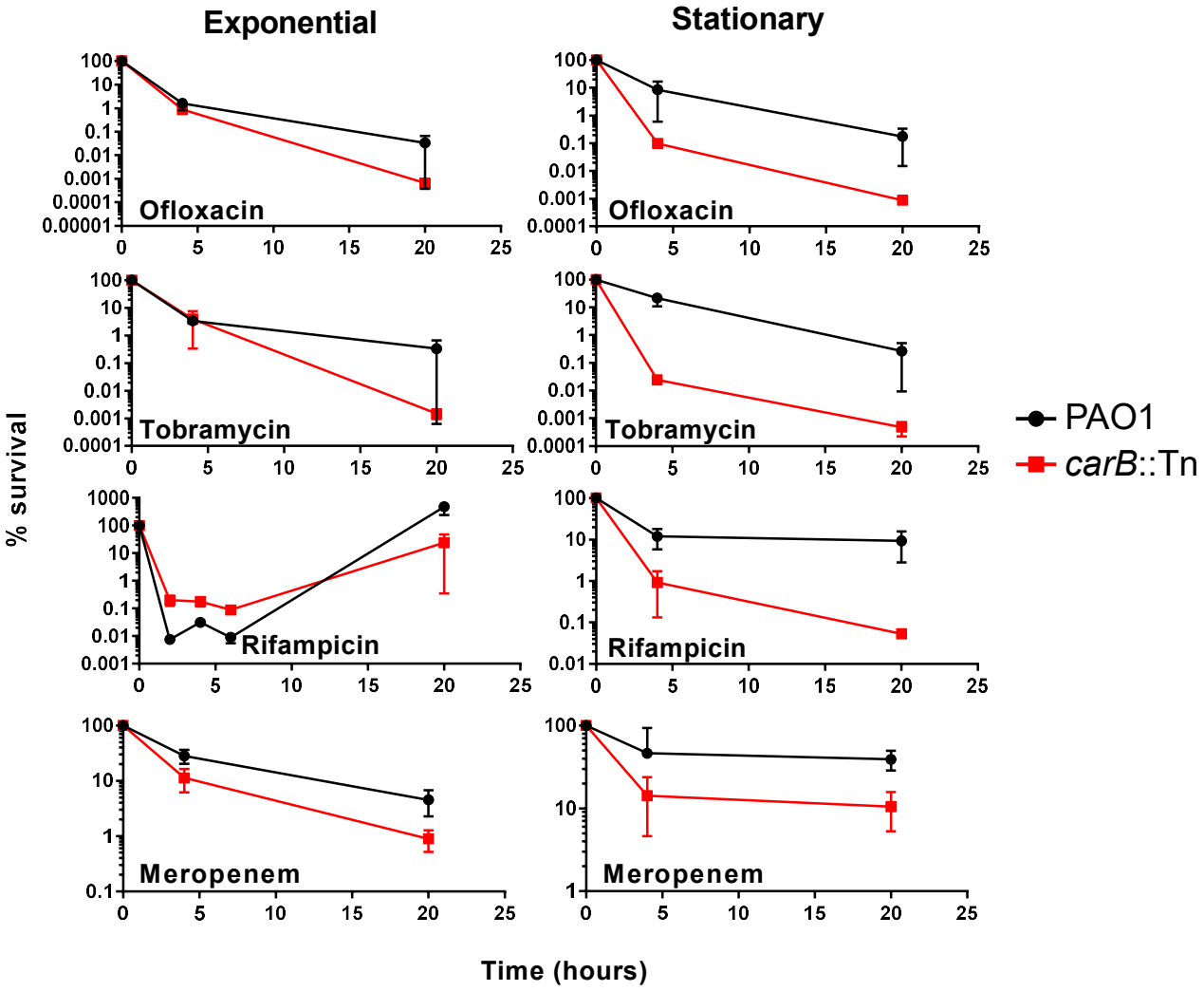


**Figure S1. Antibiotic time-kill experiments comparing survival of the *P. aeruginosa* wild-type PAO1 (black) and *carB*::Tn (red).** Cells were grown in LB media at 37ºC with aeration for either 4.5 hours (exponential) or 16-18 hours (stationary) then exposed to either ofloxacin (20 µg/ml), tobramycin (10 µg/ml), rifampicin (320 µg/ml) or meropenem (5 µg/ml). Survival was determined at 4 and 20 hours post treatment via serial dilution and colony counting. Data are the mean of at least three biological replicates, bars represent SEM.

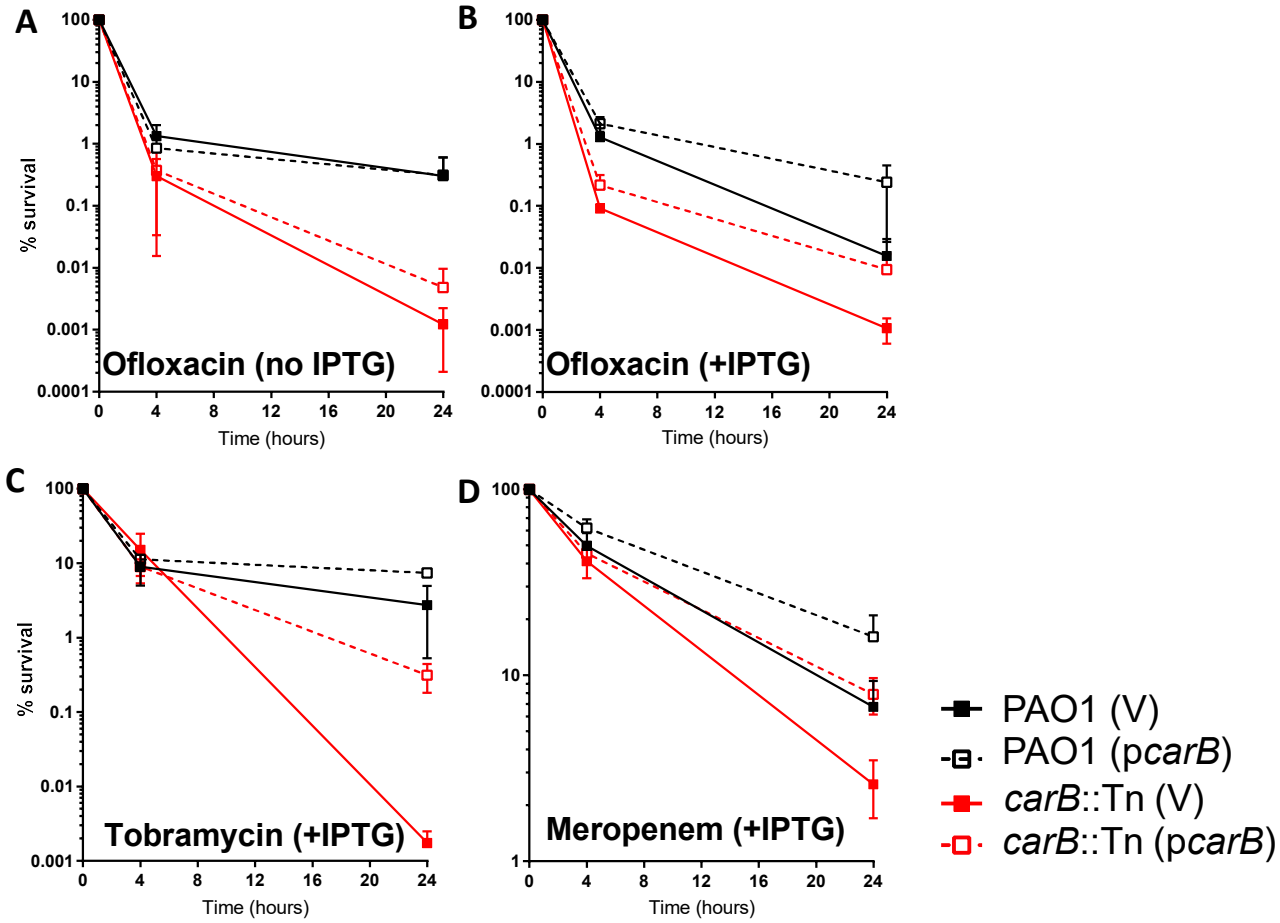


**Figure S2. Ectopic expression of *carB* under the control of an inducible promoter increases antibiotic tolerance. (A)** Cultures were grown in LB media at 37ºC with aeration for 16-18 hours then exposed to either ofloxacin (20 µg/ml), tobramycin (10 µg/ml), or meropenem (5 µg/ml). **(B-D)** For induction of $P_{tac}$, media was supplemented with 1 mM IPTG. Survival was determined at 4 and 20 hours post treatment via serial dilution and colony counting. Data are the mean of at least three biological replicates, bars represent SEM.

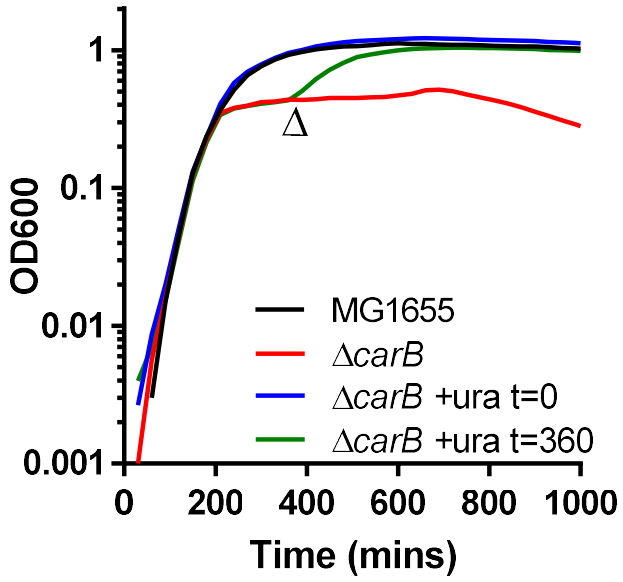


**Figure S3. Pyrimidine supplementation restores growth of the *E. coli* Δ*carB* mutant**
*E. coli* wild-type MG1655 (black) and Δ*carB* mutant (red) were grown in LB media at 37° C. To determine the growth effect of pyrimidine supplementation, 2.5mM uracil (ura) was added at t=0 (blue) or during stationary phase (green, uracil added at open arrow). Data represented is the mean of triplicate measures.