

Pre-treatment with cell-wall targeting antibiotics and aminoglycosides does not confer cross protection to cell-wall targeting antibiotics in *Escherichia coli* despite upregulation of *rprA*

Josh Bowman, Robin Couput, Anthony Pookpun, Kendrew Wong

Department of Microbiology and Immunology, University of British Columbia, Vancouver,
British Columbia, Canada

SUPPLEMENTAL MATERIAL

TABLE S1. The effects of inoculum size on the penicillin MIC.

Strain	Minimum Inhibitory Concentration ($\mu\text{g/mL}$)		
	1/100	Dilution 1/500	1/1000
DH300 (WT)	> 67	67	33
Penicillin-treated DH300 (WT)	67	67	33

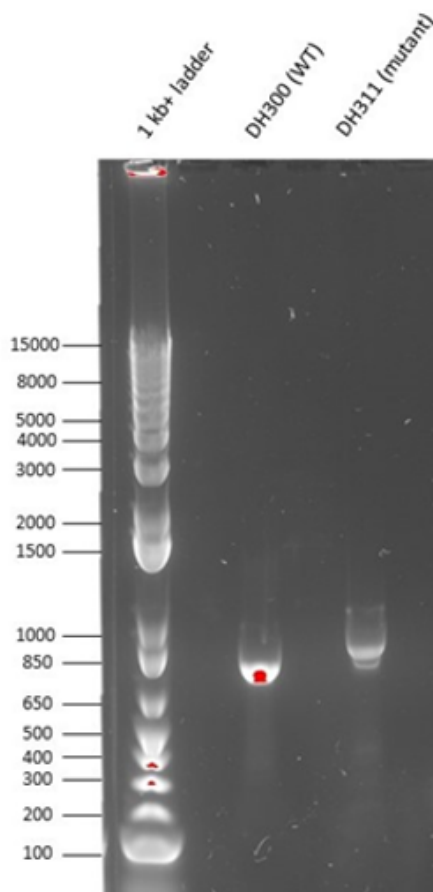


FIG. S1 PCR amplicons generated with primers flanking the *rscB* sequence in DH300 (WT) and DH311 ($\Delta rscB$) visualized on 1.0% agarose gel run at 110 V for 1 hour in 1X TBE buffer.

A
 5'GACATATGAACGTAATTATTGCCGATGACCATCCGATAGTCTTGTCCGGTATTCGCAAATCACTTGAGCAAATTGAGTGGGT
 GAATGTTGTCGGCGAATTTGAAGACTCTACAGCACTGATCAACAACCTGCCGAACTGGATGCGCATGTGTTGATTACCGA
 TCTCTCCATGCCTGGCGATAAGTACGGCGATGGCATTACCTTAATCAAGTACATCAAGCGCCATTTCCCAAGCCTGTGCATC
 ATTGTTCTGACTATGAACAACAACCCGGCGATTCTTAGTGCGGTATTGGATCTGGATATCGAAGGGATCGTGTGAAACAA
 GGTGCAACCGACCGATCTGCCGAAAGCTCTCGCCGCGCTGCAGAAAAGGGAAGAAATTTACCCCGAAAAGCGTTTTCGCCT
 GTTGAAAAAATCAGTGTGTTGTTACGGTGCACAAGCGTCTCTGCCAAAAGAGAGTGAAGTTCTGCGCTGTTTTCGGG
 AAGGCTTCTGGTGACCGAGATCGTAAAAAGCTGAACCGCAGTATTAACCATCAGTAGCCAGAAGAAATCTGCGATGA
 TGAAGCTGGGTGCTGATGAATCCCTAATGATTTTACCAAATCATTAGGGGATTCATCAGAGCTGGGTGTCGAGAACGA
 TATCGCCCTGCTGAATTATCTCTCTCAGTGACCTTAAGTCCGGCAGATAAAGACTAATCACCTGTAG 3'

B
 5'GTCCTGANNAGANNNNN**TCAGCAGNGGGGATATCGTTCGGA**CACCCAGCTCTGATGAATCCCCTAATGATTTTGGTAA
 AAATCATTAGGGGATTCATCAGCACCCAGCTTTCATCCTCGCAGATTTCTTCTGGCTACTGATGGTTTAATACTGCGGTTTCAG
 CTTTTTAGCGATCTCGGTACCAGGAAGCCTTCCGCAAAACAGGCGCAGAACTTCACTCTCTTTTGGCGAGAGACGCTTGCA
 CCGTAACCACCAAGCACTGATTTTTTCAACAGGCGAGAAACGCTTTCGGGGTAAATTTCTCCCTTTCTGCAGCGGGCGA
 GAGCTTTCGGCAGATCGGTGCGTGCACCTTGTTCAGCACGATCCCTTCGATATCCAGATCCAATACCGCACTAAGAATCGC
 CGGGTGTGTTTATAGTGCAGAAACATGATCGACAGGCTTGGGAAATGGCGCTTGATGACTTGATTAAGGTAATGCCATC
 GCCGTAATTCGCCAGGCATGGAGAGATCGGTAATCAACACATCGCATCCAGTTTCGGCAGGTTGTTGATCAGTGTCTGT
 AGAGTCTTCAAATTCGCCGACAACATTCACCCACTCAATTTGCTCAAGTATTTGCGAATACCGAACAAGACTATCGGATGG
 TCATCGGCAATAATTACGTTTCAATGNTCATGTAATAGGCTACCTTGCTACAGCAAGCTCTTGACATNACTG 3'

FIG. S2 Sequences of forward and reverse DH311 amplicon reads. A and B represent the sequences of the forward and reverse reads, respectively, with the highlighted and underlined region indicating the insert sequence.

A Met NN Met NVIIADDHPIVLF GIRKSL EQIEWVNVVGEFEDSTALINNLPKLD AHVLIITDLS Met PGDK
 YGDGITLIKIKRHFPSLSIIVLT Met NNNPAILSAVLDLDIEGIVLKQGAPTDLPKALAALQKGGKF
 TPESVSRLLLEKISAGGYGDKRLSPKSEVLRFLFAEGFLVTEIAKKLNRSIKTISSQKKSAMet Met K
 LGVENDIALNLYLSSVTLSPADKD Stop

BH Met NVIIADDHPIVLF GIRKSL EQIEWVNVVGEFEDSTALINNLPKLD AHVLIITDLS Met PGDKY
 DGITLIKIKRHFPSLSIIVLT Met NNNPAILSAVLDLDIEGIVLKQGAPTDLPKALAALQKGGKF
 PESVSRLLLEKISAGGYGDKRLSPKSEVLRFLFAEGFLVTEIAKKLNRSIKTISSQKKSAMet Met K
 LGADESPNDFYQNH Stop GIHQSWVSRITISPC Stop IISLQ Stop P Stop VRQIKTNHL Stop

CX Met SRACCSKVAYY Met XN Met NVIIADDHPIVLF GIRKSL EQIEWVNVVGEFEDSTALINNLPK
 DAHVLIITDLS Met PGDKYGDGITLIKIKRHFPSLSIIVLT Met NNNPAILSAVLDLDIEGIVLKQGA
 PTDLPKALAALQKGGKF TPESVSRLLLEKISAGGYGDKRLSPKSEVLRFLFAEGFLVTEIAKKLNR
 SIKTISSQKKSAMet Met KLGADESPNDFYQNH Stop GIHQSWVSRITISXLL

FIG. S3 Translation of *rcsB* nucleotide sequences from MG1655 and DH311 into their corresponding polypeptide sequences. MG1655 is the parental strain of *E. coli* from which DH311 was generated. A shows the peptide sequence of a functional RcsB from MG1655. B and C show the peptide sequences of RcsB translated from the forward and reverse sequencing reads of DH311, respectively.

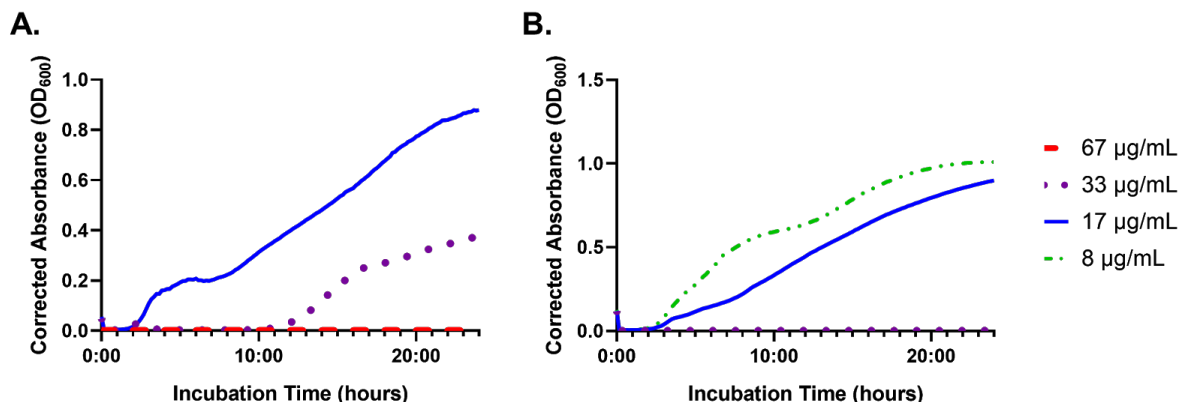


FIG. S3 Growth of A) untreated and B) penicillin pre-treated WT strains under penicillin treatment over 24 hours show no changes in absorbance for MIC. Growth of the strains were measured through sampling absorbance at 600 nm with the Biotek Synergy H1 plate reader every 10 minutes. Absorbances were corrected through subtracting a blank control. Strains were grown under different penicillin concentrations as represented by the different line patterns where concentrations used were within the range of the penicillin MICs determined in Table 3.