## Supplementary data for the article

## Targeting Superoxide dismutase confers enhanced Reactive Oxygen Species mediated eradication of Polymyxin B induced *Acinetobacter baumannii* persisters

Vineet Dubey,<sup>a</sup> Rinki Gupta,<sup>a</sup> Ranjana Pathania<sup>a\*</sup>

<sup>a</sup> Department of Biotechnology, Indian Institute of Technology, Roorkee-247667, India

\* Correspondences should be addressed to: (e-mail: ranjana.pathania@bt.iitr.ac.in)

Number of figures: 7 Number of tables: 2

## **Table of Content**

Table S1: MICs (in µg/ml) of different antibiotics against clinical isolates of A. baumannii.

**Figure S1:** Survival rate percent of *A. baumannii* AB5075, RPTC-1 and RPCT-13 against meropenem, rifampicin and tigecycline.

**Figure S2:** (A). EtBr accumulation assay of wild type *A. baumannii* AB5075 verse polymyxin B induced persister. (B). qRT-PCR analysis RND efflux genes expression in WT verse polymyxin B persister.

**Figure S3:** Schematic representation of dual fluorescent module and verification of knock-in strain.

**Figure S4:** Tolerance assay to assess the log fold reduction in CFU/ml of polymyxin B and rifampicin treatment in different clinical strains.

**Table S2:** List of primers used in this study.

**Figure S5:** Treatment of tigecycline leads to lower production of ROS as compared to rifampicin treatment in Polymyxin B induced *A. baumannii* persister.

Figure S6: Polymyxin B treatment does not eradicate rifampicin persisters of A. baumannii.

**Figure S7:** Fluorescence microscopy to assess DNA damage in cells treated with different antibiotics both alone and in combination. Scale bar for all images are  $100 \mu m$ .

	MIC (µg/ml)			
Strains	Meropenem	Tigecycline	Rifampicin	Amikacin
RPTC1	32	0.25	1	256
RPTC2	64	0.25	16	512
RPTC3	32	0.25	1	512
RPTC5	32	0.25	16	512
RPTC6	32	0.25	0.5	512
RPTC7	32	0.25	0.5	512
RPTC9	64	0.25	2	512
RPTC10	64	0.5	2	128
RPTC11	32	0.25	2	32
RPTC12	128	0.25	16	512
RPTC14	32	0.5	1	256
RPTC15	8	0.125	0.25	256
RPTC16	8	0.25	0.5	256
RPTC17	16	0.5	0.5	128
RPTC19	32	0.25	0.5	64
RPTC20	32	0.25	0.5	64
RPTC21	64	0.25	16	512
RPTC22	32	0.5	2	32
RPTC23	128	0.5	2	512
RPTC24	128	0.5	16	512
AB5075	4	0.5	1	256

Table S1: MICs (in µg/ml) of different antibiotics against clinical isolates of A. baumannii.



**Figure S1:** Survival rate percent of *A. baumannii* AB5075, RPTC-1 and AYE against meropenem, tigecycline and rifampicin.



**Figure S2:** (A). EtBr accumulation assay of wild type *A. baumannii* AB5075 verses polymyxin B induced persister. (B). qRT-PCR analysis RND efflux genes expression in WT verses polymyxin B persister.



**Figure S3:** Schematic representation of dual fluorescent module and verification of knock-in strain. P indicates strong constitutive promoter.



**Figure S4:** Tolerance assay to assess the log fold reduction in CFU/ml of polymyxin B and rifampicin treatment in different clinical strains.

Table S2. Primers used in this study				
Gene	Direction Sequence 5'→3'			
FPUSCysI500SalI	Forward	AAAGTCGACGAAAAAACGTATTGGTACAA		
RPUSCysI500BamHI	Reverse	AAAGGATCCACATGGCGTATGGCTAGTA		
FPDSCysI500KpnI	Forward	AAAGGTACCATACAGCTCTACCTGTGCTT		
RPDSCysI500EcoRI	Reverse	AAAGAATTCAGCCTTTTCACTTAGAAGCTA		
Up150CysIFP	Forward	AAGCCCGGATTTATCTGGGCTTTTTTTATG		
Ds150CysIRP	Reverse	TACAAAGCTTTTTTACCTTGAATGTTAGCC		
Fpsfbamh1	Forward	AAAGGATCCAATTGACGGCTAGCTCAGTCCTAGGTACAGTG CTAGCACCCGTTTTTTTGGGCTAGAAATAATT		
Rpsfxma1	Reverse	AAACCCGGGTTACTTGTACAGCTCGTCCATGCCGTG		
Fptdtxma1	Forward	AAACCCGGGACCCGTTTTTTTGGGCTAGAAATA		
Fptdtterknp1	Reverse	AAAGGTACCATTTGTCCTACTCAGGAGAGC		
FaraCpBADRBS	Forward	AAAGGATCCGTTACCAATTATGACAACTTGACGGCT		
RaraCpBADRBS	Reverse	AAACCCGGGTGTATATCTCCTTCTTAAAGTTAAAC		
fpSodBComp	Forward	AAACCCGGGTCGAGCTTATTTCTCTACACCAGCTGG		
rpSodBComp	Reverse	GGGGGTACCAGGAACCTGATTTCCAAAAAAT		
16S	Forward	TGTGAAATCCCCGAGCTTAAC		
16S	Reverse	TATTAGGCCAGATGGCTGC		
sodB	Forward	GTTGTTGCAGCAGCAGTAAAT		
sodB	Reverse	GGAACAGCATGAAGCCAAAC		
sodC	Forward	CGTACCATGATGTGGGGGCTT		
sodC	Reverse	GGGCTTAATCATTACCCCTGCT		
katE	Forward	TCCTTCATCCGCCACTAAAC		
katE	Reverse	GGTAGCTCGCCATTACTTACTC		
katG	Forward	GCTCTAATCCGCTCGGTAAAG		
katG	Reverse	CCAGTCTTGGGAATCGGTTAAT		
dnaK	Forward	GATGCTGGTCTTTCGACTTCT		
dnaK	Reverse	CGTCTTTACGTGGTTCTCTACC		
recA	Forward	CCTAGTTGGTTAGTACCTTTACCGT		
recA	Reverse	TCTACGCTTCAGTTCGTTTAGA		
groEL	Forward	CCAACCGAACAGGCTTATGT		
groEL	Reverse	AGCACCTTGCCGTAGAAGAA		
aceA	Forward	TACCGCCACGGATTTCTTTAC		
aceA	Reverse	CACACTGCTGCTCTTTCTACTC		
adeB	Forward	CCGCATCACCTTGAACATAAAC		
adeB	Reverse	GGTGCTATGGGCGTTAGTATT		
adeJ	Forward	TCCATTGCTTTCATGGCATCACCAGA		
adeJ	Reverse	AGCCGTATGATGCCTGAAGACTTA		
adeG	Forward	CCGGTCGTTTAGAAGCAATG		
adeG	Reverse	TTGCGGTATATGTTACCTGTGC		
tolC	Forward	CTCGCTACTGCATCTATCTGTG		
tolC	Reverse	GCTCGATGAGTGGTTAGGATTAG		
umuC	Forward	CTTTATATAAAACATCAATTTGCTGCA		
umuC	Reverse	TCACCTTAACCGGGCACAT		
umuD	Forward	CAGTGAAATCATTATCGATAAGTGC		
umuD	Reverse	GCAAGCACTCGATTTAAATGAA		



**Figure S5:** Treatment of tigecycline leads to lower production of ROS as compared to rifampicin treatment in Polymyxin B induced *A. baumannii* persister.



Figure S6: Polymyxin B treatment does not eradicate rifampicin persister of A. baumannii.



Figure S7: Fluorescence microscopy to assess DNA damage in cells treated with different antibiotics both alone and in combination. Scale bar for all images are  $100 \ \mu m$