

1 SUPPLEMENTAL MATERIAL

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3 **Mutation to *ispA* produces stable small colony variants of *Pseudomonas aeruginosa* that have**
4 **enhanced aminoglycoside resistance**

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26 **Keywords:** SCV, antibiotic resistance, tobramycin, gentamycin, burn wound infection.

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Table S1. Strains and plasmids used in this study

Strains/Plasmids	Description	Phenotype	Source
<i>P. aeruginosa</i>			
MP02	Clinical isolate	NCV	This study
MP10	Clinical isolate	SCV	This study
MP02 Δ <i>ispA</i>	MP02 mutant with <i>ispA</i> in-frame deletion	SCV	This study
MP02 <i>ispA</i> ^{Δ267-270}	MP02 mutant with <i>ispA</i> harboring the 12-base pair deletion identified in MP10	SCV	This study
MP10 <i>ispA</i> ::WT	MP10 mutant complemented in <i>cis</i> with wild-type <i>ispA</i>	NCV	This study
<i>E. coli</i>			
DH5 α	Cloning strain		Lab stock
SM10 λ pir	Conjugative strain for biparental mating		(1)
Plasmids			
pEX18Gm	Gene replacement vector, oriT+, sacB+, MCS from pUC18, GmR (=gentamicin resistance)		(2)
pEX Δ <i>ispA</i>	pEX18 derivative used to delete <i>ispA</i>		This study
pEX <i>ispA</i> ^{Δ267-270}	pEX18 derivative used to produce <i>ispA</i> ^{Δ267-270}		This study
pEX <i>ispA</i> ::WT	pEX18 derivative used to introduce wild-type <i>ispA</i>		This study

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Abbreviations: NCV, normal colony variant; SCV, small colony variant.

36 **Table S2. Antibigrams for MP02 (NCV) and MP10 (SCV)**
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Antibiotics	MP02	MP10
piperacillin/tazobactam	R	R
ceftazidime	R	R
cefepime	R	R
imipenem	R	R
meropenem	R	R
aztreonam	R	R
amikacin	R	R
gentamicin	R	R
tobramycin	I	R
colistin	S	S
trimethoprim/sulfamethoxazole	R	R
ciprofloxacin	R	R
levofloxacin	R	R

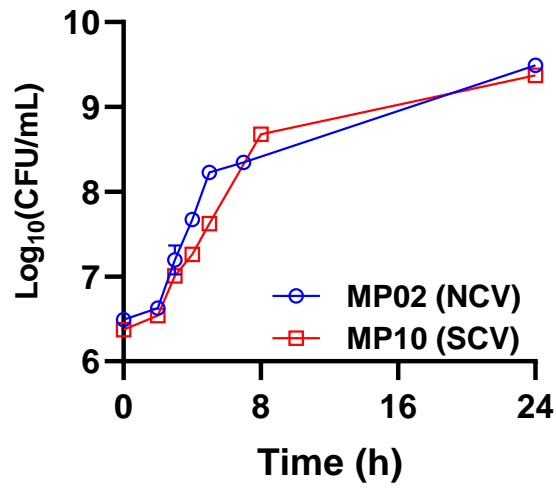
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39 NCV, normal colony variant; R, resistant; I, intermediate; S, susceptible; SCV, small colony variant
40 Susceptibility profiles as determined by the Institute of Microbiology of the University of Lausanne.

41 **Table S3.** Primers used for mutagenesis
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Name	Sequence (5' → 3')	Characteristics
ispA_up_fwd	ccct <u>ctaga</u> ccggcgtaacgggaaagg	Forward primer to delete full-length <i>ispA</i> gene (upstream region)
ispA_up_rev	<i>agttgctgcgctgatcatgcctcgtgccttc</i>	Reverse primer to delete full-length <i>ispA</i> gene (upstream region)
ispA_down_fwd	gacgcaactgaccgacgg	Forward primer to delete full-length <i>ispA</i> gene (downstream region)
ispA_down_rev	ccc <u>gaattcgctcgg</u> tgaggatcttgtgc	Reverse primer to delete full-length <i>ispA</i> gene (downstream region)
ispA_267- 270_del_fwd	ccct <u>ctaga</u> ggaacatgccgtctgtctgg	Forward primer to delete amino acids from 267 to 270
ispA_267- 270_del_rev	ccc <u>gaattcggatctt</u> gtgcggataggcc	Reverse primer to delete amino acids from 267 to 270
ispA_ins_fwd	cgcatcatcagg tcccagg	Forward primer to confirm mutagenesis
ispA_ins_rev	ggtgctg tactcgtctctg	Reverse primer to confirm mutagenesis

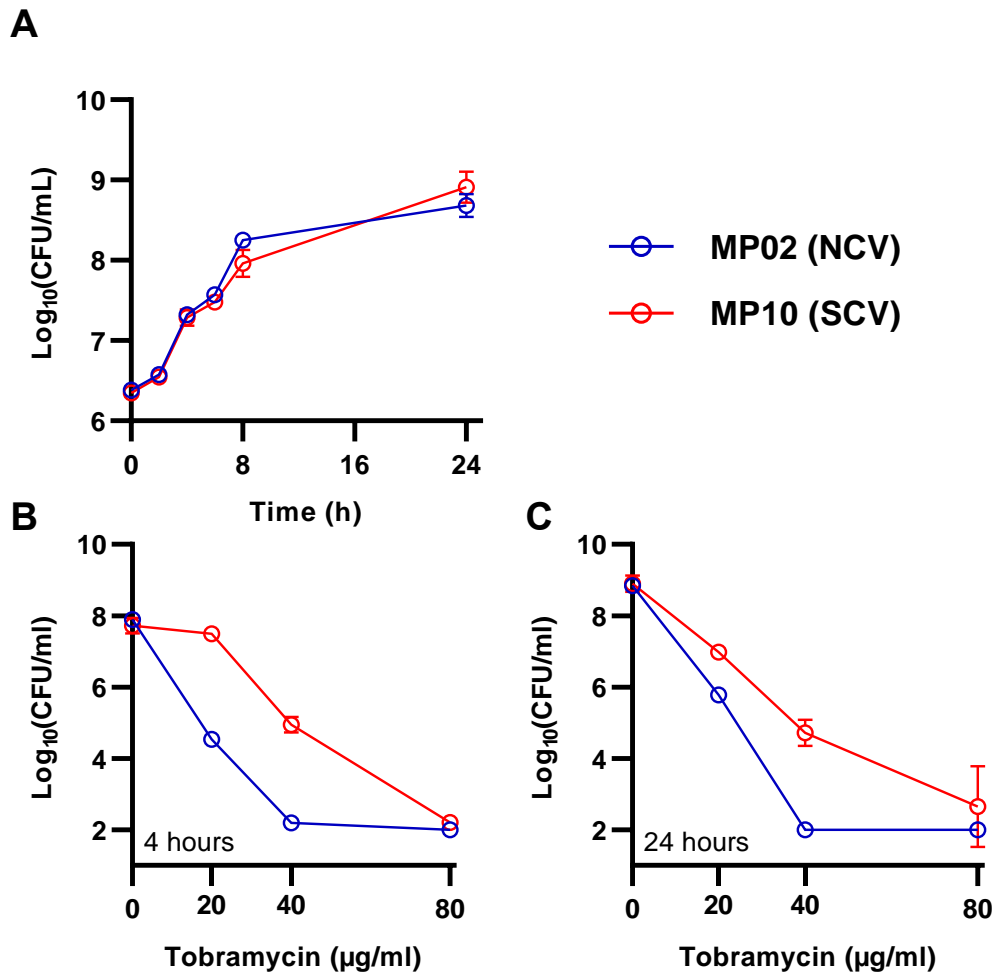
43 Regions of homology to the target amplicons are in **bold**, regions of reverse complementarity are
 44 *italicized* and restriction sites are underlined.
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Figure S1. Growth of MP02 and MP10 in LB media at 37°C with constant shaking. CFU, colony formatting unit; NCV, normal colony variant; SCV, small colony variant.

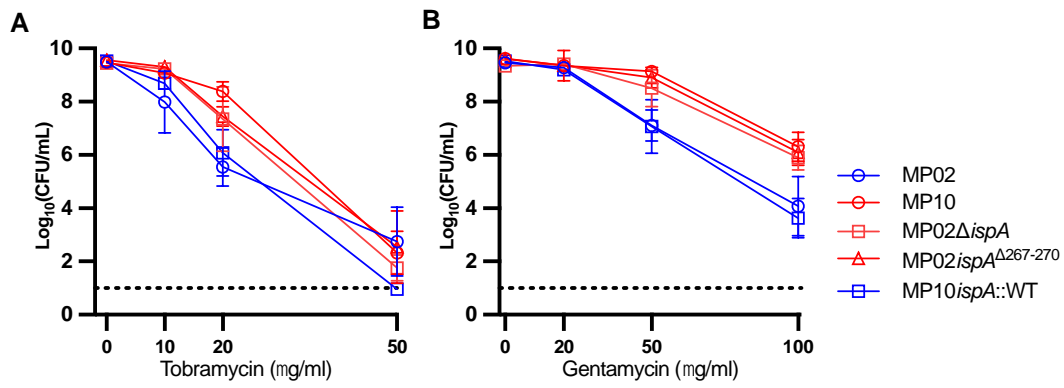


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57 **Figure S2. Mutations in *ispA* decrease tobramycin-induced killing in M9 minimal medium**
 58 **supplemented with 20 mM glucose. (A)** Growth curves of MP02 and MP10 incubated in M9 medium
 59 at 37°C under constant shaking. **(B, C)** Bacteria were grown to mid-exponential phase in M9 at 37°C
 60 before treatment for 4h **(B)** or 24h **(C)** with increasing concentrations of tobramycin. CFU, colony
 61 formatting unit; NCV, normal colony variant; SCV, small colony variant.

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Figure S3. Mutations in *ispA* decrease aminoglycoside-induced killing in LB media. Bacteria were grown to mid-exponential phase in LB at 37°C and then treated for 24h with either tobramycin (**A**) or gentamicin (**B**). CFU, colony formatting unit; NCV, normal colony variant; SCV, small colony variant.

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