

Repurposed antimicrobial combination therapy: tobramycin-ciprofloxacin hybrid augments activity of the anticancer drug mitomycin C against multidrug-resistant Gram-negative bacteria

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Supplementary Table 1 Evaluation for synergy of combinations consisting of TOB-CIP and anticancer drugs against antibiotic susceptible strains of *P. aeruginosa*, *A. baumannii* and *E. coli*.

Drug	Organism	MIC _{Drug} [MIC _{combo}], µg/mL	MIC _{TOB-CIP} [MIC _{combo}], µg/mL	FIC index	Interpretation
Mitomycin C	<i>P. aeruginosa</i> PAO1	2 [0.125]	32 [2]	0.125	Synergy
	<i>A. baumannii</i> ATCC 17978	16 [4]	>128 [16]	0.250<x<0.375	Synergy
	<i>E. coli</i> ATCC 25922	0.5 [0.062]	8 [1]	0.250	Synergy
Etoposide	<i>P. aeruginosa</i> PAO1	512 [256]	32 [1]	0.531	Additive
	<i>A. baumannii</i> ATCC 17978	512 [256]	>128 [0.25]	0.500<x<0.502	Additive
	<i>E. coli</i> ATCC 25922	512 [32]	8 [2]	0.312	Synergy
Camptothecin	<i>P. aeruginosa</i> PAO1	256 [256]	32 [0.5]	1.016	Additive
	<i>A. baumannii</i> ATCC 17978	256 [256]	>128 [0.25]	1.000<x<1.002	Additive
	<i>E. coli</i> ATCC 25922	256 [256]	8 [0.125]	1.016	Additive
5-Fluorouracil	<i>P. aeruginosa</i> PAO1	64 [16]	32 [0.5]	0.266	Synergy
	<i>A. baumannii</i> ATCC 17978	256 [128]	>128 [0.25]	0.500<x<0.502	Additive
	<i>E. coli</i> ATCC 25922	32 [16]	8 [2]	0.750	Additive
Cisplatin	<i>P. aeruginosa</i> PAO1	128 [32]	32 [2]	0.312	Synergy
	<i>A. baumannii</i> ATCC 17978	512 [512]	>128 [0.5]	1.000<x<1.004	Additive
	<i>E. coli</i> ATCC 25922	256 [256]	8 [0.125]	1.016	Additive
Doxorubicin	<i>P. aeruginosa</i> PAO1	256 [64]	32 [2]	0.312	Synergy
	<i>A. baumannii</i> ATCC 17978	256 [256]	>128 [0.5]	1.000<x<1.004	Additive
	<i>E. coli</i> ATCC 25922	256 [64]	8 [2]	0.500	Synergy

Supplementary Table 2 Evaluation for synergy of combinations consisting of TOB-CIP and mitomycin C against antibiotic susceptible and efflux-deficient strains of *P. aeruginosa*.

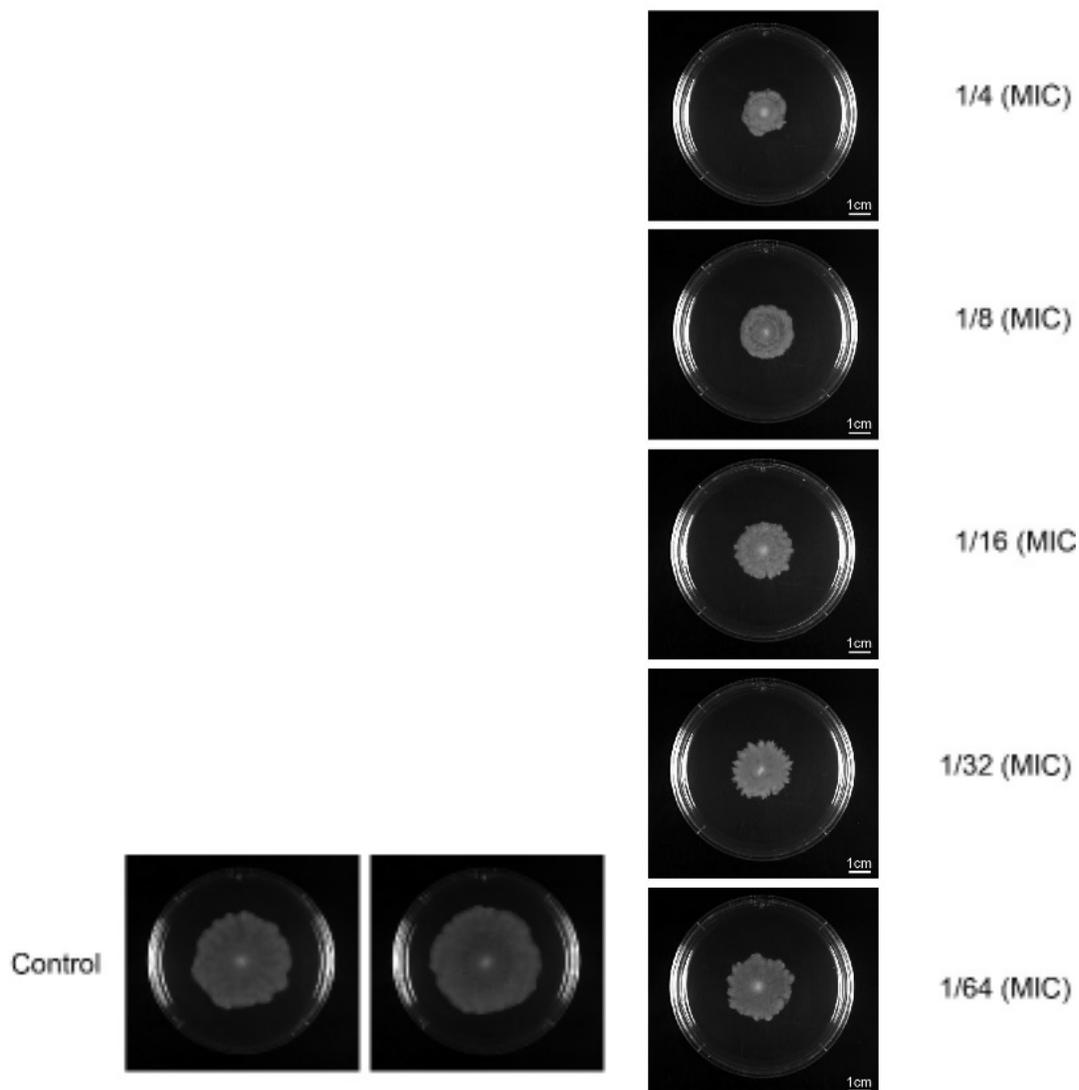
Drug	Organism	MIC _{Drug} [MIC _{combo}], µg/mL	MIC _{TOB-CIP} [MIC _{combo}], µg/mL	FIC index	Interpretation
Mitomycin C	<i>P. aeruginosa</i> PAO1	2 [0.125]	32 [2]	0.125	Synergy
	<i>P. aeruginosa</i> PAO200	0.062 [0.031]	2 [0.5]	0.750	Additive
	<i>P. aeruginosa</i> PAO750	0.031 [0.016]	2 [0.5]	0.750	Additive

Supplementary Table 3 Evaluation for synergy of combinations consisting of mitomycin C and either TOB-CIP or commercially-used amphiphiles/surfactants against antibiotic susceptible strains of *P. aeruginosa*, *A. baumannii* and *E. coli*.

Organism	MIC _{Mitomycin C} [MIC _{combo}], µg/mL	Amphiphile	MIC _{Amphiphile} [MIC _{combo}], µg/mL	FIC index	Interpretation
<i>P. aeruginosa</i> PAO1	2 [0.125]	TOB-CIP	32 [2]	0.125	Synergy
	2 [2]	Benzalkonium Chloride	64 [1]	1.016	Additive
	2 [2]	Benzethonium Chloride	32 [16]	1.500	Additive
	2 [2]	Cetrimonium Bromide	256 [2]	1.008	Additive
<i>A. baumannii</i> ATCC 17978	16 [4]	TOB-CIP	>128 [16]	0.250<x<0.375	Synergy
	16 [4]	Benzalkonium Chloride	16 [8]	0.750	Additive
	16 [4]	Benzethonium Chloride	8 [4]	0.750	Additive
	16 [2]	Cetrimonium Bromide	64 [32]	0.625	Additive
<i>E. coli</i> ATCC 25922	0.5 [0.062]	TOB-CIP	8 [1]	0.250	Synergy
	0.5 [0.125]	Benzalkonium Chloride	8 [4]	0.750	Additive
	0.5 [0.25]	Benzethonium Chloride	8 [1]	0.625	Additive
	0.5 [0.5]	Cetrimonium Bromide	64 [2]	1.031	Additive

Supplementary Table 4 Evaluation for synergy of combinations consisting of mitomycin C and either TOB-CIP or polymyxin B nonapeptide (PMBN) against antibiotic susceptible strains of *P. aeruginosa*, *A. baumannii* and *E. coli*.

Organism	MIC _{Mitomycin C} [MIC _{combo}], µg/mL	Amphiphile	MIC _{Amphiphile} [MIC _{combo}], µg/mL	FIC index	Interpretation
<i>P. aeruginosa</i>	2 [0.125]	TOB-CIP	32 [2]	0.125	Synergy
PAO1	2 [0.062]	PMBN	128 [0.016]	0.047	Synergy
<i>A. baumannii</i>	16 [4]	TOB-CIP	>128 [16]	0.250<x<0.375	Synergy
ATCC 17978	16 [4]	PMBN	>256 [32]	0.250<x<0.375	Synergy
<i>E. coli</i> ATCC	0.5 [0.062]	TOB-CIP	8 [1]	0.250	Synergy
25922	0.5 [0.0311]	PMBN	256 [8]	0.094	Synergy



Supplementary Figure 1. Motility of *P. aeruginosa* PAO1 is greatly reduced in the presence of sub-MIC concentration of TOB-CIP. Swimming plates without conjugate serve as a control.

Swimming Motility Assay was performed as described in Yang, X et al. *J. Med. Chem.* 2017, 60, 3913–3932. In brief, the medium was composed of Trypticase Peptone (5 mg/mL), NaCl (2.5 mg/mL), and 0.3% (w/v) agar. The motility plates were prepared by mixing the molten media with the desired concentration of the agent which was allowed to dry for an hour. 2.5 μ L of an overnight grown culture of *P. aeruginosa* PAO1 was diluted in sterile PBS to an OD of 1.0. The culture was then point inoculated onto the motility plate and incubated for 20 h at 37 $^{\circ}$ C.

Supplementary Table S5 Susceptibility profiles of MDR Gram-negative bacterial clinical isolates used in the study

a) *Pseudomonas aeruginosa* isolates

Stock #	PTZ	A/C	AZT	FOX	CFZ	CTR	CPM	CAZ	IMI	MER	DOR	ETP	CIP	MOX	TOB	GEN	AMK	TGC	MIN	DOX	CST	CAM
100036	8	>32	16	>32	>128	32	4	8	8	4	16	>32	>16	>16	128	>32	32	32	16	64	2	1024
PA259-96918	64	>32	32	>32	>128	>64	>64	512	32	1024	>1024	>32	>16	>16	256	>32	>64	32	32	32	1	1024
PA260-97103	128	>32	64	>32	>128	>64	16	32	32	16	16	>32	16	>16	128	>32	4	16	16	16	1	128
PA262-101856	64	>32	32	>32	>128	64	32	16	32	32	16	>32	>16	>16	1024	>32	>64	32	64	1024	1	2048
PA264-104354	256	>32	64	>32	>128	>64	32	128	32	64	16	>32	>16	>16	128	>32	8	32	32	64	1	4096

b) *Escherichia coli* isolates

Stock #	PTZ	A/C	AZT	FOX	CFZ	CTR	CPM	CAZ	IMI	MER	ETP	CIP	MOX	TOB	GEN	AMK	TGC	DOX	CST	SXT
ATCC 25922	2	8	≤0.12	4	1	≤0.25	≤0.25	≤0.25	0.12	≤0.03	≤0.03	≤0.06	≤0.06	2	≤0.5	≤1	0.12	1	0.5	≤0.12
94393 (<i>mcr-1</i> +ve)	≤1	4	≤0.12	4	1	≤0.25	≤0.25	≤0.25	0.25	≤0.03	≤0.03	0.5	1	≤0.5	≤0.5	2	0.25	4	4	≤0.12
94474 (<i>mcr-1</i> +ve)	16	>32	≤0.12	16	4	≤0.25	≤0.25	0.5	0.25	≤0.03	≤0.03	>16	16	32	16	2	1	>32	16	>8
107115	>512	>32	>64	>32	>128	>64	>64	>32	8	32	>32	>16	16	8	>32	2	0.25	>32	0.5	>8

mcr = mobilized colistin resistance gene.

c) Other Gram-negative bacteria

Stock #	Organism	PTZ	A/C	AZT	FOX	CFZ	CPM	CAZ	CAZ-AVI	CTX	IMI	MER	ETP	CIP	MXF	TOB	GEN	AMK	TGC	DOX	CST	SXT
116381	<i>K. pneumoniae</i>	8	16	16	16	>128	16	8	0.5	>64	0.5	≤0.03	0.12	>16	>16	4	≤0.5	≤1	1	>32	0.5	>8
113250	<i>K. pneumoniae</i>	4	4	≤0.12	1	1	1	0.5	ND	ND	0.25	≤0.03	≤0.03	≤0.06	≤0.06	≤0.5	≤0.5	≤1	ND	2	>16	≤0.1
113254	<i>K. pneumoniae</i>	≤1	2	≤0.12	1	1	1	≤0.2	ND	ND	0.12	≤0.03	≤0.03	≤0.06	≤0.06	≤0.5	≤0.5	≤1	ND	2	>16	≤0.1
117029	<i>E. cloacae</i>	2	16	≤0.12	>32	>128	≤0.25	0.5	0.25	≤0.25	0.25	≤0.03	≤0.03	≤0.06	≤0.06	2	≤0.5	2	0.5	>32	0.25	>8
118564	<i>E. cloacae</i>	2	>32	≤0.12	>32	>128	0.25	0.5	ND	ND	ND	0.12	ND	0.06	0.12	1	1	2	ND	4	>16	ND
121187	<i>E. cloacae</i>	1	8	≤0.12	>32	32	0.25	0.5	ND	ND	ND	0.06	ND	0.25	1	32	>32	1	ND	>32	>16	ND
92247	<i>A. baumannii</i>	<1	ND	ND	32	128	4	ND	ND	ND	ND	4	ND	≤0.06	ND	ND	ND	<1	0.25	ND	4	≤0.1
AB027	<i>A. baumannii</i>	512	ND	ND	ND	>128	ND	ND	ND	ND	32	16	ND	>16	8	ND	32	>64	4	ND	>256	>8
AB031	<i>A. baumannii</i>	4	ND	ND	ND	>128	ND	ND	ND	ND	0.25	1	ND	0.25	0.12	ND	<0.5	2	8	ND	16	4
LAC-4	<i>A. baumannii</i>	ND	ND	ND	ND	ND	ND	R	ND	I	S	S	ND	R	ND	R	R	I	S	S	ND	ND

PTZ: piperacillin-tazobactam; A/C: amoxicillin-clavulanic acid; AZT: aztreonam; FOX: cefoxitin; CFZ: cefazolin; CTR: ceftriaxone; CPM: cefepime; CAZ: ceftazidime; IMI: imipenem; MER: meropenem; DOR: doripenem; ETP: ertapenem; CIP: ciprofloxacin; MOX: moxifloxacin; TOB: tobramycin; GEN: gentamicin; AMK: amikacin; TGC: tigecycline; MIN: minocycline; DOX: doxycycline; CST: colistin; CAM: chloramphenicol; CTX: cefotaxime; AVI: avibactam ND: not determined; S: Susceptible; I: Intermediate; R: Resistant according to the antibiotic breakpoint guidelines of the CLSI for *Acinetobacter* spp.