

Rifabutin synergy / antagonism with SoC antibiotics in *Acinetobacter baumannii*

INTRODUCTION

Rifabutin (RBT) exerts potent antibacterial activity against *Acinetobacter baumannii* under iron-limiting conditions¹. RBT hijacks the *A. baumannii* siderophore receptor FhuE for active uptake enabling potent activity and overcoming common rifampicin (RIF) resistance mechanisms (Figure 1)².

Here we investigated the activity of RBT in combination with standard of care (SoC) antibiotics against *A. baumannii* clinical strains.

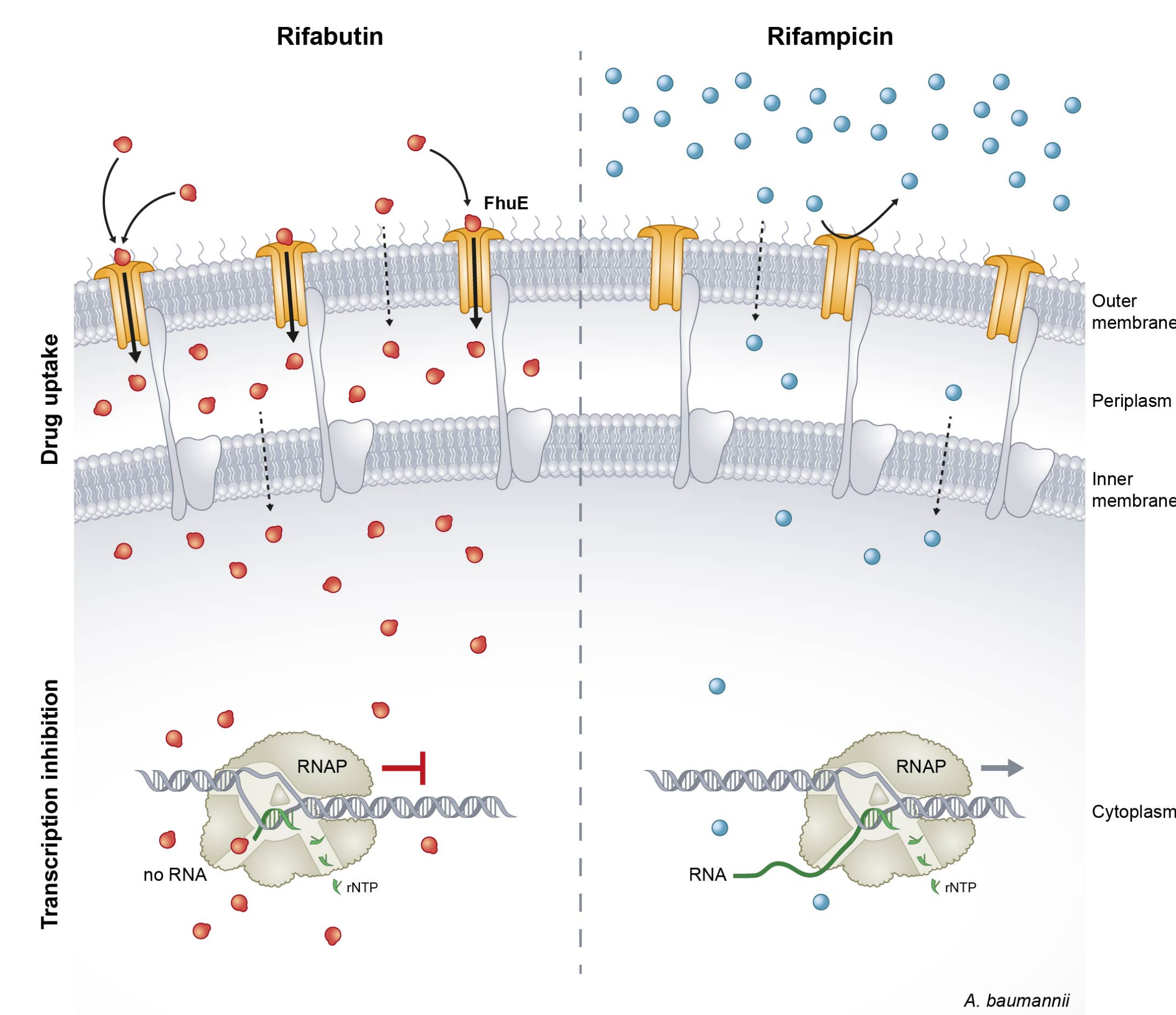


Figure 1. RBT and RIF mode of action against *A. baumannii*. Rifabutin is transported by FhuE in iron limited medium, allowing high intracellular concentration and potent activity in contrast to rifampicin.

RESULTS

1) RBT synergy with SoC antibiotics.

RBT synergy with SoC antibiotics was assessed using checkerboard assay on the representative *A. baumannii* LAC-4 strain.

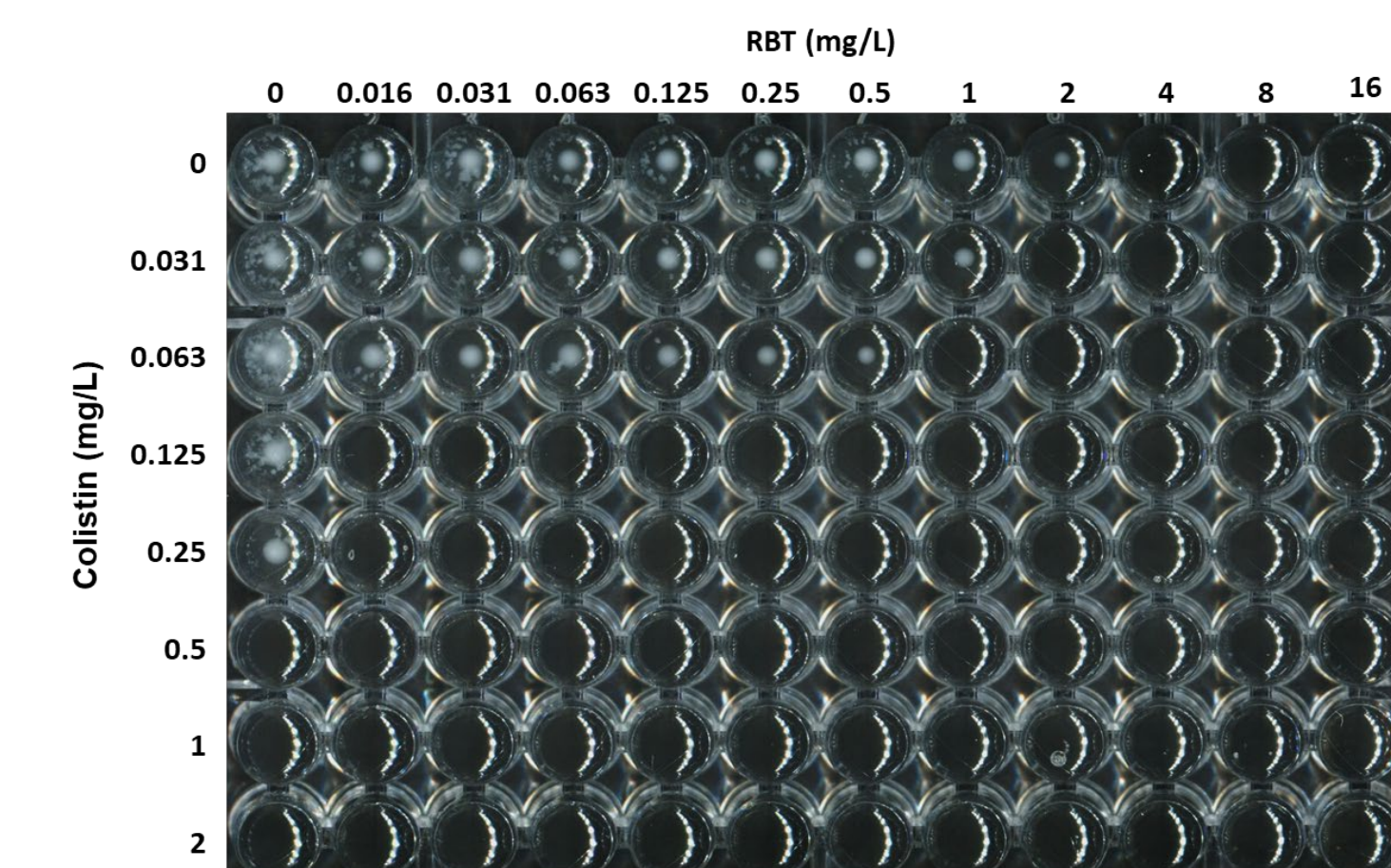


Figure 2. Checkerboard MIC illustrating RBT/COL synergy.

Table 1. RBT combination against *A. baumannii* LAC-4 strain determined in CAMHB.

Combination antibiotics	FICI	Interpretation
Colistin (COL)	0.254	synergy
Meropenem	1	indifferent
Cefotaxime	1.5	indifferent
Ciprofloxacin	1.5	indifferent
Tobramycin	1.5	indifferent
Cefiderocol* (FDC)	0.5	synergy
Eravacycline	1	indifferent
Minocycline	1	indifferent

* Determined in iron-depleted CAMHB

- No antagonism between RBT and SoC
- RBT synergizes with COL and FDC

2) RBT synergy with cefiderocol and colistin.

RBT synergy with cefiderocol and colistin was further studied on a panel of 17 *A. baumannii* isolates with elevated RBT MIC. The iron limiting RPMI + 10% FCS medium induces FhuE mediated RBT uptake, in contrast to the rich CAMHB medium where FhuE expression is not permissive. Iron-depleted CAMHB was not used to prevent skipped MIC wells².

Table 2. Antibiotic MICs and combination activities determined against a panel of *A. baumannii* clinical isolates.

Strain	Mutations RpoB	FhuE ^a	RBT MIC (mg/L)			COL MIC (mg/L)		RIF MIC (mg/L) ^c	FDC MIC (mg/L) ^c	Combination			
			b	c	b	c	RBT/FDC ^c			RBT/COL ^b	RBT/COL ^c	RIF/COL ^c	
HUMC1	-	-	0.002	4	0.25	0.5	4	2	indifferent	indifferent	synergy	synergy	
UNT091-1	-	-	0.001	8	0.25	0.5	4	4	synergy	indifferent	synergy	synergy	
IHMA690517	-	LAC-4	2	8	0.5	16	4	0.25	indifferent	synergy	synergy	synergy	
IHMA863866	-	Δ	2	4	0.5	> 32	2	4	synergy	synergy	synergy	synergy	
IHMA919656	-	LAC-4	4	4	1	> 32	2	0.125	indifferent	synergy	synergy	synergy	
IHMA1013816	-	LAC-4	2	8	0.063	16	2	0.06	indifferent	synergy	synergy	synergy	
ACC00535	-	LAC-4	2	16	0.5	0.5	> 32	16	synergy	synergy	synergy	synergy	
LAC-4	-	LAC-4	1	4	0.25	0.5	2	1	synergy	synergy	synergy	synergy	
UNT238-1	-	Δ	1	8	0.5	0.25	2	4	indifferent	synergy	synergy	synergy	
UNT191-1	-	LAC-4	2	16	0.125	2	4	16	synergy	synergy	synergy	synergy	
UNT239-1	-	Δ	0.25	4	0.5	1	2	4	synergy	synergy	synergy	synergy	
UNT087-1	-	Δ	2	16	0.06	1	4	0.5	indifferent	synergy	synergy	indifferent	
402292-17	H535C	-	0.125	> 32	0.125	1	> 32	> 32	indifferent	indifferent	synergy	indifferent	
402608-17	H535C	-	0.25	128	2	1	> 32	8	synergy	indifferent	synergy	synergy	
IHMA867231	H535N, L542F	LAC-4	> 32	> 32	1	32	> 32	1	indifferent	synergy	synergy	synergy	
401046-18	S521T, H535Q	LAC-4	32	64	0.125	0.5	> 32	128	indifferent	synergy	synergy	synergy	
401255-18	S521T, H535Q	LAC-4	8	128	0.125	0.25	> 32	16	indifferent	synergy	synergy	synergy	

^a FhuE LAC-4: variant not transporting RBT, FhuE Δ: disrupted FhuE. ^b Determined in RPMI + 10% FCS. ^c Determined in CAMHB.

Table 2.

- RBT synergizes with FDC against 41% of the tested strains.
- RBT synergizes with COL against 100% of the tested strains, regardless of resistance level.
- No RBT/COL synergy in strains with active uptake (FhuE) in RPMI + FCS (in line with COL MoA).
- RIF synergizes with COL against 88% of the tested strains.

Figure 3.

- RBT MIC fold decrease in combination is more pronounced with COL than FDC.
- In combination with COL, RBT MIC fold decrease is more pronounced than RIF MIC fold decrease.

Figure 4.

- RBT/COL combination overcomes both RBT and COL resistance, while RIF/COL does not.

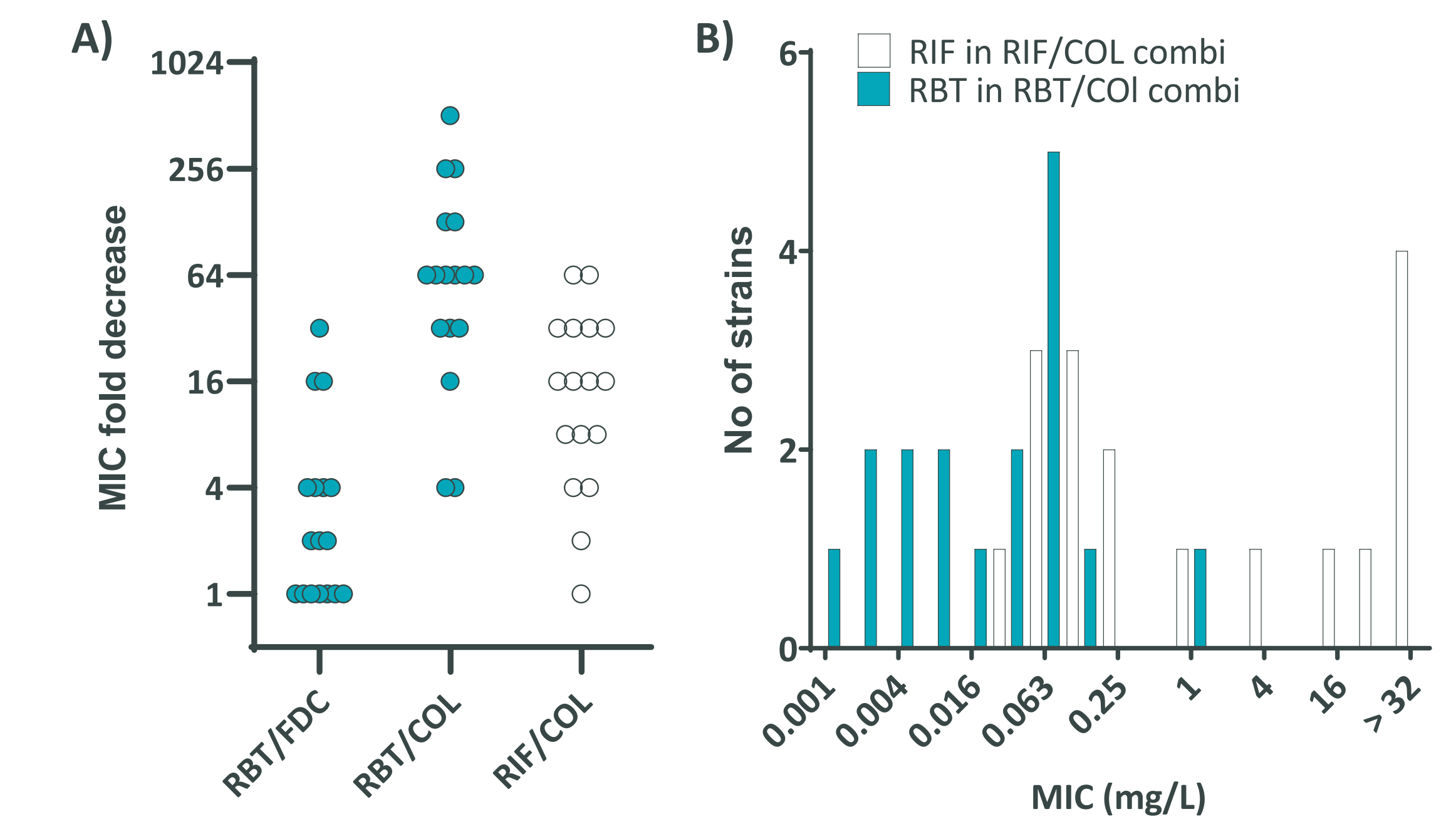


Figure 3. RBT (turquoise) and RIF (white) combination MIC fold decrease (A) and combination MIC distribution (B) determined in CAMHB against 17 *A. baumannii* isolates.

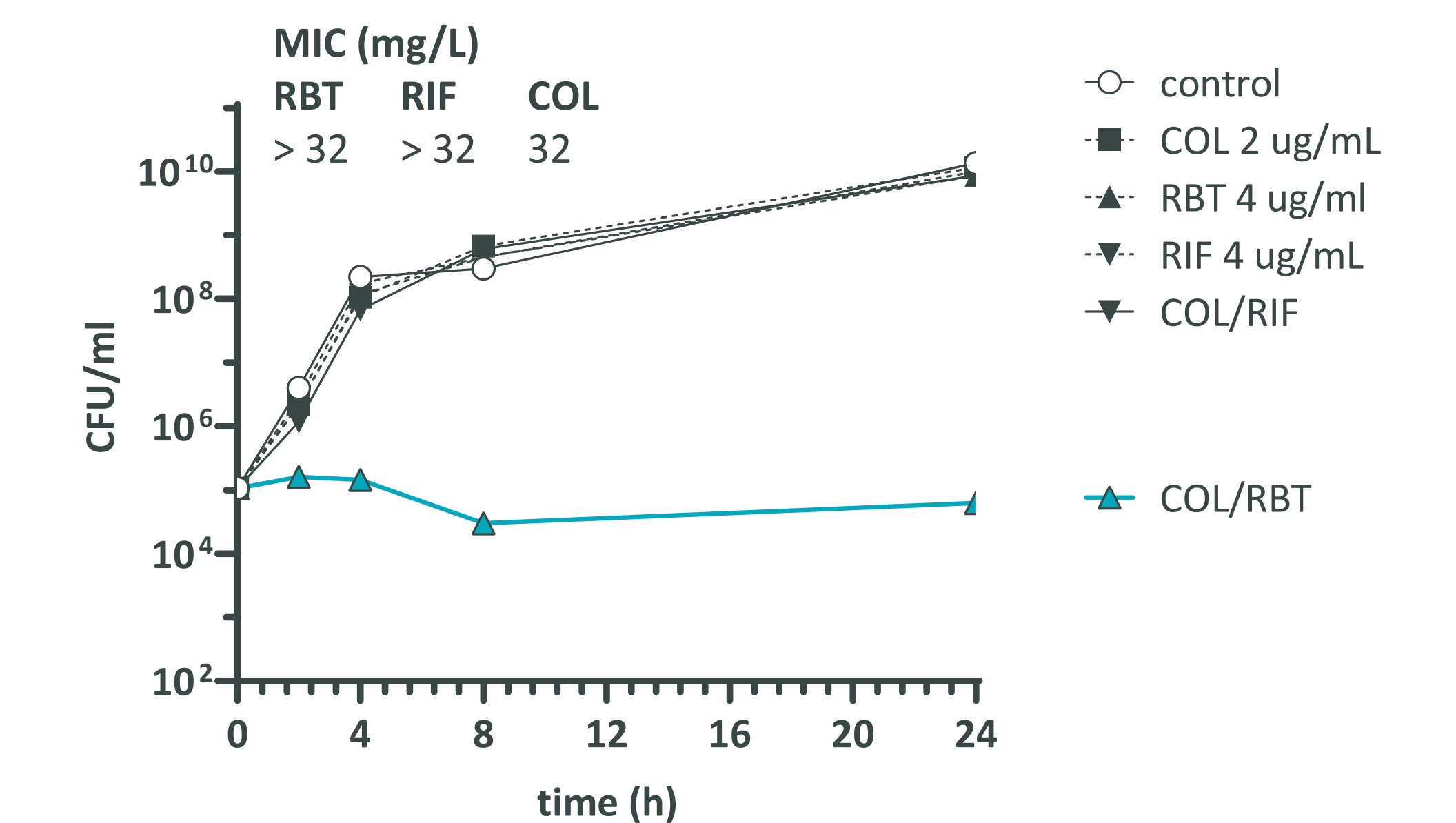


Figure 4. Time kill of RBT and RIF alone or in combination with COL against the *A. baumannii* IHMA867231 strain in CAMHB medium.

METHOD

Synergy / antagonism was evaluated on *A. baumannii* clinical isolates with elevated RBT MIC using checkerboard and time-kill curve in diverse media. Fractional inhibitory concentration index (FICI) were calculated as follow:

$$FICI = (MIC_{combination A+B} / MIC_{antibiotic A}) + (MIC_{combination A+B} / MIC_{antibiotic B})$$

synergy (FICI ≤ 0.5); indifferent (FICI > 0.5 and ≤ 4); antagonistic (FICI > 4).

CONCLUSIONS

RBT synergizes with FDC and COL against *A. baumannii*. COL synergy is potent and conserved in *A. baumannii*, regardless of initial resistance level / mechanism, overcoming both RBT and COL resistance mechanisms. In contrast, COL synergy with RIF does not allow to overcome resistance. RBT combination with COL or FDC may have the potential to improve the treatment of infections caused by highly resistant *A. baumannii* strains.

REFERENCES

1. Luna, B. *et al.* A nutrient-limited screen unmasks rifabutin hyperactivity for extensively drug-resistant *Acinetobacter baumannii*. *Nat. Microbiol.* 1–10 (2020)
2. Trebosc, V. *et al.* In vitro activity of rifabutin against 293 contemporary carbapenem-resistant *Acinetobacter baumannii* clinical isolates and characterization of rifabutin mode of action and resistance mechanisms. *J. Antimicrob. Chemother.* **75**, 3552–3562 (2020).