



An ultrashort antibacterial lipopeptide acts as an antibiotic adjuvant to combat multi-drug resistant Gram-negative pathogens

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Problem Statement

- » Antimicrobial Resistance (AMR) is the ability of microorganisms to resist the action of antimicrobials.
- » It threatens the safety and efficacy of common surgical procedures and immunosuppressive chemotherapy.¹
- » WHO declared AMR among TOP 10 GLOBAL HEALTH THREATS
- The WHO critical priority pathogens include multi-drug resistant (MDR) Gram-negative bacteria (GNBs) *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and members of the *Enterobacteriaceae* family.
- ~4.95M deaths were associated with bacterial AMR in 2019²
- By 2050, 10 M lives/year (2M in India alone) & \$10¹⁴ global GDP is at risk³
- » There is dire need of new antibiotics with low propensity of resistance and no cross-resistance with existing members of its class.

What are Ultrashort cationic Lipopeptides (USCLs)?

USCLs: Lipopeptides with ≤7 amino acid residues & fatty acid chain(s)⁴

- » Amphiphilic character due to balance of hydrophobic fatty acid chain & hydrophilic peptide moiety⁴
- » Antimicrobial activity of reported USCLs similar to Antimicrobial peptides (AMPs) & Lipopeptides (LPs) but fewer against GNBs
- » In the present study,

Fatty acid conjugated tetrapeptides of chemical formula:



were designed and screened against MDR bacterial pathogens, where, R¹ ranged from C₈–C₁₆ chain alkyl, X denoted the cationic tetrapeptide and R² was selected from –COOM and CONH₂ where M is H or (C₁–C₆) alkyl.

USCL ‘SVAP9I’ exerts broad-spectrum antibacterial activity

Bacteria (No. of Strains)	MIC (μg/mL)			
	SVAP9I	Levofloxacin	Meropenem	Minocycline
<i>E. coli</i> (27)	8	0.015-32	0.06-0.125	0.25-32
<i>Enterobacter cloacae</i> complex (ECC) (15)	8	0.5-32	0.25->64	2->64
Carbapenem resistant <i>A. baumannii</i> (4)	64	8	8	2
Carbapenem resistant <i>K. pneumoniae</i> (1)	8	64	>64	ND
<i>P. aeruginosa</i> (1)	32	1	ND	ND

- » Among the tested Lipopeptides, SVAP9I most potent, best against *E. coli* among GNBs with a favourable selectivity index of >25

- » Antibacterial activity stemmed from adding alkyl chains in a length-dependent manner to the inert tetrapeptide

- » SVAP9I's antibacterial activity is not chiral specific

‘SVAP9I’ is bactericidal with no detectable resistance generation in *E. coli*

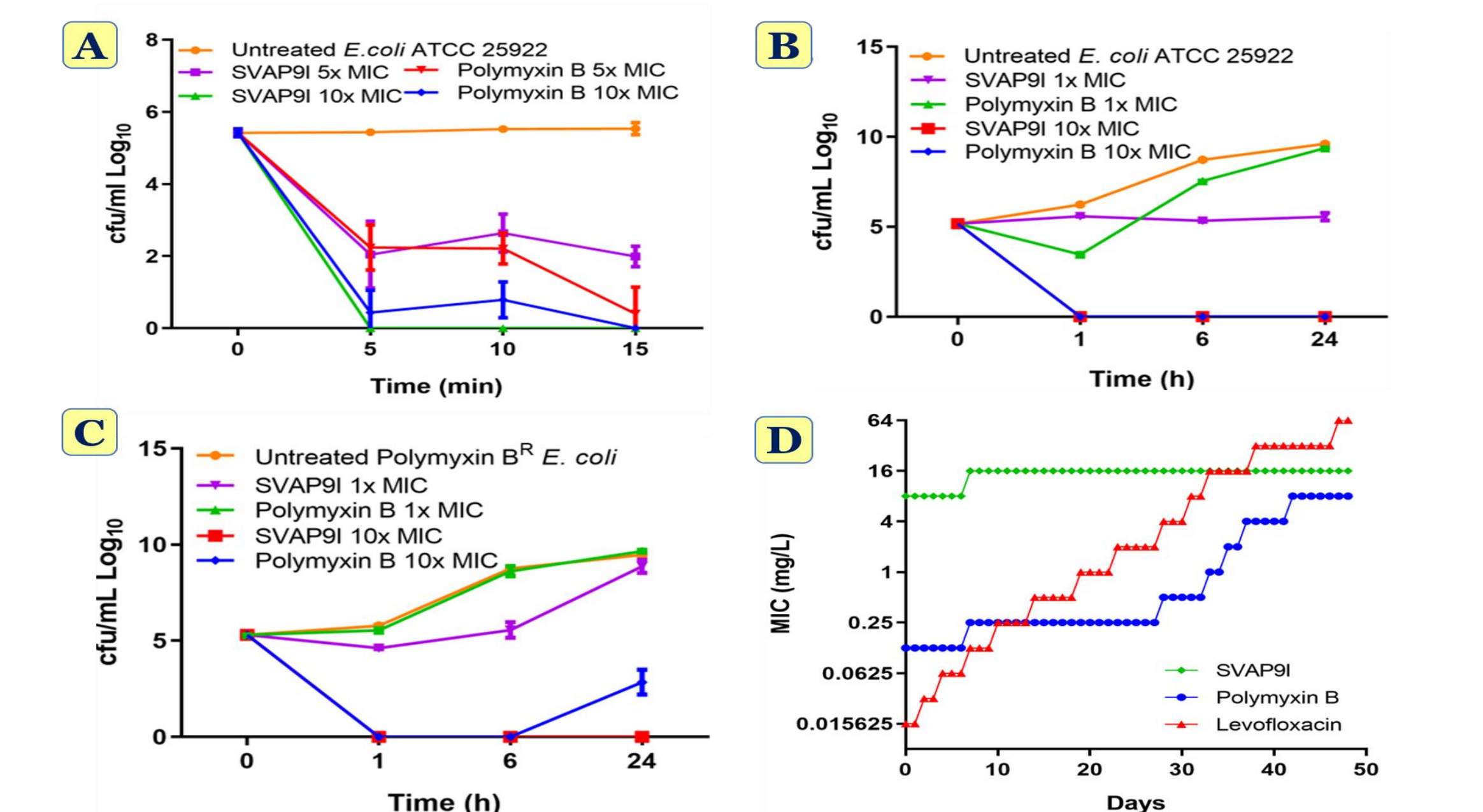


Figure 1. (A) & (B) 1 h & 24 h time-kill Kinetics of SVAP9I demonstrated rapid conc. dependent bactericidal killing, complete eradication of *E. coli* (EC) within 5 min at 10x MIC, no regrowth up to 24 h.; (C) 24 h Time-kill Kinetics against PMB^R EC were SVAP9I killed PMB^R EC (MIC 0.125» 8 mg/L) with no regrowth. In comparison, PMB treatment led to regrowth; (D) No detectable resistance induced against SVAP9I in EC over 50 days exposure while MICs of controls PMB and Levofloxacin increased ~64 and ~4096 fold.

‘SVAP9I’ targets the dual bacterial membranes of GNBs

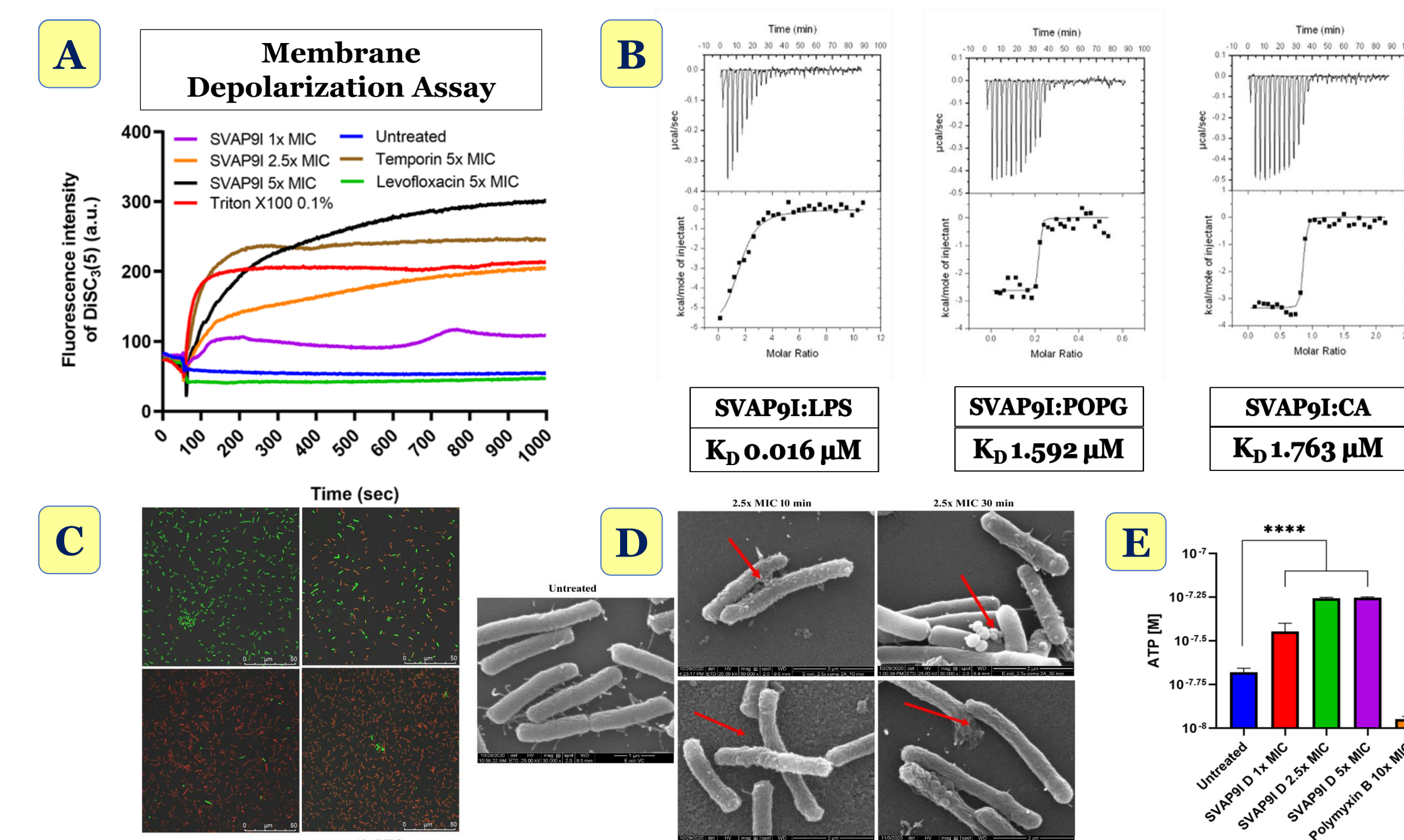


Figure 2. (A) *E. coli* inner membrane (IM) depolarization assay; (B) ITC analysis of SVAP9I with LPS, POPG & CA, present in the outer (OM) & IM of *E. coli*; (C) Confocal microscope images of SVAP9I treated *E. coli*; (D) Scanning Electron microscope (SEM) images of SVAP9I treated *E. coli*; (E) Extracellular ATP determination upon SVAP9I treatment to *E. coli*.

‘SVAP9I’ sensitized MDR pathogenic GNBs towards narrow-spectrum antibiotics

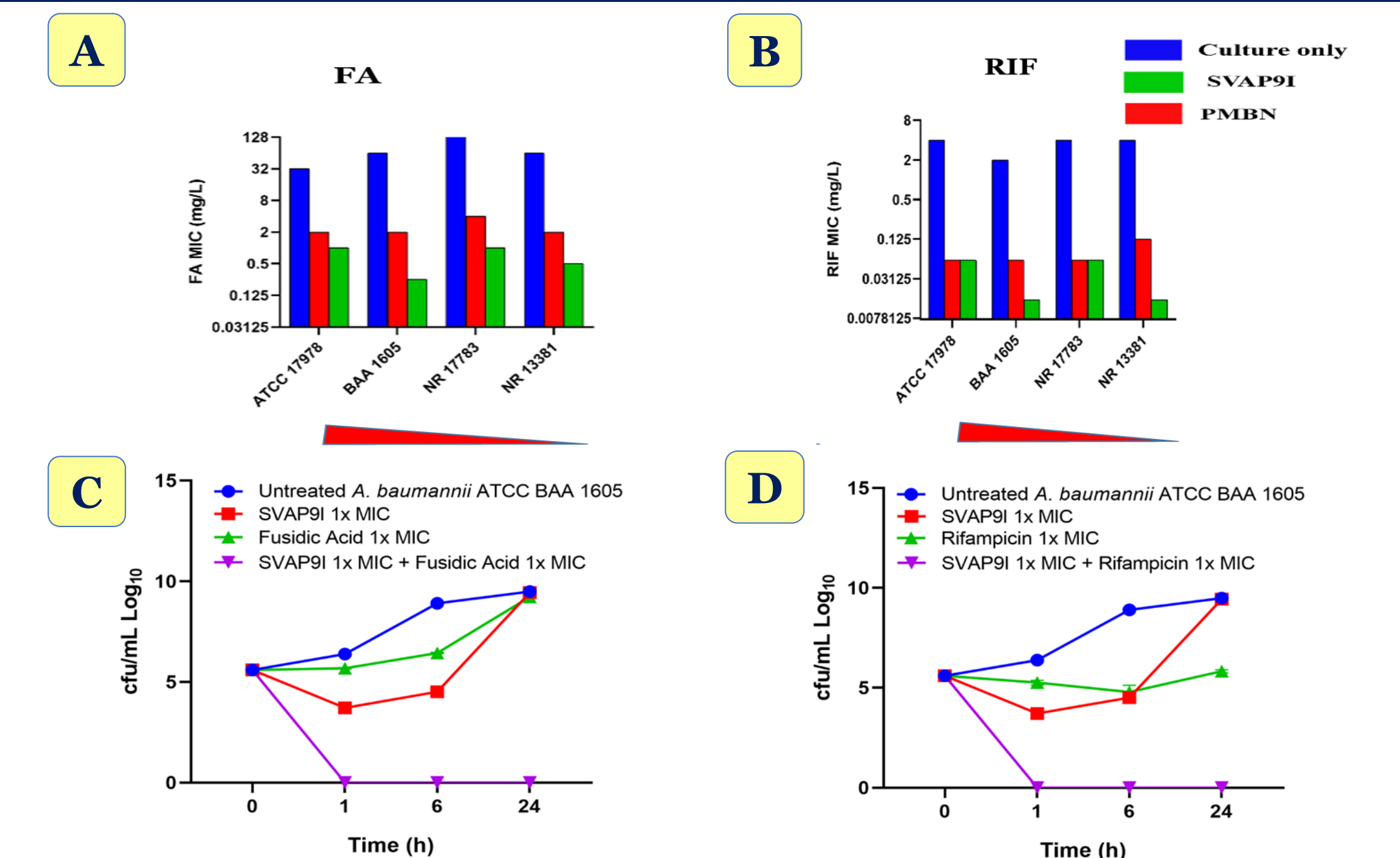


Figure 3. (A) & (B) Potentiation of Fusidic acid (FA) & Rifampicin (RIF) respectively against MDR and XDR *A. baumannii* isolates at sub-inhibitory concentrations of SVAP9I; (C) & (D) 24 h Time-kill kinetics of SVAP9I against MDR *A. baumannii* in combination with FA and RIF respectively resulting in complete eradication

‘SVAP9I’ acts as an antibiotic adjuvant to Rifampicin in vivo against MDR A. baumannii infections

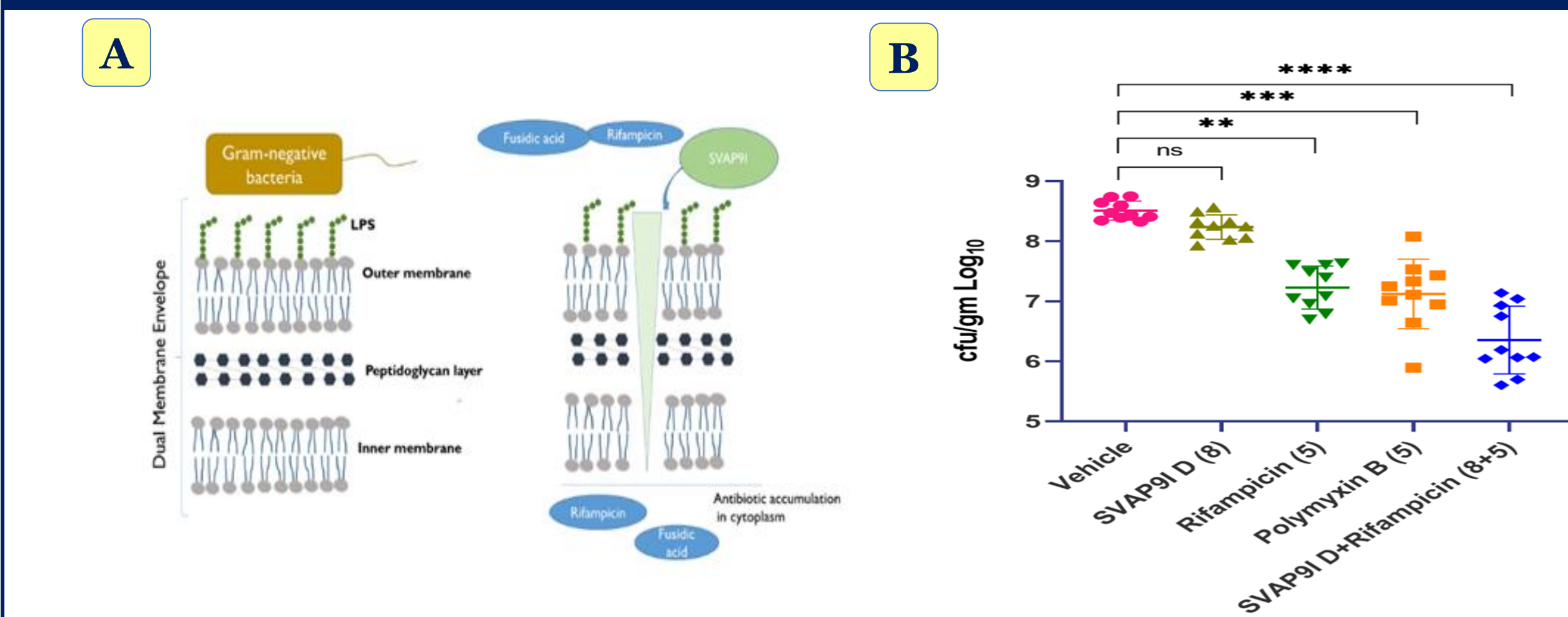


Figure 4. (A) Schematic representation of SVAP9I as an antibiotic adjuvant; (B) *In vivo* evaluation of SVAP9I's significant adjuvant activity in a neutropenic murine thigh infection model, effective dose (8 mg/kg) of SVAP9I used in combination was 12.5 fold lower than its maximum tolerable dose (MTD) (~100 mg/kg).

Conclusions

- » A novel USCL ‘SVAP9I’ was designed and identified:
 - Active against MDR & XDR GNBs with no detectable resistance
 - Targets bacterial dual membrane barrier
 - An excellent antibiotic adjuvant
- » SVAP9I has the potential to act as an adjuvant to clinically used antibiotic rifampicin against MDR *A. baumannii* infections.

Acknowledgements

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References

1. Cancer and Antimicrobial Resistance Challenges and the Path Forward, IDSA, Schmieder and Edwards, 2012.
2. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022
3. Tackling drug-resistant Infections globally: Final report and recommendations. The Review on Antimicrobial Resistance, 2016
4. Ultrashort cationic lipopeptides—effect of N-terminal amino acid and fatty acid type on antimicrobial activity. *Molecules*, 2020