

Efficacy of Antimicrobial Photodynamic Therapy (aPDT) Alone and in Combination with Antibiotics against Resistant Klebsiella pneumoniae: An Experimental In Vitro Study

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1. INTRODUCTION

- Multidrug-resistant Klebsiella pneumoniae is a major Gram-negative pathogen associated with severe healthcare-associated infection.
- Antimicrobial photodynamic therapy (aPDT) uses a photosensitizer plus visible light to generate reactive oxygen species with multitarget antibacterial activity.
- Combining aPDT with antibiotics may accelerate bacterial killing and provide an adjunctive strategy for resistant local infections.

2. OBJECTIVE

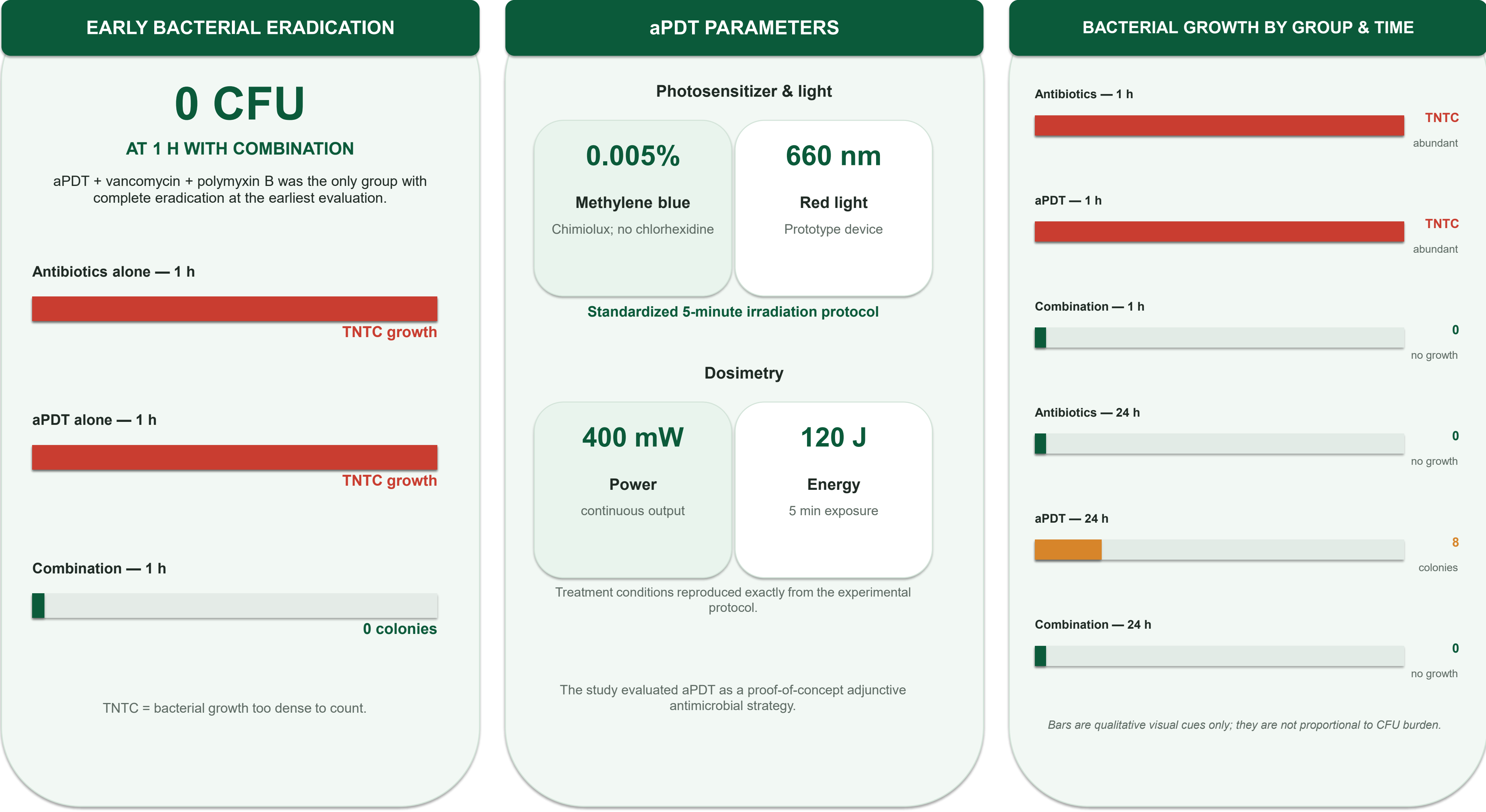
To evaluate the in vitro efficacy of methylene blue-mediated aPDT, alone and in combination with vancomycin plus polymyxin B, against a multidrug-resistant Klebsiella pneumoniae strain.

3. MATERIALS & METHODS

- Experimental in vitro study using multidrug-resistant K. pneumoniae strain N29.
- Three groups: (1) vancomycin 10 mg/mL + polymyxin B 10 mg/mL; (2) aPDT alone; (3) aPDT + both antibiotics.
- Photosensitizer: methylene blue Chimiolux 0.005%, without chlorhexidine.
- Red-light irradiation: 660 nm, 400 mW, 120 J, 5-minute application.
- Samples were plated after treatment and bacterial growth was assessed by CFU/mL at 1 h and 24 h.

Primary experimental readout: viable bacterial growth at 1 h and 24 h after intervention.

4. KEY FINDINGS

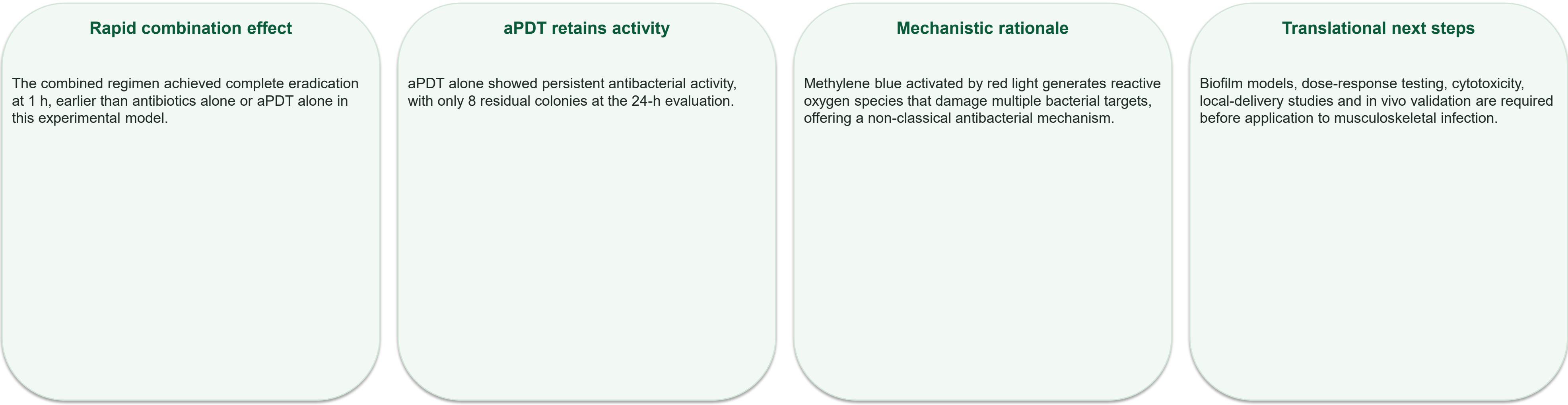


5. RESULTS

EXPERIMENTAL CONDITION	1-HOUR READOUT	24-HOUR READOUT	INTERPRETATION
Antibiotics alone VAN + PMB	Abundant growth TNTC	No bacterial growth	Delayed eradication
aPDT alone	Abundant growth TNTC	Residual growth 8 colonies	Delayed antimicrobial effect
aPDT + antibiotics	No bacterial growth 0 colonies	No bacterial growth	Rapid + sustained eradication
Photosensitizer	Methylene blue 0.005%	No chlorhexidine	Phenothiazinium PS
Light protocol	660 nm • 400 mW	120 J • 5 min	Red-light aPDT
Model / readout	MDR K. pneumoniae N29	CFU/mL at 1 h & 24 h	In vitro proof-of-concept

At 1 h, complete bacterial eradication occurred only with aPDT + antibiotics. At 24 h, antibiotics alone and combination therapy showed no growth, whereas aPDT alone showed minimal residual growth (8 colonies). No formal synergy analysis was reported.

6. DISCUSSION



7. CONCLUSION

In this in vitro model, methylene blue-mediated aPDT showed antibacterial activity against multidrug-resistant K. pneumoniae. The combination with vancomycin plus polymyxin B produced complete bacterial eradication at 1 h, whereas antibiotics alone required 24 h and aPDT alone left minimal residual growth at 24 h. These preliminary findings support further investigation of aPDT as an adjunctive local strategy for resistant Gram-negative musculoskeletal infection.

TAKE-HOME: aPDT + antibiotics achieved complete eradication at the earliest evaluation (1 h).

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