

ANTIMICROBIAL PHOTODYNAMIC THERAPY AGAINST EXTENSIVELY DRUG-RESISTANT (XDR) GRAM-NEGATIVE ISOLATES WITH NOVEL ANTIBIOTIC RESISTANCE FACTORS

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INTRODUCTION

Antimicrobial resistance (AMR) poses an urgent global public health threat, annually resulting in 1.27 million deaths worldwide¹. The emergence and worldwide spread of extensively drug-resistant (XDR) microbes is highly concerning, as infections caused by these pathogens are virtually untreatable². Antimicrobial photodynamic therapy (aPDT, photodisinfection) can be deployed to combat these drug-resistant microbes, without upregulating resistance. aPDT utilizes a photosensitizer (PS) combined with a specific wavelength of light to produce reactive oxygen species (ROS) that can rapidly eradicate a wide variety of microbes. Our novel photosensitizer formulation, Steriwave™ (Ondine Biomedical Inc., Canada), combines 0.01% methylene blue (MB) and 0.25% chlorhexidine gluconate in an aqueous excipient matrix to enhance aPDT substantivity and efficacy.

OBJECTIVE

To compare the efficacy of 0.01% MB alone to a commercial PS formulation (Steriwave™, Ondine Biomedical Inc., Vancouver, BC) against XDR Gram-negative strains *in vitro*.

METHODS

Gram-negative bacterial strains were received from the CDC & FDA Antibiotic Resistance Isolate Bank³. All tested strains exhibited extensively-drug-resistant phenotypes (Table 1), including the expression of KPC, IMP, NDM, VIM, and OXA carbapenemases. aPDT was applied to planktonic suspensions of each strain with either MB alone or Steriwave™ formulation. Illumination was performed via 670 nm diode laser adjusted to 150 mW/cm². First, a study was conducted to evaluate the effect of the commercially available PS components against two representative strains under a 60s treatment (Figure 3). Following, a light-dose-response study was performed exposing suspensions for 20, 40, and 60 seconds, and compared against a non-irradiated control sample (Figure 2). Serially diluted samples were plated on TSA for quantification by CFU counting.

Table 1. Antimicrobial classes with confirmed drug-resistance

Aminoglycosides	Carbapenems	Quinolones	Nitrofurans
Cephems	Monobactam	Fluoroquinolones	Lipopeptides
Penicillin	Tetracyclines	Sulfonamides	Fosfomycin

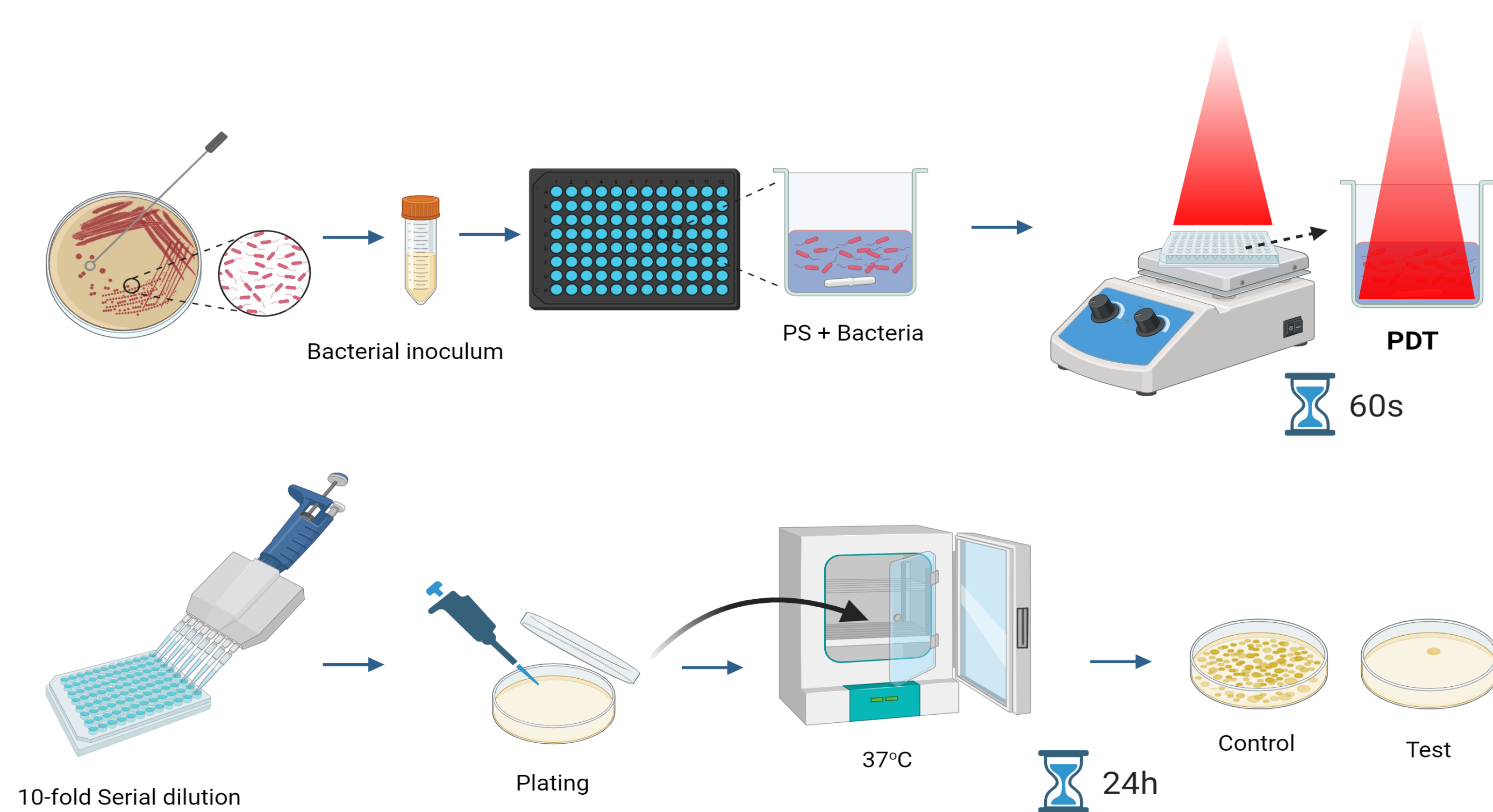


Figure 1. Planktonic method used for dose-response photodynamic inactivation assessment of XDR Gram-negative isolates.

References

- [1] Cassini A, Högberg LD, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European economic area in 2015: a population-level modelling analysis. *Lancet Infect Dis.* 2019;19:56–66.
- [2] CDC. COVID-19: U.S. Impact on Antimicrobial Resistance, Special Report 2022. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2022.

RESULTS

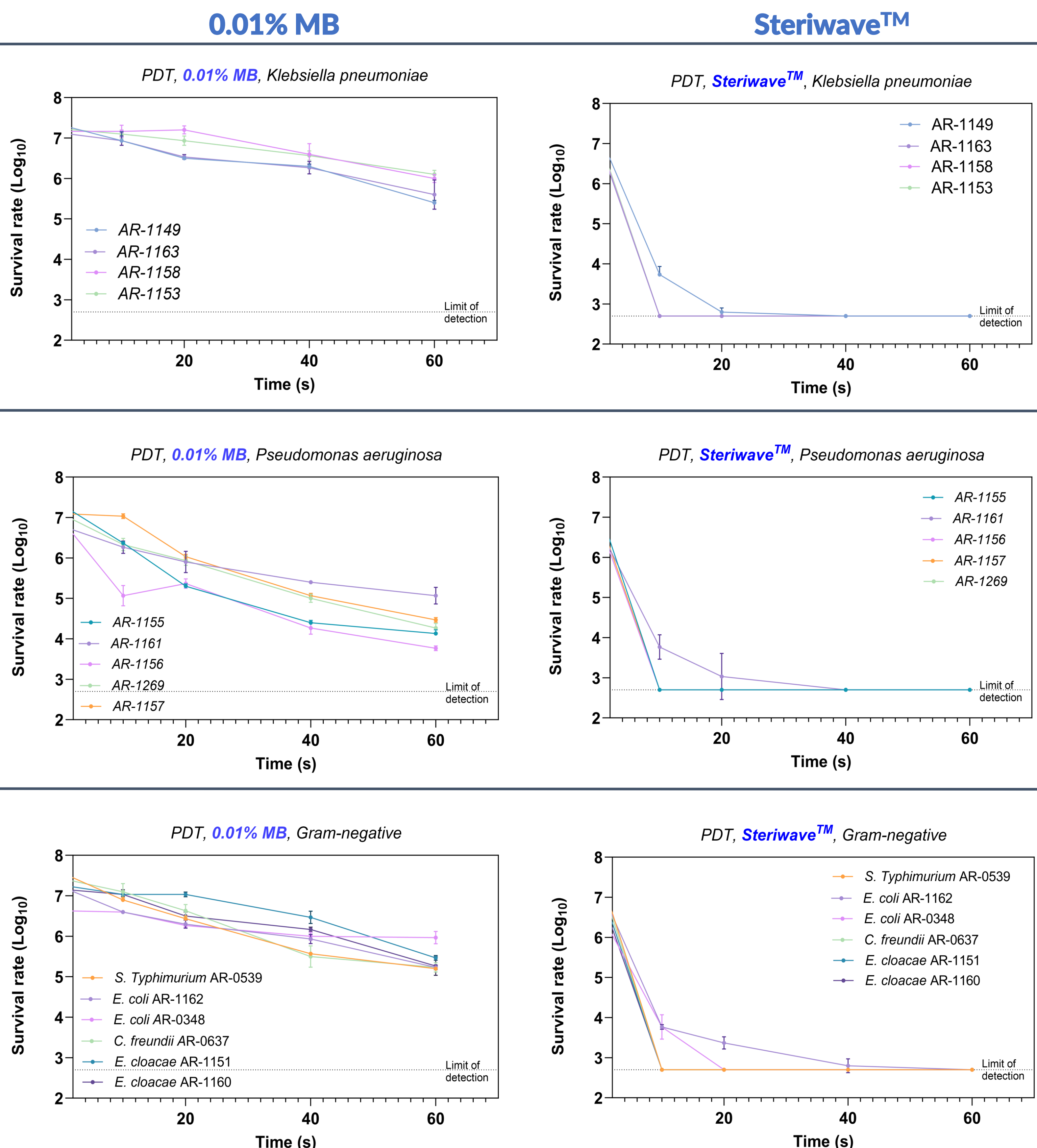


Figure 2. Dose-response PDT assessment of XDR Gram-negative isolates using 0.01% MB PS or commercially available PS.

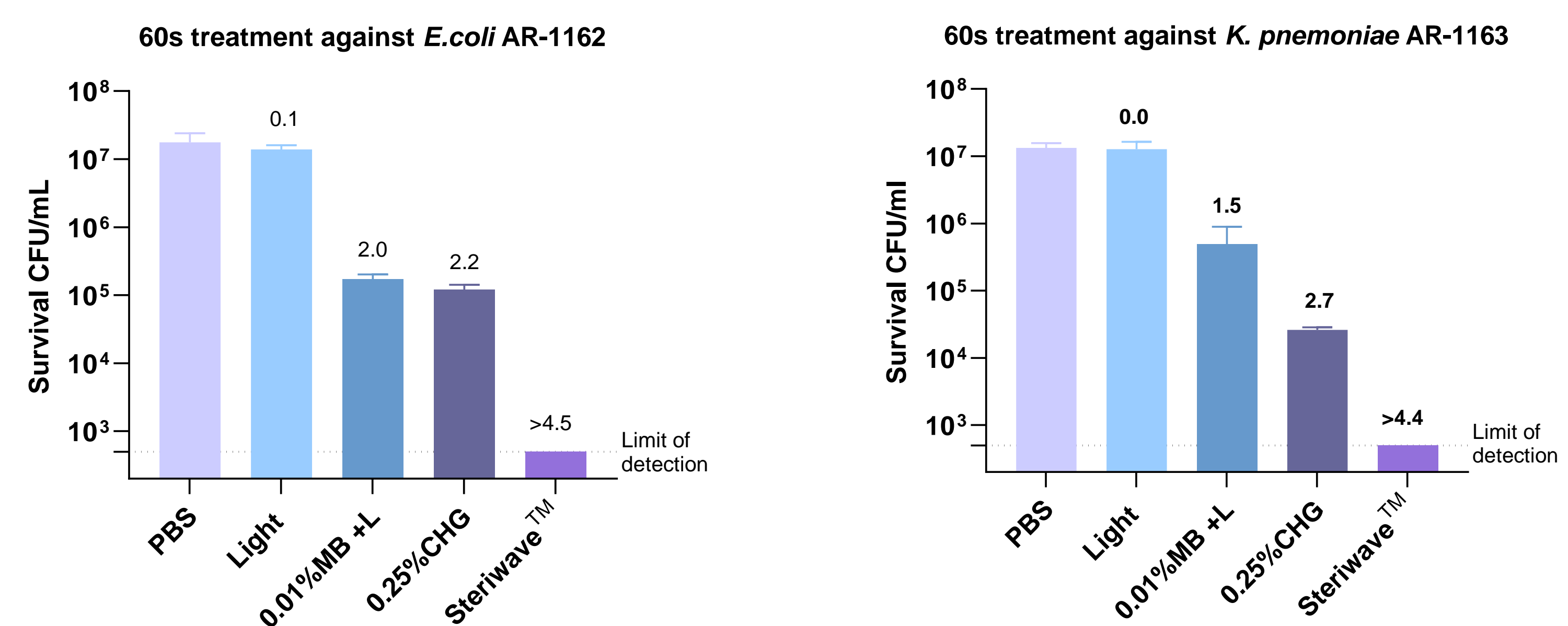


Figure 3. Effect of Steriwave™ components against *E. coli* and *K. pneumoniae*. (L = light). Data on bars represent Log₁₀ reduction with respect to control (PBS).

CONCLUSIONS

- aPDT with Steriwave™ formulation is highly effective against all tested XDR Gram-negative strains within less than 20 seconds of treatment.
- aPDT with Steriwave™ formulation promoted greater and faster bactericidal effects when compared to treatment by CHG alone or aPDT with MB alone, indicating a synergistic combination of both active components.
- aPDT provides a rapid, broad-spectrum, bactericidal alternative to antibiotics.

