# USE OF BACTERIAL OUTER MEMBRANE VESICLES IN COMBINED ANTIBIOTIC AND PHOTODYNAMIC THERAPY AGAINST ACINETOBACTER BAUMANNII

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*Acinetobacter baumannii* is a Gram-negative opportunistic pathogen, which emerged as a prevalent cause of nosocomial infections. Due to the rise of multidrug-resistant strains and high morbidity and mortality, *A. baumannii* has been added to the World Health Organization priority list of drug-resistant bacteria [1].

Emerging strategies to address this challenge include advanced drug-delivery systems. Outer membrane vesicles (OMVs) naturally shed by Gram-negative bacteria could aid in the efficient transfer of antibiotics due to their structural similarity to bacterial membranes [2]. Another approach is antimicrobial photodynamic therapy (aPDT), in which a visible-light-activated photosensitizer generates reactive oxygen species (ROS) that cause lethal oxidative damage to microbial cells. Because ROS target multiple biomolecular structures in a non-specific manner, including membranes, proteins, and nucleic acids, classical antibiotic resistance mechanisms are effectively bypassed, minimizing the likelihood of developing further resistance [3].

In this study, we compared the susceptibility of two *A. baumannii* strains to aPDT and antibiotic therapy used in combination with OMVs. Gentamicin, a widely prescribed aminoglycoside, was used on both strains. The antibiotic was loaded into OMVs using sonication. Photosensitizers for aPDT were chosen to be water soluble and non-toxic: chlorophyllin – a semi-synthetic chlorophyll derivative, and riboflavin - also known as vitamin B2. Samples were irradiated using an LED light source (350 $W/m^2$), with activation wavelengths of 402 nm for chlorophyllin and 440 nm for riboflavin. Photosensitizer concentrations used for biofilms were tenfold higher than those applied to planktonic cells. The effects of aPDT and OMV-mediated delivery of antibiotics on bacterial viability were assessed by counting colony-forming units (CFUs).

The results demonstrate a 1.58 and 1.77 $log_{10}$ CFU/mL decrease in planktonic cell viability after 4 h incubation with gentamicin encapsulated in OMVs compared to free gentamicin. The combination of aPDT and antibiotics reduced viability more (1.00–1.67 $log_{10}$ CFU/mL) than either aPDT (0.02–0.87 $log_{10}$ CFU/mL) or antibiotics alone (0.21–0.50 $log_{10}$ CFU/mL). Gentamicin administration either before or after aPDT produced comparable effects. In contrast, no additional benefit of OMV-mediated antibiotic delivery was observed in biofilm-associated cells, which may be due to extracellular matrix interference with OMV penetration. These findings indicate that OMV-based antibiotic delivery is more effective against planktonic cells than biofilm-associated cells, whereas aPDT provides the most significant benefit in combined treatments targeting biofilms.

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