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Antibacterial and anti-biofilm mechanisms of 1,8-cineole against colistin-resistant *Acinetobacter baumannii*: an integrated in vitro, gene expression, and in silico study

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The rise of colistin-resistant *Acinetobacter baumannii* (ColR Ab) poses a major clinical challenge, underscoring the urgent need for alternative antimicrobial agents. This study evaluated the antibacterial and anti-biofilm mechanisms of 1,8-cineole against ColR Ab using in vitro, transcriptional, and in silico approaches. Minimum inhibitory concentrations (MICs) and anti-biofilm activities were determined against three ColR Ab isolates. Membrane integrity was assessed via protein and nucleic acid leakage assays. Scanning electron microscopy (SEM) revealed bacterial morphology and biofilm structure, and expression of biofilm-associated genes was analyzed by qRT-PCR. Additionally, molecular docking analysis was employed to evaluate the binding affinity of 1,8-cineole against key target proteins of *A. Baumannii*. The inhibition zone in the disk diffusion method and MIC were 10.3 ± 0.8 mm and 3.6 mg/mL, respectively. 1,8-cineole also caused significant protein and nucleic acid leakage and, at 4× MIC, significantly disrupted the mature biofilm structure. SEM confirmed bacterial cell membrane rupture, intracellular content leakage, and ultrastructural damage following treatment. qRT-PCR validation demonstrated that 1,8-cineole significantly downregulated the expression of the *bfmR*, *bap*, *csuE*, *ompA*, and *pgaB*, while *abaI* expression was not significantly affected. Molecular docking simulations predicted favorable binding of 1,8-cineole to quorum-sensing regulators AbaR and LasR. 1,8-cineole exhibits significant bactericidal and anti-biofilm activity against ColR Ab. Its mechanism of action may involve disruption of cell membrane integrity and potential interference with bacterial key quorum-sensing regulators. This study provides a theoretical basis for developing 1,8-cineole as a potential therapeutic strategy against ColR Ab infections.

Keywords 1,8-Cineole, Eucalyptol, Colistin-resistant, *Acinetobacter baumannii*, Biofilm

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Acinetobacter baumannii is a major Gram-negative, aerobic, and non-motile microorganism responsible for healthcare-associated infections. Notably, this non-fermentative bacterium is characterized by its prolonged environmental persistence on solid surfaces and extensive natural and acquired antibiotic resistance profiles[1]. Multidrug-resistant (MDR) *A. baumannii* has emerged as a formidable clinical threat, frequently causing recalcitrant skin and soft tissue infections. This issue is especially problematic among burn victims, where the pathogen triggers severe secondary complications, including impaired wound healing, skin graft rejection, and life-threatening sepsis[2]. As a leading cause of healthcare-associated infections, this pathogen exhibits rapid dissemination within clinical environments, significantly elevating morbidity and mortality rates, especially among critically ill individuals. With MDR phenotypes emerging in up to 70% of clinical isolates in specific geographic regions, the World Health Organization (WHO) has designated *A. baumannii* as a top-tier critical priority pathogen, underscoring the pressing need for innovative therapeutic strategies[3,4].

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