

Terpinen-4-ol combined with colistin effectively impairs *Pseudomonas aeruginosa* biofilm formation

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Due to widespread and indiscriminate use of conventional antibiotics, bacteria have acquired a resistant phenotype in response to those antimicrobials pressure. Therefore, microbial infections associated with biofilms have become hard to treat with conventional therapy. The development of microbial drug resistance and drug-related toxicity has promoted the search for new alternatives, to control, mostly, healthcare-associated infections. Terpinen-4-ol is the major component of tea tree essential oil and has shown strong antimicrobial properties as a single agent against planktonic cultures. Colistin is an antimicrobial peptide with great antimicrobial activity, essentially against Gram-negative bacteria, such as *P. aeruginosa*.

The aim of this study was to explore the synergism between terpinen-4-ol and colistin, using low doses of each natural antimicrobial, to control the establishment of *P. aeruginosa* biofilms. Biofilms were formed in the presence of both antimicrobials, alone or in combination, being after characterized by total biomass, through crystal violet, and number of cultivable bacterial cells (log CFU/cm²).

Data related with the individual antimicrobial activity of terpinen-4-ol revealed that the biomass of *P. aeruginosa* biofilms was significantly reduced for 0.19 % (v/v), though it not affected the viability of cells even for the highest concentration tested (0.38 % (v/v)). On the other hand, colistin promoted a significantly reduction of the biofilm mass but only for concentrations higher than 1 µg ml⁻¹. The number of viable cells entrapped within the biofilms was only affect for colistin doses higher for 4 µg ml⁻¹. The association of terpinen-4-ol (0.19 % (v/v)) with colistin revealed to be a very efficient prophylactic strategy, as it impaired significantly biofilm formation. In fact, it was observed biofilm mass reductions closed to 100 % and significant decreases of the numbers of viable biofilm-cells (3-5 log of reduction) even for the lowest colistin concentration tested (0.5 µg ml⁻¹).

These data highlighted the promising antibiofilm activity of association of natural compounds, as antimicrobial peptides and secondary metabolites of essential oils, suggesting that this combination may have prophylactic potential for the prevention of *P. aeruginosa* biofilm-associated infections.

Keywords terpinen-4-ol; antimicrobial peptides; colistin; biofilms, prophylactic strategies, antimicrobial synergism

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