SYNERGISTIC EFFECT OF MONOTERPENE 1,8-CINEOLE WITH CIPROFLOXACIN ON CLINICAL ISOLATES OF *Klebsiella pneumoniae* IN PLANKTONIC AND BIOFILM GROWTH

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INTRODUCTION

Antimicrobial resistance is considered by the World Health Organization as one of the greatest threats to global health. In particular, the increase of antibiotic-resistant *Klebsiella pneumoniae* strains with the ability to form biofilms places this pathogen in the critical priority group for the development of new therapeutic strategies. The combined use of antibiotics, such as Ciprofloxacin (CPR), with natural compounds constitutes a promising alternative to address infections caused by this microorganism. The phytochemical 1,8-cineole (1,8-C) is a strong candidate as it exhibits antimicrobial activity against clinical strains of multi-drug-resistant (MDR) *K. pneumoniae,* both in planktonic and biofilm states.

OBJECTIVE: To evaluate the combined action of 1,8-C with a commonly used antibiotic (ciprofloxacin) against clinical isolates of *K. pneumoniae*, both in planktonic cultures and biofilms, in search of synergistic effects.

MATERIALS & METHODS

Bacterial strain: Clinical isolate of antibiotic-sensitive *K. pneumoniae* (Kp010) from urinary tract infection.

Minimal inhibitory concentration (MIC): Determined by measuring bacterial growth (OD_{600nm}) through the broth microdilution method using MH medium. **Inoculum**: $1x10^7$ UFC/ml.

Fractional inhibitory concentration (FIC): FIC of drug = MIC in combination / MIC alone.

Fractional inhibitory concentration index (FICI): FICI = FIC of antibiotic + FIC of phytochemical.

Synergistic interaction: FICI ≤ 0.5; Additive interaction: 0.5 ≤ FICI ≤ 1.0; Indifferent interaction: 1.0 ≤ FICI ≤ 2.0; Antagonistic interaction: FICI > 4.0.

Combenefit software: Used to visualize, analyze and quantify drug combination effects in terms of synergy, additivity and antagonism. Data processed using classical Synergy model BLISS for non-exclusive drug interactions.

Anti-biofilm effect: Assessed on pre-formed biofilms (24 h), which were challenged with increasing concentrations of CPR (ranging from 1/5 to 5 x planktonic MBC) and 1,8-C (ranging from ¼ to ½ x MBC), either alone or in combination. Biomass was measured by crystal violet staining, and cell viability was quantified by CFU counting.

RESULTS

Growth inhibition by ciprofloxacin and 1,8-C against *K. pneumoniae* planktonic growth

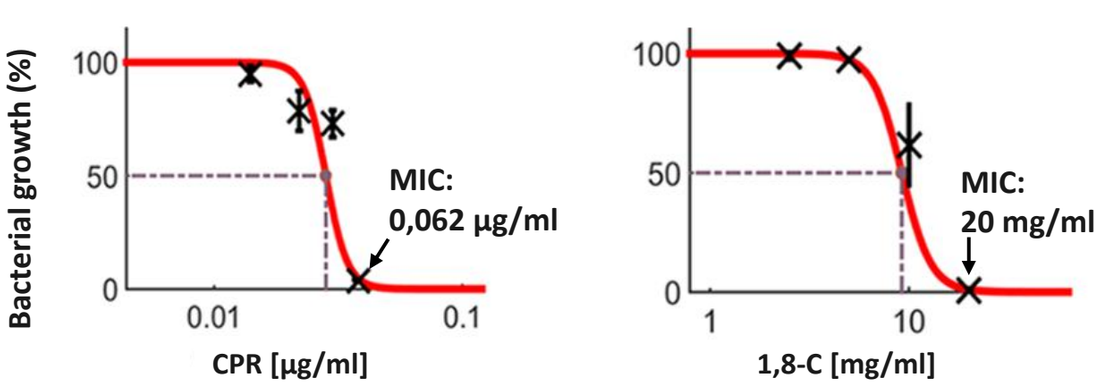

Figure 1: Ciprofloxacin and 1,8-C growth inhibition curves.

Synergistic effect of 1,8-C with ciprofloxacin

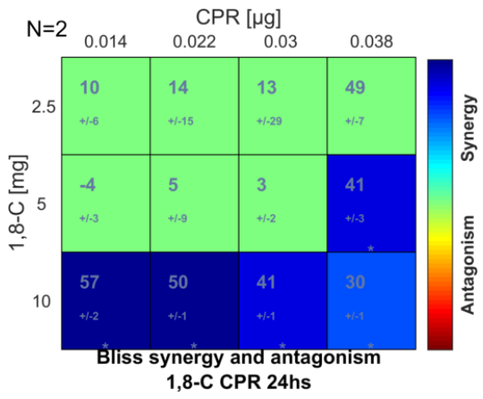

Figure 2: Ciprofloxacin and 1,8-cineole interaction matrix. The most remarkable synergistic effect occurred using 0,014 µg/ml CIP in combination with 10,0 mg/ml 1,8-C (97% of growth inhibition).

Organism	Agent	MIC alone	MIC in combination	FIC	FICI	Interaction
Kp010	CPR	0,062 µg/ml	0,014 µg/ml	0,226	0,351	**Synergistic**

Antibiofilm effect of 1,8-C in combination with ciprofloxacin

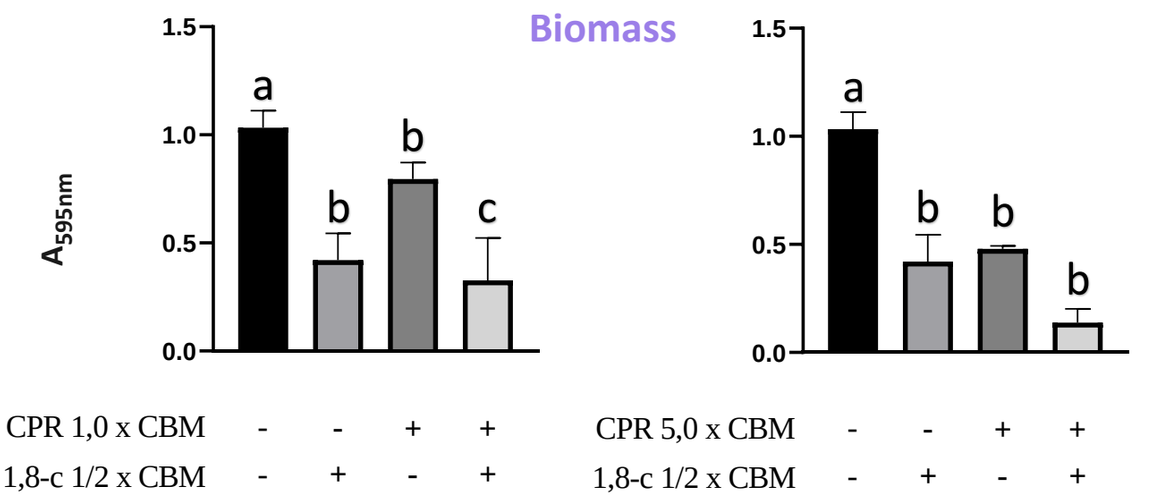

Figure 3A: Biomass quantification of biofilms challenged with different concentrations of CPR, combined or not with ½ x CBM of 1,8-C.

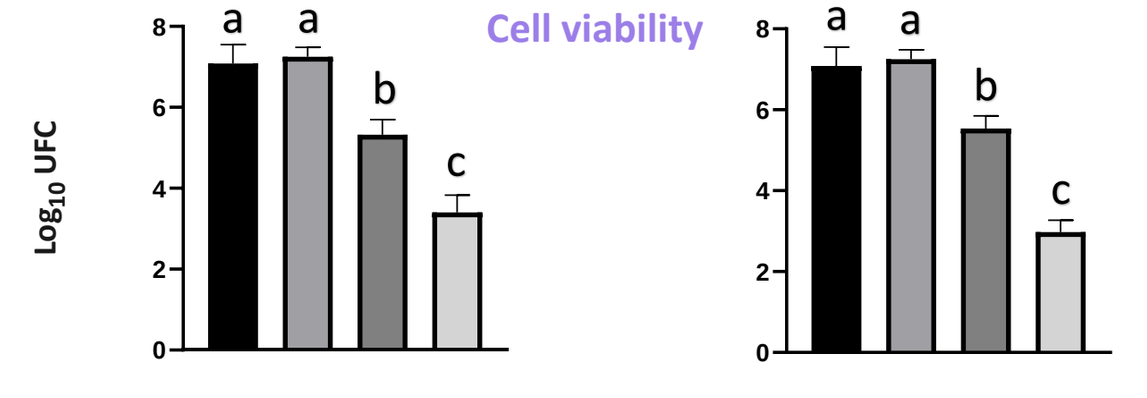

Figure 3B: Cell viability quantification of biofilms challenged with different concentrations of CPR, combined or not with ½ x CBM of 1,8-C.

CONCLUSIONS

- The CPR MIC was reduced fourfold when combined with 1,8-C under planktonic growth conditions. Furthermore, a synergistic interaction between ciprofloxacin and 1,8-C was demonstrated.
- The combination of ciprofloxacin and the phytochemical exhibited significant antibiofilm activity, resulting in a 90% reduction in biomass and a bactericidal effect (4 log_{10} reduction in CFU), compared to the individual compounds.
- These findings suggest that 1,8-C is a promising candidate for its combined application with clinically relevant antibiotics in the treatment of infections caused by *K. pneumoniae.*

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