

TITLE: COMBINED ANTIBIOFILM ACTIVITY OF TERPENE TERPINEN-4-OL AND CEFTAZIDIME AGAINST EFFLUX PUMP-PRODUCING *Klebsiella pneumoniae* ISOLATES

AUTHORS: AGRELES, M. A. A.; CONFESSOR, M. V. A.; CAMPOS, L. A. A.; CAVALCANTI, I. M. F.

INSTITUTION: INSTITUTO KEIZO ASAMI FROM UNIVERSIDADE FEDERAL DE PERNAMBUCO (ILIKA/UFPE), RECIFE, PE (PROF. MORAES REGO AVENUE, 1235, CEP 50670330, RECIFE – PE, BRAZIL)

ABSTRACT:

The irrational antibiotics use has led to bacterial resistance emergence and dissemination of its correlated genes, promoting high disease rate and increased hospitalization length. Some of the most threatening microorganisms are ESKAPEE group members, including *Klebsiella pneumoniae*, an important Gram-negative opportunistic bacterial pathogen, known to cause several infections, such as urinary tract infections, pneumonia, and blood infections. In these strains, efflux pumps expression is observed as one of the main resistance mechanisms, also playing an influential role in bacterial biofilm formation. There is a particular difficulty when treating infections associated with biofilms, hence the bacterial cells incorporated in the biofilm matrix can resist both immune defenses and antibiotics much more effectively. Terpenes are a broad and diverse class of hydrocarbons produced by a wide variety of plants that already have several proven pharmacological activities, such as antibacterial, antifungal, anti-inflammatory, and antitumor activity. Thus, this work aimed to investigate the terpene terpinen-4-ol action alone and in combination with the antibiotic ceftazidime (CAZ) against biofilms of efflux pump-producing *K. pneumoniae* resistant clinical isolates. Initially, the identification of isolates carrying the *acrB* gene was performed through PCR. Subsequently, the Minimum Biofilm Inhibitory Concentration (MBIC) and the biofilm eradication percentage of the compounds were determined by the crystal violet method. Four of the five isolates identified showed biofilm production ranging from moderate to strong, being selected for further experiments. The MBIC of CAZ for these isolates ranged from 16 to 512 µg/mL, while the MBIC of terpinen-4-ol ranged from 500 to 2000 µg/mL. The maximum biofilm eradication percentages at 16xMIC of the compounds in these isolates were 1, 15, 18 and 27% for CAZ and 15, 34, 51 and 52% for terpinen-4-ol. When CAZ was combined with terpinen-4-ol, the MBIC was reduced to 2 µg/mL for CAZ and 4 µg/mL for terpinen-4-ol against the isolates tested. Additionally, after combination, the maximum biofilm eradication percentages were 51, 59, 62 and 65%. Thus, the association of CAZ with terpinen-4-ol caused MBIC values reduction and greater activity against mature biofilms, indicating that this compound can act synergistically with antibiotics, enhancing their antibiofilm activity.

Keywords: synergy, biofilm, bacterial resistance, Gram-negative bacteria.

Development Agency: This study was partially funded by Notice Propesqi No. 09/2021 - Qualified Production Support Notice of the Federal University of Pernambuco (UFPE) and Scientific Initiation Scholarship Program of the Pernambuco Foundation for Science and Technology.