

TITLE: ANTIMICROBIAL POTENTIAL OF EXTRACELLULAR METABOLITES FROM *Burkholderia pyrrocinia* RV1R2 STRAIN AGAINST HUMAN PATHOGENS RELATED TO HOSPITAL-ACQUIRED INFECTIONS

AUTHORS: AFONSO, L.; NAVARRO, M.O.P.; SIMIONATO, A. S.; DEALIS, M.L.; GIONCO-CANO, B.; ANDRADE, G.

INSTITUTION: UNIVERSIDADE ESTADUAL DE LONDRINA, LONDRINA, PR (RODOVIA CELSO GARCIA CID, KM 380, S/N, CEP 86057-970, LONDRINA – PR, BRAZIL).

ABSTRACT:

Antimicrobial resistance is occurring globally, compromising our ability to treat infectious diseases. The low effectiveness of the antimicrobials suggest needs of new compounds for the control of important human pathogens, mostly those related to hospital infections, such as *Staphylococcus aureus* (Sa), *Klebsiella pneumoniae* (Kpn) and *Candida albicans* (Ca), considered of high prevalence. The research of new antimicrobials is crucial and natural products of microbial origin are attractive. *Burkholderia* is a genus of Gram-negative bacteria able to occupy different habitats due its extraordinary metabolic capacities modulated by the environments where are located. The purpose of this study was to evaluate the antimicrobial potential of metabolites from *Burkholderia pyrrocinia* RV1R2 strain against the pathogens Sa, Kpn and Ca. RV1R2 was isolated of Rio Verde - Itararé/SP-Brazil and identified by 16S rRNA gene sequencing in previous studies. The bacteria were cultured in 1 L of nutrient broth using inoculum of 1% v/v (1.5×10^8 CFU/mL) and incubated for 168 hours, 28°C and 170 rpm. Supernatant was obtained by centrifugation and then dried. Metabolites were separated by partition with ethyl acetate (2:1), performed three times. The ethyl acetate extract (EAE) was concentrated and resuspended in dimethyl sulfoxide. Antimicrobial activity was evaluated by broth microdilution method to define minimum inhibitory concentration (MIC) in a 96 well plate according to the CLSI protocols. Were tested the concentrations ($\mu\text{g/mL}$): 1024, 512, 256, 128, 64, 32, 16, 8, 4 and 2, against Sa (ATCC 29213), Kpn (ATCC 700603) and Ca (ATCC 26790) cultivated on Mueller Hinton agar (MHA) for 24 hours and 37°C. The inoculum was adjusted to 5×10^5 CFU/mL, positive and negative growth controls were established and incubation was performed for 48 h at 37°C. After incubation, 10 μL aliquots were used to verify bactericidal or bacteriostatic activity on plates containing MHA, incubated for 24 h at 37°C. EAE demonstrated MICs of 4 $\mu\text{g/mL}$ for Sa, 32 $\mu\text{g/mL}$ for Kpn and 128 $\mu\text{g/mL}$ for Ca. MIC for all microorganisms exhibited microbicidal activity and no microbiostatic effect was observed. The metabolites of *Burkholderia pyrrocinia* demonstrated antibacterial and antifungal activity, being a promising microorganism for development of bioactive molecules against human pathogens, mainly Sa, and these results open possibilities for characterization and antimicrobial activity studies of these metabolites.

Keywords: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, minimum inhibitory concentration.

Development Agency: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).