TITLE: SYNERGISTIC ACTIVITY OF RIFAMPICIN COMBINED WITH MINIMAL EFFECTIVE ANTIBIOTIC CONCENTRATION OF POLYMYXIN B AGAINST EXTENSIVELY DRUG-RESISTANT (CARBAPENEM- AND POLYMYXIN B-RESISTANT) *Klebsiella pneumoniae* CLINICAL ISOLATES

AUTHORS: ABICHABKI, N.[1]; GASPAR, G. G.[2,3]; POCENTE, R. H. C.[2]; LIMA; D. A. F. S.[2]; FREITAS, N. A.B.[2]; MOREIRA, N. C.[1]; DARINI, A. L. C. D.[1]; BELLISSIMO-RODRIGUES, F.[3]; BOLELLA, V. R.[2,3]; ANDRADE, L. N.[1]

INSTITUTIONS: [1] SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO – UNIVERSITY OF SÃO PAULO; [2] UNIVERSITY HOSPITAL, RIBEIRÃO PRETO MEDICAL SCHOOL – UNIVERSITY OF SÃO PAULO; [3] RIBEIRÃO PRETO MEDICAL SCHOOL – UNIVERSITY OF SÃO PAULO.

ABSTRACT:

Introduction: Polymyxin B (PB) has been used to treat infections due to extensively drug-resistant (XDR), including carbapenem-resistant, Gram-negative bacilli (GNB); however, acquired resistance to polymyxins has been increasingly detected in GNB. Rifampicin (RIF) is often used for treating *Mycobacterium* and Gram-positive cocci infections, although it is not used against GNB due to low RIF outer membrane penetration, which reflects in high minimal inhibitory concentrations (MIC). **Objective:** In this context, the objective of this study was to investigate the *in vitro* antibacterial activity of the combination RIF + PB against XDR (carbapenem- and PB-resistant) *Klebsiella pneumoniae* clinical isolates. **Material and methods:** We evaluated ten bacteria isolated from inpatients with bloodstream, pulmonary, and urinary tract infections. All isolates were resistant to PB (non-*mcr* carriers) and to carbapenems (KPC, CTX-M, and SHV producers; among which one strain co-produced NDM and was also resistant to ceftazidime-avibactam), harbored many other resistant determinants to different classes of antibiotics, showed XDR phenotype, and belonged to ST16, ST11, ST258, and ST437. We used the standard broth microdilution method to determine the RIF and PB MIC values, and the checkerboard assay to evaluate the fractional inhibitory concentration index (FICI) of RIF + PB as well as to investigate the lowest concentrations of RIF and PB, that combined (RIF + PB) have antibacterial activity. **Results:** PB MIC (32 – 256 µg/mL) and RIF MIC (32 – 1024 µg/mL) were determined for the isolates studied. For all isolates evaluated, FICI (<0.5) indicated a synergistic effect for the combination RIF + PB. Our results showed that low concentrations of PB (≤0.25 – 1 µg/mL) outstandingly favor rifampicin (≤0.03 – 0.125 µg/mL) to reach the bacterial target and exert antibacterial activity. The low concentrations of PB correspond to the minimal effective antibiotic concentration (MEAC), defined as the sublethal (subinhibitory) concentration that produces any effect on bacterial cells (e.g., outer membrane destabilization, but not killing). **Conclusion:** The combination RIF (≤0.125 µg/mL) + PB (≤1 µg/mL) showed *in vitro* antibacterial activity against XDR (carbapenem- and PB-resistant) *K. pneumoniae* clinical isolates and could be further explored as potential combination therapy in GNB infections.

Keywords: antibiotic combination, polymyxin B resistance, rifampicin.

Development Agency: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. CAPES Grant 88887.670254/2022-0 (NA).