TITLE: EVALUATION *in vitro* OF ANTIBIOTIC THERAPY AGAINST CLINICAL ISOLATES OF *Klebsiella pneumoniae* CARRIERS OF *bla*$_{NDM,}$ *bla*$_{KPC}$, *aac(6)-Ib* and *aph(3)-VI* GENE AND POLYMYXIN-B-RESISTANT

AUTHORS: FIRMO, E. F.;[1] LIMA, F. C. G.;[1], OLIVEIRA-JÚNIOR, J.B.;[1] BELTRÃO, E.M.B.;[1], OLIVEIRA, E.M.;[1], ALVES, L. C.; [1,2] BRAYNER, F. A.[1,2]; VERAS, D.L.[1,2], LOPES, A. C. S.;[1].

INSTITUTION: [1]UNIVERSIDADE FEDERAL DE PERNAMBUCO (AV. PROF. MORAES REGO, 1235 - CIDADE UNIVERSITÁRIA, RECIFE/PE, CEP 50670-901, BRAZIL). [2]INSTITUTO AGGEU MAGALHÃES – IAM/FIOCRUZ-PE (AV. PROF. MORAES REGO, S/N - CIDADE UNIVERSITÁRIA, RECIFE/PE, CEP 50670-420, BRAZIL).

ABSTRACT:
Multidrug-resistant *Klebsiella pneumoniae* and carriers of *β*-lactam antibiotics resistance genes, such as *bla*$_{NDM}$ e *bla*$_{KPC}$, cause healthcare-associated infections worldwide, and are highly relevant due to therapeutic limitations. In the search for effective treatment options, the use of combination therapy based mainly on the use of polymyxins in combination with aminoglycosides or carbapenems has been indicated by the Agência Nacional de Vigilância Sanitária (Anvisa) in Brazil. Therefore, the aim of this study was to evaluate *in vitro* the polymyxin-B (PB) activity combined with meropenem (MEM), amikacin (AMK) and gentamicin (GEN) in two clinical isolates of *K. pneumoniae* carriers of *bla*$_{NDM,}$ *bla*$_{KPC}$, *aac(6)-Ib e aph(3)-VI* genes from patients at a hospital in Recife-PE, Brazil. The combinations of antimicrobial (PB/MEM, PB/GEN and PB/AMK) were tested in two clinical isolates by the checkerboard. The variation of antimicrobial concentrations was determined from the Minimum Inhibitory Concentration (MIC) in all assays. MIC was defined as the lowest concentration of combinations, in which no visible growth was observed and the Fractional Inhibitory Concentration (FIC) was determined. The FIC values $\leq$ 0.5, 0.5 - 4 and $\geq$ 4 were considered synergistic, indifferent and antagonistic, respectively. Two isolates showed resistance to gentamicin, meropenem and polymyxin-B, and sensitivity to amikacin. Synergisms of PB/MEM, PB/GEN and PB/AMK were observed in both clinical isolates, in addition, there was a decrease in MIC, physiologically achievable concentrations. In both isolates, the combination of polymyxin-B with amikacin was more efficient. Accordingly, the use of these combinations may contribute to the therapy of patients with infections caused by multidrug-resistant *K. pneumoniae*, particularly carriers of *bla*$_{NDM}$ e *bla*$_{KPC}$. genes.

Keywords: NDM, checkerboard, synergism, *Klebsiella pneumoniae*

Development Agency: Programa de Pesquisa para o SUS (PPSUS) – Ministério da Saúde – Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).