TITLE: POLYMYXIN RESISTANCE AND HETERORESISTANCE IN CARBAPENEMASE PRODUCING *Klebsiella pneumoniae*

AUTHORS: QUEIROZ, P.A.; SILVA, B.R.; GIRARDI, L.M.; PÁDUA, R.A.F.; CALEFFI-FERRACIOLI, K.R.; SCODRO, R.B.L.; CARDOSO, R.F.; SIQUEIRA, V.L.D.

INSTITUTION: UNIVERSIDADE ESTADUAL DE MARINGÁ, AVENIDA COLOMBO, 5790, CEP: 87020-900, MARINGÁ, PARANÁ, BRASIL.

ABSTRACT:

Antibiotic resistance is a growing crisis and a grave threat to human health. It is projected that antibiotic-resistant infections will lead to 10 million annual deaths worldwide by the year 2050. *Klebsiella pneumonia* is emerging as an important nosocomial pathogen due to its rapidly increasing multidrug resistance, which has led to a renewed interest in polymyxin antibiotics, such as polymyxin B and colistin, as antibiotics of last resort. However, resistance to polymyxins is increasing and heteroresistance (i.e., the presence of a sub-population of resistant bacteria in another wise susceptible culture) may hamper the effectiveness of polymyxin treatment in patients. The aim of the present study was to detect the presence of polymyxin resistance and heteroresistance among *K. pneumonia* carbapenemase producers. We evaluated 75 clinical isolates (72 carried *bla*$_{KPC}$ and 3 carried *bla*$_{NDM}$ genes) identified in the Laboratory of Teaching and Research in Clinical Analysis of State University of Maringá, Paraná. The polymyxin B minimum inhibitory concentration (MIC) were determined by a broth microdilution method using cation-adjusted Mueller-Hinton broth and interpreted according EUCAST breakpoints. Isolates with a MIC ≤2 mg/mL were considered susceptible. For heteroresistance detection, resistance induction was performed in cultures with increasing concentration of polymyxin B sulfate. The MIC was confirmed by broth microdilution method and heteroresistant isolates were submitted to the subculture on agar in the absence of polymyxin B. Among the 75 isolates, 11 (14,67%) were resistant to polymyxin and showed variable levels of resistance, ranging 8 to >128 µg/mL. Ten isolates with an initial polymyxin MIC = 0.5 µg/mL were selected for heteroresistance research. After induction, five isolates presented heteroresistant profiles, with levels of resistance ranging 64 to 128 µg/mL. None of the isolates tested reversed their resistance profile after cultivation in the absence of the polymyxin. Heteroresistance may cause *in vivo* treatment failure during *K. pneumonia* infection. These data sound the alarm for rates of polymyxin resistance in *K. pneumonia* producing carbapenemases and for use of caution in interpreting polymyxin susceptibility test results, as isolates identified as susceptible may in fact resist antibiotic therapy and lead to unexplained treatment failures.

Keywords: *Klebsiella pneumoniae*, antibiotic resistance, heteroresistance, polymyxin.