TITLE: BIOFILM FORMATION OF MULTIDRUG RESISTANT KPC-2-PRODUCING *KLEBSIELLA PNEUMONIAE* RECOVERED FROM SEVERAL BRAZILIAN REGIONS

AUTHORS: ARAUJO, B.F.[1]; FERREIRA, M.L.[1]; CAMPOS, P.A.[1]; ROYER, S.[1]; GONÇALVES, I.R.[1]; BATISTÃO, D.W.F.[1]; BRITO, C.S.[1]; MELO, G.N.[1]; GONTIJO-FILHO, P.P.[1]; RIBAS, R.M.[1]

INSTITUTION: 1. FEDERAL UNIVERSITY OF UBERLÂNDIA – UFU, UBERLÂNDIA/MG (Av. Pará, 1720, Umuarama, Uberlândia-MG. CEP: 38400-902). Brasil.

ABSTRACT

Bacterial biofilm has a significant impact due to their rapid growth in medical devices, their resistance to antimicrobials and the ability to disseminate infections. This study evaluated the biofilm formation ability of 22 *Klebsiella pneumoniae* clinical strains, isolated in majority from blood and urine, from different regions of Brazil (Minas Gerais, Rio Grande do Sul, Paraná, Goiás, Paraíba, Rio Grande do Norte and Distrito Federal). The strains presented different molecular characteristics and Pulsed Field Gel Electrophoresis (PFGE) patterns. The initial adhesion, biofilm formation and biomass were examined by quantitative assays in replicates, in three independent experiments. The comparison among the strains was performed using the Kruskal-Wallis statistical test and the Dunn's multiple comparison tests, considering significant $P \leq 0.05$. The beta-lactamase resistance genes *bla*$_{KPC}$, *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ were evaluated by polymerase chain reaction (n=13) or by whole-genome sequencing (n=9). All the strains showed the ability to adhere to the polystyrene surface, but only 50% of them had significance in adhesion when compared to the control. Although we observed an unusual behaviour of the strains recovered from different Brazilian regions when compared to Uberlândia, in relation to viable cell counts in biofilm formation, the same was not observed regarding the biomass production. Surprisingly, 59.1% of the strains were characterized as strong or moderate biofilm producers, although only 27.3% produced more biomass when compared to the control, none of which presented significant statistical values of viable cell numbers (CFU/mL) compared to control. No relationship was observed between resistance genes and KPC-production compared to biofilm formation, except for colistina-resistant strains, which were all classified as strong biofilm producers, belonging to a single clone (clone A). There was no relationship between the ability to biofilm formation and the multidrug resistance phenotype. This study shows that biofilm producers were present in most of the analyzed strains independently of origin, phenotype and clone, suggesting that biofilm plays an important role in the virulence and persistence of highly resistant strains from the hospital environment.

Keywords: *Klebsiella pneumoniae*, biofilm, resistance, PCR, whole-genome sequencing

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