Title: QUANTITATIVE ANALYSIS OF BIOFILM FORMATION IN MULTIDRUG RESISTANT *Acinetobacter baumannii* AND *Klebsiella pneumonia* STRAINS: GENOTYPIC CHARACTERISTICS AND CLONAL PROFILES

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Abstract:

This study evaluated the biofilm formation ability of clinical and environmental *A. baumannii* and *K. pneumoniae* strains, isolated from various sources and presenting different molecular characteristics, resistance profiles and PFGE patterns. Fifty-three isolates, 23 *A. baumannii* and 30 *K. pneumoniae*were recovered from 2009 to 2014 in a Brazilian university hospital. In *A. baumannii* the following genes were evaluated $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-143}}$, IS*Aba1* (Insertion Sequence), IS*Aba1*/$bla_{\text{OXA-23-like}}$, IS*Aba1*/$bla_{\text{OXA-51-like}}$, *carO* (29kDa) and OMP 33–36 kDa; and in *K. pneumoniae* the resistance genes bla_{KPC}, bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{ampC} and the virulence genes *khe*, *fimH*, *iucC*, *mrkD*, *rmp*, *wabG*, *ecpA*, *fimA* were evaluated. Investigation of biofilm formation was performed for ten strains of each species assessed by an initial adhesion assay, biofilm cell concentration and biofilm biomass, evaluated by quantitative assays. All strains of *A. baumannii* were able to attach to polystyrene plates, although two strains adhered to a lesser degree than the control (P<0.001). *K. pneumoniae* strains showed opposite behaviour, where only three strains adhered significantly when compared to the control (P<0.001). Regarding the amount of cells in the biofilm, in general, both *A. baumannii* and *K. pneumoniae* strains showed a large amount of cells compared with the control. Quantitativeevaluation revealed that in five *A. baumannii*and four *K. pneumoniae* isolates the biomass production could be characterised as moderate. None of the isolates were strong biofilm producers and only one strain of *K. pneumoniae* was a non-biofilm producer. Although 85% of the strains evaluated had been characterised as multidrug resistant, there was no significant association between this phenotype and the production of biofilm, and there was no association between the pulsotype and the ability to form biofilms. Our results demonstrate: (1) biofilm formation is a heterogeneous property amongst *A. baumannii* and *K. pneumoniae* clinical strains and it was not associated with certain clonal types; (2) No relationship between multidrug resistance and biofilm production was observed; (3) more virulent *K. pneumoniae* strains tended to present higher production of biofilm.

Keywords: *Acinetobacter baumannii*, *Klebsiella pneumoniae*, biofilm, resistance.

Funding Source: FAPEMIG.