TITLE: ADHESION AND BIOFILM FORMATION BY KPC PRODUCING *Klebsiella pneumoniae* ASSOCIATED TO URINARY TRACT INFECTIONS IN A BRAZILIAN TERTIARY HOSPITAL

AUTHORS: TOMAZ, F. M. M. B. [1]; ANDRADE, B. S. [1]; ANDRADE, L. K. [1]; CASELLA, T. [1] ; GAZAL, L. E. S. [3]; KOBAYASHI, R. K. T. [3]; MARTINS, E. R. [1]; RIBEIRO, T. R. M. [2]; MOREIRA, C. G. [2]; NOGUEIRA, M. C. L. [1]

INSTITUTION: [1]FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO, SÃO JOSÉ DO RIO PRETO, SÃO PAULO (AVENIDA BRIGADEIRO FARIA LIMA, 5416, CEP15090-000, SÃO JOSÉ DO RIO PRETO SÃO PAULO-SP, BRAZIL); [2]UNIVERSIDADE ESTADUAL PAULISTA JÚLIO DE MESQUITA FILHO, FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE ARARAQUARA, SÃO PAULO (ROD. ARARAQUARA, JAÚ KM 1 – MACHADOS, CEP 14800-901 ARARAQUARA, SÃO PAULO – SP, BRAZIL); [3]UNIVERSIDADE ESTADUAL DE LONDRINA, CENTRO DE CIÊNCIAS BIOLÓGICAS, PARANÁ (RODOVIA CELSO GARCIA CID, PR-445, KM 380 - CAMPUS UNIVERSITÁRIO, CEP 86057-970, PARANÁ-PR, BRAZIL)

ABSTRACT:

In the last decade, the emergence and fast dissemination of KPC producing *K. pneumoniae* became an important clinical challenge, since infections by these bacteria are difficult to treat and lead to poor patient outcomes and high mortality. Hereby, we present the characterization of KPC-2 producing *K. pneumoniae* identified as agents of urinary tract infections in patients admitted to 980 beds tertiary hospital in São José do Rio Preto, São Paulo – Brazil, in 2015 and 2016. Ninety one isolates were submitted to PCR using previously described primers and protocols to investigate the presence of several virulence genes (*fimH*, *kpn, mrkD, magA*, *k2A*, *wcaG*, *wabG*, *uge*, *ycfM*, *entB, iroN, iutA*, *hly*, *cnf-1, magA*, *rpmA*) and were and submitted to molecular typing by XbaI-PFGE. After this, 19 isolates of different pulsotypes and presenting the wider combination of virulence genes were submitted to Multilocus Sequence Typing (MLST) and evaluation of its ability to produce biofilms in an abiotic surface. Also, 17 of these isolates were analyzed for the capacity to produce adhesion in Vero cells. For evaluation of biofilms isolates were previously cultivated in LB broth at 37°C and placed in 96-wells plates at 1:100 in DMEM. Wells were stained with crystal violet and O.D. was measured at 550_{nm}. For the adhesion assays Vero cells were cultiveted in DMEM containing 10% of bovine fetal serum in 24-well plates. Overnight bacterial cultures grown shaking at 37°C were used for Vero cell infection at a multiplicity of infection (MOI) of 100:1 and after incubated at 37°C in an atmosphere of 5% CO2 for 3 hours. After 3 hours of incubaton, monolayers were washed with PBS, fixed with methanol, stained with May Grünwald-Giemsa stain and examined by light microscopy. All phenotypic assays were performed in triplicate. For the biofilm formation assays, an strong biofilm producer (enteroagregative *E. coli* - EAEC 042) and a weak biofilm producer (*K. pneumoniae* ATCC BAA 700603) were used as controls and for comparison purposes. The *ycfM* gene was identified in 100% of isolates followed by *entB* and *wabG* in 98, 9%, *fimH* and *mrkD* in 95, 6%, *kpn* in 92,3% and *uge* in 81,3%. Two isolates (Kp01 and Kp02) formed a thick biofilm similar to EAEC 042, while others presented significant reduction in ability for biofilm formation ($P<0,0001$ and $P< 0,01$). Six isolates (Kp01, Kp02, Kp04, Kp07, Kp09, Kp13) formed a strong biofilm when compared to Kp ATCC BAA 700603 ($P<0,001$). The KP04 showed an intermediate capacity for biofilm formation ($P<0,05$). The other strains did not present significant statistical differences. Thirteen isolates were identified as ST11, 1 as

ST340, 1 as ST437 and 1 as ST 1647. It was interesting to notice that all 17 isolates showed a remarkable agregative pattern of adhesion in Vero cells. KPC-2 producing *K. pneumoniae* associated to UTI harbor important virulence genes, present strong biofilm production and adhesion in Vero cells capability. Further studies should be performed to verify the invasion capacity of these isolates.

Keywords: Virulence, *Klebsiella pneumoniae*, Biofilm.

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