TITLE: IDENTIFICATION AND RESISTANCE GENES CHARACTERIZATION OF KPC-2-PRODUCING K. VARIICOLA AND K. QUASIPNEUMONIAE ISOLATED FROM HOSPITALS OF SAO PAULO STATE, BRAZIL**.**

AUTHORS: BUENO, M.F.C[1,2].; FRANCISCO, G.R.[1,2]; GARCIA, D.O.[1]

INSTITUTION: [1]INSTITUTO ADOLFO LUTZ, SÃO PAULO, SP, BRAZIL; [2]PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS. CENTRO DE CONTROLE DE DOENÇAS (CCD)-SECRETARIA DE SAÚDE DO ESTADO DE SÃO PAULO, BRAZIL. **;**

ABSTRACT:

Klebsiella pneumoniae is an opportunistic pathogen associated with nosocomial infections and resistance to multiple antibiotics being previously subdivided into phylogenetic groups named KpI (*K. pneumoniae*), KpII-A (*K. quasipneumoniae* subsp. *quasipneumoniae*), KpII-B (*K. quasipneumoniae* subsp. *similipneumoniae*) and KpIII (*K. variicola*). *K. pneumoniae* was shown to be associated with cromossomal *bla*$_{SHV}$, whereas *K. quasipneumoniae* with *bla*$_{OKP}$ and *K. variicola* with *bla*$_{LEN.}$ Adonitol and L-sorbose fermentation and the ability to utilize tricarballylic acid as a single carbon source can be considered as differential for the identification of *Klebsiella* species. The presence of KPC, NDM and CTX-M in *K. variicola* and *K. quasipneumoniae* has already been reported. In a previously study, 100 KPC-2-producing *Klebsiela* spp. strains isolated in the period of 2009 and 2011 from hospitals at Sao Paulo State were characterized as ESBL-producing. Three strains (Kp259/09, Kp492/11 and Kp672/11) were negative for cromossomal *bla*$_{SHV}$ and were selected for further investigation. The aim of this study was to identify and to characterize ESBL and carbapenemases resistance genes in *Klebsiella* spp. strains negative for cromossomal *bla*$_{SHV.}$ Identification of *Klebsiella* spp. by biochemical tests, detection of *bla*$_{SHV}$, *bla*$_{LEN}$ and *bla*$_{OKP}$ by multiplex PCR and 16S rRNA gene and *rpoB* sequencing was performed. ESBL and carbapenemases genes detection were assessed by PCR and DNA sequencing. Kp259/09 was adonitol-negative and L-sorbose-positive while Kp492/11 and Kp672/11 were adonitol-positive and L-sorbose-negative. All strains were positive for tricarballylic acid utilization. Multiplex PCR detection of *bla*$_{SHV}$, *bla*$_{OKP}$ and *bla*$_{LEN}$ resulted in the presence of *bla*$_{LEN}$ in Kp259/09 and *bla*$_{OKP}$ in Kp472/11 and Kp672/11 identifying along with biochemical tests Kp259/09 as a *K. variicola* and Kp492/11 and Kp672/11 as *K. quasipneumoniae.* These results were confirmed by *rpoB* and 16S rRNA gene sequencing. Co-production of KPC-2 and CTX-M-8 were observed in all strains. *K. variicola* and *K. quasipneumoniae* isolates have been shown to have the same antimicrobial resistance genes and capacity to cause infections as *K. pneumoniae*. Difficulty in *Klebsiella* spp differentiation may lead to an underestimated infection prevalence and improvement of *K. variicola* and *K. quasipneumoniae* detection tools will contribute to a better understanding of the epidemiological and dissemination profile of this *Klebsiella* species.

Key words: *K. variicola, K. quasipneumoniae,* KPC

FINANCIAL SUPPORT: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)