TITLE: DEVELOPMENT OF A ONE-STEP MULTIPLEX PCR FOR THE PROPER IDENTIFICATION OF *KLEBSIELLA PNEUMONIAE*, *KLEBSIELLA VARIICOLA*, AND *KLEBSIELLA QUASIPNEUMONIAE* IN THE CLINICAL ROUTINE

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ABSTRACT

The opportunistic pathogen *Klebsiella pneumoniae* has been classified in three phylogenetic groups KpI, KpII-A/KpII-B, and KpIII, and the chromosomal class A β-lactamase bla_{SHV}, $bla_{OKP\text{-}A}$/$bla_{OKP\text{-}B}$ and bla_{LEN} genes had been directly associated with each of these groups, respectively. Recently, it has been shown that these groups corresponded to the species *K. pneumoniae* (KpI), *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* (KpII-A), *Klebsiella quasipneumoniae* subsp. *Similipneumoniae* (KpII-B) and *Klebsiella variicola* (KpIII). However, several of the current phenotypic tests are unable to distinguish efficiently these *Klebsiella* species, leading to an underestimation of *K. variicola* and *K. quasipneumoniae* infections, since they are often misidentified as *K. pneumoniae.* Therefore, the routine identification of *Klebsiella* species remains difficult to be determined. Due to the impact of *K. variicola* and *K. quasipneumoniae* on clinics, and the lack of a suitable identification approach for microbiological routine, we proposed here a one-step multiplex PCR scheme targeting the chromosomal *bla* genes for rapidly and accurately distinguishing *Klebsiella* species. Alleles of bla_{SHV}, bla_{LEN} and bla_{OKP} were retrieved from GenBank and from specific databases and a multiple alignment was performed using BioEdit software. Based on this alignment, universal primers were designed using the Primer3 software. These primers targeted each specific *bla* and the chromosomal gene flanking them (*deoR*) in the three *Klebsiella* species. This strategy avoids the recovery of plasmid-borne bla_{SHV} alleles, and only the chromosomal species-specific *bla* gene would be amplified. The primers were applied in a multiplex PCR scheme for the identification of *K. pneumoniae*, *K. variicola* and *K. quasipneumoniae* according to the obtained amplicon size. The specificity and reproducibility of the PCR scheme were tested by amplifying the *bla* genes from 150 *K. pneumoniae* and *Klebsiella sp.* isolates previously identified by VITEK2. The proposed PCR strategy showed to be specific and reproducible and the identity of amplicons was confirmed by sequencing. These results demonstrated the reliability of the PCR scheme proposed here, and the occurrence of *K. variicola* and *K. quasipneumoniae* misidentification as *K. pneumoniae* based on the biochemical tests, calling attention to the current underestimation and impact of the former species in human infections.

Keywords: Class A β-lactamase, *Klebsiella* species, microbiologic diagnostic, molecular identification, species misidentification.

Development Agency: FAPERJ, PNPD-CAPES and CNPq.