## Abstract

*Klebsiella pneumoniae* serotype K2, belonging to Enterobacteriaceae family, is one of the common gram negative pathogen. Due to the high prevalence of serotype K2, it is essential to provide the precise, effective and exclusive method to detect the bacteria. In the last few years, molecular methods such as PCR, gold nanoparticles biosensors have been developed for rapid, precise, easy, inexpensive with high specificity and sensetivity detection of human, plant and animal pathogens diagnosis. In this study, in order to detect the *Klebsiella pneumoniae* serotype K2, *K2A*, *rmpA* and *orf10* genes were used. Primers were designed and identification of bacteria was performed by PCR for each gene and Multiplex PCR for *orf10* and *K2A* genes. *K2A* gene sequencing was done and thiolated oligonucleotide were designed based on the sequencing result. Synthesis of gold nanoparticles with approximately 20 nm diameter was carried out and *Klebsiella pneumoniae* serotype K2 genomic DNA was used for detection using gold nanoparticles and probes. A 532 bp fragment of *K2A* gene with sensitivity of 1 pg/µL and 309 bp fragment of *orf10* gene with sensitivity of 0.07 ng/µL were amplified by PCR and multiplex PCR, however no band was observed for *rmpA* gene. Change in color of gold nanoparticles in the presence of K2 serotype genomic DNA and oligonucleotide probes was achieved and maximum changes in wavelength occurred at 550 to 650 nm. Therefore the method could be introduced as a rapid, accurate, inexpensive technique with high specificity compared with the biochemical and molecular techniques for detection of *Klebsiella pneumoniae* serotype K2.

Keywords: *Klebsiella pneumoniae*, serotype K2, Multiplex PCR, gold nanoparticles, *K2A*, *rmpA* and *orf10* genes

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# Specific detection of *Klebsiella pneumoniae* serotype K2 by gold nanoparticles probe

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