Abstract

*K. 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 *K. 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 *K. 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 *K. pneumoniae* serotype K2.

Keywords: *K. pneumoniae*, serotype K2, Multiplex PCR, gold nanoparticles, K2A, rmpA and orf10 genes
Specific detection of *Klebsiella pneumoniae* serotype K2 by gold nanoparticles probe

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