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Establishment of a PCR-based diagnostic method for the identification of *Klebsiella pneumonia*

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Klebsiella pneumoniae is one of the significant causative agents for nosocomial infections. Rate of infections by this bacterium have increased dramatically and thus searching for quick and sensitive molecular methods for its detection became a must. Our research aims at validating a sensitive and specific PCR based method for diagnosis of infections caused by *Klebsiella pneumoniae.* Our work started by using online bioinformatics tools to find a unique gene that can be used for detection of *Klebsiella pneumoniae* and designing specific primer for its amplification. *In silico* studies results in choosing the rcsA gene, regulating colanic acid capsular biosynthesis activation protein A, as a unique gene for *Klebsiella pneumoniae*. Two sets of primer pairs (named K1 and K2) were designed to target the specific gene, followed by determining primers sensitivity and specificity in *Klebsiella pneumoniae* detection. Both primer pairs K1 and K2 were able to amplify targeted gene sequence in standard and clinical strains. K1 and K2 primer pairs were able to amplify target gene in diluted DNA template concentration up to 10^{-1} ng/µl, and $1x10^{-23}$ picogram/µl, respectively, indicating the higher sensitivity of primer pair K2. K1 primer pair worked well on bacterial culture and amplified target genes in bacterial cultures diluted to 101 CFU/ml. K1 primer pair failed to amplify genomic DNA template of common Gram negative uropathogens including *Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*, thus indicating its specificity. In conclusion, rcsA gene is a unique gene that can be used for detection of *Klebsiella pneumoniae* in clinical samples.

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