

## Optimization of a Multiplex PCR for the Identification of *Acinetobacter* Species Isolated from Clinical Specimens: A Preliminary Study

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*Acinetobacter* species (spp.) are an important cause of nosocomial infections associated with high morbidity and mortality in the hospital settings. Two medically important *Acinetobacter* spp. include *A. baumannii* and *A. nosocomialis*. Differentiation of these two species using phenotypic tests are difficult due to their similar phenotypic characteristics. Therefore, this study aimed to establish a multiplex PCR to differentiate *A. baumannii* and *A. nosocomialis* in *Acinetobacter* clinical isolates.

Fifty-one clinical isolates of *Acinetobacter* spp. were collected from endotracheal tube secretions, sputum, wounds and blood of hospitalized patients. DNA extraction of above isolates was done using GenoLyse 1.0 version Bacterial DNA extraction kit. Three primer pairs were selected based on available literature; a common primer pair P-rA1(5'-CCTGAATCTTCTGGTAAAAC-3') and P-aA2(5' GTTCTGGGCTGCCAAACATTAC-3') to amplify a 425 bp region of *recA* gene of *Acinetobacter* spp, primers sp4F(5'-CACGCCGTAAGAGTGCATTA-3') and sp4R(5'-AACGGAGCTTGTCAGGGTTA-3') to amplify a 294 bp region of *gyrB* genes of *A. baumannii* and *A. nosocomialis*, and PAb-ITSF(5'-CATTATCACGGTAATTAGTG- 3') and PAb-ITSB(5'-AGAGCACTGTGCACTTAAG- 3') primers to amplify a 208 bp ITS region of *A. baumannii*. Optimum PCR conditions were determined. ATCC strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used to test for primer specificity. Optimized PCR conditions were applied to identify *Acinetobacter* spp. in the clinical isolates. *A. baumannii* isolate confirmed by sequencing was used as a positive control.

The optimum PCR reaction conditions of multiplex PCR were performed in a 25 µL reaction mixture consisted of 1X buffer (Sigma Aldrich, USA) with 0.2 mM dNTPs, 0.2 µM of forward and reverse primer, 1.25 U of Taq polymerase (Sigma Aldrich, USA) and 2 µL of nucleic acid. The PCR conditions were 94°C for 5 min for initial denaturation, followed by 35 cycles of 94°C for 1 min, 54.8 °C for 30 sec and 72°C for 30 sec and a final elongation step of 72°C for 10 min. The multiplex PCR yielded three bands for *A. baumannii* having 425 bp, 294 bp and 208 bp, while *A. nosocomialis* gave two bands at 425 bp and 294 bp. *A. baumannii* was identified as the predominant pathogen (50/51). This preliminary study suggests that multiplex PCR can be used successfully to identify *A. baumannii* and *A. nosocomialis* from clinical isolates.

**Keywords:** *Acinetobacter Baumannii*, *Acinetobacter Nosocomialis*, Multiplex PCR

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