Abstract

Klebsiella pneumoniae is an opportunistic pathogen of *Enterobacteriaceae* family and is resistant to many antibiotics presently available, especially the beta-lactams due to extensive production of beta-lactamases. The enormous capacity of such beta-lactamases to hydrolyse majority of beta-lactam antibiotics including, monobactams and carbapenems has created alarming situation in global healthcare. Moreover, horizontal transfer of genes encoding the beta-lactamases within normal bacterial strains further dispenses the antimicrobial resistance to a wider level. The alterations among the different class of beta-lactamases active-sites further limit single inhibitor-based therapy against all beta-lactamases. However, Amino acid substitutions outside the active-site cleft reportedly affect beta-lactamase activities. Interestingly in our study amino acid substitution in omega-loop of SHV-14, a serine beta-lactamase (SBL) and putative omega-loop region of New Delhi Metallo-beta-lactamases (NDM) variables have drastically affected the susceptibility profile of bacterial cells harbouring such mutant genes. Our study includes biochemical characterisation of $bla_{\text{SHV-14}}$, $bla_{\text{NDM-5}}$, $bla_{\text{NDM-7}}$, and their various mutants nearing the active (putative omega-loop) to comprehend the role of substituted amino acid residues (glutamate and serine) in maintaining resistance profile compared to the strains carrying wild-type proteins. Both full-length and soluble construct of the genes were expressed for cellular expression and *in vitro* experimental studies, respectively. Minimum inhibitory concentration (MIC) was assessed for both the strains expressing wild-type and mutated proteins to check the cellular level expression of the genes. The soluble constructs of the genes were expressed and their protein products were purified to homogeneity. Protein molecular mass and protein folding patterns were assessed via mass spectrometry and circular dichroism (CD) spectra analysis. Thermal stability of the mutant proteins was assessed by CD spectra analysis at various temperatures to comprehend the impact of the mutation on protein secondary structure stability. Antibiotic susceptibility test emphasized the significance of mutated residues in maintaining the resistance profile. Majority of the strains carrying mutated proteins became sensitive to all the antibiotics tested in the study as compared to the strains carrying wild-type beta-lactamases. Besides, kinetic studies were in synchrony with the antibiotic susceptibility data. Furthermore, the drastic decrease in the enzyme affinity (K_m), the turnover number (k_{cat}) and enzyme efficiency (k_{cat}/K_m) were observed for the mutated proteins. The thermal stability assay revealed a decrease in beta-sheet stability of the mutant proteins compared to their wild-type counterparts suggesting a crucial role of mutated residues in maintaining the protein stability.

Keywords-Beta-lactam, Antibiotic resistance, Carbapenemase, Serine beta-lactamases, Metallo-beta-lactamases, Site directed mutagenesis, Omega-loop.