Chapter 7

Summary and Future Scope

7.1 Summary of the work

The worldwide escalation and augmentation of antibiotic resistance has resuscitated the attention of scientific community to develop new and effective antimicrobial agent. Compounds that locally kill or inhibit the growth of microorganism without affecting the nearby tissues are called as antimicrobial agents. Considering the antibacterial potential of metals, in recent years, Ag NPs have been reported as an auspicious contrivance for the improvement of biomedical field. Ag NPs have attracted much interest in the scientific community because of their knowingly innovative and improved physical, chemical and biological properties attributed to their varying physico-chemical properties viz. variation in size, shape, surface capping and synthesis methods. Ag NPs are presently used at various concentrations as an antimicrobial agent in wound dressings, textiles, food storage containers, and personal care appliances. Plenty of studies have been done to measure the antimicrobial potential of silver species (bulk, ionic and nano). However, the precise molecular mechanism of their mode of action has remained unclear, which is a major obstacle towards their commercialization as antibacterial agent and in drugs. Systematic studies are required to understand the detailed mechanism behind antibacterial action of Ag NPs.

The present work discusses the synthesis of Ag NPs with variation in their physico-chemical properties viz. size and surface capping. Variation in the size of Ag NPs was achieved by the chemical reduction method by which particles with an average size of 5, 10, 20, and 50 nm were synthesized. Ag NPs of all the sizes were screened for their antibacterial potential against *Escherichia coli* K12 and based on the best antibacterial activity, NPs of smaller size range (5-20 nm) were selected to study the effect of various surface capping agents. Citrate, fucose, lysozyme, and fungal proteins were used for capping of Ag NPs as chemical agent, sugar, commercial protein, and fungal protein, respectively. Characterization of all the synthesized Ag NPs were performed by UV-visible spectroscopy, TEM, SAED, EDS, XRD, and FTIR analysis.

The minimum inhibitory concentration assay results showed that lysozyme coated Ag NPs (L-Ag NPs) with an average size of 5 nm were best antibacterial agent among all the tested Ag NPs. It was observed that, physico-chemical properties of Ag NPs have tremendous impact on their antibacterial activity and silver ions dissolution rate. Further, the detailed mechanistic action of L-Ag NPs was analysed by the biochemical and selective gene expression studies against *E. coli* K12 in comparison to silver ions. The obtained results revealed the reservoir

nature of L-Ag NPs, which slowly release silver ions. Transcriptomic analysis of *E. coli* K12 and *K. pneumoniae* MGH78578 under exposure to L-Ag NPs, confirmed the role of porin protein in the entry of silver ion in bacterial cells. RNAseq data confirmed the ROS generation in the form of hydrogen peroxide involvement of SoxRS system in the sensing of silver ions inside the cell. Upon exposure to L-Ag NPs, bacterial cell activated its defence system, but the level of activation was found to be depended on the concentration of silver ions inside the cell. In *E. coli* K12, activation of bacterial efflux pumps CusCFBA & Cue system was observed whereas in *K. pneumoniae* MGH78578, silver specific *sil* system was found to be involved to efflux the silver ions from cells.

Overall, this study suggests various options for the generation of target specific silver based antibacterial drugs. However, considering the extensive, uncontrolled and unregulated use of silver and silver based products as antibacterial agents, further studies need to be done to find out the development of resistance against silver and the mechanism involved. To the best of our knowledge, this is the first report where the effect of silver nanoparticles (lysozyme coated) was studied on *E. coli* K12 and *K. pneumoniae* MGH78578 by high throughput transcriptomic profiling.

7.2 Future prospective of the work

The current work discusses the effect of size and surface capping on the antibacterial potential of Ag NPs, but the effect of shape and crystallinity of Ag NPs also need to be disclosed in order to find out the more efficient silver based antibacterial agent. Since silver specific efflux pump get activated upon the exposure to silver species in Gram-negative bacteria, it is imperative to confirm whether exposure of silver could induce resistance or persistence. To study the full mechanism behind the resistance or persistence, silver resistant and persistent cells should be generated in the laboratory.

Further studies needs be performed to understand the mechanism behind the activation of efflux pumps by generating the mutants of highly up-regulated genes like *soxS*, *cusC* & *copA*/*silC*, which will decipher the mechanistic aspects of silver resistance in bacterial species.