Name: **Firdoos Ahmad Gogry**
Supervisor: **Dr. Qazi Mohd Rizwanul Haq (Professor)**
Department: **Department of Biosciences**

"Polymyxin resistance in bacteria: a study on genetic factors involved and their diversity among environmental isolates of bacteria"

Keywords: Gram-negative bacteria; colistin resistance; *mcr*-1; carbapenemase; aquatic environment; Lipopolysaccharide and electrostatic interaction

This study was carried out to determine the prevalence of colistin resistance from sewage treatment plants and Delhi stretch of River Yamuna. Genetic determinants conferring resistance to colistin, carbapenemase and ESBLs, Co-occurrence and co-transfer of *mcr*-1 plasmid mediated gene and other genetic resistant variants were studied. In this study, we obtained 370 non-duplicate bacterial isolates from sewage water and the river Yamuna in Delhi, India. Of the 59 positive isolates, colistin resistance gene *mcr*-1 was detected among 10 isolates. Plasmid Based Replicon Typing (PBRT) was performed for the presence of Inc groups using a specific set of 18 primers. Three to seven different incompatibility type plasmids were found in all the *mcr*-1 positive bacterial isolates. Chromosomal-based genes phoPQ, pmrAB and mgrB were amplified from 5 resistant isolates of *Klebsiella pneumonia*, sequencing confirmed 4 isolates with wild-type genotype but 1 isolate revealed a missense mutation in mgrB and phoQ of phoPQ two-component system. Moreover, carbapenem-resistant genes *bla*NDM-5, OXA-1 and OXA-9 were detected in *mcr*-1 positive bacterial isolates. ESBL determinants *bla*CTX-M, *bla*SHV and *bla*TEM were present in colistin-resistant bacteria. Whole Genome Sequencing (WGS) of isolates harboring colistin resistance and carbapenemase genes confirmed the presence of multiple antibiotic and metal resistant determinants. The results also confirmed the presence of mobile genetic elements like transposons and integrons which aid in the dissemination and incorporation of different resistance genes. The virulence factors were confirmed through the WGS that assists these resistance genes for virulence in the host bacteria. The plasmid-borne *mcr*-1 *ge*ne has been found integrated by ISI in a plasmid, which provides genetic stability of *mcr*-1 gene. Antibiotic susceptibility test of all isolates against 9 different classes of drugs revealed multidrug-resistant phenotype with high MIC values. *In vitro* conjugation studies showed successful transfer of *mcr*-1, carbapenemase and ESBL genes. Results of our conjugation studies further highlight the risk for dissemination of *mcr*-1 gene to other bacteria

including clinically important pathogens. Biophysical and biochemical analysis confirmed membrane modification with cationic phosphoethanolamine in colistin resistant Gram negative bacteria. Membrane sensitivity and permeability studies showed that colistin resistant bacteria has lesser sensitivity and permeability as compared to susceptible bacteria Zeta potential measurements demonstrated less negative charge at mid-logarithms of colistin resistant bacteria as compared to sensitive control. However, zeta potential measurement was not statistically significant in stationary phase of each strain. AFM study revealed smooth, featherless and deformed membrane structure in treated sensitive cell. However treated resistant strains exhibited lesser smoothness even at higher colistin concentrations. NMR measurements confirmed line broadening in amide region of NMR spectra by increasing colistin: LPS aggregates mass ratio of susceptible strains. Contrary to this line broadening was not recorded for the resistant strains, even at the highest colistin: LPS mass ratio. The findings of this study suggest that the colistin resistant strains can block the electrostatic contact between the cationic peptide colistin and anionic lipid A component that drives the first phase of colistin action, thereby preventing hydrophobically driven second-tier action of colistin on the outer lipopolysaccharide layer.