Conclusion

The aim of the present study is isolation and identification of *Klebsiella* spp. and to investigate antibiotic resistance and virulence factors involved in the pathogenesis of *Klebsiella* spp. This study was performed on clinical isolates of pathogenic *Klebsiella* spp. isolated from patients suffering from UTIs.

Isolation and identification of *Klebsiella* spp. using biochemical and morphological characteristics could be tricky as it is difficult to discriminate between *Klebsiella* and Enterobacter spp.; these spp. share similar biochemical characteristics. Further, due to the high resemblance between *Klebsiella* and Enterobacter spp., species level identification of *Klebsiella* spp. could be done only using MLST or whole-genome sequencing approaches. Single locus based identification such as 16s *rRNA* or *wzi* sequencing could be used to identify the genus, but are not adequate to identify individual species of *Klebsiella*. *Klebsiella pneumoniae* is the most prevalent species found in the collected clinical isolates of UTIs. Despite belonging to the same genus and source (human originated; UTIs), the collected clinical isolates of *Klebsiella* demonstrated variations in their phenotypic characteristics such as lactose-fermenting ability and mucoidity. Exhibition of two types of colonies (yellow and blue-white) by a single isolate in a pure culture is the result of mutative fermentation. Majority of the clinical isolates of *Klebsiella* spp. were encapsulated; however, thickness of the capsule varies among the isolates.

Investigation of antibiotic resistance in this study represents a blueprint of phenotypic and genotypic characterization of PDR, XDR, MDR strains of *Kp*. Global comparative analysis of clinically prevalent lineages such as ST147 and ST231 was also performed. The collected isolates were successfully categorized into four categories: MDR, XDR, PDR and susceptible using antibiotic susceptibility testing. The isolates showed variation in phenotypic parameters such as production of ESBLs, and MBLs. Multiplex PCR exhibited the differences in the carriage of carbapenemase genes by MDR, PDR and XDR strains; PDR isolate carried more number of carbapenemase genes than XDR and MDR isolates. The PDR isolate produced both, ESBLs and carbapenemase enzymes; it also exhibited the highest expression of *bla*NDM-1 among all isolates. The genomic analysis concluded that number of resistance genes, category/type of resistance genes and their expression along with chromosomal

mutations in resistance determinants present in genome collectively contribute to their resistance phenotype (PDR, XDR, MDR) of the isolate. Mutation in *ramR* and *mgrB*, integration of *bla*NDM-5 into chromosome along with presence of other resistance genes to various antimicrobial categories are responsible for the PDR phenotype of DJ. Whereas, the absence of resistance genes and lack of expression of the resistance genes present are responsible for the susceptibility of M25 against the antimicrobial agents. Chromosomal integration of *bla*NDM-5 in PDR strain like DJ is threatening because it could be spread and disseminated both horizontally and vertically within the lineage, making future population of ST147 resistant to carbapenems. XDR strains like M2 and M6 are also worrisome as they are on the verge to evolve as PDR strains in future. The integration of carbapenemase and ESBL producing genes into chromosome of high-risk clones such as ST147 and ST231 suggests the potential for maintenance and dissemination of these genes vertically within these clones and thus represent a novel antibiotic threat. The analysis of ST147 and ST231 suggest the associations between resistance genes and plasmids to specific lineages. *bla*NDM was well-spread in ST147 genomes and occurrence of *bla*OXA-48-like was more prevalent in ST231 lineage. Further, resistant genes associated with ST are also different at different geographical locations of the world. Extensive use/misuse of fluoroquinolones and carbapenems in past few years contributed as a selective pressure in the rise of extremely resistant high-risk clones in clinically prevalent lineages. Almost all XDR strains in the presented study were susceptible only to colistin and tigecycline. Therefore, now, the wise use of colistin and tigecycline is vital to limit the evolution of colistin and tigecycline susceptible XDR strains into PDR strains. Further, continuous and rigorous surveillance, data-sharing and rapid reporting of *Kp* strains circulating in hospital and clinical settings are crucial both nationally and internationally. This will help us to track the emergence and dissemination of clinically important variants as well as guide us in building combat strategies to fight the battle of antibiotic resistance.

The virulence factors of *Klebsiella* spp. such as CPS, EPS, *rmpA* and biofilms were studied. The role of CPS, EPS, *rmpA* and string phenotype in virulence of *Klebsiella* was evaluated based on the amount of phagocytosis occurred. Based

on our observations we concluded that the there is no correlation between the amount of CPS and EPS produced by clinical isolates of pathogenic *Klebsiella* spp. Amount of CPS produced by the isolate or expression of the virulence gene, *rmpA* does not determine the string phenotype of the isolate. However, what determines the string formation of an isolate still remains unclear and needs further investigation. Further, interestingly, string positive isolate with K39 (M20; non K1/K2) with low amount of CPS, no EPS and *rmpA* showed higher resistance to phagocytosis compared to the K2 isolates. Hence, string positive phenotype of an isolate is seeming to have influence on resistance to phagocytosis. This phenomenon seems to be more extensive than valued in the literature. No association between amount of CPS and inhibition of phagocytosis was found in UTI isolates of *Kp*. In case of study of biofilms, we found heterogeneity in biofilm formation by clinical isolates. All the clinical isolates from UTIs were not strong biofilm producers. Latex catheters used in healthcare settings of developing countries due to its cost effectiveness seem to contribute to the high prevalence of biofilm associated infections. Silicone catheters or silicone-coated latex catheters should be used in clinical settings to prevent the risk of biofilm associated CAUTIs. High eDNA, protein, EPS, cell adhesion, and unusual cell death were found to be associated with the strong biofilms. It is evident that increased eDNA, protein, and RNA in strong biofilm matrix is a consequence of cell death.