CHAPTER 2:

Rationale and Objectives

P. aeruginosa is an opportunistic pathogen causing 7-10% of UTIs in hospital settings. Patients with underlying illnesses, such as those with indwelling urinary catheters, are more likely to get these UTIs. Further, *P. aeruginosa* can adhere to the surface of the catheters and form a biofilm, which restricts antibiotic diffusion and also assists in the formation of antibiotic-resistant strains. Biofilms are more difficult to treat owing to high antibiotic resistance than their planktonic cells. The bacterial cells within the biofilm are shielded from harsh environmental conditions such as antibiotic stress. Detailed studies on *P. aeruginosa* biofilm are restricted to CF-causing *P. aeruginosa.* Scanty information is available on biofilm formation and resistance in biofilms formed by clinical isolates of *P. aeruginosa* causing UTIs. Major studies of biofilm formation on catheters have been done with type strains PAO1 and PA14. There is a need to study clinical isolates causing UTIs and their biofilm formation abilities. To the best of our knowledge, the reports regarding what contributes to strong biofilm formation in *P. aeruginosa* are fewer. Further, the study of biofilm matrix components of strong and weak biofilm producers are scanty. It is known that for initial attachment on the surface T4P twitching motility is required for irreversible attachment. But the same attachment and biofilm formation rate for clinical isolates causing UTIs are not available.

Other major components of *P. aeruginosa* biofilm are eDNA, extracellular protein (CdrA, ecotin), exopolysaccharides (pel, psl and alginate), etc. In type strain cell death mechanism is well studied whereas the cell death mechanism in clinical isolates of *P. aeruginosa* is still under warrant. Further, reports of CdrA protein, eDNA which helps in stability in biofilm with psl and pel polysaccharide are not present in clinical isolates of *P. aeruginosa.* So, there is a need to characterize and quantitate biofilm matrix components within UTIs causing *P. aeruginosa* isolates.

P. aeruginosa infections are challenging to treat due to resistance to multiple antibiotics and the global rise in multi- and pan-drug resistant strains. Also, WHO has listed *P. aeruginosa* under the priority pathogen list. The bacterial cells within biofilm are shielded from harsh environment and many molecular mechanisms contribute to biofilm-specific antibiotic resistance. While Persister cells are one of the reasons for relapse of the infection. Most of the reports on PC formation are from *E. coli* and with type strain PAO1 and PA14 of *P. aeruginosa* in the planktonic stage. For PC formation activation of stringent response (RelA, SpoT, DksA, and Lon) and TA system is required which is well known in the typed strain of *P. aeruginosa*

but not for clinical *P. aeruginosa* strains. Scare information is available on PC formation in biofilm at a supra-MIC level in *P. aeruginosa*. Recent studies on biofilm-mediated resistance showed that discrete genetic determinant elements such as BrlR are specifically upregulated in the biofilm stage of *P. aeruginosa* affecting the biofilm susceptibility against antimicrobials. The SagS modulates the expression of *brlR* gene which in turn regulates the efflux pump expression in the biofilm stage thus resulting in biofilm tolerance. However, there are no reports regarding the effect of mutation on repressors and activators in the biofilm stage. There are reports in the planktonic stage effect of mutation on negative regulators (NalD, NalC, etc.) leads to increased expression mexAB-oprM in *P. aeruginosa* thus resulting in antibiotic resistance. These data are warranted in the biofilm stage.

Hence, there is a need to study the biofilm-associated antibiotic resistance with respect to different supra-MIC concentration antibiotics for PC formation in clinical isolates of *P. aeruginosa*. Detailed studies on the mechanism responsible for the recalcitrance of biofilms towards antibiotics would help to develop new treatment strategies against persistent infections. Hence, this study would shed light on the biofilm-associated resistance mechanism in clinical isolates of *P. aeruginosa.*

Objectives for this study

1. Collection, isolation, and identification of isolates from UTIs pathogenic Pseudomonas aeruginosa
2. Quantification and characterization of biofilm components
3. Antibiotic resistance in biofilm
    a. To study the effect of antibiotics in persister cell formation
    b. To study the biofilm-mediated antibiotic resistance through *brlR*