Summary and Conclusions
1. In the present study, predominant serotypes, amongst 50 strains of *P. aeruginosa* isolated from urine of patients suffering from catheter associated urinary tract infections, were found to be O11, OII, O6, O1, O8, O7/8, O3, O4 and O15.

2. 50 uroisolates of *P. aeruginosa* were screened for production of virulence determinants namely alginate, pyochelin, pyoverdin, protease, elastase, phopholipase C, cell free and cell bound hemolysin. Majority of isolates were found to be high producers of all these virulence factors (VFs) but strain to strain variation in production of these was observed. In addition, when screened for production of quorum sensing molecules, it was observed that 90% of the isolates were positive for these signals.

3. Based on the maximal elaboration of different virulence traits by the tested strains, 5 uroisolates and 1 standard strain (PAO) were selected for further studies. These strains were found to have the ability to form biofilms *in vitro* on Foley’s catheter surface which was checked by scanning electron microscopy and crystal violet staining. With increasing days, there was increase in formation of glycocalyceal matrix of biofilm cells of all the 6 strains.

4. Biofilm cells were checked for elaboration of all the above mentioned virulence determinants at different days (1 to 7) of biofilm formation. It was observed that 4 day old biofilms produced maximal levels of all the virulence factors.

5. Comparison between production of virulence factors by planktonic and 4 day old biofilm cell forms grown in nutrient broth revealed that *in vitro*, biofilm cells were significantly higher producers of all the virulence determinants.

6. In order to study the effect of selective environmental conditions prevalent in the milieu of urinary tract on production of virulence
traits by \textit{P. aeruginosa}, both cell forms were grown \textit{in vitro} in different variable conditions of pH, osmolarity, absence and presence of iron, Tamm-Horsfall protein (THP) and secretory products of macrophages in medium like nutrient broth, M9 and RPMI-1640.

7. Elaboration of virulence factors by planktonic and biofilm cells of \textit{P. aeruginosa} when checked in pH range of 5.0 to 8.0 revealed significant enhancement in elaboration of alginate and exoenzymes at pH 8.0 whereas production of siderophores, PLC and hemolysin was higher at pH 5.0. However effect was more pronounced with biofilm cell forms of all the strains for the tested virulence factors.

8. With increase in osmolarity in M9 medium (200 mOsmol to 300 mOsmol), significant increase in elaboration of all the virulence factors by planktonic and biofilm cells of all the strains was observed but further increase in osmolarity to 350 mOmol lead to significant decrease in production of all the virulence determinants in both the cell forms.

9. Enhanced production of all the virulence traits was observable in all the strains following growth of planktonic and biofilm cells of \textit{P. aeruginosa} in iron deficient medium containing 100 to 300 \textmu M dipyridyl whereas there was significant decrease in production of these virulence factors with addition of ferrous sulphate in varying concentrations of 0.5 to 5 \textmu g/ml.

10. Production of virulence traits when checked in presence of THP showed significant rise in production of all the virulence factors by planktonic as well as biofilm cells with maximal effect observable at 50 \textmu g/ml concentration. Further increase in THP concentration to 70 \textmu g/ml lead to significant fall in elaboration of all virulence traits by all the six strains in both cell forms.
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11. MSPs were prepared following interaction of biofilm and planktonic cells of *P. aeruginosa* with murine peritoneal macrophages. Maximum production of virulence factors observable at 30% concentration. Further increase in MSP concentration to 50% and 70% lead to significant decrease in elaboration of all the virulence determinants.

12. For *in vivo* studies, acute ascending UTI was induced in mice by intraurethral inoculation of six selected *P. aeruginosa* strains. Assessment of overall virulence was based on bacterial load, pathology induced and biochemical as well as immunological parameters. Maximal bacterial load in bladder was observed on 10th post-infection hour and peak bacterial load in urine was observed on 3rd post-infection day in case of both planktonic and biofilm cells.

13. Neutrophil recruitment in urine (assessed by counting cells in Neubauer's chamber) and bladder tissue (assessed on the basis of MPO assay) was maximal at 3rd post-infection day with both the cell forms. However neutrophil influx was significantly higher in mice infected with biofilm cells as compared to planktonic cell infected mice showing maximum increase on 5th post-infection day. Following infection, there was gradual increase in MDA production in urine and bladder tissue till 7th post-infection day indicating tissue damage.

14. Bacteriological examination of renal tissue of experimental animals at different post-infection time intervals showed peak bacterial load on 3rd post-infection day with planktonic cells and on 5th post-infection day with biofilm cells. Peak neutrophil influx was observed on 3rd post-infection day in case of planktonic cell infected mice whereas maximal neutrophil recruitment was observed on 5th post-infection day in biofilm cell instilled mice.
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With increasing post-infection period, there was increase in renal MDA levels with both planktonic and biofilm cells.

15. Based on severity score assessment of the tissues, lesions in renal and bladder tissue induced by biofilm cells were more as compared to planktonic cells. Renal and bladder severity scores varied from 0 to +5 and 0 to +2 respectively in planktonic cell infected mice whereas these scores ranged from +1 to +8 and +1 to +3 respectively in biofilm cell instilled mice.

16. More severe acute infection of urinary tract was caused by *P. aeruginosa* grown under conditions of high osmolarity (300 mOsmol), iron depletion (300 μM dipyridyl), THP coating (50 μg/ml) and presence of MSPs (30%). This was indicated by higher bacterial load, pathology induced, neutrophil influx and MDA formation.

17. Peak production of RNI in renal and bladder tissue as well as urine was observed on 3rd post-infection day leading to decrease on 7th post-infection day in case of both planktonic and biofilm cell instilled mice. However, biofilm cells produced more reactive nitrogen intermediate radicals as compared to planktonic cells.

18. ROS production was assessed in terms of superoxide dismutase (SOD). Significant decrease in SOD levels was observed till 3rd post-infection day showing increase on 5th and 7th post-infection day. Biofilm cells were significantly higher producers of reactive oxygen species based on lower detectable levels of SOD, as compared to planktonic cell infected animals.

19. Peak production of pro-inflammatory cytokine, MIP-2 was observed on 6th post-infection hour followed by decrease in 24 post-infection hour in urine, bladder and renal tissue in case of both biofilm and planktonic cell infected mice. In contrast, maximal production of TNF-α was observed on 4th post-infection
hour and on 3\textsuperscript{rd} post-infection day. Highest levels of IL-1\(\beta\) were detectable in renal tissue on 3\textsuperscript{rd} post-infection day in planktonic cell infected mice and on 5\textsuperscript{th} post-infection day in biofilm cell instilled mice. IL-10, an anti-inflammatory cytokine, was produced in significantly lower amounts in renal tissue of mice infected with biofilm cells as compared to those infected with planktonic cells. Peak production of IL-10 was observed on 7\textsuperscript{th} post-infection day with both the cell forms.

20. Standard Quorum sensing (QS) producer strain PAO1 of \textit{P. aeruginosa} was more virulent in the urinary tract infection model induced in mice as compared to its mutants PAOJP1 (\textit{lasI} mutant) and PAOJP3 (\textit{lasR rhlR} double mutant) and non-producer clinical isolates as assessed by bacterial load, pathology induced, neutrophil influx and MDA formation.

21. In chronic ascending UTI, induced by inoculating \textit{P. aeruginosa} thrice, bacteria were demonstrable in the kidneys of mice infected with planktonic cells till 12\textsuperscript{th} post-infection day whereas biofilm cells persisted till 15\textsuperscript{th} post-infection days following first dose of the inoculum. Following instillation of second dose (at 15\textsuperscript{th} post-infection day), planktonic cells persisted for 20 days whereas biofilm cells were demonstrable in renal tissue till 24\textsuperscript{th} post-infection day. At all these time periods, renal bacterial counts were found to be significantly higher (p<0.001) in animals inoculated with biofilm cells of \textit{P. aeruginosa} as compared to the planktonic cells. When third dose was administered (at 120\textsuperscript{th} post-infection day), kidneys of mice infected with planktonic and biofilm cells were found to be sterile on 123\textsuperscript{rd} and 125\textsuperscript{th} post-infection day respectively.

22. Evaluation of pathology of renal tissue in chronic model revealed that renal lesions were significantly more with four day old
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biofilm cells as compared to those induced with planktonic cells at all time intervals. Severe inflammation, abscess, crowding of glomeruli and destruction of tubules was observable during experimental chronic infection. Increased neutrophil influx and constant production of MDA was observed during entire course of infection.

23. This study highlights the host parasite interaction in relation to pathogenesis of catheter associated urinary tract infections caused by biofilm cells of *P. aeruginosa*. Comparison of planktonic and biofilm cell forms of *P. aeruginosa* brings out that preformed biofilm cell forms are more virulent *in vivo* in terms of establishment of UTI in mouse model which may be due to enhanced elaboration of virulence factors as observed *in vitro*. Besides this, environmental condition prevalent in the host's milieu like variations in osmolarity, iron depletion, presence of macrophage secretory products and presence of ubiquitous urinary protein, THP have the potential to alter the course of acute infection. This study is unique in highlighting the importance of quorum sensing molecules in an experimental animal model of UTI.