Introduction
Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span (Kucheria et al., 2005; Chang and Shortliffe, 2006). Incidence in women in the age of 20-40 years ranges from 25-30% whereas in older women above 60 years of age it ranges from 4-43% (Kunin, 1987; Hooton et al., 2004; Williams and Schaeffer, 2004). UTIs can be classified as uncomplicated or complicated. The recognized predisposing factors in complicated UTIs are anatomic defects, vesicoureteric reflux (VUR), obstruction, surgery, metabolic diseases like diabetes mellitus and generalized immunosuppression especially in patients of organ transplant (Warren et al., 1982; Read et al., 1988; Bonadio et al., 1999; Munoz et al., 2001; Geerlings et al., 2002; Leone et al., 2003). Catheterization of urinary tract is one of the most common factor which predisposes the host to complicated UTIs (Reid, 1999; Sabbuba et al., 2003; Smith et al., 2003; Saint and Chenoweth 2003; Bass et al., 2003; Shaw et al., 2005). Instillation of catheter may lead to damage of mucosal layer, which disrupts the natural barrier and allows bacterial colonization (Logan, 2003). Organisms can gain entry via extraluminal route (Kalsi et al., 2003) by moving across the outer lumen of catheter or by intraluminal route by directly entering the interior of catheter (Dickinson and Bisno, 1989).

The organisms most commonly responsible for catheter associated UTIs are *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis* (Chang et al., 1990; Jarvis and Martone, 1992; Hootan, 2001; Fluit et al., 2001). In case of *E. coli*, the epidemiological, experimental and clinical studies have established the role of multiple virulence factors of *E. coli* like adhesins operative through type-I fimbriae and P fimbriae, O serotypes, K1 capsule, serum resistance, hemolysins, cytotoxic nectrotizing factor (CNF) and siderophores (enterochelin and
Introduction...

aerobactin) in relation to uncomplicated and complicated UTIs (Johnson et al., 1992; Kucheria et al., 2005).

*Pseudomonas aeruginosa* is the third most common pathogen associated with hospital acquired catheter associated UTIs (Jarvis and Martone, 1992). Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell-associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phospholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores (Matheson et al., 2006; Yates et al., 2006; Zulianello et al., 2006). These factors have been shown to play an important role in pathogenesis of *P. aeruginosa* induced infections like respiratory tract infections, burn wound infections and keratitis (Woods et al., 1986, Woods et al., 1997; Lysczak et al., 2000; Vance et al., 2005; Smith et al., 2006). In addition to elaboration of virulence factors, *P. aeruginosa* has a tendency to form biofilms on the surface of indwelling catheters in catheterized patients. Growth of *P. aeruginosa* begins in the form of microcolonies, which later coalesce together to form biofilms (Hoiby et al., 2001; Klausen et al., 2003; Kuchma et al., 2005). Biofilms are resistant to antimicrobial agents as well as to host defense mechanisms and hence are difficult to eradicate. Biofilms contribute towards pathogenicity of *P. aeruginosa* as these often lead to persistent and recurrent infections (Donlan, 2001; Drenkard, 2003; Boles et al., 2004).

Recently it has been proposed that *P. aeruginosa* controls production of virulence determinants and conversion to biofilm cell mode through chemical signals known as quorum sensing signals operative through autoinducers mainly acylhomoserine lactones (AHLs) (Lerat and Moran, 2004; Juhas et al., 2005; George et al., 2005; Heurlier et al., 2006). In *P. aeruginosa* two types of quorum-
sensing systems, *las* (Gambello and Iglewski, 1991) and *rhl* (Raychaudhuri *et al.*, 2005) have been reported which consist of two signal-generating synthetases (*LasI*, *RhlI*) and two cognate transcriptional regulators (*LasR*, *RhlR*). Role of these quorum-sensing signals in virulence and pathogenicity of *P. aeruginosa* has been demonstrated in models of respiratory tract infections, burn wound infections and keratitis (Rumbaugh *et al*., 1999, Pearson *et al*., 2000, Wu *et al*., 2001; Lesprit *et al*., 2003; Zhu *et al*., 2004). No such study highlighting the role of these signal molecules in the pathogenesis of urinary tract infections is available.

While establishing in the urinary tract, presence of urine, which is a complex medium, exposes invading organism to conditions like varied osmolarity, pH and THP as well as variability of ions such as iron (Shand *et al*., 1985; Suh *et al*., 1999; Kim *et al*., 2003). Urine is subject to change in pH and osmolarity depending on host’s diet and clinical situation. Environmental conditions prevalent in the host milieu may bring about certain changes in organism like change in OMP profile, porin size (Srichyochati and Cox, 1986; Lamont *et al*., 2002) and adhesive ability operative through lectins (Winzer *et al*., 2000; Suh *et al*., 1999). In addition, invading organism in the urinary tract is exposed to glycoprotein, Tamm-Horsfall protein, which is a major component of urinary slime (Kokot and Dulawa, 2000; Devuyst *et al*., 2005). THP, which has been proposed to play dual role, both defensive and offensive (Kuriyama and Silverblatt, 1986; Bates *et al*., 2004; Weicchart *et al*., 2005), may not necessarily act as host defense component (Hawthorn *et al*., 1991). Exposure of organism to environmental changes may play an important role in deciding the ultimate outcome of the infection.

Besides environmental factors, the host also plays an important role in the establishment of an infectious process. In this regard the innate immunity provides a first line of defense in which macrophages
and neutrophils play an important role. Macrophages, coming mostly from circulation, form one of the initial line of defense in the urinary tract and offer resistance against infection. These macrophages interact with invading pathogen leading to elaboration of biochemical substances referred to as macrophage secretory products (MSPs). MSPs have been recognized to contain peptide hormones, complement components, enzymes, bioactive oligopeptides and lipids, reactive oxygen and nitrogen species as well as cytokines (Nathan, 1987). In addition to these, PMNs also provide defense operative through phagocytosis as well as elaboration of cytokines. Cytokines like TNF-α, MIP-2, IL-6 and IL-1β have been reported to be produced in urinary tract following infection with uropathogenic E. coli which help in transepithelial migration of phagocytes from blood to the site of infection (Godaly et al., 2000; Olszyna et al., 2001). Role of all these factors during evolution of urinary tract infection caused by biofilms of P. aeruginosa needs to be looked into since such infections, which may lead to persistence and chronicity, pose a threat for a treating clinician. There is paucity of literature in relation to pathogenesis of catheter associated UTIs caused by biofilms of P. aeruginosa. The present investigation therefore was planned with following aims and objectives:

1. a) To isolate and identify 50 Pseudomonas aeruginosa strains from the urine of patients suffering from catheter associated UTI.
   b) To procure reference strain, a genetically characterized strain (PAO) of Pseudomonas aeruginosa expressing all the recognized virulence factors.
2. To check the ability of all the isolates in planktonic cell form in terms of elaboration of following virulence factors:
   a) Extracellular Polysaccharide (Alginate Production).
   b) Adhesive and Hydrophobic interactions.
c) Exoenzymes (Proteases, Elastase, PLC) Production.
d) Iron mopping ability through hemolysin as well as siderophores (Pyochelin and Pyoverdin).
e) Qualitative and quantitative assessment of quorum sensing signals.

3. Based on elaboration of maximal virulence factors, selection of wild type isolates (5) for further studies. To employ selected 6 strains of *P. aeruginosa* including reference strain (PAO) for generation of biofilms on Foley’s catheter in the laboratory (for period extending upto 7 days).

4. To establish acute and chronic ascending urinary tract infection in mice by planktonic and biofilm cells of selected *P. aeruginosa* strains.

5. To compare virulence of planktonic and biofilm cells of selected (6) strains of *P. aeruginosa* under variable conditions of pH, osmolarity, iron content, Tamm-Horsfall protein and macrophage secretory products (*in vitro* and *in vivo*).

6. To assess role of quorum sensing systems in mouse model of ascending UTI by employing standard *P. aeruginosa* quorum sensing producer strain PAO1 and its isogenic mutants PAOJP1 (lasI mutant) and PAOJP3 (lasR rhlR double mutant) along with quorum sensing negative uroisolates.

7. To study molecular mechanisms of pathogenicity in the urinary tract infection model induced by selected strains of *P. aeruginosa* in planktonic and biofilm cell forms *in vivo* in terms of production of:
   a) Myeloperoxidase (MPO)
   b) Malondialdehyde (MDA)
   c) Reactive nitrogen intermediates (RNI)
   d) Oxygen free radical (OFR)
   e) Cytokines (TNF-α, MIP-2, IL-1β and IL-10)