Chapter 1

Introduction

Urinary tract infections (UTI) are common bacterial infections across the world, which pose a major burden economically on the public health care system (Foxman and Brown, 2003). UTI starts as an infection of the bladder (cystitis) and develops into an acute infection of the kidneys characterized with inflammation caused due to invasion and colonization of the pathogen, resulting in renal scarring and failure (Ulet et al., 2013). If it is not diagnosed and treated at the right time, it can lead to sepsis, which can result in death due to multiple organ failure (Dimitri and James, 2008). When compared to men, women are 30 times more susceptible to UTI (Naber et al., 2009), and one-fourth of this population suffers from recurrent infections every year (Rann, 2008). Approximately 60% of the women suffer from an episode of UTI once in their lifetime, raising this condition to be the most common diagnosis for treatment across the field of healthcare. An acute episode of infection results in approximately 145 h of symptoms, 54 h of work relieve and 60 h spent for recouping in bed (Foxman, 2002). The increased mortality and co-morbidity rate due to bacteraemia (Rebelo et al., 2011), increased uptake of antibiotics resulting in severe sepsis (Johnson et al., 2011), lack of a clear picture on the pathogenic mechanisms of the uropathogens in the host and escalating costs of treatment, have propelled research pertaining to UTI vital.

1.2. SYMPTOMS OF UTI

The common symptoms of UTI are dysuria, urinary urgency, pain in the supra-pubic region and pelvic pain. The other clinical manifestations of UTI include fever, abdominal pain, and tenderness during micturation, nausea, vomiting, pyuria and hematuria accompanied by foul smelling and cloudy urine. The common risk factors that are responsible for 90% of the uncomplicated UTI are sexual intercourse, use of spermicide-based contraception, delayed post-coital micturation and history of recent UTI (Bent et al., 2002). Thrombocytopenia, acute renal failure, disturbance of consciousness and shock after initial presentation, worsen treatment and often leads to high risk of mortality (Huang and Tseng, 2000).
1.3. CLASSIFICATION OF UTI

Colonization of the pathogen based on the site, broadly classifies UTI as urethritis (infection of the urethra), cystitis (urinary bladder), or pyelonephritis, when the bacterial infection affects one or both kidneys in the pelvis, calyx or the parenchymal region (Foxman, 2003). Pyelonephritis is classified as obstructive or non-obstructive, depending on whether the normal renal function is affected. Pyelonephritis is subdivided further as acute, chronic and emphysematous infections of the kidney.

Acute infections of the kidney occur at the ureter, the renal pelvis and the parenchyma; the infection spreads via the blood stream or by the ascending microorganisms along the urinary tract. The inflammatory process is focal and generally irregular, and occurs in the pelvis, calyces and medulla. Medullary infiltration of neutrophil’s is accompanied by inflammation, renal oedema and purulent urine. The released phagocytic lysozymes, oxygen radicals, and inflammation bring about damage in the tubular cells. During severe infection, though sparing of the glomeruli has been reported, abscesses are evident in the medullary and cortical regions. The tubules are severely affected and show signs of extensive damage with vacuolation along with the necroses of the papillae. After an acute phase of infection, healing occurs by the deposition of scar tissue and atrophy of affected tubules with the decrease in the number of bacteria in the urine, until it becomes sterile. Acute pyelonephritis rarely causes renal failure (Kooman et al., 2000).

A persistent and recurrent episode of acute pyelonephritis leads to chronic pyelonephritis where a fibrotic kidney with a shrunken appearance is noticed. To confirm the extent of damage to the renal tissues imaging techniques are utilized and generally, one or both the kidneys are involved. The urine from patients suffering from chronic pyelonephritis when analyzed microscopically, have been identified to contain less number of leukocytes and microorganisms (Rubin et al., 1996).

Emphysematous pyelonephritis (EP) occurs in patients with Diabetes mellitus, with or without the obstruction of the urinary tract. Patients suffer with severe suppurative infection if the kidney with emphysema formation are noticed in the
pelvicalceal region. Women above 60 years of age are affected more commonly when compared to men of the same age group (Ivangi et al., 1988). In most cases, only the left kidney is affected (Pontin et al., 1995) and rarely both are affected (Lowe and Poage, 1991). EP are considered dangerous due to the involvement of secondary infection by gas forming bacteria’s, presence of excess glucose levels in the tissues, impaired tissue perfusion and defective immune response (Huang and Tseng, 2000).

When a patient presents with clinical manifestations of the infection such as flank pain and fever as in the case of acute pyelonephritis or dysuria, increased frequency and urgency of urination as in the case of cystitis, it is referred to as symptomatic bacteriuria. However, in women at most times, the clinical manifestation of the infection is absent, although significant numbers of bacteria are present in the clean-voided, midstream urine specimen obtained from the patient twice at regular intervals. This condition is widely referred to as asymptomatic or covert bacteriuria (Zelikovic et al., 1992).

Based on the severity of the infection, they are further grouped as complicated (due to structural and functional abnormalities or presence of foreign objects such as catheters, nephrostomy tubes or stents or medical conditions such as polycystic kidneys, vesicoureteral reflux, surgical reconstructions, obstructions, enlarged prostate, renal calculi, renal failure, and diabetes mellitus) and uncomplicated infections. Depending on the number of episodes a person suffers annually, UTIs are classified as first infections or as recurrent infections (Chang and Shortliffe, 2006).

After successful eradication of the initial infection, the chances of a recurrent infection are the highest, during the next two weeks. These recurrent infections are subdivided as unresolved bacteriuria, bacterial persistence and re-infection to aid in easy diagnosis and treatment. Unresolved bacteriuria occurs due to inadequate antimicrobial therapy as a result of noncompliance, malabsorption, suboptimal drug metabolism, drug resistant uropathogens (Pewitt and Schaeffer, 1997), while bacterial persistence occurs due to improper eradication of the uropathogen residing in protected sites of the kidney such as infected urinary calculi (Abrahams and Stoller, 2003), necrotic papilus, urethral stents (Kehinde et al., 2004) or catheters due to insufficient administration of antimicrobial therapy (Schlager et al., 2001).
Identification of bacterial persistent UTI is necessary, as surgical intervention is required to prevent the permanent damage of kidney function.

Re-infection in contrast to bacterial persistence is due to infection by a variety of uropathogens in the peri-urethral region (Schoen et al., 2000) or by fecal-perineal-urethral route (Yamamoto et al., 1997) that has been documented with each new bout of UTI, after the complete eradication of the previous pathogen. It can also occur due to the resistance of the bacterial species to the antibiotics administered or the emergence of a secondary bacterial resistant strain, poor excretion of the antibiotic in the urine due to renal insufficiency, or papillary necrosis from analgesic abuse.

1.4. INCIDENCE OF UTI

1.4.1 Children

The incidence of UTI during childhood varies with age, gender and other factors such as sanitation, febrile illness etc. During the first year of a child’s life, the incidence of UTI is the highest (1%) in both genders. This ratio decreases in the case of male infants due to various reasons such as circumcision, congenital and functional abnormalities of the urinary tract (Marra et al., 2004). UTI in infants are manifested by the presence of high-grade fever, with subtle and non-specific clinical presentations (Gorelick and Shaw, 1999). If unidentified, it leads to kidney scarring and hypertension at a later age, which ultimately leads to renal failure (Brooks, 1990). Paediatric UTI if, not diagnosed and treated, can result in permanent kidney damage and chronic kidney disease (Rehman et al., 2008).

The identification of UTI in children is often difficult as the clinical symptoms and signs are non-specific and urine collection for interpreting results is not easy (NICE, 2007). During pre-schooling and in the later years, the incidence of UTI is higher in girls (1-3%) when compared to boys (0.1%). Most of the infections are asymptomatic and are detected only by screening. In girls, recurrent infections are common, and close monitoring is necessary to prevent renal scarring and further injury. A study of the Indian population reported that the overall prevalence ratio of UTI among children to be 65.3%, where the incidence ratio in girls was found to be 59.6% when compared to boys (40.3%) (Choudhury et al., 2013). In a study carried
out among the primary school students in Maiduguri, Nigeria, the incidence rate of significant UTI was found to be higher among the girls (19.5%), when compared to the boys (12.5%) (Isa et al., 2013).

1.4.2. Women

The lifetime risk of an UTI in women is approximated to be 30% and around 71% of the reported cases with UTI were identified to be women (MacVane et al., 2014). Women are five times more likely to experience an acute pyelonephritis infection when compared to men and 11% of the women annually experience at least one diagnosis of UTI (Mulvey, 2002). The mature vagina under the influence of the oestrogens maintains an acidic pH, in which \textit{Lactobacillus} exists as the commensal species. The pH’s of the prepubertal and postmenopausal vagina are maintained close to neutral and the commensal flora consists of \textit{Enterobacteriaceae} (Smith et al., 2014).

1.4.3. Pregnancy associated UTI

During pregnancy, 4 – 12.7% of the women suffer from asymptomatic bacteriuria (Chandel et al., 2012; Almushait et al., 2013). This often progresses to symptomatic UTI, postpartum UTI or pyelonephritis (Andriole and Patterson, 1991, Patterson and Andriole, 1997). UTI during pregnancy when left untreated has been reported to be associated with low birth weight in the infant, premature birth, increased foetal mortality and at times infection of the kidneys during birth (Romero \textit{et al.}, 1989). The severity of acute pyelonephritis increases with age and up to 2% of the pregnant women experience an episode of pyelonephritis with a subsequent risk for miscarriage (Papanicolaou and Pfister, 1996).

1.4.4. Men

Men with structural, obstructional (enlarged prostate, calculi, neurogenic bladder), functional and anatomical abnormalities and those above 50 years of age are generally more prone to a complicated UTI (Wagenlehner \textit{et al.}, 2014). This population equates to 10% of the entire population suffering from UTI. Out of this
10% of the population, 30% are at a higher risk to die due to complications of UTI (Munro et al., 1990).

1.4.5. Elderly

Elderly women and men who are non-institutionalized and are active members in a community dwelling, have an UTI incidence rate of 6 – 30% and 11 – 13%, respectively. However, the rates of an UTI in the institutionalized elderly who are 30 – 70% immobilized have a very high rate of UTI, and the incidence ranges from 25 – 50% (Lee et al., 2014).

1.4.6 Complicated UTI

UTI’s are 3 times more common in diabetics than in non-diabetic women and it increases with age. The improper maintenance of blood glucose levels (Geerlings et al., 2000), damage to the genitourinary system due to diabetic neuropathy that leads to dysfunctional bladder (ADA, 2013) has been identified to be the major factor for developing infections of the urinary tract. Diabetics are predisposed to develop acute pyelonephritis and renal papillary necrosis. The presence of high glucosuria often directly relates to the high bacterial counts in the diabetics (Chen et al., 2009; Funfstuck et al., 2012) and favours the development of severe urinary complications (Brown et al., 2005; Turan et al., 2008). The age of the patient, metabolic control and duration of diabetes and impaired immune function are responsible for the progressive renal damage than the infection by itself (Geerlings and Hopelman, 1999).

1.5. DIAGNOSIS

1.5.1 Quantitative analysis

The diagnosis of an UTI is based on three criteria: (i). Clinical symptoms at the time of presentation in a health care facility (ii). Detection and signs of infection in the urine provided to the laboratory and (iii). Detection and identification of the pathogen in the urine. To diagnose asymptomatic bacteriuria and symptomatic UTI many tests are available. A plated culture of the mid-stream urine (ECLM, 2000) provides detailed documentation of the bacterial infection with regards to the causative at the end of 24 h and its antibiogram after a period of 48 h (Palmqvist et
al., 2008) with 80% sensitivity and 90% specificity in detecting UTI. The presence of 
10^3 colony-forming units per millilitre (cfu/mL) defines the presence of a significant 
UTI (Warren et al., 1999). Although being the “gold standard technique” with the 
merits of being a cheap and highly accurate and reproducible technique, it has the 
disadvantages of requiring skilled and technical resource personnel and time involved 
for initiating treatment in high risk patients, implying the need for rapid diagnostic 
techniques (Cocht et al., 1998). The other recent methods, along with the urine culture 
are chromogenic agars such as CLED agars (Pappas, 1991)

1.5.2 Microscopic analysis of urine

The sediment obtained from the centrifugation of a fresh urine sample is 
examined microscopically to check for the presence of erythrocytes, leukocytes, cells 
derived from the kidney and urinary tract. The sediment includes the presence of 
THP casts, fat and pigmented particles. An increase in erythrocytes or casts implies 
hematuria due to infections of the urinary tract. Leucocytes or casts indicate the 
presence of white cells in the tubular region of the kidney. Inflammation of the upper 
urinary tract results in the presence of polymorphonuclear casts. Whereas, when an 
inflammation of the lower urinary tract is present, casts are not visible. In individuals 
suffering from bouts of acute infection, hematuria may lead to colouration of the 
urine, with the presence of large numbers of erythrocytes and leukocytes as the 
duration of the infection increases, the presence of sediments in urine also rapidly 
increases (Mohkam et al., 2014).

1.5.3 Chemical and enzymatic methods

Various tests such as the triphenyltetrazolium chloride reduction test, the 
detection of urinary glucose and protein concentration, detection of nitrite, bacterial 
ATP and endotoxin have been used to identify UTI. Pyuria is detected by the 
leukocyte esterase activity (Shah et al., 2014).

1.5.4 Semi-quantitative analysis

One of the semi-quantitative methods widely followed are the leukocyte 
esterase and nitrite dipstick method, commercially available through vendors, which
proves to save time in delineating patients with a possible infection (Lohr, 1991 and USPSTF, 1990). The major disadvantage of this method lies in the fact that an accurate diagnosis of an UTI depends on vital factors such as urine collection, handling, the presence of substances that could produce a false positive result (Beer et al., 1996), specificity of the infection being an UTI and dipstick storage and handling (Gallagher et al., 1990; Edwards and Granier, 1996).

1.5.5. Automated methods

Photometric methods that measure the light scattered by the bacteria, depending on their growth rate and incubation time, are utilized, but are often less reliable for slow-growing pathogens. Bioluminescence based methods that measure the bacterial ATP are rapid, but are less preferred due to the high false positive results and poor specificity. The other automated methods utilized depend on colorimetry, electrical impedance, filtration, fluorescence and radiometry of $^{14}$CO$_2$ released as a by-product of bacterial metabolism from the isotope labelled substrates and flow cytometry (Bonkat et al., 2014).

Chromatographic methods such as Gas-liquid chromatography and High-pressure liquid chromatography are useful in classifying clinically significant strains of infection (Rossello et al., 2014). Identification of the strains is based on the principle that anaerobic bacteria cannot oxidize nutrients to carbon dioxide and water, instead they form intermediate products of short-chain fatty acids, organic acids and alcohols that are relatively distinct for the specific groups of anaerobic bacteria. The major disadvantage of this method is that it cannot be utilized to differentiate the gram-negative pathogens of the urinary tract (Krijt et al., 2013).

1.5.6. Molecular Biology based methods

Real-time PCR is used as an alternative to conventional culture methods for the diagnosis of UTI, to shorten the time period involved to identify the pathogen. The drawback of this method lies in the fact that it can be utilized to screen only one pathogen or its Gram status in a single run (Hinata et al., 2004 and Shigemura et al., 2005). Multiplex real-time systems that detect up to 25 bacteraemic pathogens have been introduced to investigate and quantitatively identify urinary pathogens
The overlap of the primer binding sites helps in the discrimination of more than 20 pathogens. However, the modified and emerging uropathogens cannot be detected through this method (Grude et al., 2001) due to the alterations in their genetic material.

The real-time PCR based methods save close to 43 hours compared to the culture based identification methods of the uropathogens and have a sensitivity of 82% and specificity of 60% (Hansen et al., 2013). It has to be taken into consideration that even though these primer based methods are employed to identify the pathogen, only empirical antibiotic treatment is initiated to relieve the patients from discomfort and to avoid further complications due to urosepsis. The results of the antibiogram by conventional methods are necessary to prescribe the needed antibiotic regimen to eradicate the infection (Lehmann et al. 2011).

1.5.7. Antibiogram testing of the isolates

Antibiogram analysis of the pathogen to the commonly administered drugs is an important procedure to determine the success of the treatment options for treating an UTI. Based on the results of the standard procedures outlined by clinical and laboratory standards institute (CLSI), the pathogens are interpreted as susceptible, intermediate, or resistant to the drugs under investigation (AST, 2013).

1.5.8. Non-invasive methods of localization of infection

Infections of the upper urinary tract are identified through differential culture of the urine and detection of antibody-coated bacteria in the urine, by fluorescein-labelled anti-IgG. Recent advancements in this field utilize monoclonal antibodies that are fluorescein conjugated. The major drawback of this method lies in the fact that dead organisms and cell debris in the media interfere and provide false-positive results (Fracchiolla et al., 2013).

Serum antibody titration utilizing antibodies raised against the O antigens of the gram-negative bacteria by indirect haemagglutination (detects IgM antibodies) and direct bacterial agglutination (detects IgG antibodies) are utilized to localize
upper UTI. In addition, detection of circulating THP has also been used to localize infection (Iorember and Vehaskari, 2014).

Urinary $\beta_2$ microglobulin, procalcitonin, proadrenomedullin and urinary enzymes of renal origin have been used to detect renal damage (Daure et al., 2013). Measurement of C-reactive protein helps to distinguish upper and lower infections (Page et al., 2014). Measurement of renal concentrating ability and osmolalality provides an idea on the effect of treatment (Williams, 2013).

1.5.9. Invasive methods of localization

Excretion urography, contrast-enhanced ultrasonography, cytoscopy, bladder washout, Pyelography, micturating cystography, urethrocystography, stamey test and ureteric catheterization have been utilized to identify the extent of damage to the urinary tract and due to infection and vesicoureteral reflux. Renal biopsy is at times carried out to identify the damage due to bacteriuria (Pezzlo, 2014).

1.6. EPIDEMIOLOGY

The sterility of the normal urinary tract is partly attributed to perputial (in men), urethral, periurethral, introital, vaginal and faecal commensal flora that play a significant role in pathogenesis and resistance against UTI (Zasloff, 2013). The distal urethra has a sparse but complex variable microflora comprising of various aerobic and anaerobic bacteria such as coagulase negative Staphylococci, Streptococci, Enterococci, and non-pathogenic strains such as Neisseia spp., Bacteroides, Fusobacterium, Peptostreptococcus, Eubacterium, Clostridium spp. etc. Commensal mycobacteria and mycoplasmas have also been reported in certain races of population (Summanen et al., 1993).

Under the oestrogenic influence, the vagina provides an acidic environment (pH 5.0) in which the Lactobacillus spp. predominate along with the aerobic cocci, non-sporing anaerobic bacilli and Clostridia that are present (Smith et al., 2014). In prepubertal and postmenopausal women, the pH is maintained close to neutral and the microflora is predominantly Lactobacilli and Enterobacteria (Hooton and Stamm, 1996). During initial infections, the causative pathogen are present in the perineum of
the urinary tract. However, in the case of recurrent infections and in patients with structural or functional or anatomical abnormalities or with urinary catheters, the predisposing factors vary.

The aetiology of UTI range from the gram negative to the gram positive spectra of the bacteria: *Escherichia coli* (80%), *Klebsiella* (5%), *Proteus mirabilis* (5%), *Staphylococcus aureus* (8%), and *Staphylococcus saprophyticus* (1 -2%) are known to be the major players in both clinical and community settings (Gupta *et al.*, 2001). The other species that are responsible for causing an infection are *Streptococcus* spp., *Micrococcus* spp., *Actinomyces* spp., *Prevotella* spp. Fungal infections of the urinary tract appear to be apparently uncommon, yet, *Candida* being the common pathogen if an infection were to occur (Boedeker and Kilzer, 2001). Multiple fungal species have been reported to colonize the urinary tract, and form an obstructive fungal ball that leads to the formation of a symptomatic UTI in urolithic (Burgues *et al.*, 2003) and immuno-compromised patients (Kale *et al.*, 2002). Annually, due to parasitic infections of the urinary tract, 200 million are affected with the major pathogen, *Schistosomiasis*. They adhere to the host urethral region, and lead to inflammation and scarring of the urinary tract, ultimately leading to urothelial malignancies (Baisoum, 2003).

In India, among children, *Escherichia coli* is responsible for 53.0% of the infections in the paediatric population followed by *Proteus* spp. (16.8%), *Klebsiella* spp. (15.3%) (Choudhury *et al.*, 2013). The prevalence of *E.coli* is predominant and equal in the age groups ≥ 12 months and ≥ 12months, in both the sexes. In case of male infants, *Proteus* spp. was found to be predominant in infants < 12 months of age, when compared to infants ≤ 12 months. *Klebsiella* spp. was found to be equally distributed among male children of different age groups. However, in girl infants, *Proteus* spp. and *Klebsiella* spp. occur predominantly in the <12 months age group compared to ≥ 12 months. The infections due to gram-positive bacteria, although very less, was predominantly due to *Enterococcus* spp. (5.6%) followed by Coagulase negative *Staphylococcus* (4%), and *Staphylococcus aureus* (2.5%) (Choudhury *et al.*, 2013).
From the study carried out in UK, it was identified that Extended spectrum β-Lactamases (ESBL) producing E.coli were the predominant causative (85%) of the cases followed by Klebsiella pneumonia (10%) and the other species contributed to 5% of the cases (Enoch et al., 2012). From the samples analyzed in Nigeria, it was noticed that Pseudomonas was the major pathogen (12.08%), followed by E.coli (11.39%) and S.aureus (13.56%) of the cases (Okwori et al., 2011). Similarly in Greece, E.coli was responsible was 68.9% of the infections of the urinary tract, followed by P. mirabilis (6.8%), K. pneumoniae (6.4%) and Enterococci in 6% of the cases (Maraki et al., 2013).

From the study to monitor antimicrobial resistance trends (SMART) study carried out across 11 countries in Asia during 2009 -2010, Enterobacteriaceae were responsible for 86% of the infections (E. coli – 56.5% and K. pneumoniae 13.8% of the infections (Lu et al., 2012). In Mexico, E. coli (77%), was found to be the predominant player among sexually active and pregnant women. However, among the pediatric population, K. pneumoniae was more common (22%). In a similar study carried out in France, E. coli was responsible for 28.7% of the infections followed by S. aureus (17.4%), K. pneumoniae (14.9%), and Enterobacter aerogenes in 10% of the cases. From the North American urinary tract infection collaborative alliance (NAUTICA) study carried out across North America and Canada, it was found that E.coli (57.5%), Klebsiella pneumonia (12.4%), Enterococcus spp. (6.6%), Proteus mirabilis (2.7%) Psuedomonas aeruginosa (2.9%), Citrobacter spp (2.7%), Staphylococcus aureus (2.2%), Enterobacter cloacae (1.9%), Coagulase negative Staphylococci (1.3%), Staphylococcus saprophyticus (1.2%), Klebsiella spp. (1.2%), Enterobacter aerogenes (1.1%) and Streptococcus agalactiae (1%) were responsible for UTI in the 1990 isolates included in the study (Zhanel et al., 2005).

1.7. UROPATHOGENIC Escherichia coli

Escherichia coli are common inhabitants of the gastro-intestinal tract with a beneficial symbiotic relationship to the host. They play an important role in maintaining the intestinal homeostasis and the stability of the microflora in the luminal region of the gastro-intestinal tract (Yan and Polk, 2004). Strains of pathogenic E. coli emerge from the commensal cohorts, by acquiring pathogenic
nature by the horizontal transfer of transposons from other bacterial genome, plasmids, bacteriophages and pathogenicity islands (Oelschlaeger et al., 2002), which provide the emerged pathogen with the ability to adapt to new niches and cause a broad spectrum of diseases (Wiles et al., 2008).

*Escherichia coli*, being facultative, motile, gram-negative rods, that are generally 0.6 – 1 µm wide and 2 – 3 µm long and hold many facets, commonly referred to as virulence factors (VFs), which enable it to be an etiologic agent of various intestinal and extra-intestinal infections, in the otherwise well protected and immunologically highly effective host milieu (Johnson and Russo, 2005). Uropathogenic *E. coli* have been reported to belong to the phylogroup B2 (Johnson and Russo, 2002 and Ewers et al., 2007). The roles of these VF, although mostly putative, have been widely accepted to play specific roles during pathogenesis by aiding in mucosal colonization, subverting systemic host defences, acquisition of nutritional requirements, invasion of host tissues and for the stimulation of inflammatory host response beneficial to the invading pathogen (Johnson and Russo, 2005). The expression of these VF depends on the immune competence of the host, geographical location of isolation, pathotype and virulence potential of strain causing the infection (Tenaillon et al., 2010).

1.8. VIRULENCE MECHANISMS IN UPEC

VF are subdivided as surface virulence factors and secreted virulence factors based on the effect they have during the establishment of an infection (Emody et al., 2003). The surface VF are fimbriae that aid in the adsorption and adhesion of the uropathogen to the host tissues within the urinary tract. They act in three ways: (i) activate the host and bacterial cell signalling pathways, (ii) facilitate the delivery of bacterial products across the host tissues, and (iii) contribute to bacterial invasion (Mulvey, 2002). The adherence of the pathogen to the urothelium and the erythrocytes are mediated through the Mannose - sensitive fimbriae (Hughes et al., 1983) and the mannose-resistant fimbriae (Jann et al., 1981), capable of haemagglutination present on the surface (Waters and Crosa, 1988). The various fimbriae which have been implicated to play a role in the adhesion of UPEC are type 1 fimbriae, S and F1C fimbriae, P fimbriae, Fimbrial Dr and afimbrial Afa adhesions, Lipopolysaccharide
and Flagella (Fig.1.1). Toxins that are secreted by UPEC form the VFs that are responsible for the inflammatory response by the host. The commonly encountered toxins are α-haemolysin, cytotoxic necrotising factor 1, secreted autotransporter toxin. It has been discovered that there are non-pilus adhesins present on the bacterial cell wall that aid in the adhesion process of the bacteria to the extracellular matrix of the host (Tomme et al., 1995).

1.8.1. Type 1 Fimbriae

Type 1 fimbriae (T1F) were identified to be potential virulence factors from the UTI models generated in animals. Their role in human pathology remains yet to be elucidated (Bergsten et al., 2007) as these fimbriae are expressed in both pathogenic and non-pathogenic strains with similar frequency (Plos et al., 1991). In murine models of UTI, these fimbriae have been implicated to play a role in enhancing bacterial survival rate in the host tissues, initiate mucosal inflammation, and promote invasion (Martinez et al., 2000) and growth of intracellular bacterial communities (Anderson et al., 2003).

The T1F are hydrophobic in nature and are capable of mannose-resistant haemagglutination through non-specific hydrophobic interaction (Brinton, 1959) and a mannoside binding region (Falkowski et al., 1986). The T1F adhesins, PilE - FimH, are located laterally or at the tip, sparsely along the length of the fimbriae (Abraham et al., 1987). The rod-shaped adhesins are stable (Salit and Gotschlich, 1977) and require extreme physiochemical conditions for depolymerisation and polymerize into intact fimbriae under appropriate conditions (Eshdat et al., 1981). The T1F expression is promoted by growth at limiting oxygen concentrations (Brinton, 1959), subinhibitory concentrations of cephalosporins, while the vancomycin, chloramphenicaol, aminoglycosides, and tetracyclines have been identified to reduce its expression (Klien et al., 1985). The receptors for these fimbriae are present on erythrocytes (Duguid et al., 1979), buccal epithelial cells (Ofek et al., 1977), intestinal cells (Wold et al., 1988), vaginal cells (Falkowski et al., 1986) and in urothelial cells (van den Bosch et al., 1980).

The binding of T1F to the mannosylated integral membrane proteins, uroplakins Ia and IIIa and THP, present on the surface of the urothelium through the
FimH adhesin (a 30 kDa protein) subunits located at the fimbrial tip. These host cell-contacting pili have been identified to be 0.12 µm in length in contrast to the non-contacting pili, that are 1 – 2 µm in length, suggesting that there could be an interplay wall that aid in the adhesion process of the bacteria to the extracellular matrix of the host (Tomme et al., 1995). The bacteria utilise the pili retracting mechanism to enhance tight bacteria – host cell apposition, for the subsequent internalization of UPEC (Mulvey et al., 1998). The swapping of the arginine and serine residues at the 70 and 78 positions in the 300 residue sequence of FimH pili, has been identified to be responsible for the varying length of the adhesion to the host cells (Sokurenko et al., 1995).

The T1F promote adherence of the UPEC to human polymorphonuclear leukocytes through a 150-kDa glycoprotein, CR3, located on its surface and promote phagocytosis of the pathogen, depending on its cell surface hydrophobicity and state of opsonisation. The killing of the pathogen also depends on the phagocytic granules released in response to bacterial binding or a result of the toxic products released due to respiratory burst. The bacteria that are phagocytised survive within the lysozymal vacuoles until they are further opsonised due to the increased myeloperoxidase activity in the lysozymes (Thumbikat et al., 2009).

Mannose sensitive (MS) adherence, if facilitated by the presence of T1F (Duguid and Gillies, 1957) which aid in binding to the THP that is rich in D-mannose subunits on its branched oligosaccharides, upon binding form a pseudo-capsule (Duguid and Olds, 1980) around the microorganism and thus protect it from forced expulsion (Orskov et al., 1980). When the concentration of THP falls, bacterial adhesion to the urothelial cells is promoted (Duncan, 1988). The presence of receptors for T1F on Polymorpho-Nuclear Lymphocytes (PMNLs) thus aid in phagocytosis of the pathogens depending on their state of opsonisation and hydrophobicity (Weinstein and Silverblatt, 1983). Some cells that evade the PMNLs are killed due to the toxic products released from the respiratory burst of the extracellular phagocytes (Stenqvist et al., 1983) and are known to survive in the lysosomes where the activity of myeloperoxidases are low (Goetz et al., 1987).
Virulence factors associated with the pathogenesis of Uropathogenic *Escherichia coli*

They aid in the adhesion of the pathogen to the mucosal and tissue matrix of the host cell, activating the production of cytokines (Godaly *et al*., 2000).

1.8.2 P fimbriae

P fimbriae are the second common VFs, which play an important role in the pathogenesis of ascending UTI and pyelonephritis due to UPEC in humans (Plos *et al*., 1995). The papA-K gene operon encodes the P fimbriae and is composed of heteropolymeric fibres, composed of different subunits that constitute the fimbrial protein PapA, that recognizes the Gal α (1 – 4) determinant of the renal glycosphingolipids with the aid of the papG adhesin (Wulit *et al*., 2000). The allele variant II of papG adhesion is generally present in pyelonephritic patients, while the allele variant III is present in women and children. This binding occurs due to the bend in the disaccharide chain, where the hydrophobic surfaces are exposed on the convex side that acts as the epitope. The binding of the adhesin to the receptor releases ceramide, that activates the toll-like receptor 4 (TLR4), which in turn activates the immune cell response (Fischer *et al*., 2007) and leads to local inflammation and pain associated with UTI (Bergsten *et al*., 2005). P Fimbriae
synergistically play a role with T1F *in vivo* where P fimbriae mediates early colonization of the tubular tissues and T1F mediates colonization of the centre of the tubules to aid in bacterial binding and biofilm formation. The formed bacterial communities have a significant role in the obstruction of the nephron function, leading to the exhibition of the patho-physiological features of pyelonephritis (Melican *et al.*, 2011).

1.8.3. S Fimbriae and F1C fimbriae

These fimbriae bind to the epithelial and endothelial cells of the urinary tract and kidney which facilitate in bacterial dissemination and aid in sepsis (Mulvey, 2002).

1.8.4. Dr Fimbriae and Afa adhesins

They play a role in gestational pyelonephritis and recurring cystitis (Servin, 2005). Dr adhesins at the tip of the fimbriae bind to type 4 collagen and activate the decay accelerating factor in the kidney (Nowicki *et al.*, 2001) and exhibit tropism to the basement membrane of the renal tissues (Goluszko *et al.*, 1997) along with the Afa adhesins (Bouguenec, 2005).

1.8.6 Lipopolysaccharide (O-antigen) and capsule

Lipopolysaccharide (LPS), an outer membrane constituent of bacterial cell wall, is a potential endotoxin, mediates a number of deleterious effects upon the host. Smooth type LPS, richly coated with glycolipids are highly pathogenic and O groups O1, O2, O4, O6, O8, O16, O18 and O75 are predominantly present in the UPEC (Lindberg *et al.*, 1975; Waalwijk and DeGraf, 1983; Jorgenson *et al.*, 1976). The antigens produced by LPS are highly toxic. They are not easily identified by the host immune cells as they mimic the receptors of the local proteins. Therefore they can easily activate the complement pathways and release the mediators that are responsible for the characteristic UTI symptoms (Johnson, 2003). Capsular polysaccharides of UPEC capsule protect them from the commensal *E. coli* in the host and interfere with the O-ag detection, phagocytosis and lysis mediated by the complements (Johnson, 2003). The polysaccharides expressed on UPEC are of the
gp2 or gp3 type and renders poor immunogenicity, which is attributed to the terminal 
sialic acid residues, which are identical to the host glycoproteins (Johnson, 1991). 
LPS being an integral component of the cell wall of gram negative bacteria and are 
known to activate the host response to induce nitric oxide and cytokine production 
(Backhed et al., 2001).

1.8.7 Flagella

Flagella are responsible for the motility of the UPEC and are involved in the 
interaction of the strain with the epithelial surface of the kidney and in its ascension in 
the kidney (Schwan, 2008). Flagellated UPEC strains are responsible for 70-90% of 
UTI. Flagellated strains are known to play a role in the invasion of the collecting duct 
cells through the flagellin, which acts as an invasion in the process (Pichon et al., 
2009).

1.8.8 Cytolysins

UPEC encode a variety of toxins. **Cytolethal distending toxin** is encoded by 
cdtABS that blocks the G2 phase in cell cycle (Scott and Kaper, 1994). During the 
block, the mitosis promoting factor is kept in an inactive form. Cytotoxic necrotising 
factor has two variants, CNF1 and CNF2 that are encoded by the chromosomes and 
the plasmids, respectively. They act by inducing multi-nucleation and necrosisand 
also by enhancing the activity of Rho-GTPases (Rippere-Lampe et al., 2001).

**SPATE** (Serine Protease Auto-transporters of Enterobacteriaceae) are another 
group of toxic proteins secreted by UPEC. Two types of SPATE have been identified 
till date: **SAT** (Secreted Autitransporter Toxin) (Parham et al, 2004) and **PicU** (Guyer 
et al, 2000). All the SPATE are characterized by the presence of a serine protease 
active site motif in the passenger domain. SAT functions by creating vacuolation in 
the host cells (Guyer et al., 2000). Cytolysins are the most commonly encountered 
toxins. They are active due to the expression of the α-haemolysins and belong to RTX 
(repeats in-toxin) family of exoproteins produced by gram-negative pathogens. They 
exhibit target cell specificity, post-translational modification, C-terminal calcium 
binding domain of acidic glycine rich repeats, which has a consensus sequence 
GGXGXDX[L/I/V/W/Y/F] and secretion by type I system (Lilie et al., 2000). The
post translation modification and calcium binding are vital for the cytotoxic activity of the toxin.

1.8.9. Haemolysin

Haemolytic strains of Uropathogenic *E.coli* (UPEC) are encountered in 51% of the upper UTI, 30% of lower UTI, 49% pyelonephritis, 40% Cystitis and 20% asymptomatic bacteriuria secrete α haemolysins, a cytolytic toxic protein (Cavalieri *et al.*, 1984; Caprioli *et al.*, 1987). The secreted haemolysin lyse erythrocytes with the help of calcium to form haemolysin aggregates; these aggregates enter into the phospholipid membranes, cause inflammation, tissue injury and impaired host defence and changes in the PMNL’s (Bhaksi *et al.*, 1988). Research is being carried out on the production of vaccines using hemolysin with Gal – Gal pili against pyelonephritis (Guyer *et al.*, 2002; Johnson *et al.*, 2002).

The RTX toxin operon consists of four genes hlyC, hlyA, hlyB and hlyD. hlyC is responsible for the post-translational modification of HlyA by acylation of the two lysine residue epsilon amino groups (K564 & K690) (Henderson *et al.* 1996). Inactive HlyA is produced by hlyA; hlyB and hlyD are essential for HlyA secretion. HlyB, is an inner membrane traffic ATPase containing an ATP binding cassette, that supplies energy for the secretion and translocation of HlyA across the inner membrane (Holland and Blight, 1999). HlyD, is a member of the membrane fusion proteins family, and is anchored in the inner membrane and forms a channel connected by HlyB (Johnson and Church, 1999) and TolC, an outer membrane protein (Koronakis *et al.*, 2000, Anderson et al, 2001) and helps in the translocation of HlyA (Zgurskaya and Nikaido, 2000). The hly determinants are located either on the chromosome or the pathogenicity islands, and can be transmitted by horizontal gene transfer among the gram-negative bacteria. The receptors for α-haemolysin are two polypeptides CD11a and CD18, to which HlyA binds. These receptors have been identified on erythrocytes, glycoproteins and integrins (Cortajarena *et al.*, 2003).

1.8.10 Cytotoxic necrotising factor 1

Cytotoxic necrotising factor 1(CNF1), are present in a third of the UPEC strains that cause pyelonephritis and are involved in kidney invasion (Landraud *et al.*, 2002).
They stimulate the stress fibre formation and membrane ruffle formation in actin through the Rho-GTPase pathway (Bower et al., 2005). It has also been reported to play a role in phagocytosis by polymorphonuclear cells and apoptosis of the bladder epithelial cells (Fiorentini et al., 1997) leading to enhanced access to the underlying tissues (Mills et al., 2000).

1.8.11 Secreted autotransporter toxin and Cytolethal distending toxin

Secreted autotransporter toxin and Cytolethal distending toxin of the UPEC strains have a toxic effect on the bladder cells (Guyer et al., 2002) and play are speculated to have a role in the bacterial survival in the renal system (Cirl et al., 2008).

1.8.12 Siderophores

Host iron is complexed with proteins such as lactoferrin, transferrin, haemoglobin that restrict the iron source from being available to the invading pathogens by chelation with proteins, shifting them for intracellular storage and by decrease in intestinal absorption (Weinberg, 1990). Microbes sequester these iron sources into the medium by producing two types of siderophores, Enterocholins (phenolates) and aerobactin (hydroxamates), which actively complex with ferric ions and transport them inside the bacterial membranes (Rosenberg and Young, 1974). UPEC contains the hydroxamate type of siderophores (Warner et al., 1981). Recent studies have shown that siderophores not only sequester iron but also promote adhesion to the epithelial cells of the host (Johnson et al., 2000). Thus, production of aerobactin serves as an important virulence factor (Stuart et al., 1982; Sharma et al., 1991; Sharma et al., 1995).

1.9. INNATE IMMUNITY OF THE KIDNEY

The innate immune system plays an important role in the defence of the urinary tract through its effectors such as antimicrobial peptides (AMP’s), proteins that rapidly neutralize the pathogen and the chemokines (Zasloff, 2013). These defences are clinically silent and operate at the basal level before the onset of an infection. They are expressed at higher levels during microbial invasion thus
preventing the microbes from gaining a foothold in the urothelium by killing them or inhibiting their growth (Brogden, 2005). The AMP’s are widely distributed in certain bacteria, protozoa, fungi, plants, and multicellular animals (Zasloff, 2002). The expression of these AMP’s reflect the microbial challenges of the niche the organism and its commensal inhabitants occupy.

1.9.1 Primary defence in the kidney

The sterility of the urinary tract is primarily due to the flow of urine, which removes the non-attached or the weakly adherent microbes from the bladder surface (Sobel, 1997). Glucosamine secreted by the transitional epithelium protect the urinary tract from bacterial adherence by forming the mucin layer (Fukuoka and Kobayashi, 2001). Low pH, presence of salts, urea and organic acids reduce bacterial survival within the urinary tract (Cavallone et al., 2001).

AMP’s are amphipathic, have a net positive charge due to the presence of lysine and/or arginine residues. The presence of cationic charge and amphipathic nature are largely responsible for their activity. They are generally composed of about 20 to 60 amino acids and are selective for Gram-positive and negative bacteria (Brogden and Brogden, 2011). Their amphipathic nature renders it possible for them to attain high concentrations in the aqueous environments and also within the cells (Zasloff, 2007). The rate of action of the AMP’s on the microbes is rapid i.e. they act in regulated steps within 15 – 90 minutes to kill the micro-organisms lethally (Matsuzaki et al., 1995).

The difference in the composition of the lipids that surround the urothelial cells and that of the microbes is responsible for the specificity of the AMP’s. Urothelial cells are composed of zwitter ionic phospholipids on the outer surface of the membranes and anionic phospholipids on the inner surfaces; while the microbes contain negatively charged phospholipids on their membranes (Matsuzaki et al., 1995). Due to this reason the micro – organisms lack the ability to develop resistance to these AMP’s, which would require the complete redesigning of their membrane lipid composition and its topology (Zasloff, 2002).
Defensins and cathelicidins, the most studied AMP’s, probably play a vital role along with Tamm-Horsfall protein (THP), lactoferrin, and lipocalcin, in maintaining the normal milieu of the urinary tract (Zasloff, 2002). The α and β defensins present in high concentrations on the neutrophil's are differentiated based on their manner of folding; they contain three sets of disulphide bonds. Defensin’s have the ability to permeabilize vesicles that mimic the lipid composition of the bacterial membranes; provide the neutrophil's with their non-oxidative microbial activity. The defensins gene clusters present on chromosome 8p23.1 exhibits a copy number polymorphism of 2 – 12 in a diploid genome (Lehrer, 2013).

Cathelicidin a linear peptide is expressed on all epithelial surfaces and by circulating white cells, including neutrophil’s, monocytes, natural killer cells, and T cells due to local injury or infection. It is expressed in low levels in urine (Zasloff, 2007). In vitro studies show that the mRNA encoding for cathelicidins sharply rose upon exposure to E.coli in renal explants, thus leading to increased secretions of the peptide into the surrounding medium (Nielsen et al., 2014). Along with defensins, it acts as a chemo-attractant for the immune cells against the pathogens and is expressed along the urinary tract in humans. The role of these peptides have not yet been proved as anti – infective protein beyond the membrane models thus remaining a mystery which requires the proof of how these peptides interact with the micro-organism’s, to remove before adhesion onto the urinary tract. The role of the neutrophil produced cathelicidins in maintaining the urogenital tract homeostasis is yet to be studied (Zasloff, 2007).

Tamm Horsfall Glycoprotein (THP), the most abundant protein next to albumin in urine; although not antimicrobial in nature, serves as an anti – infective protein by preventing the adherence of uropathogens to the epithelium, by interacting with the mannosylated residues on the fimbriae of the pathogens, and thus contributing to their elimination via urine (Mo et al., 2004; Pak et al., 2001). The immunomodulatory role of THP lowers the activation threshold of the innate immune cells against the THP – bound uropathogens (Saemann et al., 2001; Saemann et al., 2005).
Lactoferrin and lipocalin play an important role as AMP’s by restricting the availability of iron to the microbes, which serves as an essential nutrient for microbial growth. Lactoferrin acts by chelating iron and brings about membrane damage due to the interactions effected by the cationic amphipathic sequences (Abrink et al., 2000). Research by Singh et al. has shown that lactoferrin effectively blocks the biofilms developed by opportunistic pathogens (Singh et al., 2002). Lipocalins, designed to capture the iron – saturated organic siderophores that bacteria utilize to scavenge their iron requirement from the surrounding environment (Goetz et al., 2002), exhibit bacteriostatic activity against the siderophores – secreting organisms (Flo et al., 2004). The mechanism by which lipocalin is inducted to act against these microorganisms remains to be identified.

The secreted AMP’s also activate the toll – like receptors (TLR’s) that aid in recognition of the distinct pathogen receptors such as lipopolysaccharides (LPS) (Fischer et al., 2006) also aid in maintaining the host – microbial relationship in the urinary tract (Perron et al., 2006). The activation of TLR’s and its co- receptors leads to the production of the various inflammatory cytokines and chemokines through the specific intracellular signalling cascades in the innate immune cells of the urinary tract (Saemann et al., 2007). More studies on THP, based on the modifications, its interactions with phospholipid models that shed insight into its anti-microbial nature have proposed its nature of interaction with the microorganisms and its role in the prevention of UTI especially with special emphasis to the diabetic population (Taganna et al., 2011).

1.9.2. Inflammatory host response

The continued presence of UPEC in the urinary tract triggers the exfoliation of the infected bladder cells and leads to inflammation. Exfoliation of the urothelial cells is FimH dependent and occurs through the apoptosis pathway leading to disruption of urothelium integrity (Klumpp et al., 2001). However, the bacteria manage to escape the exfoliated cells and infect the underlying tissue that regenerate slowly and aids in the persistence of the bacteria for a long period (Mulvey et al., 2001).
Adherence of the bacteria to the urothelium and the bacterial VF’s trigger rapid innate and adaptive immune responses in the host (Anders and Patole, 2005). This leads to the activation of pro-inflammatory cytokines and chemokines, interleukins (IL-6, IL-8, IL-17A), that effect local tissue damage (Jantausch et al., 2000). Subsequent to the activation of the pro-inflammatory markers, the recruitment of the neutrophil’s to the site of infection and the production of acute phase proteins occur leading to the production of renal scars. The innate immunity of the host serves not only to eradicate the pathogenic bacteria but also leads to tissue damage and permanent scarring (Gabay, 2006).

The bacterial components LPS and capsule, activate the signalling pathways in the host by binding with the Toll like receptors (TLR) present on the urothelial surface (Andersen-Nissen et al., 2007). Activation of the various TLR leads to the translocation of the activated NF-κβ and IRF3, which aid in the antibacterial defence and immunoregulation (Song et al., 2007).

1.10. PATHOPHYSIOLOGY OF UTI

The bacteria invade and rapidly replicate to form intracellular bacterial communities and filamentous form of the pathogen that are essential for the further colonization of the underlying transitional epithelium and the kidney (Fig. 1.2). The interaction of the pathogen and its lipopolysaccharide with the urothelium results in the activation of the host’s immune system, which leads to the infiltration of polymorphonuclear leukocytes, macrophages and cytokines to the site of infection. This invasion of the urothelial cells also results in the apoptosis of the infected cells or their exfoliation into the formed urine, thus leading to protein loss from the kidneys (Hunstad and Justice, 2010).

Fimbriation of UPEC aids in the adhesion of bacterium to the bladder epithelium, and colonization of the sterile urinary tract (Schilling et al, 2002). The bacteria internalize by penetrating the lipid rafts of the urothelium and reside before they produce an infection (Duncan et al., 2004) and thereby silently evade the innate immunity of the host (Mulvey et al., 2001). At this stage the pathogens are resistant to
the antibiotic regimen thus paving way for recurrent UTI in the bladder and the kidneys (Mates et al., 1999). The entry and re-emergence of the UPEC from the urothelium, leads to the increased levels of ROS and RNS in the body; it also results in inflammation due to activation of NF-κβ (Brown and London, 2000). This results in the damage of the surrounding macromolecules (Rahmann et al., 2008) and the sloughing of the infected bladder and renal epithelial cells resulting in the loss of the various glycoproteins lining the epithelial surface such as Caveolin-1, THP, Transferrin, and Lipocalin etc (Hawthorn and Reid, 1990).

**Figure 1.2. Mechanism of invasion and persistence of Uropathogenic *Escherichia coli* within the bladder and kidney of tissues of the host**

![Mechanism of invasion and persistence of Uropathogenic Escherichia coli](image)

The innate immune response impedes the invasion of the UPEC in the urinary tract within minutes, by recruiting neutrophil’s and macrophages to the site of action. This increased neutrophil presence was noticed in the pelvi-uretic section of the mice. Invasion of the uropathogen increases cytokine levels, due to which inflammatory receptors such as TNF-α and NF – κβ are expressed at the cell surface and lead to urothelial inflammation (Grover et al., 2011).
1.11. TREATMENT OF UTI

The non-specific and specific defences of the body although effective are not perfect and the bacteria have identified and developed mechanisms to circumvent them. The major advancement of the past century was the discovery of natural defences that have been supplemented by the externally provided chemical defences, viz., the antiseptics, disinfectants and the antibiotics.

Antibiotics are low molecular weight compounds that act by killing or inhibiting the growth of the pathogen. They are ingested or injected into the host body to produce the desired effect. Most antibiotics that are currently used are secondary metabolites produced by the bacteria or signalling molecules in the bacterial system. Since bacterial infections are non-specific in their clinical presentation, broad-spectrum antibiotics are normally prescribed to avoid septicaemia and septic shock (Khasawneh et al., 2014).

1.11.1. Mechanism of action

The bacterial targets for various antibiotics are either not available in the host or it is different from the same molecule or process in eukaryotic cells that there is no or less cross-reactivity. The commonly used targets are inhibitors of peptidoglycan synthesis, protein synthesis, DNA synthesis and folic acid synthesis. The key concept of antimicrobial therapy (AMT) lies in the difference between the action of the bactericidal (antimicrobial compound aims to kill the pathogen) and bacteriostatic drugs, that merely stop or slow the growth of the bacteria. In patients with an intact immune system, bacteriostatic compounds can be very effective as the innate defence system of the host is capable of eliminating them. However, in people with defective immune defences, there is much reliance on the bactericidal antimicrobial compound to effect a cure for the infections.

The properties of the bacteria also affect the mechanism of action of antibiotics due to the formation of bacterial biofilms, where a bactericidal antibiotic rendered bacteriostatic or completely ineffective by the growth state of the bacteria. The pharmacokinetics of the drug determines the bioavailability, its susceptibility pattern to the commonly administered drugs and the side effects of those drugs.
(allergic reactions, hearing loss etc.) are to be identified before resorting for an AMT for UTI.

Most antimicrobial compounds provided as treatment for UTI are excreted in the urine in high concentrations, but the concentration in the substance of the kidney is related to their concentration in the bloodstream. An AMT to treat cystitis and uncomplicated UTI requires high concentrations in the urine, while the AMT for pyelonephritis, needs to be present in high concentrations in the bloodstream. Therefore, the treatment strategy should be prescribed depending on the dose of the antimicrobial agent that produces an therapeutic concentration at the site of infection and the course of treatment should not be longer that it is necessary to sterilize the urine. This approach limits the possibility of unwanted effects of treatment and reduces the risk of bacterial resistance, super-infection and drug related toxicity.

1.11.2. Cell wall synthesis inhibitors

Antibiotics belonging to this class belong to four groups: β-lactams, glycopeptides, phosphonomycin and bacitracins.

β-lactams: these class of antibiotics contain a four-membered β-lactam ring. The antibiotics in this class include the penicillins, cephalosporins, carbapenams and the monobactams. Although these drugs are widely used, the toxicity due to penicillins and cephalosporins especially as an allergic reaction due to the formation of β-lactam/serum protein conjugate is a major problem. Monobactams are used as alternatives in such situations. β-lactams act by inhibiting the last step of peptidoglycan synthesis i.e. the transpeptidation reaction that cross-links the peptide side chains of the polysaccharide-peptidoglycan backbone or by binding and inhibiting the action of other inner membrane proteins (transpeptidases / penicillin binding proteins), that play a role in peptidoglycan synthesis, thereby triggering endogenous enzymes that degrade peptidoglycan. The endogenous enzymes function in the presence of peptidoglycan, therefore they allow the bacteria to grow and divide. The intake of β-lactams, removes the checkpoints that keep these enzymes repressed in activity and stimulates their attack on peptidoglycan.
The peptidoglycan cell wall prevents the bacteria from bursting in response to high osmotic strength of the cytoplasmic contents relative to the Na\(^+\) content of the external medium and its destruction leads to cell lysis. \(\beta\)-lactams are generally bactericidal. The action of \(\beta\)-lactams vary with the high osmolarity of the tissue of action/effect, toxicity imparted, stability in the host, rate of clearance from blood, lode of ingestion and the ability to penetrate the blood-brain barrier. The commonly prescribed \(\beta\)-lactams for the treatment of UTI include Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Amoxicillin-Clavulunate, Ampicillin-Sunbactam, Cefixime, Cefdinir, Cefditoren, Ceftotaxime, Cefpodoxime and Ceftazidime (especially for UTI caused by \textit{P. aeruginosa}).

\textit{Glycopeptides}: The commonly administered glycopeptides are vancomycin and teichiplanin, that act as peptidoglycan synthesis inhibitors. They are currently the last drugs that are active against gram negative pathogens such as \textit{Staphylococcus aureus}. These drugs bind to the D-Ala-D-Ala portion of UDP-muramyl-pentapeptide after its transferred out of the cell cytoplasm. The binding inhibits trans-glycosylation and trans-peptidation.

\textit{Phosphohomycins} are used to treat methicillin resistant \textit{Staphylococcus aureus} infections and they disrupt the peptidoglycan synthesis by inhibiting the conversion of UDP-NAG to UDP-NAM. \textit{Bacitracin} and \textit{colistin} are commonly used to treat bladder infections by interfering with the recycling of bactoprenol.

1.11.3. Protein synthesis inhibitors

\textit{Aminoglycosides}: the commonly prescribed aminoglycosides are gentamicin, amikacin, and netilimycin. Aminoglycosides are trisaccharides with NH- groups that target the bacterial ribosome as it differs from mammalian ribosome’s. These antibiotics act by binding to the 30S subunit. Although binding does not prevent the transfer of the bacterial tRNA to the P site, it does prevent the protein synthesis, as the 50S subunit does not bind to the 30S subunit to form an active ribosome. Aminoglycosides are bactericidam in nature and the common side effects noticed due to the ingestion are loss of hearing and impairment of kidney function, which calls for their judicious usage.
**Tetracyclines** contain four fused cyclic six member rings, target the bacterial ribosome, and bind to the 30S subunit. The binding distorts the A site and prevents alignment and finally amino-acetylated tRNA with the codon on the mRNA. They are bacteriostatic in action and cautious usage is necessary as they are toxic at very high dose and causes yellowing of teeth in young children accompanied by nausea and phototoxicity.

**Macrolides** due to their side effects do not find wide spread usage. They act by binding to the 50S subunit of the ribosome at the 23S rRNA looped segment and inhibit bacterial protein synthesis and translocation. They are bacteriostatic in nature and used to treat UTI due to Streptococci spp.

1.11.4. Quinolones

They act by inhibiting DNA replication and effective in treating UTI due to their bactericidal nature. The major drawback is that these drugs act against the commensal flora in addition to the pathogen. The commonly prescribed quinolones are ciprofloxacin, gatifloxacin, levofloxacin, nalidixic acid norfloxacin and ofloxacin.

1.11.5. Trimethoprim and Sulfonamides

They are inhibitors of enzymes in the bacterial pathway for the production of tetrahydrofolic acid. Trimethoprim is structurally similar to dihydrofolic acid and acts as a competitive inhibitor of dihydrofolate reductase. Sulfonamides are similar to p-aminobenzoic acid and prevent the tetrahydrofolic acid pathway at a earlier step.

1.11.6. Duration of treatment

In a community acquired UTI, a short treatment course of not more than 3 days is usually sufficient to reduce the symptoms of infection that usually resolve after the first few doses, and longer courses of treatment offer no advantage. Single dose therapy is often effective only in uncomplicated UTI, but even with a high dose the rates of cure are very less and care should be taken to administer the dose at night, as it maintains a very high concentration of the drug in the bladder for a longer period. Covert bacteriuria in adults should not be treated with antimicrobial agents except
during pregnancy, and the infections resolve due to the innate defence mechanism. In young children as the symptoms of UTI are non-specific and bacteriuria may be significant which requires treatment with an antibiotic regimen.

In diabetics and in those undergoing surgery, treatment for bacteriuria is essential, as it is a major risk for serious complication. Recurrent infection in the absence of an underlying abnormality should be treated according to the probable precipitating cause. In women, recurrence may be associated with sexual intercourse and prophylaxis may be appropriate. In men with recurrent infection due to prostatic dysfunction in whom surgical operation is undesirable or not possible, a single dose may prevent recurrence.

A similar regimen is followed in women with UTI due to stress induced incontinence, pelvic floor weakness or disturbance of bladder emptying, or when persistence of the infection may lead to progressive renal damage. In long-term therapy, the dose should be minimum to maintain the sterility of the urine and prevent side effects; regular monitoring of urine is necessary. Complicated UTI are often difficult to treat and the pathogens often show a wide spectrum of antimicrobial resistance. In such cases, a prolonged and high course of therapy is necessary to prevent further renal involvement. The organisms responsible for hospital acquired UTI are often resistant to a variety of antimicrobial agents and systemic treatment with aminoglycosides or newer penicillins or recent generation of cephalosporins, may be necessary. Quinolones provide an effective means of oral therapy for infections caused by multidrug-resistant bacteria (Baelyer, 1996, Riden and Schaeffer, 1996).

1.12. ANTIMICROBIAL RESISTANCE

The use of broad-spectrum antibiotics in the empirical treatment against uropathogens, destroys not only the pathogen in consideration but also affects the micro-flora of the host. Destruction of the micro-flora allows further colonization by the pathogen, and this eventually leads to antibiotic resistance. The misuse of various antibiotics (WHO, 2000) and their synthetic analogues such as Amoxicillin, Cefotaxime, Fluoroquinolone, Fosfomycin, Trimethoprim – Sulphamethoxazole,
Nitrofurantoin, and Ciprofloxacin usages have increased by 40% in the span of four years from 2005-2009 (GARP, 2011). The modification of the antibiotic target in the bacteria genome or protein or ribosome, the reduced uptake of the antibiotic across the cytoplasmic membrane, restricted access of the antibiotic to the target the failure to activate the antibiotic due to lack of enzymes as in the case of aminoglycosides, and enzymes that inactivate the antibiotic by hydrolyzing or adding a chemical group to it (β-lactams), have lead to the emergence of broad-spectrum resistance disease pathogens (Dimitri and James, 2008).

1.12.1. Antimicrobial resistance pattern across India

A clear pattern on the antimicrobial resistance across the country has been on the rise as valid documentation provided by the scientific community. Although it is not available for every state in the country, the current evidence provides a comprehensive idea on the menace (Fig. 1.3).

**West Bengal:** The degree of susceptibility of *E. coli* towards gentamicin, amikacin and third generation cephalosporins like ceftazidime were 94.5%, 88.2% and 73.0% respectively. Nitrofurantoin had the highest level of activity against *E. coli* with cent percent susceptibility. *Klebsiella* sp. showed the highest percentage of resistance against tetracycline (80.8), followed by ciprofloxacin (76.0) and 100% percent sensitivity towards nitrofurantoin (Choudhury et al., 2013).

The resistance of *Proteus* spp. against ciprofloxacin and co-trimoxazole were 73.5% and 72.8%. *Proteus* spp. showed 100% susceptibility towards imipenem. Gram-positive bacteria like *S. aureus* were highly resistant to ampicillin, followed by ciprofloxacin. Nitrofurantoin was found to be effective against the Gram-positive bacteria like *S. aureus*, *Enterococcus* sp. There was a fall in the sensitivity levels of the uropathogens from 2009 through 2010 to 2011 for a number of antibiotics. The percentage of resistance of *E. coli* against tetracycline, for example, increased from 85.6 in 2009 to 87.0 in the next year to 89.0 in 2011, of Ampicillin from 67.5 in 2009 to 69.0 in 2010 to 69.7 in 2011 (Choudhury et al., 2013).

**Karnataka:** In a study carried out in Karnataka, the uropathogens were found to be 100% resistant to Ampicillin, 90% resistant to netilmicin and cephalixin, 80 – 85%
resistant to cotrimoxazole, gentamicin, amoxyclav, ceftoxime and tobramicin and 60 – 70% resistant to norfloxacin, ciprofloxacin and ofloxacin, while the isolated strains were susceptible to imipenem and nitrofurantoin. From the results of another study in Bangalore, Karnataka, it was evident that three-fourths of the uropathogens were resistant to Quinolones, 74% to ciprofloxacin, norfloxacin and ofloxacin, 49% to gentamicin, and were susceptible to nitrofurantoin and amikacin (72%) (Eshwarappa et al., 2011; Ashok et al., 2013). to ceftazidime and ceftriaxone among the UPEC isolates were noticed.

**Orissa:** In a study carried out in this state, out of 1169 samples collected the uropathogens were susceptible to drugs such as nitrofurantoin (10%) and amikacin (6%) (Dash et al., 2013).

**Figure 1.3. The antibiotic resistance patterns among the clinical strains of Uropathogenic Escherichia coli isolated across the various states in India**
1.12.2. Antimicrobial resistance pattern across the countries

Across the world too, the problem of antimicrobial resistance has escalated by leaps and bounds and making the identification of novel drug targets a necessity. In Nepal 75% of the strains were resistant to Ampicillin, 71% to ciprofloxacin, 58% to cotrimoxazole and 44% to norfloxacin (Neupane et al., 2013). In another study carried out across the Asia – Pacific under the SMART across 11 countries in 38 centres, where 1762 strains were analyzed during the period 2009 – 2010, the UPEC strains were 92% resistant to amikacin, 87% resistant to ertapenem and imipenam, 85% resistant to piperacillin and tazobactam 50 – 70% resistance to 3rd and 4th generation cephalosporins and approximately 50% resistant to cefoxitin, ciprofloxacin and levofloxacin (Lu et al., 2012).

In two different independent studies carried out across Africa in Nigeria and Egypt, it was noticed that 47% of resistant rates towards Ampicillin and 42% resistant rates were observed towards gentamicin in Nigeria (Okwori et al., 2011); while in Egypt 40 – 89% resistance rates to the β-lactam antibiotics, followed by 63 -66% resistance to Quinolones and around 11 -57% resistance to the prescribes aminoglycosides (Mogeeb, 2014).

In Germany, the uropathogens were resistant to trimethoprim (18%) and ciprofloxacin (9%) and susceptible to the drugs such as fosfomycin (96%) and nitrofurantoin (98%). In Greece, a significant increase in the resistance to the β-lactams, monobactams, aminoglycosides, Quinolones and cotrimoxazole were noticed. UPEC strains showed the highest susceptibility to nitrofurantoin, but were however resistant to Ampicillin, amoxicillin clavulunic acid and cephalosporins (schmiemann et al., 2012). A similar trend was also observed across France, where the highest resistance was towards amoxicillin (79%) followed by TMP/SMX (78%), tetracycline (76%) and the strains were highly susceptible to netilimicin (97%) followed by cefotaxime (86%) (Moroh et al., 2014).
1.13. ALTERNATIVE TREATMENT STRATEGIES

The under- , over- and the mis-usage of the various antibiotics (WHO, 2000) and their synthetic analogues such as Amoxicillin, Cefotaxime, Fluoroquinolone, Fosfomycin, Trimethoprim – Sulphamethoxazole (TMP-SMX), Cephalothin, Nitrofurantoin, and Ciprofloxacin usages have increased by 40% in the span of four years from 2005-2009 (GARP, 2011). This has lead to the emergence of broad-spectrum resistant disease pathogens (Dimitri and James, 2008). To prevent generation of antibiotic resistant microbes, to reduce the cost of the synthetic analogues and to reduce the side-effects that incur, WHO, recommended the search, for safer and alternative medication for human infectious diseases. This lead to a resurgence in the search of active drug leads from plants, which are both superior in action and similar in structure to the existing molecules (WHO, 2000).

The ancient medicine system, Siddha, followed in India is based on the preparation of drugs and formulations from the plant parts and they offer an array of chemicals, with synergistic interactions (Barnes, 1999) for treating ailments. The plant products are widely utilized as home remedies by the rural population as a cost effective form of treatment, over the counter drugs and raw materials in the pharmaceutical industry. The modern concept of industrialization has led to revitalization of plant products as sources of new drugs (Astin, 1998). Most herbs in their natural state are not fit for treatment in their natural state and are converted into a consumable form through the various methods of preparation outlined in the pharmacopeia and these formulations lack in vitro and/or in vivo evidence to support the occurrence of synergism between constituents in herbal extracts, and clinical evidence on the effect, toxicity and extend of the active principle (Fontanarosa et al., 2003). The therapeutic potential of the herbal drugs depend on its form: the parts of the plant used, the methods of extraction and the active constituents present in the extract.

Chandraprabhavati is one such poly herbal drug, which has been prescribed for various urinary problems, anaemia, renal calculi, to strengthen the kidney and for general malaise for the past 40 years in India. Most herbal drugs are mixtures of various plants and the active principles are mostly unknown as plant materials are
chemically and naturally variable. The phyto – compounds present in the drugs depend on the climate, temperature, methods of extraction, storage, processing and the polarity of the solvent utilized during extraction. Also to date neither the exact mechanism nor the active principles responsible for the activity of the herbal drugs remain a mystery (AYUSH, 2003).

This study with the background of the AMP’s and the current scenario of antimicrobial resistance and the development of multi-drug resistant uropathogens and thrust on the re-emerging field of alternative treatment therapies, was designed to study the regulatory role of urinary antimicrobial proteins and inflammatory markers upon treatment with Chandraprabhavati and cardamom extracts in experimentally induced pyelonephritic mouse models.

In order to achieve the above aim in a phased manner the following objectives were laid out

- Isolation and characterization of uropathogenic Escherichia coli (UPEC) from urinary tract infected subjects and standardization of mouse model for pyelonephritis
- Efficacy of Siddha drug, Chandraprabhavati against UPEC RRL - 36, in vitro and in vivo
- Exploration of the effect of cardamom seed and pod extracts on UPEC RRL - 36
- Synergistic effect of Chandraprabhavati and cardamom extract on antimicrobial proteins and inflammatory markers in treating UTI