1. INTRODUCTION

1.1 General introduction

Human body needs elimination of waste products and maintenance of fluid / salt balance for its proper functioning. It is done by the urinary system otherwise known as renal system that consists of paired kidneys with ureters, a urinary bladder, two sphincter muscles, prostate (in men) and urethra. In the urinary system of kidneys collect wastes and extra water from blood to produce urine. The ureters carry the urine from kidneys to bladder. Bladder stores the urine and squeezes it out when full. The prostate adds fluid to semen. The urethra carries the urine out of the bladder (NIDDK, 2007).

A group of infections that occur in the urinary tract is known as Urinary tract infections (UTIs) and it may be in any part of the urinary tract. It causes more financial burden on society. Commonly occurring UTI is cystitis, which occurs in the bladder, where urine is stored. Other UTIs involve the kidneys (pyelonephritis), bladder (cystitis), prostate (prostatitis), urethra (urethritis) or urine (bacteriuria).

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span (Steven et al., 2006 and Kucheria et al., 2005). UTI is the third most common cause of admission to hospitals in India. It has been estimated that about 6 million patients per year are visited worldwide for UTI out of which around 30,000 are treated in the wards (Bano et al., 2012). UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (Stamm, 2002 and Weinstein et al., 1997). UTI also varies based on severity (i.e., complicated versus uncomplicated). Complicated UTI is the infections in urinary tracts with structural or functional abnormalities or the presence of foreign objects (eg. placing urethral
catheter). There is also a practice of categorizing UTI into first infection and recurrent infection. Recurrent infections are again subdivided into unresolved bacteriuria, bacterial persistence, and reinfection. Pain, fever, and discomfort are the common manifestations due to the urinary tract infections and it can be easily treated. Treatment becomes tough when the infection spread to the kidneys (Hvidberg et al., 2000).

First infection is the initial documentation of UTI by a proper urine culture. There may be several reasons for the recurrence of UTI. This is commonly caused due to inadequate antimicrobial therapy. Therapeutic agents like antimicrobials may not function properly because of noncompliance, deficiency in absorption, improper drug metabolism, and uropathogens becoming resistant and unresponsive to the therapy provided. This can be cured altering the therapy according to antimicrobial sensitivity tests carried out by a proper urine culture. Bacterial persistence and reinfection occur when the route cause of infection in the urinary tract is not eradicated. The uropathogens escape for the antimicrobial therapy by hiding themselves where the therapeutic agents cannot reach like urinary calculi and foreign objects (Steven et al., 2006).

The majority of UTIs develop in the normal urinary tract and are therefore termed ‘uncomplicated’. Symptoms of lower urinary tract infection include frequency, painful urgency and haematuria (haemorrhagic cystitis). Other causes of symptoms of lower tract irritation will need to be considered. It is customary to separate sexually transmitted disease from UTI on the basis of pathogens and site of infection and yet the distinction between urethritis, vaginitis and cystitis may be very indistinct symptomatically. Moreover, pathogens which are more familiar populating the urethra and genitalia, e.g. Chlamydia, ureaplasma, gardnerella and other unfamiliar or fastidious organisms may cause cystitis but are otherwise undetected by standard urine culture techniques. Nevertheless, they have a variable contribution to statistics on epidemiology of UTI (Engel and Schaeffer, 1998).
1.2 Common Symptoms

1.2.1 For bladder infections

- Frequent urination along with the feeling of having to urinate even though there may be very little urine to pass.
- Nocturia: Need to urinate during the night.
- Urethritis: Discomfort or pain at the urethral meatus or a burning sensation throughout the urethra with urination (dysuria).
- Pain in the midline suprapubic region.
- Pyuria: Pus in the urine or discharge from the urethra.
- Hematuria: Blood in urine.
- Pyrexia: Mild fever
- Cloudy and foul-smelling urine
- Increased confusion and associated falls are common presentations to Emergency Departments for elderly patients with UTI.
- Some urinary tract infections are asymptomatic.
- Protein found in the urine.
1.2.2 For kidney infections

- All of the above symptoms.
- Emesis: Vomiting is common.
- Back, side (flank) or groin pain.
- Abdominal pain or pressure.
- Shaking chills and high spiking fever.
- Night sweats.
- Extreme fatigue.

1.3 Urinary pathogens

Pathogens responsible for community acquired cystitis and pyelonephritis comprise 70% *E. coli* and the remainder a variable contribution from *Proteus, Klebsiella, Enterococcus and Pseudomonas*. In the summer and autumn *Staphylococcus saprophyticus* is a relatively common isolate on culture (5–10%) in young adult females. Somewhat different proportions of organisms are found in isolates of urine from patients treated in hospitals and institutions. The differences reflect the greater incidence of complicated Coli to vaginal and buccal mucosa in women susceptible to UTI (Fowler and Stamey, 1997 and Schaeffer *et al.*, 1981) Antimicrobial resistance surveillance is necessary to determine the size of problem and to guide empirical selection of antimicrobial agents for treating infected patients. The goal of this study was to determine the current prevalence of urinary tract infection and the find out a solution to tackle the urinary tract infection causing microbes using plant derived natural drugs.
1.4 Pathogenesis

Virtually all infection in the urinary tract originates in a reservoir of uropathogenic \textit{E. coli} (UPEC) in the large bowel reservoir. Several virulent factors distinguish these strains and facilitate colonisation, adhesion and invasion (Mulvey \textit{et al.}, 2000). One of the most important host defense mechanisms is a sequence of hydrodynamic factors allowing dilution, rinsing and elimination of bacteria through the addition of fresh urine into the bladder and adequate voiding. Artificial in vitro systems were developed early on to model these processes and as little as 20 ml of residual urine could be significant in bacterial proliferation. The initial adhesion to urothelium is achieved through a variety of organelles some of which can preferentially bind to receptors on so called uroplakin plaques on the superficial umbrella cells of the bladder epithelium. The bacteria can then become internalised. The host response is first induction of apoptosis and then the shedding of infected cells into the urine which is then voided. The bacteria may escape this process by invading the deeper epithelial cells and assuming a non-planktonic form (e.g. plasmids) in the midst of a biofilm which can develop wherever infection becomes chronic. Alternatively, they can escape into the urine as free bacteria and become adherent elsewhere before they are voided. In addition, infected umbrella cells stimulate an acute inflammatory response through production of IL6 and IL8. Polymorphs then migrate through the bladder wall and into the urine. It is notable that diabetic women with asymptomatic bacteriuria have a lower urinary cytokine and leucocyte count compared with nondiabetics.
1.5 Urinary tract infection in women

Urinary tract infections are common among women. It affects one in five women during their lifetime (Foxman, 2002). UTIs are not as common in men under the age of 50, but they are prone to complications like stone or enlarged prostate. About 20% of women who have one infection will have a recurrence. Of this group, 30% will have a third occurrence, and of this group, 80% have additional recurrences. It is mostly due to antibiotic resistance (Kathleen Head, 2008).

UTIs are common during pregnancy period. About 8% of pregnant women experience with UTI (Delzell and Lefrvre, 2000). Increased bacterial population in vagina is the main reason for UTI in both pregnant (Sharami et al., 2007) and non pregnant women (Harmanli et al., 2000). UTI may spread to kidney during pregnancy due to the part of urethral dilation and hydronephrosis.

Vaginal epithelium becomes thin in the case of postmenopausal women and the amount of glycogen and estrogen gets decreased. This helps to increase of pH level in vagina which paves way for colonization of uropathogens (Gupta and Stamm, 1999). There are also some other factors responsible for UTI certain blood-group antigens which increases the attachment of P-fimbriated E. coli to glycolipids on vaginal and uroepithelial cells.

UTIs are dangerous when it occurs in older, pregnant women and diabetic patients. UTI is more frequent in women because they have short urethra. So the bacteria can reach the bladder quickly. The main symptoms seen in the affected UTI are suprapubic pain, increased frequency and urgency of urination, dysuriya, and nocturia, hematuria, cloudy urine and foul or strong urine odor. The other constitutional symptoms are fever, chills, malaise, nausea, vomiting, weight loss, flank or back pain. UTI is predominantly a disease
of females. 50 to 80% of the women’s acquire at least one time during their life time (Randhir Puri and Jaideep Malhotra, 2009).

1.6 Urinary tract infection in children

In the life time approximately 1% of boys and 3% of girls experience with UTI before the age of 11. In children UTI may associated with some congenital abnormality of urinary tract. If it does not get proper treatment in time, permanent kidney damage may be the result. Males are more prevalent to UTIs under one year of age and female after one year of age. When the first attack is below 5 years, it is the indication of initiation for continues as risk of recurrences (Kumud Mehta, 1996).

Children below 5 years should be treated carefully otherwise UTI may persist and develop chronic pyelonephritis. But asymptomatic bacteriuria need not be treated in above 5 years (Leung and Robson, 1991).

1.7 Nosocomial Urinary tract infection

UTIs are mostly hospital-acquired (nosocomial) infections. Previous studies reported that nosocomial UTIs are responsible for 1, 00,000 Persons/Year (Burke and Zavasky, 1999), 40% of Infections/Year is pyelonephritis (Warren et al., 1999 and Foxman, 2002), and catheterization is the reason for 80% of Infections/Year occurring due to instrumentation (Asher et al., 1986). Patients who need a urinary catheter for more than 7 days, has daily risk of 5% to acquire urinary tract infection.

Urinary tract infections are among the most common infectious diseases increasing in outpatient as well as in hospitalized patients and can vary according to geographical and regional location (Mathai et al., 2001 and Karlowsky et al., 2002).
1.8 Urinary tract infection in adults

Several studies reported that sexual activity and birth control usage is the most effective cause for UTI in both symptomatic (Hooton et al., 1996) and asymptomatic bacteriuric (Hooton et al., 2000). Anatomic and genetic factors are also having role in non-behavioral UTI. It is proved by maternal history of UTI before the age of 15 (Scholes et al., 2001).

1.9 Urinary tract infection in diabetic individuals

Other important factors are diabetes mellitus and previous history of UTIs (Hooton, 1996). In a study conducted by Boyko et al. (2005) asymptomatic bacteriuria was 6.7 and 3.0 % for every 100 Persons / Year in diabetics and non-diabetics, respectively.

1.10 Establishment of UTI and biofilm formation

Bacteria can establish colonization of a patient’s bladder within three days of their introduction onto the inner or outer surface of urinary catheters (Donlan, 2001). The introduction of bacteria with urinary catheter use is often associated with catheter-related biofilms. Biofilms are complex structures that include bacteria, host cells and cellular by-products. Biofilm formation within invasive medical devices is proposed as a primary mechanism in the development of certain diseases, including catheter associated UTI. Once a biofilm has developed on the inside or outside surface of a urinary catheter, the only way to eliminate the risk of UTI is to remove the catheter.
1.11 Microbial Etiology of UTI

Urinary tract is normally a sterile; bacteria when infects the urinary tract, it can move upwards from rectum or vagina and reach urethra. Normally, there is UTI when the midstream urine has $10^5$ organisms/mL of urine. The most common bacterial agent that causes UTI is Gram negative bacilli especially *E. coli*. Other Gram negative bacilli that cause UTI are *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter aerogens*. Gram positive bacteria also sometimes cause UTIs and the agents are *Staphylococcus aureus* and *Enterococci*.

Most frequently UTI is caused by endogenous microorganisms that are present in one’s own bowel. Though the *E. coli* and *Proteus* spp. are common causative agents, they can be easily treated by their sensitivity to most of the antibiotics. When *E. coli* is the causes for primary infection of UTI, there is more chance of recurrence of infection by different bacteria or by the same *E. coli* within next 6 months (American college of obstetricians and gynecologists, 2008 and Foxman *et al.*, 2000). UTIs are frequently caused by more resistant Gram-negative spp. like *Klebsiella* and *Pseudomonas* (Department of Health, 2001; Society for Healthcare Epidemiology of America, 2001 and Huang *et al.*, 2004). *E. coli* is the leading cause in uncomplicated UTIs and Gram-negative, Gram-positive and multidrug resistant organisms are responsible in complicated UTIs (Florian Wagenlehner and Kurt Naber, 2006). Fungal and viral pathogens also account for UTIs (Cattell, 1996).

*E. coli*, *Enterobacter*, *Klebsiella*, *Enterococci*, and *Proteus* spp. are endogenous intestinal microflora and are common pathogens of the urinary tract. Urinary catheters are potentially colonized by these organisms. When the equipment and hands of healthcare workers have deficiency in proper decontamination, it may introduce environmental and
common skin bacteria during insertion or maintenance of the urinary catheter. Because of that, *Pseudomonas*, *Serratia*, coagulase-negative *Staphylococci*, *Acinetobacter*, and few other non-intestinal microbes or environmental microbes can also may responsible for healthcare-associated UTI. Patients with long-term indwelling catheters often have polymicrobial bacteriuria. *Candida* species are a common organism isolated from urine in the intensive care unit (ICU) setting. The use of antifungal drugs and of broad-spectrum antibiotics for empiric therapy has led to increasing prevalence of drug-resistant fungi and bacteria in intensive care and long-term care settings.

Several studies have shown that *E. coli* associated UTIs occur in 80% of cases (Kasper *et al.*, 2005 and University of Michigan Health system, 2005); *E. coli* associated UTI is both community and hospital acquired (Gruneberg, 1994; MacGowan *et al.*, 1993 and Barret *et al.*, 1999).

Bacteria that are rarely associated with UTI are *Lactobacillus*, *Corynebacterium* spp., *Streptococcus* spp., Coagulase negative *Staphylococci*, (other than *S. saprophyticus*). Pathogens involved in complicated UTI are *Enterococcus* spp., *Pseudomonas aeruginosa*, *Proteus* spp., antibiotic-resistant *E. coli* (Morgyn Warner, 2009).

There are many different serotypes in *E. coli*, and studies shows that recurrent *E. coli* UTI is reinfection and not bacterial persistence. Serotyping (or careful examination of antimicrobial sensitivity profile) helps in diagnosis of reinfection in equivocal situations when the pathogenesis of UTI is by some element. Similar to bacterial persistence in abnormal conditions with reinfection such as fistulae, the source of infection can be corrected by surgery (Steven *et al.*, 2006).
1.12 Treatment for UTI

If a UTI is suspected, the best practice is removal of the old catheter before obtaining the specimen in order to eliminate the confounding factor of possible catheter biofilm. If an indication for urinary catheterization still exists in a patient suspected of having a UTI, obtain the urine specimen after replacing the old one. Specimens collected from an indwelling urine catheter must be noted on the laboratory requisition or in the urine culture order.

Historically, acute uncomplicated cystitis in women has been treated with longer (7-10 days) courses of antibiotics. Recent studies showed that shorter courses (3-5 days) of oral antibiotics are effective as traditional courses. A review of 28 treatment trials of adult women with uncomplicated cystitis concluded that no benefit was achieved by increasing the length of therapy beyond 5 days. Decrease in the expense of antibiotics, improvement in patient compliance and decrease in adverse effects of antibiotic treatment (e.g., amoxicillin associated vaginitis) are the advantages of shorter course therapy. When comparing the different treatment strategies, single dose regimens are less efficient at eradicating bacteriuria, than 3-5 day regimens. Beta-lactam antibiotics are more effective, with cure rates of 77 to 92%, if given greater than nitrofurantoin are recommended. There is no benefit in increasing the duration of trimethoprim/sulfamethoxazole (TMP/SMX) or trimethoprim (TMP) more than 3 days; with 3-day therapy, the cure rates achieved is from 82 to 85%. Adverse effects increase when the treatment is continued after 3 days. For 3-day regimens, TMP/SMX is more effective and less expensive than nitrofurantoin, cefadroxil, or amoxicillin for treatment of uncomplicated cystitis in women. Quinolones is also effective for 3-day courses; however cost is higher than TMP combinations. Ciprofloxacin, 100 mg BID for 3 days, appears to be the most cost effective quinolone regimen/SMX. Therefore, the optimal treatment of uncomplicated UTI in patients who are not allergic or sensitive is 3-days of TMP/SMX. Longer courses of therapy should be used in women who are diabetic, pregnant (quinolones contraindicated), have had symptoms longer than 7 days, or have
other evidence for complicated UTI. In general, older women with lifelong history UTI and no history complicating factors are categorized as uncomplicated UTI. Theoretically, antibiotics can alter hormone levels, suggesting that backup contraception could be advisable when using antibiotics and oral contraception. However, in practice no cases of oral contraceptive failure have been definitely related to antibiotic use for UTI (University of Michigan Health System, 2005).

*Lactobacilli* are used as probiotics in the treatment of UTI by intravaginal or oral route of administration. Very good results are received when using well characterized strains only (Florian Wagenlehner and Kurt Naber, 2006). Probiotics provide proper treatment when they are well established into the bladder. Clinical studies have revealed that when *Lactobacillus rhamnosis* GR1 is administered vaginally either *L. reuteri* B 54 or RC 14 combination is needed for better results.

There are number of clinical studies that revealed many natural substances which may provide effective prophylaxis for recurrent infection. Nutrients and plant materials that have shown greatest efficiency in treating UTIs include cranberry, berberine, and probiotics. *uva ursi*, vitamins C and A, mannose, and estriol cream have been reported for the same purpose, but they need additional research, clinical evidence and study. There are many alternate medicines that include nutrients and other non-plant sources without clinical research, but proved to be successful use in the treatment of UTIs. While most clinical research has examined the effect of natural substances for prevention of UTIs, the mechanisms of action (primarily anti-adherence) and clinical experience of health care practitioners demonstrate effectiveness when used acutely, particularly at the first sign of infection. Plant sources and Plant material extracts are particularly useful for acute use, but cannot be recommended for long-term use, include berberine and *uva ursi*; but cranberry, mannose, probiotics, and estriol can be recommended for long-term prevention (Kathleen Head, 2008).
It is suggested that the antibiotic selected for treating UTI reoccurrence should be based on the knowledge of local prevalence of bacterial organism and antibiotic sensitivities rather than universal guidelines (Rupinder Kaur et al., 2012).

1.13 Antibiotic resistance

Antibiotics are used to treat the infections. To effective full course require antibiotics even if feeling better, because some bacteria may still remain active and antibiotic should be prescribed continuously if necessary. Antibiotics have been used since 1930s. Antibiotics can prevent bacteria from multiplying (bacteriostatic) or kill them (bactericidal). Both types of prevention are equally effective. When the immune system is weak, a bactericidal is more effective to compensate (faqs.org, 2012). New antimicrobial substances was developed more in number in the past and it balanced the emergence of antibiotic resistance. But now the medical field is facing a risk of antibiotic treatment loosing the effectiveness in the future.

Since 1990, there has been a steadily increasing rate of resistance to TMP/SMX, reaching >30% in some areas. The average resistance rate to TMP/SMX for E. coli in the US is 18%. Worldwide, resistance rates to quinolones is increasing at alarming rates, reaching >20% in Spain. Quinolone resistance in E. coli in the US remains less than 5%. Similarly, nitrofurantoin resistance is less than 5%. Cost effectiveness analyses have shown cost savings for quinolones over TMP/SMX when resistance to TMP/SMX exceeds 22%. This does not take into account potential detrimental effects of antimicrobial resistance to quinolones. When resistance rates are less than 10-20%, TMP/SMX remains the most cost-effective therapy. Factors associated with increased likelihood of resistance to TMP/SMX include: recent hospitalization, recent antibiotic use of any kind in the last month or use of TMP/SMX in the last 3-6 months, or the presence of diabetes mellitus or other complicating factors (University of Michigan Health System, 2005).
Antibiotics resistance by organisms is basically by 3 mechanisms. They are malfunctioning uptake and efflux, inactivation of drug and target change (Lambert, 2002). When the organisms become antibiotic resistance, the treatment becomes ineffective and leads to health problems. The main drawback of current antibiotic therapies is the emergence and rapid increase in resistant organisms (Fred Tenover, 2006).

Drugs can only modify the inherent functions of the concerned tissues or the cells like stimulating or depressing cellular activity, replacing deficient substances, causing irritation, or killing or weakening the invading foreign organisms. Otherwise do not create new functions. Antibacterial drugs penicillins, cephalospoins, carbapenems, and monobactams are the β-lactams drugs that affect the synthesis of bacterial cell wall and vanomycin and teicoplanin are glycopeptides involved in same process (Neu, 1992 and McManus, 1997). By interfering with the enzymes required for the peptidoglycan layer synthesis, the β-lactams agents inhibit bacterial cell wall synthesis. Both Gram positive and Gram negative bacteria can be treated by this kind of antibiotics.

β-lactamase is present in E. coli as virulence factors. It protects the bacteria against β-lactam antibiotics such as pencillins, cephamicins, and carbapenems by hydrolyzing the β-lactam ring which has 4 atoms in the antibiotics. The structure of the antibiotic is collapsed by this process (Neu, 1969).

The mechanisms how the bacteria skip the drug action are not only intrinsic impermeability; it also involves plasmids, transposons and mutations.

E.coli that produces Extended-spectrum β-lactamases (ESBL) is rapidly increasing through genetic transformation, by which the resistance character from resistance organism is transferred to susceptible one. This kind of enzyme causes pain and burning when urinating and also symptoms like frequent urination and fever.

TEM, CTXM, OXA and SHV are genes that expresse β-lactamase. They are in the plasmid of bacteria belonging to the family Enterobacteriaceae. The enzyme bla-TEM is
usually expressed in Gram negative bacteria. Ninety percent of ampicillin resistance observed in *E.coli* is due to *bla-TEM*. *K.pneumonia* also has this enzyme.

The enzyme *bla-SHV* and *bla-TEM* are structurally similar and shows 68% similarity. The enzyme *bla-SHV* is found commonly in *K.pneumonia* and is responsible for up to 20% ampicillin resistance. The enzyme *bla-CTXm* is greatly responsible for the antibiotic cefotaxime resistance. *bla-CTXm* is expressed commonly in *Salmonella enteric*, *Typhimurium* and *E.coli* and is also present in other *Enterobacteriaceae* species. It is also present in a rare pathogen *Kluyvera* species. These enzymes have only 40% similarities with *bla-TEM* or *bla-SHV*. More than 80 CTXm enzymes have been revealed right now.

Molecular study of antibiotic resistance gene from *Staphylococcus aureus* its amplification and sequencing of mecA gene which responsible for most of the β-lactams antibiotics resistance including methicillin will give insight in to design new synthetic drugs to control UTIs. Pantosti *et al.*, (2007) also reported that the same enzymatic inactivation of antibiotics in *Staphylococcus aureus*.

The enzyme *bla-OXA* is scarcely found in *E.coli*, *K.pneumoniae* and other Enterobacteriaceae and mainly in *P.aeruginosa* and provide ampicillin and cephalothin resistance. Sometimes it is also responsible for resistance to ceftazidime. *PER*, *VEB*, *GES* and *IBC* are the other β-lactamase which are very rare and expressed mostly in *P.aeruginosa*. Geographical location plays role in presence of these enzymes. For example, *PER-1* isolates in France, Turkey and Italy, *VEB-1* and *VEB-2* in Southeast Asia, and *GES-1*, *GES-2* and *IBC-2* in South Africa, France and Greece (Gerhard Weldhagen *et al.*, 2003 and Ronald Martin, 2012).

Multi drug resistant organisms have emerged because of the extensive use of antimicrobial substances, and number of urinary tract infections have increased and have made UTIs clinically complicated (Angelescu, 1998 and Banciu, 2005).
A combination of several antibiotics has to be tried to treat multidrug resistance (Health Protection Agency, 2009). Hospitals are the main source for UTI infections caused by antimicrobial resistant bacteria because it is a place where antibiotics are most extensively used.

Multiple antimicrobial resistances found commonly among gram-negative organisms causing urinary tract infections are a problem which lies in medical field for long time.

The prevention of emergence of resistance in human medicine entails several strategies

- decrease of antibiotic consumption
- antibiotic cycling
- new dosing strategies for antibiotics
- combination of two classes of antibiotics

All strategies are not specifically designed for antimicrobial therapy of UTI may have to be adjusted accordingly.

Since the antibiotic resistance by bacteria differs according to the type of infection, medical facility provided, region, and time, the purpose of this study is to define the antibiotic resistance pattern and investigate the presence of genes encoding several virulence factors in test isolates obtained from different urine samples of humans. The study also focuses on the possible correlations between the virulence factors and antibiotic resistance possessed by the strains with their source of isolation.

Medical attention should be given to UTI immediately because they may cause acute morbidity and also result in long term medical problems, including secondary hypertension and reduced renal function. Identification of the infection, treatment, and evaluation of risk for kidney damage is possible only by medical attention.