3. REVIEW OF LITERATURE

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it. When bacteria get into the bladder or kidney and multiply in the urine, Urinary tract infections (UTI) are worldwide incidence of approximately 150 million cases annually. About 40% of women and 12% of men experience at least one symptomatic UTI during their lifetime, and approximately 25% of affected women show recurrent UTI (RUTI) (Foxman, 2010). Therefore, RUTI represents a substantial burden to the health-care system. Uropathogenic Escherichia coli (UPEC) are responsible for 75–90% of uncomplicated UTIs; UPEC strains exhibit virulence factors that facilitate invasion, colonization, and survival in the urogenital tract (Hunstad and Justice, 2010). Colonization can lead to the establishment of a quiescent intracellular reservoir in the bladder. Activation of this quiescent intracellular reservoir results in recurrent UTIs (Anderson et al., 2003).

Urinary tract infections in children are particularly important because their occurrence may be associated with some congenital abnormality of the urinary tract or an error in management. If not corrected, these may lead to recurrent infections causing damage to the urinary tract. Infection may occur at many places along the genitourinary tract: urethra, bladder, ureter, renal pelvis, or renal parenchyma (Feld et al., 1989 and Kalantar et al., 2008). The prevalence of urinary tract infection varies markedly with sex and age. Symptomatic UTI occur in about 1.4 per 1000 newborn infants, with a slight male preponderance (Anis-ur-Rehman et al., 2008 and Kalantar et al., 2008).

The clinical and epidemiologic spectrum of 175 cases of community-acquired urinary tract infection (UTI) was evaluated at a university hospital by Finkelstein et al., (1998). Patients were grouped in five different categories of which complicated UTI was
the most common (39%). Bacteraemia was detected in eight patients (18%) of this group and in five (12%) with acute uncomplicated pyelonephritis. A single organism was isolated in 166 cases (95%). The rate of *Escherichia coli* bacteria ranged from 60% (asymptomatic bacteriuria) to 94% (uncomplicated cystitis). Of the 184 isolates, 92% were susceptible to ciprofloxacin and significantly high rates of resistance were found for ampicillin, cefazolin, cefuroxime, and co-trimoxazole. Isolates causing uncomplicated UTI had significantly high rates of resistance to ampicillin, amoxycillin-clavulinate and co-trimoxazole and those causing complicated UTI, had significantly high rates of resistance to most oral antibiotics tested, except quinolones and nitrofurantoin. Community-acquired UTI requiring hospital evaluation occurs in a complex group of patients, and current patterns of antibiotic resistance make it difficult to suggest empiric oral treatments in this setting.

After birth, the periurethral area, including the distal urethra, becomes colonized with aerobic and anaerobic microorganisms that appear to function as a defense barrier against colonization by uropathogens (Anis-ur-Rehman et al., 2008). In early childhood, enterobacteria and enterococci are part of the normal periurethral flora. *Escherichia coli* are the dominant gram-negative species in young girls, whereas *E. coli* and *Proteus* spp. predominate in boys. Children as old as about 5 years are predisposed to have urinary tract infections, partly because of periurethral colonization by *E. coli*, *Enterococci*, and *Proteus* spp. (Naylor, 1984; Modarres and Oskoii, 1997; Anis-ur-Rehman et al., 2008 and Shaikh et al., 2008).

These potential uropathogens usually diminish in the first year of life and are rarely found in children older than 5 years. Studies of girls and women prone to urinary tract infection showed that periurethral colonization occurs with the specific bacterium that causes the next infection (Fischer, 2010).
Sanderson and Alshafi (1995) have examined the bed sheets, floor and bedside chairs of 33 patients with urinary tract infections for organisms causing urinary infection. In ten patients the causative organism was recovered from the under sheet, and in two of these it was also recovered from the floor and bedside chair. *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Enterococcus faecalis* were recovered from sheets, while *Haemolytic streptococci* and *Candida tropicalis* were also found on floors and chairs.

Catheter-associated urinary tract infections (CAUTI) are the commonest nosocomial infections worldwide. While they are often asymptomatic and frequently cost less than nosocomial surgical site infections or nosocomial pneumonia, they are major reservoirs of antimicrobial resistant pathogens. Numerous strategies have been devised in an attempt to reduce the incidence of CAUTI but few have proven effective. Novel technologies such as the potential use of antiseptic or antimicrobial coatings on catheters hold promise for possibly reducing these infections in the fight against antimicrobial resistance (Paul Tambyah, 2004).

The introduction of antimicrobial drugs most notably penicillin was thought to herald the beginning of the end of bacterial infections. Unfortunately the rapid recognition of penicillin resistance within a year of its introduction disabused the physician of this notion (Furuya and Lowy, 2005). Multiple antibiotic resistances to useful classes of antibiotics including the beta-lactams, aminoglycosides and quinolones has generally emerged and this has been increasingly observed among a number of gram-negative pathogens such as the Enterobacteriaceae bacteria (Yah et al., 2006). Initially infections caused by antimicrobial resistant bacteria occurred mainly in hospital settings, where antibiotic use was most extensive. Thus bacteria carrying antimicrobial resistance genes had survival advantage that facilitated dissemination in this setting (Dzidic and Bedekovic, 2003). However, Furuya and Lowy (2005) observed a disturbing trend on the spread of
antimicrobial resistant bacteria within the community. The foremost reason for this trend is the increasing volumes of antimicrobial usage and studies indicate correlation between usage and the extent of antimicrobial resistance (Goossens, 2005). Antibiotic resistance in bacteria develops either by mutation or acquisition of new genes through a process known as horizontal gene transfer. This involves the transfer of resistance genes among pathogens which are often facilitated by the localization of these genes on plasmids, particularly those associated with integrons and transposons (Fred Tenover, 2006). Numerous studies have shown that resistance genes could be transferred from one bacterium to the other both in vitro and in vivo. Transfer of resistance genes between Shigella spp. and Escherichia coli has been observed (Hooper, 2000 and Fred Tenover, 2006). Aluyi and Akortha (2002) reported invitro transfer of resistance genes among enteric bacteria of diarrhea origin. Shoemaker et al., (2001) observed an extensive transfer of tetracycline resistant determinants among bacteroids and other enteric bacteria in the human colon.

The high incidence of Recurrent UTI (RUTI) caused by multidrugresistant Uropathogenic Escherichia coli (UPEC) is consistent with the high prevalence of antibiotic resistance. Antibiotics must be used with prudence to treat RUTI effectively. The correlations between resistance spectrums, virulence factors, and recurrence rates are of great clinical value for the clinical diagnosis, treatment, and predictive prognosis of RUTIs (Shiwei Liu et al., 2013).

To assess the appropriateness of antibiotic prescription for urinary tract infections in several hospital emergency services and to evaluate the variability of antibiotic prescription among these services Maria et al., (2007) conducted a cross-sectional study in the emergency services of 10 hospitals from different Spanish regions. The sample was composed of patients diagnosed with acute urinary infection, aged less than 14 years. A Consensus Conference, held by a panel of experts, established first-choice, second-choice
and inappropriate antibiotic treatments for each type of urinary tract infection, based on the available scientific evidence. All the observed prescriptions in the study were classified according to this pattern. The main variables were: type of urinary infection, antibiotic prescription, urine culture request, co-morbidity and hospital admission. A sample of 3797 acute urinary tract infections was studied. Eighty-one percent were lower urinary tract infections. The most commonly used antibiotics were ciprofloxacin and amoxicillin. The global percentages of first-choice, alternative-choice and inappropriate antibiotic prescriptions were: 42.4% (95% CI: 40.8-43.9), 44.1 (95% CI: 42.5-45.7) and 13.6% (95% CI: 12.5-14.7), respectively. A significant variability was observed in appropriateness of antibiotic prescriptions among the participating centers (p < 0.001). From the study, the Physicians at Spanish emergency rooms prescribe an excessive number of second choice antibiotics for urinary tract infection treatment. There exists a high variability in antibiotic prescription among hospitals from different regions.

Reid (2001) reported that, in healthy women, occurrence of urinogenital microflora is depending upon reproductive stages. In premenopausal the vaginal flora are lactobacilli which protects against colonization of uropathogens and certain properties of lactobacilli such as adhesive ability and production of acids, bacteriocins, hydrogen peroxide, and bio surfactants, appear important in conferring protection to the host. Efforts to artificially restore an unbalanced flora with the use of probiotics have met with mixed results and they could well provide a reliable alternative treatment. This can prevents routine use of antibiotics in the future.

Studies by Kathleen Head (2008) showed that Lower UTIs are common in young women (particularly who are sexually active), during pregnancy and in peri- and postmenopausal women and were often prescribed with long-term antibiotics results antibiotic resistance.
Secretory IgA (sIgA) is an important parameter in the predisposition to recurrent urinary tract infection (UTI). Ahmet Nayir et al., (1995) investigated whether sIgA and frequency of UTI could be positively influenced by intramuscular vaccination with inactivated uropathogenic bacteria (Solco-Urovac). Ten otherwise healthy girls aged from 5 to 11 years (mean 9 ±1.7 years) with recurrent UTI were immunized with Solco-Urovac by injections three times at weekly intervals. A booster injection was given after 6 months. Urinary sIgA secretory component (SC) concentration was determined by radial immunodiffusion assay. Ten other age-matched girls with UTI were not immunized. Immunization therapy caused a significant reduction in the frequency of infection and an increase in urinary sIgA SC, while in the non-vaccinated group the values remained constant.

Truls et al., (2006) obtained data from two internet-based studies on Nosocomially acquired urinary tract infections (NAUTI) in hospitalized urological patients: the Pan European Prevalence (PEP) study, which was a 1-day prevalence study in November 2003; and the Pan Euro-Asian Prevalence (PEAP) study, which was carried out in November 2004. Overall, 93 and 101 hospitals from the two studies, respectively, completed the hospital questionnaires and provided patient information for the study. NAUTI was diagnosed according to the Centres for Disease Control and Prevention (CDC) criteria in 727 of the 6033 patients hospitalized on study days in urological departments. The most commonly reported pathogen was Escherichia coli (31%), followed by species of Pseudomonas (13%), Enterococcus (10%), Klebsiella (10%), Enterobacter (6%) and Proteus (6%). Candida spp. and Pseudomonas spp. occurred significantly more frequently as causative agents in urosepsis than in other types of infections. The resistance of E.coli, Klebsiella and Proteus spp. was below 45% for the most commonly used antibiotics. Enterococcus spp. and Pseudomonas spp. however, had resistance rates above 70% to most
antibiotics. A total of 56% of the hospitalized urological patients were receiving antimicrobial therapy on the study day; 46% for prophylaxis, 26% for microbiologically proven UTI, 21% for only clinically suspected UTI and 7% for other infections. The most commonly used antibiotics were fluoroquinolones (35%), cephalosporins (27%), penicillins (16%), aminoglycosides (15%), and co-trimoxazole (9%). Differences between countries and regions were highly significant. There is an urgent need for continuous surveillance of NAUTI and improvement of antibiotic policy to counteract the widespread increase of antimicrobial resistance.

A repeated course of antibiotics is often prescribed for the treatment and prevention of RUTIs, which selects for resistant strains of uropathogenic Escherichia coli (UPEC) (Blango and Mulvey, 2010) investigate resistance, virulence factors, and their correlations with recurrence, 205 UPEC strains isolated separately from RUTI patients from five hospitals between January 2008 and September 2010 were included in this study. Most of these strains were resistant to multiple antibiotics: 159 (77.56%) strains were resistant to at least three antibiotics (multidrug resistant) and 114 (55.60%) were resistant to at least five antibiotics (pandrug resistant). Piperacillin showed the highest resistance rate (87.32%), followed by gentamicin (75.12%), levofloxacin (70.73%), and cefazolin (61.95%). The strains exhibited similar sensitivity to amikacin (9.31%), piperacillin/tazobactam (5.88%), and ceftazidime (9.76%), as well as a complete sensitivity to imipenem and meropenem (Shiwei Liu et al., 2013).

Polymerase chain reaction analysis of seven virulence factors in E.coli strains (Johnson and Stell, 2000) showed that the most common virulence factors were minor component of type 1 fimbriae (fimH) (90.3%), conjugal transfer surface exclusion protein (traT) (80.6%), aerJ (62.3%), the P fimbrial adhesion papGIII (32.2%), pathogenicity island (PAI) (26.9%), pesticin/ yersiniabactin receptor protein (fyuA) (20.4%), and p pilus adhesin
PapG protein (papG) (11.8%). The isolates often harboured multiple virulence factors: 82.7% had more than three virulence factors, and 63.4% had more than five factors. Correlation analysis showed that the number of virulence factors, the resistance spectrum, and the rate of recurrence were positively correlated (p< 0.000). The more virulence factors a UPEC strain contains, the more antibiotics it shows resistance to which in turn leads to a higher recurrence rate. Single virulence-factor-based correlation analysis showed that the presence of PAI, fyuA, or papGIII was related with higher rates of resistance and recurrence (p< 0.05). The b-lactam antibiotics are one of the most common types of antibiotics. Extended spectrum b-lactamases (ESBLs) were detected in 41 (20.00%) strains, which showed significantly higher resistance (10.69 [95% CI, 9.30–12.07] vs 5.77 [95% CI, 4.96–6.57]; p < 0.000), higher recurrence rates (9.88 [95% CI, 8.24–11.51] vs 5.04 [95% CI, 4.34–5.74]; p < 0.000), and more virulence factors (4.00 [95% CI, 3.42–4.58] vs 3.09 [95% CI, 2.75–3.43]; p < 0.05).

Joseph DiPersio and Michael Dowzicky (2007) reported on the activity of tigecycline and comparators against multidrug-resistant (resistant to ≥3 antimicrobial classes; MDR) Enterobacteriaceae from the USA collected between January 2004 and January 2006 as part of the Tigecycline Evaluation and Surveillance Trial (TEST).

Genomic DNA from 70 demographically matched geographically diverse pairs of urinary isolates of antimicrobial-susceptible and multidrug-resistant Escherichia coli was restricted using XbaI and analyzed by pulsed-field gel electrophoresis. Antimicrobial-susceptible isolates demonstrated limited genetic relatedness, whereas 2 epidemiologic clusters containing a total of 40 isolates (57.1%) were identified among the multidrug-resistant isolates (James Karlowskya et al., 2007).
300 urine samples with significant bacteriuria collected from 3 hospitals in Mubi were analysed for presence of Enterobacteriaceae bacteria. 187 urine samples comprising 68.9% female and 31.1% male yielded Enterobacteriaceae bacteria growth. The isolates include *Escherichia coli* 51.5%, *Klebsiella pneumonia* 24.4%, *Klebsiella oxytoca* 3.1%, *Enterobacter aerogenes* 9.7% and *Citrobacter freundii* 10.9%. Antibiotic resistance profile revealed high resistance of isolates to ampicillin 37.5%, ciprofloxacin 36.4% and co-amoxyclav 21.3%. Streptomycin, nalidixic acid, cephalaxin and gentamicin highly inhibited growth of the organisms tested. Gentamicin resistance rate of 17% was obtained while curing of selected donor isolates showed that gentamicin resistance in 75.8% of the isolates were plasmid mediated or located on mobile genetic element. Transfer rates of 34.8% and 41.1% respectively were obtained for inter-generic and intra-species transfer of gentamicin resistance genes (Gmr) among the Enterobacteriaceae isolates. Evidence of transferability of Gmr *in vitro* concurs to the assertion that under favorable conditions conjugal transfers of gentamicin resistance determinants and hence R plasmid could occur *in vivo* (Akortha and Filgona, 2009).

Transfer of resistance and virulence genes was demonstrated between *Salmonella* spp. inside epithelial cells. This dissemination of antibiotic resistance genes has led to rapid emergence and spread of antibiotic resistance among bacteria populations and thus pathogens involved in urinary tract infections. Gentamicin resistance genes aac (3)-IIa and aac (3) – VIa have been reportedly detected in gram-negative bacteria in the clinical settings. Sequencing and PCR experiments have confirmed that these genes are present on mobile genetic element that can facilitate their horizontal transfer among bacteria (Diaz et al., 2006).
Mark (2008) reported that *E. coli* strains resistant to ciprofloxacin were detected in the digestive tracts of villagers from rain forest community in Guyana, despite that they had never been given the drug. Most of the villagers however had been given chloroquine, a drug closely related to ciprofloxacin. In view of the wide usage of ciprofloxacin, resistance to this antibiotic and other fluoroquinolones could constitute an important public health problem in area where malaria is endemic. Therefore there is need to prevent malaria using integrated approach coupled with development of effective vaccine so that humans will not end up creating more problems in an attempt to solve one. Another possibility of resistance development is by horizontal transfer of the resistance determinants which have been shown to be common among members of Enterobacteriaceae (Osterblad *et al.*, 2000; Lawrence, 2005 and Fred Tenover, 2006). Series of researches have shown that dissemination of resistance genes mediated by plasmids among bacteria can occur by conjugation (Aluyi and Akortha, 2002; Diaz *et al.*, 2006; Shoemaker *et al.*, 2001 and Ferguson *et al.*, 2002). Sequencing and PCR experiments confirmed that Gmr genes are present on mobile genetic elements that can facilitate their horizontal transfer among bacteria (Heuer *et al.*, 2002 and Diaz *et al.*, 2006). Ferguson *et al.*, (2002) reported that gene transmission between bacteria can occur inside animal cells by conjugation and the conjugation in cell may be as efficient as on agar plate. An *in vitro* intra-species transfer of Gmr by conjugation was observed in *E. coli, K. pneumoniae, E. aerogenes* and *C. freundii*; suggesting that the Gmr determinant(s) in these isolates may be located on conjugative plasmids. However, transfer of gentamicin resistance determinant(s) was not observed in some isolates that had their Gmr genes on plasmids. This is an indication that the Gmr genes in these isolates are probably located on non conjugative plasmids. On the contrary, transfer was observed in some isolates that had their Gmr genes on the chromosome. It is most probable that the Gmr genes were located on transposable element or integron thus resulting in transfer function. Inter generic transfer of Gmr was also observed with high transfer rate from donor isolates to *E. coli*. This finding
underscores the flexible ease with which *E. coli* accepts exogenous genes as also evident in its ability to acquire various virulent factors from other bacteria (e.g. *Shigella* spp.). The average intra species and inter generic transfer rates from this study were 41.1% and 34.8% respectively. Aluyi and Akortha, (2002) reported transfer efficiency of 33% from some enteric bacteria of diarrhea origin to *E. coli* (UB5201).

The most common organism implicated in UTIs (80—85 %) is *E. coli*, while *Staphylococcus saprophyticus* is the cause in 5—10 % (Nicolle, 2008). During cystitis, uropathogenic *Escherichia coli* (UPEC) subvert innate defenses by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial communities (IBCs) (Justice *et al*, 2006). By working together, bacteria in biofilms build themselves into structures that are more firmly anchored in infected cells and are more resistant to immune system assaults and antibiotic treatments. This is often the cause of chronic urinary tract infections.

Drug pattern in *E.coli* isolated from UTI was explained by Asad Khan and Mohd Zaman (2006). Among 168 samples, 102 patients were identified *E.coli*. Among 102 samples 17% were males and 83% were females. In 83% of females 59.8% were pregnant.

Florian Wagenlehner and Kurt Naber (2006) in their work, highlights the current and future treatment options for UTIs. There are two aims preferred in treatment of UTI. One is rapid and effective treatment with care to prevent the repetition of infection. Second aim is to prevent the emergence of resistance to chemotheraphy. In recurrent UTIs low dose of antibiotic is effective and suggested vaccination may be important in future.

Kumud Metha (1996) revealed that, uncomplicated UTI can be treated by drugs like co-trimoxazole, nitrofurantoin and cephalosporins. But the treatment period may be depending on, where the infection occurs and selection of antibiotic is based on proper
diagnosis of UTI. Collection of urine sample from mid stream catch and microbial count should be $10^5$ or more/ml is necessary for diagnosis of UTI.

Fred Tenover (2006) reported that, due to antibiotic resistance bacterial infection becomes complicated. Antibiotics act on microbes by some specific actions. They are interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis and inhibition of metabolic pathway. Susceptible bacteria acquire resistance gene from resistance bacteria through various genetic transformation and get ability to produce an enzyme which destroy or prevent the antibiotic from action. *E.coli* contained *bla*-TEM-1 indicated low level ceftazidime resistance whereas the *E.coli* contained the second *bla*-SHV-1 indicated high level resistance to third-generation cephalosporins.

In women with acute uncomplicated cystitis, antibiotics are prescribed for longer (7-10 days) course. But Norrby’s (1990) study concluded that, in adult women with the same above mentioned problem there is no benefit with increasing the length of therapy more than 5 days. The curer rate of different treatment was studied in 28 trials. Cure rate of single dose treatment (3-5 days) is 23-81% and long-term treatment (7-10 days) is 77.91%, when β-lactam antibiotics were used ($> 5$ days) it reached 77-92%. Usually nitrofurantoin recommended for 7 days of therapy. Use of trimethoprim and/or sulfamethoxazole for 3 days resulted in 82-85% of cure rate and there is also no benefit using more than 3 days.

Claudia Mladin et al., (2010) demonstrated that, *E.coli* strains are the top of microbes causing UTI. Among studied *E.coli* strains, 118 *E.coli* strain highly resistance to aminopenicillin and multiple resistances to β-lactam aminoglycosides, fluoroquinolones and co-trimoxazole. Double disk synergism test (DDST) indicated 65.2% are β-lactamase producers and these strains are resistance to clavulanic acids but susceptible to sulbactam.
The number and types of β-lactamases was detected based on isoelectric point and PAGE. This revealed the presence of *bla-TEM, bla-CTXm, bla—AmpC* and *bla—SHV*. PCR and sequencing methods indicated the presence of *bla-TEM* and group I *CTXm* genes in 75% of test isolates.

Jankoska *et al.*, (2008) reported that some important virulence factors in UTI causing agents are haemolysin, enterococcal surfacr protein (Esp), aggregation substance and gelatinase. 50 strains of *Enterococcas faecalis* were isolated and analyzed. Formation of zone for β-haemolysis around steak on Columbia CAN agar, determined the haemolysin production. Gelatinase was detected by tripticase soy agar with 1.5% of skim milk and Esp was detected by PCR method. Among 50 isolates 38 were Esp positive and it was the most frequently determined factor. In 16 isolates (32%) all the tested virulence factors were present. Two virulence factors were found in 19 isolates (38%) and only one in 11 isolates. four isolates had no virulence factors. In case of antibiotic sensitivity test using ampicillin, ceftriaxone, vancomycin, nitrofurantoin and ciprofloxacin, all the isolates were susceptible to vancomycin whereas, in resistance pattern nitrofurantoin, 12 isolates were resistant to ampicillin, 17 to ceftriaxone and 14 to ciprofloxacin. Hence this work highlights that, there is no relationship between virulence factors and antibiotic resistance pattern of test isolates.

Johnson (1991) has studied that, the common uropathogen *E.coli* are characterized by their properties, products or structure called virulence factors. These factors are responsible for causing disease in host. Several virulence factors are importance in pathogenesis of UTI such as adhesion, the aerobactin system, hemolysin, K capsule and resistant to serum killing. These factors are most common in genetically related strains of *E.coli*. The strains which express more virulence factors were more severe in causing infection. These virulence factors are mostly causing pyelonephritis and also develop cystitis and asymptomatic bacteriuria. Though these factors are found in wild-type of strains.
can’t able to spread into non-virulence organism. Use of immunological and biochemical anti-virulence factors are effective in animal models of UTI and this will help in human in future.

The correlation between antibiotic resistance and virulence factors was evaluated by Baylan et al., (2011) in Enterococcus strains of inpatient (Jan 2008- Jun-2010). Ninety one Enterococcus strains (59 were E. faecalis, 31 were E. faecium and 1 was E. gallinarum) were isolated from urine cultures on inpatients. Presence of the genes for some virulence factors such as aggregation substance (asal), enterococcal surface protein (esp) and hyaluronidase (hyl) were studied by molecular method. In Vancomycin resistance genes (VanA and VanB) test result showed 8 (25.8%) E. faecium isolates were glycopeptides resistance, among this 7 had VanA and in one it was neither VanA nor VanB. In antibiotic sensitivity test E. faecium and E. faecalis showed high level of resistance against gentamicin (E. faecium= 74.2%; E. faecalis=22%) and streptomycin (E. faecium=. 61.3%; E. faecalis=27.1%). hyl gene was higher in E. faecium, esp and asl genes productivity, haemolysin production and gelatinase activity were higher in E. faecalis. Aggregation substance (26.7%) and enterococcal surface protein (25.6%) were the frequent virulence factors. Asal gene positive strains were more resistant to ciprofloxacin, norfloxacin and levofloxacin than Asal negative strains. esp positive E. faecalis strains were resistant to doxycycline than esp negatives and hyl gene positive E. faecium strains were more resistance to nitrofurantoin.

Health Protection Agency (2006) reported that, E.coli is a common gut flora and lives harmlessly. ESBL producing E.coli was more likely to cause UTIs. ESBL bacteria spread to non-pathogenic organism by faecally contaminated hands or unclean equipments used for UTI treatment. There are many way to stop the spreading of ESBL producing
bacteria such as personal hygiene. One of the important mode to reduce antibiotic resistance is to prescribed antibiotics only when needed.

In both hospital and community acquired infection the spread of ESBL is successful due to mobilization and evolution of the genetic elements. Antibiotic resistance test were observed in 285 isolates includes 172 E.coli; 84 Klebsiella spp.; 20 Enterobacter spp.; 5 Salmonella spp.; 4 Citrobacter spp.; The susceptible rates for meropenem, imipenem, tigecycline, amikacin and piperacillin-tazobactam were observed as 100%, 100%, 97.5%, 93.3% and 93% respectively. Tigecycline was 4-256-fold more active than doxycycline and minocycline. The $bla-CTX_m$s were the prevalent (61.4%) in both hospital (58.6%) and community–acquired (65.8%) isolates. The $bla-SHV$ present in 22.8% and $TEM$ in 15.8% of isolates. The resistance rate of isolates associated with $CTX-M-9$, for other antibiotics were observed as follows gentamicin, 27.4%; tobramycin, 27.4%; amikacin, 6.7%; and chloramphenicol, 29.1% Sulfonamide (61.7%), trimethoprim (52.3%), streptomycin (50.5%) and ciprofloxacin (37.2%) (Morosini et al., 2006).

In the study conducted by Vervoort et al., (2012), two new $CTX_m$ types of ESBL variants were found in E.coli from stool samples of 2 elderly admitted patients in Tel Aviv Sourasky Medical Center, Israel. Both patients underwent treatment with cephalosporins prior to isolation of the test E. coli strains. ESBL were detected by Double Disk synergy test (DDST) and PCR used for sequencing of $\beta$-lactamase genes. The $bla-CTX_m$ genes were cloned into the pCR-BluntII-TOPO vector in E. coli TOP10 electrocompetent cells. By using PCR $bla-CTX_m$. Bla-SHV and $bla-TEM$ were detected. Two new $bla-CTX_m$s ($CTX_m-94$ and $CTX_m-100$) were identified and amplified using the same primers of $CTX_m-25$. Both new variants differed from $bla-CTXM-25$ by substitution of V77A and from $bla-CTXm-39$ by D240G. $CTXm-94$ differed from all $CTXm-25$ group enzymes by substitution of F119L.
Nuno Mendonca et al., (2007) conducted a study in 3 Portuguese regions. 181 unduplicated *E.coli* strains were isolated in 9 different hospitals. Among them, 119 were observed as *bla-CTXm* producers and they were identified as causative agents in community–acquired infections (56%), UTIs (76%) and affecting more than 60 years old peoples (76%). In minimum inhibitory concentration (MIC) test the resistant rate of all 119 test isolates were 100% to cefotaxime, 92% to ceftazidime, 93% to quinolones, 89% to aminoglycoside, and 26% to trimethoprim-sulfamethoxazole. In case of, sensitive rates 100 to carbapenems, and 92% of the strains had a multidrug resistance phenotype were observed. Molecular tests indicated 92% of isolates had *bla-CTXm-15* gene, only one strain had *bla-CTXm-32* gene which is the first identified in the country and 9 strains had *bla-CTX14* gene. And also among 119 isolates 85% of *bla-TEM1B* and 84% 0f *bla-OXA-30* were observed. The strains of *E.coli* were producing the both *bla-CTXm* and *bla-OXA* enzymes increasing resistance to β-lactamase inhibitors (<24% resistance to clavulanic acid and tazobacta; >98% to sulbactam). *CTXM* producing *E.coli* strains especially with *CTXM-15* is common in Portugal. Cabapenems are regarded as the agents of choice for severe infections caused by ESBL producing *E.coli*.

ESBL producers were obviously enabled to hydrolysis penicillins, broad-spectrum cephalosporins and monobactams. In general *CTXm* genes derived from *TEM* and *SHV*-type enzymes. ESBL is increasing world- wide; 10-40% strains of *E.coli* and *Klebsiella pneumoniae* express ESBLs. ESBL-producing organisms are clinically important and these need use of appropriate antibiotics. But it is complicated by multidrug resistance. Though ESBL-producing organisms are resistance to aminoglycoside and fluoroquinolone, they are inhibited by some β-lactamase inhibitors like clavulanic acid. Recently Carbapenem is the better choice for ESBL, but it may cause emergence of carbapenem-resistant bacterial species such as *Stenotrophomonas* spp. or *Pseudomonas* spp. (Rupp and Fey, 2003).
*E.coli* is the important in UTI. In ESBL, the *CTXm* are cefotaximase. A total 250 clinical isolates of *E.coli* were collected from 3 university hospitals in Tehran. The phenotypic tests for confirmation of ESBL were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Presence of *bla-CTXm* genes were determined by multiplex PCR which is the fast and rapid method to detect sequencing the genes. In primary phenotypic tests among 250 isolates 140 (56%) were ESBL producers. But in confirmatory tests using clavulanic acid, only 135 isolates were considered as ESBL producers. In 135 isolates the genotypic results indicated the presence of *bla-CTXm-1* in 50 isolates, *bla-CTXm-9* in 5 isolates and *bla-CTXm-25/26* in one isolate of test *E.coli* (Mohsen Mirzaee *et al.*, 2009).