INTRODUCTION

Urinary tract infection (UTI) is one of the common infections that are encountered as community-acquired or nosocomial infections (**Stapleton, 2005**). About 150 million people are diagnosed with UTI each year (**Akram *et al*., 2007)**.

Catheter-associated UTI is the most frequent source of Gram-negative sepsis in hospitalized patients (**Stamm, 1991**).

Urinary tract infections can be classified as cystitis, pyelonephritis, urosepsis, catheter-related infections, as well as asymptomatic bacteriuria, which requires medical management among certain, more vulnerable hosts. A remarkable estimate detected that about 250,000 cases of pyelonephritis occur annually in the United States, many of which require hospitalization (**Stapleton, 2005**).

Pregnancy confers an increased risk of pyelo-nephritis, and UTI is associated with adverse obstetrical and newborn outcomes. Patients with diabetes are also at increased risk of pyelonephritis (**Stapleton, 2002**).

Urinary tract infection (UTI) is one of the most commonly acquired bacterial infections in ambulatory and

hospitalized populations; 11% of women aged 18 years and older had experienced at least one physician-diagnosed UTI each year **(Foxman *et al.,* 2000).**

Escherichia coli is the causative agent in 90% of the uncomplicated UTIs and in 78% of the complicated UTIs (**Arslan *et al.*, 2005**).

Uropathogenic *E.coli* (UPEC) strains are responsible for the majority of uncomplicated urinary tract infection, which can present clinically cystitis or pyelonephritis (**Lioyd *et al.,* 2007**)**.**

A complicated urinary tract infection is a urinary infection occurring in a patient with a structural or functional abnormality of the genitourinary tract. *E.coli* is the most common organism isolated, but is isolated more frequently in women than in men **(Nicolle, 2005).**

E.coli that cause UTI and other uropathogens are distinguished from related members of their genus and species by the presence of specific virulence determinants, microbial adaptations promoting success in the urinary tract (**Johnson, 2003**).

Other clinical factors affect the incidence of UTI in a given host, such as acquired and intrinsic differences in host susceptibility, genetic factors, and behavioral exposures.

However, several studies have indicated that virulence determinants of uropathogenic bacteria enhance the ability of these organisms for entering the urinary tract and establishing disease, particularly among otherwise healthy hosts (**Stapleton, 2005**).

The virulence determinants of uropathogenic *E.coli* (UPEC) have been most extensively studied. These include adhesins, such as P, type 1, S, Dr, and F1C fimbriae; toxins and cytolysins, such as cytotoxic necrotizing factor, secreted autotransporter toxin, cytolethal distending toxin, and hemolysin; iron acquisition mechanisms, including aerobactin, enter-obactin, and yersiniabactin; and surface components such as capsule, flagellum and lipopolysaccharide (LPS) **(Oelschlaeger *et al.*, 2002; Emody *et al.,* 2003 and Johnson, 2003)**.

Some other adhesins do not form fimbriae: among these are the AFA afimbrial adhesive sheaths (AFAs) that are encoded by the *afa* gene clusters **(Le Bouguénec, *et al.,* 2001).**

Urovirulence factors of *E.coli*, analyzed by multiplex PCR, are useful markers for detection of uropathogenic *Escherichia coli* **(Yamamoto *et al.,* 1995 and Johnson *et al.*, 1997).**

Aim Of Work

The objectives of the present investigation were:

- Isolation, characterization, and determination of nucleotide sequencing of pyelonephritis-related gene (*pap* or P fimbriae) in *E.coli*.

These objectives were achieved by the following:

- Isolation and identification of some pathogenic bacterial isolates of *E.coli*. Identification was carried out using manual methods (including morphological, physiological and biochemical characters), and molecular biology methods including polymerase chain reaction (PCR).
- Screening the identified isolates for their resistance to different antibiotics.
- DNA sequencing of the target gene which responsible for encoding pilus associated with pyelonephritis caused by *E.coli.*

LITERATURE REVIEW

I. CLASSIFICATION AND CHARACTERIZATION OF *ESCHERICHIA COLI*

According to Bergy's manual of systemic bacteriology **(Holt and Krieg, 1994)**, *E.coli* belongs to family Enterobacteriaceae.

E.coli may be recovered from various sites of the body either as normal flora or as causative agents of a variety of infections (gastrointestinal tracts, urinary tract and wound infections, meningitis, septicemia, etc…). Specimens from normally sterile body sites are plated on the usual nonselective media such as blood, chocolate, or nutrient agar. If materials are likely to be contaminated or contain different organisms (e.g. specimens from the respiratory or the urinary tract, or from wound infections), media of low selectivity such as MacConkey, CLED (cysteine, lysine electrolyte deficient), or eosin-methylene blue (EMB) agar should be included in the set of plating media **(Holt and Krieg, 1994).**

E.coli is Gram-negative forming rod shaped cells of 2.0-6.0μm in length and 1.1-1.5μm in width with rounded ends **(Qrskov and Qrskov, 1984)**. The shape may vary from coccal to long filamentous rods; in some strains one

of these forms may prevail but others may show a wide diversity (**Gross and Holmes, 1990).**

E.coli strains, with exception of the "inactive" types, are usually motile by a set of peritrichous flagella. These protinaceous structures form long, slender appendages of 19-24nm diameter which extend about 15- 20μm from the cell surface **(Lawn *et al.,* 1977; Silverman and Simon, 1977).**

E.coli produce different kinds of fimbriae that vary in structure and antigenic specificity. These are filamentous, proteinaceous, hair like appendages surrounding the cell in varying numbers. Capsules or microcapsules made of acidic polysaccharides are common in *E.coli* **(Qrskov and Qrskov, 1984).**

E.coli strains are facultatively anaerobic organisms. They are chemo-organotrophic, having both a respiratory and a fermentative type of metabolism, but growth is plentiful under anaerobic conditions. The optimal temperature is 37˚c at which they grow well on ordinary media containing 1% peptone as carbon and nitrogen source **(Holt and Krieg, 1994).**

E.coli exerts pronounced metabolic activity between 15 and 45˚c **(Gross and Holmes, 1990)**; under optimal conditions the generation time is 20 min. Exotoxins such as

enterotoxins and hemolysins are best produced at about 37˚c **(Bockemühl, 1992).**

E.coli ferments lactose and produces indole at 44˚c. This combination has been used for identification in food and water bacteriology. After 18-24hrs of incubation at 37˚c *E.coli* forms large (2-3mm), circular, convex and non-pigmented colonies on nutrient and blood agar; hemolysin is produced by a number of strains. *E.coli* strains are resistant to low concen-trations of bile salts (e.g., 0.05 percent sodium deoxycholate) and grow as large red colonies on MacConkey agar. *E.coli* is more heat resistant than most other species of enterobacteriaceae and survives at 60˚c for 15 min or at 55˚c for 60 min **(Gross and Holmes, 1990)**.

All strains of *E.coli* are methyl-red positive and ferment D-mannitol and D-mannose; they do not ferment meso-inositol; they give negative reactions in the vogas-proskauer, phenylalanine deaminase test, they are generally urease negative; they do not produce H_2S in triple-sugar-iron (TSI) agar. Most strains form gas from glucose (**Collee *et al.,* 1996).**

The outer layers of *E.coli* consists of the outer membrane with phospholipids, lipid A and proteins, from which protrude the polysaccharide (LPS) chains, overlaid by capsular polysaccharides (CP) **(Jann and Jann, 1987).**

Both LPS and CP are the chemical basis of O and K antigens, respectively, and contribute to the pathogenicity of the organism.

E.coli strains are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigens. More than 170 different O antigens serogroups are currently recognized (**Nataro *et al.,* 1998 and Lioyd *et al.,* 2007**).

E.coli is a component of the normal intestinal flora of both humans and worm-blooded animals (mammals and birds). The organism is excreted with the feces and may survive in the environment. However, it appears that there is no independent existence outside the body. Accordingly, *E.coli* is considered an indicator organism for fecal contamination and is an important parameter in food and water hygiene. In organs outside the intestinal tract, *E.coli* may cause a variety of disease, but the responsible strains as well as strains causing enteritis in human and mammals, are characterized by the presence of specific virulence factors. Infections with such strains develop either by the endogenous route (e.g., urinary tract or gall bladder infections, septicemia), or they are spread in the hospital via contaminated equipment and by the hands of the nursing staff (urinary and respiratory tract infections,

wound infections, septicemia, meningitis) **(Holt and Krieg, 1994)**.

II. PATHOGENICITY OF *ESCHERICHIA COLI*

E.coli is able to colonize both the intestinal and extra-intestinal environments in humans **(Siegfried and Kmeťová, 1997).**

A balanced relationship exists between the human immune system and strains of *Escherichia coli,* which normally allows them to occupy the host without causing illness. However, in some specific circumstances (e.g., immediate immune deficiency or because of individual host susceptibility), these apparent commensals may cause diarrhea or extra-intestinal infections **(Kmeťová and Siegfried, 1999).**

Certain strains of *Escherichia coli* can cause enteritis or gastroenteritis by six distinct mechanisms, resulting in six different clinical syndromes. These include enterotoxigenic *E.coli* (ETEC), entero- patho-genic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), enterohemorrhagic *E.coli* (EHEC), enteroaggregative *E.coli* (EAEC) and diffusely adherent *E.coli* (DAEC). EPEC, EAEC, DAEC isolates are characterized by their distinct patterns of adherence to epithelial cells in vitro. EPEC strains bind to host cells in a pattern called localized adherence, in which microcolonies form on the surfaces of the cells. EAEC isolates bind in an aggregative adherence pattern that is

charac-terized by a stacked - brick - like arrangement on the surfaces of the cells. DAEC strains are defined by a pattern of diffuse adherence in which the bacteria uniformly cover the entire cell surface **(Nataro *et al.,* 1998).**

E.coli strains capable of causing disease outside the gastrointestinal tract considered to be a different group of isolates referred to as extraintestinal pathogenic *E.coli* (ExPEC) (**Russo and Johnson, 2000; Johnson and Russo, 2005**).

ExPEC strains are responsible for a variety of diseases, including urinary tract infections (UTIs), newborn meningitis, septicemia, nosocomial pneu-monia, intra-abdominal infections, osteomyelitis and wound infections (**Eisenstein and Jones, 1988; De Louvois, 1994; Russo and Johnson, 2000; Johnson and Russo, 2002**).

When the strains cause urinary tract infection, such strains usually emerge from the host's own intestinal flora **(Hooton and Stamm, 1996**). Such strains ascend the urethra and colonize the bladder, resulting in cystitis, and in severe cases, infection may spread up to the kidneys, causing pyelonephritis (**Ikaheimo *et al.,* 1993**).

Certain O:K:H serotypes and virulence factors occur more frequently in urinary isolates than in fecal isolates,

suggesting that uropathogenic *E.coli* strains are different from normal bowel inhabitants (**Johnson, 1991**).

E.coli isolated from infected urinary tracts often express specific properties that are not prevalent among strain from the commensal fecal flora **(Svanborg-Eden and De Man, 1987).** These properties include the expression of adhesins mediating attachment to specific receptors of uroepithelial cells, production of hemolysin, serum resistance, release of aerobactin, and presence of particular surface antigens. These factors enable these isolates to overcome host defense and cause infection (**Lioyd *et al.,* 2007**).

Pathogenic *E.coli* cells, which cause intestinal and extraintestinal infections in humans, generally adhere to mucosal epithelia early in the colonization of host tissues (**Garcia and Le Bouguenec, 1996)**. These bacteria produce a wide variety of adhesive proteins and organelles. Adhesins are often assembled into hair like fibers called fimbriae and are classified based on their adhesive properties (**Mulvey *et al.,* 1998**).

Each adhesin of UPEC recognizes specific receptor (s) on the surface of the uroepithelial cells (**Kallenius *et al.,* 1981a**).

Urinary tract infection due to *E.coli* can progress to bacteremia, which is associated with significant mortality. *E.coli* is recognized as the one of the two most common causes of bacteremia (**Lau *et al.,* 2008).**

In some cases, Uropathogenic *E.coli* strains may be acquired by sexual transmission. These exposures, by facilitating entry *E.coli* into the bladder, may initiate events leading to cystitis, pyelonephritis, or both (**Brown and Foxman, 2000**).

Urogenital infections, including asymptomatic bacteriuria, cystitis and pyelonephritis, are the most common and frequently encountered medical complications of pregnancy and the primary cause of maternal and fetal morbidity and mortality due to infections. It has been observed that urogenital infections are associated with low birth weight and preterm labor. The most frequent etiologic agent of urogenital infections is uropathgenic *E.coli*, accounting for 65% to 90% of cases **(Millar and Cox, 1997).**

III. EPIDEMIOLOGY OF *ESCHERICHIA COLI*

E.coli was the causative agent in 90% of the uncomplicated UTIs and in 78% of the complicated UTIs in Turkey. 17% percent of *E. coli* strains isolated from uncomplicated cases and 38% of *E. coli* strains isolated from complicated UTI were found to be resistant to ciprofloxacin (**Arslan *et al.,* 2005).**

Another study in Moscow demonstrated that the prevalence of *E.coli* in community urinary tract infections (cUTI) (acute and chronic cystitis, chronic pyelonephritis and acute pyelonephritis were most frequent cUTI), in such study *E.coli* was present in complicated and uncomplicated cUTI in a percentage of 53 and 80.9%, respectively (**Rafal'skiĭ *et al.,* 2007**).

In Spain, a study discussed the urinary tract infection in renal transplant recipients. UTIs were elevated in kidney recipients transplanted, The most common clinical features were uncomplicated acute bacterial cystitis, (77%), and acute pyelonephritis, (23%). Microbiological isolation was confirmed in 63% of isolates. Bacterial infections were the most frequent etiologies: gram-negative bacilli in (90%), gram-positive cocci in (7%), fungal in (3%), and one viral BK virus (2%) infection. The causative microorganisms were *E.coli* as the principal isolated agent in 71% of cases **(Valera *et al.,* 2006).**

Pan European Prevalence (PEP) study and Pan Euro-Asian Prevalence (PEAP) study, were carried out on the prevalence of nosocomial urinary tract infections (NAUTIs) in hospitalized patients. NAUTI was diagnosed in patients hospitalized and the most commonly reported pathogen was *E.coli* (31%), followed by species of *Pseudomonas* (13%), *Enterococcus* (10%), *Klebsiella* (10%), *Enterobacter* (6%) and *Proteus* (6%) **(Johansen *et al.,* 2006).**

In United state, A study was carried out on community urinary tract infections in adults, out of 650 patients (417 women, 233 men; age range, 18-94 years) have been diagnosed for cUTIs. It was found that the most common pathogen was *E.coli* (65.6%) (**Peterson *et al.,* 2007).**

Another study on the urinary tract infections in children was done in Taiwan. 68% of patients were 1 year old or younger. Boys predominated infant cohort (68.1%). The results showed that the most common pathogen was *E.coli* (74.7%), followed by *Proteus* spp. (6.7%), and *Klebsiella* spp. (6.4%) **(Wu *et al.,* 2004).**

In another study in European countries and in Brazil, It was found that *E.coli* was most frequent (76.7%) in patient suffered from uncomplicted cystitis, followed by *Enterococcus faecalis* (4.0%), *Staphylococcus saprophyticus*