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Molecular Analysis of *Fim H* Gene from Uropathogenic *Escherichia.coli*

A thesis submitted in partial Fulfillment of the Requirements for the Master's Degree of Science in Biology

By

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***Fim H* Gene from Uropathogenic *E.coli*)**

By

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Has been prepared under my supervision at the College of Sciences/ Al Muthanna University as a partial fulfillment of the requirements for the Degree of Master of Science in Biology.

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In view of the available recommendations, I forward this thesis for debate by the Examining Committee.

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We are certified as an examining committee, that we have read the thesis entitled **(Molecular Analysis of *Fim H* Gene from Uropathogenic *E.coli*)** and examined the student **" Sabreen Fleih Nino"** In 14\12\2020 and found that the thesis meets the standards for the degree of Master of Science in Biology.

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Dedication

To

The Prophet Mohammed and his progeny (peace be upon them).

The person who always be there for me, with his love, Tenderness and support.. My Dearest Husband.

The meritus and my strength, who removed the thorns from my path to pave my way to the science and gave me love, cooperation and support … My Dear family and to my daughters.

sabreen

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Sabreen

Abstract

Urinary tract infection (UTI) is defined as presence of microbial pathogens in the urinary tract with associated symptoms. The infection affects both lower and upper urinary tracts and is known as acute cystitis and polynephritis respectively. This study done to detect the variation of single nucleotide in *FimH* gene and its correlation with the pathogenicity of uropathogenic *Escherichia coli* caused the urinary tract infection. The study group included 150 patients with Urinary tract infection. There were 120 (80%) female and 30 male (20%) their age ranges between (5-55) years, collected in AlHussein Teaching Hospital for the period from January 2019 to March 2019. Urine samples were collected from the patients. Bacterial isolation was identified by microscopic examination and culture characteristic on selective media MacConky agar, Eosin Methylene Blue and Chrome agar. The isolated were diagnosis by Vitek®2 system. Bacteriological results showed that 62(41.3%) isolates were characterized as *Escherichia coli.* Genomic DNA was extracted from the bacterial culture for further molecular study to reveal C640T and T591A *fimH* gene . Polymerase chain reaction-restriction fragmentlength polymorphism (PCR-RFLP) technique was used for this purpose and digestion of the amplified DNA products by ScrFI1 and BstNI endonuclease respectively, the results gave fragments with different molecular sizes which express certain genotypes. This study detected that the prevalence rate of single nucleotide polymorphism(SNP) C640T *FimH* was (85.48%), endonuclease enzyme only affects the C allele sequence and it does not have any influence on the T allele sequence. The prevalence rate of single nucleotide polymorphism T591A *fimH* gene was (80.64%),endonuclease enzyme only affects the T allele sequence and it does not have any influence on the A allele sequence.

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List of abbreviations

Abbreviations	Whole phase
UPEC	Uro Pathogenic Escherichia Coli
ECEC	Enteric Escherichia coli
EXPEC	Extra Intestinl Pathogen Escherichia Coli
UTI	Urinary Tract Infection
GBS	Group B Streptococcus
AT	Anto Transporter Proteins
SNPS	Single Nucleotide Polymorphisns
EPEC	Extraintestinal Pathogen Escheri Coli
EHEC	Entero Haemorrhagic Escherichia Coli
ETEC	Entero Toxigenic Escherichia Coli
EAEC	Entero Aggregative Escherichia Coli
EIEC	Entero Invasive Escherichia Coli
DAEC	Diffusely Adherent Escherichia Coli
(A/E)	Attaching And Effacing
TD	Traveler's Diarrhea
STs	Stable Temperature
LTs	Labile Temperature
AAFs	Aggregative Adherence Fimbriae
STEC	Shiga Toxigenic E. Coli
VTEC	Vero Toxigenic E. Coli
HUS	Hemolytic Uremic Syndrome
DEC	Diarrheagenic group of E. Coli
HEP-2	Hela Epithelial
SEPEC	Sepsis – associated Pathogenic E. Coli
NMEC	Neonatal Meningitis Associated E. Coli
LPS	Lipopolysaccharide
LD	Lectin Domain
PD	Pillin Domain
NHANES	National Health And Nutritional Examination Surveys
ASB	Asymptomatic Bacteriuria
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
UV	Ultra Violate
EMB	Eosin Methylene Blue
TBE	Tris base Boric acid EDTA

Bp	bace pairs
DNA	Deoxyribonucleic Acid
LE	Leukocyte Esterase
ATN	Acute Tubular Necrosis

1.1Introduction

Escherichia coli is a ubiquitous bacterial species commensal of humans and warm blooded animals, nevertheless some strains have evolved the capability to cause both intestinal and extra intestinal infections (Nataro *et al*., 2011). It is categorized into two foremost categories, enteric *E. coli* (ECEC) that mostly causes infections restricted to the mucous lining of the intestine and extra intestinal pathogens (ExPEC) which have the capability of spreading from the intestine and inflicting infections in other parts of the body (Xie *et al*., 2006) Urinary tract infection (UTI) is one of the most widespread infections in humans which causes extreme morbidity and considerable expenses (Minardi *et al.*,2011). UTIs have an effect on different components of the urinary tract and are classified into a number disease corporations based on infection sites. UTIs are classified into cystitis (the bladder), pyelonephritis (the kidney) and bacteriuria (the urine) (Barber *et al.*, 2013). Urinary tract infection are induced by means of each Gram-negative and Gram positive bacteria, as well as by certain fungi. The most frequent causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in occurrence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp.(Foxman, 2014). For complicated UTIs, the order of occurrence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae, Candida* spp., *S. aureus*, *P. mirabilis* and *P. aeruginosa*(Flores-Mireles*etal*.,2015) Uropathogenic *Escherichia coli* (UPEC) strains are regarded as a primary pathogen in about 80% of patients with UTIs (Bien *et al.*, 2012). UPEC has many virulence factors that assist its colonization, invasion, and survival within the host urinary system (Behzadi *et al.*, 2016). These factors include adhesins, siderophores, toxins, capsule production, and proteases.

In addition, several autotransporter (AT) proteins, which are typically associated with phylogeny (Zude *et al*., 2014), are correlated with virulence and have been identified in UPEC (Zalewska-Piatek *et al.,* 2015). Among adhesions of UPEC, the adhesive subunit of type 1 fimbriae, *FimH*, is a most important determinant, which has high tropism for urinary tract receptors; thus, *FimH* adhesion is vital in colonizing different niches of *E. coli* (Hojati *et al.,* 2015). Researchers pointed out *FimH* adhesin as a key player in *E. coli* mediated urinary tract infections. This detail persuaded the researchers to the development of a *FimH* based anti‑urinary tract infection vaccine. The feasibility of this approach to the vaccine preparation has shown promising results in experimental animal urinary tract models(Yun Mei Lai *et al*., 2015). single-nucleotide polymorphism (SNP) analysis of *FimH* is a screening tool for epidemiological typing of UPEC (Dias *et al*., 2010).

1.2 Aim of the study

The study aimed to investigate the role of nucleotide polymorphism in *FimH* gene in patient infected with urinary tract infection. the objectives of the study as the follow:
1- Isolation and identification of UPEC from urine of patients with UTI
2-To investigate the association between SNP (C640T and T591A) of *FimH* gene and its effect on the severity of pathogenicity of UPECs to cause UTIs.

2. Literature Review

2.1 *Escherichia Coli*

2.1.1 Taxonomy and Description of *E. coli*

Theodor Escherich first reported the isolation and characterization of slender short rods from infant stool, which he named Bacterium coli commune, in his 1885 publication (Croxen *et al*., 2013).

Escherichia coli is a rod-shaped, Gram-negative bacterium, and classified as a member of the family Enterobacteriaceae within the Gammaproteobacteria class. *E. coli* is among one of the well-studied bacteria. *E. coli* can grow rapidly under optimal growth conditions, replicating in ~20 min. Many gene manipulation systems have been developed using *E. coli* as the host bacterium, producing countless enzymes and other industrial products. Genome sequence analysis of *E. coli* was first reported in 1997. Since then, more than 4800 *E. coli* genomes have been sequenced. The fast growth characteristics of *E. coli* make it suitable to study the evolution of micro-organisms and a long-term experimental evolution study of *E. coli* involving more than 50 000 generations is ongoing (Tenaillon *et al*. 2016) *E. coli* and other facultative anaerobes make up about 0.1 percent of gut flora and the major route through which pathogenic strains of the bacterium cause disease is fecal – oral transmission. Cells can survive outside the body for a limited period of time, making them potential indicator organisms for testing fecal contamination environmental samples (Thompson and Andrea, 2007). Commensal *E. coli* primary habitat in the large intestine of the digestive tract and mainly in the caecum and colon. They are populating the mucus layer that covers the epithelial cells throughout the digestive tract. The mucus is degraded and thrown into the lumen of the intestine where it is excreted in the feces (Tenaillon *et al*., 2010).

2.1.2 Growth Characteristics of *E. coli*

E. coli is gram negative facultative anaerobic, motile bacteria,. Its capability to grow in temperatures ranging from 7°C to 50°C and tolerate acidic condition down to pH 4.4 is reflected in its ability to survive various environmental conditions and it can be detected contaminating different surfaces from hands and clothes to soil and underground water (Kaper *et al.* 2004; Kramer, Schwebke & Kampf 2006;). In the presence or absence of oxygen, it can be easily recovered from clinical specimens using simple culture media incubated at 37 oC (Garrity 2005; Weintraub 2007).

2.1.3 Pathogenic Classification of *E. coli*

Pathogenic *E. coli* can be divided into two main categories: diarrhoeagenic *E. coli* and extraintestinal *E. coli* (ExPEC). There are sets of strains known as pathotypes within each of these broad groups known as pathotypes that share common virulence factors and produce similar pathogenic effects (Marrs *et al.,* 2005).The majority of diarrhoeagenic *E. coli* can further be divided into six pathovars: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). (Lukjancenko *et al*., 2010)**.**

2.1.3.1 Enteropathogenic *Escherichia Coli* (EPEC)

Until the 1970s serotyping was the only means of distinguishing EPEC strains from those of normal flora, since no biochemical, microbiological or animal tests were available for their differentiation. The 12 serogroups originally recognized by the World Health Organization as EPEC or the classical EPEC were; O26, O55¸ O86, O111, O114, O119,

O125, O126, O b127, O128, O142 and O158 (Hernandes *et al*., 2009). Current classification of EPEC however, is based on the presence of specific virulence genes, which the use of molecular techniques has shown to be present in serogroup/serotypes other than classical ones as well (Ochoa *et al*., 2008). The distinctive histopathology induced by this group of *E. coli* is termed attaching and effacing (A/E) lesions and is caused by the intimate attachment of bacteria to the intestinal epithelial cells and effacement of enterocyte microvilli. Formation of the micro ulcers and exfoliation of the cells at the site of EPEC attachment was first described in experimentally infected pigs and subsequently in biopsies from infected infants (Chen & Frankel. 2005). The initial step includes adherence to the host cell. After attachment, a type III secretion system would be used to inject virulence factors in the host cell. Finally, an intimate bacterial attachment and pedestal formation is observed. (Jia & Alfredo. 2015). Diarrheal cases caused by EPEC varied from subclinical to fatal infections. In addition, EPEC may lead to severe malabsorption of nutrients, which would progress to nutritional aggravation and persistence of diarrhea(Arenas-Hernandez *et al*., 2012).

2.1.3.2 Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) is an important cause of childhood diarrhea with fatal consequences for children under 5 years of age (Croxen and Finlay, 2010). ETEC is pathovars the most common pathogen causing traveler's diarrhea (TD) worldwide, being responsible for up to 30 to 60% of all TD cases (82). ETECmediated diarrhea has been described by the secretion of characteristic toxins: the heat-stable enterotoxins (STs), the heat-labile enterotoxin (LT) or a combination of these (Croxen and Finlay, 2010) . Other ETEC strains produce heat-stable enterotoxins (STs) in addition to or instead of LT. They are classified into two distinct groups (STa and STb) or STI, and genes for ST are found on plasmids, although some have been found in transposons (Gillespie and Hawkey, 2006).

STs have a similar but distinct mode of action to that of LT. STa activates guanylate cyclase activity, causing an increase in cyclic guanosine monophosphate, which results in increased fluid secretion (Mims *et al*., 2008).

2.1.3.3 Enteroaggregative *E. coli* (EAEC)

Enteroaggregative Escherichia coli (EAEC) has been associated with persistent diarrhea in both children and adults. The EAEC is characterized by its aggregative adherence fimbriae (AAFs) that enable the bacteria to adhere to the intestinal epithelium and form a persistent biofilm. The EAEC is described as a very heterogeneous group with respect to its virulence genes and their regulation (Hebbelstrup Jensen *et al*., 2018). The main features of EAEC pathogenesis include attachment of bacteria to the intestinal mucosa, production of various toxins and cytotoxins, and stimulation of mucosal inflammation (Estrada-Garcia &Navarro Garcia. 2912: Navarro-Garcia & Elias.2011). These include toxins such as Pet, Sat, SigA, SepA, and Pic, which can be involved in loosening of cellular tight junctions, mucosal damage, and hypersecretion, and promotion of biofilm form (Navarro-Garcia *et al*., 2010; Al-Hasani *et al*., 2000; Coron *et al*., 2009)

2.1.3.4 Enterohaemorrhagic *E. coli* (EHEC)

Shigatoxigenic along with verotoxigenic *E. coli* (STEC), (VTEC) are strains of *E. coli* that are known to produce Shiga toxin and Shiga-like toxin (verotoxin) respectively. The strains that cause ailments in humans are commonly known as enterohemorrhagic *E. coli* (EHEC). The terms mentioned above are often used interchangeably. EHEC serotype O157:H7 is a human pathogen found to be responsible for bloody diarrhea outbreaks and hemolytic uremic syndrome (HUS) worldwide. (Marder *et al*., 2018) The infections caused

by *E. coli* O157:H7 range from asymptomatic to severe. Few individuals can develop potentially fatal complications like hemolytic uremic syndrome (HUS). Humans are known to acquire *E. coli* O157:H7 in multiple ways, for example, contaminated food and water and direct contact with infected animals and humans. Consumption of contaminated food like ground beef, dairy products, and fresh produce is responsible for the majority of the outbreaks (Beauvais *et al*., 2018).

2.1.3.5 Enteroinvasive *E. coli* (EIEC)

Enteroinvasive *E. coli* (EIEC) EIEC are etiological agents of bacillary dysentery in humans, particularly in low-income countries (Croxen *et al*., 2013; Gomes *et al*., 2016).It causes keratoconjunctivitis in experimental guinea pigsand invades human colon cells, causing an infection similar to that caused by *Shigella* sp. The complex process in colonization and EIEC survival in the gastrointestinal barrier depends on the presence of a large plasmid of about 220 kb (*pinv*), very similar to that found in*shigella*. In this process, multiple bacterial genes are involved, both chromosomal and plasmidial. Bacteria with-out the virulence plasmid do not cause keratoconjunctivitisin guinea pigs, being considered nonvirulent (Gibotti *et al*., 2004).

Enteroinvasive *E. Coli* and Shigella spp. Share several phenotypic and genotypic characteristics, often making the discrimination between the two genera challenging especially in case of shared serogroups (Pavlovic *et al*., 2011; van den Beld and Reubsaet, 2012). As a matter of fact, both EIEC and Shigella spend much of their life cycle within the eukaryotic cells, possessing the ability to use nutrients coming from the host environment. Similarly to Shigella, most EIEC strains are unable to decarboxylate lysine, lack the ability

to ferment lactose, and are generally nonmotile, with the exception of strains belonging to a few serogroups (Tozzoli and Scheutz, 2014).

2.1.3.6 Diffusely Adherent *E. Coli* (DAEC)

Diffusely adherent *Escherichia coli* have been considered a diarrheagenic group of *E. Coli* (DEC). They are characterized by the diffuse adherence pattern on cultured epithelial cells hela or hep-2 (Mansan-Almeida *et al.,* 2013). It has been shown that the relative risk of diarrhea associated with DAEC increases with age of children from 18 months to 5 years. The intestinal carriage of these strains has also been reported to be widespread in older children and adults. The consequences of this persistence are unknown, but several observations have suggested a potential role in the development of chronic inflammatory intestinal disease (Jafari *et al.,* 2012). Some of the investigations on DAEC have shown that the bacterium may be present in children without clinical symptoms, while other studies have shown a relationship between DAEC infection and the presence of symptoms. DAEC-related acute diarrhea is a relatively new topic that is of public health significance (Abbasi *et al*., 2017).

2.1.4 Extra intestinal pathogenic *Escherichia coli*

Compared with enteric *E. Coli*, expec has not yet been well categorized. According to the kind of disease they cause, the expec are differentiated into: Uropathogenic *E. Coli* (UPEC); Sepsis-associated *E. Coli* (SEPEC); and Neonatal meningitis associated *E .coli* (NMEC) (Johnson *et al.* 2003 ; Johnson & Russo 2005). They have also been characterised by phylogenetic group (predominantly groups B2 and D) and the presence of specific virulence factors: P-fimbriae, type 1 fimbriae, haemolysin, yersiniabactin, aerobactin, type II capsule,

serum resistance proteins and the virulence-associated pathogenicity island *malx* (Johnson *et al*. 2012). However, *E. Coli* is known to cause infections in other anatomical sites. It has been recently suggested that the use of an inclusive term, such as expec, rather than the restrictive terms such as UPEC, SEPEC and NMEC, would be useful in reflecting their broad infectious capacity (Johnson *et al.* 2003; Johnson & Russo 2002).

2.1.5 *Escherichia coli* as an Uropathogen

UPEC is the primary cause of community-acquired utis (Foxman, 2014; Flores-Mireles *et al*., 2015). *E. Coli* strains, which are capable of colonizing within the urinary tract and making themselves safe from the host immune system, become uropathogenic *E. Coli*. UPEC is responsible for>80% of UTI (Wurpel *et al*., 2013). Infections of the urinary tract are very common and about 10% of people (Farajnia *et al.,* 2009), and half of all women (at least once) get infected throughout their lives (Simmering *et al.,* 2017). Infections can occur in both upper and lower urinary tracts. Lower urinary tract infection is known as cystitis, and is called pyelonephritis in the case of upper urinary tract infection. (Müller *et al.,* 2009). However, UTI in addition to UPEC can be caused by *Klebsiella pneumonia* (about 7%), *Proteus mirabilis* (about 5%), and *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Streptococcus bovis*, and the fungus *Candida albicans* (Palou *et al.,* 2013; Hof, 2017).

2.1.5.1 Virulence Factors of Uropathogenic *E. Coli*

UPEC strains encode a number of virulence factors, which allow the microorganism colonize the urinary tract and persist in face of highly effective host defense. UPEC isolates exhibit a high degree of genetic variety due to the possession of specialised virulence genes located on cellular genetic elements called pathogenicity islands (Wiles *et al*., 2008). Virulence factors of E. Coli that have been potentially implicated as important to establish utis can be divided into two groups: (i) virulence factors associated with the surface of bacterial cell and (ii) virulence factors, which are secreted and exported to the site of action (Embody *et al*., 2003).

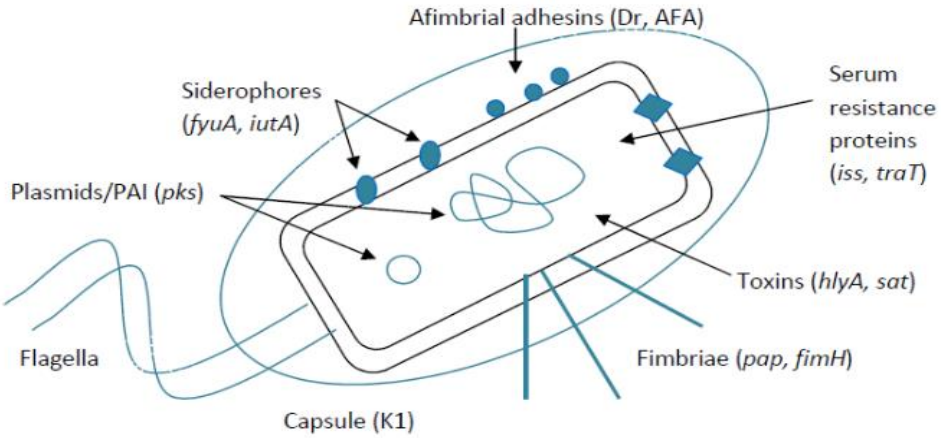


Figure (2.1): Genetic Elements and Virulence Factors Possessed by Extraintestinal Pathogenic *Escherichia coli*

2.1.5.1.1Adhesins

Each microorganism either pathogen or non-pathogen needs to be adhered for colonization. Indeed, colonization of pathogenic microorganisms results in pathogenesis within human body's host. For this reason, UPEC has a range of superficial proteins and adhesins . However the hair-like structured are invaluable virulence factors which enable UPEC pathotypes to have successful attachment, colonization, biofilm formation and virulence

(Jahandeh *et al.*, 2015; Donnenberg, 2013). The presentation of adhesive molecules (adhesins) by UPEC is the most important determinant of pathogenicity. UPEC adhesins can contribute to virulence in different ways: (i) directly triggering host and bacterial cell signaling pathways, (ii) facilitating the delivery of other bacterial products to host tissues, and (iii) promoting bacterial invasion (Mulvey *et al*.,2002).

1-Type 1 Fimbriae

Type 1 fimbriae are important virulence factors and have a peripheral arrangement upon the microorganisms' surfaces with a number of 1–5 hundred. Type 1 fimbriae with up to 10 nm width and up to 2 μm length are able to perform haemagglutination. The fimbriae of type 1 are encoded by the highly preserved geneoperon consistingof nine genes of fimBEAICDFGH. The main adhesive is the *FimH* protein that is located at the top of Type 1 fimbria. *FimG*, *FimF* and *FimA* protein molecules are, respectively, situated under the *FimH* molecule. *FimC* and *FimD* play their roles as chaperone and usher proteins, respectively. The recombinase enzymes of *FimB* and *FimE* activate two-way switching molecules for turning on and/or turning off the cluster gene expression. The activities of *FimB* and *FimE* are directly associated with environmental factors (Matuszewski *et al*., 2016; Kot, 2017) . Type 1 pili are highly conserved and extremely common among both UPEC and commensal isolates and have come to be considered one of the most important virulence factors involved in the establishment of a UTI. (Bahrani-Mougeot *et al*., 2002). *FimH* contains a binding pocket that recognizes mannose-containing host glycoprotein receptors (Hung *et al*., 2002). The integral membrane glycoprotein uroplakin 1a, which is abundantly expressed on the apical surface of the bladder, appears to be a key receptor for the *FimH* adhesin, although *FimH* can also bind many other host proteins(Eto *et al*., 2007). In particular, our laboratory recently showed that α3β1 integrin subunits expressed by many host cell types,

including bladder epithelial cells, are bound by *FimH* and mediate uptake of type 1 piliated *E. coli.*

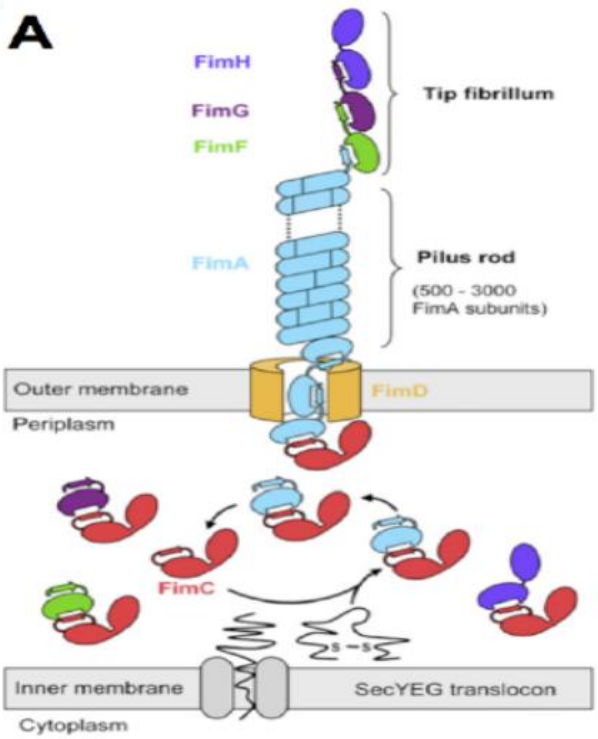


Figure (2.2) :Schematic representation of the type 1 pilus. (Puorger *et al.*, 2008).

2-P-Fimbriae

UPEC are endowed with a unique type of pili encoded by the *pap* genes that are known as pyelonephritis-associated pili or P fimbriae. P fimbriae are required for mannose-resistant agglutination of human erythrocytes and PapG is the fimbrial-tip adhesin that renders specificity to P fimbriae-mediated adherence. (Subashchandrabose & Mobley , 2015). Although P fimbriae are associated with adherence to renal cells, their contribution to the virulence of UPEC remains equivocal (Lane &Mobley, 2007). P fimbriae are required for attachment of UPEC to Bowman's capsule in a live animal. P fimbriae mediated adhesion

minimizes the impact of shear stress induced by the flow of primary filtrate within Bowman's space (Melican *et al*., 2011).

P fimbriae have been shown to enhance early colonization of the tubular epithelium, while the type 1 fimbriae mediate colonization of the center of the tubule via a mechanism that involves interbacterial binding and biofilm formation. The heterogeneous bacterial community within the tubule subsequently affects renal filtration leading to total obstruction of the nephron. The obstruction contributes to the full pathophysiology of pyelonephritis (Melican *et al.*, 2011).

3- S Fimbriae

S fimbriae are associated with those ExPEC strains causing neonatal meningitis and UTI. Most of the ExPEC isolates causing cystitis are found to express S fimbriae (Mabbett *et al.* 2009). Morphologically, S fimbriae are similar to type 1 or P fimbriae of *E. coli*. Epithelial cells of the proximal and distal tubules, collecting ducts, and glomerulus, renal interstitium, and renal vascular endothelium are known to be the binding site for S fimbri.

4-F1C-Fimbriae

F1C is a virulence factor responsible for urinary tract infections, which is encoded by an operon of seven genes, i.e., *focAICDFGH*, where FocA is the major subunit and FocH is the tip adhesin . F1C receptors are present in bladder endothelium and muscular layer. They cannot bind to the epithelium. They bind to glomeruli, distal tubules, collecting ducts, and vascular endothelial cells. Studies show that F1C fimbriae and pyelonephritis are correlated though there is a little difference in the prevalence of type 1 fimbriae in UTI strains and normal fecal isolates. Prevalence of F1C fimbriae in normal fecal isolates is 10% which is 16% in UTI strains (Spurbeck & Mobley, 2013).

5-F9-Fimbriae

The F9 fimbriae encoded by *f9* gene operon including *c1931–c1936* are detectable in 78% of UPEC populations. The C1931 protein is the major subunit identified in F9 fimbriae. The genetic and structural characteristics of F9 fimbriae are very close to Type 1, F1C and S fimbriae. Gal-beta-(1-3)-Glc-NAc and lacto-Ntetraose glycans are recognized as the main F9 fimbriae receptors (Wurpel *et al.*, 2014).

2.1.5.1.2 Toxins Produced by Uropathogenic *E. coli*

Most hemolytic UPEC strains secrete a heat-labile cytolytic protein toxin known as alpha hemolysin (Kucheria *et al.*, 2005), which is encoded by a polycistronic operon, consisting of four genes arranged in the order of hly-CADB (Burgos & Benton, 2010). The product of hlyC is important in the activation of the hemolytic toxin, which is the product of the *hlyA* gene. The gene products of *hlyB* and *hlyD* together with TolC are involved in secretion of the hemolysin through the bacterial cell wall (Holland *et al.*, 2005). In addition to its cytolytic activity, HlyA is also involved in the communication between host and pathogen (Dhakal & Mulvey, 2012). While most UPEC strains carry one copy of the hemolysin operon, pyelonephritogenic strains harbor two copies of the *hly* operon and both loci are required for the full virulence (Subashchandrabose & Mobley , 2015). However, several in vitro and in vivo studies showed that this protein interferes with polymorphonuclear phagocytosis and evokes apoptotic death of bladder epithelial cells and may lead to bladder cell exfoliation and to enhanced bacterial access to underlying tissue [Mills *et al* ., 2000].

2.1.5.1.3 Iron Acquisition Systems for Uropathogenic *E. coli*

Bacteria and the host compete for available iron, which is needed for oxygen transport and storage, DNA synthesis, electron transport, and metabolism of peroxides .Pathogenic bacteria, including UPEC, have devised ways of accessing iron by producing siderophore mediated iron transport systems. UPEC exhibit multiple mechanisms for extracting iron from the host, mainly siderophoresiderophore receptor systems, but also heme uptake (Garcia *et al.,* 2011). siderophores as high-affinity ferric iron chelators, including the catecholates enterobactin, salmochelin, the hydroxamate aerobactin, and yersiniabactin . Although both heme and siderophore iron acquisition contribute to the ability of UPEC to infect the urinary tract, evidence for distinct roles of specific systems exists. Heme uptake via ChuA was shown to play a role in bladder and kidney colonization , as well as in the formation of intracellular bacterial communities within superficial bladder epithelial cells . Mutants lacking the salmochelin receptor IroN, which were defective for urothelial cell invasion , were outcompeted by the wild-type strain in the bladders of infected mice, but kidney colonization was unaltered. Consequently, different iron acquisition systems appear to have differing contributions to bladder and kidney infection (Erin *et al.,* 2011)

2.1.5.1.4 Capsule

The capsule and the O antigen represent two cell-surface associated polysaccharides that contribute to UPEC virulence (Billips *et al.,* 2009). These extracellular polysaccharides protect UPEC against phagocytosis by immune cells such as neutrophils and inhibit complement-mediated killing. Bacterial lipopolysaccharide (LPS) plays a primary role in mediating UPEC resistance to the bactericidal activity of human serum . LPS consists of the highly conserved lipid Acore and repeating O antigen subunits that differ greatly between

strains based on the sugar residues and their linkage patterns within the repeating subunits (Sarkar *et al.,* 2014). capsule classification scheme has since been described which includes four capsular groups (1-4) composed of O-antigens and/or K antigens. Not all strains of *E. coli* express K antigens, but the majority of ExPEC isolates are capsulated, supporting the notion of capsules as an important virulence factor (Mahjoub-Messai *et a.l* 2011).

Although LPS of UPEC is important in activation of proinflammatory response in uncomplicated UTIs, it is not clear whether LPS plays a role in mediating a renal failure and acute allograft injury in patients with ascending UTIs. It has been demonstrated in an animal model that the acute renal failure due to LPS depends on the systemic response to LPS and does not depend on expression of functional LPS receptor (Bien *et al.,* 2012).

2.1.5.2 Fim H gene

FimH is located at the tip of type 1 fimbriae expressed by Gram-negative pathogens, including *E. coli, Salmonella enterica* and *Klebsiella pneumonia* (Stahlhut *et al.,* 2009). The type 1 pilus consists of five parts. They are, starting from the cellular body, *FimD* (inserted in the outer membrane), *FimA* (pilus rod), and *FimF*, *FimG*, and *FimH* (tip fibrillum). Recently, it has become clear that pilus type 1 exhibits several different phenotypes, due to allelic variation of the genes *FimA* and *FimH*, and that these phenotypes are differentially distributed among *E. coli* isolates (Iebba *et al.,* 2012). *FimH* occurs in two alternative conformations depending on interaction of its two structural domains, i.e., lectin domain (LD having mannose binding pocket) and pillin domain (PD connecting *FimH* with other pillin minor subunits like *FimG*). LD and PD are connected *via* a short linker chain and on their close interaction LD exists in twisted and compressed conformation that leads to an opening of mannose binding pocket. Hence, as a result, close interaction between LD and

PD leads to much lower affinity for mannose binding than in separated domain conformation. The binding of mannose to *FimH* is allosterically regulated that leads to shuffling between its two alternative conformations (Tchesnokova *et al.,* 2011). The mannose binding domain (LD) of the *FimH* is mainly responsible for adherence, which is the first step of infection (Zuberi *et al.,* 2017). In recent years, many SNPs have been discovered in different regulatory and structural genes of bacterial pathogens. Scientists have focused on the association between different SNPs of bacterial pathogens and its effect on the severity of pathogenicity of pathogens to cause disease (Barber *et al.,* 2013). The *FimH* phenotypic variants are mainly the result of SNPs in *FimH gene* (Molaie *et al.,*2016). The naturally occurring of these variants was investigated through different studies. These studies reported that the naturally occurring *FimH* variations significantly alter the tissue tropism of *E.coli*. Furthermore, the selection of genetic variations in the *FimH* gene as well as acquisition of virulence genes, results in the shifting from commensal to virulent phenotype (Tarchouna *et al.,* 2013).

2.2Urinary Tract Infections (UTIs)

Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting 150 million people each year worldwide (Flores-Mireles *et al*., 2015). UTIs are defined as the presence of bacteria in urine along with symptoms of infection. The infection is important clinically because of many organelles of the urinary tract may involve such as urethra, bladder, uterus, and kidney. All age groups are susceptible to this type of infection, but the frequency in women is higher than men (Tan & Chlebicki, 2016) . The reason may be attributed to many factors including short urethra, the absence of prostates secretion, pregnancy and easy contamination of the urinary tract with fecal flora. Additionally, during

pregnancy, the physiological increase in plasma volume decreases the concentration of urine up to 70%. As a result, pregnant women develop glucosuria, which encourages bacterial growth in the urine (Mohammad *et al.,* 2018)

2.2.1 Classification of Urinary Tract Infections

The infection is named based on the site of infection. The infection of urethra and ureter are referred to as urethritis and ureteritis respectively whereas cystitis and phylonephritis corresponds to bladder and kidney infections.(Vasudevan, 2014) Cystitis is a common type of infection whereas the infection associated with the renal damage is an issue of serious concern. Therefore the infection of bladder and urethra are referred as the infection of the lower urinary tract whereas the kidney and ureter infection is an indication of upper tract infection. Generally UTIs are classified based on the factors that trigger the infection and the nature of occurrence, uncomplicated or complicated (based on the factor that triggers the infection) and Primary or recurrent (depending on the nature of occurrence) uncomplicated UTIs occur without a clear causative factor, while complicated UTIs occur in patients with immunosuppression, diabetes mellitus, an anatomically or functionally abnormal urinary tract, or patients at the extremes of age (Nitzan *et al.*, 2015, Arshad and Seed, 2015) Approximately 20–30% of women with acute cystitis go on to develop recurrent UTI (rUTI) (Foxman , 2014).

2.2.2Epidemiology of Urinary Tract Infections

All individuals are susceptible to UTIs; however the prevalence of infection differs with age, sex and certain predisposing factors. Urinary tract infections are the most frequent bacterial infection in women (Dielubanza, & Schaeffer, 2011). According to the National Health and Nutritional Examination Surveys (NHANESIII), the incidence is 53,067 per 100,000 adult women per year (Clement, 2017). They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives. Recurrences are common, with nearly half of people getting a second infection within a year (Stapleton *et al.,* 2012). elderly men are at increased risk of developing UTI due to factors such as kidney stones or prostate problems. Any abnormxality of the urinary tract that interferes with the flow of urine set the stage for increased risk of complicated UTI (John *et al.*,2016). Rates of bacteriuria increases with age from two to seven percent in women of child bearing age to as high as 50% in elderly women . Among the most common infectious diseases, urinary tract infections (UTIs) are commonly encountered diseases by clinicians in developing countries. They are important complications of diabetes, renal disease, renal transplantation and structural and neurologic abnormalities that interfere with urine flow and a source of bacteremia in these patients (Naber *et al*., 2008).

2.2.3 Etiology of Urinary Tract Infections

Urine is generally considered to be sterile and is believed to be germ free. Any source of possible infection occurs through urethra which initiates the incidence of the infection. The predominant pathogen responsible for UTI is *E. coli* which constitutes up to 80-85% and is followed by *Staphylococcus saprophyticus* which accounts to 5-10% (Nicolle *et al.,* 2005).

The occurrence of the infection due to viral or fungal agents is a rare phenomenon. In addition to the above mentioned bacterial species, *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter* are associated with UTI. Pathogens like *E. coli* and *S. saprophyticus* are associated with population acquired acute uncomplicated infection whereas *Klebsiella*, *Enterococcus*, *Proteus Species*, *Enterobacter*, *Bacillus*, *Shigella* are known to confer uncomplicated cystitis and phylonephritis that are sporadic (Vasudevan *et al*, 2014). Gram positive cocci play a lesser role in urinary tract infections. However, *Staphylococcuss aprophyticus* novobiocin resistant, coagulase-negative specie accounts for 10 to 15% of acute symptomatic urinary tract infections in young females while Enterococci occasionally cause acute uncomplicated cystitis in women (Nicolle , 2008).

2.2.4 Symptoms of urinary tract infection

The clinical symptoms of UTI are dependent on age, stage of infection, host response and type of bacteria causing the infection. Young infants often present with unspecific symptoms like fever, irritability, vomiting, lethargy, or poor feeding. As the children grow older, and in adults, more explicit symptoms like pain upon voiding and increased frequency are present in lower UTI. Upper UTI on the other hand is associated with flank pain and fever. Recently, a meta-analysis evaluated the diagnostic accuracy of UTI signs and symptoms in children. For older children, abdominal pain and fever, back pain, new onset of urinary incontinence and dysuria, were most reliable (Shaikh *et al*. 2007). In a proposed algorithm for evaluating women with symptoms of UTI, a combination of several clinical symptoms reached a probability of UTI of 90% without laboratory testing .

2.2.5 Pathogenesis of urinary tract infection

In most cases the uropathogens originate in the rectal flora and colonization of the perineum and periurethral area precedes the development of infection. Colonization is inhibited by the normal bacterial flora, including Staphylococcus epidermidis, lactobacilli and corynebacteria, which are disrupted by antibiotics and postmenopausal oestrogen deficiency (Sheerin , 2015) Pathogen adherence is a key event in initiating each step in UTI pathogenesis (Flores-Mireles *et al*.,2015). Appendages in the urethra such as flagella and pili allows colonization of the periurethral mucosa. In the bladder, uropathogens survive by binding directly to the bladder epithelium, which is composed of umbrella cells, intermediate cells, and basal cells. The consequences of complex host-pathogen interactions ultimately (Clement, 2017). Determine whether uropathogens are successful in colonization or elimination Multiple bacterial adhesins recognize receptors on the bladder epithelium (also known as the uroepithelium) and mediate colonization. Uropathogens such as UPEC survive by invading the bladder epithelium, producing toxins and proteases to release nutrients from the host cells, and synthesizing siderophores to obtain iron. By multiplying and overcoming host immune surveillance, the uropathogens can subsequently ascend to the kidneys, again attaching via adhesins or pili to colonize the renal epithelium and then producing tissue-damaging toxins Consequently, the uropathogens are able to cross the tubular epithelial barrier to access the blood stream, initiating bacteraemia (FloresMireles *et al*, 2015).

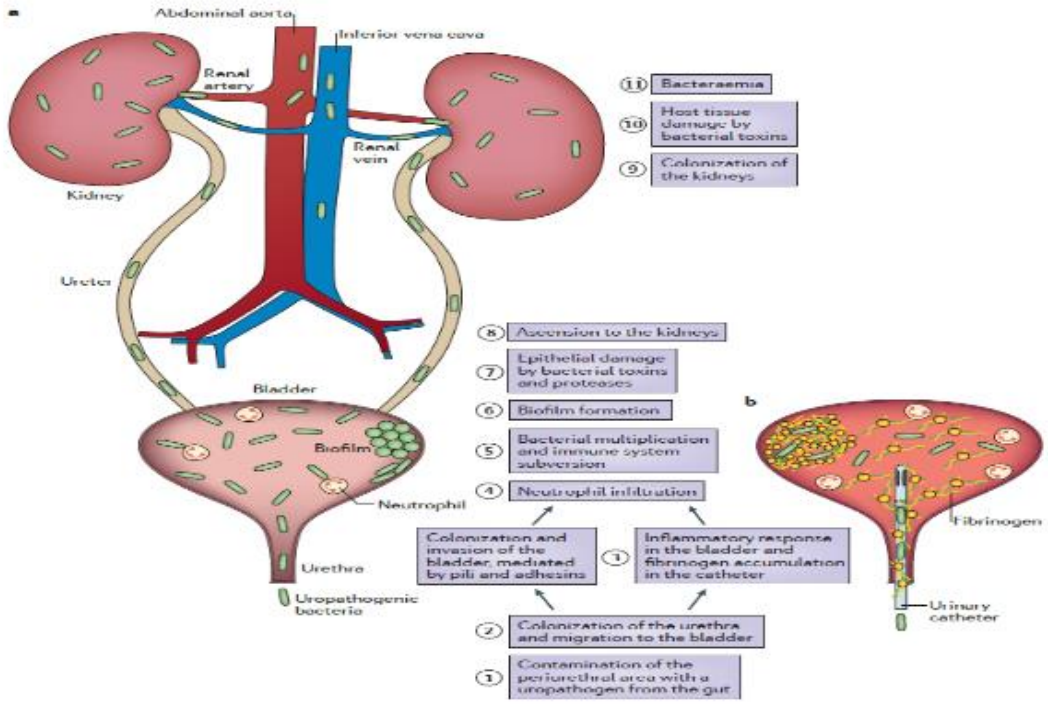


Figure (2.3): Steps of pathogenesis for UPEC strain in UTIs. a. Uncomplicated UTIs b. Complicated UTIs (Flores-Mireles *et al*., 2015)

2.2.6 Urinary Tract Infection Antibiotic Susceptibility and Resistance

The first step in treating UTIs is to classify the type of infection, such as acute uncomplicated cystitis or pyelonephritis, acute complicated cystitis or pyelonephritis, asymptomatic bacteriuria (ASB), or prostatitis (Coyle 2017) The efficacy of antibiotic treatment depends on the identification and antimicrobial resistance pattern of uropathogens responsible for UTI (Bartoletti *et al.,* 2016). The production of various virulent factors and developing drug resistance in UPEC. Antibiotic resistance may provide a substantial advantage to the survival of the pathogen. The drug resistance among UPEC is on rise therefore the selection of appropriate antibiotics (after antibiotic susceptibility testing) is must for proper treatment of patients and to avoid emergence of drug resistance (Foxman, 2010).

3. Materials and Methods

3.1. Materials

3.1.1. Instruments And Equipment's

The instruments and equipment's used in this study were listed in table (3-1).

Table (3-1): Instrument & Equipment's with their remarks:

No.	Equipment's / Instruments	Company	Country
1	Beakers	AMSCO	Germany
2	Centrifuge	Hettich EBA20	Japan
3	Cylinders	AMSCO	Germany
4	Deep freezer	GFL	Germany
5	Eppendorf tubes	Bioneer	Korea
6	Digital camera	Sanyo	Japan
7	Exispin centrifuge	Bioneer	Korea
8	Flasks	AMSCO	Germany
9	Incubator	Memmert	Germany
10	Micropipette injector	Eppendrof	Germany
11	PCR tubes	Bioneer	Korea
12	Plain tubes	AF co -Dispo	Jordan
13	Vortex	Stuart scientific	UK
14	Water bath	Kottermann	Germany
15	Gel electrophoresis system	Consort	Belgium
16	Microcentrifuge	Lab Tech	Korea
17	Tips	Sterellin Ltd	UK

18	Water distillatory	Lab Tech	Korea
19	Thermocycler PCR	BioRad	USA
20	Al-Muthanna gel documentation system		Iraq

3.1.2 Chemicals And Biological Material

The chemicals and biological materials used in this study are listed in table (3-2).

Table (3-2): Chemicals and biological materials with their remarks

NO	Chemicals &biological materials	Companye4	Country
1	Agarose	Promega	USA
2	PCR water	Bioneer	Korea
3	Restriction enzyme Buffer	Biolab	USA
4	Absolute ethanol	Pharmacia	England
5	Ethidium bromide	BDH	UK
6	Loading dye	Bioneer	Korea
7	Restriction enzyme	Biolab	USA
8	Free nuclease water	Biolab	USA
9	Gram stain reagents	Syrbio	Syria
10	Molecular ladder 100bp	Promega	USA

3.1.3 Polymerase Chain Reaction Kits

In table (3-3) chemical materials that were used in PCR-RFLP work to this study with their companies and countries of origin were listed.

Table (3-3): PCR kits with their remarks

No.	Kit	Company	Country
1	Genomic DNA extraction kit	**favorgen**	**Korea**
2	**PCR PreMix**	**Biolabs**	**England**
3	DNA Ladder	Promega	USA

3.1.4. Primers

The gene primers which were used in REFLP-PCR for detection of C640T and T591A SNPs was provided from Biocorp company , as following table (Molaie *et al.*, 2016).

Table (3-4): Primer with their sequence used in this study (Hojati *et al.*, 2015).

Primer		Sequence	PCR product size
C640T	**F**	GTG CCA ATT CCTCTT ACC GTT	164
	R	TGG AAT AAT CGTACC GTT GCG	
T591A	**F**	CCG TTA CTC TGCCGG ACT ACA C	76
	R	CCC AGG TTT TGGCTT TTC GCA CAA T	

3.1.5. Media

The following ready to use media are listed in Table (3.6).

Table (3.6): Ready to use media

NO	Medium	Company	Country
1	Brain heart infusion broth	Oxoid	England
2	Eosin methylene blue agar	Mast group	England
3	MacConkey agar	Oxoid	England
4	Chrom agar	Pronadisa	Spain

These media were prepared as recommended by manufacturing company.

3.2. Methods

3.2.1. Collection Of Specimens:

The current study was conducted on 150 patients (120 females, 30 males) were seen Al-Hussein Teaching Hospital from January 2019 to March 2019. The patients were diagnosed clinically by physician as having UTI infection . Urine samples were taken from both male and female using sterile containers. The samples were directly activation on Brain heart infusion broth and then streak on MacConkey agar, Eosin methylene blue agar , Chrom agar and incubated at 37° C for 24 hrs .

3.2.2 Preparation the Culture Media and Sterilization

3.2.2.1 Preparation the Culture Media

All culture media were prepared according to their manufacturing company instructions. The prepared media were then distributed into sterile tubes or Petri dishes then kept at 4°C until they used, listed in Table (3.2).

3.2.2.2 Sterilization

The prepared media were sterilized by autoclaving at 121oC/1 pound for 15min then distributed into sterile tubes or Petri dishes then kept at 4°C until they used. All Eppendorf and tips that used in DNA extraction and PCR amplification were sterilized by autoclaving at 121oC/1 pound for 15min and then left to dry in closed sterile place for 24 hours to ensure evaporation of water from the walls. Then exposed to UV-light for 20min using UV sterilization cabinet.

Instruments used in PCR have been sterilized before applying the process using Decontamination nucleases solution and UV-light for 20min inside the UV sterilization cabinet where the work is done.

3.2.3 Isolation of Uropathogenic *E. coli*

After incubation, a loopfull from Brain heart infusion broth was cultured on MacConkey agar, a loopfull of lactose fermented colonies from MacConkey agar was sub cultured on EMB agar and chrom agar for a further identification and incubated at 37°C for 24 hour.

3.2.4 Identification of Uropathogenic *E. coli*

3.2.4.1. Morphological Characteristics

Colonies able to grow on selective media were further identified by studying their morphological characteristics beginning by staining with Gram stain and studying their characteristics under a microscope (size, colour, shape, arrangement, spore formation).

3.2.4.2 Vitek2® System test:

All isolates of *E. coli* were identified by using Vitek2® system according to steps of manufacture Company

3.2.5 Maintenance of Uropathogenic *E. coli* isolates

Maintenance of bacterial isolates was performed according to Maniatis *et al*. (1982)

1. Short-term storage

Bacterial isolates were maintained for few days on MacConkey agar lates that were wrapped tightly with parafilm, and stored at 4C°.

2. Medium-term storage

Bacterial isolates were maintained for few months by streaking on brainheart infusion agar slants in a glass universal with tightly closed screw cap containing 5-8 ml of the medium and stored at 4°C .

3.2.6 Molecular Methods

3.2.6.1 Solution Preparation

A- Tris - Borate - EDTA (TBE) buffer

TBE buffer (10X) this solution is prepared by mixing 100 µl of the supplied TBE-10x buffer with 900 µl of distilled water then placed in the microwave for two minutes to obtain homogeneous TBE (1X) buffer and stored at room temperature until used for agarose gel electrophoresis (Sambrook and Russell, 2001).

B- Agarose Gel Preparation

Agarose gel is prepared by melting 1 gm of agarose powder in 100 ml of TEB (1X) buffer dissolved in the microwave oven for intermittent intervals until reached to the boiling point, then the solution left at room temperature to cooling for 50oC. Ethidium

bromide (5µl of a 10mg/ml stock solution per 100 ml) added with mixing to the agarose gel to obtain 1% agarose gel electrophoreses poured out into the gel jar to prevent bubble formation, then cooled to 20oC. When agarose gel is poured, several wells were carefully made with a comb at one side of the gel about 5-10 mm away from the end of the gel, after final solidification. The comb is carefully removed; the jar was put in the electrophoresis tank to be used in electrophoreses process (Sambrook and Russell, 2001).

C- Primer Preparation

The lyophilized primers were spined using microcentrifuge prior to opening to prevent loss of pelleted oligonucleotide, The primers are prepared according to manufacturer instructions that include the volume of nuclease-free water to be added to the lyophilized primers to get the stock solution with a concentration of 100 pmol/ µl, for each primer.

Ten microliters of the stock solution of each primer were placed in a number of 1ml microcentrifuge tube to avoid contamination of The stock solution during the uses and also to be ready For each forward and reverse primer combination by added 90 µl of nuclease-free water, for preparing the mixed primer by added equal amount of each primer. Gently pipette the entire volume up and down 10 times to mix thoroughly to get a final concentration of 10 pmole/µl and kept at -20°C for storage

3.2.6.2 DNA Extraction and Purification

Genomic DNA from culture cell were extracted by using Favorprep Genomic DNA extraction kit (blood , culture cell) favorgen. Korea, and done according to company instruction as following steps:

Step 1-Sample Preparation

Transfer the appropriate number of bacterial cell (up to 1 x) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 minute.

Then discard the supernatant. 200 µl of FATG Buffer added and resuspend the pellet by vortex or pipetting. Incubate for 5 minutes at room temperature. Follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

Step 2 –Cell Lysis

Volume of 200µl of FABG Buffer was added to the sample and vortex for 5 seconds.

Incubate for 10 minutes at 70ºC or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70ºC water bath

Step 3 – Binding

Volume of 200µl ethanol was added (96~100%) to the sample and vortex for 10 seconds. (Pipetting if there is any precipitate.)

FABG Column placed to a 2ml collection tube. Transfer the sample mixture (including any precipitate) carefully to FABG Column. Centrifuge for 5 minute at full speed (14,000 rpm or 10,000 x g) and discard the 2ml collection tube. Place the FABG Column in a new 2ml Collection tube.

Step 4 – Washing

FABG Column was washed with 400µl W1 Buffer. Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.

The FABG Column was placed back in the 2ml Collection tube. Wash FABG Column with 600µl Wash Buffer (ethanol added). Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) and discard the flow-through. --Make sure that ethanol has been added into Wash Buffer when first open. The FABG Column was Placed back in the 2ml Collection tube. Centrifuge for an additional 3 min at full speed (14,000 rpm or 10,000 x g) to dry the column. --Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Step 5 – Elution

Place the dry FABG Column to a new 1.5ml microcentrifuge tube.

Add 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column. Stand FAGB Column for 3~5 min or until the buffer is absorbed by the membrane. Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely. Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) to elute the DNA . --Standard volume for elution is 100 µl. If sample has low number of cells, reduce the elution volume (30 µl - 50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl. The DNA sample was stored at 4ºC or -20ºC.

3.2.6.3 Preparation of PCR Master Mix

Amplification reaction was conducted in 0.2 ml tube of PCR Premix tube according to the corporation's instruction as summarized in table (3-7).

Table (3-7): PCR master mix reaction

Component	50 µl Reaction	Final Concentration
OneTaq Quick-Load 2X Master Mix with Standard Buffer	25 µl	1X
10 µM Forward Primer	1 µl	0.2 µM
10 µM Reverse Primer	1 µl	0.2 µM
Template DNA	variable	<1,000 ng
Nuclease-Free Water	to 50 µl	

The PCR tube vortexed until the lyophilized pellet dissolved, and then PCR tube was entered into PCR Sprint-Thermal-Cycler for thermocycling condition . After that, these PCR master mix components that are mentioned above placed in standard PCR PreMix Kit that containing all other components which are needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye). Then, all the PCR tubes. placed in PCR Thermocycler.

3.2.6.4 PCR Thermocycler Conditions

PCR thermocycler conditions were done by using convential PCR thermocycler system as the following table:

Table (3-8): PCR Thermocycler Conditions for C640T

PCR step	Temp.	Time
Initial Denaturation	94°C	30sec.
Denaturation	94°C	15-30sec.
Annealing	56°C	15-60 sec
Extention	68°C	1 min/kb
Final extension	68°C	5min
Hold	4-10°C	Forever

Table (3-9): PCR Thermocycler Conditions for T591A

PCR step	Temp.	Time
Initial Denaturation	94°C	30sec.
Denaturation	94°C	15-30sec.
Annealing	66° C	15-60 sec
Extention	68°C	1 min/kb
Final extension	68°C	5min
Hold	4-10° C	Forever

3.2.6.5 PCR Product Analysis

The PCR products was analyzed by agarose gel electrophoresis following steps:

1. Dissolving of 1% Agarose gel in 1X TBE and boiling in Microwave at 100 °C for 15 minutes, and, left to cool to 50°C.

2. Then 3µ of ethidium bromide stain were added into agarose gel solution.

3. Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 5µl of PCR product were added in to each comb well and 5ul of (100bp Ladder) in one well.

4. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt for 15 min and decrease to 50 volt for 40 min.

5. PCR products (164 bp) as specific for C640T gene and (76 bp) for T591A were visualized by using UV Transilluminator.

3.2.6.6 REFLP PCR Master Mix Preparation

Polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) master mix was prepared for detection gene mutation in urine samples of UTI patients by using restriction endonuclease (ScrFI) that digestion of the 164 bp PCR product of C640T FimH gene and restriction endonuclease (BstNI) that digestion of the 76 bp PCR product of T591A FimH gene this master mix done according to company instructions as following table (2-12):

Table (3-10): Mixture for genotype

REFLP-PCR Master mix	Volume
PCR product	10ul
Restriction enzyme buffer 10X	2ul
Restriction enzyme (10 unit)	1ul
Free nuclease water	7ul
Total volume	20ul

After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 2 minutes, then transferred into incubation at 37°C for 5-15minute . After that, REFLP-PCR product was analyzed by agarose gel electrophoresis methods that are mention in PCR product analysis.

4. Results and Discussion.

4.1. Subjects Demographic Characteristics

A total of 150 urine specimens were collected from patients suffering from urinary tract infections in Al-Hussein Teaching Hospital during the period from January 2019 to March 2019. The result show only 62 isolates (41.3%) were given typical morphological characteristics and biochemical tests related to *E. coli*, while the rest 88 isolates (58.6%) belonged to pathogenic bacteria from different genera.

Results of isolation in this study was agreed with result study Shahab *et al*., (2017) revealed that (35%) from infection causes by *E. coli*, and also agreed with Hussein *et al*., (2017) thier result showed that (50 %) of infection caused by *E. coli*, the high prevalence of this bacteria to being one of the normal bacteria present in the human gut and be infection.

Table (4.1): Distribution of patients according to age group intervals location of collection.

Age (year)		Samawa		Warkaa		Rumaitha		Total	
		Female	Male	Female	Male	Female	Male	N	%
8-23	N	17	2	1	0	2	0	22	35.48%
	%	77.27	9.09	4.55	0	9.09	0		
24-39	N	22	5	1	0	1	1	30	48.39%
	%	73.33	16.67	3.33	0	3.33	3.33		
40-45	N	5	4	1	0	0	0	10	16.13%
	%	50.00	40.00	10.00	0	0	0		
Total	N	44	11	3	0	3	1	62	100
	%	70.97	17.74	4.84	0	4.84	1.61		

from a total of 62 isolates that identified as UPEC, 50 isolates (80.65%) were isolated from female and 12 isolates (19.35%) were from male , and this result was agreement with a study done by Ojo and Anibijuwon (2010). They reported that regarding the demographic distribution, there were a high percent of urinary tract infection among the age group between 21-25 years and 26-30 years.

Also the lowest incidence was among the 40-55 years old age group (16.13%) whereas the highest incidence was among the 24-39 years old age group (48.395).

The high ratio of UPEC isolated from females were agreed with Neamati *et al*., (2015) they collected 150 urine samples from both genders. The most common isolated pathogen was UPEC (82.6%), from those 78% of the isolates were from females while 22% of them were from males, Also a study in a Al-Karkh Surgery Hospital in Baghdad city by Kareem and Rasheed, (2011) revealed that out of 311 urine samples that were collected from patients suffering from UTIs, (68.75%) of the isolates were identified to UPEC, 100 isolates were from females and 25 isolates were from males.

Urinary Tract Infection (UTIs) were more common in women because of their anatomy; the shorter urethra and the relative proximity of the urethra to the anus. Several other factors have also been shown to increase the risk of UTI in women: particularly sexual intercourse and the use of spermicide,(Scholes *et al*., 2000) which is thought to affect the vaginal microbial flora resulting in a reduction in lactobacilli allowing for an increased proportion of potentially pathogenic Gram negative bacteria, such as *E. coli* to colonize the genital tract (Walsh and Collyns, 2017). Incidence of infection in females increases directly with sexual activity and child-bearing. Most of the women will have a history of incidence of UTI in their lifetime and the risk of occurrence increases in postmenopausal women (Pulipati *et al*., 2017).

Urinary tract infection is less common in men than in women because the male urethra is long, making it is difficult for the bacteria to spread to the bladder (Sheerin, 2011). Risk factors for UTIs in young men may include sexual transmission by an infected female partner, anal intercourse and preputial obliteration or may be associated with a kidney stone (Johnson and Delavari, 2002). Urinary tract infection usually happens in older men who have an enlarged prostate or when a catheter was used to drain the bladder. An enlarged prostate can cause a urinary tract infection by preventing the urine from draining out of the bladder completely. A less common cause is urethral stricture, which is when the urethra becomes narrower because of scar tissue forming inside it.

4.2 Urine analysis

4.2.1 Specific Gravity

The specific gravity in all urine sample in these study were in the normal range in all location and age group.

The location has effect on specific gravity as shown in figure (4-1) the specific gravity 1.030 it was higher level in all study area 87.27,100.00,75.00 in Samawa , Warkaa , and Rumaitha , respectively , and note the specific gravity 1.025 gave high value in Samawa 9.09 while absent in Warkaa and Rumaitha , the specific gravity 1.020 gave high value in Rumaitha 25.00 and small percentage in Samawa 3.64 while absent in Warkaa.

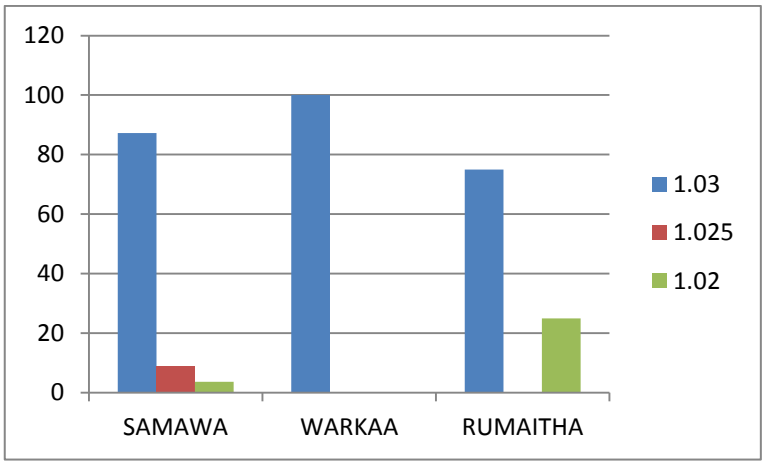


Figure (4.1): corelation of Locations with Sp. Gravity

The age has effect on specific gravity show in figure (4-2) and the specific gravity 1.030 has higher level in all level age 81.82, 90.00, 80.00 respectively while the specific gravity 1.025 and 1.020 give lower value.

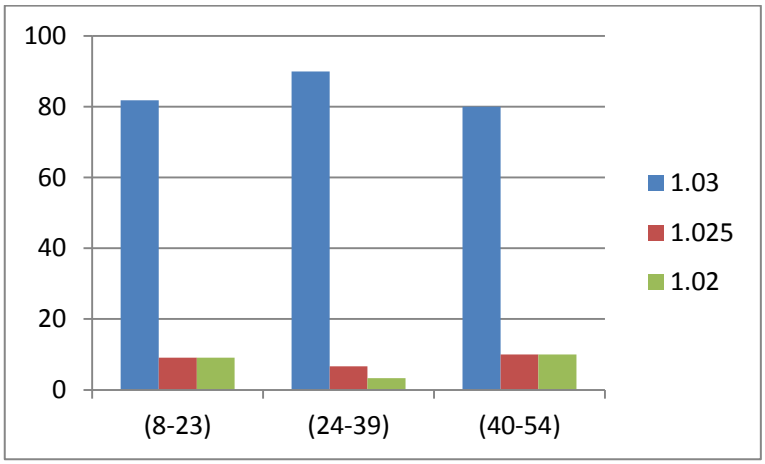


Figure (4.2): corelation of Age with Specific Gravity

4.2.2 pH

The pH of Urinary normally can range from 4.5 to 8 and slightly acidic (i.e., 5.5 to 6.5) because of metabolic exercise. Alkalinity may indicate a UTI with certain types of bacteria such as *Proteus mirabilis*, *Klebsiella* or *Pseudomonas* (Higgins, 2007). Most of the cases

in this study are acidic pH , this is probably due to the above reason and the *E. coli* is the most common of infection in all the cases of study.

The location has effect on PH as shown in figure (4.3) the acidic (PH) were recorded in all study area 94.55, 100.00, 75.00 in Samawa , Warkaa , and Rumaitha respectively, while the alkaline (PH) give high value in Rumaitha 25.00 while no appear in Warkaa and recorded 5.45 in Samawa.

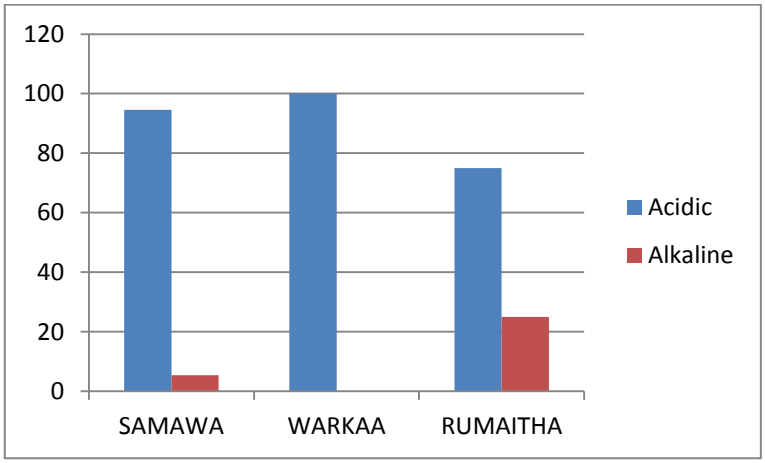


Figure (4.3) : corelation of Locations with pH.

The age has effect on PH show in figure (4-4) the acidic (PH) has higher level in all level age 90.91 , 93.33 , 90.00 respectively while the alkaline (PH) give lower value 9.09 , 6.67 , 10.00 respectively.

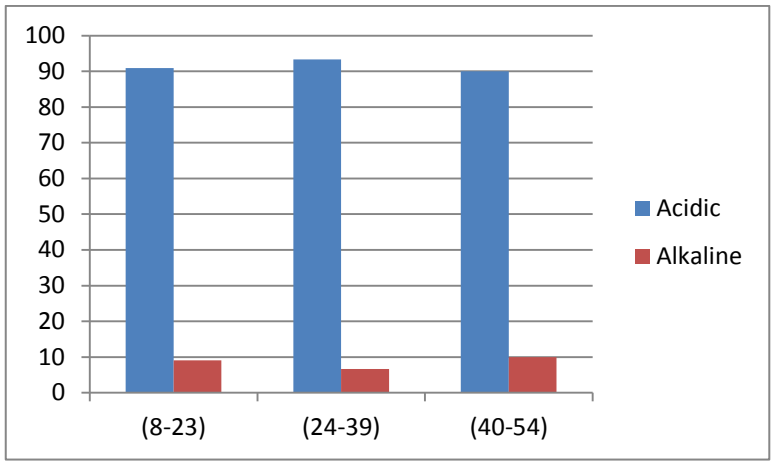


Figure (4.4): Relationship Between Age with pH

4.2.3 Albumin

Proteinuria is describe as excretion of albumin in urine about 300 mg per day . UTI is often associated with proteinuria . however, relationship between proteinuria and UTI remains incompletely understood . As many as 60%-80% of all urine specimens received for culture not contain etiological agent or contain only contaminants (Tanisha *et al*., 2017). The result show there is not found for the albumin in the urine of the patient in high percentage of sample (75.85%) and found in various amount in (24.24%) of patient.

The proteinuria might be caused by the great impact of *E. coli* bacteria on glomerular membrane which leads to increased glomerular membrane permeability for proteins, and the effect of other bacteria on renal tubules which result in decrease of renal tubular re-absorption (Dilista *et al*.,2010). proteinuria is not a sufficiently strong indicator of UTI as a single parameter but may have a good predictive power when combined with the other urinalysis parameters and clinical presentation in the diagnosis of UTI.

The albumin has effect on location as show in figure (4.5) the albumin has higher level in all study area 74.54,66.67,100.00 in Samawa , Warkaa , and Rumaitha , respectively.

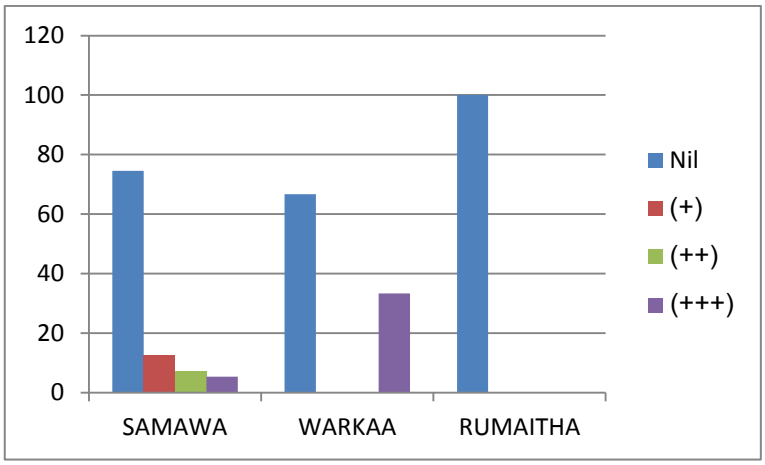


Figure (4.5): corelation of Locations with Albumin.

The results revealed their due age was effect on albumin as shown in figure (4.6) in our study we recorded several value at different age stage.

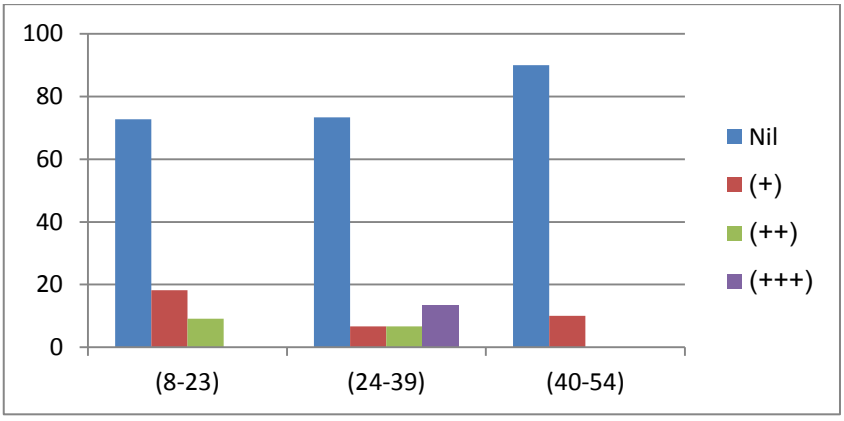


Figure (4.6): corelation of Age with Albumin

4.2.4 Pus cell

The study result showed the high number of pus cells were appear in the age group (8-23) followed by the age group (24-39) while low percint were appear age group (40-45).

The term “pyuria” means “pus in the urine” but, in common usage, the focus is not on the presence of pus but on the number of white blood cells (WBCs) or amount of leukocyte

esterase (LE) that exceeds a threshold and suggests a urinary tract infection (UTI). (Roberts 2016). Pyuria is an indication of microbial infections since pus cells are white blood cells that have succumbed in defense of the body against pathogens that invade it (Uju *et al*.,2014). The pus cell has effect on age as shown in figure (4.7) the higher level of pus cell recorded in (8-23) age about 13.5682 , in (24-39) age recorded 12.2667 and in(40-45) gave 9.9000.

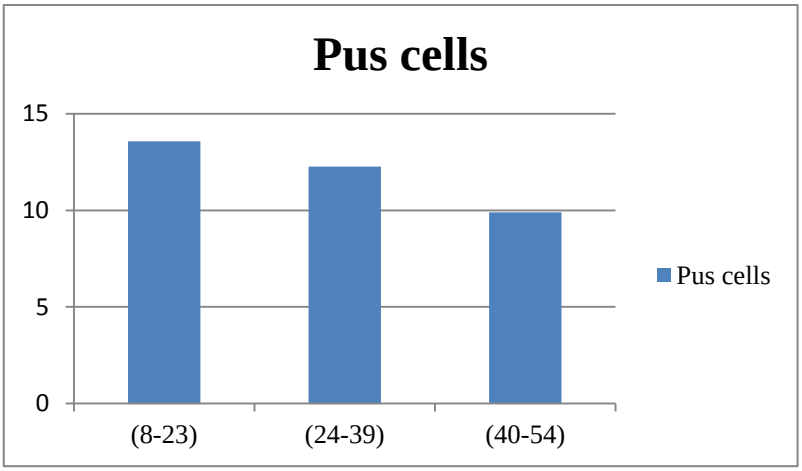
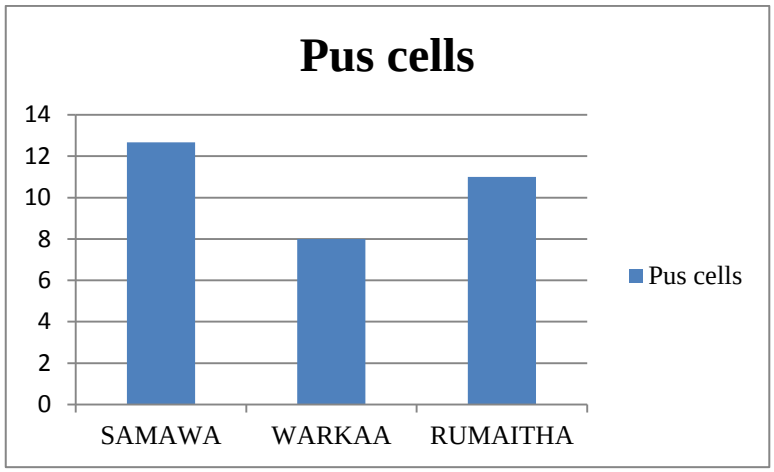


Figure (4.7): corelation of Age with Pus Cells.

The effect of location on pus cell was appear in figure (4.8) the pus cell were higher level in all study area they recorded 12.6727, 8.0000, 11.0000 in Samawa , Warkaa , and Rumaitha , respectively.



Figure(4.8): corelation of Locations with Pus Cells.

4.2.5 Ketone Bodies

The effect of location Ketone Bodies was show in figure (4.9).

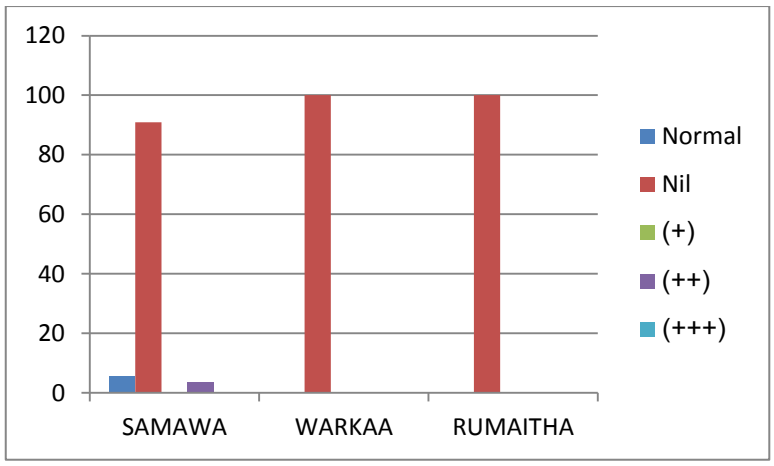


Figure (4.9): corelation of Locations with Ketone Bodies.

The effect of age on Ketone Bodies had been show in figure (4.10).

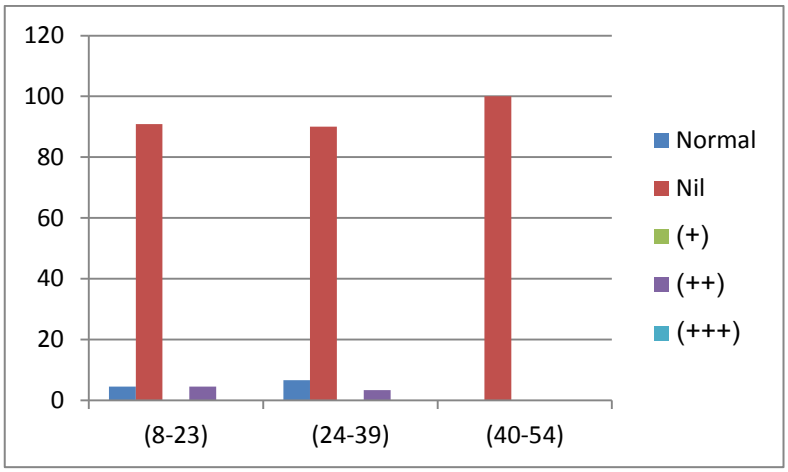


Figure (4.10): corelation of Age With Ketone Bodies.

4.2.6 Red Blood Cell

According to the American Urological Association, the presence of three or more red blood cells (RBCs) per high-powered field (HPF) in two of three urine samples is the generally accepted definition of hematuria (Jeff et al., 2005). Blood in the urine may also be indicative of a blood clotting problem or a side effect of anticoagulant drugs. Contamination of the urine with menstrual blood may also occur. In young male infants, haematuria may result from the formation of crystals in the urethra. hematuria may be caused by non-infective pathological conditions of the urinary tract or by renal mycobacterial infection, with or without associated pyuria (Whitfield, 2006). If the hematuria is associated with the lower urinary sickness, red blood cells usually maintain their normal look. However, if the disease process occurs higher in the urinary tract, the look is often dysmorphic. The R.B.Cs has effect on location as show in figure (4.11) the RBCs has different level in all study area 4.0364, 2.6667, 1.7500 in Samawa , Warkaa , and Rumaitha , respectively.

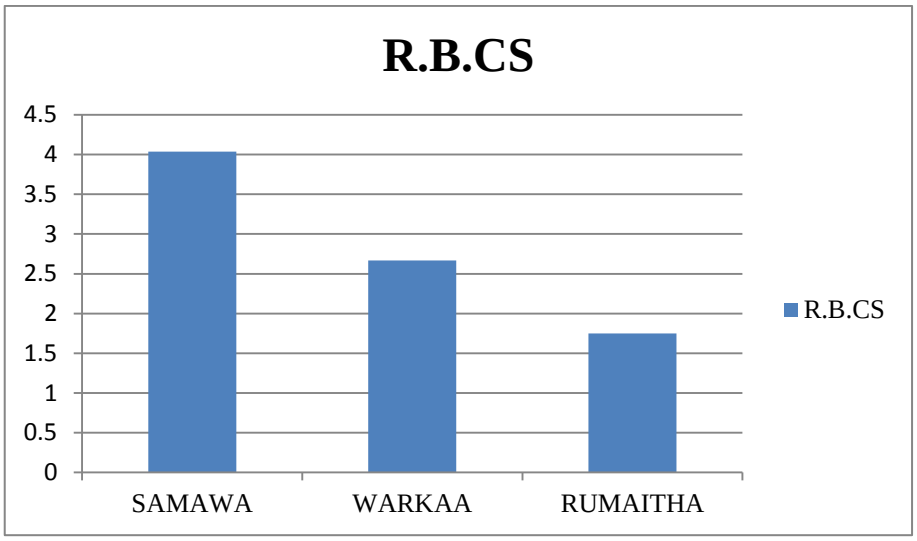


Figure (4.11) corelation of Locations with R.B.CS.

The RBCs has effect on age as show in figure (4.12) in our study we recorded higher value in (8-23) age stage and lower value in(40-45) age stage.

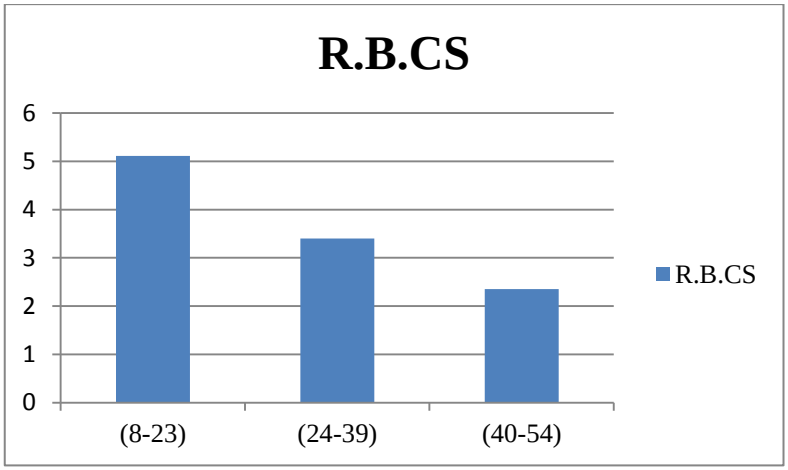


Figure (4.12): corelation of Age with R.B.CS.

4.2.7 Epithelial Cell

Epithelial cells are ranging from 12 µm to 20 µm in diameter. Proximal tubular cells are oval or egg-shaped, and tend to be larger than the cuboidal distal tubular cells, but because their size varies with urine osmolality, they cannot be reliably differentiated. A few tubular cells may be seen in a normal urine sample. More commonly, they indicate

tubular damage or inflammation from acute tubular necrosis (ATN) or interstitial nephritis (Taal et al., 2012). The epithelial cells has effect on location was show in figure (4.13) and the epithelial cells has different level in Samawa , Warkaa , and Rumaitha.

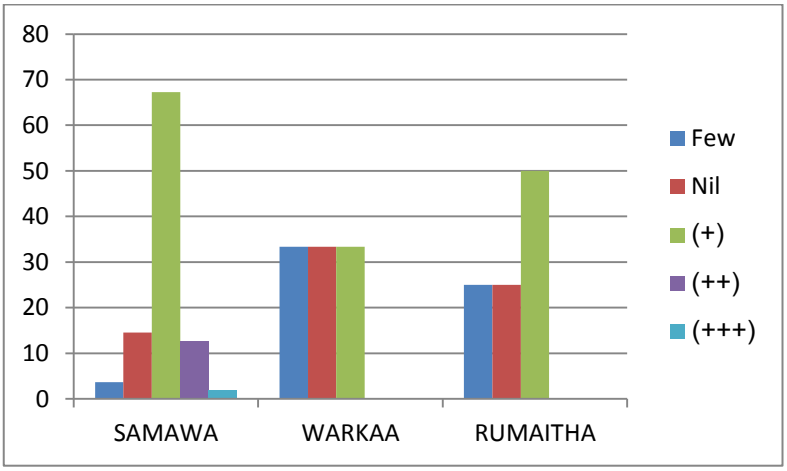


Figure (4.13): corelation of Locations with Epithelia Cell

The epithelia Cell has also effect on age as show in figure (4.14).

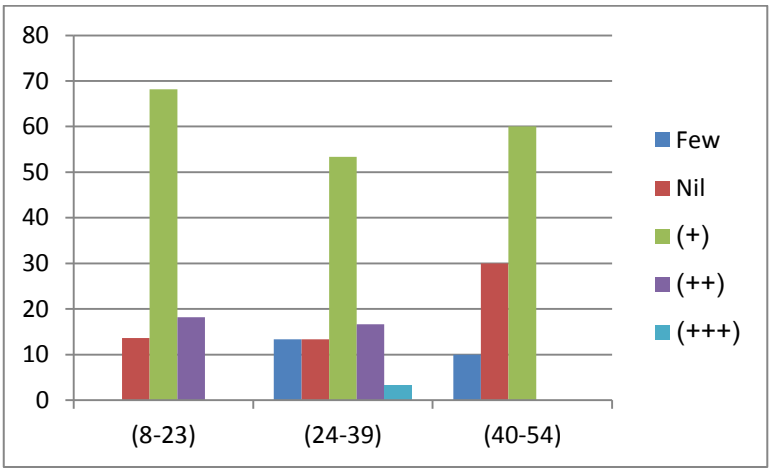


Figure (4.14): corelation of Age with Epithelia Cell.

4.3 Isolation and identification of Uropathogenic *E. coli*

Primary identification of *E. coli* isolates was based on cultural, morphological characteristics, microscopically diagnosis , while the confirmatory identification test was based on selective Chrome agar medium and according to Vitek®2 system. After activation on Brain Heart Infusion broth at 37◦C for 24 hours Bacterial colonies developed on plate agar, were studied; on MacConkey Colonies appeared as a deep purple colour due to lactose fermentation. It is also appeared as circular, flat and moist with entire margin on this medium. MacConky agar is a selective plating medium used for the isolation of Enterobacteriaceae and related to gram negative rods.

On EMB agar Black, Dark colour colonies with green Metallic sheen, the aniline dyes (eosin and methylene blue) in this medium combine to form a precipitate of green metallic sheen at acidic pH serving as indicators of acid production from lactose (Leininger *et al*., 2001).while On CHROM Agar medium the isolates give metallic blue.

Microscopic examination after staining with gram stain is showing that the cells arrangement, appeared as pink rods, slender, or oval shaped organisms, short to medium length, straight or slightly curved, non-sporulating occurring as singles or in pairs.

Identification of all 62 suspected isolates of E, coli was confirmed by Vitek®2-automated system. The results demonstrated that there were full similarity between biochemical tests results, chrome agar and Vitek2 system results.

4.4 Genetic Study:

4.4.1 Genomic DNA Extraction:

In order to perform a genetic detection of virulence factor genes in UPEC isolates from UTIs patients, the DNA of 62 strain of UPEC extracted and purified using genome DNA purification Kits. The results were detected by electrophoresis on 1% agarose gel and exposed to U.V light in which the DNA appeared as compact bands as shown in figure . (4-19) .

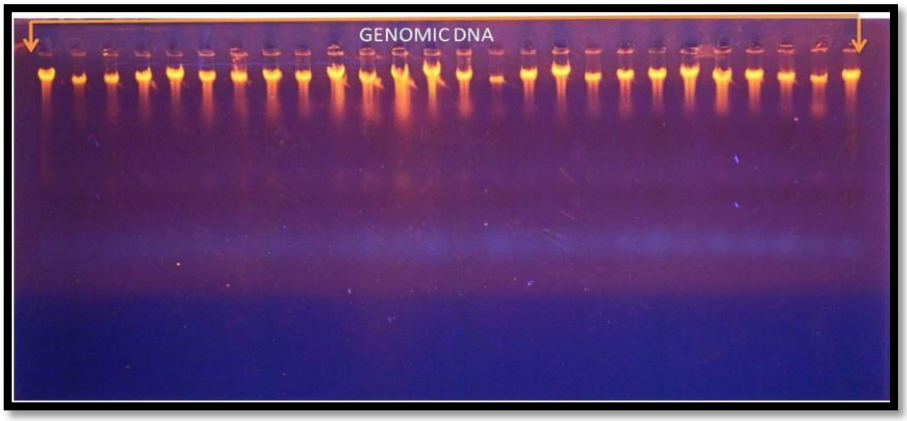


figure (4.19): Ethidium bromide stained agarose gel electrophoresis appearance that displays genomic DNA was extracted from uropathogenic *E. coli*

4.4.2 DNA Amplification

The products of PCR amplification between the extracted DNA and specific primers of C640T and T591A *fimH* gene were detected by gel electrophoresis analysis using DNA marker and the products size was 164 bp and 76 bp respectively . The presence of the *fimH* gene was confirmed by PCR and the results indicated that the *fimH* gene was present in 62 UPEC isolates; Apart from investigation on evaluation of the *FimH* gene in UPEC strains, this gene has been detected in other strains of *E. coli*. The products of successful

binding between the extracted DNA and specific primers for FimH gene are detected by gel electrophoresis analysis using DNA marker and the products size is 164bp as shown in figure (4-20).

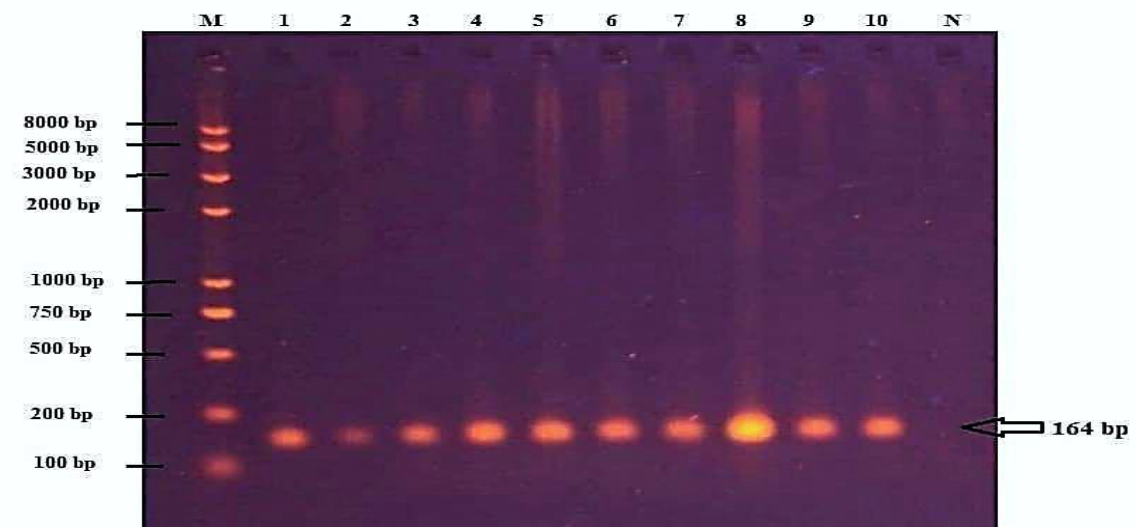


Figure (4.20): Gel Electrophoresis of PCR Amplified 164bp of C640T *fimH* gene. Lane (M):DNA molecular size marker, Lane (N): negative controls

The products of successful binding between the extracted DNA and specific primers for FimH gene are detected by gel electrophoresis analysis using DNA marker and the products size is 76bp as shown in figure (4-21).

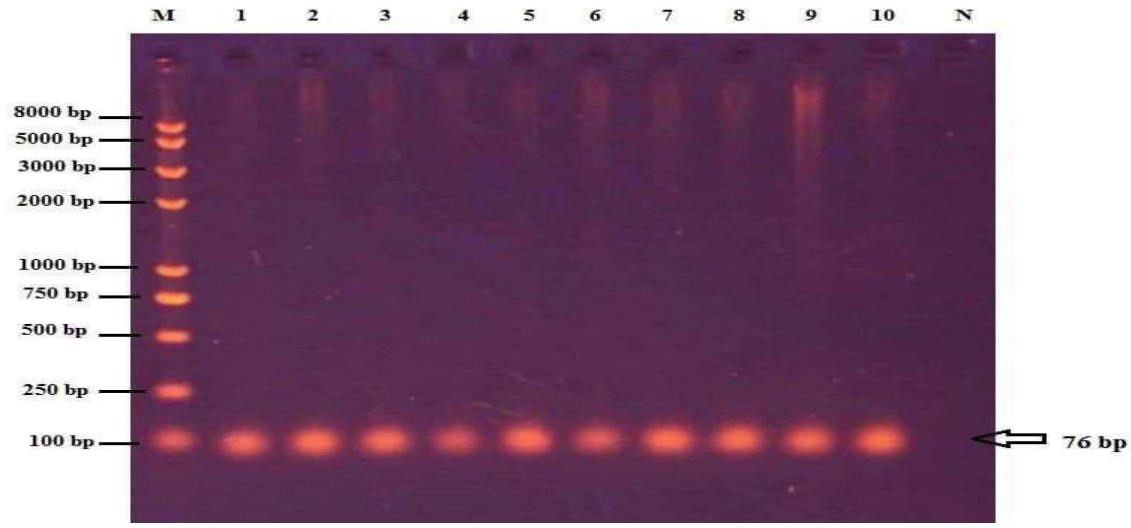


Figure (4.21): Gel Electrophoresis of PCR Amplified 76bp of T591A *fimH* gene . Lane (M):DNA molecular size marker, Lane (N): negative controls.

Positive PCR shows the presence of the virulence gene, but anegative PCR does not point to the absence of the corresponding operon.

In Iran, a study reported by (Rahdar et al. 2015), aimed to determine the occurrence of *FimH* and pap genes among 100 UPEC isolates collected from patients with UTIs. The results showed that the genes encoding fimbrial adhesive systems (fimH) were detected in 95% and 57% of UPEC isolates, respectively. " In another study in Tunisia reported by (Tarchouna et al. 2013) aimed to evaluate the prevalence of different operons coding for virulence factors among 90 UPEC strains isolated from the urine of patients with UTI. The results showed that the genes encoding virulence factors genes, (fimH) were detected in 68% , 41% and 19% of UPEC isolates, respectively. The results of a study by (Hojati et al. 2015) in Iran indicated that more than 90% of uropathogenic E. coli isolates harbored fimH gene, the high binding ability of fimH may lead to increased pathogenicity of E. coli strains. Thus, fimH could be used as a possible diagnostic marker"." In another study in

Mexico, "clinically diagnosed women with UTIs were further screened to identify the presence of virulence genes, fimH was detected in (86.1%) of UPEC isolates , papC was in (62%) and HlyA was in (7.4%)of them, respectively. The high incidence of papC gene indicated that UPEC isolates have the ability to attach the kidneys cell and cause pyelonephritis (López-Banda et al., 2014).

from 62 DNA samples of UPEC were subjected to a molecular detection by PCR amplification of the *fimH* gene using a specific primer, 53 isolates (85.48%) gave positive results of C640T *fimH* gene and 50 isolates (80.64%) gave positive result of T591A *fimH* gene. The same results was found by Abdallah *et al*., (2011) , they found the incidence of *fimH gene* were found in (80%) of *E. coli* strains isolated from china patient, Usein et al.,(2001) found the incidence of *fimH gene* in *E. coli* strains isolated from Romanian adult with UTI was 86%. A study of salih *et al*. (2015) and Hojati et al., they reported a that 92.8 % of UPEC isolates which harbored *fimH* gene. *fim*H gene were detected in 91.07% of UPEC isolates among 112 UPEC isolates obtained from outpatients suffering from UTIs in seven Iraqi hospitals. the high frequency of *fimH* gene in all studies relating to UTIs indicates the critical roles of this virulence factor in *E. coli* pathogenesis

4.4.3 Detection of Genes Polymorphism

Genotyping is performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique . After amplification , PCR products are digested using specific restriction enzymes (ScrFI,BstNI) each restriction enzyme identifies a specific sequence of nucleotides (between four and eight bases) and makes cut in both strands of the double-stranded DNA.

Distribution of *fimH* gene C640T polymorphism was detected by PCR-RFLP technique, the enzyme digested the 164bp segment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths . The PCR products are directly analyzed on 1% agarose gel by electrophoresis, and each allele is recognized according to its size figure (4-22) , and the enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths for the T591A *fimH* gene figure (4-23), these results agree with the result study of (Molaie *et al.*, 2016).

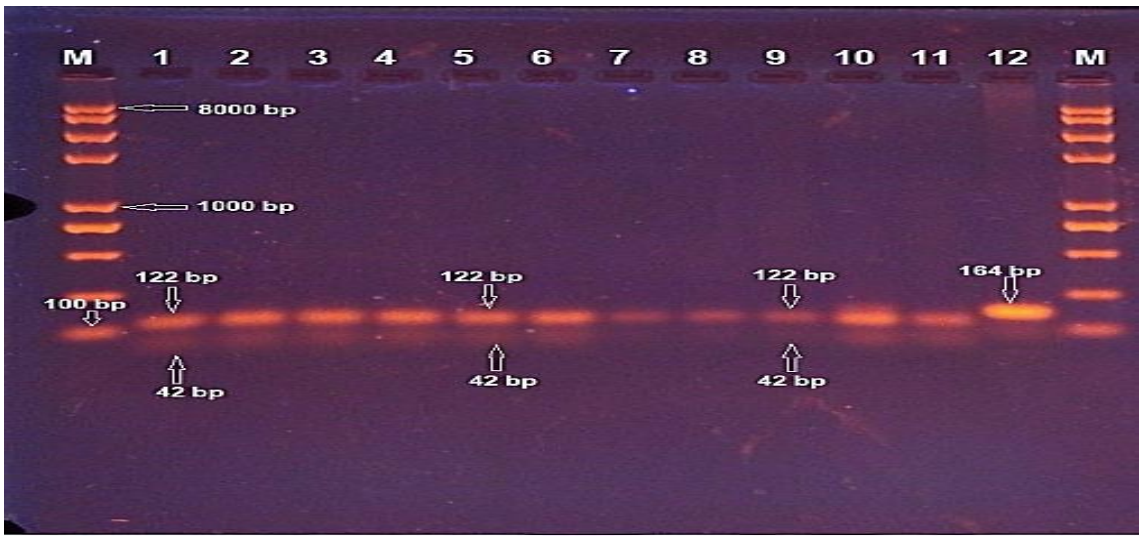


Figure (4.22): Genotyping analysis of C640T SNP. ScrFI enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths. . lane M: Marker (8000-100 bp); lanes (1-11) digested PCR products, lane 12 un-digested PCR products;on 1% agarose gel at 100 voltages for 15 min then at 50 voltages for 45 min.

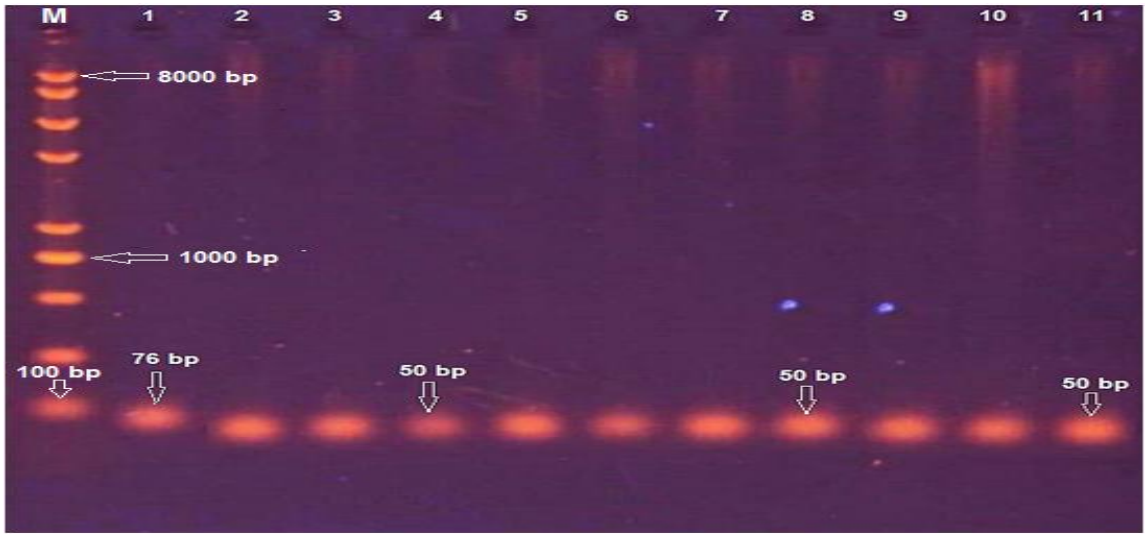


Figure (4.23): Genotyping analysis of T591A SNP. BstNI enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths. The 26 bp fragment was removed from gel because of the small size of fragment . lane M: Marker (8000-100 bp);lane1 un-digested PCR products ,lanes (2-11) digested PCR products. On 1% agarose gel at 100 voltages for 15 min then at 50 voltages for 45 min.

4.4.4 Distributions of *FimH* Genotypes and Alleles

Specific amplification of *fimH* gene, consist of C640T and T591A polymorphic site results in a 164 bp and a 76 bp PCR products, respectively. Genotyping was accomplished using the ScrFI and BstNI restriction enzymes for C640T and T591A SNPs, respectively. Allelic frequencies were expressed as a percentage of the total number of alleles. The nucleotide change from C to T at 640 position in the *FimH* gene creates a restriction site for ScrFI restriction enzyme. The wild type allele of *FimH* gene is C, and the Mutant is T. The C640T substitution generates as ScrFI restriction Recognition sequence, and restriction digestion gives fragments of the following sizes: 122+42bp (C allele) table(1). The

nucleotide change from T→A at 591 position in the *FimH* gene creates a restriction site for BstNI restriction enzyme. The wild type allele of *FimH* gene is T. And the mutant is A. The T591A Substitution generates as BstNI restriction recognition sequence, and restriction digestion gives fragment of the following sizes: 50+26bp (T allele). Table(2). These results agree with the reports of other studies such as the study of chou et al.,(2003).

Table 1:Genotype of C640T and T591A polymorphism.

Genotype	No. (%)	P. value*
CC	53(100)	
CT	0(0)	0.0369
TT	0(0)	
Allelic frequency**		
C	53(100)	0.0255
T	0(0)	

* P. value were calculated by X2 test.
** Allelic frequency of C=CC + 1/2 CT

Table 2 : allelic frequency of C640T and T591A polymorphism .

Genotype	No. (%)	P. value*
TT	50(100)	
TA	0(0)	0.0299
AA	0(0)	
Allelic frequency**		
T	50(100)	0.0217
A	0(0)	

* P. value were calculated by X2 test.
** Allelic frequency of T=TT + 1/2 TA

Type 1 fimbriae were produced by more than 80% of all UPEC, it was very well established that the production of type 1 fimbriae by *E. coli* is a determining virulence trait for pathogenic strains(Merza, 2017). Studies have shown only minor sequence variation within the *fimH* genes, which renders the *fimH* alleles feasible for use in high-resolution subtyping of multilocus sequence typing (MLST)-based *E. coli* clonal groups (Roer et al., 2017). Phenotypic variants of *fimH* were predominantly the product of SNPs in *fim*H. SNPs that contribute to the ability of pathogens to cause disease confer a selective advantage during the course of a single infection, epidemic spread or longterm evolution of virulence (Weissman et al., 2003) . In a previous study, Tartof et al.(2007) explored *fim*H single-nucleotide polymorphism (SNP) analysis as a screening test for the epidemiological study of Uropathogenic *E. coli*, UPEC as well as other strains of *E. coli*, to the extent that the *FimH* gene was detected in more than 90% of the *E. coli* strains. The high binding ability of *FimH* could result in increased bacterial binding to target cells and increased pathogenicity of *E. coli* thus, *FimH* could be used to design vaccine for prevention of *E. coli* infections by bloking the bacterial attachment and colonization. In addition, *FimH* could be used as a tool for the extension of rapid detection-based assays.

5-1 Conclusions

1- *FimH* is one of the virulence factors of UPECs.

2- *FimH* C640T polymorphism with CC genotype and C allele are mainly expressed among UPEC isolated from patient with UTI , whereas T allele are not affected by the restriction enzyme.

3- *FimH* T591A polymorphism with TT genotype and T allele are mainly expressed among UPEC isolated from patient with UTI , whereas A allele are not affected by the restriction enzyme.

5.2 Recommendations

1. To investigation more virulence factors that may be participate in increase urinary tract infection.

2. To study more develop effective therapeutic approaches capable of eliminating the virulence of *FimH*.

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Part 1:Patients' Questionnaire Form

Name :

Age :

Sex:

Address:

Part 2: Result of urine examination

Color :

Reaction :

Albumin :

Specific gravity :

Albumin :

Pus cell:

Ketone Bodies:

Appendix

Red Blood Cell: 

Epithelial cell: 

Sugar: 

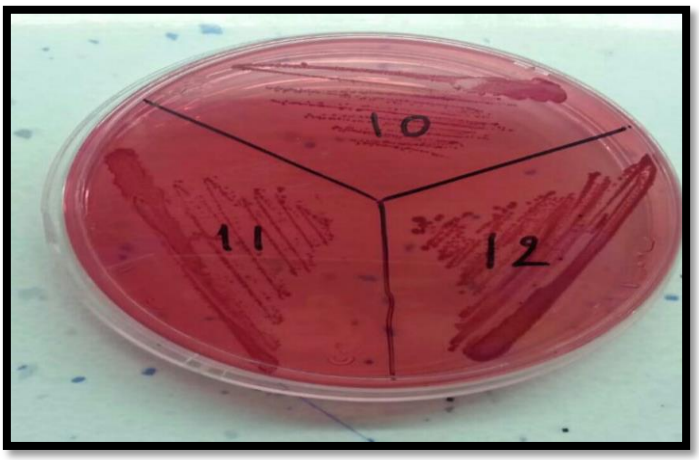

Figure (4.15): Growth of *E. coli on Macconkey agar.*

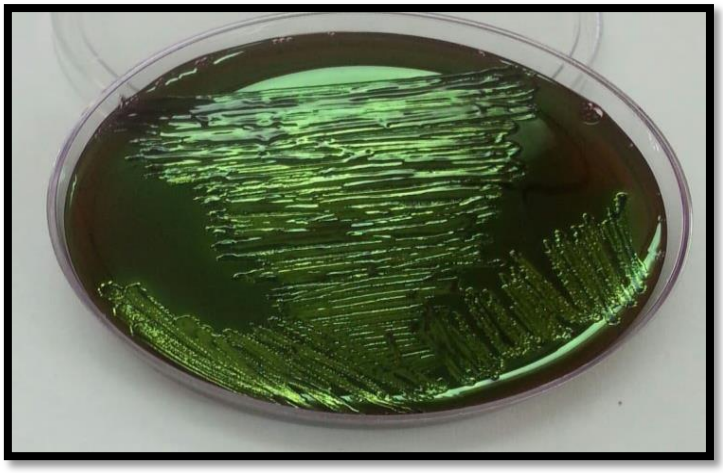

Figure (4.16): Green metallic sheen of *E. coli growth on EMB agar.*

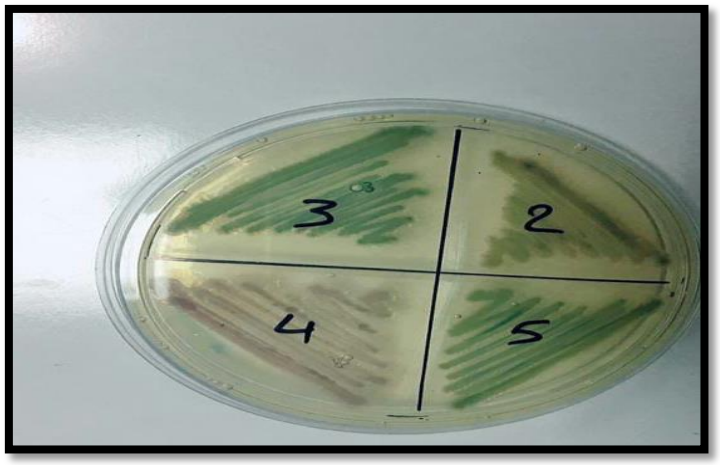

Figure (4.17): *E. coli* growth on CHROM agar.

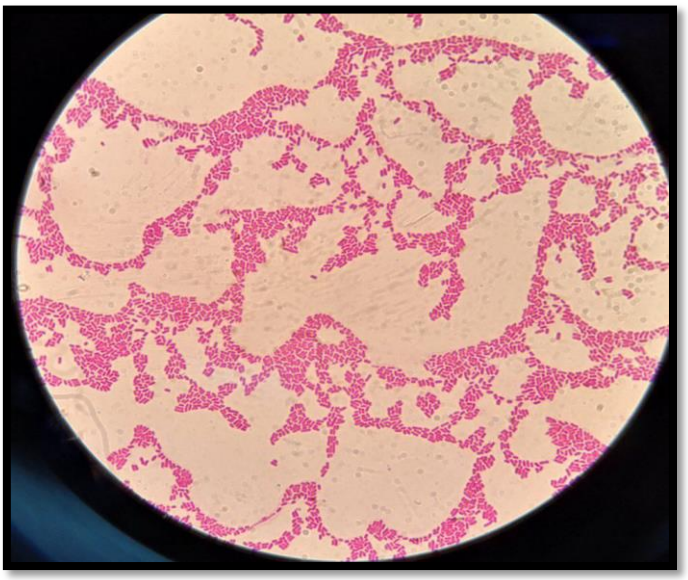

Figure (4.18): *E. coli* under microscope.(Oil immersion).

الخلاصه

تعرف عدوى المسالك البولية على أنها وجود مسببات الأمراض الميكروبية في المسالك البولية مع الأعراض المرتبطة بها. تصيب العدوى كلا من المسالك البولية السفلية والعلوية وتُعرف بالتهاب المثانة الحاد و التهاب الحويضة والكلية على التوالي. أجريت هذه الدراسة للكشف عن تباين الجينات FimHوارتباطها مع إمراضية الإشريكية القولونية المسببة لالتهاب المجاري البولية .شملت مجموعة الدراسة ١٥٠مريضا بالتهاب المسالك البولية. كان هناك ١٢٠إناث ٣٠ ذكور تتراوح أعمارهم بين ٥٥-٥سنة في مستشفى الحسين التعليمي للفترة من كانون الثاني ٢٠١٩ إلى اذار ٢٠١٩ المرضى الذين يعانون من التهاب المسالك البولية الناجمة عن uropathogenic *Escherichia coli* تم العثور عليها في الفئة العمرية من ٢٤-٣٩سنة. تم جمع عينات البول من المرضى.تم تشخيص العزلات البكتيرية عن طريق الفحص المجهري ودراسة الخصائص المظهرية على الأوساط . تم تأكيد التشخيص Chrome agarو Eosin Methylene Blueو MacConky agarانتقائية بواسطة نظام Vitek®2.أظهرت النتائج البكتريولوجية أن ٦٢عزلة (*Escherichia coli*.) تم استخراج الحمض النووي الجيني من العزلات البكتيرية لمزيد من الدراسة الجزيئية للكشف عن تعدد الأشكال الجينية C640T fimHو T591A fimH.تم استخدام تفاعل PCR وتقنية PCR-RFLP لهذا الغرض وهضم منتجات الحمض النووي المتضخم بواسطة انزيم ScrFIو BstNI endonuclease والتي أعطى اجزاء بأحجام جزيئيه مختلفه التي تعبر عن أنماط جينية محدده .كشفت هذه الدراسة أن معدل انتشار C640T FimH SNPكان ٨٥.٤٨% ، حيث اثرت انزيمات الهضم فقط على تسلسل أليل Cولم تظهر أي تأثير على تسلسل الأليل T.كان معدل انتشار تعدد الأشكال النوكليوتيد الوحيدة ، ٨٠.٦٤%T591A fimH حيث اثرت انزيمات الهضم فقط على تتابع الأليل T وليس له أي تأثير على تسلسل أليلA.

الإقرار المتعددة الأشكال

نحن نشهد كأعضاء لجنة مناقشة قد اطلعنا على هذه الرسالة الموسومة ب **(التحليل الجزيئي لجين Fim H من اشريشيا القولون المسببة لاتهاب المجاري البولية)** وقد ناقشنا الطالبة (صابرين فليح نينو) بتاريخ 2020/12/14. ووجدنا بأن الرسالة تفي بمستوى الحصول على درجة الماجستير علوم/ علوم حياة.

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قسم علوم الحياة

التحليل الجزيئي لجين *Fim H* من *اشريشيا القولون*

المسببة لألتهاب المجاري البولية

رسالة مقدمة الى قسم علوم الحياة كجزء من متطلبات نيل درجة الماجستير في

علوم الحياة

من قبل

صابرين فليح نينو الفتلاوي

بكلوريوس علوم الحياة /2009

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2020م 1441هـ