TURKISH REPUBLIC OF NORTH CYPRUS
NEAR EAST UNIVERSITY
HEALTH SCIENCE INSTITUTE

Antibiotic susceptibilities of Uropathogenic *Escherichia coli* strains isolated from Urinary tract infections

NIZAR HAZIM WALI WALI

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

SUPERVISOR

PROF. DR. NEDIM ÇAKIR

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The Directorate of Health Sciences Institute,

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STATEMENT (DECLARATION)

Hereby, I declare that this thesis study is my study, I had no unethical behaviors in all stages from the planning of the thesis until writing there, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list; and, had no behavior of breaching patent rights and copyright infringement during the study and writing of this thesis

NIZAR HAZIM WALI WALI

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ABSTRACT

Aim: This study was conducted to determine Mannose-Resistance hemagglutination (MRHA) from pathogenic *E. coli* strains that isolate from urine samples and study their antimicrobial susceptibility testing.

Materials and Methods: The current study was conducted in the microbiology laboratory at the near east university hospital in the Turkish Republic of Northern Cyprus (TRNC). A total of 105 samples for the study was executed Between (July 2012 and August 2020) from urine samples of hospitalized patients from various hospital departments, without taking an antibiotic for at least 3 days, in order to detect and evaluate uropathogenic *E. coli* strains by microbiological culture method, in addition, Antibiotic-susceptibility and mannose-hemagglutination testing.

Results: A total of 105 samples of the uropathogenic *E. coli* strains the result of the microbiological culture method show there are 36.2 % of the samples are belong to UPEC, and 63.8 % was Non-UPEC. In addition mannose-hemagglutination test performed, the result was 24.8% to MRHA and 11.4% showed as a MSHA, following, performed Antibiotic resistance test to the Amikacin (0.0%), Ampicillin/Sulbactam (18%), gentamicin (1.9%), ciprofloxacin (15.2%), Trimethoprim/ sulfamethoxazole (13.3%), Ceftriaxone (17.1%), Meropenem(0.0%).

Conclusions: These findings will certainly help understand the pathogenicity and proper management of UTI patients, thus decreasing the improper use of antibiotics.

Keywords: E. coli, Urine sample, Culture, Susceptibility test, Mannose Hemagglutination tes

ÖZET

Nizar Hazim Wali WALI

PROF. DR. Nedim Çakır

Amaç: Bu çalışma, idrar örneklerinden izole edilen patojenik *E. coli* suşlarından Mannoz Dirençli hemaglütinasyonu (MRHA) belirlemek ve bunların antimikrobiyal duyarlılık testlerini incelemek için yapılmıştır.

Gereç ve Yöntemler: Bu çalışma, Kuzey Kıbrıs Türk Cumhuriyeti'nde (KKTC) yakın doğu üniversite hastanesinin mikrobiyoloji laboratuvarında gerçekleştirildi. Üropatojenik E coli suşlarının mikrobiyolojik kültür ile tespiti ve değerlendirilmesi amacıyla çeşitli hastane bölümlerinden hastanede yatan hastaların idrar örneklerinden en az 3 gün antibiyotik alınmadan (Temmuz 2012 - Ağustos 2020) arasında toplam 105 örnek gerçekleştirildi. yöntem ilavesi Antibiyotik duyarlılık ve mannos-hemaglütinasyon testi.

Bulgular: Mikrobiyolojik kültür yöntemi sonucu üropatojenik *E. coli* suşlarından toplam 105 örnek, örneklerin% 36,2'sinin UPEC'e ait olduğunu ve% 63,8'inin UPEC olmadığını gösterdi. Ek olarak mannos-hemaglütinasyon testi yapıldı, sonuç MRHA'ya% 24.8 ve MSHA olarak% 11.4 olarak gösterildi, ardından Amikasin (% 0.0), Ampisilin / Sulbaktam (% 18), gentamisine (% 1.9) antibiyotik direnç testi yapıldı, siprofloksasin (% 15,2), Trimetoprim / sülfametoksazol (% 13,3), Seftriakson (% 17,1), Meropenem (% 0,0).

Sonuçlar: Bu bulgular kesinlikle İYE hastalarının patojenitesini ve uygun yönetimini anlamaya yardımcı olacak ve böylece uygun olmayan antibiyotik kullanımını azaltacaktır.

***Anahtar Kelimeler*:** *E. coli*, İdrar örneği, Kültür, Duyarlılık testi, Mannoz Hemaglütinasyon testi

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LIST OF ABBREVIATIONS

ETEC	**Enterotoxigenic *E. coli***
EIEC	**Enteroinvasive *E. coli***
EPEC	**Enteropathogenic *E. coli***
EHEC	**Enterohemorrhagic *E. coli***
EAEC	**Enteroaggregative *E. coli***
DAEC	**Diffuse Adhering *E. coli***
UPEC	**Uropathogenic *E. coli***
NON-UPEC	**Non-Uropathogenic *E. coli***
MR	**Mannose-Resistant**
MS	**Mannose Sensitive**
InPEC	**Intestinal Pathogens *E. coli***
DEC	**Enteric/Diarrheagenic *E. coli***
ExPEC	**Extra-Intestinal Pathogenic *E. coli***
ABU	**Asymptomatic Bacteriuria**
MSHA	**Sensitive Haemmagglutination**
MRHA	**Mannose-Resistant Hemagglutination**
CAUTI	**Catheter Associated Urinary Tract Infection**
AUC	**Acute Uncomplicated Cystitis**
AUP	**Acute Uncomplicated Pyelonephritis**
NMEC	**Neonatal Meningitis *E. Coli***
NTD	**N-Terminal Domain**
CTD	**C-Terminal Domains**

CU	**Chaperone-Usher**
TLR	**Toll-Like Receptor**
hPMNLs	**Human Polymorphonuclear Leukocytes**
MPL	**Monophosphoryl Lipid**
EMB	**Eosin Methylene Blue**
AK	**Amikacin**
CN	**Gentamicin**
SAM	**Ampicillin/Sulbactam**
CRO	**Ceftriaxone**
MEM	**Meropenem**
CIP	**Ciprofloxacin**
SXT	**Trimethoprim/Sulfamethoxazole**
AST	**Antibiotic Susceptibility Test "**
ESBL	**Extend Spectrum Beta-Lactamase**
MDR	**Multi Drug Resistance**
G-ve	**Gram-Negative**
UTIs	**Urinary Tract Infection**
µm	**Micrometers**
LPS	**Lipopolysaccharide**
IL	**Inter-Luken**

CHAPTER ONE: INTRODUCTION

1.1. INTRODUCTION

Escherichia coli (also known as *E. coli*) is a very complex bacterial species that appears naturally in the human intestinal tract and usually lives in the intestinal flora of warm-blooded organisms (Cho et al., 2018; Marrs et al., 2005). *E. coli* is a gram-negative, optionally anaerobic bacterium of the genus Escherichia. Cells are usually rod-shaped and are 0.25-1.0 μm in diameter and approximately 2.0 micrometers (μm) long with a cell volume of 0.6-0.7μ. The optimum production of *E. coli* occurs at 98.6 °F (37 °C), although certain laboratory strains can replicate at temperatures of up to 49 °C (120.2 °F). Development may be driven by aerobic or anaerobic respiration utilizing a broad range of redox pairs, including oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and reducing substrates such as oxygen, nitrate, fumaric acid, dimethyl sulfoxide, and trimethylamine N-oxide. *E. coli* is categorized as an optional anaerobic food. It uses oxygen when it's current and usable. It will, however, continue to develop in the absence of oxygen using fermentation or anaerobic respiration. *E. coli* strains are Gram-negative since their cell wall is made of a thin peptidoglycan layer and an outer membrane. During the staining phase, the colour of the counterstain safranin is taken up by *E. coli*, which stains pink. A resistance to such antibiotics such as *E. coli* is established by the outer membrane protecting the cell wall. Penicillin does not affect *E. coli* (Redorbit , & Retrieved , 2013).

Although most strains of *E. coli* are harmless, Some strains are pathogenic that cause diseases such as watery diarrhea, bleeding diarrhea, inflammation of the urinary tract, meningitis, and sepsis that may lead to death (Cho et al., 2018; Gyles, 2007). Harmless strains are part of the usual gut flora and may assist their hosts by developing vitamin K2 and resisting intestinal invasion by pathogenic bacteria

(Hudault et al., 2001; Sciences, 1982; Sprunt & Leidy, 1988). Pathogenic forms are described by strains of particular serogroups with a complex range of virulence factors that are responsible for diverse clinical manifestations defined by *E. coli* infections. Pathogenic *E. coli* induces severe human diseases, including several forms of diarrhea, inflammation of the urinary tract, sepsis, and meningitis. *E. coli* strains have been categorized into six major groups that induce human diarrhea with varying severity: Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enteroaggregative *E. coli* (EAEC), and Diffuse adhering *E. coli* (DAEC). *E. coli* strains classified as uropathogenic *E. coli* are known to induce urinary tract infections. though *E. coli* K1 is also involved for forms of sepsis (Kaper, 2005). For almost all the pathogenic *E. coli*, fimbriae or pili mediate colonization of a specific host surface, also referred to as colonization factors. The urinary tract is normally sterile, apart from the anterior surface of the urethra. UPEC can live in the colon and be inserted in the urethra afterwards. UTIs arise from the growing invasion of the urinary tract by these virulence factors. Infections may occur in the urethra (urethritis), bladder (cystitis) and kidney (pyelonephritis), and microorganisms may reach the bloodstream under some conditions (Kaper, 2005).

Escherichia coli's significant virulence factors can be classified predominantly into two groups: the bacterial cell surface and the secreted virulence factor. Virulence Factors of bacterial cell surface most frequently involve fimbriae, such as generally type 1fimbriae and P fimbriae. Such fimbriae assist in the adhesion of the host cell membrane, tissue invasion (which is essential in UPEC pathogenesis causing UTIs) (Shah et al., 2019). The capacity of the strain to agglutinate erythrocytes and bind to epithelial cells tends to be consistent with the involvement of pili, or some bacterial surface factor associated with pili, on urinary *Escherichia coli*, however, non-piliated strains with these attachment functions have also been identified (Gander et al., 2014). *E. coli,* that enter the urinary tract, are likely to be flushed out and unable to develop themselves until they can adhere to the urinary

epithelium, and Recently , Much attention has been placed on the skill of *E. coli* In the urinary tract, and intestines bind to the host cells. Adhesion requires an association between the invading organism and the host receptors, and research into this process will shine a flashlight not just on bacterial virulence causes, but also on the sensitivity of the host to infection. Cell surface materials that mediate binding are called adhesins, and the most studied of these are fimbrial adhesins. Their involvement is often seen by the agglutination of red cells that could be mannose susceptible (MSHA) or mannose resistant (MRHA) to the presence of tiny quantities of mannose. MRHA is essential in urinary tract infection since several strains with this property bind to the P blood group antigen, which is widely spread. P Fimbriate of *E. coli* is present in upper urinary tract infections. Other MRHAs bind to various receptors, but the importance of these adhesins in urinary tract infection has not been fully evaluated. MSHA strains bind to mucus and their function is ill-understood in urinary tract infection. In fact, their removal can be helped by mucus binding (Cooke, 1985). Acute pyelonephritis is associated with P fimbria, a mannose-resistant adhesive of uropathogenic *Escherichia coli* (UPEC) (Lane & Mobley, 2007). Several studies have shown that the development of type 1 fimbriae results in virulence and lack of expression, culminating in loss of identity, but that their existence cannot be linked to UTI, because typical fecal strains often have similarly expressed type 1 fimbriae. However, a key stage for cystitis is type 1 fimbriae-mediated attachment. Adhesins of this fimbriae are mannose sensitive (Nielubowicz & Mobley, 2010). described two kinds of *E. coli* hemagglutinins, designated mannose-resistant (MR) and mannose sensitive (MS) (Gander et al., 2014). It is difficult to quantify adhesion to epithelial cells, whereas red blood cell agglutination is a quickly calculated response that in clinical microbiology is widely used. The phenomenon of adhesion mentioned here were shown to correlate with erythrocyte agglutination exceptionally well. Both adhesion and haemagglutination of type 1 fimbriae adhesion are mannose-sensitive (a-methyl-Dmannopyranoside inhibited), suggesting that a structure(s) containing mannose on both erythrocytes and epithelial cells may act as a receptor. *E. coli*

consistent with pyelonephritis, on the other side, causes human red blood cells to have mannose-resistant hemagglutination (MHRA) (Tallgren et al., 1981).

Based on the nature of structural or neural urinary tract anomalies (Terlizzi et al., 2017). The distinction is essential among complicated and uncomplicated infections. Complicated infections are those that often arise in or after obstructive uropathy, including parenchyma (pyelonephritis or prostatitis). Kidney damage is predisposed to obstruction, stones or high-pressure vesicoureteric reflux, perinephric abscess, sepsis, or a mixture of life-threatening sepsis (Huland & Busch, 1984, Saldanha et al., 2009).

Episodes can be refractory to treatment, sometimes leading to relapses, frequently leading to severe sequelae such as sepsis, metastatic abscess, and sometimes severe kidney disease. An uncomplicated disease is a case of cysto-urethritis after ureteral and bladder mucosal bacterial colonization. It is considered that this kind of infection is uncomplicated because sequelae are uncommon and exclusive in a subset of women since, contrary to the significant morbidity with reinfection. A subset of patients with pyelonephritis (acute, uncomplicated pyelonephritis) can also have a low incidence of sequelae in young women who respond well to therapy (Saldanha et al., 2009). Antimicrobial resistance in globally, *E. coli* and rising resistance levels among *E. coli* was established. Increasing bacterial resistance to antibiotics complicates the treatment of infections in both developed and developing countries, is a growing issue. In general, without bacteriological examination, up to 95% of patients with serious symptoms are treated. Profiles of frequency and sensitivity of *E. coli* As well as major disparities between different cultures and conditions, exhibit significant regional variations (Kibret & Abera, 2011). The prevalence and antimicrobial resistance patterns of *E. coli* were carried out in this research from urine samples

1.2. AIM and SCOPE

The aim of this research to determine Mannose-Resistance hemagglutination(MRHA) from pathogenic *E. coli* strains that isolate from urine samples and to detection their antimicrobial susceptibility testing.
In order to:

1. Prevalence of Uropathogenic *E. coli* in Near East hospital
2. Determine of Mannose resistance hemmagglutination(MRHA)
3. Detection of antibiotic susceptibility of UPEC strains

2. GENERAL INFORMATION

2.1. Taxonomy and History

The bacterium *E. coli* was found in 1885 in the feces of healthy people by the German-Austrian pediatrician Dr. Theodor Escherich (1857–1911) and named it Bacterium coli commune because it is found in the colon. He performed the growth of the intestinal flora in neonate meconium and feces of breast-fed infants. Five-day old neonate meconium and feces, respectively, contained bacterial strains with a broad phylogeny, highly represented in adults. He detected "slender short rods" of 1-5 μm in length and 0.3-0.4 μm in width in the preparations of meconium and stool samples under the microscope. In addition, he cultivated these bacteria on agar and blood serum plates, where these bacteria grew as white, non-liquidating colonies. He also showed that, as a consequence of acid formation, these bacteria slowly cause milk to be clotted, and showed that these bacteria have fermentative capacity. He also conducted the Gram staining method and revealed that with all aniline dyes, these bacteria quickly take color but lose color after potassium iodide and alcohol treatment. Bacterium coli was placed in a genus based on its motility and shape by

early prokaryotic classification. The bacterial classification of Ernst Haeckel later placed bacteria in the kingdom of Monera (Adamczyk & Reed, 2017; Escherich, 1885; Olusegun et al., 2012). Later In 1895 Migula reclassified bacteria in the genus Escherichia and in 1919, the bacterium was renamed by Castellani and Chalmers after its discoverer and became *Escherichia coli*,(Adamczyk & Reed, 2017; Méric et al., 2016; Olusegun et al., 2012)**.** This genus belongs to the bacterial group formally named " coliforms "which are part of the " the enterics" classified as Enterobacteriaceae family (Adamczyk & Reed, 2017; Brenner et al., 2005).

An important phase in the efficient urinary tract colonization and pathogenesis of UTI is the ability of UPEC to bind to host uroepithelia. In 1976, it was proved that *E. coli* in patients with acute symptomatic pyelonephritis, isolated from the urine adhered to exfoliated uroepithelial cells in larger numbers than with *E. coli* Patients of asymptomatic bacteriuria have isolated from their urine. Two years later, the ability of UPEC to adhere to human uroepithelial cells was attributed to the presence of fimbriae (or pili), which appear as hair-like appendages that protrude from the surface of bacteria. These fimbriae were determined to be distinct from normal fimbriae (otherwise known as type 1 fimbriae) by mediating uroepithelial cell adherence in the presence of mannose, a known type 1-mediated adherence inhibitor(Lane & Mobley, 2007; Svanborg Edén et al., 1976; Svanborg Eden & Hansson, 1978). The most widely researched adhesin, and it is the first virulence-associated factor found for UPEC is P fimbria. P fimbriae are greatly prevalent in UPEC strains causing pyelonephritis, encoded by the pap (pyelonephritis-associated pili) genes (Källenius et al., 1981). and are characterized by their mannose-resistant adherence to Gal(a1–4) Galb moieties present in the globoseries of membrane glycolipids on human erythrocytes of the P blood group and on uroepithelial cells (Leffer & Svanborg-Eden, 1981; Lelffler & Svanborg-edrnn, 1980).

E. coli's meteoric development and exalted biological status stem from how simple it is to recognize and work with it. Virtually any individual can be isolated

from hardy, nonpathogenic, and flexible strains that grow rapidly on several different nutrients. These traits have made *E. coli* is a staple of laboratory microbiology teaching collections. Consequently, as microbiologists cast around for a model organism in the early 20th century, *E. coli* one of the most commonly available choices was. Those who have elected to work with *E. coli* Bordet and Ciuca (1921), Werkman (1927), Wollman (1925), Wollman and Wollman (1937) and Bronfenbrenner and Korb (1925), Bronfenbrenner (1932) were among those who carried out pioneering research on bacterial physiology, viruses and genetics (Blount, 2015; Daegelen et al., 2009). By the 1940s, *E. coli* was firmly established by its use in many fundamental studies. At the beginning of the molecular biology revolution in the 1950s, was the bacterial model organism of choice, making it the obvious organism to work with. As a result, it became the organism in which the most important aspects of life, including the genetic code, transcription, translation, and replication, were first worked out included Crick (1961), Nirenberg (1965), Judson (1996), for an fantastic history of early molecular biology and *E. coli*'s role in it (Crick, 1961; Blount, 2015; Nirenberg et al., 1965; Renneberg et al., 2017). It is not hyperbole to say that *E. coli* is now the most important model organism in biology (Blount, 2015).

The urinary tract of a human is being a hollow organ which is highly prone to infection. Its main function is to first collect, and then transport, store, and finally eliminate urine out of the body regularly. The whole process is highly controlled. And as an end result, the human body makes sure that the products of metabolism and toxic substances are entirely removed by the kidneys through the urinary tract. Urine flowing in the upper part of the tract and being eliminated from the lower part, has an important role in cleaning and removing the microbes which might have invaded the tract already. At the time that urine is not being eliminated, the urinary tract is like a closed system, in which microorganisms will not be able to reach it. Proximally, the urinary tract is made up of; papillae, pelvis, ureters, bladder, and urethra. Each of which has a distinct function and, a particular anatomical feature.

2.2. *E. coli* classification

There are many subdivision systems of E. coil. Here, we are mentioning the common subdivision that is not in accordance of serotype relateadness but based on surface antigens O antigen part of lipopolysaccharide layer H. however, the only important one to be mentioned is the serogroup, for example, the O-antigen. The numbers of serogroups that are identified recently are around 190. In the laboratory, the strain that is noticeable is usually associated to the alternation that constrains creation of an O antigen. Taxonomically speaking, the kingdom and domain of both bacteria and *Escherichia coli* exactly matches, and the reason is these fellows are cellular microorganisms. To be continued, the phylum of both proteobacteria and *Escherichia coli* matches and the reason is this group fellows are Gram-negative bacterium with having membrane outside that is made of lipopolysaccharides mainly. However, in terms of class, the class of both the Gammaproteobacteria and the *Escherichia coli* matches since this group follows are anaerobic G-ve bacterium, and also the Enterobacteriales and bacteria *Escherichia coli* order fits well into each other and this is because these set fellows are anaerobic G-ve bacterium with rod-shapes. Moreover, the Enterobacteriaceae and the family *Escherichia coli* are matched since it has motile capacity via peritrichous flagella that can expand fine at 37oC, is Catalase positive, Oxidase negative, and diminishes nitrates. Furthermore, the genus of both *Escherichia coli* and Escherichia matches because fellows of this group are taking immediate advantages and it settles in the mammal's colonic area, the species bacteria is one of five ones known under the Genus Escherichia. The natural actions that mark *E. coli* measured exceptional (owns lysine decarboxylase, ferments lactose, is Vogus-Proskauer negative, creates indole, doesn't raise on nitrate, and doesn't create (H2S) (http://bioweb.uwlax.edu/bio203/s2008/moder_just/classification.htm, Donnenberg, M), (Samaranayake, L., 2018).

2.3. Epidemiology

One of the most common bacterial infections nowadays considered to urinary tract infection (UTI), could be one of the very usual bacterial infections. 150 million people will be victims of this infection annually. A few years ago in the US, about ten million patients visited clinics for UTI symptoms, and two to three million presented to the ED. Health managements and losing the ability to work costs the UTI patients about four billion every year. This infection is one of the noteworthy causes of agony in male infants, all females and old men. Complications of UTI may include; recurrent infections, pyelonephritis, permanent kidney damage, antibiotic resistance due to regular use of antibiotics, and Clostridium difficile colitis. Some studies show: low rates of ESBL-producing Enterobacteriaceae which accounts about 3 to 8 percent, seen in: Singapore, Japan, and Sweden. Moreover the higher rates are noted in: i. Portugal - 34 percent. ii. Italy - 37 percent. iii. USA - 44 percent. iv. Latin American countries – 30 to 60 percent. v. Saudi Arabia - 8 to -38 percent. vi. Kuwait – 31 percent. United Arab Emirates – 41 percent.

Escherichia coli is a common microorganism present in any individual's gastrointestinal tract, where it generally forms part of the normal gut (Turck et al., 2016). They constitute about 0.1% of normal flora of gut (Adamczyk & Reed, 2017; Raman et al., 2005). *E. coli* has a successful symbiotic association with its host and plays a major role in supporting the equilibrium of normal flora the luminal which preserve the intestinal homeostasis (Bien et al., 2012; Yan & Polk, 2004). Rather, *E. coli* stays harmlessly confined to the intestinal lumen, like a commensal, which seldom induces disease. However, in the weakened or immunosuppressed host, non-pathogenic-commensal forms of *E. coli* are often present or may cause infections when the gastrointestinal boundaries are breached (Bien et al., 2012; Kaper et al., 2004). Some *E. coli* strains are taking on a more pathogenic character, will diverge from their common cohorts. These strains acquire unique factors of virulence that

give the bacteria an improved capacity to adjust to new niche areas and allow them to improve their performance to cause a large range of diseases. Many *E. coli* strains are microbes that are commensal, although certain strains may be pathogens and other hosts (Kaper & Nataro, 2016).

Pathotypes of *E. coli* that are able to cause disease in stable people. Infection with one of these pathotypes will progress to three general clinical syndromes: enteric/diarrhoeal disorder, urinary tract infections (UTIs), and sepsis/meningitis. Among the intestinal pathogens, *E. coli*(InPEC) there are six well-described types: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E.coli*(DAEC). And the most specific two pathotypes extra-intestinal pathogenic *E. coli* (ExPEC) are neonatal meningitis *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC). Extra-intestinal pathogenic *E. coli* (ExPEC) is the most prevalent Gram-negative bacterial pathogen. In all age groups, It is linked with multiple clinical illnesses, including bacteremia (septicemia *E. coli*-SPEC), neonatal meningitis (neonatal meningitis *E. coli*-N EMEC) and up to 80% of uncomplicated urinary tract infections (UTIs) acquired in the population (uropathogenic *E. coli*-UPEC) (Kaper et al., 2004; Poolman & Wacker, 2016; Russo & Johnson, 2003). The effect of UTIs is uropathogenic *E. coli*(UPEC), which is the most predominant extraintestinal *E. coli* diseases and the most prominent source of UTI is *E. coli* (Bien et al., 2012; Campos et al., 2004; Johnson, 1991; Kaper et al., 2004; Lamprecht et al., 2014; Rojas-Lopez et al., 2018).

The primary cause of human extraintestinal *E. coli* Infections is strains of ExPEC. Although historically referred to as uropathogenic *E. coli* (UPEC) Because of their connection with UTI, such strains are now known as more generally pathogenic, leading to the more inclusive term ExPEC being commonly used (Russo & Johnson, 2000; Vila et al., 2016) . However, *E. coli* strains which are able to occupy and defend themselves from the host immune system inside the urinary tract, become

uropathogenic *E. coli*. From UPEC causes > 80percent of UTI (Wurpel et al., 2013). Infections of the urinary tract are very popular and 12% of males and 10% to 20% of females report acute symptomatic UTI and a still larger percentage establish asymptomatic bacteriuria (ABU). Thus, around 10% of individuals and half of all females become sick in their lives at least once. UTIs are the most widespread diseases following upper respiratory tract infections. Infections can be symptomatic or asymptomatic, and any type of infection can result in severe sequelae if left untreated. While UTIs can be caused by many different microorganisms, like fungi and viruses, the major causative organisms are bacteria and more than 95 percent of UTI cases are responsible. *Escherichia coli* is the most predominant UTI etiologic agent and is directly responsible for over 80 percent of these infections. In order to shorten the course of the disease and avoid the increase of the inflammation of the upper urinary tract and kidney failure, an correct and prompt diagnosis of UTI is necessary (Bonadio et al., 2001; Delanghe et al., 2000; Farajnia et al., 2009; Johnson, 1991; Kandemir et al., 2012). More than 100,000 people in the United States are admitted annually leading to urinary tract infections, according to a study (J.R. & W.E., 1989). And in 2011, 400,000 people were hospitalized and the aggregate expense was about USD 2.8 billion (Simmering et al., 2017). Disease of the lower urinary tract (bladder and urethra) is referred to as cystitis, and in the situation of infection of the upper urinary tract (kidney), is referred to as pyelonephritis and untreated pyelonephritis leading to sepsis. In order to induce inflammation, without distinction of location, the causative organism must first escape the host's immune system and recolonise the urinary tract (Müller et al., 2009). An order to cause diseases in the bacterial community, many different virulent factors are needed. Pathogenic *E. coli* strains, for example, In sites where they normally do not reside, express Adhesion factors that shape pili or fimbriae of different kinds for their connection. There are causes of structural virulence that involve, in general, P fimbriae and type 1 fimbriae. In host cell surface adhesion, tissue invasion, these fimbriae assist (which is important in pathogenesis of UPEC causing UTIs). Virulence factors that promote the binding of *E. coli* are fimbrial adhesins such as

PapG and CsgA (Bien et al., 2012; Kaper et al., 2004; Luna-Pineda et al., 2018). In addition, UPEC can affect the host immune system in a number of ways (Olson & Hunstad, 2016).

These are both haemmagglutination adhesion: type 1 fimbriae is mannose-sensitive haemmagglutination(MSHA) and mannose-resistant hemagglutination (MRHA) is P fimbriae (Johnson, 1991). Such as processes of iron acquisition and toxins, and these are considered factors of secreted virulence. The production of these virulence factors by UPEC will induce an inflammatory reaction that provides a probable mechanism for symptoms of UTI (Bien et al., 2012). However, both the host and the uropathogenic *E. coli* strain has different functions in the formation and colonization mechanism of the urinary tract (Winberg, 1984) . P fimbriae and type 1 fimbriae will be mentioned here in this research as the important virulence factors of uropathogenic *E. coli* From.

The most common bacterial infection in children is considered to be UTI. During the first 6–12 months of life, up to 30% of infants and children who experience recurrent infections have UTI. Symptoms vary among patients in many ways. For instance, there is a higher incidence among very young infants, especially in male. It is accepted that *Escherichia coli* is the most serious sign of disease, however, in the first year of life, *Enterobacter*, *Klebsiella pneumonia*, *Pseudomonas*, and *Enterococcus* are much more frequent than later in younger age, with a higher risk of urosepsis compared with adulthood. Main causative factors for UTI in the community are age, diabetes, history of UTI, and sexual activity. Records show elevation in UTIs with age in men. In contrast, with women, there is a decrease in UTIs at middle ages, but an increase after the sixties. Among people who are not committed to an institution, the incidence is about 14 percent in women and 10 percent in men. There is a record showing that one third of women who are older than 85, are diagnosed within one year, and two thirds are diagnosed in 5 years. History of previous UTI in young women could be related by an increased risk of developing a CAUTI (Catheter Associated Urinary Tract Infection). A study shows that after menopause and up to 75 years old, subsequent UTIs with a frequency of more than

five times were the strongest factor of a new UTI. A major, independent, confirmed risk factor for UTI is having sexual intercourse in the previous 48 hours, in women of ages. A recent study carried out among young women presented that the rate of physical intercourse, is strongly proportional with the increased incidence of this type of infection.

2.4. Etiology

The urinary tract infections (UTIs) are deemed severe complications that impact healthcare worldwide. The most common UTI bacteria that affect human beings are *K. pneumonia, E. coli, E. faecalis, P. aeruginosa, S. marcescens, S. aureus, Proteus mirabilis*, and *S. saprophyticus*. The *E. coli* represents about 50% of hospital-acquired UTIs and nearly 85% of community-acquired UTIs, and considering other factors like gender, age, urological instruments, and immuno-suppression probably affect the spread of UTIs. One of the most dangerous health risks is Catheter-associated UTIs which causing 34% of all infections associated with healthcare. The outgrowth of broad-spectrum beta-lactamases was dangerously affecting the experimental use of ciprofloxacin and cephalosporins. There are various mechanisms by which Microorganisms use to develop resistance to medications, as horizontal gene transfer, the chromosome level when foreign recombining the DNA of the bacteria, also genetic material alteration. The Resistance of microorganisms pattern varies among different countries, governorate to governorate, small hospitals compared to large hospitals, and even community to hospital (Sabir, Anjum et al. 2014).

In the city of Erbil, the problem of antibacterial resistance might be due to the overuse and misuse of antibiotics as an experimental therapy for UTIs. Higher resistance in Multi-drug showed to be less prevalence among non-ESBL-producers than ESBL-producing *E. coli*. Other findings were similarly reported among several recent studies. In fact, ESBL, which produce by organisms of Enterobacteriaceae's family were basically considered as MDR started in the hospitals. The last few years

ago an increase of such ESBL-producers was detected in the settings of outpatient, specifically those connected to UTIs, thus having choices to reduce treatment for a restricted number of antibiotics. It's difficult to Treat ESBL-producing organisms infections. This is due to opposition to other agents of antimicrobial coded by plasmids and also associated with the opposition to the extended-spectrum cephalosporins itself (Aka and Haji 2015). When dealing with UTI causing pathogens, it's essential to test for their resistance to commonly prescribed antibacterial medications in clinical setups to enhance the effectiveness of experimental treatment. The aim of the current thesis is to figure out the opposition form of *E. coli* isolates and to highlighting the bacterial etiology of UTIs (Sabir, Anjum et al. 2014).

2.5. UTI Pathogenesis

Pyelonephritis is referred to as the infection that attacks the upper urinary tract while cystitis is referring to the lower urinary tract infections. The most repeated bacterial pathogen that causing pyelonephritis and cystitis is *E. coli* and it's related to 85–90% of the cases. The origin of Uropathogenic *E. coli* (UPEC) is known to come through the fecal flora, then spreading in the perineum, then later to entering the bladder from the urethra. Investigation extensively of UPEC pathogenesis intermediate cystitis has been done from the two perspectives of the pathogen and host. The interested reader can be referred to the reviews recently addressed on this topic (Becknell, Schober et al. 2015). UTI's are not only highly recurrent but also common. Specifically, the elderly, women that sexually active and also pre-pubertal children are highly exposed to chronically recurrent UTI, which results in negatively affecting the quality of life and increased use of antibiotics. About 20-30 percent of adult women with an original UTI will suffer a recurrence within 3-4 months. About one in three children having a UTI by the age of one will also have a recurrence over the next three years in reality about 18 percent will have a recurrence within a few months (O'Brien, Hannan et al. 2016).

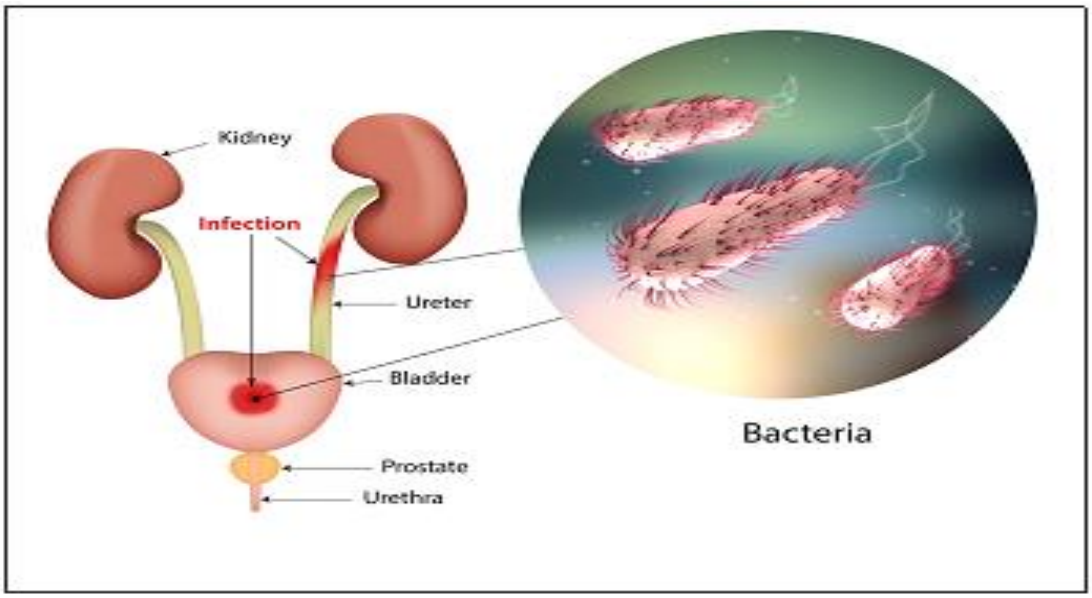

Figure: 1.1. The UTI and infection area

2.6. Complicated and Uncomplicated Urinary Tract Infections

A complicated UTIs can be identified as UTIs associated with other factors that compromise the host's defense system or the urinary tract, and those factors include the obstruction of the urinary tract, neurological disease induced urinary keeping, transplantation of renal, pregnancy, immunosuppression, renal failure and the being of foreign bodies presence like in residency catheters, calculi or different drainage devices (Flores-Mireles, Walker, et al. 2015). In outpatient facilities, uncomplicated urinary tract infections (UTIs) have the largest rate of infections. And this is why antibiotics are prescribed because, after respiratory tract infections, they are the most prevalent reason. Uncomplicated UTIs composed of both acute uncomplicated cystitis (AUC) and also acute uncomplicated pyelonephritis (AUP). Lately, according to the degree of resistance of uncomplicated UTI, the pathogens have significantly increased. Considering this rising, the world of medicine has turned out to be more cautious about the direct effects of antibiotics that are applied systemically. The use of various antibiotic agents is exerting different selective

pressures on both the innocent observer botany at the same site and the infection-causing pathogens. Understanding this fact has led to re-evaluation an internationally the therapeutic recommendations of antibiotics in uncomplicated UTI (Wagenlehner, Hoyme et al. 2011).

2.7. UPEC Virulence factors

The Commensal, *E. coli* instead, remains healthy in the colonic lumen and since it is harmless, it rarely induces illness. However, in the case of having weak immune hosts, or having the gastrointestinal barriers desecrated, even nonpathogenic-commensal strains of *E. coli* will have ability to cause contagion (Kaper, Nataro et al. 2004). It is important to know that some of *E. coli* strains may depart from their partners, starting an added pathogenic nature. These strains of *E. coli* require exact reasons via DNA horizontal transfer of transposons, bacteriophages, pathogenicity island and plasmids that let the bacteria having more capacity to lead to a wide-ranging illness with having the capacity to adjust to new positions. The strains of pathogenic *E. coli* are generally categorized as these two which are enteric *E. coli* or (ExPEC) extraintestinal *E. coli*. ExPEC type contained six different *E. coli* "pathotypes" and they contain (EHEC) which is short for enterohemorrhagic *E. coli*. (EPEC)enteropathogenic *E. coli* ,(ETEC) enterotoxigenic *E. coli* , (EIEC) enteroinvasive *E. coli* ,(EAEC) enteroaggregative *E. coli* , and (DAEC)diffusely adherent *E. coli* . The two well known ExPEC pathotypes are (UPEC) short for uropathogenic *E. coli* and (NMEC) short for neonatal meningitis *E. coli*. There are numerous pathotypes of enteric *E. coli* that produce gastroenteritis, even though it is unusual for them to cause disease outside of the colonic area. Nevertheless, the extraintestinal *E. coli* strains can live and stay in the gut and not giving an outcome, but to not forget that they could distribute and attack other host places like the blood, and the vital nervous system, and of course causing infection (Bien, Sokolova et al. 2012).

2.8. Adhesion virulence factors of UPEC

According to the urinary discharge and antimicrobial activity of uric acid, urine from an uninfected person is sterile. Regular urine flow would not cause microorganisms inside the urinary tract to be colonized. Attachment, though, to *E. coli* In uroepithelial cells, helps mitigate the effect of the flow of urine. In the colonization process, it is called the first step, and both the host and *E. coli* for several pathogenic microorganisms, operates in this phase. Adhesins are adherent molecules which enable bacteria to attach to the host surface and recognize receptors. They are the most significant determinant of UPEC pathogenicity, activating the signalling mechanisms of host and bacterial cells, serving to supply host tissues with other bacterial materials, and facilitating bacterial invasion (Kaper & Nataro, 2016; Mulvey, 2002). The ability of UPEC to colonize depends on several adhesive fimbria being expressed. UPEC represents multiple adherence variables that are necessary for attachment and are thus recognised as virulence variables for effective adherence to the surface of the host cell. In a thin filamentous structure called fimbriae or pili, some bacterial adhesins are structured, while evidence of the existence of adhesins on the surface of the bacterial cell is available. During the attachment phase, Fimbrial-type adhesins are required (Parvez,& Rahman, 2018; Emody et al., 2003; Johnson, 1991; Riegman et al., 1988;). Fimbriae, also referred to as pili, are long hair-like structures found on the cell surface in bacteria that identify particular molecules of the target host cells that are usually carbohydrates. Pili are the short version of fimbriae which can be used interchangeably with fimbriae. Oligomeric pilin proteins are composed of fimbriae. These proteins are structured in such a way that they create a helical cylindrical structure and are both thinner and shorter than flagellum. In uropathogenic strains with *E. coli* these protein structures are expressed which are known as virulence factors (Parvez,& Rahman, 2018; Winberg, 1984). Carbohydrates are the bulk of the receptors for these fimbriae. They contain fimbriae of form 1 fimbriae and P fimbriae (Parvez,& Rahman, 2018).

The Fim operon encodes type 1 pili (expressing mannose-sensitive hemagglutination) in UPEC, while the pap operon encodes P- or Pap-pili (which are able to interact with the digalactoside unit in the P-blood group antigen). In clinical isolates of UPEC, Fim operon is constitutive, while pap is part of a PAI that is often responsible for other determinants of putative virulence. In general, all types of pili are heteropolymeric, consisting of a main pilus protein subunit that supplies the stalk of the pilus and many minor proteins at the distal end of the subunit, reflecting PapG and FimH as the actual adhesins. Two domains consist of PapG and FimH, the first facilitating copolymerization and forming a pilin domain, while the second is a lectin domain capable of binding carbohydrates. (Kline et al., 2009; Terlizzi et al., 2017). Several bacterial pathogens can create a number of these adhesins, and inhibition of a single adhesin can often cost the bacterium enough to lose its virulence. Pili or fimbriae roles are not restricted to adhesion only and may enable the microbe in several other essential pathways thrive and avoid the immune system of the host. In tissue tropism, the development of several adhesive types plays a part. In gram-negative bacteria including UPEC, adhesins are exposed by the chaperone-usher-assisted pathway. In this pathway, two proteins are involved, one is a periplasmic chaperone, while the other is a protein named usher. The structure is focused on Usher, and the chaperone's role is to fold and recruit the subunits. In the absence of the chaperone, pilin proteins are damaged and misfolded and therefore cannot be arranged in the form of a mature pilus. On the other side, Usher enables mature the fimbriae and their transport through the outer membrane's outer integrity. An N-terminal domain (NTD), 24-stranded beta-barrel channel, a plug domain, and two C-terminal domains are the constituents of usher proteins (CTD). From uropathogenic *E. coli* strains, More abundant are chaperone-usher family fimbriae (Klemm & Schembri, 2000; Volkan et al., 2014).

Pili assembles the chaperone-usher (CU) pathway. Over 1,000 copies of the FimA major pilin shape the sort 1-pilus rod, while the pilus tip contains the FimH adhesin at its distal end, accompanied by single copies of the subunits of the FimG and FimF adaptor. In a Rho GTPases (Rac1)-mediated host actin cytoskeleton rearrangement-dependent fashion, mannosylated proteins located on the bladder

epithelium bind to FimH (Eto et al., 2007). This eventually contributes to cystitis production due to bacterial invasion (Hahn et al., 2002). Besides that, phase variance is strictly regulated by the expression of type 1 pili, which reversibly varies between the active expression of type 1 pili (Phase-ON, piliated cells) and the absence of expression (Phase-OFF, non-piliated cell) (Schwan, 2011). Molecular pathways involved in reversible switching between ON-OFF modes are strictly regulated by environmental signals inside the urinary tract, such as acidic pH and salt growth conditions. The P pilus consists of six distinct subunits that are separated into two separate subassemblies (the tip fibrillum and the pilus rod). At the distal end, the tip fibrillum consists of one PapG adhesin, accompanied by subunits of PapF and PapE. The pilus rod is created by more than 1,000 copies of the PapA subunit. At the base of the pilum, which is a superhelical structure, the PapK connector subunit connects the above subunits to the PapA rod (Busch & Waksman, 2012; Terlizzi et al., 2017).

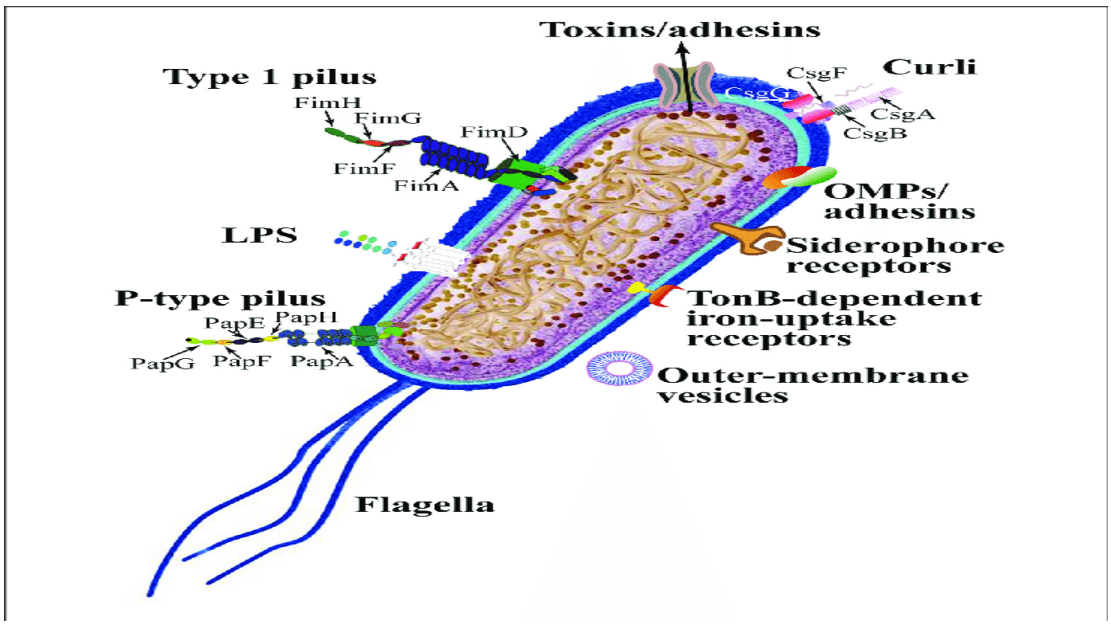

Figure 1.2. Adhesins and harboring/motile structures of *Escherichia coli* (Terlizzi et al., 2017)

The uninfected people's pee is clean because of the pee stream and healthy process of lithic corrosive. The normal stream of pee prevents microbes to settle in the renal system. Nevertheless, the bond of the *Escherichia coli to* the layer of lining cells

would make them to submerge the outcome of the urine stream. For most harmful microbes, this is regarded to be the first step in the invasion procedure (Parvez and Rahman 2018). Anyhow the Bacterial attachment to the organism tissues is a complicated procedure that mostly engagement of a number of definite adhesins are included, in which all of them may work together at the same time or differently according to the stages of invasion (Ofek and Doyle 2012). Additionally, the Pili is a collection of moderators between bacteria and biofilm establishment, or moderate distinct identification of invaded-cell receivers (Rendón, Saldaña, et al. 2007). Mostly carbohydrates are receptors for fimbriae. Their fimbriae make fimbriae first type, P pili, and flimsy collection of fimbriae (Parvez and Rahman 2018). The structural part which makes the first extension of harmful bacteria to the lining cells and succeeding successful invasion of the organism are polymeric sticking fibers termed ‘‘fimbriae’’. as for commensal bacteria it has been obvious that the pili accomplish the biological function of settling distinct recess and controlling organism’s normal opening methods. This is thought that a number of harmful *E. coli* breed utilize fimbriae type one or *E. coli* to invade living organisms’ cell groups. However, the genomic series scanning *Escherichia coli* with some hostile *Escherichia coli* types has been demonstrating the availability of different putative pili operons, recommending the formation of other pili by harmful and regular greenery *Escherichia coli* inside organisms gastrointestinal (Rendón, Saldaña et al. 2007).

2.8.1. Fimbriae

The inclusion of clinical devices innately encourages the cells that contain bacteria enter body places that are typically non-productive, and the creation of biofilms by supply an abiotic layer. The shortcomings of structured cure of biofilm-related infections are the inborn obstruction of biofilms hostile to the host resistance method and antibiotics treatment. So, one of the important guide reason for being diseased inside the environment of hospitals and care homes are biofilm-linked

invasions, making extra medical management price run over $1 billion/year in the United States (Reisner, Maierl, et al. 2014). Fimbriae moderate various responsibilities, along with attachment and biofilm creation. They are lengthy biomolecular complexes that stretch out outside of numerous microorganisms. The pieces that bacterium utilizes to aim an exact layer and show tissue tropism are Fimbrial attachment; they are regularly put at the tip of the organelle, which mostly identifies distinct receptor aim in a familiar way. Different sorts of attachment pilus are explained in thin-layer and thick-layer bacteria (Wurpel, Beatson et al. 2013). although, the link is typically moderated by attachment pilus, that is filament-like supplement set outside of the microbes, most of the polypeptide pattern, that develop distinct holding on the receiver to the host cells which is Eukarya, they can create biofilms on sterile layers like an as flexible tube (Garcia and Le Bouguénec 1996), and its availability is dedicated to serious human contamination (Barnhart and Chapman 2006). Fimbria has the capability to specially work with the organism's complex polypeptide like cold-insoluble globulin, domain IV, and endothelial to start the procedure of attaching and settling the host. Gram-negative bacteria show substance combination to indicator color as stated by Hammar et al., and this quality is utilized to specify attachment pilus availability in the attachment examination phenotypically (Barnhart and Chapman 2006). The urinary tract invasion capability of UPEC and turning into the disease is mostly linked with the declaration of fimbriae attachment (Klemm, Hancock, et al. 2010). UPEC attachment to the lining cells disables it to allow efficient approach strength of pee movement along with activating organisms and ways that signal cells containing bacteria, and accomplish invasion. between the specialized heterogeneous group linkages and Gram-negative bacteria correspond along with lining cells with moderate holding to a special receiver inside the renal system (Wullt, Bergsten, et al. 2000). UPEC P fimbriae are generally involved in the settlement of the upper part of the urinary tube (Roberts, Marklund, et al. 1994), with heterogeneous group stretches that make kidney swellings normally send number of duplicates of pyelonephritis pili genome collections in their gene. Pyelonephritis pili dedicate for an invasion via holding to the diary sugars

receive antigen within lipids inside carbohydrate's CD77 antigen available renal system and on Red blood cell. Pyelonephritis pili identify their receivers with the help of the cellular structures –put attachment, and they are accurately held to various antigenic. Pyelonephritis pili dedicate to surface cell attachment and placement of inside immune reactions in animals and humans. So, occupy pyelonephritis pili adhesion might stop renal system invasion by *E. coli*, and also may stop the settlement of swelling in the kidney which could cause severe harmful noticed in event of chronic (Watts, Tan, et al. 2012).

2.8.1.1. Type 1 fimbriae

By mediating adhesion to mannose-containing receptors on the uroepithelium and promoting intracellular bacterial formation, invasion and growth as a biofilm, type 1 fimbriae (also called type 1 pili) is essential for UTIs. These fimbriae identify manno-oligosaccharides normally expressed on glycoprotein molecules of the host cell surface (Emody et al., 2003; Martinez et al., 2000; Schembri & Klemm, 2001). Inside 99% of the *E. coli* strains, genes that encode type 1 fimbriae, are present and kill urinary tract cells by mediating enhanced inflammation through urinary tract infections (Parvez,& Rahman, 2018; Connell et al., 1996; Vigil et al., 2011). When entering the host cells of the urinary tract, type 1 fimbriae play a great function. Type 1 fimbriae are extraordinarily stable factors with UPEC virulence that may stabilize the bacteria's connection to different cell types in the urinary tract. Although their binding sites could not be identified in the capsules and glomerulus of Bowman, a clear affinity was observed in proximal tubules and vessel walls for type 1 fimbriae. They are strongly bound to the muscular layers of the bladder and mildly attached to vessel walls. Receptors for type 1 fimbriae were also found in the distal tubules and the collecting ducts. They can also cause their connection to the surfaces of macrophages (Avalos Vizcarra et al., 2016). Iuroplakin from urinary epithelial cells and mannoside-containing host proteins are recognised by these fimbriae. These are encoded by the bacterial backbone DNA, unlike many other significant types of

adhesins, which are primarily composed of FimA proteins along with FimF, FimG and FimH (Parvez,& Rahman, 2018; Hallstrom & McCormick, 2014). The most famous are FimA proteins, but virulence is not pivotal. Allelic variants of FimH decide the sugar specificity and the deletion of FimH results in less colonization among other subunits of type 1 fimbriae in mouse models of ascending UTI, and colonization with the FimH gene can be restored by plasmid expression. FimH alone or in conjunction with LPS can induce toll-like receptor 4 to initiate complex signaling cascades that will enable the humoral immune response (TLR4). Several experiments have shown that the development of type 1 fimbriae results in virulence and lack of expression contributes to loss of expression, but their presence should not be correlated with UTI as regular fecal strains have similarly expressed type 1 fimbriaee (Connell et al., 1996; Nielubowicz & Mobley, 2010). However, a key stage for cystitis is type 1 fimbriae-mediated connection. The adhesins of these fimbriae are mannose sensitive.

2.8.1.2. P fimbriae

P fimbriae, is a uropathogenic *Escherichia coli* (UPEC) mannose-resistant adhesin, was shown to be consistent with acute pyelonephritis (Lane & Mobley, 2007). And P-fimbriated with *E. coli* are heteropolymeric fibers encoded by the papA-K gene operon that function in humans on ascending UTI and pyelonephritis pathogenesis. And Alpha-DGalp-(1-4)-beta-D-Galp is attached to the carbohydrate structure. This fimbriae are responsible for activating cytokine production and mucosal and tissue matrix adhesion (Kaper & Nataro, 2016; Lane & Mobley, 2007; Mulvey, 2002). In patients with severe renal transplantation, P-fimbriae are the most significant virulence factor. P fimbriae tends to play a role in mediating in vivo uroepithelial cell conformity and in producing an inflammatory reaction during renal system colonization, contributing to damage to the kidney during acute pyelonephritis. Verifying the P fimbriae leads to UPEC pathogenesis during ascending UTI (and acute pyelonephritis in particular) (Lane & Mobley, 2007). They

bind tightly to the capsule, glomerulus, and endothelial cells of Bowman's vessel walls in the kidney. A minimum of six subunits make up this strongly organized composite structure. P fimbriae has conveyed *E. coli* once. They form bacteriuria which help pass the epithelial barrier to reach the bloodstream which may induce erythrocyte hemagglutination. This type of fimbriae is encoded by the pap gene cluster (also known as fso and fst), and pap + strains remain in the intestinal flora longer than pap strains (Parvez,& Rahman, 2018Wullt et al., 2000).

P antigens are present on the cell surface of red blood cells and on the lining of numerous cells in the urinary tract. P-fimbriated UPEC receptors act as P1 (present in human glycoproteins), P, PK, and LKE antigens. *E. coli* will not be agglutinated by P-fimbriated Red blood cells that lack P antigen. Isolated P fimbriae can bind the receptor to a synthetic analog, and the infection process is impeded by experimental application of that analogue. In the pap gene family, at least nine genes are located with two restriction sites at two ends. With the regulatory portion, the following Eco R1, consisting of papI and papB, starts. PapA, papH, papC, papD, papE, papF, and papG are then found, and after these, Bam HI is present. The P fimbria, which is helically united, contains roughly 1000 subunits. The PapA (19.5 KD) protein subunit is the main constituent, and the smaller subunits are PapE (16.5 KD), PapF (15 KD), and PapG (35 KD). In the periplasmic space, PapD (27.5 KD) may be present and may also be integrated into the structure. By transporting the subunits beyond the cell part, another PapC protein, which is the highest with such a mass of 80 KD, helps the process. Although PapA is the main constituent, it is not mandatory for connection, and among several serotypes, PapA molecules exhibit clear homology with the amino acids N and C termini. PapA also has an average degree of resemblance to other *E. coli* of the cellular subunits.

E. coli fimbriae, with fimbriae of form 1 included. Tiny subunits at the tip of the fimbria decide the receptor specificity. Several mutational experiments have also shown that conformity in PapA is not affected by mutation, whereas mutation in other genes (i.e. papEFG) is not inhibited by fimbrial structural appearance. In the fine

structure of P fimbriae, which is linked by PapE subunits to PapA (bulk potion of the structure) subunits, a PapF-PapG complex is formed. Finally, PapH terminates the assembly of the fimbriae and attaches them (Collinson et al., 1992). An relevant point is that the amino acid sequence of PapG is almost similar to Shiga's toxin. Other serotypes of *E. coli* Shiga toxin has been identified. Another feature of PapG has been established in some variants of P fimbriae, which may initiate subunit polymerization. Several studies have shown that the expression of these fimbriae is not essential for infection of the urinary tract, whereas other more advanced studies have concluded that their function in pathogenesis is significant. However, in immunocompromised patients, fewer expression of P fimbriae is seen during infection, which indicates that P fimbriae is required to combat certain types of host immune attacks. To initiate inflammatory responses, P fimbriae may trigger TLR44. UPEC protects against hPMNLs (human polymorphonuclear leukocytes). Environmental conditions influence the expression of P fimbriae via the urinary tract in a constantly evolving environment. While this phenomenon has many variants, P fimbriae expression is preferred at 37 °C and inhibited at a range of 18-22 °C. Temperature-dependent expression is regulated by the pap gene cluster region close to papB (Parvez,& Rahman, 2018; Hunstad & Justice, 2010).

2.8.2. Hemagglutination of uroepithelial-cell adherence

Hemagglutination (HA), reactions have been classified as Mannose sensitive or resistant, It depends on whether D-mannose, its derivatives or may inhibit HA (Shareef et al., 2010; Simi et al., 2002). It was first recognised in the late 1970s the strains of *E. coli* Despite the involvement of mannose (mannose resistant hemagglutination [MRHA]), allows UTI to typically agglutinate human erythrocytes and bind to human uroepithelial cells (Edén et al., 1978; Green & Thomas, 1981; Johnson, 1991; Wilson, 1979). Adherence to uroepithelial cells is also normally not impaired by mannose (mannose-resistant adherence) and is more prevalent in mannose-resistant hemagglutination (MRHA) strains than in mannose-sensitive

hemagglutination strains (Johnson, 1991; Paris, 1986). The strong relationship in individual strains between epithelial-cell adherence and MRHA was clarified by the discovery that fimbriae mediates both properties among most urinary isolates (Edén et al., 1978; Johnson, 1991; Valvano & Crosa, 1988). The discovery that fimbriae mediate both MRHA and epithelial-cell adherence is consistent with the findings of studies by Duguid et al. They identified that erythrocyte agglutination was carried out by clinical isolates of *E. coli* is due to the bacterial connection through thin fiber-like appendages to and cross-linking of erythrocytes, which these researchers called fimbriae (from the Latin word for threads or fringe). Brinton later named these structures pili (from the Latin word for hairs) and shown that when sheared and distilled from bacteria, they maintained their hemagglutinating ability (Frey JW & M, 1967; Johnson, 1991; Wilson, 1979).

2.9. Mannose sensitive (MS) and mannose resistant (MR) adhesion.

Bacterial adhesion is subdivided into mannose-sensitive (mannose-inhibited adhesion) and mannose-resistant (adhesion not inhibited by mannose). Mannose sensitive adhesion is common for non-pathogenic and pathogens in both *E. coli* and is regulated by fimbriae of type 1 (Andreoni, 1994; Winberg, 1984). But it is not further described as it has not been seen so far to play any part in the initiation of human pyelonephritis. Although mannose resistant is the type of adhesion used in most *E. coli* clinical isolates, this is a rather crude description of adhesive, showing us just that type 1 fimbriaeee is not regulated by adhesion (Kjkllenius & Mollby, 2000; Winberg, 1984).

The mannose-resistant adhesins of *E. coli* strains exhibiting MRHA is complex, As shown by the variety of patterns in which the erythrocytes are agglutinated from different organisms and classes of blood, MRHA These adhesins may be known to detect antigens of the blood group P (P fimbriae) (Evans et al., 1980; Johnson, 1991; Vosti, 1979). But because of type 1 fimbriae, mannose-sensitive adherence induced

by *E. coli* strains. Mannose-sensitive hemagglutination is commonly understood to indicate the existence of type 1 fimbriae (Johnson, 1991; Kjkllenius & Mollby, 2000).

2.10. Immunology

2.10.1. Innate Immunity

Both male's and female's generative areas are vital to the internal mucosal facing of the human body, and those mucosae with those of lung mucosa and gastrointestinal are alike, they also have the ability to increase a complete range of immune replies. In both men and women, the generative area's immune system has different alterations in order to see the serious physiological tests of both well keeping complete guard against microbial invasion. The typical structures of the male and female genital areas are categorized of immunity system and replies of mucosal immune. The features that regulate immune replies result in a generative area contain cellular relations with mechanisms that create the generative tract immune system also the resident microenvironment which is conquered by a matchless microbiome and sex hormones (Wira, Fahey, et al. 2005, Kaushic, and Ferreira et al. 2010). The immune system of humans is basically distributed into two main divisions, which are called the adaptive (specific) and the innate (non-specific) immune system. Though those two both have a defensive job in contradiction of invading pathogens, they vary in the period to respond or react, the cells included, type, effector devices, and specificness of receptors. The inborn system of the immune establishes the first reply to contagion and joins quickly after working with contagious agents, because of this cause it has a crucial part in host protection (Janeway Jr and Medzhitov 2002, Wira, Fahey, et al. 2005). The stable responsibility of the system of immune is to protect the surfaces of epithelial to discriminate and execute pathogenic microorganisms. Basic responses are produced by pathogen-acknowledgment receptors ligation in and on

cells of epithelial and started by cells of the immune. These results begin and resist phagocytes, particularly neutrophils and macrophages (polymorphonuclear leukocytes), that has ability to finish bacterial pathogens at path point. One of the ways to become ill and repel the immune recognition, the dissimilar types of microorganisms all of them have gotten an collection of molecular devices to decline and prevent mammalian basic immunity. The authority of these microorganisms is normally diverse between Gram-negative bacteria. several kinds of pathogens even these three, Yersinia, Salmonella, and Vibrio; actual proteins interfering with the hint of the cell of a host like post-translational modification, kinase activation, cytoskeletal reorganization, and dissimilar actions are assumed to the host cell by discharge type III. Later on, we goal the core cause for urinary tract infections, that is the specific immune modulation actions of (UPEC) short for UroPathogenic *Escherichia coli* (Olson and Hunstad 2016). The growing level of overview of UroPathogenic *Escherichia coli* (UPEC) or pathogenic microorganisms inside the mammal's urinary area reasons an inflammatory response, fundamentally happened by rouse of (TLRs) Toll-like receptors. Stimulation of TLRs by (LPS) short for bacterial lipopolysaccharides passes the path of NF-κB, activating the announcement of neutrophil chemo attractants and cytokines including IL-6 , for example, IL-8 (CXCL1), that has ability to be predicted in human and mice urine by using UTI (Hedges, Anderson et al. 1991, Samuelsson, Hang et al. 2004). Uroepithelial cyclic AMP increment, rouse by TLR4-induced growths in intracellular [Ca2+], the result is independent NF-κB- development of IL-6 and IL-8 appearance (Song, Duncan et al. 2007). Bacterial flagellin energized TLR5 has the ability to penetrating irritation throughout UTI (Andersen-Nissen, Hawn et al. 2007, Smith, Varley, et al. 2011). neutrophil response broadcast may be determined by cytokines, e.g. IL-17, that has a significant career in joining inborn to versatile immunity (Peck and Mellins 2010) and it proceeds duty of the innate response through the UTI test (Sivick, Schaller, et al. 2010). A minor, cationic antibacterial peptide, that is recognized as human cathelicidin LL-37 is visible in the urine of humans in cystitis, and mice lacking in its orthology (CRAMP) prove enlarged vulnerability to pyelonephritis (Chromek,

Slamová, et al. 2006). Though it may paradoxically promote bladder contagion (Danka and Hunstad, et al.2015). From the defensive class of antimicrobial peptides, some different entities are formed locally, during UTI (Becknell, Spencer, et al. 2013, Nielsen, Dynesen, et al. 2014), and the presence of these available molecules in host-pathogen talk remains a fertile zone for learning. Neutrophil to the bladder is a central outcome of the soluble inflammatory reply. Furthermore, a diagnostic hallmark of human UTI is the discovery of neutrophils located in the urine. The phagocytic boundary of neutrophils shows an elementary part in governing UTI and UPEC, as displayed in dissimilar inquiries (Olson and Hunstad 2016).

2.10.2. Adaptive Immunity

When the wide innate immune responses of the urinary area are extra open to infection, adaptive immune responses, especially in the bladder, are typically forced to occur. UTI that advancement to the kidneys has the ability to contribute to the formation of antibody for the specific infection. Diseased patients confined to the bladder unpredictably disregard to make an antibody response (Abraham and Miao 2015). This seeming fault in the antibody reply of the bladder may perhaps be a main cause for the amazing reappearance of UTIs. This thought clinically was lately replicated in mouse in which UTIs that were severely restricted to the bladder made no or tiny antibody reply to the contaminating bacteria, while a considerable antibody reply was made during contagions of kidneys and the bladder both. The fundamental origin of the incapability of the bladder to stand an adaptive reply was related to develop local IL-10 creation, as mice IL-10-deficient displayed considerable antibody replies to bladder contagion. Mast cells are a primary basis of IL-10 in the bladder that occurs in bacterial contagion, as it is deliberated above. Since such secretory cells play an important role in initiating an immune response in the recent stages of urinary contagion, mast cells seem to resist their action around 6 hours after infection by switching to IL-10 development to complete this response (Chan, John et al. 2013). Mast cell-generated IL-10 has capacity to stop the appearance of molecules of co-

stimulatory on DCs and thus bound their ability to task as actual antigen-presenting cells while they traffic to demanding lymph nodes. Therefore, steady with the part of mast cell-derived IL-10 in weakening inborn immune reply, the incapability of the bladder to stand an antibody reply to bacterial contagion possibly will be a by-product of its effort to stop damaging adaptive immune replies to the insides of urine, also to ease the fast renewal of its epithelium succeeding infection-induced harm (Abraham and Miao 2015).

2.10.3. Immune escape

To settle the area of host urinary successfully , UPEC strains direct a range of virulence aspects. These contain flagella to ease bacteria movement and adhesins for add-on to the uroepithelium, permitting struggle to the movement of stable urine . Iron-acquisition systems for example, siderophores are doing a job which is permitting UPEC to gain iron in order to guarantee their development. Also, some type of toxins with some aspects like creating a capsule, gives allowance to the bacteria to effect the host immune reaction to flee (Bower, Eto et al. 2005, Dhakal, Kulesus et al. 2008). For the perseverance of contagion, UPEC create biofilms for example, for attachment to bladder catheters or even create intracellular colonies (Anderson, Palermo et al. 2003). It is bettet for the intracellular UPEC to be safe from antibiotics and cells of immune , allowing their perseverance (Dhakal, Kulesus et al. 2008). In oposition, it is important know that the system of immune is not inactive, and it has devices like to prevent the avoidance UPEC strategies. Based on the pathogens discovery by (TLR4) short for toll-like receptor 4 and other form appreciation receptors, a number of soluble aspects are unknown like for example, (antimicrobial proteins and peptides, plus chemokines). Moreover, the bacterial contagion encourages caspase-dependent apoptosis of epithelial cells with sickness to decrease the bacterial weight. Lastly, immune cells of local sentinel for example, (mast cells, , dendritic cells, natural killer cells and macrophages) feel the contagion and hide several cytokines to employee other immune cells from the blood flow,

particularly neutrophils, to remove the contagion (Abraham and Miao 2015). This battle that is happening between both the bacteria and the host organism matches the evolutionary hypothesis of Red Queen. focusing on one specific species and improving it assure co-evolution in terms of a difficult and varied guard and preventing other species. These researches was done on the immune against UTI give the clearance and put a spot on UTI pathogenesis (Schwab, Jobin et al. 2017).

2.10.4. Immunomodulation of urinary tract

Continues in persons who in the usage of the type of antibiotic known as broad-spectrum consumes may manage to occur the development of some type of antibiotic-resistant among various bacteria which mostly related to the urinary tract as a significance, the managing of viable type of contagions establishes a severe in addition to developing medical challenge. In order to control both controlling types of innate in addition to adaptive human body resistant organizations pertaining in urinary tract region might be possibly taking significant therapeutic insinuations for the fix the condition which related to UTIs. Since the lining of the urinary tract is exceedingly enhanced in TLR4 particles, regulating TLR4 particular ligands straightforwardly to the UTI seem trigger TLR4 intervened type of immune response termed as innate immune reactions subsequently improving nearby reactivity and resistance to disease. (Ashkar, Yao, et al. 2004, Nurkkala, Nordström, et al. 2007). These perceptions recommend stimulating of the TLR4 flagging passageway within UTI that can be in effect restorative operators in contradiction of current contagions. Besides, the fundamental and it is in order to utilize TLR4 ligands in the stimulus of promoting to the beginning of the passageway needed. Indeed in the event that TLR4 ligands which utilized for treatment use and it is impossible that LPS to be a ligand of special ever since LPS has inherent toxicity. Moreover, the TLR4 ligand per enormously moved forward protection outlines as monophosphoryl lipid (MPL) may be used in place (Evans, Cluff, et al. 2003). Since the immune element, both types of B in addition to T cells facilitate the type of immune which term adaptive immunity

disapprovingly reliant on indications resulting after the type of innate immune of human being, modulators that improvement the type of innate immune answers may be of value in increasing major of the adaptive immune responses (Pasare and Medzhitov 2005, Parker, Prince et al. 2007) Certainly, the innate immune defense at present regularly being combined as ‘adjuvants’ to improve immunity to vaccines (Ishii and Akira 2007, Tse and Horner 2007).

2.11. Clinical significance of *E. coli*

Escherichia coli has long been used as a model organism for exploring gene regulation in bacteria and has become the most thoroughly studied organism in the world. *E. coli* is a gram-negative enteric rod belonging to the family of Enterobacteriaceae. *E. coli* peritrichous flagella are usually motile, while some strains are nonmotile. The bacterium is a facultative anaerobe that can grow on a variety of complete laboratory media. However, *E. coli* can produce in minimal media by utilizing glucose as a sole carbon source to make all macromolecules necessary for growth (Reuter 2012; Ronald 2002). Moreover, there is an indication for an abdominal environment for uropathogenic bacteria infection (Goetz, Mahmood, et al. 1999), as a type of strains of human uropathogenic *E. coli* consumes been originating in the colonic microflora and there are new molecular has recognized 29 virulence genes of *E. coli* bacteria (Johnson and Stell 2000). Moreover, there is much research associating with UTI to non-immunocompromised, low immune and renal complication individuals establish *E. coli* was the greatest frequently inaccessible pathogen to all groups (Kärkkäinen, Ikäheimo, et al. 2000). Moreover, the virulence issues of bacterial *E. coli* are more public between non-immunocompromised versus immune-compromised individuals and it is recommended less infectious *E. coli* can reason for the UTI additional regularly in low immune patients with renal complication (Ronald 2002). .

2.12. Prevention

Hypothesized components for the presence and it is supply resistance of the antimicrobial in clinics incorporate taking after (i) Presentation of safe living being an already helpless populace. (ii) Securing type resistance and is related to susceptible strain (through unconstrained transformation and/or genetic exchange). (iii) Appearance relates with directed resistance as of now presents within the populace. (v) Determination of a resistant subpopulation. (iv) Dispersal or spread of safe living beings (Murthy 2001).Whatever the ICUs give a singular setting, encouraging the development and blowout of resistance for many explanations: (1) Near the quarters or in height recurrence of the health worker deal with patient interaction give chance to increase patient to patient connection. (2) The way to the transmission of pathogens agent due to deficiency of hand halogen and handwashing degrees decrease through expanded assignment). (3) Substantial determination weight by broad-spectrum anti-microbial utilize. (4) Natural defilement giving encourage occasion for the transmission of pathogens through polluted tools and health hospital care specialists (Murthy 2001).

2.13. Treatment

Different research on the assessment of antibiotic resistance in UPEC extracted from UTI patients in different countries are routinely conducted, and their findings have been reported in numerous journals. Through analyzing these published papers, it can be inferred that various patterns of resistance to antibiotics and susceptibility to treatment have been identified, based on the time of research and the geographic region. In addition, antibiotic treatment of various types of UTIs The routine treatment of UTIs is dependent on the use of antibiotics, and suitable antibiotics should be chosen by taking into account features such as causative isolate antibiotic susceptibility pattern, form of infection (community-acquired or hospital-acquired infection), patient situations, including age, gender, history of allergies, underlying illnesses, past utilization of medicines, other medical items, history of former UTIs, UTI locations (bladder, kidney or prostate) as well as pathogenic or

natural causative isolate flora. Selective medications for the care of uncomplicated UTIs have been trimethoprim, β-lactams and nitrofurantoin antibiotics in several countries. The usage of fluoroquinolones is, among the selective medications in certain countries for the treatment of complicated and uncomplicated UTIs. Fosfomycin and nitrofurantoin medications have been approved by the Infectious Diseases Society of America for the care of patients with uncomplicated UTIs, particularly those with resistant strains that cause UTI. In certain complex UTIs, care with antibiotics is challenging and certain forms of infections are treated according to the nature of the illness and the patients. Antibiotic treatment has long been debated for chronic UTIs, although if not properly chosen, not only can antibiotics not kill bacterial reservoirs, they may also serve as a shield for the existence of bladder cell bacteria. Despite the absence of systematic research, several research are proposed to evaluate the efficacy of antibiotics and their behavior in removing IBC types of UPEC as a major cause of recurrent UTIs, which in the future will definitely be beneficial in managing UTI patients (Terlizzi et al., 2017; Walker, E., et al, 2016; Asadi Karam, et al, 2019).

2.14. Vaccine

Related to the rising antibiotic resistance and its adverse effects on the natural flora of the human body, the decreased efficacy of antibiotics has contributed to the continuation of studies to discover alternative medicinal methods against UTIs, the most significant of which is the production of successful vaccines. The production of an efficient UPEC strain vaccine would play a significant role in lowering patients' mortality and morbidity rates, as well as decreasing economic costs. For many factors, developing a successful UPEC vaccine is difficult. (i) Variety of strains from UPEC. (ii) The risk of adverse effects on normal bowel flora. (iii) Dependence of UTIs on the presence of some UPEC strains' virulence factors. Therefore, because UPEC strains use a range of virulence factors, it seems that an appropriate vaccine should be able to provide a defensive immune response against virulence factors expressed at various phases of the growth of UTIs, such as occupation, invasion, and IBC reservoir

creation. It is possible to divide vaccines which have so far been established against UTIs into two classes. (i) Vaccines depending on cells (killed or live-attenuated vaccinations) and (ii) vaccines dependent on antigens, include subunits, toxin-based and conjugate vaccines. There are several benefits and disadvantages of both of these vaccination classes. The lack of good and long-term immune responses that will improve their immunogenicity by utilizing the components called adjuvants is one of the disadvantages of modified antigens relative to whole-cell-based vaccines (Choubini et al., 2018; Skwarczynski and Toth, 2016; Asadi Karam, et al, 2019).

CHAPTER TWO: MATERIAL AND METHOD

2.1. Material

2.1.1. Devices and Tool

Equipments	Company	Country
Incubator	WTB-Binder	Germany
Oven	Memmert	Germany
Medical Refrigerator	Sanyo	Japan
Autoclave	Sakura	Japan
Sensitive balance	Shimaduz	Japan
Centrifuge	Hettich	Germany
Microscope	BH2	Japan
Disposable Petri dishes plate	The Science	USA
Disposable test tube 10 ml	The Science	USA
Safety cabinet II	DALTON	Japan
Slides	The Science	USA
Cover slip	The Science	USA
Inoculation loops	The Science	USA
H.A Powder	D-mannose Nak	USA

2.2. Design of Study

The current study was conducted in the microbiology laboratory at the near east university hospital in the Turkish Republic of Northern Cyprus (TRNC). A total of 105 random samples for the study were executed Between (July 2012 and August 2020) from urine samples of hospitalized patients from various hospital departments.

The study will be performed among to urophaogenic *E. coli* infection indidividuals, the sample was collecting from those who expect from suffering urinary tract infection in both gender (male and female), following, the collecting information's according to the special questioners that related to the current study. Detection and evaluation uropathogenic E coli strains by microbiological culture method inaddtion Antibiotic-susceptbility and mannos-hemagglutination test, Morerover, the patient's demographic features (age, sex, sample date, sample type, isolated UPEC, NON-UPEC strains, determine MRHA, MSHA, Antibiotic Susceptibility test "AST") were recorded and analyzed.

2.3. Samples Collection

Urine samples will collect randomly from 105 patients who have a history of urinary tract infection in Cyprus-Nicosia city and without taken antibiotics for at least 3 days. During the study, urine samples were received in the microbiology laboratory at the NEAR EAST UNIVERSITY. These samples were categorized by gender (male and female), sample type only (urine). Urine samples were obtained from patients who were symptomatic for UTI. The isolates were identified by standard microbiological methods. The following study after collected each sample were cultured on Blood agar (Merck, KgaA, Germany) and Eosin Methylene Blue (EMB) agar (Becton Dickinson, Sparks, MD 211 52 USA) and incubated for 24-48 hours at 35°C to get pure colonies. then all samples of isolated *E. coli* strains were kept in bacteria storage tubes (OR-BAK, Ankara, Turkey) at -80°C until they were used.

2.4. Samples Culturing

The stored samples of *E. coli* strains isolates were cultured on nutrient agar for Haemagglutination test (HA) and also on Blood agar and Eosin Methylene Blue (EMB) agar to prepare for Mueller-Hinton Agar (MHA) for Antibiotic Susceptibility Testing. Blood agar, Eosin Methylene Blue (EMB) agar, and nutrient agar were prepared as per the manufacturer's directions as follows:

2.4.1. Preparation of Nutrient Agar

1. Suspend 28 grams of purified/distilled water per 1000 ml.
2. Heat to a boil to totally remove the medium.
3. Sterilize at 15 lbs by autoclaving. (121°C) pressure for 15 minutes.
4. To 45-50 ° C, cool. Mix well and drop into sterile Petri plates and leave the plates before the agar has solidified on the sterile surface.
5. Replace each Petri dish's lid and stack the plates in a fridge.

2.4.2. Preparation of Blood Agar

1. 1000 ml of purified/distilled water is applied to suspend around 40 grams of the prepared medium.
2. Heat to a boil to totally remove the medium.
3. Sterilize at 15 lbs by autoclaving. (121°C) pressure for 15 minutes.
4. The medium is then withdrawn from the autoclave and cooled to around 40-45 °C.
5. The sterile defibrinated blood with 5 percent v / v is applied aseptically and well mixed.
6. Then the media is mixed well and poured into sterile Petri dishes.
7. Replace each Petri dish's lid and stack the plates in a fridge.

2.4.3. Preparation of Eosin methylene blue agar (EMB)

1. Using 1000 ml of purified/distilled water to suspend 36 grams of EMB agar.
2. Heat to a boil to completely dissolve the medium.
3. Sterilize at 15 lbs by autoclaving. (121°C) intensity for 15 minutes.
4. To oxidize the methylene blue and to suspend the flocculent precipitate, cool to 45-50 ° C and shake the media in.
5. To allow plates to warm to room temperature, pour into sterile Petri plates.
6. Replace each Petri dish's lid and stack the plates in a fridge.

2.5. Haemagglutination test (HA)

The haemagglutination assay was observed by the haemagglutination of erythrocytes by bacterial fimbriae in the presence of D-mannose to assess haemagglutination of mannose resistance. The haemagglutination test has been done two times, by using (slide, normal slain, blood group O RH+, and 1% D- mannose). The first time it has done to isolate UPEC from NON-UPEC strains without using mannose in the procedure. Then the second time it has done only for UPEC strains with using mannose to determine mannose resistance haemagglutination (MRHA). The absence of occur haemagglutination was taken negative (-) for NON-UPEC in the first time and mannose sensitive haemagglutination (MSHA) in the second time. The Presence of occur haemagglutination was taken positive (+) for UPEC in the first time and mannose resistance haemagglutination (MRHA) in the second time. The procedure was conducted according to the test-slide method of direct bacterial haemagglutination. One full drop of bacteria was suspension with 2 drops of normal slain on the slide. Then add 2 drops of erythrocytes blood group (O RH+) without adding mannose to isolate UPEC from NON-UPEC strains. After isolated UPEC from NON-UPEC, the same procedure was repeated only for UPEC strains but with adding mannose this time to determine MRHA.

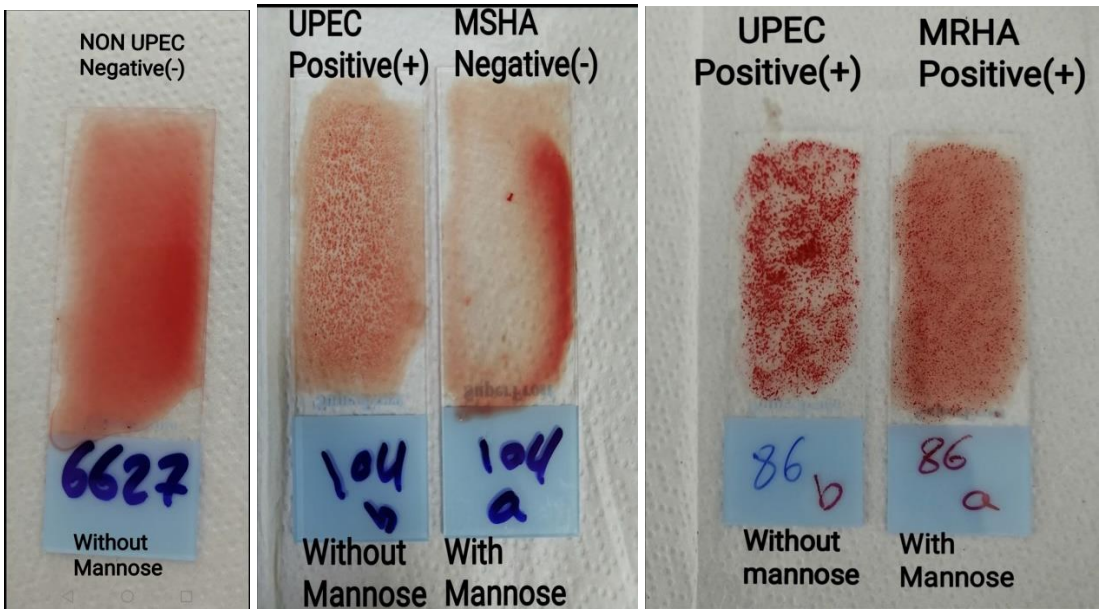

Figure: 2.1. Slide method of haemagglutination test

2.6. Antibiotic Susceptibility Testing

In this study, the disc diffusion method has been performed for the antibiotic susceptibility test. The process of disk diffusion used for bacteria (Mueller-Hinton agar) was augmented by 2% glucose and 0,5 µg/mL methylene blue coloring with a pH range of 7,2 to 7,4. The presence of glucose provides the bacteria with sufficient development, while the presence of methylene blue dye enhances the description of the zone edge (Fothergill, 2012).

Mueller-Hinton agar was processed as follows, combined with 2 percent glucose and 0,5 µg/mL methylene blue dye (MH-GMB) (Espinel-Ingroff & Cantón, 2007).

1. For 1000 mL of Mueller-Hinton agar, apply 100 µL of methylene blue dye.
2. For 1000 mL of Mueller-Hinton agar, apply 20 g of glucose.
3. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
4. To 45-50 ° C, cool. Mix well and drop into roughly 4 mm diameter, sterile Petri dishes.
5. Stored at refrigerator temperature 2 to 8°C and used within 7 days of preparation.

2.7. Antibacterial Discs

In this study, the antibacterial agents used in the disc diffusion method are shown in Table: 2.1.

Table 2.1.: Antibacterial discs (Bioanalyse, Ankara, Turkey)

Antibacterial Agent	Symbol	mcG
Amikacin	AK	30
Gentamicin	CN	30
Ampicillin/Sulbactam	SAM	20
Ceftriaxone	CRO	30
Meropenem	MEM	10
Ciprofloxacin	CIP	10
Trimethoprim/sulfamethoxazole	SXT	25

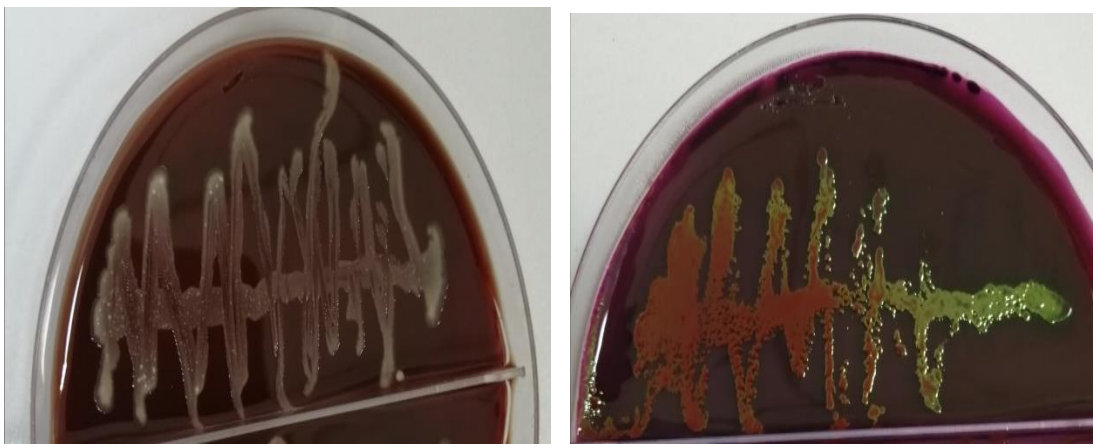

Figure: 2.2. *E .coli* growth on blood media and EMB media

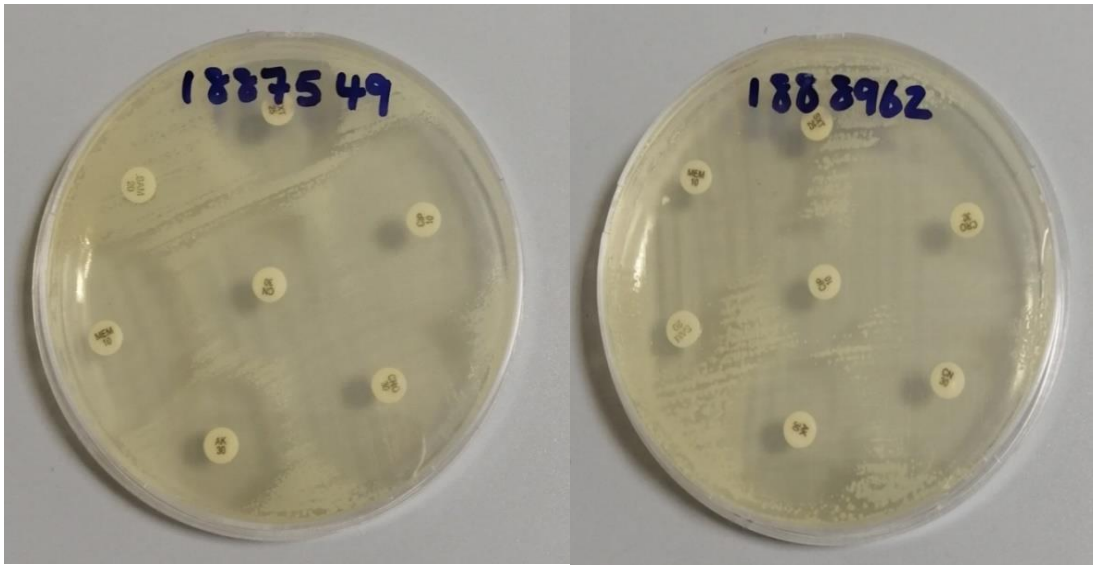

Figure:2.3. Bacterial susceptibility on Mueller-Hinton Agar

2.8. Storage and Usage Instructions

As the manufacturer's instructions, place the discs at -20 to +8 ° C after receipt. The expiry date is only valid for unopened blister packs which are kept under appropriate conditions. If a cartridge is open, it is best to store it for no more than 7 days. Allow room temperature to come in containers before opening to prevent condensation as this can reduce the antimicrobial agent's potency. If opened, the discs should be placed in the container given or any appropriate opaque airtight desiccated container within the dispenser to protect the discs from moisture. Containers will be placed in the refrigerator inside the dispenser and allowed to reach room temperature before opening to avoid condensation from the formation. Return unused discs to the refrigerator once the discs have been applied. First, use the oldest discs. Discard discs that have expired.

2.9. Bacterial Inoculum

From the primary isolation medium, five colonies showing similar morphology are either taken by direct colony suspension method and suspended by using a flamed loop or by using a sterile cotton swab, bacteria are extracted and suspended in a clean saline solution of 4-5 ml (0,85 percent NaCl). For 15 seconds, combine the cell

suspension using a vortex mixer. The turbidity is then noticeable and the density of the suspension is modified with the usage of a spectrophotometer. The turbidity needs to be balanced to 0,08 to 0,10 at an absorbance of 625 nm for the 0,5 McFarland standard. It needs to be used about an hour after the standard suspension has been prepared.

2.9.1. Inoculation into Agar Plates

Inoculation has been done according to the Kirby-Bauer method as follows:

1. Mix the prepared bacterial suspension well with a sterile cotton swab, and by gently pushing and spinning the swab within the container, the excess fluid of the swab is extracted above the fluid level.
2. Three times streak the whole agar surface of an MH-GMB layer, turning the plate 60 ° between streaks to achieve even inoculation.
3. For preventing the excessive wetness of the medium, allow Petri dishes to dry for 3 to 5 minutes, a maximum of 15 minutes, at room temperature.

2.9.2. Application of Antibacterial discs to Inoculated Agar Plates & Incubation

1. The cartridge is opened under the flame and then discs are discharged from the cartridge onto a clean Petri dish with the help of a flamed and cooled forceps.
2. The discs have to be distributed into the agar plates not less than 24 mm from the center to the center and 12 mm away from the edge of the agar plate within 15 minutes.
3. Once in touch with the surface of the agar, do not transfer the disk.
4. In an incubator set at 35 °C (± 2 °C), put the plates in an inverted position for one night in an aerobic atmosphere within 15 minutes of the application of the disks.
5. Incubate all plates for 20 to 24 hours.

2.9.3. Reading Inhibition Zones and Interpretation of Results

1. During overnight incubation, test the plates (20-24 hours). If the plate has been satisfactorily streaked and the inoculum is right, the resultant inhibition zones across the disk are circular, with a semi confluent growth area, uniformly clear.
2. Keep the plate over a dark, non-reflective backdrop illuminated with reflective light only a few centimeters above.
3. Measure the diameter of the zone to the nearest full millimeter at the point where there is a noticeable reduction in growth. (The emergence of pinpoint micro-colonies at the outside of the zone or large colonies within the inhibition zone must be overlooked).
4. If there is insufficient growth after 24 hours of incubation, re-incubate the plates and read them at 48 hours of initial incubation time.
5. Accordingly, the results obtained are either evaluated as susceptible (S), intermediate (I), or resistant (R) for clinical application.

2.10. Statistical Data Analysis

The data analysis was performed using SPSS version (25.0). The evaluation of the total percentages number analyzed by the Friquency test with the crosstable test, in order to investigate the real amount of each data percent in the study.

CHAPTER THREE: RESULT

3. RESULTS

3.1. Study Population

The contemporary study was conducted in the microbiology laboratory of the near east hospital in the Turkish Republic of Northern Cyprus (TRNC). There was a total of 105 samples for the study was implemented Between (July 2012 and August 2020) from human urine samples of the hospitalized patients from various hospital departments and without taken antibiotic at least 3 days. Moreover, the study was performed among the uropathogenic *E. coli* infection individuals, also, the sample was collecting from those individuals who expect from suffering urinary tract infection, In addition, the collecting information's according to the special questioners that related to the current study such as gender (Male 29 %, Female 76 %), age (Children 17.1 %, Adult 22.9 %, Elder 60.0 %), and type of patients was divided in to In-patient 28.6 % and Out-patient 71.4 %. Moreover, the evaluation of the uropathogenic *E. coli* strains by microbiological culture method (UPEC 36.2 %, Non-UPEC 63.8 %). In addition, Antibiotic-susceptibility and mannose-hemagglutination test performed and the features of the determination of MRHA, MSHA, Antibiotic Susceptibility test "AST") were recorded and analyzed.

Table 3.1: The distribution of patients within gender groups.

Gender	No. of Patient	Percent
Male	**29**	**27.6**
Female	**76**	**72.4**
Total	**105**	**100.0**

Table 3.2: The distribution of patients within age groups.

Age	No. of Patient	Percent
Child (1-17)	**18**	**17.1**
Adults (18-49)	**24**	**22.9**
Elder (≥ 50)	**63**	**60.0**
Total	**105**	**100.0**

Table 3.3: The in-patient and out-patient distribution of the specimens.

Patient Type	No. of Patient	Percent
In- Pateint	**30**	**28.6**
Out-Pateints	**75**	**71.4**
Total	**105**	**100.0**

Table 3.4: The distribution of the specimens from UPEC and NON-UPEC strains.

E. Coli	No. of Patient	Percent
UPEC	**38**	**36.2**
NON UPEC	**67**	**63.8**
Total	**105**	**100.0**

Table 3.5: The distribution of Mannose resistance hemagglutination(MRHA) and Mannose sensitive hemagglutiation (MSHA)

E. Coli		Total No. of Patient	Percentages
Non-UPEC		**67**	**63.8%**
UPEC	**MRHA**	**26**	**24.8%**
	MSHA	**12**	**11.4%**
Total		**105**	**100.0%**

Table 3.6: Antimicrobial Susceptibility Testing Pattern.

Antibiotics 105	Non UPEC 67 (63.8%)		UPEC				Total No. (%)	
			MRHA 26 (24.8%)		MSHA 12 (11.4%)			
	S	R	S	R	S	R	S	R
Amikacin	67 (63.8%)	-	26 (24.8%)	-	12 (11.4%)	-	105 (100%)	-
Gentamicin	48 (45.8%)	19 (18%)	24 (22.9%)	2 (1.9%)	7 (6.65%)	5 (4.75%)	79 (75.35%)	26 (24.65%)
Ampicillin/ Sulbactam	21 (19.95%)	46 (43.9%)	7 (6.65%)	19 (18%)	2 (1.9%)	10 (9.5%)	30 (28.5%)	75 (71.4%)
Ceftriaxone	33 (31.4%)	34 (32.5%)	8 (7.6%)	18 (17.1%)	5 (4.75%)	7 (6.65%)	46 (43.75%)	59 (56.25%)
Meropenem	66 (62.9%)	1 (0.95%)	26 (24.7%)	0 (0.0%)	11 (10.45%)	1 (0.95%)	103 (98.05%)	2 (1.9%)
Ciprofloxacin	30 (28.7%)	35 (33.3%)	12 (11.4%)	16 (15.2%)	7 (6.65%)	5 (4.75%)	49 (46.75)	56 (53.25%)
Trimethoprim/ sulfamethoxazole	29 (27.58%)	37 (35.37%)	14 (13.3%)	14 (13.3%)	6 (5.7%)	5 (4.75%)	49 (46.58%)	56 (53.42%)

CHAPTER FOUR: DISCUSSION

4.1 DISCUSSION

The most widely diagnosed renal infection is urinary tract infection (UTI). It is most generally linked with morbidity in both hospitalized and outpatient patients on a continuous basis. And there are almost 50-90 percent of all uncomplicated urinary tract infections, the most widespread organism seen is uropathogenic *Escherichia coli* (UPEC). UPEC are *Escherichia coli* strains that divert from their commensal position as intestinal flora, develop and persist in the urinary tract and show a range of virulence factors and strategies that permit them to invade the urinary tract and create diseases. Such strains of *E. coli* is closely correlated with uropathogenicity and is referred to as UPEC (Hryniewcz et. al., 2001, Dasgupta et. al., 2005).

The *E. coli* that cause urinary tract infection are not all strains of the intestinal tract but a subgroup chosen by factors that enhance extra-intestinal survival. These variables include flagella motility, structural characteristics such as fimbriae or pili, and chemical adhesion. Also considered to be the most commonly distributed among uropathogenic *E. coli* are the type-1 pili. On facet cells lining the bladder or vaginal epithelial cells, *E. coli* attaches to mannose containing glycoprotein receptors and is considered to be correlated with enhanced UTI frequency. An number of other virulence factors that impart on certain fecal *E. coli* have also been reported.The capacity of *E. coli* to colonize the mucosa of the vagina and induce symptomatic urinary diseases. Such a subset of *E. coli* collectively is referred to as uropathogenic *E. coli* (UPEC) Clones (Emody et. al., 2003; Todar et. al., 2008).

These species need to be destroyed to prevent treatment failure that could contribute to the spread of virulent *E. coli* and resulting morbidity (Lescure et. al., 2001). However, the capability of *E. coli* is rapidly causing urinary tract infections, although

the convenience of managing these infections is becoming increasingly elusive leading to multidrug antibiotic resistance to first-line antibiotics such as cotrimoxazole, ampicillin, and nitrofurantoin. The recent rise in resistance to classes of fluoroquinolones such as ciprofloxacin and levofloxacin is of greater concern (Hooton, 2003; Karlowsky et. al., 2006). The growing capacity of *E. coli* induces urinary tract infections and the challenge faced by multidrug antibiotic resistance in controlling these infections involves updating the information of their drug resistance in a given setting. This is especially important in a worldwide population, particularly in certain countries where, with or without prescription, all types of antibiotics are accessible over the counter. In patients with UTI, the classification of UPEC isolates and their association with cycles of antibiotic resistance is not well established, especially in the Middle East. Understanding of virulence factors definitely helps to correctly treat UTI and, finally, to avoid antimicrobial resistance (Okeke and Lamikanra, 2001) (Olorunmola, F. et al, 2013).

Cyprus (TRNC). A total of 105 samples for the study was executed from urine samples of hospitalized patients from various hospital departments and outpatient. Moreover, the study was performed among the uropathogenic *E. coli* infection individuals, also, the sample was collecting from those individuals who expect from suffering urinary tract infection, Also, The findings of this study showed a greater percentage of female isolates (72.4 percent) than male isolates (27.6 percent). (Aiyegoro et. al., 2007: Omoregie et. al., 2008). and the age prevalency was divided into three categorical stages like (Children 17.1 %, Adult 22.9 %, Elder 60.0 %), and type of patients was divided into In-patient 28.6 % and Out-patient 71.4 %. Moreover, the evaluation of the uropathogenic *E. coli* strains by microbiological culture method (UPEC 36.2 %, Non-UPEC 63.8 %). In addition, Antibiotic-susceptibility and mannose-hemagglutination test was performed and the features of the determination of MRHA, MSHA, Antibiotic Susceptibility test (AST) were recorded and analyzed. Whatever there are different type of antibiogram where examined and the result showed various resistance and susceptibility as the following: Haemagglutination is

referring to red blood cell agglutination, generally known as fimbriae-mediated haemagglutination, which is proportionate to the capacity to bind to uroepithelial cells. The blood group antigen serves as a receptor for these species in mannose-resistant haemagglutination (MRHA) and has strengthened uroepithelial cell binding (Emody et. al., 2003). In the present study, the total percentages of the (MRHA) were about (24.8%), and it is the value is lower than the reported in India which is mention that the whole rate of the mannose resistance was about (30.9 %)(Raksha et. al., 2003). In another hand the result of (MRHA) of other research experiment show (13.9%) (Olorunmola, F. et al, 2013), which is lower than our result when comparing with each other.

In our study there was a wide range of both types of antibiogram (resistance and susceptibility) of the *E. coli* culture were isolate (Table 3.6) in all of the commonly used antibiotics in this environment, specifically, Amikacin, Ampicillin/Sulbactam, gentamicin, ciprofloxacin, Trimethoprim/sulfamethoxazole, Ceftriaxone, Meropenem.

As a result comparison between our observation and other research experimental we noted that there are some differences in statistical analysis number such as UPEC rate in our study was about 36.2%, while there was no resistance were observed in both MRHA and MSHA to amikacin drug. But in another hand, in Shah, C., et al, 2019, Resistance to 22% of UPEC was the result of the Amikacin drug, while only 10% resistance was observed and a strong association between hemolysin development and antimicrobial resistance was emulated in the sample. In 41% of hemolysin-positive strains, Amikacin was resistant, while in non-hemolysin, only 12% resistance was found. In another report, 92% were isolated as sensitive to Amikacin, which is close to our definition in the case of susceptibility (Kausar, Y., et al, 2009). The study emulated a correlation between the antibiotic resistance in both MSHA and MRHA production, moreover, the In the previous study Shah, et al, 2019, clarify that there was resistance in ampicillin/sulbactam observed in 69% of MRHA while only 32%

resistance in ampicillin/sulbactam was observed in MSHA, Nearly to our result the total number of MSHA toward ampicillin/sulbactam was 28.5%, in another hand, the MRHA was 71.4%.

Gentamicin resistance limits the possibilities for successful management of infections of the urinary tract caused by *E. coli* in the environment. This is because, leading to their extremely high efficiency against pathogens immune to other antibiotics, antibiotics have been considered life-threatening antibiotics, moreover, there was 75.35% of the antibiotic Gentamicin was sensitive and 24.65% were detected as resistant, As support, our finding, the experimental study the Fatima, N., et al, 2012, found out of 250 samples there were 103 samples sensitive to gentamicin which nearly equal to (41.2%), and 147 (58.8%)sample were found as a resistance.

Among our isolates, the resistance to ciprofloxacin was higher (53.25%) than among Eryılmaz M, et al, 2010 isolates, and is the result of ciprofloxacin resistance observation was (15%). Moreover, the Sanchez et al, 2010, proposed that an improvement in ciprofloxacin resistance among *E. coli* strains were the result of the extensive usage of this antibiotic in the early 2000s in the treatment of uncomplicated UTIs.

In our study also the antibiogram test performed to Trimethoprim/ sulfamethoxazole, and as a result of comparation between our observation and other research experimental we noted that there are some differences, the total number of mannose resistance hemagglutination (MRHA) was 53.42%, while in the Shah, et al, 2019, the MRHA was 67%, which is particularly similar to our result surveillance. Following the MSHA in our data was about 46.58%, but Shah, et al, 2019, mention the MSHA rate was 24%, which have dissimilar to each other.

Jadhav, S., et al, 2011, mention out of 150 samples of UPEC patterns the antimicrobial susceptibility test perform toward Ceftriaxone, and the result shows there was 48% are resistant, while, others are sensitive 52%. In another hand,

antibiogram performed by Fatima, N., et al, 2012, there was also 108(43.2%) found as sensitive, and 142(56.8%) were observed as resistant. In our experimental study and among to the UPEC, the antibiotic susceptibility result show there are 46(43.75%) samples are sensitive, and 59(56.25%) were showed as a resistance, finally, the other two experimental study idea are very similar to our result of both type of antibiotic resistance and sensitivity.

Resistance to antimicrobials among *E. coli* causing UTIs is rising in several countries around the world. In the previous study Shah, et al, 2019, explain that there was resistance in meropenem observed 23% of MRHA while about 50% resistance in meropenem was observed in MSHA, But our experimental study result shows a different amount of mannose-sensitive and resistance, in our result the total number of MSHA toward resistance of meropenem was 0.95%, in another hand, the MRHA was zero.

Haemagglutination refers to red blood cell agglutination, generally known as fimbriae-mediated haemagglutination, which is proportionate to the ability to bind to uroepithelial cells. The blood group antigen serves as a receptor for these species in mannose-resistant haemagglutination (MRHA) and has strengthened uroepithelial cell binding (Emody et. al., 2003). The maximum percentages of the (MRHA) is approximately (24.8%) in the current study, and the value is lower than that recorded in India, which reports that the total rate of the (MRHA) was approximately (30.9%) (Raksha et. al., 2003). In another hand the result of (MRHA) of other research experiment show (13.9%) (Olorunmola, F. et al, 2013), which is lower than our result when comparing with each other.

It has also been shown that the empirical treatment of *E. coli* UTI in this environment is no longer running. Until the antibiotic is chosen for treatment, culture and susceptibility checks should be conducted on infecting bacteria in order to prevent treatment failure. This will prevent the indiscriminate use of antibiotics and reduce the

selective pressure that could contribute to the spread of uropathogenic *E. coli* that is virulent and resistant in the cultural environment (Olorunmola, F. et al, 2013). Therapy should be advocated as far as possible after culture and sensitivity have been conducted, in view of the emerging drug resistance among UPEC. Which would not only help to manage patients better, it would also prevent the indiscriminate use of antibiotics and avoid bacterial drug resistance from growing further.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The current study was the design of Study in order to evaluate the UPEC and it is the production of mannose-hemagglutination with antibiotic susceptibility. A total of 105 samples for the study was executed Between (July 2012 and August 2020) from urine samples of hospitalized patients from various hospital departments. Moreover, the study was performed among to uropathogenic *E. coli* infection individuals, and the detection and evaluation uropathogenic E .coli strains by microbiological culture method, in addition, Antibiotic-susceptibility and mannose-hemagglutination test, Moreover, the determine MRHA, MSHA, Antibiotic Susceptibility test "AST") were recorded and analyzed.

In conclusion, analysis of the antimicrobial susceptibility shows a different level of both types of resistance and susceptibility to the Amikacin, Gentamicin, Ampicillin/ Sulbactam, Ceftriaxone, Meropenem, Ciprofloxacin, Trimethoprim/ sulfamethoxazole, After the assessment of MRHA and MSHA between UPEC virulence factors and UPEC antimicrobial resistance. Routine checking and co-relation of HA of these factors is recommended. These results would definitely help to clarify the pathogenicity and careful treatment of patients with UTI, thus minimizing the usage of antibiotics improperly.

5.2. RECOMMENDATION

These results would definitely help to clarify the pathogenicity and careful treatment of patients with UTI, thus reducing the usage of antibiotics improperly.
Moreover:

1. The randomly prescribing of antibiotics should be avoided.
2. For all patients who has UTI, a culture examination should be done.
3. Randomly taking antibiotics by individuals should be avoided.

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Biology teacher for high school level	In Kurdistan school	2017-2018
Working in Laboratory	In Kalakchi hospital	2016-2017
Working In Microbiology Lab / Near East Hospital	Near East Hospital	2019-2020

Courses and Certificate

Name	Name of the Institution where take place	Year
Microbiology Laboratory Practical Training	Near East University, North Cyprus	2019
Rare Disease Day Symposium in Health Certificate (Attendance)	Faculty of Medicine, Near East University	2020
9th National and 2nd International Congress of Hydatidology Certificate (Attendance)	DESAM Institute, Near East University, Nicosia	2018
Mathematical Modeling in Health Certificate (Attendance)	DESAM Institute, Near East University, Nicosia	2019
Parasitology Academic Course: Essential and Application	Turkish Microbiology Society, TMC-KKTC Microbiology Platform, Nicosia	2020
Certification of Completing (16 Hours) of an online "Media Course Correspondent"	At Macos Organization for Education & Learning-Innovation & Training Iraq-Kurdistan	2019
Certification of participating (10 Hours) of an online	At knowledge Academy & K.N.N.G.O Iraq-Kurdistan	2020

Computer Knowledge

Program	Use proficiency
SPSS	Good
Python Programming Language	Excellent
Common Computer Programs and Skills	Excellent

Other languages

Languages	Speaking	Writing	Reading
Arabic	Excellent	Excellent	Excellent
English	Excellent	Excellent	Excellent
Turkish	Good	Beginner	Beginner