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MOLECULAR CHARACTERISATIONS OF SOME VIRULENCE GENES OF *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN THE SOUTH OF IRAQ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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ZAINAB FAKHRI ABDULHASAN ALSURAIFI

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August 2023

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ABSTRACT

MOLECULAR CHARACTERISATIONS OF SOME VIRULENCE GENES OF *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN THE SOUTH OF IRAQ

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Master of Science in Biology

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Acute urinary tract infection (UTI) is a common health concern affecting individuals worldwide. Prompt and accurate diagnosis is crucial for effective treatment, as UTIs can lead to severe complications if left untreated. This study aims to investigate the prevalence of *Escherichia coli* (*E. coli*) in UTI patients and assess their antibiotic susceptibility patterns. Additionally, the presence of specific genes associated with antibiotic resistance in *E. coli* isolates is examined. A total of 80 urine samples were collected from patients with clinical features indicative of UTI who visited Al-Hussein Teaching Hospital from January 2022 to June 2022. Fifty healthy individuals were included as the control group. UTI patients were diagnosed based on clinical criteria, such as dysuria, frequency, urgency, fever, and loin pain. The age and gender distribution of UTI patients was analyzed. Among the UTI patients, females exhibited a significantly higher prevalence, with 49 female patients (61.25%) compared to 31 male patients (38.75%). The percentage of UTI cases was highest in females above 60 years (15.0%) and in females aged 50-59 years (13.75%), while males in these age groups recorded 8.75% infection rate. *E. coli* was the most prevalent bacterium, accounting for 35.0% of the patient samples, followed by *Pseudomonas aeruginosa* at 15.0%. All 28 *E. coli* isolates were identified through standard gram-negative card characteristics, showing positive results for catalase, methyl red, and lactose fermentation tests, but negative results for oxidase, citrate, and vogas-proskauer tests. Antibiotic susceptibility testing revealed that 7 antibiotics displayed resistance to *E. coli*, 2 antibiotics were

sensitive, and 3 antibiotics demonstrated moderate resistance. Furthermore, the prevalence of three genes (*hlyA*, *vat*, and *mcr-1*) associated with antibiotic resistance in *E. coli* isolates was investigated using Real Time PCR, with positive rates of 26.25%, 23.75%, and 12.50%, respectively. This study provides valuable insights into the prevalence and antibiotic susceptibility of *E. coli* isolates in UTI patients from Al-Hussein Teaching Hospital. The high occurrence of antibiotic resistance genes highlights the importance of continuous surveillance and appropriate antibiotic stewardship to combat UTI infections effectively.

2023, 56 pages

Keywords: *Escherichia coli*, Urinary tract contaminations, Virülance genes

ÖZET

GÜNEY IRAK'TA İDRAR YOLLARI ENFEKSİYONU OLAN HASTALARDAN İZOLE EDİLEN *ESCHERICHIA COLI* 'NİN BAZI VİRÜLANS GENLERİNİN MOLEKÜLER ÖZELLİKLERİ

Zainab Fakhri Abdulhasan ALSURAIFI
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Eş Danışman: Dr. Öğr. Üyesi Ahmed Khassaf ATYA
Ağustos 2023

Akut idrar yolu enfeksiyonu (İYE), dünya genelinde bireyleri etkileyen yaygın bir sağlık sorunudur. Etkili tedavi için zamanında ve doğru teşhis, İYE'nin ciddi komplikasyonlara yol açabileceği göz önünde bulundurulmalıdır. Bu çalışma, İYE hastalarında *Escherichia coli* (*E. coli*) yaygınlığını ve antibiyotik duyarlılık örüntülerini araştırmayı amaçlamaktadır. Ayrıca, *E. coli* izolatlarında antibiyotik direnci ile ilişkili belirli genlerin varlığı incelenmektedir. Ocak 2022 ile Haziran 2022 arasında Al-Hussein Öğretim Hastanesini ziyaret eden, İYE'nin klinik özelliklerini gösteren hastalardan toplam 80 idrar örneği toplandı. Kontrol grubu olarak 50 sağlıklı birey dahil edildi. İYE hastaları, disüri, sık idrara çıkma, aciliyet, ateş ve bel ağrısı gibi klinik kriterlere göre teşhis edildi. İYE hastalarının yaş ve cinsiyet dağılımı analiz edildi. İYE hastaları arasında, dişi hastalarda belirgin bir şekilde daha yüksek yaygınlık gözlendi; 49 kadın hasta (%61.25) ve 31 erkek hasta (%38.75). İYE vakalarının en yüksek yüzdesi, 60 yaşın üzerindeki kadınlarda (%15.0) ve 50-59 yaş aralığındaki kadınlarda (%13.75) görüldü, bu yaş gruplarında erkeklerde enfeksiyon oranı %8.75 olarak kaydedildi. *E. coli*, hastaların %35.0'ini oluşturan en yaygın bakteriyi temsil ediyordu, onu %15.0 ile Pseudomonas aeruginosa izliyordu. Tüm 28 *E. coli* izolatı, katalaz, metil kırmızı ve laktoz fermentasyon testlerinde pozitif sonuçlar verirken, oksidaz, sitrat ve vogas-proskauer testlerinde negatif sonuçlar verdiği standart gram-negatif kart özellikleriyle tanımlandı. Antibiyotik duyarlılık testi, *E. coli*'ye karşı 7 antibiyotiğin dirençli olduğunu, 2 antibiyotiğin duyarlı olduğunu ve 3 antibiyotiğin ılımlı dirence

sahip olduğunu ortaya koydu. Ayrıca, *E. coli* izolatlarında antibiyotik direnci ile ilişkilendirilen üç genin (*hlyA, vat* ve *mcr-1*) yaygınlığı, Real Time PCR yöntemiyle incelendi ve sırasıyla %26.25, %23.75 ve %12.50 oranlarında pozitif saptandı. Bu çalışma, Al-Hussein Öğretim Hastanesi'ndeki İYE hastalarında *E. coli* izolatlarının yaygınlığı ve antibiyotik duyarlılığına dair değerli bilgiler sunmaktadır. Antibiyotik direnç genlerinin yüksek sıklığı, etkili bir şekilde İYE enfeksiyonlarıyla mücadele etmek için sürekli gözetim ve uygun antibiyotik yönetiminin önemini vurgulamaktadır.

2023, 56 sayfa

Anahtar Kelimeler: *Escherichia coli*, İdrar yolu kontaminasyonları, Virülans genleri

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Zainab Fakhri Abdulhasan ALSURAIFI
Çankırı-2023

CONTENTS

LIST OF SYMBOLS

/	Divide
g	Gram
>	Greater than
<	Less than
L	Liter
µL	Microliters
mL	Milliliter
-	Minus
min	Minute
%	Percent
+	Plus
rpm	Revolutions per minute

LIST OF ABBREVIATIONS

APC	Antigen presenting cells
APEC	Avian-pathogenic *E. coli*
CNF1	Cytotoxic necrotizing figure 1
DAEC	Diffuse-adhering contains *E. coli*
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EF	Edema factor
EIEC	Enteroinvasive *E. Coli*
EPEC	Enterohemorrhagic *E. Coli*
ETEC	Enterotoxigenic *Escherichia coli*
ExPEC	Extraintestinal pathogenic *Escherichia coli*
GUE	General urine Examination
LF	Lethal factor
LPS	Lipopolysaccharide
MDR	Multidrug-resistant
NMEC	Neonatal meningitis *E. coli*
PA	Protective antigen
PMCR	Plasmid-Mediated Colistin Resistance
SEPEC	Sepsis *E. coli*
SPATE	Serine protease autotransporters of the *Enterobacteriaceae*
SPATEs	Serine protease autotransporters
TCR	T cell receptor
UPEC	Uropathogenic *E. coli*
UTIs	Urinary tract infections

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1. INTRODUCTION

Escherichia coli (E. coli) could be a Gram-negative, rod-shaped bacterium within the Enterobacteriaceae family, this kind of bacteria is found within the typical greenery of the intestinal tracts of some animals and people. *E. coli* commonly causes urinary tract contaminations (UTIs) in people. Women and children are the gather most influenced by UTIs. In women, UTI can be seen for the most part amid pregnancy, menopause, or hormonal changes amid the ripe period. Anti-microbials utilized in the treatment of bacterial diseases in veterinary and pharmaceutical settings can cause serious public health issues by supporting the development of resistant strains. In this context, "supporting" means that the use of antibiotics encourages the growth and spread of antibiotic-resistant bacteria, making them stronger and more difficult to treat. This is a significant concern as it reduces the effectiveness of antibiotics, making it harder to combat bacterial infections in both animals and humans (Braoios *et al.* 2009).

Pathogenic *E. coli* strains are separated into two classes: enteric/diarrheal *E. coli* (DEC) and extraintestinal *E. coli* (ExPEC), there are six diverse as illustrated in below *E. coli* pathotypes are:

1- EPEC.
2- EHEC.
3- ETEC.
4- EAEC.
5- EIEC.
6- DAEC.

ExPECs incorporate neonatal meningitis *E. coli* (NMEC), avian-pathogenic *E. coli* (APEC), and uropathogenic *E. coli* (UPEC) (Russo and Johnson 2000, Kaper *et al.* 2004). Among the ExPECs, uropathogenic *E. coli* (UPEC) is the foremost common cause of human UTIs. Avian pathogenic *E. coli* (APEC) strains share a few destructiveness characteristics with ExPEC strains from human diseases and have been related with extraintestinal diseases of poultry such as airsacculitis (aggravation of the

discuss sac), pericarditis (aggravation of the pericardium), cellulitis, and septicemia". ExPEC strains contain numerous harmfulness markers that play a part in extraintestinal diseases (Dho-Moulin and Fairbrother 1999).

For UPEC to colonize the urinary tract and create particular harmfulness components that encourage its attack into have cells (Donnenberg 1996). These components incorporate adhesins, capsule, aerobactin, poisons, and proteases, which help in convenience in mucosal tissues, restrain resistant defense, and advance the intrusion of regularly sterile urinary tract and tissues (Bauer *et al.* 2002). UPEC strains have a better predominance of harmfulness variables than commensal *E. coli* (Guyer *et al.* 2000). Be that as it may, half of all UPEC separates have few of the destructiveness components that have been characterized so distant, and in this way other uncharacterized bacterial factors play a vital part within the pathogenesis of UTI. Urinary tract infection, which most often affects an adult, is defined as a chronic or temporary disorder that occurs in the urinary tract system. There are many symptoms that appear in the infected person; For example: painful urination, frequent urination, sometimes blood in the urine, it can be predicted that there are 100 pathogenic bacteria of the urinary tract/mL of urine (Stamm 1982).

In truth, approximately 50% of UTIs don't come to hospitals. Alternatively, microscopic organisms within the bladder epithelium may return occasionally and cause repeat of UTI (Silverman *et al.* 2013). Contamination of the kidneys (pyelonephritis) is more serious and is demonstrated by side effects that incorporate chills, tall fever, sickness, joint and muscle hurts and flank torment. On the off chance that cleared out untreated, pyelonephritis can cause kidney disappointment, bacteremia, and sepsis (Mehnert-Kay 2005).

UPEC strains express an assortment of harmfulness variables to effectively colonize the unforgiving environment of the host's urinary tract. These incorporate different fimbrial and afimbrial adhesins, siderophores, and discharged poisons. UPECs are mindful for 80% of UTIs. UPEC can too be transmitted from individual to individual through coordinate contact with sullied nourishment, water, or coordinate contact. Generation of

poisons by UPEC is a critical destructiveness calculate since they can actuate a fiery reaction and lead to side effects of UTIs. UPEC-associated poisons incorporate α-hemolysin (*hlyA*), TosA, cytotoxic necrotizing figure 1 (CNF1), intestinal colonization-associated protease (Pic), discharged AT poison (sat), and vacuolating AT poison (*vat*). Pic, sat and *vat* are emitted proteins of ~110KDa estimate and have a place to serine protease autotransporters of the Enterobacteriaceae (SPATE) protein family (Nichols 2016).

Nearly about 50% of the total UPEC strains emit hemolysis, a cytotoxic pore-forming poison (Nhu *et al.* 2019). One of the foremost imperative destructiveness variables of UPEC is *hlyA*. *HlyA* is dependable for around 50% of UTIs that cause kidney complications. *hlyA* could be a powerful and omnipresent cytolysin. It is the prototypical part of the repeats-in-toxin (RTX) family. The part of α-hemolysin as a destructiveness figure is said to differ depending on the strain and the site of contamination within the have. *hlyA* plays a part as a harmfulness figure within the colonization of UPEC microscopic organisms within the intestinal tract in intestinal contaminations (O'Hanley *et al.* 1991, Ristow and Welch 2016).

In expansion, there's a more prominent concern with expanding anti-microbial resistance among the pathogens that cause UTIs (Flores-Mireles *et al.* 2015).

In later a long time, colistin was considering a viable treatment choice for the fast increment of MDR gram-negative pathogens. In any case, it is detailed that the predominance of the portable colistin resistance quality (*mcr-1*) in some animals and people around the world is additionally critical. It was initially found that the *mcr-1* quality is broadly plasmid-mediated. As of now, microscopic organisms creating *mcr-1* have been detailed in numerous locales in China. *mcr-1* was shown to be very common in *E. coli*. A few reports propose that the *mcr-1* quality may coexist with other resistance qualities (such as CRE/ESBL) in *Klebsiella pneumoniae* in addition to *E. coli*, conceivably causing it to end up sedate safe and increment treatment challenges. For this reason, the rise and spread of the *mcr-1* quality in people ought to be closely observed. Resistance to ciproflaxacin (47.3%), levoafsakine (43.6%) and cephalosporins

(27.6%) is as often as possible watched. Also, 63% of strains are thought to be MDR (Ramírez-Castillo *et al.* 2018).

1.1 Aim of Study

The aim of this thesis study is to perform molecular characterizations of some selected of the virulence genes of *E. coli* which were isolated from UTI patients in the south of Iraq.

2. LITERATURE REVIEW

2.1 *Escherichia coli*

Numerous strains of *E. coli* are considered a component of the typical vegetation found within the human and animal intestinal tracts. In spite of the fact that the *E. coli* strain is common, it seldom causes clinical UTIs. In any case, a few strains of *E. coli* carry destructiveness qualities that permit them to cause intestinal and extra-intestinal contaminations (Figure 2.1) (Croxen and Finlay 2010, Kaper *et al.* 2004). Concurring to the phylogenetic gathering strategy, *E. coli* is separated into four primary classes (A, B1, B2, and D.). By and large, pathogenic strains encoding harmfulness components have a place to bunches B2 and D, while most fecal *E. coli* strains having a place to groups A and B1 don't have destructiveness variables.

Among *E. coli* pathotypes, ExPEC are important etiological factors of bacteremia and other systemic infections. EXPEC is generally classified in different groups according to the anatomical site of infection, there is a relationship between UPEC and UTI (Toval *et al.* 2014).

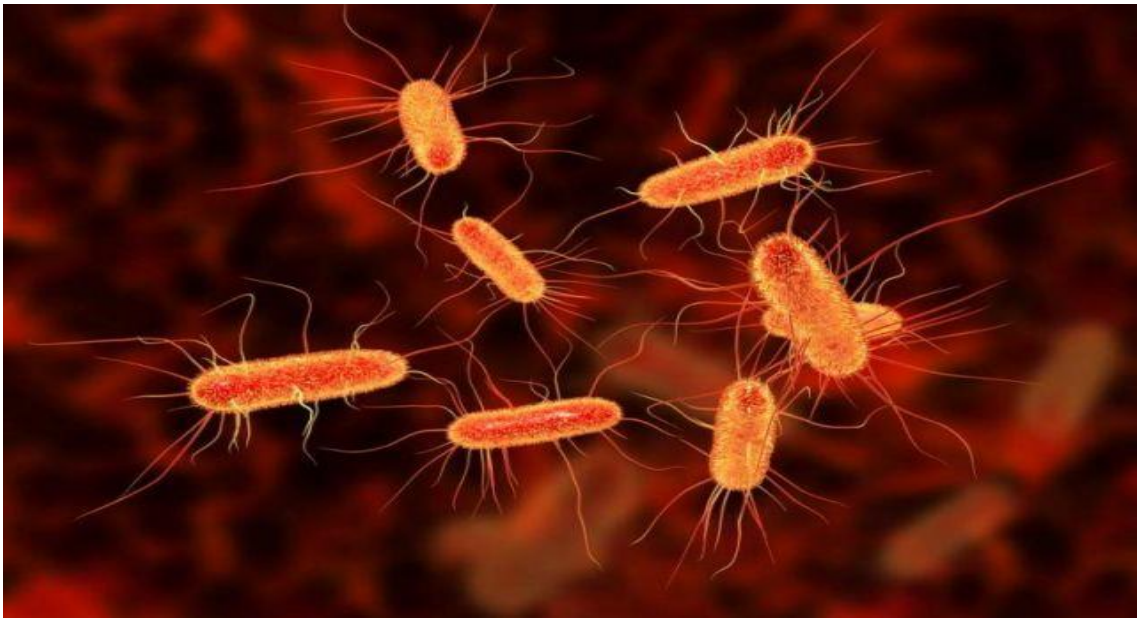

Figure 2.1 *Escherichia coli* (Croxen and Finlay 2010)

2.2 Uropatogenic *E. coli*

UPEC may be a pathotype of ExPEC and constitutes the intestine microbiome, inside the intestine, UPEC once in a while causes any complications and includes a useful advantageous relationship with the intestine microflora. ExPEC are facultative pathogens, they are portion of the typical human being's intestinal vegetation, but, the nearness most probably in relation with a few irresistible infections such as UTI, neonatal meningitis *E. coli* (NMEC) and sepsis *E. coli* (SEPEC), the foremost common being UTIs (Figure 2.2) (Foxman 2014, Sarowska *et al.* 2019).

UPEC has the capacity to spread and colonize other have situations for instance; the urinary tract and bloodstream. Destructiveness variables, such as poisons, can to cause harm whereas altering the have to advance contamination (Flores-Mireles *et al.* 2015) may be a pathotype and constitutes the intestine microbiome. Within the intestine, UPEC once in a while causes any complications and encompasses an advantageous advantageous relationship with the intestine microflora. ExPEC are facultative pathogens that are portion of the ordinary human intestinal vegetation, but their nearness may be related with a few irresistible illnesses such as UTI, neonatal meningitis *E. coli* (NMEC) and sepsis *E. coli* (SEPEC), the foremost common being UTIs (Figure 2.2) (Foxman 2014, Sarowska *et al.* 2019).

UPEC has the capacity to spread and colonize other have situations such as the urinary tract and circulation system. Harmfulness components such as poisons can moreover cause hurt whereas adjusting the have to advance contamination (Flores-Mireles *et al.* 2015).

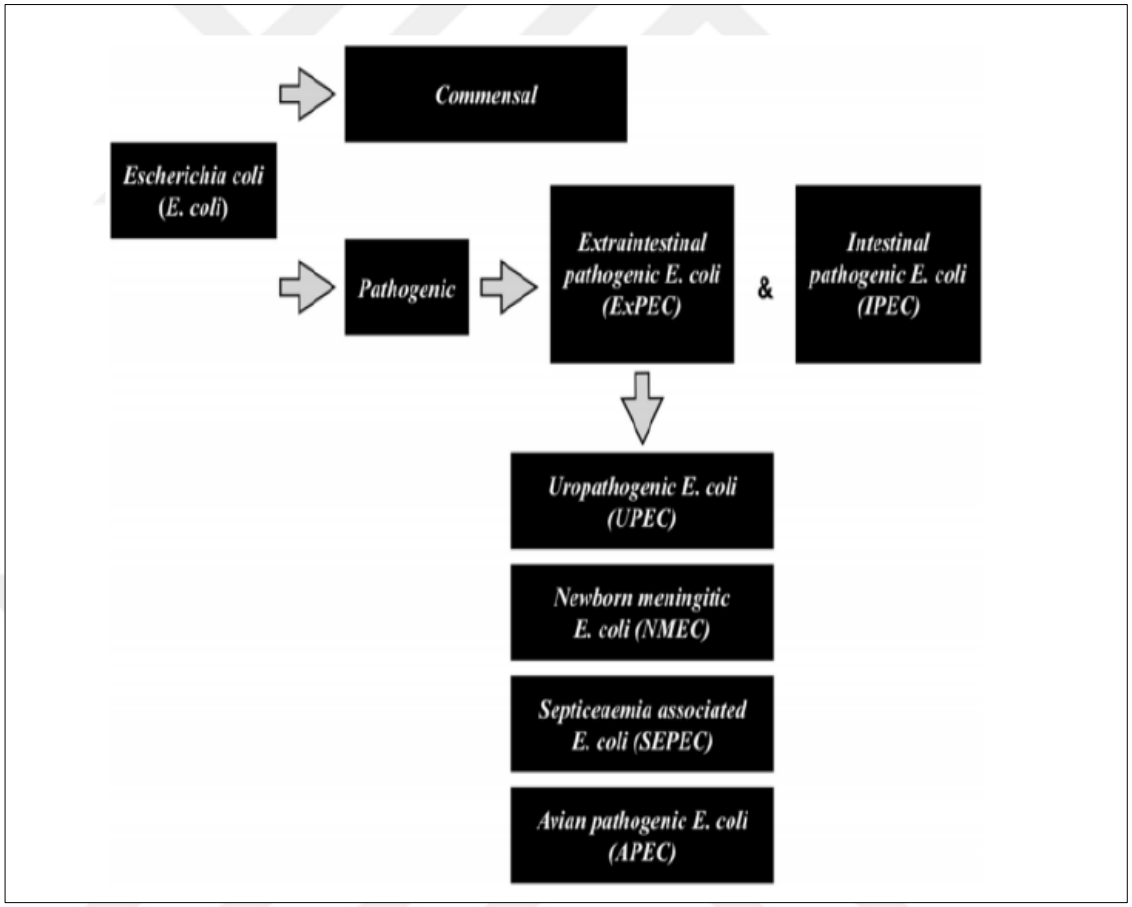

Figure 2.2 Pathogenic diversity of *E. coli* strains (Sarowska *et al.* 2019)

UPEC is the causative microbes in most UTIs. UPEC cells follow to bladder epithelial cells, attack them and cause uropathogenicity. Obtrusive UPEC cells can survive as a stationary intracellular store for removal into the extracellular environment or to outlive as an intracellular bacterial community and along these lines cause diseases. The capacity of UPEC to follow to bladder epithelial cells is considered the foremost basic calculate in uropathogenicity. UPEC alludes to multiple pili frameworks to get a shield within the urinary framework and encourage connection to the shallow bladder epithelial cell layer (Figure 2.3) (Mann *et al.* 2017).

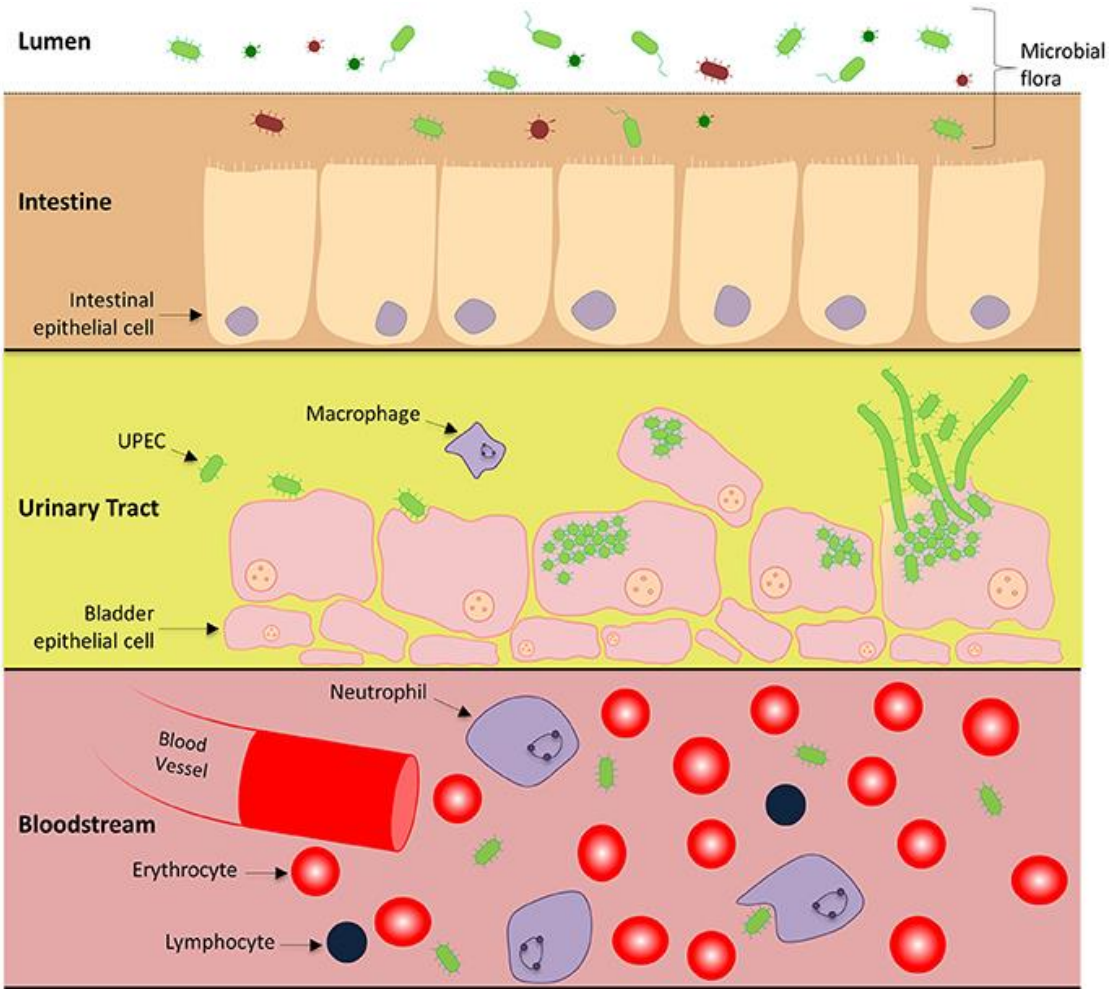

Figure 2.3 UPEC pathogenesis in multiple microenvironments (Mann *et al.* 2017)

Whereas urethra contaminations are ordinarily intense and can be effectively treated with anti-microbials, they can end up inveterate with serious harm to the bladder and kidneys. Diligent and incessant UTIs are amazingly troublesome to treat, in portion due to the torpid nature of intracellular UPEC, where have epithelial cells can "stir" to start a moment wave of contamination (Gilbert *et al.* 2017). These are each: cystitis (bladder), pyelonephritis (kidney), and bacteriuria (pee) (Foxman 2003).

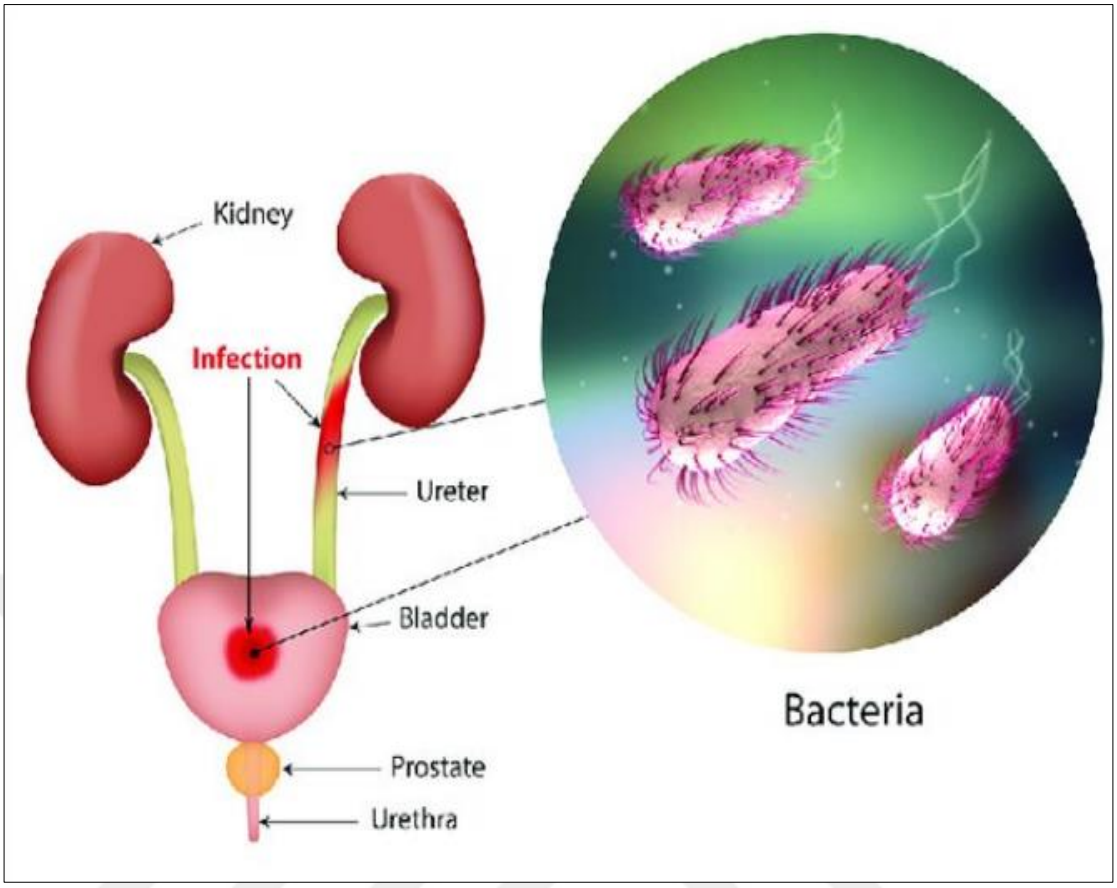

Figure 2.4 Urinary tract and sites of infection (Gilbert *et al.* 2017)

2.3 Virulance Factors

Pathogenic microorganisms (infectious or disease-causing agents, pathogens) vary widely in their severity, degree or strength (virulence) to cause disease in humans and animals (Brooks 1991).

1) Exotoxins: These types of toxins are usually heat-sensitive and soluble substances of protein character and are synthesized by toxigenic microorganisms. Exotoxins can be secreted in vivo and in vitro. There are many aerobic, anaerobic, spore or non-spore-forming bacteria and fungi that can synthesize exotoxins (Barbosa *et al.* 2010).

Toxins can cause not only infections but also death in living things, depending on their amount and activity. Among the most effective bacterial toxins to date, the exotoxin of

Clostridium botulinum has been reported. 1 MLD of *Clostridium botulinum* A for mouse (minimum lethal dose) 2. 5x10-5 mcg (purified toxin); Toxin of C. tetani 1 MLD for mouse 4x10-5 mcg; It has been stated that 1 MLD of diphtheria toxin for guinea pigs is 6x10-2 mcg and 1 MLD of *S. aureus alpha* toxin for rabbit is 5 mcg (Spanu *et al.* 2012).

Some properties of exotoxins are briefly as follows:

a) Exotoxins, plasmids in some microorganisms (B. anthracis, C. tetani); They are specific by bacteriophage (prophage) in *Corynebacterium diphtheriae* and *Clostridium botulinum*, and by genomic DNA (chromosome) in some. If the plasmid or phages are removed or removed from the bacteria, the microorganisms become atoxigenic or apathogenic (Barth and Aktories 2011).

b) Exotoxins are of protein character and are generally sensitive to heat (60-80°C) (thermolabile, TL). In contrast, enterotoxins of S. aureus and *E. coli* are resistant to temperatures above these degrees (100° C) (thermostable, TS).

c) Even small amounts of exotoxins have virulence in the susceptible host. After a certain incubation period, intoxications occur in susceptible experimental animals, which are characterized by specific disease manifestations, according to the mechanism of action of the toxin.

d) Exotocins are also immunogenic. They stimulate the synthesis of specific antibodies in the body (antitoxic antibodies, antitoxins). These antibodies neutralize the toxin in vivo or in vitro, thereby removing its ability to cause disease ((Barth and Aktories 2011).

e) Some physical (heat) and chemical substances (formaldehyde, iodine, etc.) inactivate the toxin, eliminating its ability to cause disease and causing it to become toxoid. Although toxoids do not have virulence, they can stimulate antibody synthesis when

administered to living organisms. Therefore, they have immunogenicity and are used as vaccines (Brooks 1991).

Exotoxins are divided into several categories according to the tissues and/or organs they affect in the body. Neurotoxins (*S. aureus*, *C. tetani, and Clostridium botulinum*), Enterotoxins (*E. coli, S. aureus*, *V. cholerae*, *C. perfringens, S. dysenteriae*, *Klebsiella sp*, etc.) and Cytotoxins (synthesized by many microorganisms), hemolysin, leucocidin, dermonecrotoxin, hepatotoxin, etc.) (Spanu *et al.* 2012).

However, just as a microorganism synthesizes more than one type of toxin, a toxin can affect several tissues or organs. Therefore, this basic classification may change over time and when necessary.

Although exotoxins are different from each other in character and activity, some of them are similar in structure. This similarity is often described as the "A-B model". This model is also known as the structural dimeric model. Accordingly, some toxins are composed of two subunits. One of them is the A fragment, which has an enzymatic feature and produces a toxic effect on the cells of the human's host, and the other is the B fragment, which allows the toxin to bind to specific receptors on the host cell surface. Although the isolated A subunit has a toxic effect, it does not have the ability to bind to cells. The B subunit can bind to cells but is nontoxic and biologically inactive. Two main mechanisms are suggested for the entry of the toxin molecule into the cell. One of them, the B subunit of the toxin, binds to specific receptors on the cell surface. A lysis occurs on the cell surface and through the specific channels formed, fragment A enters and reaches the cytoplasm. Fragment B stays out. The other view is that after the B subunit of the toxin is attached to the cell, the entire molecule (fragments A and B) is internalized by endocytosis. This type of entry is somewhat similar to pinocytosis. In this second mechanism, the entire molecule is collected in vesicles and then the B subunit is separated from A and brought to the cell surface. The A subunit enters the cytoplasm and shows its activity by going to the target area from there (Brooks 1991).

Even with both mechanisms, the important thing is that the A fraction reaches the cytoplasm. Here, toxins exert three main types of effects at the molecular level. 1) Inhibition of protein synthesis in cells, 2) Impairment of nerve snaps function, and 3) Disruption of the cytoplasmic membrane and disruption of the membrane transport system (Barth and Aktories 2011).

Brief information about some important A-B models, exotoxins and their mechanisms of action is given below.

Diphtheria toxin: This potent exotoxin (MA:62000 A and 38000 B) is specified by the prophage (beta phage) found in Corynebacterium diphtheriae. After the toxin (A-B model) enters the cell, the A fragment binds to the ribosome, which is the target region, and especially with EF2 (elongation factor 2), which has an important function in chain elongation, preventing the elongation of the polypeptide chain and thus preventing protein synthesis. Antitoxins against diphtheria toxin can neutralize the toxin and eliminate its effectiveness. If binding to cells has occurred, neutralization cannot occur. If the antitoxin is administered before the B fraction of the toxin binds to the ganglioside Gml on the cell surface, this subunit can be neutralized, thereby preventing the toxin from binding. Considering this situation in the treatment, an effort is made to give antitoxic serum as early as possible (Meinel *et al.* 2014).

Botulinum toxin: Exotoxins with 7 different activities and all host specificity are synthesized by Clostridium botulinum types. Of these, humans are sensitive to toxins A, B, E, F, and cattle are susceptible to C and D. Of these, Clostridium botulinum C toxin is specific by the bacteriophage (prophage). All of these toxins cause paralysis of varying severity. The toxin inhibits the production of acetylcholine, which is synthesized by nerve cells, which plays a very important role in muscle contraction and transmits signals from nerves to muscles in areas where nerves and muscles join. Thus, when the signals cannot reach the muscles, they cannot make the necessary reactions and contractions and paralysis occurs. Rather, the toxin binds to axons close to the neuromuscular region, preventing acetylcholine synthesis in cells in this region. If the

resulting paralysis extends to the chest muscles and diaphragm, death occurs due to respiratory failure. Botulinum exotoxin fits model A and B (Molloy *et al.* 2011).

Tetanus toxin: The exotoxin synthesized by C. tetani, which grows under anaerobic conditions in deeply contaminated wounds with foreign bodies on the body surface, is specific by a plasmid. The toxin has two main effective components. One of them causes spasm by affecting the nerves (tetanospasmin). The other is tetanolysin, which breaks down red blood cells. When the exotoxin synthesized by C. tetani, which grows in wounds, reaches the brain, it prevents the synthesis of glycine, an amino acid, in the cells. This leads to simultaneous contractions of muscles that have opposite functions in the body. Thus, tetanus spasms occur. These contractions can be so severe that muscles can tear and sometimes bones can break. Inability to control muscle contractions also leads to respiratory disorders. The toxin affecting the nerve is a single polypeptide molecule with a molecular weight of 150000. The molecule, which is inactive when first synthesized, is separated into two fractions by proteolytic enzymes (one, H chain, MA; 100000, and the other L chain, MA 50000). These two fractions are joined by one or two disulfide bonds. Fits Toxin A-B model (Hauser 2011).

Anthrax toxin: An exotoxin that leads to the bad effects (disease) for the both of humans in addition to the animals, it is synthesized by B. anthracis, is of plasmid origin. The toxin is protein in character and weakly antigenic and consists of mainly 3 parts PA, EF, and LF. These three toxin genes are encoded by plasmid pX01. Of these, amino acid PA 735, LF 776 aa and EF consists of 767 aa. The toxin causes hemorrhages by disrupting the permeability of blood vessels. These 3 fractions are not fully effective alone, but at least two of them (PA + LF) are lethal together. The toxin fits models A and B (Moayeri and Leppla 2004).

A second plasmid found in B. anthracis (pX02, 60 MDa) has codes for capsule formation.

2) Superantigens: Superantigens are T cell mitogens that have the ability to stimulate T cells even at a much lower concentration (picomolar level) than the immunogens

identified so far. Some exotoxins synthesized by staphylococci, streptococci, *Pseudomonas aeruginosa* and *M. arthritis* are considered in this group of substances. The important differences of these antigens (super antigens) from other antigens are removed to the surfaces of APCs together with the MHC II molecule and presented to T cells (T4 or T8) from there, without being processed by APC (antigen presenting cells). They combine with the TCR (Variable region of the beta chain of the T cell receptor (VB)) on the surface of T cells by making a direct connection. Thus, T4 cells are stimulated very strongly and at the same time they start to synthesize various cytokines (Brooks 1991).

Superantigens are divided into 4 main categories according to their origin.

3) Endotoxins: Endotoxins are a structural component of the cell wall (outer membrane) of Gram-negative bacteria with Lipopolysaccharide (LPS) character. LPS consists of three main parts. One of them, the lipid portion (lipid A), has a toxic character. It is bound by central polysaccharides and O specific carbohydrates (0 antigen). Since LPS has a structural feature, they cannot be secreted like exotoxins. However, they pass into the environment when the bacteria lyse. LPSs are also known as endotoxins. In the activity of lipid A, the alternative way of activation of complement and the stimulation of cytokine synthesis play a large role (Brooks 1991).

Some properties of endotoxins are briefly mentioned below.

a) They should be given in high doses (compared to exotoxins) in order to cause a toxic effect (lethal effect) in experimental animals.

b) They are thermostable and their antigenicity is weak.

c) When given to the body in large amounts, they produce nonspecific clinical symptoms (fever, septic shock, weakness, diarrhea, blood coagulation, intestinal hemorrhages, inflammatory reactions and fibrinolysis).

d) Endotoxins have weak specific affinities for cells or tissues.

e) They cannot be converted to toxoid.

f) Endotoxins have the character of lipopolysaccharide.

g) When they enter the body, they do not have a certain incubation period.

Not all gram-negative microorganisms can form LPS with the same chemical structure. There are differences between them. For example, some of the 0-specific carbohydrates are short and also of varying nature. In some (sprirocheta) it is present in the outer membrane, lipoprotein as well as LPS (Figure 2.5) (Brooks 1991).

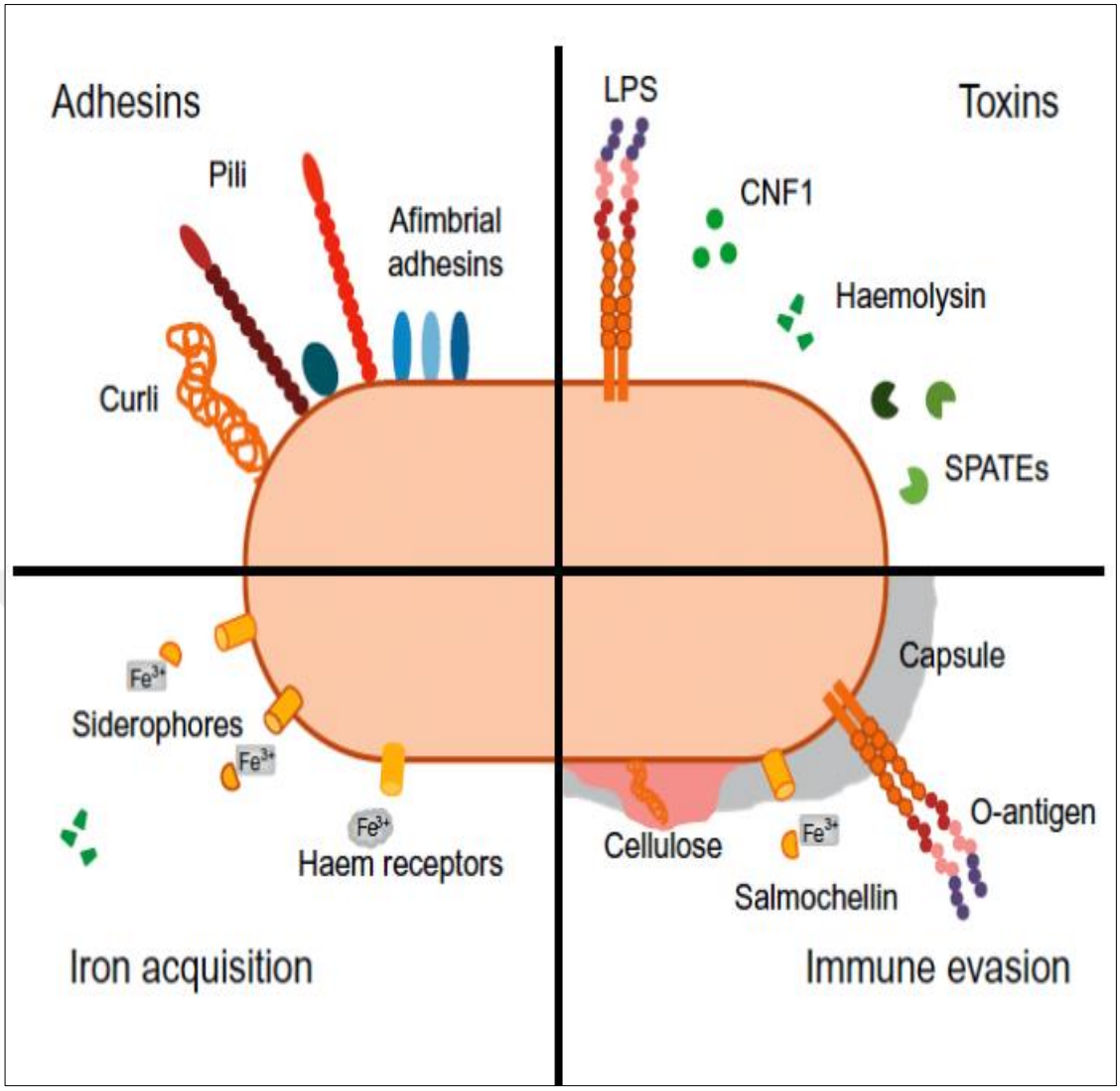

Figure 2.5 Virulence factors of uropathogenic *E. coli* (Shenouda *et al.* 2020)

2.3.1 *hlyA* gene

E. coli α-hemolysin (*hlyA*) is an imperative destructiveness figure for a few bacterial species included in human beings extraintestinal illnesses for instance; UTIs, peritonitis, meningitis, and septicemia. The title hemolysis is given since it causes the breakdown of hemolyzed ruddy blood cells. *hlyA* is accepted to act basically by assaulting the host's safe framework cells, extremely disabling cell capacities, regularly without causing cell lysis (Figure 2.6) (Coote 1996).

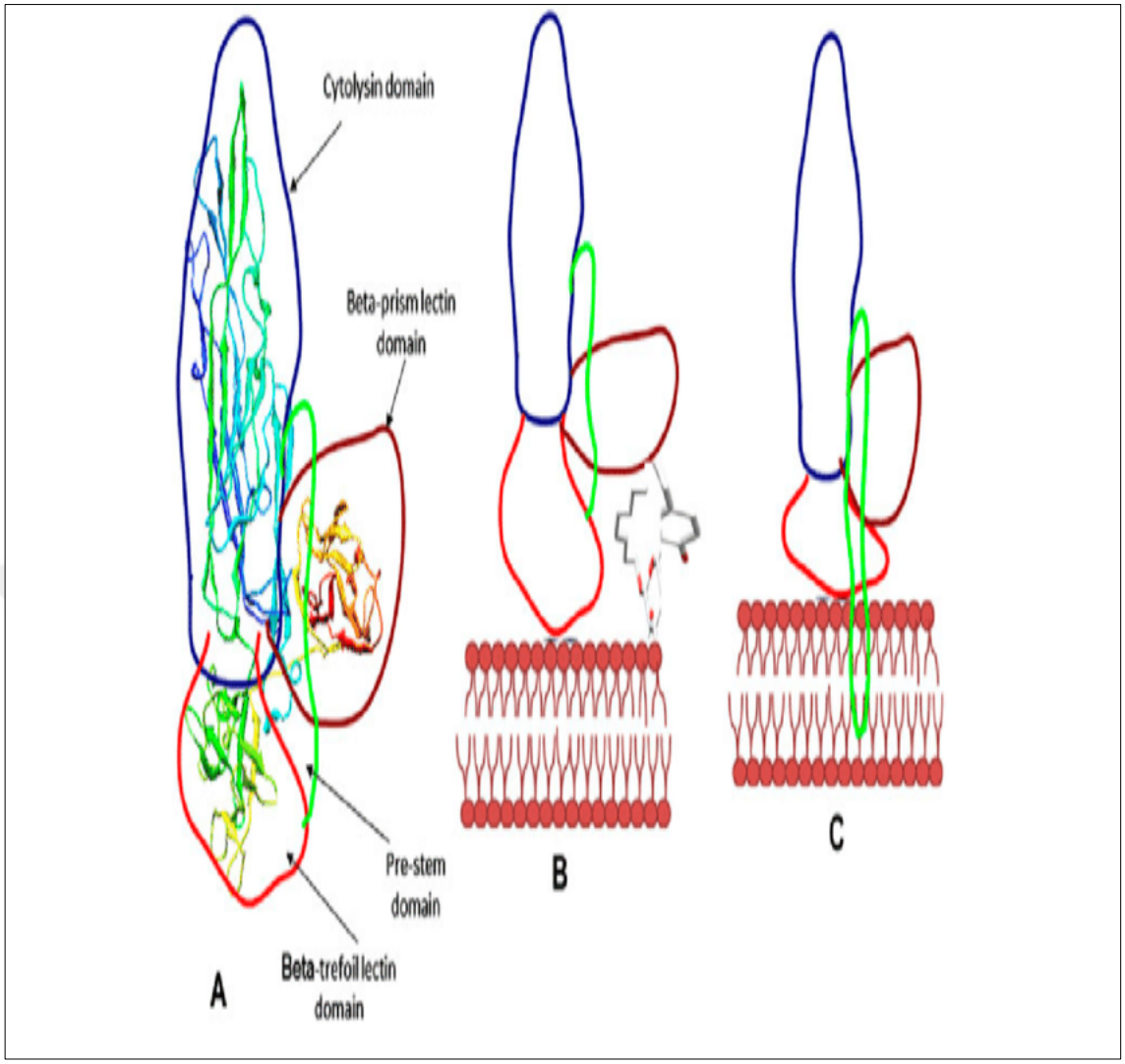

Figure 2.6 *hlyA* insertion into target cell membrane (Coote 1996)

The instrument of cytotoxicity of *E. coli* hemolysis is broadly accepted to result within the arrangement of layer pores (Welch 2016). At first, analysts separated this hemolytic *E. coli* into two types. *hlyA*-producing *E. coli*, which incorporates a genuine extracellular, filterable hemolytic activity, and the other, beta-hemolysin-producing *E. coli*, have detailed a hemolytic action that's not free in cell-bound and sifted culture supernatants (Figure 2.7) (Welch 2016).

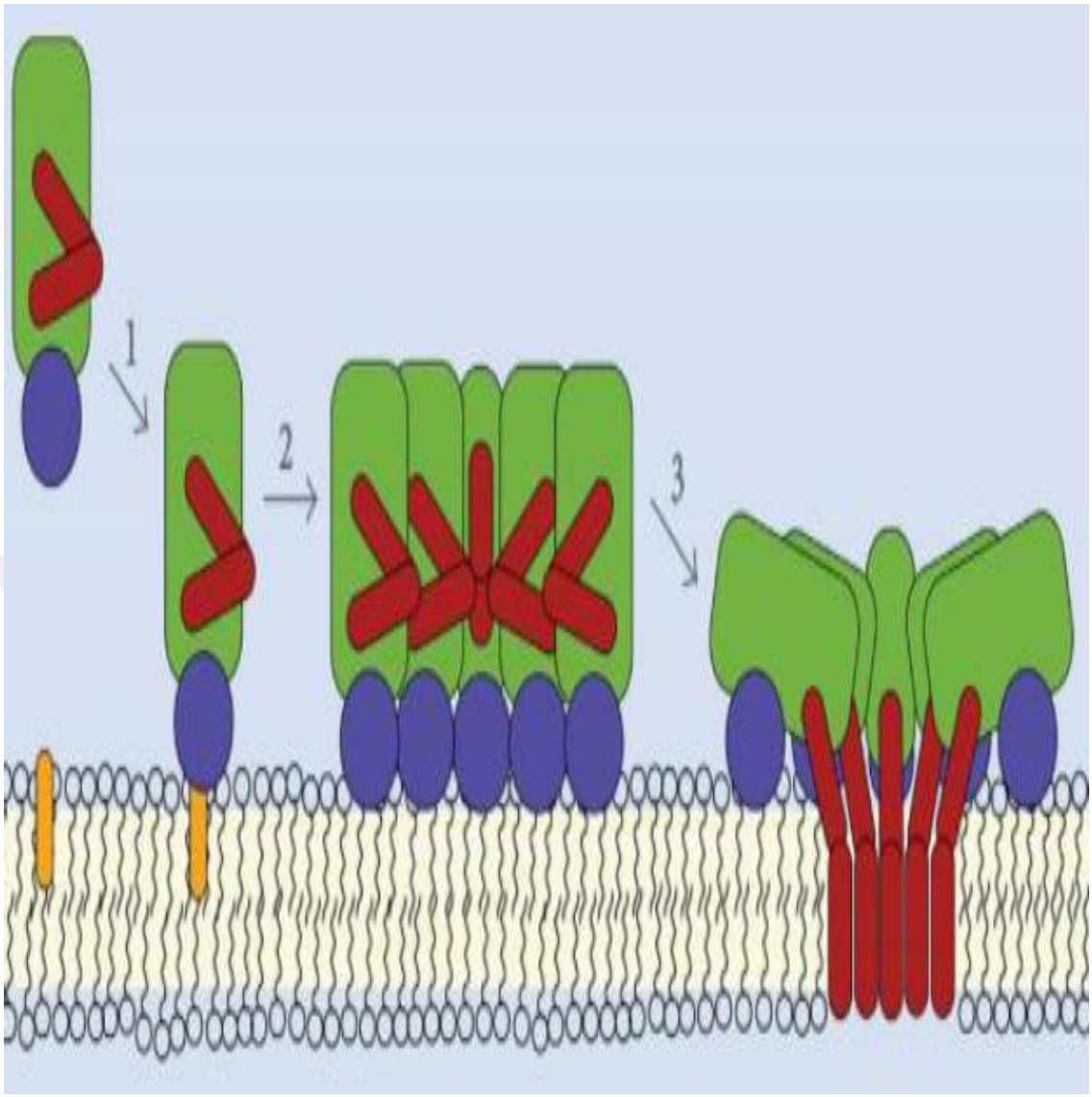

Figure 2.7 The toxin is secreted by the bacterial pathogen into the extracellular environment in a water-soluble form, usually as a monomer (Sonnen and Henneke 2013)

2.3.2 *Vat* gene

Autotransporters are emitted proteins with numerous capacities created by different Gram-negative microscopic organisms. In Enterobacteriaceae, this autotransporter could be a subgroup of SPATEs of the Enterobacteriaceae. SPATEs play a critical part within the survival and harmfulness of pathogens for instance; Shigella spp. In addition to *E.*

coli, thet are cause extra-intestinal infections in addition to the intestinal (Pokharel *et al.* 2019).

The vacuolating autotransporter poison (*vat*) could be a 140kDa lesson II SPATE found to be encoded on a pathogenicity island from APEC *E. coli* Ec222 and has been detailed to contribute to respiratory disease, cellulitis, and septicemia in poultry (Figure 2.8) (Parreira and Gyles 2003, Pokharel *et al.* 2019).

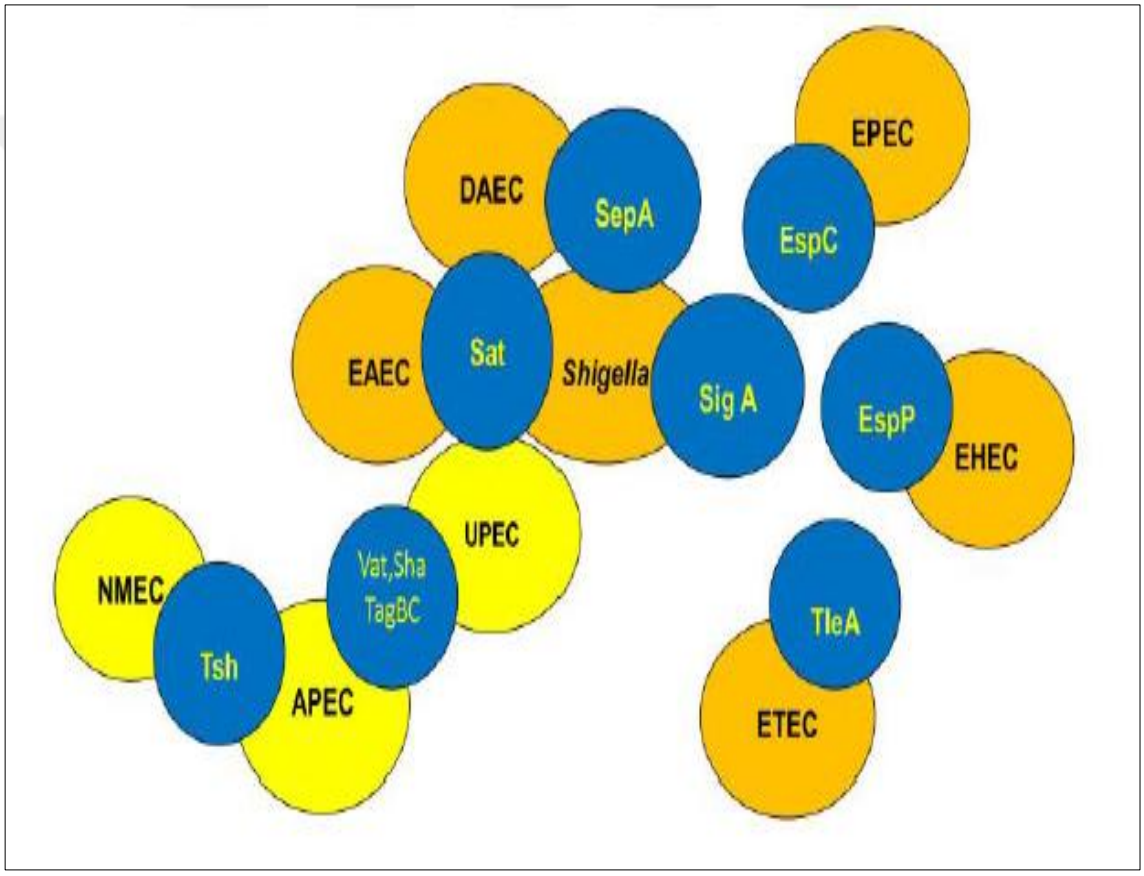

Figure 2.8 Distribution of SPATEs among intestinal and extraintestinal pathogenic *E. coli* (Pokharel *et al.* 2019)

Vat could be a chromosome-encoded autotransporter vacuolating poison found in both UPEC and APEC segregates (Spurbeck *et al.* 2012). In spite of the fact that numerous harmfulness components are related with ExPEC, no grouping of harmfulness variables can clearly recognize between ExPEC subgroups. Not at all like diarrheic *E. coli* (DEC) strains, gut-hardened pathogens, ExPEC strains can abruptly colonize the human

intestine; in this manner, the intestinal tract can act as a supply for extraintestinal *E. coli* (Köhler and Dobrindt 2011, Spurbeck *et al.* 2012).

As *E. coli* colonizes and produces its poisons, it causes a provocative reaction, which could be a conceivable pathway for UTI side effects. *Vat* makes a difference systemic disease of UPEC. *Vat*-specific antibodies were recognized in plasma tests of urosepsis patients tainted with *vat*-containing UPEC strains and were communicated amid the disease period of *vat* (Lores-Encarnación *et al.* 2019).

2.3.3 P fimbria

In the second half of the 1970s, *E. coli* bacteria were also shown in cases of acute pyelonephritis. It has the ability to adhere to the urinary system epithelium. This character is frequently seen in cases with cystitis and asymptomatic bacteriuria. In the following years, this characterization enabled it to bind to P-type adhesins on the cell surface.

It is a “mannose resistant” fimbria with the ability to agglutinate human erythrocytes. It is named P fimbria because it specifically attacks P blood group antigens on erythrocytes and uroepithelial cells. This type of fimbriae is made only by certain strains of *E. coli* under certain environmental breeding conditions. P fimbriae do not adhere to the uromucoid but adhere to uroepithelial cells with high capacity.

2.4 Resistance Genes

2.4.1 *mcr-1* gene

The PMCR *mcr-1* quality is an irresistible quality thatwhich leads to the inactivation of drugs that cause lipopolysaccharide adjustments by administrative frameworks, connected with phospholipids, and deregulate the structure of cell films. Microbial resistance is for the most part related with chromosomal transformations, but when colistin implies it frequently happens through even quality exchange (Mello *et al.* 2018).

A PMCR quality *mcr-1* encoding phosphoethanolamine transferase was to begin with depicted in Enterobacteriaceae disconnected from people and some animals in China in 2015 (Figure 2.9) (Li *et al.* 2018). Since at that point, plasmid-mediated polymyxin resistance of *mcr-1* has been detailed around the world in animals, nourishment, and people (Li *et al.* 2018, Poirel *et al.* 2017).

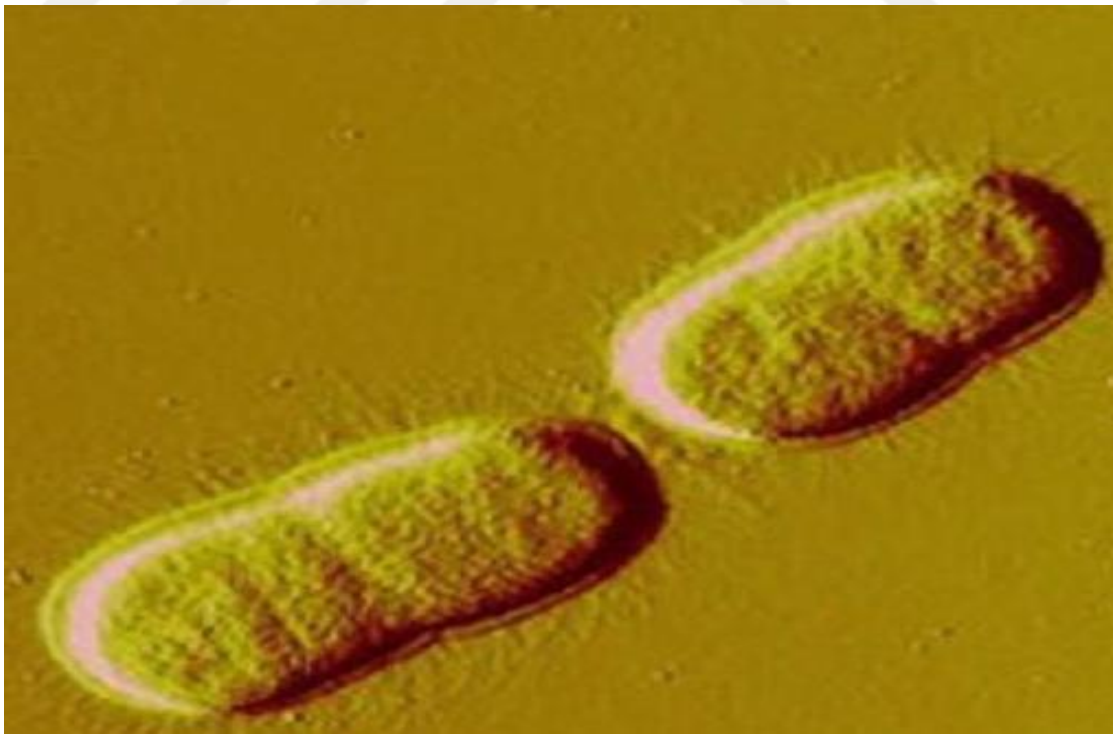

Figure 2.9 Dangerous antibiotic-resistant bacteria (Li *et al.* 2018)

The nearness of antimicrobial resistance (MDR) pathogens in nourishments of animal beginning such as drain, meat and poultry has expanded essentially over the past few a long time (Figure 2.10) (Muloi *et al.* 2018, Perez-Rodriguez and Mercanoglu-Taban 2019). Mindfulness of the potential dangers of AMR could be a developing concern for the nourishment industry due to the seriousness of mawomen that MDR zoonotic pathogens can cause, which seem lead to misfortune of shopper compliance and ensuing lessening in nourishment request. To control the spread of MDR pathogens through nourishment of animal root, sources of defilement must be distinguished. These safe microbes and/or AMR qualities have an assortment of complex irresistible pathways all through the nourishment chain. In any case, the relative commitment of nourishment to the worldwide burden of disease caused by MDR pathogens has not however been assessed (Likotrafti *et al.* 2018, Perez-Rodriguez and Mercanoglu-Taban 2019).

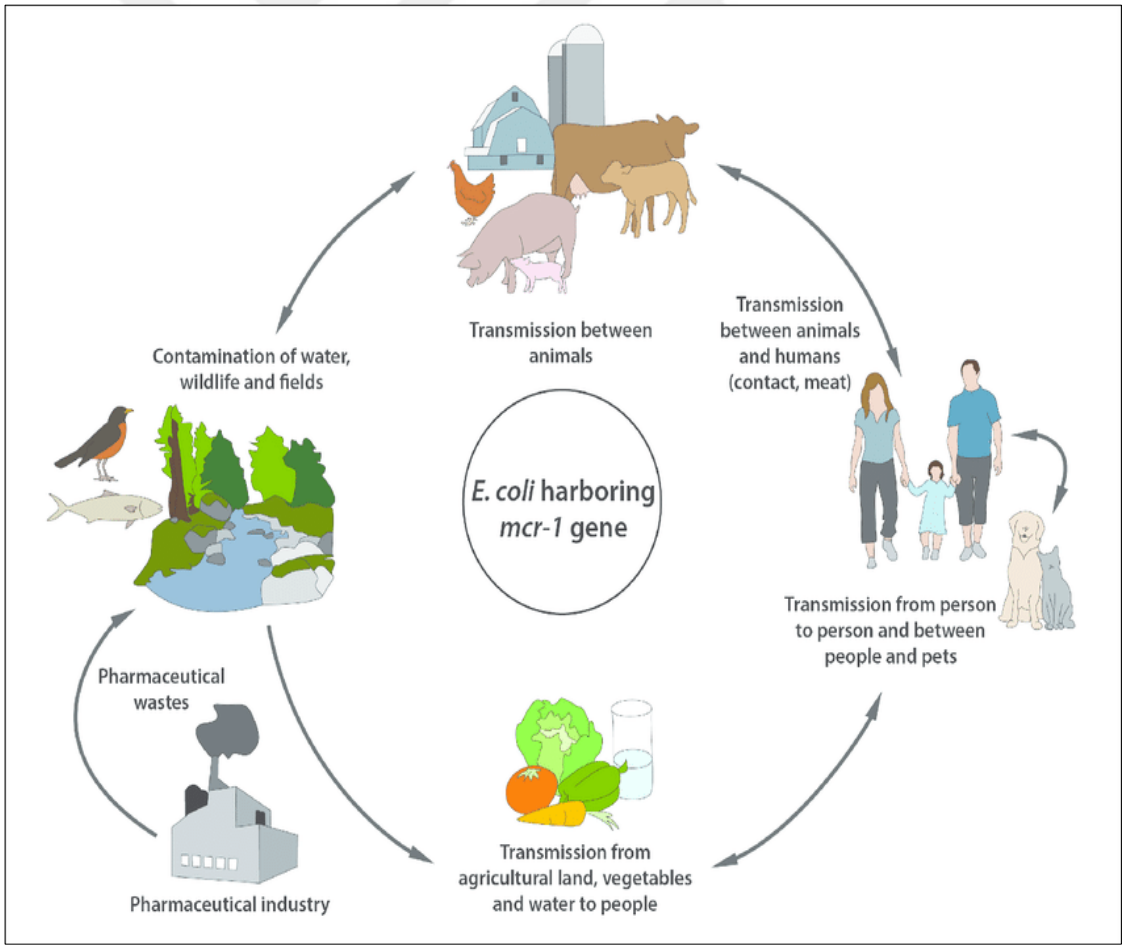

Figure 2.10 Circulation of colistin-resistant *Escherichia coli* harboring the *mcr-1* gene among animals-environment-food and humans (Rhouma *et al.* 2016)

An add up to of 110 *E. coli* multi-drug resistance, antibiograms and a few harmfulness variables have been distinguished in Mexico, 79 of which were confined from community and 31 hospital-acquired UTIs. It has been detailed that the foremost influenced gather is girls and 20% of the overall test is *vat* quality positive and 9.1% *hlyA* quality positive. It has been appeared that these quality proportions are higher in hospital-acquired UPECs than in community-acquired separates (Ramírez-Castillo *et al.* 2018).

An add up to of 110 *E. coli* confines gotten from pee tests of people analyzed with UTI were explored within the Microbiology and Immunology research facility of the Ljubljana Organized of Medication. The nearness of poison encoding qualities was screened by PCR. Among the filtered qualities, it was decided that the *hlyA* quality was considered among a few qualities encoding poison and its predominance was 25.45%. In rundown, the predominance of toxin-encoding harmfulness qualities among *E. coli* strains confined from UTIs in Slovenia is said to be comparative to that obtained in ponders in several topographical locales (Rijavec *et al.* 2006).

In another comparable consider, the relative predominance rates of the *hlyA* quality in an add up to of 156 UPEC strains disconnected from pee tests of outpatients and inpatients performed at the Pasteur Established of Iran were found to be 2.2 to 3.5 times higher in outpatients than inpatient confines. It has been decided that the poison *hlyA* quality is common at a rate of 30.8% in UPEC separates (Tabasi *et al.* 2016).

Between October 2014 to June 2015, 250 *E. coli* confines were collected from diverse patients with UTIs in Kerman, Iran, and identified broad-spectrum beta-lactamases (ESBLs) utilizing the plate dissemination strategy and the combined plate strategy to decide the anti-microbial helplessness profile. *hlyA* and cnf1 qualities were identified by PCR strategy. 44.8% of the overall segregates delivered wide range beta-lactamases and *hlyA* and cnf1 qualities were recognized in 28.8% and 29.2%, individually. (Hashemizadeh *et al.* 2017).

The prevalence of the *vat* gene with different virulence genes was analyzed among 100 *E. coli* isolates which sampled from UTI patients to evaluate the data on the prevalence of virulence factor in different geographical regions in Zabol of Iran. Using PCR with primers specific for UPEC virulence genes, 99 (99%) of 100 UPEC samples were collected to carry virulence genes. Among other genes, the prevalence of wattage has been identified as 18%. It has been stated that the detection of these genes as primary controllers of UPEC virulence may help to better manage related infections (Shookohi and Rashki 2016).

During six months, 350 urine samples were collected from three hospitals in Tehran, Minoodasht and Abhar cities and 297 *E. coli* isolates were identified among them. As a result, it was shown that 225 strains (75%) contained the sat gene and 106 strains (36%) contained the *vat* gene. An investigator proved that the *vat* gene may be necessary for *E. coli* to enter the bloodstream or for its survival, and strains with the *vat* gene are more dangerous than other UPEC pathotype strains (Saraylu *et al.* 2012).

In the study they aimed to isolate the prevalence of *mcr-1* gene from about of 700 *E. coli* samples strains isolated from a Chinese university hospital from August 2014 to August 2015, they found that only 4 strains (0.6%) were *E. coli* producing *mcr-1* (He *et al.* 2017).

They screened a total of 908 bacterial isolates of clinical colistin-resistant Enterobacteriaeae collected between 2014 and 2016 in terms of mcr genes. As a result, 27 *E. coli*, 1 K. pneumonia and 1 Enterobacter cloacae were found to be mcr positive. It has also been reported that they show the highest activity against antimicrobial agents (Wise *et al.* 2018).

In a think about conducted to explore the nearness of *mcr-1*/mcr-2 in clinical Enterobacteriaceae separates from diverse locales of Turkey, the nearness of *mcr-1* and mcr-2 qualities was decided in 329 Enterobacteriaceae segregates from 22 centers utilizing PCR strategy. Of all considered segregates, 217 (66%) *Klebsiella pneumoniae*, 75 (22.8%) *Salmonella spp*., 31 (9.4%) *E. coli*, 3 (0.9%) Enterobacter cloacae, 2 (0.6%)

Klebsiella oxytoca and 1 (0.3%) *Enterobacter aerogenes* and these qualities were not found in any of these confines. This shows that these resistance instruments don't exist in our nation however. Be that as it may, analysts recommended that these instruments ought to be taken after up and carefully explored in non-clinical separates (animals, crude meat, separates of natural tests, etc.) that appear clinical and special significance in numerous resistance profiles (Sarı *et al.* 2017).

In arrange to examine the predominance and atomic characteristics of the *mcr*-1 quality in an add up to of 144 *E. coli* separates gotten from all patients with circulatory system contamination in a Chinese educating clinic for one year, they were collected in a educating clinic in Changsha, China, between January and December 2016, and the *mcr-1* quality was decided. nearness was assessed by PCR. Three (2.1%) of 144 *E. coli* segregates were positive for the *mcr-1* quality. MLST comes about appeared that three *E. coli* separates were alloted to three diverse grouping sorts: ST457, ST101 and ST1413, individually (Zhong *et al.* 2019).

Urinary samples of 368 pets (224 dogs, 144 cats) with suspected UTI were collected at the I-Vet laboratory in Brescia (Italy) between May and October 2017 and investigated the presence of *mcr-1* and ESBL producers among UPEC. 142 specimens (99 dogs, 43 cats) were positive for bacterial growth ESBL (34.55%) and *mcr-1* (8%). It has been shown to confirm the role of *E. coli* in UTIs in both humans and animals (Yamaji *et al.* 2018).

3. MATERIAL AND METHOD

3.1 Materials

3.1.1 Instruments

The following instruments that used in this study illustrated in Table 3.1.

Table 3.1 Instruments of the study

No.	Appratus	Origin	Company
1	AURA TM PCR Cabinet	England	Tecnoplast
2	Microspin 12, High-speed Mini-centrifuge	Latvia	BioSan
3	Vortix mixer	India	Bionics scientific
4	Electrophoreses	USA	Apple
5	UV transmission analuzer	Japan	Labsphere
6	DNA analyser	England	Thermo Fisher scientific
7	Gen Amp PCR system 9700	USA	Applied Biosystemic
8	Balance	India	Indiamart
9	Laboratory centrifuge	India	India mart
10	Incubator	Koria	Fine Tech
11	Compaound microscope	Autria	Micros
12	Water path	Germany	Memmert
13	Vitex 2 compact system	France	Bio Merieux

3.1.2 Kits

The following kits were used in this study are illustrated in Table 3.2.

Table 3.2 The kits for the current study

NO	Material	Cat #	Company/ Origin
1	Agarose	8100.11	Conda / USA
2	Red safe staining souluion	21141	Intron / Korea
3	6X Loading dye	21161	Intron / Korea
4	Ladder 100 bp	24073	Intron / Korea
5	Pre mix pcr	25025	Intron / Korea
6	TBE buffer 10 X	IBS.BT004	Conda / USA
7	Primer	3124	Alph DNA/USA/Canada
8	i-genomic BYF DNA Extraction Mini Kit	17045	Intron biotechnology /Korea
9	Vitex® 2G	423948	France Bio Merieux
10	Vitex® 2AST-N419	21341	France Bio Merieux

3.1.3 Media used in this study

The media for the current medical job are shown in Table 3.3.

Table 3.3 The media that used for the current study

Media	Company	Origin
MacConkey agar medid	IndiaMart	India
Blood agar media	IndiaMart	India
MR-VP broth media	IndiaMart	India

3.1.4 Urine samples collection

About 80 urine samples were taken from the patients' urine of with a feature of acute of urinr truct infecion (UTI) who came to the Al-Hussein Teaching Hospital in Thi-Qar Governorate /Iraq from January 2022 to June 2022 and we choose 50 healthy persons as control group. Patients selected in this study (urinary tract patients) were diagnosed according to some approved clinical criteria, as well as the selection was made based on several clinical symptoms and signs, such as dysuria, frequency, urgency, fever, flank pain, and other constrictive symptoms.

The medical laboratory critieria were included the GUE, define the turbidity of urine and presence of Isolation and identification of *E. coli,* albumin, leukocyte, bacteria seen, and other bacterial isolates.

All urine isolates were isolated and identified in accordance with their morphological characteristics of colonies, biochemical tests and vitek 2 system. We were placed the urine samples in sterile urine tubes then cultured on MacConkey agar by using the streaking method, then inoculated the tubes at 37 °C for 24 hours. The bacterial colonies were identified based on morphological charateristics of colonies and biochemical tests based on Bergy' Manual of Systematic Bacteriology 2nd edition (Garrity 2005) and vitek 2 system to identified the colonies shapes, and the morphological charateristics of *E. coli* colonies that includes color, size and edge of colony were recorded after 18-24

hours after incubation the tubes at 37 °C. To study the gram stain, we were fixed a single colony of each sample on a glass slide under compound microscope at power 100X.

3.1.5 Vitek 2 system

We were used a vitek 2 system to confirm *E. coli* and other bacterial species from a diagnostic group specific to the system bu utilizing a diagnostic card specific to gram negative bacteria including 64 slots and each solt consist of dried color indicator. The indicator is reacted with the sample and the vitek 2 system records this change that happen through bacterial growth on the slot and identified the bacteria according to guidance given by Bio Merieux (Pincus 2011).

Firstly, place the kit cards at room temperature and choose platic tubes for identified the bacterial isolates, prepare three plastic tubes namely distiiled water tube, bacteria identified tube and sensitivity tube.

Put 3 mL of sterile saline in each tube and then put one pure colony of bacterial culture (18-24 hours age) in each tube and mix well by vortex mixer then read the turbidity of the bacterial suspension by Densi Chek instrument, zero the machine by place the D.

Water tube in the machine then put the second tube (bacteria identified tube) in the machine and read turbidity (turbidity must be record 0.5 – 0.63 Mcfarland units), then transfere the rack in the first chamber and push start, the machine will make a pressure that lead to transfere bacterial suspension from tubes to pits of the kit cards until filled the pits.

After this step we were transfere the rack to the second chamber to identified the bacterial species and in the next spet the machine will read the biochemical card to identified the bacteria.

3.2 Methods

3.2.1 Biochemical tests

All the bacterial isolates of *E. coli* were subjected to the biochemical tests as mentioned by MacFadden (2000) as following:

3.2.1.1 Oxidase test

We were performing this test by using filter paper moisered with a few drops of a freshly solution prepared of tetrammethyl –p-phenylenediamine dihydrochloride, then picked up a clump of alls from bacteria growth of the slant and smeared on the paper. The development of the color (violet or purple) within 10 seconds indicates a positive result.

3.2.1.2 Catalase test

We were taken a single colony of each isolate and smeared on clean slide and then flooded three drops of 3% of H_2O_2. The presence of gaseous bubbles indicates a positive result.

3.2.1.3 Methyl red test

Inoculate one colony of each isolate in tube containing MR-VP broth then incubate the tube for 24 hours at 37 °C, add three drops of methyl red reagent to the tube. Convert the color of medium to red color indicates a positive result.

3.2.1.4 Citrate utilization test

Firstly, we were streaked a loopful of colony in a slant contining simmon citrate agar, incubated the slant at 37 °C for 48 hours. The changing in color of the medium to blue indicates a positive result.

3.2.1.5 Vogas-proskauer test

Inoculte one colony of bacteria in a slant containing MR-VP broth and incubate the slant for 24 hours at 37 °C, add two drops of Vp1 and four drops of VP2. The changing in color or medium to red color after 15 minutes indicates a positive result.

3.2.2 Identification of *E. coli* by Gram stain method

We were performed Gram stain procedure that used by Merchant and Packer (1969) to identified the shape, arrangement, and size of *E. coli* and we revealed the bacteria was gram negative, colony that is pink, rod-shaped in appearance, arranged in pairs/ or singles, or that is suspected to be *E. coli*.

3.2.3 Motility test

We were used the hanging drop technique as described by Cowan (1985) to identified the motility bacteria from non- motile one. This technique was prepared by broth culture and examined under compound microscope 100X power objective. The motile bacteria were suspected as *E. coli*.

3.2.4 DNA isolation of *E. coli* strains

Strains produced on blood agar were grown at the desired density by inoculation into 5 ml liquid culture. The culture was precipitated by centrifugation at 10,000 rpm for 2 min and the supernatant was discarded. The precipitate was suspended in 567µL TE buffer. 10% SDS and proteinase K were added and incubated at 37˚C for 1 hour. After adding CTAB/NaCl solution, it was incubated in a hot water bath at 65˚C for 10 min. An equal volume of chloroform/isoamylalcohol was added and centrifuged at 12,000 rpm for 5 minutes. The upper phase was taken into a separate eppendorf tube, (300) µL of isopropanol was added, and the vortexing and centrifugation steps were repeated. The DNA was centrifuged again at 12000 rpm at +4 oC for 5 minutes by placing 75% ethanol on the tube, on which the supernatant was poured (Ausubel *et al.* 1991).

3.2.5 Amplification of *hlyA, vat,* and *mcr-1* genes of *E. coli* strains by PCR

First, a master mix was prepared containing 2.5 µL of 10x Reaction Buffer, 2.0 µm dNTP mix, 1 µL of each of the primers (Table 3.1), and 0.15 µL of Taq polymerase. 23 µL of master mix and 2 µL of template DNA were added to each tube so that the total volume in the PCR tubes was 25 µL. Initial denaturation at 95 ^{o}C for 15 min. 30 cycles, at 94 ^{o}C for 30 s, annealing at 60 ^{o}C for 30 s, extension at 72 ^{o}C for 2 min., at 72 ^{o}C for 10 min. A final elongation step has been carried out.

Table 3.4 Primer pairs used for PCR applications

The genes studied	5' and 3'	PCR product size bp	References
hlyA gene	Forward AACAAGGATAAGCACTGTTCTGGCT Revers ACCATATAAGCGGTCATTCCCGTCA	1177	Tabasi *et al.* 2016
vat gene	Forward AACGGTTGGTGGCAACAATCC Revers AGCCCTGTAGAATGGCGAGTA	420	Restieri et al, 2007
mcr-1 gene	Forward AGTCCGTTTGTTCTTGTGGC Revers AGATCCTTGGTCTCGGCTTG	320	Rebelo *et al.* 2018

3.2.6 Agarose gel of PCR products

We used the agarose gel electrophoresis to assert the integrity and presence of DNA that extract by PCR technique, 1.5 g of agarose was added to 100 mL of TAE and dissolved in the microwave, when the temperature of 50-55° C was reached, 10 µL of ethidium bromide was added and poured slowly into the gel electrophoresis tray, and the comb to form the wells was placed.

After the gel had frozen, the comb was carefully removed and placed in the gel tank. TAE buffer was added to cover the gel, and then the PCR product and loading dye were mixed and loaded.

It was carried out at 100 Volts for 40 minutes and photographed with a DNA imaging system under UV light. The molecules of DNA were moved from cathode to anode poles and finally we used the gel imaging system to watch the stained gel bands.

3.3 Statistical Analysis

Crosstab (Chi-Square) Statistics program was applied to determine the relationship between *vat* and *hlyA* positive groups and *mcr-1* positive groups according to the results obtained for the studied genes.

4. RESULTS AND DISCUSSION

4.1 Age Distribution of the Patients with Urinary Truct Infection (UTI)

Female patients showed a significantly higher prevalence of UTI compared to male patients. Among the total UTI cases, 61.25% were females (49 patients), while 38.75% were males (31 patients). In the age group above 60 years, the percentage of female UTI cases was 15.0%, and for males, it was 8.75%. In the age range of 50-59 years, female UTI cases reached 13.75%, while male cases were 8.75%. For both genders aged 10 years and above, the percentage of infection was 2.5% (Table 4.1, Figure 4.1).

Table 4.1 Age and gender distribution of the patients with urinary tract infection (UTI)

Range of patients age	**No. of patients infected with UTI**			
	Male	**Percentage of male infected with UTI**	**Female**	**Percentage of female infected with UTI**
≥ 10 years	2	2.5	2	2.5
11-19 year	2	2.5	4	5.0
20-29 year	3	3.75	5	6.25
30-39 year	5	6.25	7	8.75
40-49 year	5	6.25	8	10.0
50-59 year	7	8.75	11	13.75
≤ 60 years	7	8.75	12	15.0
Total	31	38.75	49	61.25

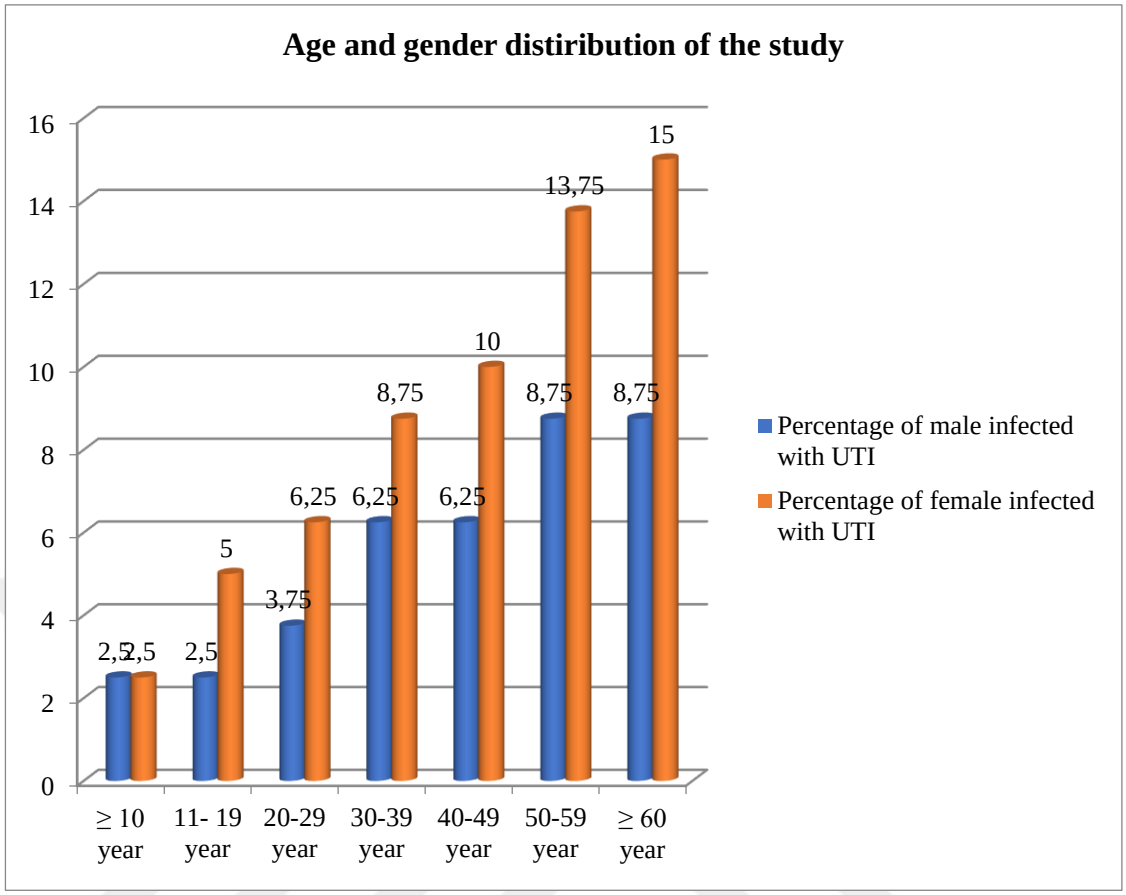

Figure 4.1 Age and gender distribution of patients with UTI

4.2 Isolation and Identification of Bacterial Cultural Isolates

Clinical samples of UTI were cultured in MacConkey agar medium; 28 isolates were found to be able in fermentation of lactose as indicates by the exist of pink color colonies in the culture medium, these morphological characteristics comes according to the corresponding cultural charasteristics of *E. coli* bacterium that mentioned by Zinnah *et al.* (2007).

The results of Table 4.2 showed that *E. coli* bacterium recorded upper percentage of patient's samples which recorded 35.0 % with 28 patients of this study while *Pseudomonas aeruginosa* recorded 15.0% with 12 patients (Table 4.2, Figure 4.2).

These results were in agreement with the results of Saadi Al-Baer and Hussein (2017) which they were isolated 10 samples of *E. coli* from 25 urine sampled from a ptients and revealed that these *E. coli* isolates gave about 96% similarity to those *E. coli* characteristics.

Table 4.2 Percentage of bacterial culture isolates

No.	Bacterial isolate	Type of bacteria	Percentage of bacterial isolates
1	Gram negative bacteria	*Pseudomonas aeruginosa* *E. coli* *K. pneumonia*	12/80(15.0%) 28/80(35.0%) 9/80(11.25)
2	Gram positive bacteria	*S. aureus* *S. pneumonia*	11/80(13.75%) 8/80(10.0%)
3	Yeast	*Candida albicans*	4/80(5.0%)

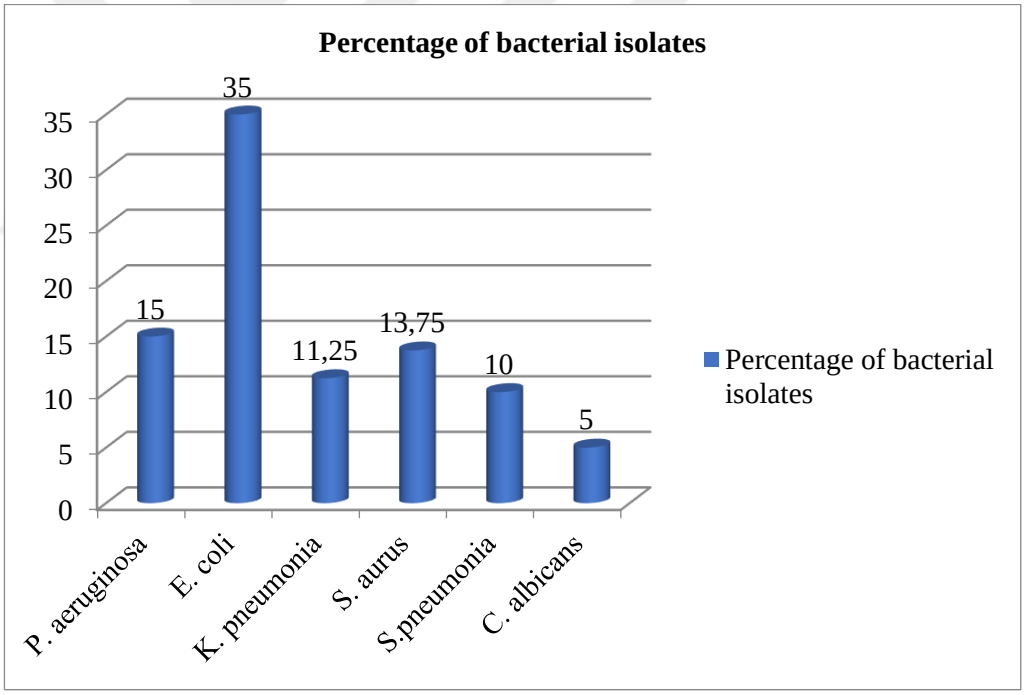

Figure 4.2 Percentage of bacterial isolates of this study

4.3 Microscopical Examination and Biochemical Characteristcs

Colonies of pure probe cultures containing these bacteria were identified based on their Gram staining as well as on some other known microscopic characteristics. The

suspected bacterial isolates of *E. coli* were subjected, based on several microscopic characteristics, to the biochemical tests used in this type of related investigation.

Table 3.4 indicates, that all 28 samples (isolates) proved a positive results for fermentation tests for catalase, methyl red, and lactose, but at the same time showed negative results for oxidase, citrate, and Vegas-Proskauer tests. These results were in agreement with the results of Saadi Al-Baer and Hussein (2017) which showed that all the tested isolates of *E. coli* had given positive results for catalase, methyl red and latose ferintation tests and negative results for oxidase, citrate utilization and vogas-proskauer tests.

Table 4.3 Biochemical tests of *E. coli* isolates

No. of isolate	Oxidase test	Catalase test	Methyl red test	Citrate utilization test	Lactose fermintation	Vogas-prokauer test
E2	-	+	+	-	+	-
E4	-	+	+	-	+	-
E7	-	+	+	-	+	-
E8	-	+	+	-	+	-
E10	-	+	+	-	+	-
E12	-	+	+	-	+	-
E15	-	+	+	-	+	-
E20	-	+	+	-	+	-
E22	-	+	+	-	+	-
E24	-	+	+	-	+	-
E26	-	+	+	-	+	-
E30	-	+	+	-	+	-
E31	-	+	+	-	+	-
E32	-	+	+	-	+	-
E35	-	+	+	-	+	-
E38	-	+	+	-	+	-
E40	-	+	+	-	+	-
E42	-	+	+	-	+	-
E46	-	+	+	-	+	-
E50	-	+	+	-	+	-
E52	-	+	+	-	+	-
E58	-	+	+	-	+	-
E65	-	+	+	-	+	-
E70	-	+	+	-	+	-
E71	-	+	+	-	+	-
E72	-	+	+	-	+	-
E73	-	+	+	-	+	-
E74	-	+	+	-	+	-

+ = positive result, - = negative results

4.4 Biochemical Tests for Identification of *E. coli* by Vitex 2 System

The results of the Table 4.4 showed that the 28 isolates of *E. coli* were varied in their sensitivity to the tested 12 antibiotics, 7 antibiotics (Gentamycin, ampicillin, tetracyclin, pencillin, erythromycin, trimethoprim, and clindamycin) were showed resistance to the *E. coli* isolates, 2 antibiotics (rifamycin and fusaric acid) were showed sensitive to the *E. coli* isolates wherase 3 antibiotics (vancomycin, fostomycin, and cefuroxime) were showed moderate resistance to the *E. coli* isolates. These results gave 94% similarity to those *E. coli* characteristics as identified by the standard gram-negative card (Uribe-Beltrán *et al.* 2017).

Table 4.4 Antibiotiec tests for identification of *E. coli* by vitex 2 system

No.	Antibiotic	No. of resistance isolates	No. of sensitive isolates
1	Rifamycin	8	20
2	Fusaric acid	3	25
3	Vancomycin	12	16
4	Fostomycin	15	13
5	Gentamycin	24	4
6	Ampicilin	28	0
7	Cefuroxime	12	16
8	Tetracyclin	25	3
9	Pencillin	27	1
10	Erythromycin	28	0
11	Trimethoprim	26	2
12	Clindamycin	27	1

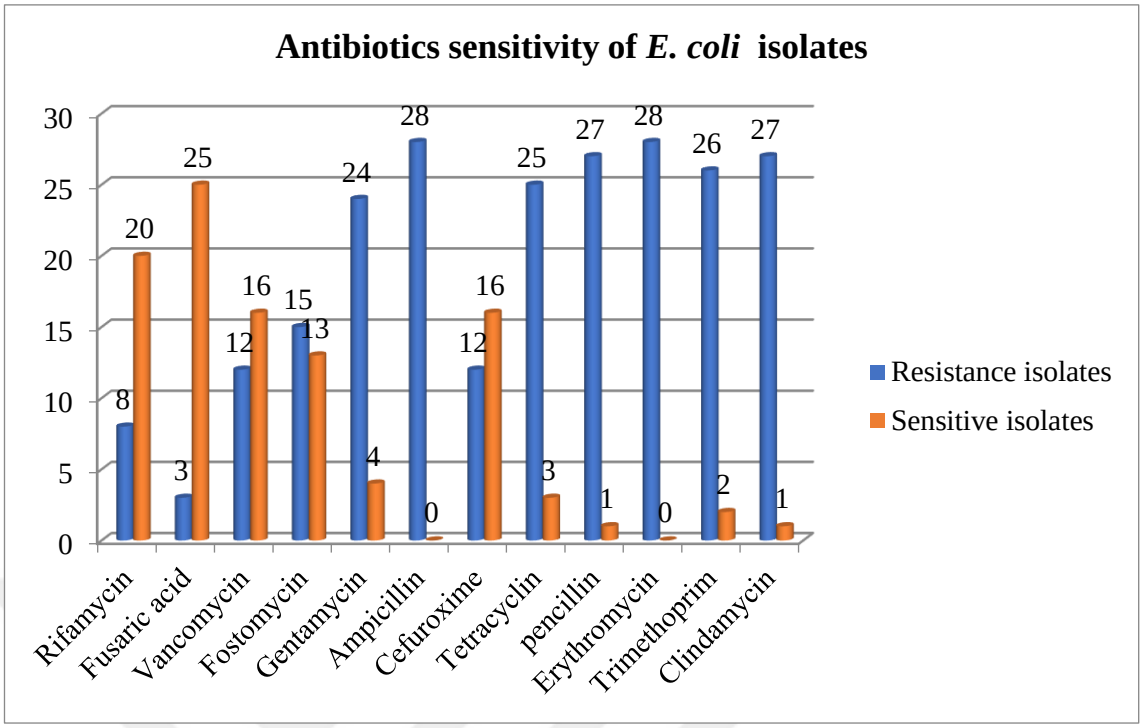

Figure 4.3 Antibiotics sensitivity of *E. coli* isolates

The *hlyA*, *vat*, and *mcr-1* gene regions of the 90 *E. coli* isolates studied were amplified by a double primer in a thermo brand gradient thermal-cycler. PCR products were observed by DNA imaging system under UV light using ethidium bromide and DNA marker in agarose gel electrophoresis.

4.5 Prevalence of *hlyA* Gene in UPEC Isolates

The *hlyA* gene was found to be positive in 21 of the 80 *E. coli* isolates studied with the percentage of 26.25 % (Tables 4.5, Figure 4.4, and Figure 4.5). The *hlyA* gene produced an amplification product of 1177 bp. There was no statistical relationship between the *hlyA* positive group and the *vat* gene positive bacteria with the bacterial samples containing the *hlyA* gene ($P > 0.050$).

Table 4.5 Gene encoding virulence factor tested by PCR of *E. coli* isolates

Isolate number	hlyA Gene	vat Gene	mcr-1 Gene
E2	+	-	-
E4	-	-	-
E5	-	-	-
E6	-	-	-
E7	-	+	+
E9	+	-	-
E12	+	-	-
E13	-	-	-
E14	-	-	-
E15	-	-	-
E16	+	+	-
E19	-	+	-
E20	-	+	+
E22	-	-	-
E23	-	-	-
E24	-	-	-
E25	-	-	-
E26	-	-	-
E27	-	-	-
E28	-	-	-
E23	-	-	-
E33	-	+	-
E34	-	-	-
E38	-	-	-
E40	-	-	-
E41	-	+	-
E42	-	-	-
E44	-	-	-
E45	+	-	+
E46	-	-	-
E47	+	-	-
E48	+	-	-
E49	-	-	-
E50	+	-	-
E51	-	-	-
E52	-	+	+
E53	-	-	-
E54	-	-	-
E56	-	-	-
E57	-	-	-
E58	-	-	-
E59	+	+	-
E60	-	+	+
E61	+	+	+
E62	+	-	-
E63	-	+	-
E64	-	+	-
E65	+	-	-
E66	-	+	-
E67	-	+	-
E69	+	-	-
E70	-	-	-

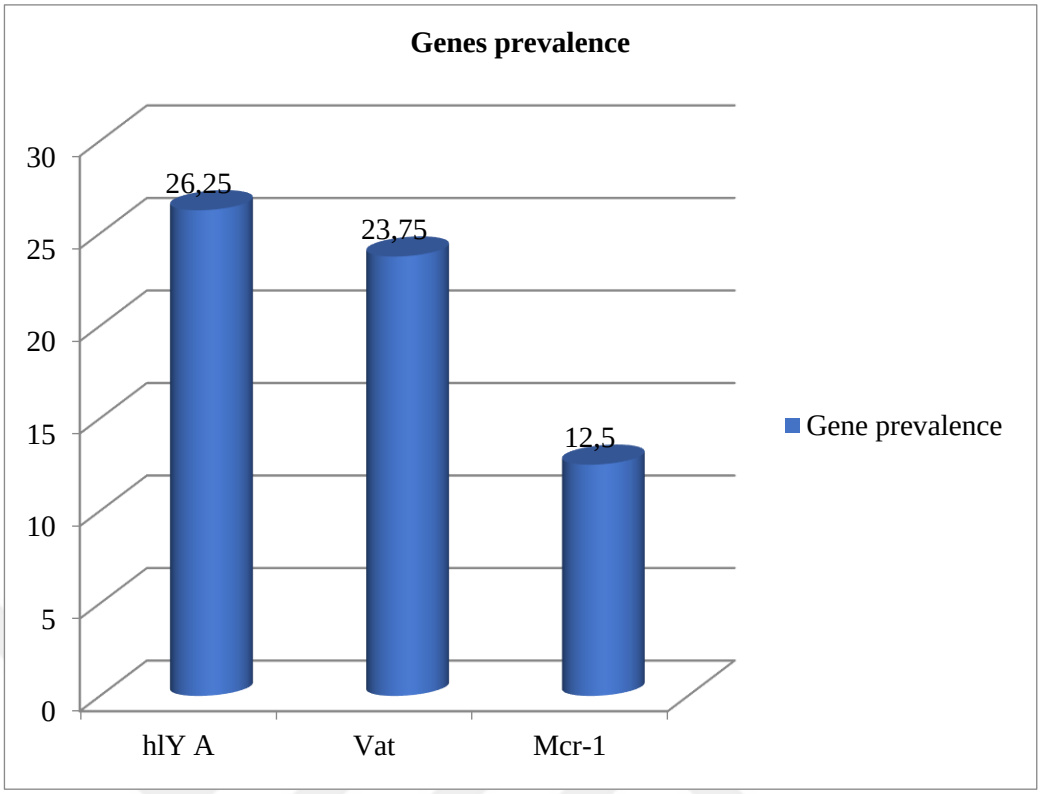

Figure 4.4 Genes prevalence of *E. coli* isolates

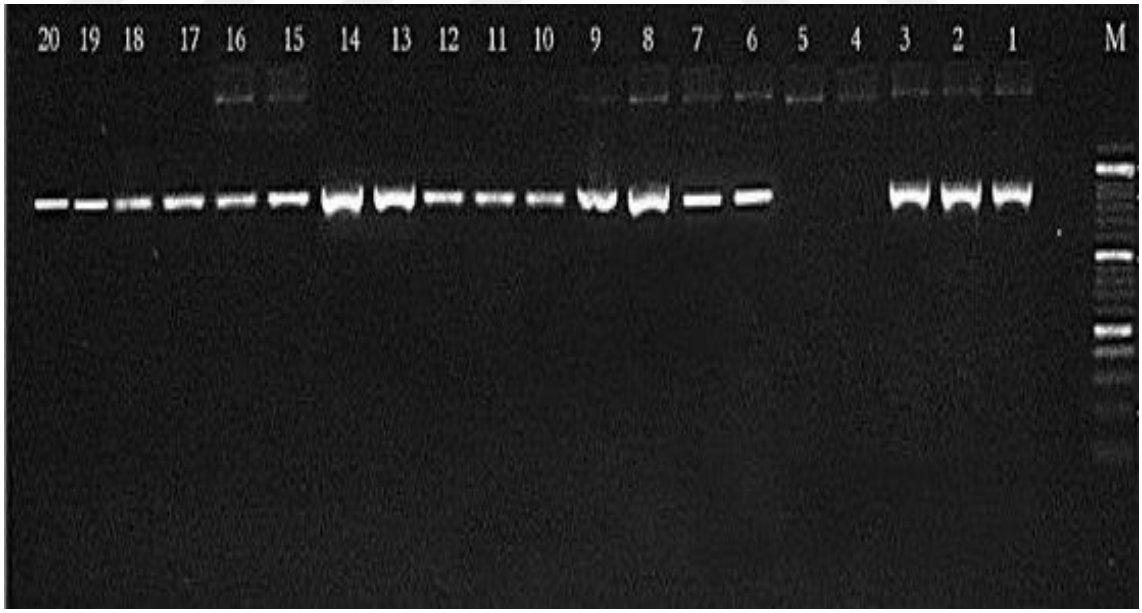

Figure 4.5 1.5% agarose gel electrophoresis image of PCR products of the *hlyA* gene region in *E. coli* isolates

4.6 Prevalence of *vat* Gene in *E. coli* Isolates

The *vat* gene was found to be positive in 19 of the 80 *E. coli* isolates studied with the percentage recorded 23.75% (Figure 4.6 and Table 4.5). The *vat* gene gave an amplification product of 420 bp. There was no statistical correlation between bacterial isolates in terms of containing both virulence genes (P > 0.050).

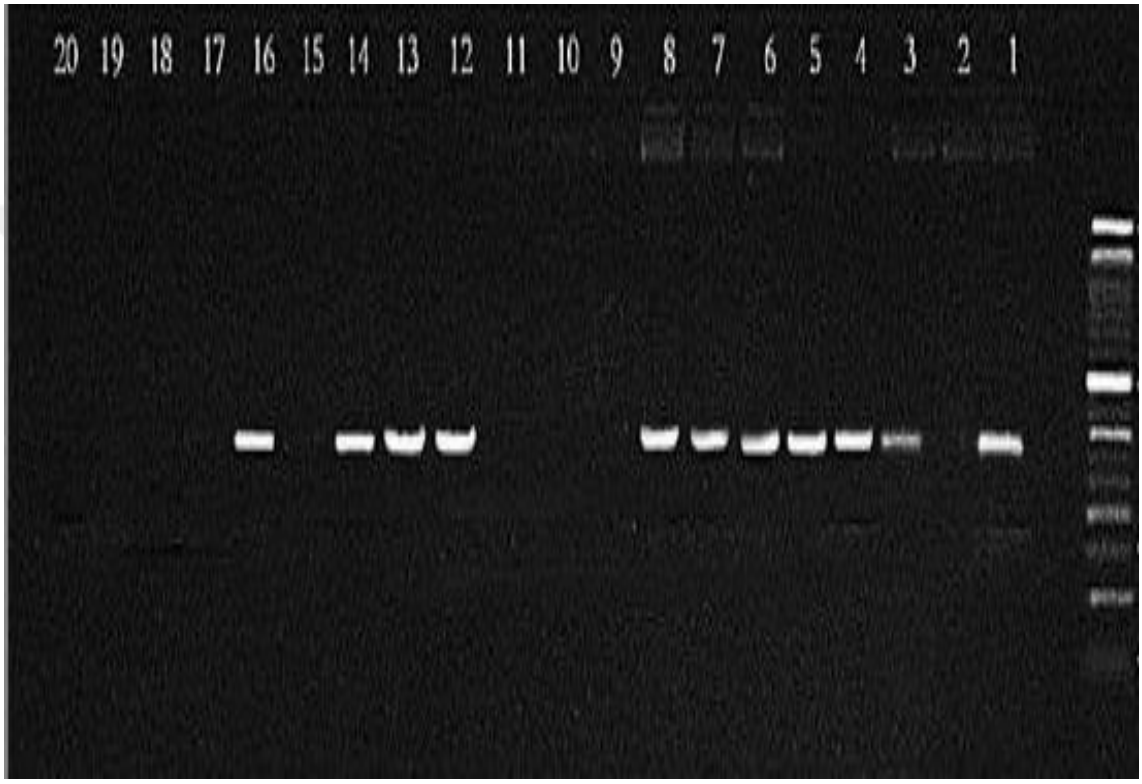

Figure 4.6 1.75% agarose gel electrophoresis image of PCR products of the *vat* gene region in *E. coli* isolates

4.7 Prevalence of *mcr-1* Gene in *E. coli* Isolates

The *mcr-1* gene was found to be positive in 10 of the 80 *E. coli* isolates studied with the percentage of 12.50 % (Tables 4.5, Figure 4.7). The *mcr-1* gene gave a 320 bp amplification product. A statistical relationship was found between *E. coli* isolates positive for *vat* and *hlyA* frequency genes and *mcr-1* positive isolates (P < 0.050).

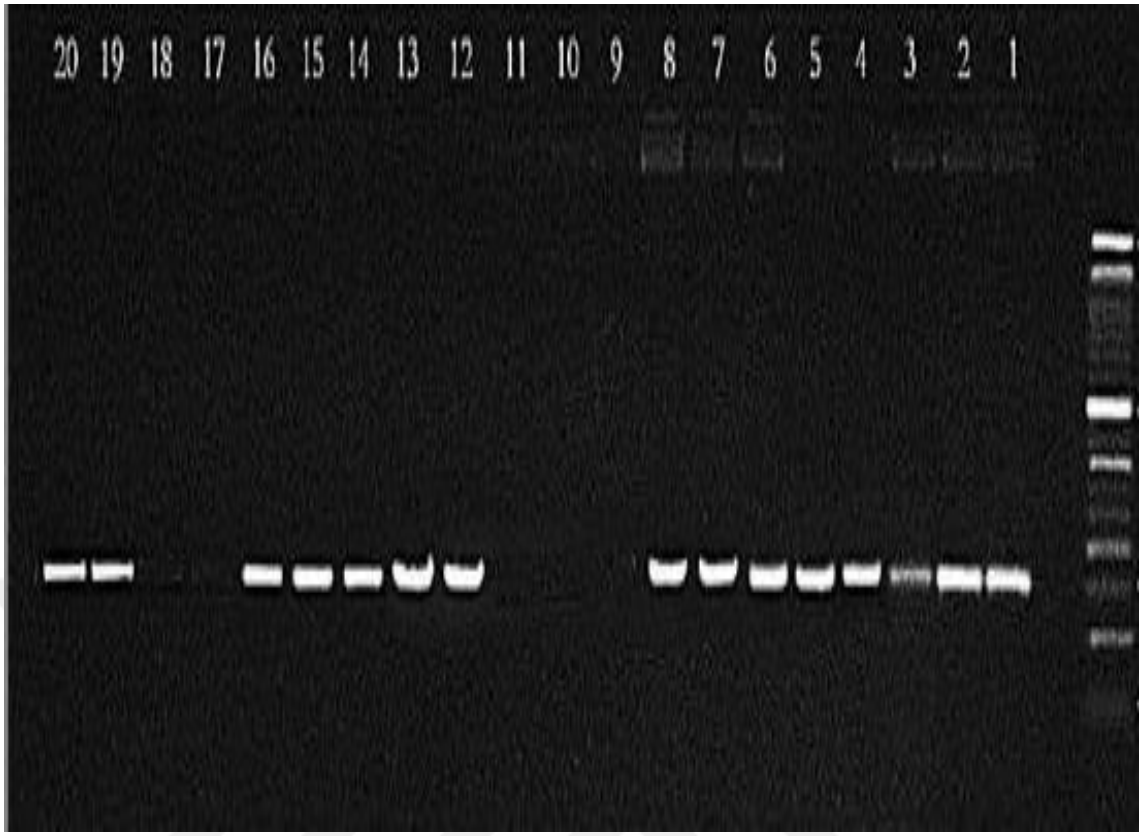

Figure 4.7 1.75% agarose gel electrophoresis image of PCR products of *mcr-1* gene region in *E. coli* isolates

Table 4.6 Percentage and number of genes that diagnostic by Real Time PCR technique in this study

Gene	Positive	Percentage (%)
hlyA	21/80	26.25
Vat	19/80	23.75
Mcr-1	10/80	12.50

The results showed that the *mcr-1* gene was found at a low rate of 12.50% in *E. coli* isolates isolated from urine samples coming to the microbiology laboratory of some hospitals, and it was noted that they developed resistance to most antibiotics, including colistin. While colistin has been considered as an effective treatment option for the rapid increase of multidrug resistance (MDR) gram negative pathogens in recent years, the high prevalence of this *mcr-1* gene in Konya poses a threat to colistin efficacy.

However, the fact that the *vat* gene, which has been proven by studies, is found especially in pathogenic *E. coli* and its strikingly high prevalence of 23.75 % in the thesis study suggests that it should be followed for effective treatment. Like the *vat* gene, the *hlyA* virulence gene contributes to its pathogenicity by avoiding the host's immune system in *E. coli* strains, but the obtained *hlyA* positive rate (26.25%) was found to be at the estimated prevalence in *E. coli*.

UPEC refers to numerous virulence factors that cause bacteria to form UTIs (Shah *et al.* 2019). UTIs, including cystitis and pyelonephritis, are one of the most common infectious diseases of humans and pets (Xia *et al.* 2011, Farshad *et al.* 2012, Nam *et al.* 2013).

Consistent with the results of this thesis, a group of researchers suggested that there is a significant relationship between UPEC virulence factors and UPEC antimicrobial resistance, and that these factors should be routinely tested and cooperated with AMR (Shah *et al.* 2019).

A group of researchers obtained lower rates in contrast to the 27.77% *hlyA* positive prevalence we found in this study. Düzgün *et al.* (2019) proved a low prevalence of the *hlyA* gene, only 3.3%, in 90 *E. coli* strains they isolated from UTI patients. Likewise, in another study in which a total of 110 *E. coli* isolates were studied, 79 of which were isolated from the community and 31 of which were isolated from hospital-acquired UTI, the prevalence of the *hlyA* gene was determined as 9.1%, and it was stated that this rate was higher in *E. coli* isolates.

Contrary to these reported studies, in line with the results of this thesis study, Tabasi *et al.* (2016) determined that the prevalence of the *hlyA* gene was 30.8% in a total of 156 *E. coli* isolates isolated from the urine samples of outpatients and inpatients, while this rate was found to be 30.8% compared to inpatient isolates. It was stated that it was 2.2-3.5 times more in those who were treated. In a few similar studies, the prevalence of *hlyA* gene was determined as 28.8% and 25.45% in strains isolated from urine samples (Hashemizadeh *et al.* 2017, Rijavec *et al.* 2006).

Consistent with this study, another study reported that this virulence gene had a relatively higher prevalence of 36% in *E. coli* isolates isolated from many samples such as feces, urine, blood, wounds, etc. of hospitalized patients (Nojoomi and Ghasemian 2017).

Contrary to the 67.85% watt-positive prevalence we found in this study, some researchers obtained lower rates. Shookohi and Rashki (2016) reported a low prevalence of only 18% of the *vat* gene in 100 *E. coli* strains isolated from UTI patients.

Saraylu *et al.* (2012) reported that the *vat* gene had a prevalence of 36% in 297 *E. coli* strains isolated from urine samples of hospitalized patients. In another study, it was reported that the prevalence of the *vat* gene was 25.5% in a total of 47 *E. coli* strains isolated from hospitalized UTI patients (AL-Hayali *et al.* 2019).

Some researchers have obtained lower rates in contrast to the 11.11% *mcr-1* positive prevalence we found in this study. He *et al.* (2017) found that the prevalence of the *mcr-1* gene was only 0.6% in a total of 700 *E. coli* strains isolated from their hospital. In another study, the *mcr-1* gene was found to be 8% in strains isolated from urine samples of 368 pets (224 dogs, 144 cats) with suspected UTI, and it was stated that *E. coli* has a role in UTIs in both humans and animals (Yamaji *et al.* 2018).

A low prevalence of only 2.1% of the *mcr-1* gene was reported in a total of 144 *E. coli* strains isolated from hospitalized patients with bloodstream infections (Zhong *et al.* 2019).

Consistent with this study, in another study, it was reported that this virulence gene had a higher prevalence of 64.9% in 739 *E. coli* strains, a total of 5,484 urinary tract samples isolated from children with suspected UTI in hospital (Parajuli *et al.* 2017).

5. CONCLUSIONS AND RECOMMENDATIONS

Female patients had a significantly higher UTI infection rate than male patients. Females accounted for 61.25% (49 patients) of total cases, while males accounted for 38.75% (31 patients). Among patients below 60 years, females had a 15.0% infection rate compared to 8.75% for males. In the age range of 50-59 years, female UTI cases were 13.75% compared to 8.75% for males. For both genders aged 10 years and above, the infection rate was 2.5%.

Clinical samples of UTI were cultured in MacConkey agar medium; 28 isolates were found to be able in fermentation of lactose as indicates by the exist of pink color colonies in the culture medium, these morphological characteristics comes according to the corresponding cultural charasteristics of *E. coli* bacterium that mentioned by Zinnah *et al.* (2007).

The results showed that *E. coli* bacterium recorded upper percentage of patient's samples which recorded 35.0% with 28 patients of this study while *Pseudomonas aeruginosa* recorded 15.0% with 12 patients.

Pure culture colonies of bacteria were identified depending on their gram staining and other microscopically characteristics. The bacterial isolates suspected to the *E. coli* bacterium depending on microscopical characteristics were subjected to the linked biochemical tests.

The results showed that all the 28 isolates were had given positive results for catalase, methyl red and lactose fermentation tests, but negative results for oxidase, citrate and vogas- proskauer tests. The results showed that the 28 isolates of *E. coli* were varied in their sensitivity to the tested 12 antibiotics, 7 antibiotics (gentamycin, ampicillin, tetracyclin, pencillin, erythromycin, trimethoprim, and clindamycin) were showed resistance to the *E. coli* isolates, 2 antibiotics (rifamycin and fusaric acid) were showed sensitive to the *E. coli* isolates wherase 3 antibiotics (vancomycin, fostomycin, and

cefuroxime) were showed moderate resistance to the *E. coli* isolates. These results gave 94% similarity to those *E. coli* characteristics as identified by the standard gram negative card.

A critical thought when creating drugs that target the digestion system prepare, or for that matter, all drugs that target basic bacterial forms, is the potential side impacts on the adjust of the microbiome within the intestine. Subsequently, in a perfect world, the target chosen for medicate improvement ought to be UPEC-specific and have negligible affect on the intestine microbiota. Since UPEC's iron availability is restricted within the urinary tract, press take-up utilizing the tonB carrier is basic for *E. coli* destructiveness. In this manner, press transporters in *E. coli* are potential medicate targets. In any case, for this target to be reasonable, encourage investigate is required to investigate the part of press transporters within the intestine survival of individuals of the intestine microbiome, as the potential sedate will moreover have suggestions for the intestine microbiome (Mann *et al.* 2017).

There's a noteworthy relationship between *E. coli* harmfulness variables and *E. coli* antimicrobial resistance. The rise of drug-resistant microorganism among UPEC isolates truly increments the risk to worldwide wellbeing (Tabasi *et al.* 2016, Shah *et al.* 2019).

Hence, information of the neighborhood predominance of *E. coli* and antimicrobial resistance is basic for ideal administration of UTI. These discoveries will offer assistance to get it the pathogenicity and fitting treatment of UTI patients, subsequently diminishing the abuse of anti-microbials (Shah *et al.* 2019).

The expanding number of MDR pathogens in animal nourishments isn't as it were putting a noteworthy burden on the worldwide nourishment industry, but moreover driving to significant and financial losses. therefore, there's a critical ought to superior get it hazard components along the nourishment chain (Perez-Rodriguez and Mercanoglu-Taban 2019).

E. coli, UTI may be a bacterium of great around the world intrigued, and UTI may be a genuine open wellbeing issue, so information of *E. coli* destructiveness components gives distant better; a much better; a higher; a stronger; an improved a higher understanding of bacterial pathogenesis. The *vat* gene quality is said to be fundamental for *E. coli* to enter the circulation system and survive, and isolates with the *vat* quality are more perilous than other *E. coli* pathotype isolates. *hlyA* contributes as a destructiveness calculate to the capacity of *E. coli* microscopic organisms to colonize the intestinal tract in intestinal diseases. In any case, the *mcr-1* quality gives resistance to a few microscopic organisms, expanding the treatment difficulties. Therefore, *E. coli* isolates got to be distinguished utilizing atomic strategies and the predominance of harmfulness qualities among *E. coli* isolates to be assessed. There's a got to control transmission of the *mcr-1* quality, and the rise and spread of the *mcr-1* quality in people ought to be closely checked.

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