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Molecular Study of Some Biofilm Formation Genes for *Escherichia coli* Isolated from Urinary tract infection

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا﴾

صَدَقَ اللهُ العَلِيُّ العَظِيم

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Certification

I certify that the preparation of this thesis was prepared by Dina Hasan Abed Abbood under our supervision at University of Babylon, College of Sciences, Department of Biology, as a partial fulfillment of the requirement for the master degree of Science in Biology. Accordingly, I Recommend this study for discussion.

Prof. Dr.

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In view of the available recommendation, I forward this thesis for debate by the examining committee.

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/ / 2023

Dedication

To...

Who removed the thorns from my path to pave my way to the science ... my father

Who surrounded me with tenderness and safety... my mother

Who helped me to overcome all the difficulties... My husband

Whose supported and encouragement me all the way since the beginning of my study...My lovely family

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DINA

Summary

Urinary tract infection (UTI) are one of the most common bacterial infections, affecting 150 million people each year worldwide . A total 117 urine samples were collected from patients with urinary tract infection from AL- Hilla Teaching Hospital and Babylon Hospital for Maternal and Pediatrics in Hilla city/Iraq, during the period from November 2021 to February 2022.

In this study, the outcome were 50 isolates identified as *Escherichia coli* depending on selective media (Eosin Methylene Blue agar) and Vitek2 system to confirm the diagnosis of bacterial isolates along with Polymeras Chain Reaction diagnosis for *16S rRNA.* The current study showed that the infection rate in females (82%) was higher than males (18%) and high risk of infection was in the age group (21-30) years .

When using the method of disc diffusion, the results of the antibiotic sensitivity test were as follows : Ampicillin 50%, Pipercillin 62%, Amoxicillin-clavulanate 98%, Cefepime 36%, Ceftazidime 72%, Cefixime 48%, Cefotaxime 68%, Aztreonam 26%, Imipenem 4%, Meropenem 0%, Amikacin 22%, Gentimicin 48%, Tobramicin 40%, levofloxacin 34%, ciprofloxacin 30%, and Trimethoprim 52%. Multiple drug resistance was present in high numbers among *E. coli* isolates, where 42 out of 50 isolates represented the MDR, and the percentage was 84%.

The productivity of the biofilm was quantified based on tube method for included all isolates and according to the results, they were classified as 5 (10%) strong adherent and 36 (72%) moderate biofilm producer, while 9 (18%) represented weak biofilm formation.

PCR technique was performed for three genes involved in stages of biofilm development for 50 Uropathogenic *E.coli* isolates. PCR technique was carried out to determine the presence of adherent factors genes in all *E. coli* isolates and the results were as follow: *FimH* 46(92%), *CsgA* 49(98%), *flu* 46(92%). In the present study, sequence analysis was achieved for PCR product for *fimH* gene of nine local isolates of *E. coli* representing three biofilm phenotypic categories (weak, moderate, and strong). This sequence analysis was performed to find out the nucleotide identity with others worldwide strains through NCBI- Blast-query nucleotide –online application. The results showed that, the identity percentages of these 9 local *E.* coli isolates with other gene bank registered strains were ranged between (96- 98%). Multiple alignment nucleotide sequences were also performed to define the variations among these local isolates by using BioEdit software along with amino acids alignment. Three positions (202, 214, and 230) of variations were repeatedly detected among strong biofilm former isolates (*FimH* 7, 8, and 9) which led to change in the amino acid types of the sequence (68,72, and 77) after changing their codons comparing with other local isolates. However, the weak biofilm formers (FimH2 and FimH3) displayed unique nucleotide variation in sequence 185 along with one isolates of moderate biofilm former, which also resulted in change of amino acid type at sequence 62.

Conclusion: The biofilm phenotype was indicated in all *Escherichia coli* isolates and that can confer virulence behavior and considered as a great challenging health problem and significant association between adherent factor' gene (fimH) and the ability to produce biofilm within *E. coli* isolates

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List of Abbreviations

Abbreviation	Terms
Ag43	Antigen 43
AR	Antibiotic Resistance
CLSI	Clinical and Laboratory Standards Institute
CsgA	Curli fimbriae
CDT	Cyclomodulins toxin
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
EMB	Eosin Methylene Blue
ExPEC	Extraintestinal Pathogenic *E.coli*
FimH	Type 1 fimbriae Adhesion Protein

Abbreviation	Terms
UPEC	Uropathogenic *E.coli*
UTI	Urinary Tract Infection
UV	Ultraviolet
VFs	Virulence Factors
WHO	World Health Organization

Units of Abbreviation

Abbreviation	Units
µg	Microgram
gm	Gram
M	Molar
µl	Microliter
ml	Milliliter
µm	Micrometer
Mm	Millimeter
hr.	Hours
min.	Minute
sec.	Second
RPM	Rotations per Minute
Pmol.	Picomol
bp.	Base pair
˚C	Celsius degree

Chapter One

Introduction

1. Introduction

Urinary tract infections (UTIs) are one of the most prevalent health issues and predominant bacterial nosocomial infections in developed countries. Every year, approximately 150 million people worldwide are diagnosed with UTI. Along with being a causal agent in different infectious disease, *E. coli* is the most prevalent etiological agent of UTI (80%), followed by the significantly less common *Staphylococcus saprophyticus* , *Klebsiella*, *Proteus*, or other Gram-negative bacteria (Fattahi *et al.*, 2015). Up to 40% of women will suffer from a UTI at some point in their lives, with a significant number among these women having recurrent UTIs. Furthermore, *E.coli* can cause both community-acquired infections and health care-related infections, and able to cause disease in all age groups (Galindo-Méndez, 2020).

Similar to other mucosal infections, uropathogenic *E. coli* (UPEC) strains have a multi-step pathogenesis process. These approaches allow it to colonize host mucosal surfaces, injuries, invade damage host tissues, evasion of hosts defense and incite a host inflammatory response (Kaper *et al.*, 2004). Bacterial persistence via the formation of a stable and complicated form known as biofilm seems to be the main pathogenesis factor and treatment failure. Biofilm formation protects UPEC from antimicrobial drugs, host immune system, and environmental stress. Adhesion to the surface, cellular aggregation, the production of an extracellular matrix, and biofilm maturation are the four basic steps in the forming of a biofilm (Vetrivel *et al.*, 2021). The development of biofilm requires effective adherence. A variety of factors have been demonstrated to be essential for a given bacterial strains to create a biofilm. Firstly, flagella-assisted motility (twitching motility) allows cells to reach surfaces, secondly, adherence compounds such as fimbriae *eaeA* and curli

(*CsgA*) supports attachment and initial colonization on the target material; and eventually, the development of exopolysaccharide matrixes aids in the formation of the three-dimensional biofilm structure (Taghadosi *et al.*, 2017). Most of coliform clinical isolates express type 1 (mannose-sensitive) and type 3 (mannose-resistant) fimbrial adhesins. Type 1 fimbria encoding by *FimH* gene is an important determinant, which has high tropism for urinary tract receptors. Thus, FimH adhesion is important for colonizing different niches of *E. coli*. The self-recognizing surface adhesin structure called antigen 43 (Ag43), encoding by *flu* gene, found in *E. coli* strains conferring the clumping and fluffing of cells and induce biofilm formation because of its typical characteristic of cell-to-cell accumulation (Mahmood and Abdullah, 2015).

In chronic wounds such as diabetic foot infections (DFIs) and urinary tract infections, biofilm formation is common. Biofilms have a major medical significance because they reduce susceptibility to antimicrobial agents and have been associated with the disruption of the efficacy of the most commonly used and well-known antibiotics to treat UPEC infections (Mahmood and Abdullah, 2015). This style of growth promotes the development of multidrug-resistant organisms (MDRO) by allowing for poor antimicrobials penetration and in horizontal transmit of virulence genes (Katongole *et al.*, 2020).

Aim of the study

The aim of this study was to determine the possible relationship between biofilm formation and susceptibility to different antibiotic groups among *E. coli* isolates from UTI patients. The aim was achieved through several objectives.

- Isolation and identification of *E. coli* from the UTI patients.
- Identification of antibiotic resistance profile of *E. coli* isolates.

- Identification and quantification of biofilm formation.

- Molecular detection of genes (*FimH*, *CsgA*, and *flu*) encoding adhesion factors implicated in biofilm formation

Chapter Two
Literatures Review

2. Literature Review

2.1. *Escherichia coli*

Escherichia coli is a Gram-negative, facultatively anaerobic bacterium, belongs to the Enterobacteriaceae family, Gammaproteobacteria class, phylum Proteobacteria , which belongs to the kingdom Eubacteria (Garrity *et al.*, 2005).

It is a rod-shaped and nonsporulating bacterium that is simple to cultivate. *E. coli* can replicate in around 20 minutes when given the optimum growth conditions, include 37°C, aeration, and a pH of 7.0 (Sharma *et al.*, 2016, Tenaillon *et al.*, 2016). It has served as a model organism for numerous investigations of industrial microbiology and biological engineering, being a microbe that extensively examined. As a host bacterium, numerous gene-manipulation systems have been created, leading to yield myriad enzymes and other industrial materials. Similarly, rapid development makes it a typical candidate of microorganisms′ evolutionary research and long-term experimental studies. The first study on *E. coli* genome sequence analysis was published in 1997, since that, the genomes of over 4800 strains have been sequenced and more than 50, 000 generations of *E. coli* are under research considerations (Jang *et al.*, 2017).

As a member of the Enterobacteriaceae family, bacterial cells of *E. coli* are able to move either by using peritrichous flagella or fibrillar proteins, which are widely spreading from the bacterial surface to the surrounding membrane (Ahsan, 2016).

Their occurrence in the intestinal tract of human and other warm blood animals were attributed to their commensalism living and they are typically considered as benign bacteria. Moreover, their colonies were also observed in the

samples of soil, water, and food due to the fecal contamination (Baylis, 2006). Within these environments, *E. coli* become as normal flora (when they are frequently isolated over the course of months or years and their genotypes are being distinct from those animal- host origin genotypes). Their occurrence has also been associated with periphytic and macrophytic algae, as well as soil, silt, and sand (Jang *et al.*, 2017). It is often colonizes the child intestine within hours of delivery and plays a significant role as a normal flora of humans' gut (Kaper *et al.*, 2004a). Fimbriae or pili, which are distinct morphological structures, are vital for the adherence of these bacteria to the mucous layers (Abdul-Ghaffar and Abu-Risha, 2017a).

2.1.1. Pathogenicity of *Escherichia coli*

Pathogenic *E.coli* can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream, and central nervous system (Croxen and Finlay, 2010). Among the intestinal pathogens, there are six well-described categories: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). UTIs are the most common extraintestinal *E. coli* infections and are caused by uropathogenic *E. coli* (UPEC). A common cause of extraintestinal infections is the pathotype responsible for meningitis and sepsis-meningitis associated *E. coli* (MNEC). The *E. coli* pathotypes implicated in extraintestinal infections have recently been called ExPEC. EPEC, EHEC and ETEC can also cause disease in animals by using many of the same virulence factors that are present in human strains as well as unique colonization factors that are not found in these strains. An additional animal pathotype known as avian pathogenic *E. coli* (APEC) causes extraintestinal infections, primarily

respiratory infections, pericarditis, and septicaemia of poultry (Servin, 2005: Lara *et al*., 2017).

Pathogenic *E. coli* strains use multi-step scheme of pathogenesis that is similar to that used by other mucosal pathogens, which consists of colonization of a mucosal site, evasion of host defenses, multiplication and host damage (Kaper *et al.*, 2004a). The biological properties which may contribute to the pathogenicity of *E.coli* includes: adhesion to epithelial cells, production of enterotoxins, invasiveness and ability to multiply within the epithelial cells, insensitivity to complement lysis and resistance to phagocytic killing (Gillen and Oliver, 2010). Pathogenicity has been correlated with the presence of gene encoding virulence factors organized on large blocks, called pathogenicity islands. It has been shown that the pathogenicity islands can disseminate horizontally between distinct *E.coli* strains whether they are located on plasmids, bacteriophages, or even the bacterial chromosome (Whittam and Bumbaugh, 2002). Only the most successful combinations of virulence factors have persisted to become specific 'Pathotypes' of *E. coli* that are capable of causing disease in healthy individuals. Three general clinical syndromes can result from infection with one of these pathotypes enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis(Cursons *et al.*, 2005).

2.1.2.Uropathogenic *Escherichia coli* (UPEC)

Each year, over 150 million people in the world get UTI, which has significant socioeconomic implications (Flores-Mireles *et al.*, 2015). A UTI is defined as having a certain amount of bacteria in the urine (often > 10^5/ml) (Sasirekha, 2017). It is divided into kidney (Pyelonephritis) and bladder (Cystitis), which can be symptomatic or asymptomatic (Prakasam *et al.*, 2012). It usually starts as a bladder infection (cystitis) but can develop to acute kidney

infection (pyelonephritis), ultimately resulting in scaring and renal failure. UTI is one of the most common bacterial infections in humans, with 40–50% of women reportedly having at least one episode of cystitis in their lives (Kaper *et al.*, 2004).This because women's urethras are shorter and closer to the anus than men's, the pathogenic germs can more easily access the bladder, increasing the likelihood of infection (Tajbakhsh *et al.*, 2015). These microorganisms have been associated with number of diseases in pregnant women due to their virulence factors that enable them to colonize the vagina and/or endocervix (Guiral *et al.*, 2011). Urinary tract infections is also a major cause of sepsis, which has a mortality rate of 25% and results in more than 36,000 deaths per year in the USA (Totsika *et al.*, 2012).

While there are many causal agents leading to UTIs, bacteria are responsible for more than of 95 %, with 85% obtained in the community and 50% of infections acquired in the hospital are caused by *E. coli* (De Souza *et al.*, 2019, Farajnia *et al.*, 2009). Patients with an indwelling urinary catheter for 30 days or longer are commonly developed catheter-associated UTI, reach up to 40% of all hospital-acquired infections (Kaper *et al.*, 2004). Uropathogenic *E. coli* evolved from non-pathogenic strains by acquiring new virulence factors (adhesins, toxins, siderophores, and polysaccharide) by DNA horizontal transfer. Virulence factors are recognized as pathogenicity islands, enabling cells to survive and grow in urine and other extraintestinal environment (Mladin *et al.*, 2009). They are expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to chronic pyelonephritis (Sarowska *et al.*, 2019a). Uropathogenic *E. coli* strains are responsible for 80-90% of community-acquired UTI and 30-50% of hospital-acquired UTI (Fattahi *et al.*, 2017).

Along with *E. coli*, the commonly isolated bacterial pathogens, was the major causative agents responsible for both uncomplicated and complicated UTIs. For the agents involved in uncomplicated UTIs, UPEC are the most common in prevalence, followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the order of prevalence for causative agents following UPEC is *Enterococcus spp*., *K. pneumoniae*, *Candida spp*., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS (Flores-Mireles *et al*., 2015a).

2.2. Virulence Factors

The most investigated and studied virulence factors of *E. coli* bacterial cells can be broadly divided into two groups: bacterial cell surface and secreted virulence factor. Bacterial cell surface virulence factors most commonly include fimbriae, mainly type 1 fimbriae and P fimbriae. These fimbriae help in adhesion to host cell surface, tissue invasion, biofilm formation, and cytokine induction which are crucial in pathogenesis of UPEC that is responsible for UTIs. Bacterial cell surface virulence factors are also included flagellum, capsular lipopolysaccharide and outer membrane proteins. Haemolysin and siderophores are secreted virulence factors. These virulence factors are necessary for enabling bacteria to colonize the urinary tract and persist despite the effective response of host defense mechanism (Shah *et al.*, 2019).

2.2.1. Toxins

Previous research has been identified toxins as a necessary virulence factor in a variety of *E. coli* mediated diseases. The production of toxins by colonized *E. coli* may cause an inflammatory response that leads to the UTI symptoms. Toxins have the ability to alter the host cell signaling cascade and modulate

inflammatory responses. Several *in vitro* and *in vivo* studies showed that toxins can also contribute to the stimulation of the host cell death and releasing of necessary nutrients, which provide the ability to access deeper tissues within the urinary tract (Sarowska *et al.*, 2019). Cyclomodulins toxin (CDT) (a bacterial virulence factor produced by several Gram-negative pathogenic bacteria) was first reported as virulent toxin in UPEC in 1987 (Pons *et al.*, 2019). This investigation was opened a new door in the research of UTI pathogenesis, and then many other UPEC toxins were identified, including α-haemolysin (HlyA), cytotoxic necrotizing factor 1 (CNF1), secreted auto transport toxin (SAT), cytolysin A, plasmid-coded toxin (PET), etc (Hozzari *et al.*, 2020).

2.2.2. Heamolysin

An enzyme that degrades red blood cells and is produced by many Gram-negative bacteria such as *E. coli*, *Serratia spp*., *Proteus spp*., *Vibrio spp*., *Pasturella spp*. and *Pseudomonas aeruginosa*. It is considered as one of the pivotal causes of bacterial infections for certain diseases. This enzyme consists of several types: alfa-hemolysin, beta-hemolysin, gamma-hemolysin, and as for the fourth type, it is known as delta-hemolysin (Pontieri, 2018). To detect enzyme production by bacteria, methods such as genetic detection and lysis on blood cells have been applied (Badouei *et al.*, 2016) .The isolates of *E.coli* bacteria that cause urinary tract infections are more hemolytic than the isolates of other diseases ((Banar *et al.*, 2016) . Uropathogenic *E. coli* secretes high concentrations of α-haemolysin, that oligomerizes cholesterol-rich cell membrane. This results in pore formation in the host membrane and lead to promotes cell lysis, which facilitates iron and nutrient acquisition by the bacteria (Flores-Mireles *et al.*, 2015).

2.2.3. Lipopolysaccharide and Capsule

Uropathogenic *E. coli* strains have a polysaccharide coating on their surface. Lipopolysaccharides (LPS), which are known as O antigens, are present on the exterior side of the outer membrane and are often covered by a capsule layer (K antigen) (Russo *et al.*, 2009). There is high antigenic heterogeneity between O and K antigens of UPEC strains. Lipopolysaccharide and capsular polysaccharides can help UPEC strains resist host immune defense processes such phagocytosis and death by antimicrobial peptides. Some capsular types, such K1 and K5, negate the function of the humoral response to urine infections by displaying a molecular resemblance to tissue components (Karam *et al.*, 2019). Additionally, it appears that LPS contributes to the bladder colonization of UPEC and antibiotic resistance (Aguiniga *et al.*, 2016)**.**

2.2.4.Siderophores

Iron is an essential component of the enzyme system in the most bacterial species. Bacteria need about 0.4 - 4 µmol/L of iron, while the abundance of free iron is too low to sustain their growth. Therefore, many Gram-negative bacteria respond to this environmental signal by de-repressing siderophore - mediated iron transport. Siderophores are small, high affinity iron-chelating molecules, that are secreted by microorganisms such as bacteria, taken up iron via specific receptors at the outer membrane (Garénaux *et al.*, 2011). Low abundance of iron acts as an environmental signal that regulate the expression of other virulence factor genes such as exotoxins. In addition, the production of siderophores is associated with the virulence capacity of many animal pathogens. However, Siderophores act as growth stimulants for plants by making iron less available to their pathogens such as fungi and Rhizobacteria, (Williams *et al.*, 1998). Bacterial siderophores and host iron-binding proteins compete for iron. Many *E.*

coli strains related to urinary tract infections produce siderophore, which binds iron and allows it to be taken by certain bacterial surface receptors and utilized by the pathogen.(Vagarali *et al.*, 2008). The results from independent epidemiological studies illustrated the importance of these iron acquisition systems for UPEC persistence in the urinary tract. Iron-related genes are highly abundant among persisting strains compared to those of sporadic infections (Ejrnæs *et al.*, 2011, Luo *et al.*, 2012). Moreover, The ferric uptake regulatory (Fur) system in *E. coli* is very essential features during UTI events (Williams *et al.*, 1998). The expression of the iron intake system using siderophores is to withdraw iron from the environment and gradually concentrate it within bacterial cytoplasm. This regulatory approach is necessary for bacteria to colonize and survive in iron-poor environments such as the urinary tract. Limiting iron is necessary for host defenses against invading bacterial pathogens (Wiles *et al.*, 2008).

2.2.5.Biofilm Formation

Microorganisms exist in nature in the form of free-floating cells or as architectural structures known as biofilms (Harmsen *et al.*, 2010). A biofilm which occurs among higher than 90% of bacteria, is defined as a group of microorganisms encased within a matrix of self-secreted polymeric extracellular material that is irreversibly attached to a biotic and biotic surface and hard to remove with a gentle rinse. Depending on the environment in which the biofilm has developed, noncellular materials such as mineral crystals, corrosion particles, clay or silt particles, and blood components may also contribute to the biofilm formation. Biofilm may be formed on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems (Donlan and Costerton, 2002). Biofilm formation

promotes encrustation and protects the bacteria from the hydrodynamic forces of urine flow, host defenses and antibiotics (Schembri *et al.*, 2001). Gram-negative pathogens adhere to biotic and abiotic surfaces by a variety of methods, including fimbriae (such as type 1, type 3, type IV, curli, and conjugative pili), cell surface adhesins (such as autotransporter proteins like antigen 43), and flagella. (Ong *et al.*, 2010).

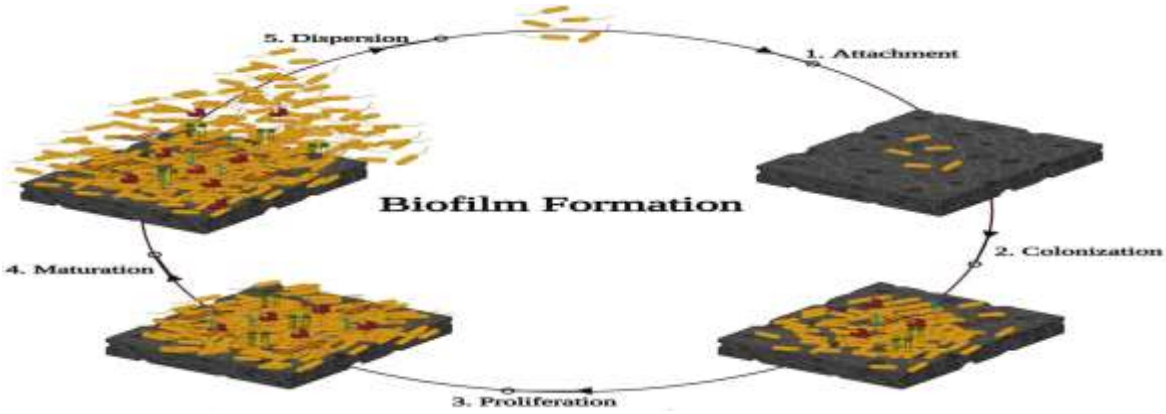
In *E. coli*, various infections were attributed to their ability of biofilm formation which confer resistant and allow it to withstand against mechanisms of bacterial eradication. The extracellular appendages are among factors involved in *E. coli* colonization on different surfaces, their function and expression are highly regulated, to control on the productive events lead to mature biofilm formation. There are four major steps involved in biofilm formation figure(2-1): first adhesion or attachment (reversible); premature development of biofilm structure (irreversible); maturation of the developed biofilm and dispersion of cells from the biofilm to restore the planktonic form (Sharma *et al.*, 2016).

- First adhesion: When nutrients are available or under proper condition, bacterial cells can aggregate to form biofilm on either biotic or a biotic surface. Interaction between *E. coli* cells and their attachment can be facilitated by flagella that responsible for cell motility. Flagella can also be responsible for the enhancement adhesion between the cells and surfaces in the first stage of contact between them (Beloin *et al.*, 2008).
- Premature biofilm development: As the adherent *E. coli* cells become sessile due to their attachment to the surface, the synthesis of flagella is suppressed and the diversion from a planktonic to a sessile state is mediated by a number of tiny molecules. The concentration of cyclic-diguanylic acid (c-di-GMP) is low during the motility stage and increases with the development of biofilms. Type 1 fimbriae and curli fimbriae are two adhesion-factor organelles of a

complex structure that are crucial during *E. coli* irreversible attachment to the surfaces and mediate bacterial adherence to host epithelial receptors (Wood, 2009). They are also serve as virulence factor helps in development of urinary tract infection (UTI), enabling bacterial attachment to the bladder cell surface glycoprotein uroplakin and other host proteins that contain mannosides (Abdul-Ghaffar and Abu-Risha, 2017a). In *E. coli*, Type 1 fimbriae or pili, are encoded by *fimH* gene, their expression is triggered by the early stages of biofilm formation. The extracellular structures that adhere to the extracellular matrix proteins are called curli fimbriae, are encoded by the *Csg* gene, facilitate cell-to-cell communication and promote adhesion to abiotic surfaces (Beloin *et al.*, 2008).

- Maturation: In the phase of maturation, bacteria produce extracellular matrix which provides a three-dimensional structure to the biofilm. Autotransporters (for cell-to-cell interaction) and EPS (for matrix formation) are both crucial for biofilm maturation. Autotransporter proteins are those that can move to the outer membrane without the assistance of accessory proteins (Beloin *et al.*, 2008). Antigen 43 (Ag43) is the key autotransporter encoded by the *flu* gene. It promotes cell-to-cell adhesion, thus facilitating auto-aggregation and three-dimensional development. The transporter proteins Aid A and Tib A are associated with virulent strains of *E.coli*, induce cell aggregation and biofilm formation (Vogeleer *et al.*, 2014).

The EPS matrix is the medium through which bacterial cells are attached to the surface and facilitate cell-to-cell as well as cell-to-surface interactions. It provides support to biofilm cells and gives the biofilm a three-dimensional architecture, thus providing a protective as well as structural role (Flemming and Wingender, 2010).



Figure(2-1) Biofilm formation stages of *E.coli* (Srinivasan *et al*,.2021)

2.3. Genes Involved in Biofilm Formation

Several genes in *E.coli* have been associated with biofilm formation, including the genes for type 1 fimbriae (*FimH*) , curli (*csgA*) , and Antigen 43(*flu*).

- ***Type 1 fimbriae (FimH)***

A first critical step in the establishment of infection is bacterial adhesion to urothelial cells under flow conditions, which is mediated by 0.1−2 μm long, proteinaceous filaments on the bacterial surface termed type 1 pili (Jones *et al.*, 1995, Hahn *et al.*, 2002). Type 1 pili are composed of up to 3,000 copies of the subunit FimA building the pilus rod, as well as the subunits FimF, FimG and FimH forming the distal tip fibrillum (Waksman and Hultgren, 2009). The adhesin FimH at the fimbrial tip specifically binds in a catch-bond mode (Thomas *et al.*, 2002) to terminal α-D-linked mannoses of N-linked glycans of the receptor uroplakin 1a on urinary epithelial cells (Zhou *et al.*, 2001). Owing to its important role in establishing infection, FimH is an attractive target for the development of anti-adhesive drugs for UTI treatment. The FimH protein is a

precursor of 300 amino acids, recognizing terminal mannoses on epithelial glycoproteins, which are later converted into 279 mature forms. FimH is a two-domain protein, it is comprised of the fimbria-incorporating pilin (Pd, 160–273 amino acids) and the mannose-binding lectin (Ld, 1-156 amino acids), which are joined by a brief linker. There is a mannose-binding pocket in the form of a barrel at the tip of Ld (MBP) (Foroogh *et al.*, 2021). Many virulence factors were highly prevalent throughout the population, such as fimH, indicating that they were either introduced into *E. coli* early in its evolutionary history and are now found in all members of the species (which is likely the case for fimH) or, if more recently acquired, are highly horizontally mobile and strongly selected for in the context of urosepsis (Johnson and Stell, 2000). According to study, the pathogenic function of the host and *E. coli* virulence factors in the development of bacteremia in patients with upper urinary tract infections was reported (UTI). The type 1 fimbrial (fimH) adhesin genetic determinant was highly prevalent (92%) (Abdul-Ghaffar and Abu-Risha, 2017) . Additionally, investigations were also conducted into the existence of adhesins (fimH) linked to the development of biofilm (Mahmood and Abdullah, 2015).

- **Ag43 Protein *(flu* gene)**

Some *E. coli* strains are able to autoaggregate, resulting in characteristic flocculation and cell settling from static liquid suspensions. Diderichsen was the first to describe this phenomenon, defining a locus flu mapping at 43 min on the *E. coli* K-12 chromosome. The flu locus appeared to control a number of most interested phenotypes, including autoaggregation and a distinctive frizzy colony morphology. Separate studies discovered that the flu locus product was identical to an outer membrane protein known as antigen 43(Ag43) (Kjærgaard *et al.*,

2000). Antigen 43 (Ag43) or fluff protein is a member of a growing family of Gram-negative bacteria self-carrier proteins. Urovirulence is also linked to the phase-variable surface-located adhesin antigen 43 (Ag43). Ag43 is belong to the family of autotransporter proteins (AT). Autotransporter proteins are distinguished by the fact that their primary sequence is enough to direct their transport across the system of bacterial membrane and the final tracking of the various passenger domain (alpha-domain) to the cell surface.(Ulett *et al.*, 2007). AT proteins are a type of afimbrial adhesin with several features in common, including an N-terminal domain leader sequence, a passenger domain responsible to define the protein's functional properties, and a C-terminal translocator or -barrel domain which found to integrate with the cell outer membrane and facilitates the transport of the passenger domain. One of these autotransporters in *E. coli* is the well-studied phase-variable antigen 43 (Ag43) protein (Martinez-Gil *et al.*, 2017).

- **Curli *(Csg A)***

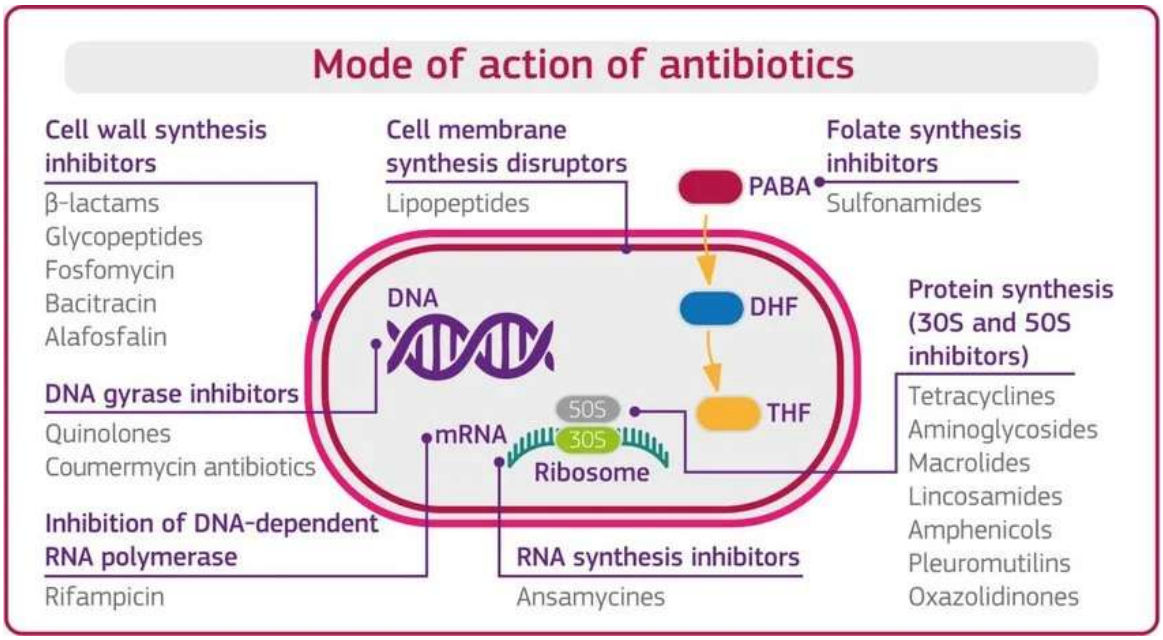
Most *E. coli* natural isolates have a transcribable curli gene, but only a few strains can assemble the subunit protein into curli (Bhoite *et al.*, 2019). Curli fimbria is a fibrous surface protein that is essential for *E. coli* and Salmonella biofilm growth, and its presence is linked to acute human infections (Cordeiro *et al.*, 2016). Curli production is relying on the csg genes "two differently transcribed operons", which encode the major structural components and auxiliary proteins. One encodes the *csgB, csgA*, and *CsgC* genes, while the other encodes the *csgD, csgE*, and *csgG* genes. *CsgA*, the major subunit of the curli amyloid fiber, is formed in the cytoplasm and transmitted to the cellular membrane as an unfolded protein, where it aggregates to form extracellular amyloid polymers (capable of self-polymerizing into beta-sheet rich amyloid

fibers that bind to amyloid binding dyes) upon interaction with the CsgB nucleator protein (Jain *et al.*, 2017). To initiate adherence to and colonization of the host, these polymers are specifically interact with host matrix proteins such as fibronectin, laminin, and plasminogen (Siddiqi *et al.*, 2017). Among the functional amyloids, Curli was the first fiber identified in *E. coli* which are highly stable, unbranched, and act as a scaffolding agent in the formation of biofilm. Curli has also been studied for its pathogenic role in avian infection by *E. coli*, and there are signs that haemagglutination activity, fibronectin binding, and curli production are co-expressed in an APEC strain. Haemagglutination and fibronectin binding are recognized as virulence factors that might be crucial for pathogenic adherence with the host surfaces. It has also been associated with bacterial internalization that leading to avian septicaemia (Antão *et al.*, 2009). In UTI patients, csgA was found in 93.3% of *E. coli* isolates, and the association between biofilm production and the presence of csgA was 100% (Jaber and Aal Owaif, 2020).

2.4. Antibiotic Resistance for *Escherichia coli*

Uropathogenic *E.coli* (UPEC) was considered as the main cause of urinary tract infections around the world. It is developed resistance towards the majority of tested antibiotics. The investigation, surveillance system, and efficient strategy can work together in order to select an appropriate treatment for controlling bacterial distribution (Assafi *et al.*, 2022). Antibiotics such as ciprofloxacin, trimethoprim, ampicillin, and sulfamethoxazole, are currently being the most commonly prescribed treatments for UTIs. However, increasing the antibiotic resistance among bacterial isolates as well as recurrence rate will significantly increase the burden for such common infection among the society (Flores-Mireles *et al.*, 2015). Bacterial resistance is strongly linked to the clinical

use of antimicrobial agents. Prolonged antibiotic treatment can cause the emergence of resistance in a microorganism that was initially susceptible to antibiotics. The onset of an antimicrobial-resistant phenotype is dependent on a host's multiple factors, including the degree of resistance expression, the ability of a microorganism to tolerate resistance mechanisms, the initial colonization site, and other factors (Giedraitienė *et al.*, 2011).



Figure(2-2) Mode of action of antibiotics (Gaskins *et al.*, 2002)

Biofilm maturation increase the antibiotic resistance among uropathogens as this type of growth act as physical barrier towards antimicrobial penetration. Realizing of specific biofilm and dispersal mechanisms in particular species is therefore essential for presenting of new therapies to stop colonization like biofilm inhibitors, anti- adhesive compounds, and compounds that stimulate bacterial dispersion. Uropathogenic *E. coli* (UPEC) forms intracellular bacterial communities (IBCs) like biofilm to protect their cellular membrane from different stresses such as neutrophils and antibiotics (Flores-Mireles *et al.*, 2015).

The presence of multidrug-resistant *E. coli* has been detected in a number of countries over the last few decades. Cephalosporin resistance increase in particular with parallel increasing of multi drug frequency which became as growing issue for *E. coli* infection treatment (Wu *et al.*, 2021). Although *E. coli* is intrinsically sensitive to about all clinically relevant antibacterial drugs, this bacterial species has a high capacity for resistance gene accumulation, primarily via horizontal gene transfer. In *E. coli*, the most corresponding problematic mechanisms is regarding to the acquiring the extended spectrum β-lactamases genes; carbapenemases; 16S rRNA methylases; plasmid-mediated quinolone resistance (PMQR) genes; and mcr genes that conferring resistance to broad-spectrum cephalosporins; carbapenems; pan-resistance to aminoglycosides; fluoro (quinolones); and polymyxins respectively (Poirel *et al.*, 2018). From January 2016 to the end of December 2018, a highly multidrug resistance percentage (80.56%) was noticed in Uropathogenic isolates of *E. coli* tested in Zakho emergency hospital (Iraq), with great resistance toward B-lactamase and macrolides antibacterial drugs (Assafi *et al.*, 2022). However, imipenem and meropenem were more sensitive. UTI *E. coli* isolates were found to be highly resistant to ceftriaxone (89.0%), ampicillin (86.6%), levofloxacin (82.9%), and cefotaxime, but comparatively less resistant percentages (2.4%, 34.1%, 35.4%) to imipenem, cefepime, and ciprofloxacin respectively (Jalil and Al Atbee, 2022). In terms of biofilm formation, UTI *E.coli* was highly resistant to Garamycine (86%) and Rifampcine (100%), with (72%) and (16%) indicating moderate and strong biofilm formation, respectively. In another study, Amoxicillin-clavulanate showed 100% resistance against *E. coil* isolates, followed by Ceftazidime, Rifampicin, and Ceftriaxone (78.72%, 72.34%, and 59.57%, respectively). Among most clinical isolates of bacteria, MDR mainly *E. coli* pathotypes, is a major healthcare problem that is associated with increased

morbidity and mortality worldwide. Several factors contribute to the rise in MDR *E. coli* strains, including self-prescription of antibiotics, which leads to overuse/misuse of these medications. (Raheem and Ali, 2022). MDR *E. coli* revealed 79% than other sensitive isolates (21%) (Almutairy *et al.*, 2016). Furthermore, a high incidence of resistance to commonly used antibiotics may play a role in the development of recurrent and complicated UTI (Raheem and Ali, 2022).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Materials

3.1.1. Equipments

Table (3-1): The devices and equipment's used in this study:

Type of equipment	Manufacture (Origin)
Autoclave	Hirayama (Japan)
Burner	Himedia/ India
Centrifuge	Universal 320 / Germany
Deep freezer	BEKO /USA
Distillator	GFL (Germany)
ELISA reader	Bio lab/England
Gel documentation system	Biometra/Germany
Incubator	Binder (Germany)
Micro centrifuge tube 1.5ml	POSI-CLICK TUBES/USA
Micro titer plate 96	Jordan
Micropipette set (0.5-1000µl)	Dragon MED/USA
Microwave	Mbiano /UK
Millipore filter	CHM CA syringe filter/China
Oven	Binder (Germany)
PCR system (Thermocycler)	TP-Professional\Biometm (Germany)
PCR tubes	Eppendorf /Germany
petri dish	Afco-Dispo Jordan
Refrigerator	Electrostar/Egypt
Sensitive balance	Memmert/ Germany

Type of equipment	Manufacture (Origin)
Spectrophotometer	PD-303UV APECO \Jaban
Standard loop 0.01 ml	Himedia/India
Swab	China
Electrophoresis	Biocom direct/UK
Vortex	Griffen and George Ltd/UK

3.1.2. Chemical Materials

Table (3-2) The chemical materials used in this study

Chemicals	Company/ Origin
Agarose	Fisher bioreagents / Germany
Crystal violet	Sigma/ Germany
DNA loading dye	Fisher bioreagents / Germany
Glycerol ($C_3H_8O_3$)	Fluka/Germany
Red safe Nucleic Acid Staining	Bioland Scientific /USA
Acetic acid glacial	Loba Chemie / India
Absolute Methanol	Srlchem /USA
Absolute Ethanol	Honeywell/Germany
TBE buffer	Himedia/ India

3.1.3 Culture Media:

Table (3-3) The culture media used in this study

Medium	Uses	Manufacturer (Origin)
Brain heart infusion broth	Long Term Storage	Himedia (India)
MacConkey agar	Isolation and identification of *E.coli*	
Muller-Hinton agar	Antibiotic susceptibility	
Luria bertani broth	Biofilm formation	
Nutrient broth	Antibiotic susceptibility	
Nutrient agar	Isolation Bacteria	
Eosin Methylene Blue agar	identification of *E.coli*	
Blood agar	Isolation and identification of *E.coli*	

3.1.4. Antibiotic Disks

Table (3-4) list of antibiotic disks

Class	Antibiotic	Symbol	Concentration (µg/disk)	Company/ Origin
Aminoglycosides	Amikacin	AK	30 µg	Bioanalyse/ Turkey Liofilchem (Italy)
	Gentamicin	CN	10 µg	
	Tobramicin	TOB	10 µg	
Folate Pathway Antagonists	Trimethoprim	TM	5 µg	

Class	Antibiotic	Symbol	Concentration (µg/disk)	Company/ Origin
Cephems (parenteral)	Ceftazidime	CAZ	30 µg	
	Cefixime	CFM	5 µg	
	Cefepime	FEP	30 µg	
	Cefotaxime	CTX	30 µg	
Monobactams	Aztreonam	ATM	30 µg	
Penems	Imipenem	IMP	10 µg	
	Meropenem	MEM	10 µg	
Penicillins	Ampicillin	AMP	10 µg	
	Piperacillin	PRL	100 µg	
Quinolones	Ciprofloxacin	CIP	5 µg	
	Levofloxacin	LEV	5 µg	
Beta-Lactam	Amoxicillin-clavulanate	TET	20/10 µg	

3.1.5. Primers

Table (3-5) List of primers used in this study

Primer Name	Primer Sequence 5′-3′	Product Size/bp	Ref.
Fim H	Forward: TGCAGAACGGATAAGCCGTGG Reverse: GCAGTCACCTGCCCTCCGGTA	508	(Abdul-Ghaffar and Abu-Risha, 2017)
flu	Forward: CGGCGGGCAATGGGTACA Reverse: CAGCTCTCACAATCTGGCGAC	384	Restieri *et al.*,) (2007
CsgA	Forward: GATCTGACCCAACGTGGCTTCG Reverse: GATGAGCGGTCGCGTTGTTACC	178	(Silva *et al.*, 2014)
16S rRNA	Forward: CCTAACACATGCAAGTCGAA Reverse: CATCTGACTTAACAAACCGC	558	this study

3.1.6 Kits and Marker

Table (3-6) Kits and marker used in this study

Name of Kit	Company	Origin
FavorPrep Genomic DNA Mini Kit	FAVORGEN	Taiwan
Taq Green master mix	Promega	USA
100 bp DNA ladder	Bioneer	Korea
gram stain	Crescent	India
primers	Bioneer	Korea

3.2. Methods

3.2.1. Preparation of Solutions

3.2.1.1. Normal saline

Ready to use, sterile normal saline (NS) 0.9 was used for the preparation of culture suspension.

3.2.1.2. McFarland's Turbidity Standard (0.5)

McFarland Standard Solution was prepared following the 0.5 McFarland's standard tube (1.5×10^8 CFU / ml) by adding 0. 5 ml of 1.175 % barium chloride solution to 99.5 ml of 1 % sulfuric acid to obtain a barium sulfate precipitate and the OD was 625 nm. A visual comparison was made between the turbidity of a suspension of bacteria the 0.5 McFarland's standard was made by using the solution (de Sousa Eduardo *et al.*, 2018).

3.2.1.3. Crystal Violet Solution

To prepare it for the biofilm formation test, 0.1 g of crystal violet were mixed with 100 ml of distilled water. The solution was then filtered using a Millipore 0.45 mm filter before being kept at room temperature in a dark bottle. (Mathur *et al.*, 2006).

3.2.1.4. Preparation of 1x TBE Buffer

This solution was used to dissolve agarose during the electrophoresis procedure and was created by diluting concentrated 10x TBE buffer. A final concentration of 1x TBE was obtained by mixing 100 ml of each 10x TBE with 900 ml of sterile distal water. (Green and Sambrook, 2012). 0.5x TBE running buffer were prepared to fill the tank of electrophoresis.

3.2.2. Preparation of Culture Media

All culture media were prepared according to the instructions of the manufactures, and then sterilized by autoclaving at 121^{o}C at 15 pound per square inch for 15 min.

1- Brain heart infusion broth: It was prepared by dissolving 37g of medium in 1 liter distilled water and then sterilized by autoclave, after it cool poured medium to petri dishes.

2- MacConkey agar: It was prepared by dissolving 51.55g of medium in 1000 ml distilled water, heating until dissolve, then sterilized by Autoclave and poured after cooling.

3- Muller-Hinton agar: This medium was prepared by dissolved 38g of media in 1liter distilled water, heating until dissolve, then sterilized by Autoclave and poured after cooling.

4- Luria bertani broth: it was prepared by dissolving 20 g of medium in 1L of distilled water, mix well and heating, the then sterilized by Autoclave and poured after cooling.

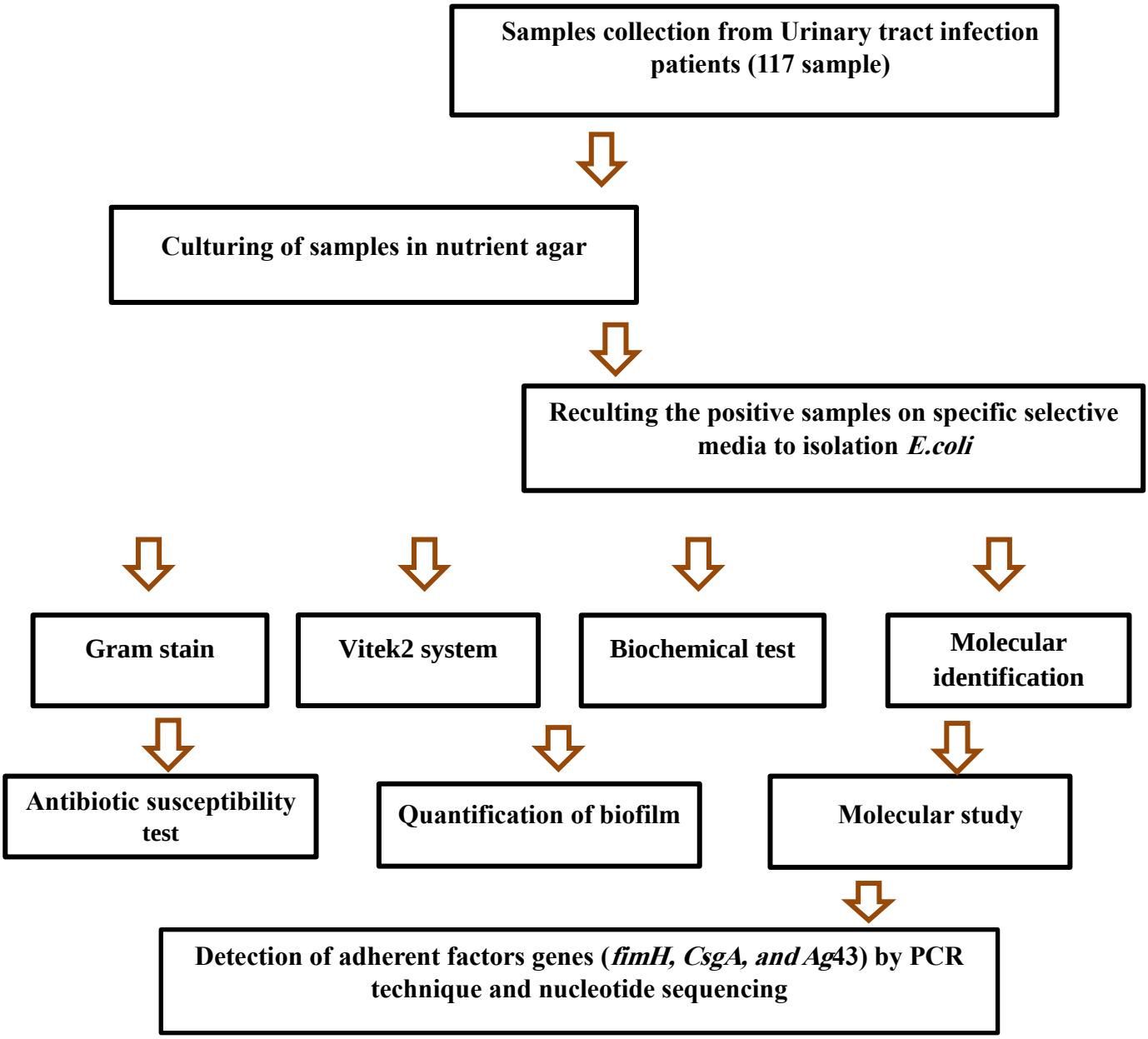
5- Nutrient broth: it was prepared by dissolving 13.0 g in 1L purified distilled water, heating until dissolve, then sterilized by Autoclave and poured after cooling.

6- Nutrient agar: it was prepared by dissolved 28.0 g in 1 L distilled water, heating until dissolve, then sterilized by Autoclave and poured after cooling.

7- Eosin Methylene Blue agar: it was prepared by dissolved of 36.0g of medium in 1 L distilled water, heating until dissolve, then sterilized by Autoclave and poured after cooling.

8- Blood agar: it was prepared by dissolving 40.0 g base medium in 1L distilled water, then sterilized, after cooling to 40-45 ºC, human blood was added to final concentration (5%) and poured into fresh sterilized petri dishes.

3.2.3. Study Design



Figure(3-1) flow chart of study design

3.2.4. Collection of Specimens

One hundred and seventeen urine specimens were collected from patients with urinary tract infection from AL- Hilla Teaching hospital and Babylon Hospital for Maternal and Pediatrics in Hilla city/Iraq, during the period from November 2021 to February 2022. Urine samples were taken from both male and female (age group1-70 years) using sterile containers. The samples were directly streaked on Nutrient, MacConkey agar, and Blood agar, then incubated at 37° C for 24 hrs.

3.2.5. Identification of Uropathogenic *Escherichia coli*

3.2.5.1. Morphological Characteristics

After incubating a single colony on the nutrient agar, MacConkey agar and blood agar, the colonies were sub-cultured in EMB agar for further identification (Md, 2018) and incubated at 37 °C for 24 h. The pure culture was obtained with homogenous colonies. The colonies were then subjected for further identification by Vitek2 system

3.2.5.2. Identification with Vitek2 System

Vitek®2 was used as an autoanalyser system in clinical microbiology to identify (ID) in clinical samples. The Vitek2® GN cards were configured in accordance with BioMérieux guidelines.

3.2.5.3.Molcular identification

Polymerase chain reaction detection by designing specific primers based on the sequence data available for *E. coli* in the database of NCBI for 16S rRNA gene (see section 3.2.10 for PCR conditions).

3.2.6. Specimens Maintenance Medium

3.2.6.1. Short Term Storage

Slants of nutrient agar were inoculated with bacterial growth, incubated for (18-24) hrs. at 37ºC and stored for a period of few weeks at refrigerator.

3.2.6.2. Long Term Storage

Medium for long storage was composed of brain heart infusion broth and 15% glycerol. Brain Heart Infusion Broth autoclaved at 121˚C for 15 min, then cooled to 37˚C and inoculated with bacteria at 37ºC for 24 hr. In micro centrifuge tube (1.5ml) we added 750 µl of 30% sterile glycerol to 750 µl culture broth, then stored at -20˚C. This medium was used to preserve the isolates of bacteria under freezing for long term storage after conformation that the identity of these isolates were related to *E.coli* (Forbes *et al.*, 2007).

3.2.7. Antimicrobial susceptibility test

The susceptibility of identified isolates were tested against 16 antibiotic (Ampicilin 10 µg, Piperacillin 100µg, Amoxicillin- clavulanate 20/10 µg, Cefepime 30 µg, Ceftazidime 30 µg , Cefixime 5 µg, Aztreonam 30µg, Imipenem 10 µg Meropenem 10 µg, Amikacin 30µg, Gentimicin 10µg, Levofloxacin 5 µg, Tobramicin 10 µg, Cefotaxime 30 µg, Trimethoprim 5 µg, Ciprofloxacin 5 µg) as described by (CLSI, 2022), using the disk diffusion method. The isolated colonies produced on nutrient agar plates were combined with 5 ml of sterile normal saline to create the inoculums for this test, which were then compared to (1.5×10^8 cl/ ml) McFarland standard tubes (0.5). From the bacterial suspension, 100 µl of inoculums were streaked equally on a Mueller-Hinton agar (Lab M, UK) plate by using a sterile micropipette, then allowed to dry. Using flamed forceps or a disc applicator the antibiotic discs were equally dispersed throughout the medium's surface and (four discs in each

plate) incubated for 24 hours at 37 ° C. To evaluate the sensitivity, inhibition zones were measured using a ruler or caliper and compared to the zones of inhibition established by the Clinical Laboratory Standards Institute (CLSI,2022).

3.2.8. Biofilm Formation Assay

The biofilm formation among *E. coli* isolates by tube method were investigated according to (O'Toole, 2011).

- Growing bacteria for biofilm: Bacterial cultures of the *E. coli* were cultivated in a rich medium (Luria bertani broth) overnight, then diluted into 1: 100 by using fresh medium. Three milliliters of diluted culture were transferred into new fresh tube for biofilm assay (all cultures were fixed initially at OD. 0.06), incubated the tubes for 4-24 hrs at 37 ° C.
- Staining of Biofilm: After incubation, the cells were got rid of by gently shaking the liquid and flipping them over. The tubes were then placed in a small tank and shaken to remove any remaining liquid. The procedure was carried out once more. Significantly less background staining was resulted from the removal of unattached cells and medium components that could be stained in the following phase.
- Each tube contained three milliliters of a 0.1% solution of crystal violet(CV), incubated for 10 to 15 minutes at room temperature. The tubes were then flipped upside down and dried for a few hours or overnight after being cleaned 3–4 times with water submerged in a tank of water as described above. The tubes can be imaged when dry for qualitative testing.
- Three milliliters of 30% acetic acid were added to each tube to solubilize the CV. solution, incubated for 15–20 minutes at room temperature. After that, a fresh tube was filled with 1 ml of the solubilized CV, and the absorbance was

measured at 550 nm, 30% acetic acid were used as the blank. The results were interpreted according to (Stepanović *et al.*, 2007) as follows:

Non - adherent if OD ≤ ODc

Weakly adherent if ODc < OD ≤ 2 x ODc .

Moderately adherent if 2 x ODc ≤ OD ≤ 4 x ODc.

Strong adherent OD > 4 x ODc.

3.2.9. Molecular study

3.2.9.1 DNA Extraction Kit for Gram - negative bacteria

Step 1

1. One milliliter of bacterial culture was transferred to a 1.5 milliliter microcentrifuge tube, and the tube was centrifuged for one minute at full speed (14,000 rpm). The supernatant was discarded.
2. Two hundred µl of FATG Buffer were added, then the pellet was re-suspended by vertexing or pipetting, incubated for 5 minutes at room temperature.

Step 2 -Cell Lysis

1. Two hundred µl of FABG Buffer were added to the sample and vortexed for 5 sec.
2. Incubated for 10 min at 70 ° C or until the sample lysate is clear. During incubation, the tube was inverted every 3 min.
3. Elution Buffer was preheated (for Step 5 DNA Elution) in a 70 ° C water bath.

Step 3 – Binding

1. The samples exposed to 200µl l of ethanol (96–100%) for 10 seconds. (Checking the pipes for any precipitation).

2. A FABG Column is attached to a 2 ml collecting tube. Carefully transfer the sample mixture, to the FABG Column. Discard the 2 ml collection tube after centrifuging at full power (14,000 rpm) for 5 min . Then use a fresh 2ml Collection tube with the FABG column.

Step 4 - Washing

1. FABG Column washed with 400µl W1 Buffer Centrifuge for 30 seconds at full speed (14,000 rpm) and discard the flow - through.
2. The FABG Column placed back into 2 ml Collection tube. Washed FABG Column with 600µl Wash Buffer (ethanol added), Centrifuged for 30 seconds at full speed (14,000 rpm) and discard the flow – through. The FABG Column was centrifuged for an additional 3 min at full speed (14,000 rpm) to dry the column.

Step 5 - Elution.

1. The dried FARG Column was placed to a new 1.5ml microcentrifuge tube.
2. One hundred µl of Preheated Elution Buffer (TE) were added to the membrane center of FABG Column. Stand FAGB Column for 3-5 min or until the buffer is absorbed by the membrane.
3. Centrifuged at high speed for 30 seconds (14,000 rpm) to elute the DNA and 100 µl of the Elution buffer were added to dissolve the DNA.

3.2.10. Polymerase chain reaction

3.2.10.1. PCR Procedure

1. Primers Dilution

To achieve the final concentration of the stock solution (100 pmol/µl) all of the primers were provided by at (Bioneer/Korea) in lyophilized form, which re-dissolved with nuclease-free water in accordance with the instruction of the manufacture. To make 10 pmol/ µl, 10 µl of stock solution were dissolved in 90 µl of N.F.W. to create the working solution.

2. PCR Components

The reaction mixture, which had a final volume of 25 µl, was made following the table provided below:

Table (3-7) Components for PCR

Components	Volume
Go Taq®Green Master Mix(2X)	**12.5** µl
Forward primer	**1** µl
Reverse primer	**1** µl
DNA template	**3** µl
Nuclease free water	**7.5** µl
Final volume	**25** µl

3.2.10.2. PCR cycling program conditions.

Depending on the tables below, the PCR for the genes *fimH*, *csgA*, *flu* and*16s rRNA* was carried out by using a PCR thermal cycler under the following conditions:

Table (3-8) Program of PCR conditions to *flu* ,*CsgA*, *fimH* and 16S rRNA genes

Gene name	Temperature (^{o}C)/Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
flu	95°C for 5 min	95°C for 30 sec	55 °C / 30 sec	72 °C for 1 min	72°C for 7 min	30
csgA	95°C for 5 min	95°C for 30 sec	60°C / 30 sec	72 °C for 1 min	72°C for 7 min	30
fimH	95°C for 4 min	95°C for 30 sec	63 °C / 30 sec	72 °C for 1 min	72°C for 7 min	30
16s rRNA	95°C for 5 min	95°C for 30 sec	60°C/30 sec	72 °C for 1 min	72 °C for 7 min	30

3.2.11. Electrophoresis

3.2.11.1. Agarose gel electrophoresis :

One (1) grams of agarose powder were dissolved in 100 ml of 1xTBE buffer to create agarose gel (pH:8). The solution was then heated in a microwave (Mbiano) for 90 seconds at full power. Red safe dye was added after allowing the mixture to cool to 50 ºC. In order to create wells for loading DNA samples, a comb was connected to one end of the gel tray and a tape was placed across the end. It took 30 minutes for the agarose to completely solidify after being carefully put into the tray. The tap was then carefully removed from the tray's

ends, followed by the comb. The latter was fixed in an electrophoresis chamber with a gel surface covered in 0.5x TBE running buffer. Transferring 5µl of each PCR product to Eppendorf tube, 1 µl of loading dye at a final concentration of 1x was then added. Then 5 µl of the mixture were transferred to the wells. The electric current was allowed at 85 volts for 1 hr., then the results photographed using gel documentary system to observe DNA bands.

3.2.12. Sequencing of *fimH* gene

Sequencing of gene was performed by macrogene company in Korea. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit software for multiple alignment of *fimH* among different isolates.

3.2.13. Statistical Analysis

SPSS version 23.0 statistical software package was used for statistical analysis by using Chi-square test for all obtained results at p value ≤ 0.05.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Isolation and Identification of *Escherichia coli*

The results of this study showed that from 117 UTI samples collected, 50 isolates were identified as *E. coli*. These isolates were collected from both sexes (figure 4.4 and table 4.1) at different ages from different hospitals and laboratories in Babylon province. The diagnosis was performed according to the steps recommended by (MacFaddin, 2000) for diagnosis, single colony from each positive culture was taken and then identified based on morphological characteristics (color production, colony shape, texture and edge). The first diagnosis of the isolates has been done by growing them on MacConkey agar, nutrient agar and blood agar medium to check the shape and color of *E. coli* colonies. Colonies are smooth, round, medium in size, convex and lactose inflamed with a pink color on MacConkey agar medium, while the colonies are large, round, gray and moist, non-hemolytic (gamma-hemolytic) or beta-hemolytic (β) colonies on Blood agar medium figure (4-1).

A

B

Figure (4-1) Morphological properties of *Escherichia coli* colonies on different agar media A: Blood agar B: MaCconkey agar C: nutrient agar

colonies were subcultured on selective EMB medium to confirm the identification, *E. coli* bacteria developed in EMB media often circular and smooth, with a green metallic sheen (4-2).

Figure (4-2) *Escherichia coli* colonies on EMB showing green metallic sheen

Additionally, the Vitek2 System (BioMerieux, France), with detailed biochemical features in (appendix 1) for *E. coli* isolates, also performed to confirm the diagnosis of bacterial isolates along with PCR diagnosis by *16S rRNA* (figure 4-3). The band size of PCR products for all isolates was approximately 558 bp which is compatible to the expected size of primers designed specifically for 16S rRNA of *E. coli* based on NCBI database. To examine the specificity of these primers towards 16S rRNA of *E. coli*, the PCR was also carried out on different *Klebsiella* isolates and the results showed no product have been found after gel electrophoresis. According to these results, these primers could be beneficial for *E. coli* diagnosis in the future studies.

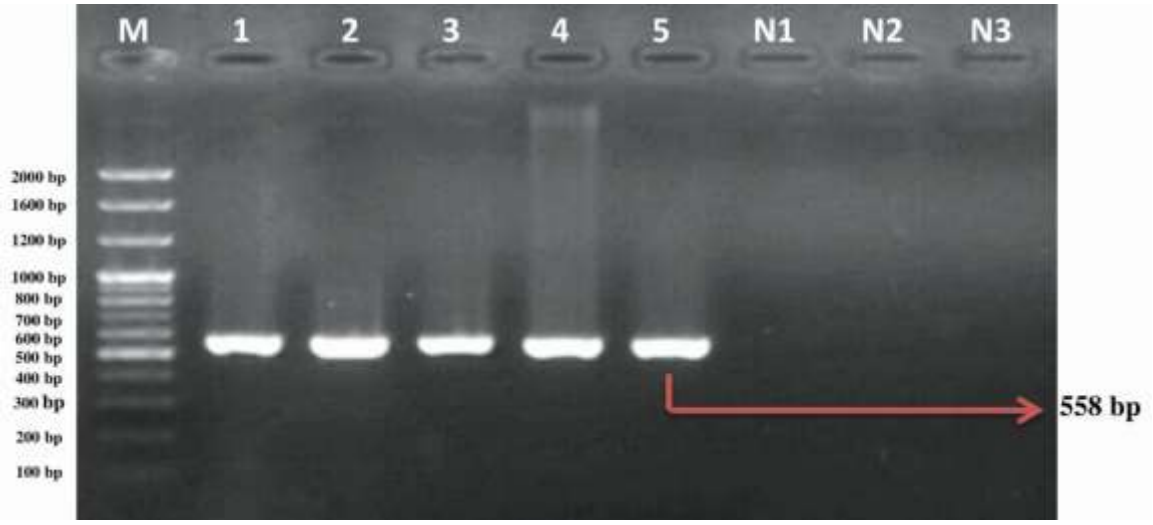


figure (4-3): Agarose gel electrophoresis of *16s rRNA* PCR product (558bp) among *Escherichia coli* isolates. M: represent 100bp DNA ladder, lane 1-5 represent the isolates, N: represent *Klebsiella pneumoniae* negative isolates at 85 volt for 60 min

The current study showed that the infection rate in females (82%) was higher than in males (18%), as shown in Figure (4.1). These results are in agreement with several studies in which the incidence of *E. coli* was higher in females than in males (Shah *et al.*, 2020a; Gunardi *et al.*, 2021; Al-Awkally *et al.*, 2022). Because both sexes are liable to infection, women are mostly susceptible as a result of their anatomy and procreant physiology. The entry of infectious pathogens into the urinary tract leads to infections that actually happen by means of the urethra. Such a case is regarded as a crucial reason for the higher rate among women than men because of the shorter length of the urethra in women making them liable to such infections. The shorter length of the urethra in women increases the pathogen's ability to occupy the bladder causing cystitis. Another reason, is the proximity of the opening of the female urethra to the anus and the change in the vaginal microflora, which plays an important role in

encouraging vaginal colonization with *E. coli* that may lead to UTI (Vasudevan, 2014).

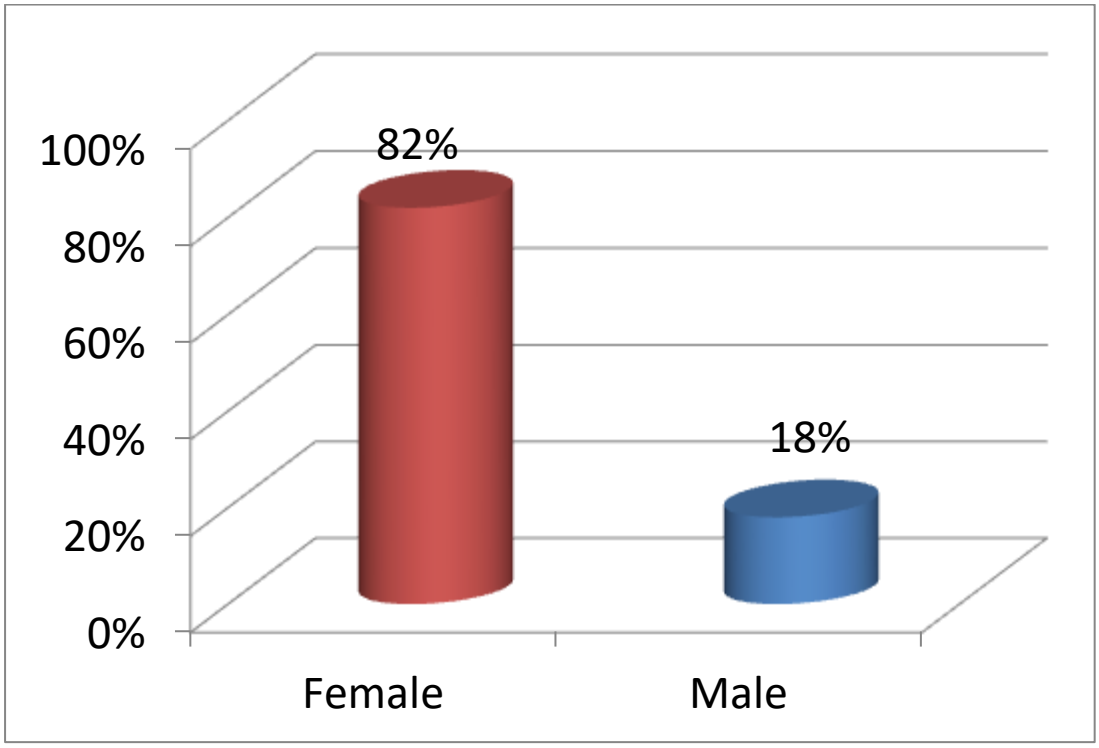


Figure (4-4) Distribution of bacterial isolates according to gender

The prevalence of high-risk increasing UTI infection was detected within age ranging from 21 to 30 years old (50%) followed by 31-40 years (24%). This finding has been similar to many previous studies FarajzadehSheikh *et al.*, (2019)and Pandit *et al.*,(2020). The percentages in other groups of study were decreased into 8% and 6% in (41-50) years and (61-70) years respectively. However, the rate of infection was equal to 4% in the following age groups 0-10 years, 11-20 years, and 51-60 years.

Table (4-1) Distribution of bacterial isolates according to age groups

Age group	Female NO(%)	Male NO(%)	Total NO(%)
1-10	2(4%)	-	2(4%)
11-20	2(4%)	-	2(4%)
21-30	24(48%)	1(2%)	25(50%)
31-40	7(14%)	5(10%)	12(24%)
41-50	3(6%)	1(2%)	4(8%)
51-60	1(2%)	1(2%)	2(4%)
61-70	2(4%)	1(2%)	3(6%)
Total	41(82%)	9(18%)	50(100%)

4.2. Antibiotics Susceptibility of *Escherichia coli*

The test for antibiotic susceptibility was conducted by using the CLSI protocol (CLSI 2022). In this work, 50 UPEC isolates of *E. coli* were evaluated using the disc diffusion method for susceptibility to 8 different antibiotic group (Penicilline, Beta-Lactam, Cephems, Monobactams, Carbapenems, Aminoglycosides, Quinolones and Fluoroquinolone, Folate Pathway Antagonists). After overnight incubation the pathogenic isolates on Muller-Hinton agar at 37 °C, the colonies displayed varying degrees of resistance and Sensitive to these antibiotics.

Table(4 -2): Antibiotic susceptibility profile for uropathogenic *Escherichia coli* isolates.

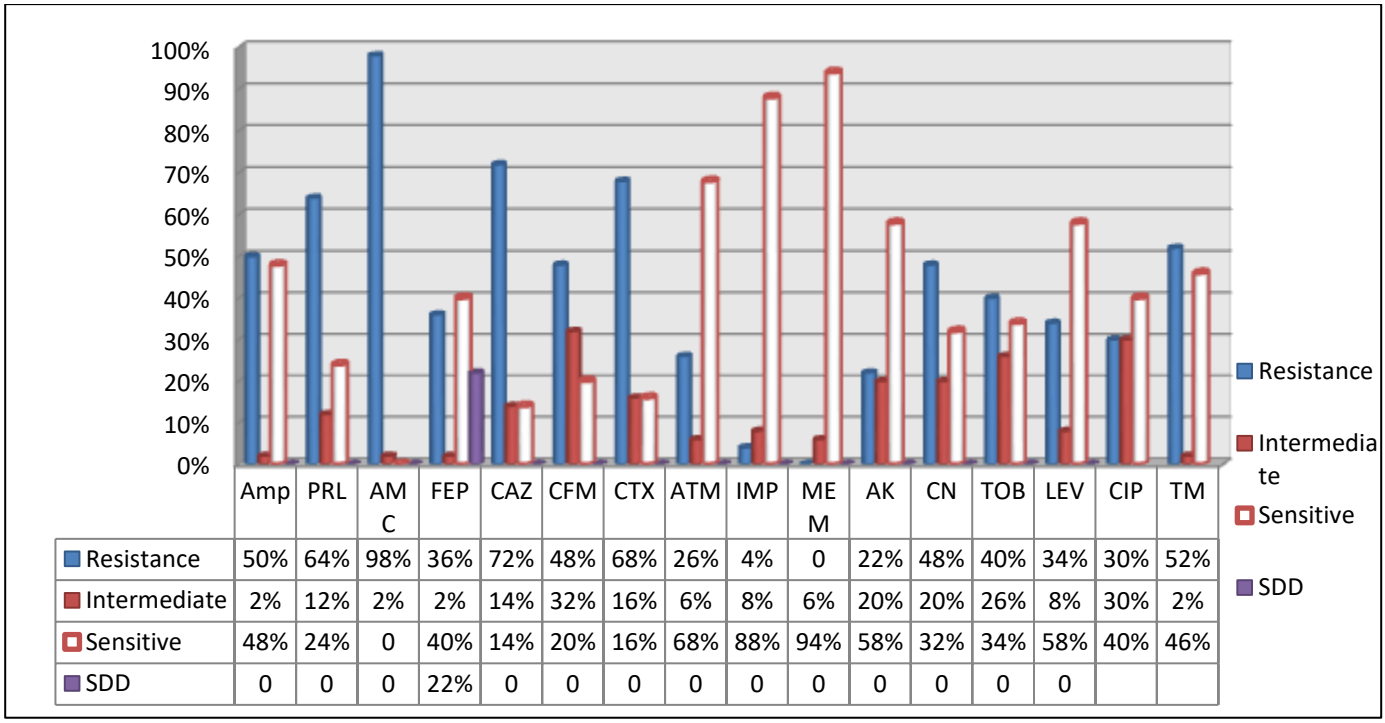
Group	Antimicrobial Agent	Resistance No.(%)	Intermediate No.(%)	Sensitive No.(%)
Penicilline	Ampicilin	25(50%)	1(2%)	24(48%)
	Pipercillin	31(62%)	7(14%)	12(24%)

Group	Antimicrobial Agent	Resistance	Intermediate	Sensitive
Beta-Lactam	Amoxicillin-clavulanate	49(98%)	1(2%)	0
Cephems	Cefepime	18(36%)	1(2%)	20(40%)
	Ceftazidime	36(72%)	7(14%)	7(14%)
	Cefixime	24(48%)	16(32%)	10(20%)
	Cefotaxime	34(68%)	8(16%)	8(16%)
Monobactams	Aztreonam	13(26%)	3(6%)	34(68%)
Carbapenems	Imipenem	2(4%)	4(8%)	44(88%)
	Meropenem	0	3(6%)	47(94%)
Aminoglycosides	Amikacin	11(22%)	10(20%)	29(58%)
	Gentamicin	24(48%)	10(20%)	16(32%)
	Tobramycin	20(40%)	13(26%)	17(34%)
Quinolones and Fluoroquinolones	Levofloxacin	34(68%)	4(8%)	29(58%)
	Ciprofloxacin	15(30%)	15(30%)	20(40%)
Folate Pathway Antagonists	Trimethoprim	26(52%)	1(2%)	23(46%)

The results of the antibiotic sensitivity test were as follows: the highest percentage (98%) of resistance was observed towards Amoxicillin-clavulanate antibiotics, however, *E. coli* isolates displayed 94% rate of sensitive against Meropenem antibiotics. Regarding to the resistant toward antibiotics groups, listed in (Table 4.2) , the resistance to Penicillines group (Ampicillin and Piperacillin) was reach up to 50% and 62% respectively, which was agreed with the study by (Muhammad and Ghareb, 2019). However, Beta-Lactam (Amoxicillin-clavulanate) showed resistance up to 98% and was compatible with

(Abass *et al.*, 2014), this can be attributed to the presence of Beta-lactamase enzymes in *E. coli* isolates.

The percentage of resistance was varied between the Cephems group (ceftazidime 72%, cefotaxime 68%, cefixime 48%, cefepime 36%), the percentages of resistance towards Ceftazidime and Cefepime were agreed with (Almutairy *et al.*, 2016, Shakhatreh *et al.*, 2019, Ghaffoori and Suleiman, 2022). For Monobactams group, the resistant to Aztreonam antibiotic was 26% and this percentage was not agreed with previous results by (Almutairy *et al.*, 2016). In Carbapenems group (Imipenem 4% and Meropenem 0%), this group showed high level of sensitive among *E. coli*. isolates, Meropenem was found to be more effective than other tested antibiotics, since all isolates were sensitive toward it and exhibited no resistance, followed by Imipenem antibiotic. The sensitivity of the isolates might be due to the presence of the enzyme metallo β-lactamases breaks down the carbapenems group of antibiotics (Al-Kharousi *et al.*,2019). The percentages of (Amikacin 22%, Gentamicin 48% and Tobramycin 40%) that belongs to Aminoglycosides group was agreement with (Karam *et al.*, 2018, Al-Taai, 2018, AL–Zubaidi, 2020). In addition, Levofloxacin and Ciprofloxacin, which both belong to the class quinolones and fluoroquinolones, showed resistance rates of 34% and 30%, respectively and the percentage of Ciprofloxacin was agreed with (Ebadi *et al.*, 2017), while the resistant value of the folate pathway antagonists Trimethoprim group was 52% (figure 4-6).



	Amp	PRL	AMC	FEP	CAZ	CFM	CTX	ATM	IMP	MEM	AK	CN	TOB	LEV	CIP	TM
Resistance	50%	64%	98%	36%	72%	48%	68%	26%	4%	0	22%	48%	40%	34%	30%	52%
Intermediate	2%	12%	2%	2%	14%	32%	16%	6%	8%	6%	20%	20%	26%	8%	30%	2%
Sensitive	48%	24%	0	40%	14%	20%	16%	68%	88%	94%	58%	32%	34%	58%	40%	46%
SDD	0	0	0	22%	0	0	0	0	0	0	0	0	0	0		

figure (4-5):percentages of antibiotic susceptibility of *Escherichia coli* isolates

Multidrug-resistant (MDR) of *E. coli* can cause life threatening infections, isolates resistant to at least three antimicrobial classes were considered as MDR isolates. In this study, *E. coli* isolates displayed a significant level of MDR. Forty-four out 50 (88%) of isolates represented the MDR (table 4-3), this was agreed with the study performed by Al-Hasnawy *et al.*, (2019); Hamza *et al.*, (2022) in Babylon province and showed percentage of 88.09% and 72% respectively. Similarly, our results were also compatible with results of Shahid and Research, (2022) in AL-Diwaniyah province, which presented 100% percentages of MDR among *E. coli* isolates. The considerable number of MDR isolates among the UPEC may be resulted from of widespread use and misuse of antibiotics in hospitals and in the community (Gholipour *et al.*, 2014). The increase in the emergence of resistant to antibiotics are constituted a very critical

situation for human health, particularly patients with the immune system problem as well as infection by multi-resistant Gram-negative bacteria, therefore the effectiveness of these antibiotics are become very low (Bassetti and Righi, 2013).

Table (4-3) Show the Multidrug-resistant (MDR) *Escherichia. coli*

No. of groups antibiotic resistant	No. of isolates	Percentage %	Total 100%
seven	7	14%	MDR 84%
six	10	20%	
five	9	18%	
four	6	12%	
three	10	20%	
two	5	10%	Non-MDR 16%
one	3	6%	

4.3. Biofilm Formation

The results revealed that quantification of biofilm formation by tube method classified the isolates: 5 (10%) as strong adherent and 36 (72%) as moderate biofilm producer, while 9 (18%) produces weak biofilm as shown in table (4-4).

Table (4-4) the percentage of Biofilm formation among *Escherichia coli* isolates

Biofilm evaluation		No.	Percentages %
Non- adherent		0	0
Adherent	**Weakly adherent**	9	18%
	Moderately adherent	36	72%
	Strongly adherent	5	10%
	Total	50	100%

The productivity of the thin biofilm layer was noticed in all isolates with variation in their intensity of formation. This result was similar to Muhammad and Ghareb, (2019) and Al-Taai, (2018) showed that their isolates were 100% biofilm producer. The biofilm productivity of all isolates is an indication that *E. coli* contains at least one adhesion factor gene within the stages of biofilm formation.

Mittal et al., (2015) defined biofilms as a virulence factor that form as a barrier, protect microorganisms from the effect of the drug. Virulence factors have an important role in preventing bacteria from unfavorable conditions, and this made treatment difficult due to increased drug resistance, Table (4-5) showed the relationship between antibiotic resistance and biofilm formation in *E. coli* isolates.

Table (4-5) the correlation between antibiotic resistance and biofilm formation of *Escherichia coli* isolates.

Antibiotic	Biofilm formation			*P. value
	Weak No.(%)	Moderate No.(%)	Strong No.(%)	
Aztreonam	2(22.2%)	10(27.7%)	1(20%)	0.000
Imipenem	0%	2(5.5%)	0%	0.04
Ceftazidime	6(66.6%)	26(74%)	4(80%)	0.000
Gentamicin	5(55.5%)	17(47.2%)	2(40%)	0.000
Meropenem	0%	0%	0%	0.000
Pipercillin	7(77.7%)	21(58.3%)	3(60%)	0.000
Amikacin	2(22.2%)	8(22.2%)	1(20%)	0.000
levofloxacin	6(66.6%)	10(27.7)	0%	0.000
Amoxicillin-clavulanate	9(100%)	35(97.2%)	5(100%)	0.000
Ampicilin	5 (55.5%)	17(47.2%)	3(60%)	0.000
Cefixime	4(44.4%)	16(44.4%)	4(80%)	0.04
Cefepime	5 (55.5%)	12(33.3%)	1(20%)	0.000
Tobramycin	3(33.3%)	15(41.6%)	2(40%)	0.00
Trimethoprim	4(44.4%)	19(52.7%)	2(40%)	0.00

ciprofloxacin	2(22.2%)	11(30.5%)	2(40%)	0.04
Cefotaxime	8(88.8%)	23(63.8%)	3(60%)	0.00

* P-value was at ≤ 0.05 via Chi- square test

The results of this study showed relatively high percentages of antibiotic resistance in the presence of virulence factor biofilm such as amoxicillin clavulanate, cefotaxime, ceftazidime, pipercillin, The reason might be attributed to the drug resistance strategy that can supported by biofilm feature to protect the microorganisms from the host immunity. This feature was included as one of the most necessary mechanisms of bacterial defense against antimicrobial drug along with acquisition of new enzymes, mutation in drug targets, insufficient concentration of antimicrobials, delayed penetration into the deep layers of biofilms, antibiotic interaction with biofilm components, high bacterial density and slower bacterial growth inside biofilm (Shrestha *et al.,* 2017; Pompilio *et al.,* 2018; Shah *et al.,* 2019). The antibiotics found to be effective against biofilm producing *E. coli* isolates were Imipenem, Meropenem, Amikacin and this was agreed with results of Shah *et al.,(*2020).

4.4. Molecular Study

4.4.1. Isolation of genomic DNA

The gDNA isolation was done for all 50 *E.coli* isolate. The effectively of the extraction was checked by loading DNA sample into electrophoresis. Showen in Figure (4-7).

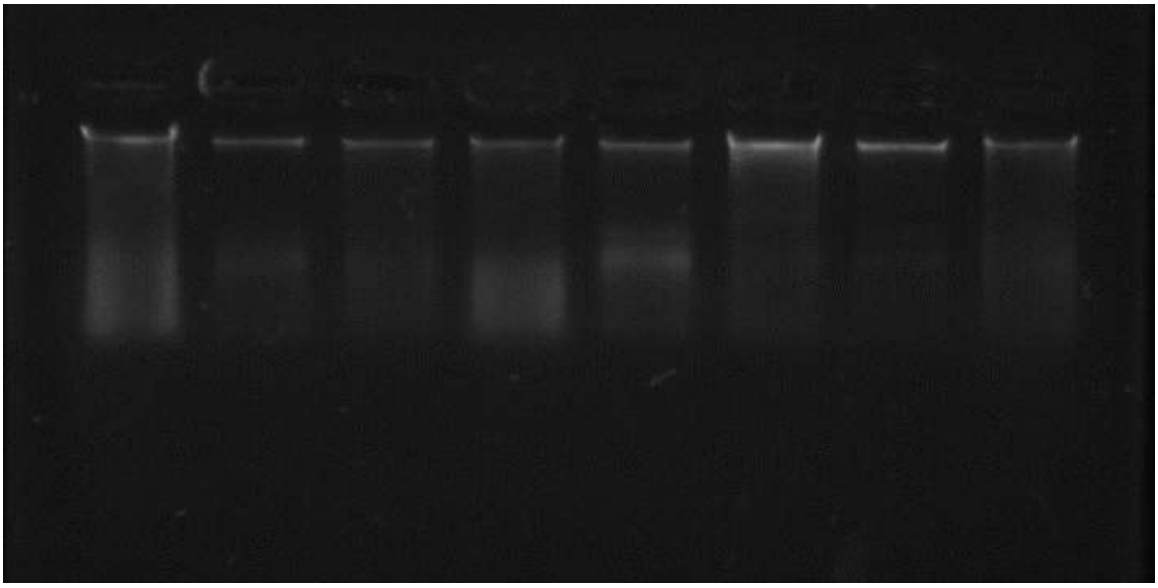


Figure (4-6) Gel electrophoresis for gDNA isolated from *Escherichia coli*. Samples were run in 1% Agarose gel for 60 minutes and 85 V.

4.4.2. Detection of adherent factors genes (*fimH, CsgA*, and *flu*) by PCR technique

4.4.2.1. *fimH* gene

Fifty UPEC isolates were subjected to PCR to investigate the adherence factors genes encoding type 1 fimbriae or pili. The first adhesion to abiotic surfaces depends on the *fimH* gene. Amplification bands with a molecular size of 508 bp were obtained from 46 isolates (92%), as shown in Figure (4-7).

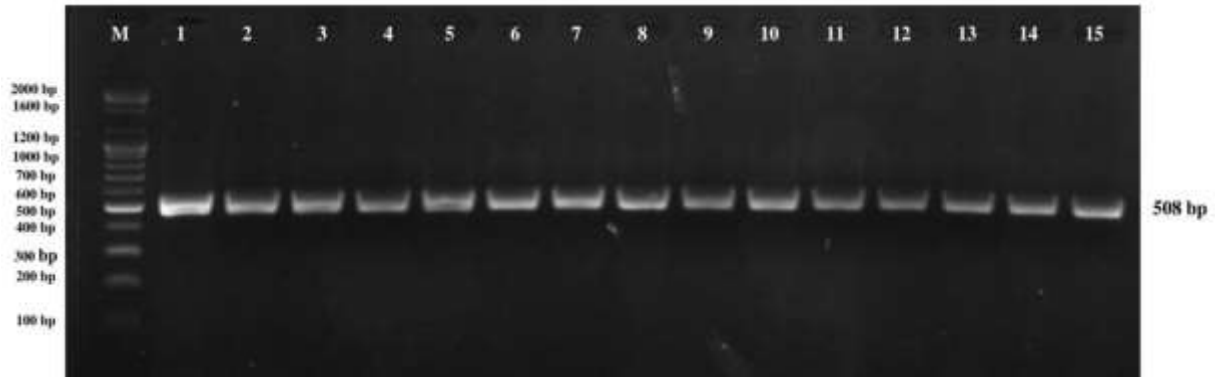


Figure (4-7): Gel electrophoresis of the PCR product for the targeted sequence of *fimH* gene among *Escherichia coli* isolates. M represent 100bp DNA ladder, lane 1-15 represent the isolates. Samples run in 1% Agarose gel for 60 minutes and 85 V. The size of the bands was around (508bp)

Previous studies were nearly compatible with our results of the adhesion factor gene *fimH* (Abdul-Ghaffar and Abu-Risha, 2017a; Merza, 2017; Muhammad and Ghareb, 2019; Fadhil, 2020) in Iraq. These studies showed that the prevalence of this gene was 88.37%,94.8%, 97.5%, 100%, respectively and that can prove the widespread of virulence genes in isolates from patients with UTI. According to Ahmed, (2016), *fimH* is one of the factors responsible for the ability of bacteria to adhere on the surfaces of epithelial cells and the mucous membranes of the host cells. The *fimH* was also responsible for one of the essential steps for bacterial colonization, as the capillaries bind to special receptors on the surfaces of epithelial cells in the urinary tract, which leads to the presence of *E. coli* in non-intestinal tissues, thus increasing the rate of biofilm formation.

4.4.2.2. *CsgA* gene

Curli fimbriae, encoded by the *CsgA* gene, provides adhesion to abiotic surfaces by promoting cell-surface interaction. The result showed that the PCR products for *CsgA* gene were demonstrated clear bands with a molecular size of

178 base pairs (Figure 4.8) and the percentage was about 49(98 %) from total isolates.

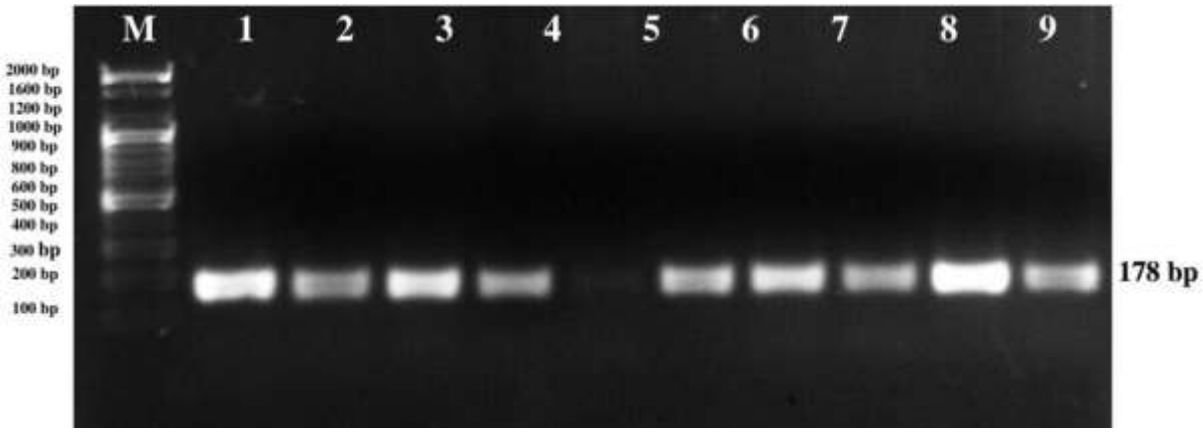


Figure (4-8) Gel electrophoresis of the PCR product for the targeted sequence of *CsgA* gene among *Escherichia coli* isolates. M represent 100bp DNA ladder, lane 1-9 represent the isolates. Samples run in 1% Agarose gel for 60 minutes and 85 V. The size of the bands was around (178bp)

According to previous studies of (Al-yasi and Al saadi, (2022) and Abd El-Baky *et al.*, (2020), the results were similar to those of our study for the *CsgA* gene. Curli fibers contribute to the development of biofilms and are responsible for the pathogenicity of bacterial cells (Azam *et al.*, 2020).

4.4.2.3. *flu*

Under ideal conditions, PCR detection of *flu* gene was carried out using the specific primers depicted in the figure (4-9). A total of 50 DNA samples were extracted, and 46 isolates produced positive results (92%) for *flu* gene encoding for primary autotransporter protein. In the Maturation phase, it encourages cell-to-cell attachment, enabling auto-aggregation and three-dimensional growth.

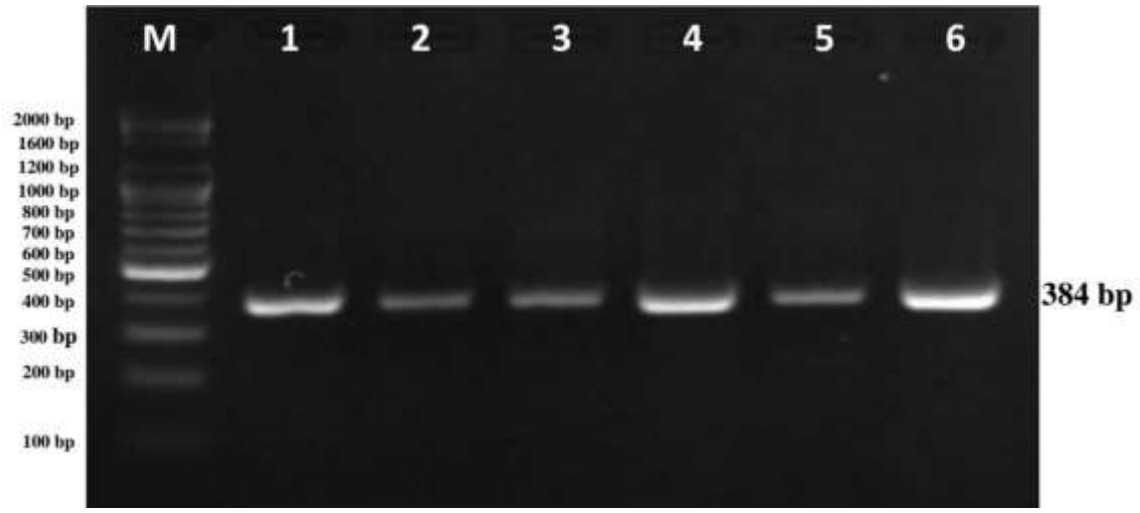


Figure (4-9) Gel electrophoresis of the PCR product for the targeted sequence of *flu* gene among *Escherichia coli* isolates. M represent 100bp DNA ladder, lane 1-6 represent the isolates. Samples run in 1% Agarose gel for 60 minutes and 85 V. The size of the bands was around (384bp)

Studies by Kadry *et al.*, (2020) and Restieri *et al.*, (2007) demonstrated that the *flu* percentages among tested biofilm isolates were 89.3% and 90%, respectively. These studies were nearly similar to the results of our study for the *flu* gene, while the results of the study done by Abdul-Ghaffar and Abu-Risha, 2017) did not agreed with the results of current study .

Our results agreed with previous studies that referred to the positive relationship between the capacity of biofilm formation and the presence adhesion factor genes. Biofilm can be responsible for the increasing the rate of bacterial virulence as well as the severity of disease, biofilm forming bacteria are more abundant in urinary tract infections and their infection was very hard to cure, Table(4-6) (Fattahi *et al.*, 2015, Muhammad *et al.*, 2019)

Table (4-6): Relationship between biofilm formation and prevalence of *fimH, csgA, flu* gene in *Escherichia coli* isolates

Biofilm formation	*Fim H*	*csgA*	*flu*	*P*. value
Weak (No. 9)	8 (16%)	9 (18%)	8 (16%)	0.000
Moderate (No. 36)	33 (66%)	36 (72%)	33 (66%)	
Strong (No. 5)	5 (10%)	4 (8%)	5 (10%)	
Total (No. 50)	46 (92%)	49 (98%)	46 (92%)	

4.5. Sequencing of *fimH* gene of UPEC local isolates:

The results of DNA sequencing were firstly examined to confirm the nucleotide identity with others worldwide strains through NCBI- Blast-query nucleotide – online application. The results showed that, the identity percentages of 9 local *E.* coli isolates with other gene bank registered strains were ranged between 96-98%. In the present study, genotypic variations were compared between 9 *E. coli* isolates that represent three biofilm phenotypic categories (weak, medium, and strong) and three replicates were chosen for each type. The percentages of alignment within different isolates were thoroughly detailed in table (4-7 and also available in Appendix in further details). It is obvious that number 9 local isolate, that represent strong biofilm former, was highly variable when compared with the rest of isolates. Multiple alignment nucleotide sequences were performed to define the variations among these local isolates by using BioEdit software (figure 4-10) along with amino acids alignment (figure 4-11). Three positions (202, 214, and 230) of variations were repeatedly detected among strong biofilm former isolates (*Fim* H 7, 8, and 9) which led to change in the

amino acid types of the sequence 68 into Tryptophan, 72 into Asparagine, and stop codon in 77 into Leucine respectively after changing their codons comparing with other local isolates. However, the weak biofilm formers (Fim2 and Fim3, see figure 4-10) displayed unique nucleotide variation in sequence 185 along with one isolates of moderate biofilm former, which also resulted in change of amino acid type at sequence 62 into Cysteine. These variations in amino acid types, resulted from nucleotide sequence alteration, insertion, and deletion might have potential role in the functional heterogeneity of fimH protein. The extended fimH mutational landscape in biofilms could reflect a natural strategy to diversify *E. coli* surface binding capacity, resist physical and chemical disruptions and potentially providing selective advantage for persistence during *E. coli* cycling between hosts and the environment (Yoshida *et al.*, 2022). The plasticity of *FimH* adhesin should therefore be considered when designing anti-biofilm strategies on medical equipment as the use of pilicides inhibiting type 1 pili production may be preferred to mannose derivatives inhibiting *FimH* specific binding to mannose (Cegelski *et al.*, 2008). From an evolutionary perspective, *FimH* mutations affecting mannose-binding capacity greatly depend on the environmental surfaces colonized by *E. coli*., fimH can undergo rapid microevolution, leading to increased nonspecific adhesion and biofilm formation independently of mannose-binding capacity (Yoshida *et al.*, 2022). Study by Sokurenko *et al.*, (1998) showed that naturally occurring *FimH* variations dramatically change the tissue tropism of *E. coli* and can be a major factor in shifting the bacterial adaptation from commensal to pathologic habitat. Thus, the transition from commensal to virulent phenotype may be mediated not only by acquisition of “virulence genes” but also by selection for genetic variations in a commensal trait that are adaptive to a pathologic environment.

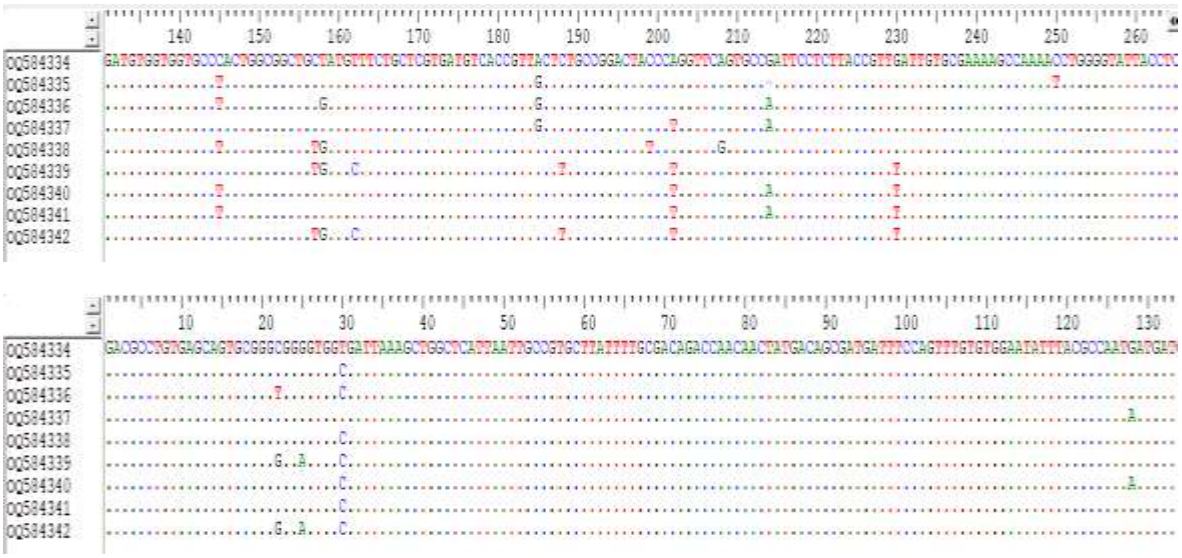


Figure (4-10) Nucleotides sequence alignment of the *fimH* gene among 9 local UTI isolates of *Escherichia coli.* Alignment carried out based on BioEdit sequence Alignment Editor software.

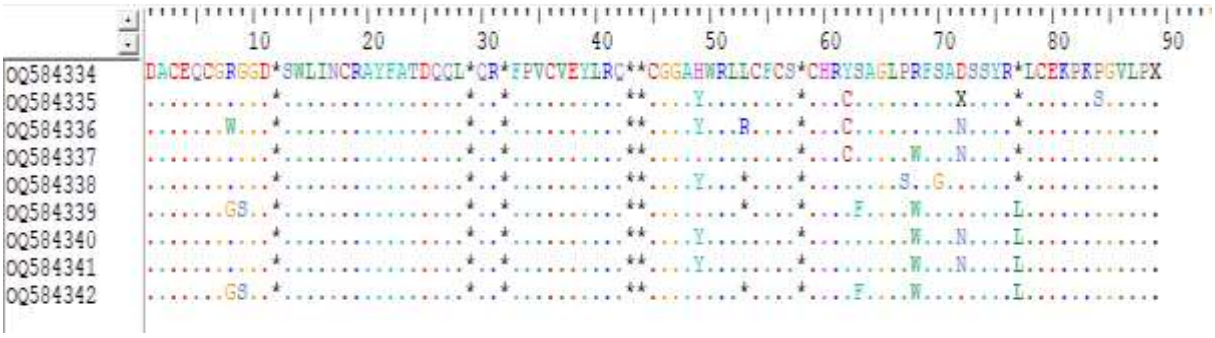


Figure (4-11) Amino acid alignment of the *fimH* gene among 9 local UTI isolates of *Escherichia coli.* Alignment carried out based on BioEdit sequence Alignment Editor software.

Table (4-7) Comparison of percentage similarity among biofilm isolates by sequences.

	Fim1-FimH-F	Fim2-FimH-F	Fim3-FimH-F	Fim4-FimH-F	Fim5-FimH-F	Fim6-FimH-F	Fim7-FimH-F	Fim8-FimH-F	Fim9-FimH-F
Fim1-FimH-F		98%	98%	98%	98%	97%	98%	98%	97%
Fim2-FimH-F	98%		98%	98%	97%	95%	98%	98%	95%
Fim3-FimH-F	98%	98%		98%	98%	96%	98%	98%	96%
Fim4-FimH-F	98%	98%	98%		96%	96%	98%	98%	96%
Fim5-FimH-F	98%	97%	98%	96%		97%	97%	97%	97%
Fim6-FimH-F	97%	95%	96%	96%	98%		97%	97%	100%
Fim7-FimH-F	98%	98%	98%	98%	97%	97%		99%	97%
Fim8-FimH-F	98%	98%	98%	98%	97%	97%	99%		97%
Fim9-FimH-F	97%	95%	96%	96%	97%	100%	97%	97%	

Conclusions and Recommendations

Conclusions

The results of this study have come out with the following conclusions:

1. This study finds out relationship between the female gender and the prevalence of UTI infection by *E. coli* isolates. The infection was dramatically increased among patients ages rang between 21- 30 years old within Al- Hillah city.
2. High percentages of resistant was identified among UTI *E. coli* isolates towards Amoxicillin-clavulanate, Ceftazidime, and Cefotaxime antibiotics in consistent with high percentages of their moderate to strong biofilm forming capacity.
3. The biofilm phenotype was indicated in all *E. coli* isolates and that might be conferred virulence behavior and considered as a great challenging towards health problems as the first critical step in the establishment of infection is bacterial adhesion to urothelial cells under flow conditions.
4. There is significant association between adherent factor's genes (*FimH*, *CsgA*, *flu*) of *E. coli* isolates and their capacity to produce biofilm.
5. This study was indicated several variations of amino acid types among three biofilm phenotypic categories of *E. coli* isolates resulted from codon change, might be responsible for structural and functional heterogenicity of type 1 fimbria D-mannose specific adhesin protein.

Recommendations

1. This study recommends to identify the genetic association of more virulence factors responsible for the pathogenicity of UTI *E. coli* isolates along with detection and evaluation of their phenotypic characteristics.
2. Investigate another biofilm corresponding genes responsible to control different stages of biofilm maturation and their relationship with antibiotic resistant degree as well as genetic variations.
3. This study suggests to evaluate the gene expression of some selected biofilm genes within UTI isolates of *E. coli*.
4. Beside all the previous suggestions, this study also recommends to detect and evaluate the cellular exudates might be secreted during biofilm formation to protect the accumulated cells and support the virulence performance or can be regulated the expression of particular virulence genes.

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Appendix

Appendix1: figures Identification *Escherichia coli* by biochemical test depending on Vitek2 system.

bioMérieux Customer: Microbiology Chart Report Printed June 16, 2022 1:05:55 PM CDT

Patient Name: 2 Patient ID: 8620229
Location: Physician:
Lab ID: 8620229 Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

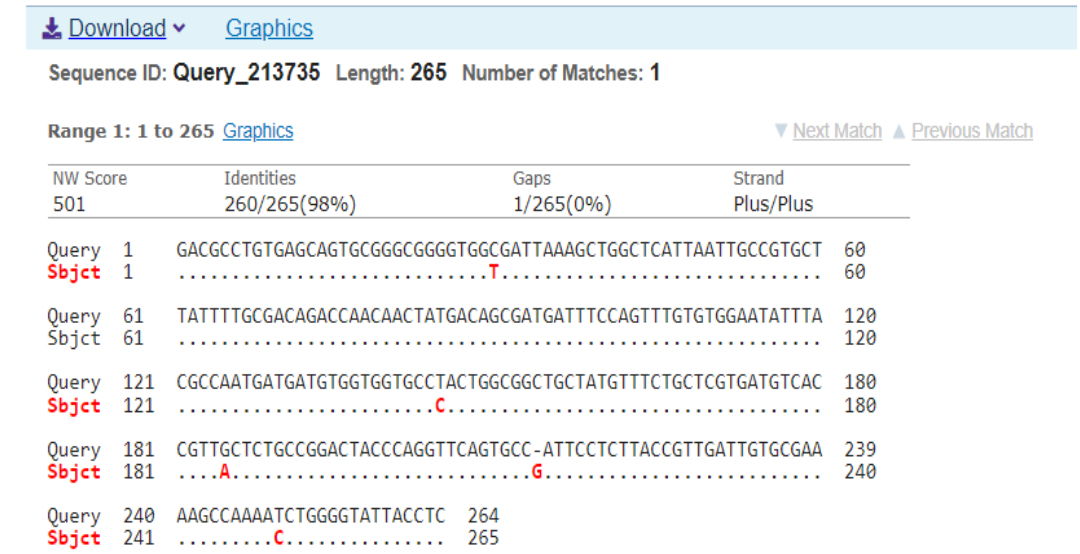
Identification Information	**Analysis Time:**	2.90 hours	**Status:** Final
Selected Organism	99% Probability	**Escherichia coli**	
	Bionumber:	0405610454026611	
ID Analysis Messages			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	+
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Appendix

Appendix2: figures show comparison of percentage similarity among biofilm isolates by sequences



Download Graphics

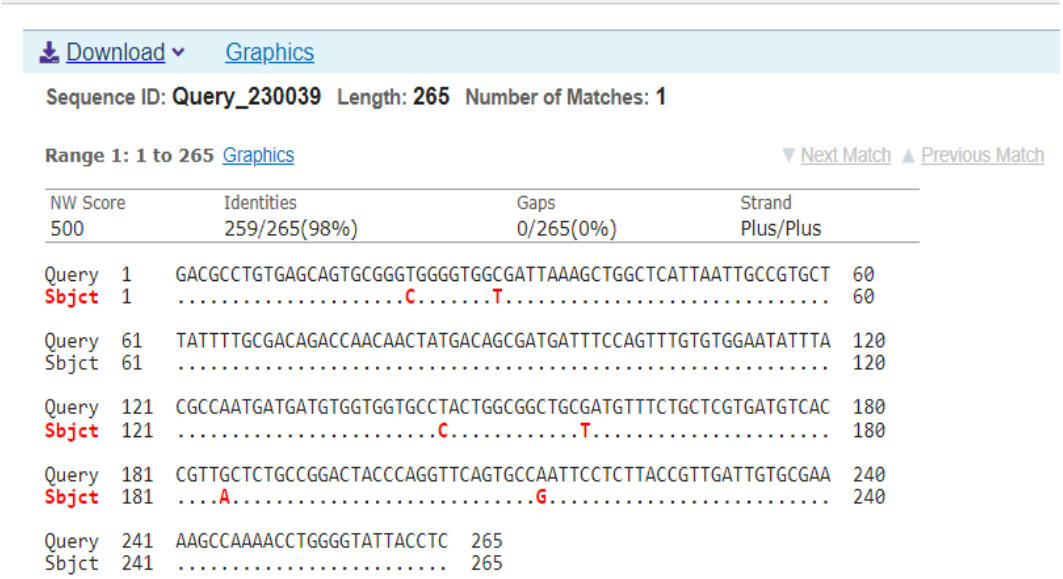
Sequence ID: **Query_213735** Length: **265** Number of Matches: **1**

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
501	260/265(98%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................C...................................  180

Query  181  CGTTGCTCTGCCGGACTACCCAGGTTCAGTGCC-ATTCCTCTTACCGTTGATTGTGCGAA  239
Sbjct  181  ....A............................G...........................  240

Query  240  AAGCCAAAATCTGGGGTATTACCTC  264
Sbjct  241  .........C...............  265
```

*fimH*1and 2



Download Graphics

Sequence ID: **Query_230039** Length: **265** Number of Matches: **1**

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
500	259/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGTGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................C.......T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCGATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................C...........T......................  180

Query  181  CGTTGCTCTGCCGGACTACCCAGGTTCAGTGCCAATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ....A............................G...........................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 3

Appendix

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Sequence ID: **Query_508019** Length: **265** Number of Matches: **1**

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
510	261/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGTGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCCACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G....................................................  180

Query  181  CGTTGCTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ....A.............A...........G..........................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 4

Download Graphics

Sequence ID: **Query_6869** Length: **265** Number of Matches: **1**

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
500	259/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T...............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGTGATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ......................C...........CT......................  180

Query  181  CGTTACTCTGCCGGACTATCCAGGTTCGGTGCCGATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ................C.........A...............................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 5

Appendix

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Sequence ID: Query_12041 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
485	256/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    .....................C..G....T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  .....................................CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......C.............A............................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 6

Download Graphics

Sequence ID: Query_36317 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
500	259/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G.............C.....................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ......................A..........G..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 7

Appendix

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Sequence ID: Query_54875 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
505	260/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................C...................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ......................A...........G..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 8

Download Graphics

Sequence ID: Query_51485 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
485	256/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................C..G....T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ....................................CT...T...................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......C.............A............................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH1*and 9

Appendix

Download Graphics

Sequence ID: Query_39495 Length: 264 Number of Matches: 1

Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
506	261/265(98%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGTGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    .....................C.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCGATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ....................................T.......................  180

Query  181  CGTTGCTCTGCCGGACTACCCAGGTTCAGTGCCAATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ................................-...........................  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T...............  264
```

*fimH2*and 3

Download Graphics

Sequence ID: Query_55087 Length: 264 Number of Matches: 1

Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
496	259/265(98%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGTGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ..............................C.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCCACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G.................T.................................  180

Query  181  CGTTGCTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ......................A............-...........................  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T...............  264
```

*fimH2*and 4

Appendix

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Sequence ID: Query_18733 Length: 264 Number of Matches: 1

Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
491	258/265(97%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGTGATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .....................................CT......................  180

Query  181  CGTTACTCTGCCGGACTATCCAGGTTCGGTGCCGATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ....G.............C........A.....-..........................  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T...............  264
```

*fimH2*and 5

Download Graphics

Sequence ID: Query_41841 Length: 264 Number of Matches: 1

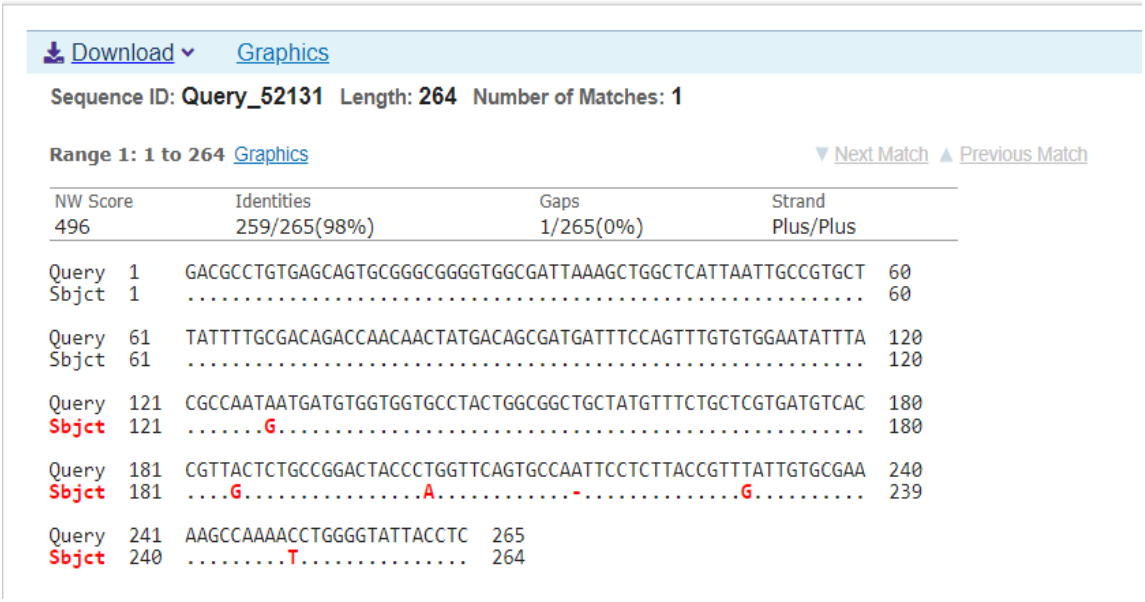
Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
466	253/265(95%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................C..G.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................T...........CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G..C.............A...........-...............G..........  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T...............  264
```

*fimH2*and 6

Appendix



Download Graphics

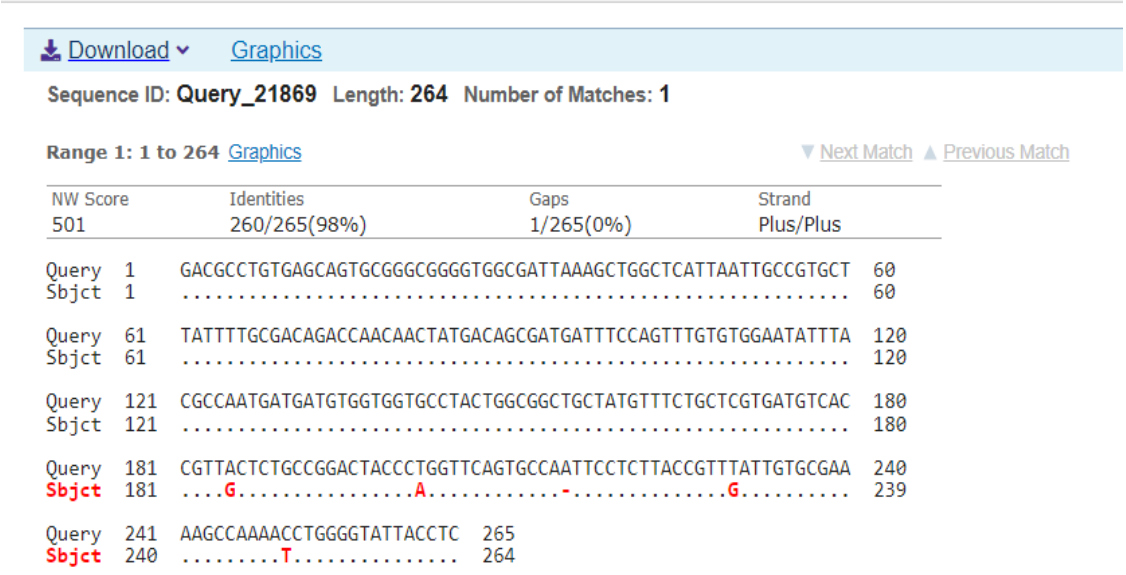
Sequence ID: **Query_52131** Length: **264** Number of Matches: **1**

Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
496	259/265(98%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G....................................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G.............A...........-.............G..........  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T..............  264
```

*fimH2*and 7



Download Graphics

Sequence ID: **Query_21869** Length: **264** Number of Matches: **1**

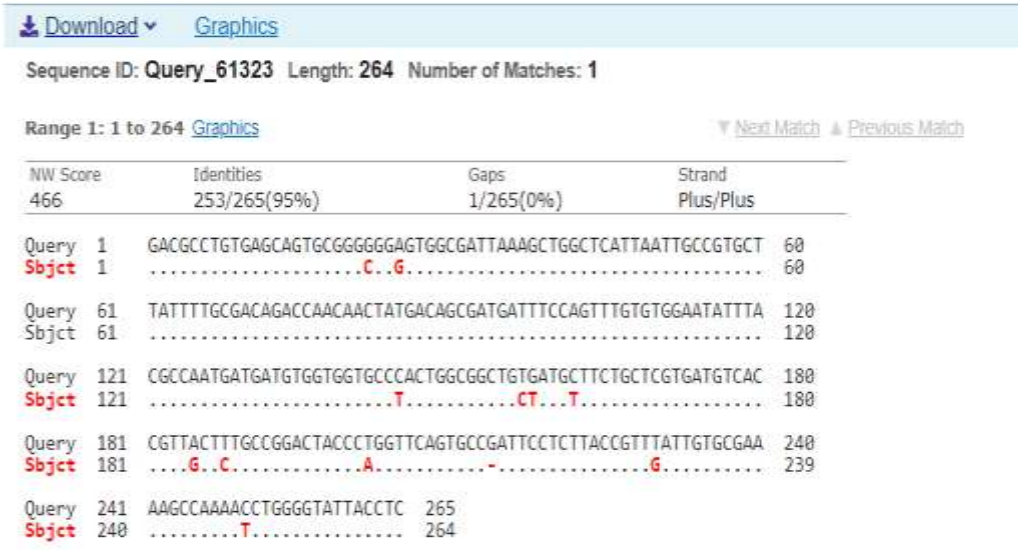
Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
501	260/265(98%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ............................................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G.............A...........-.............G..........  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T..............  264
```

*fimH2*and 8

Appendix



Download Graphics

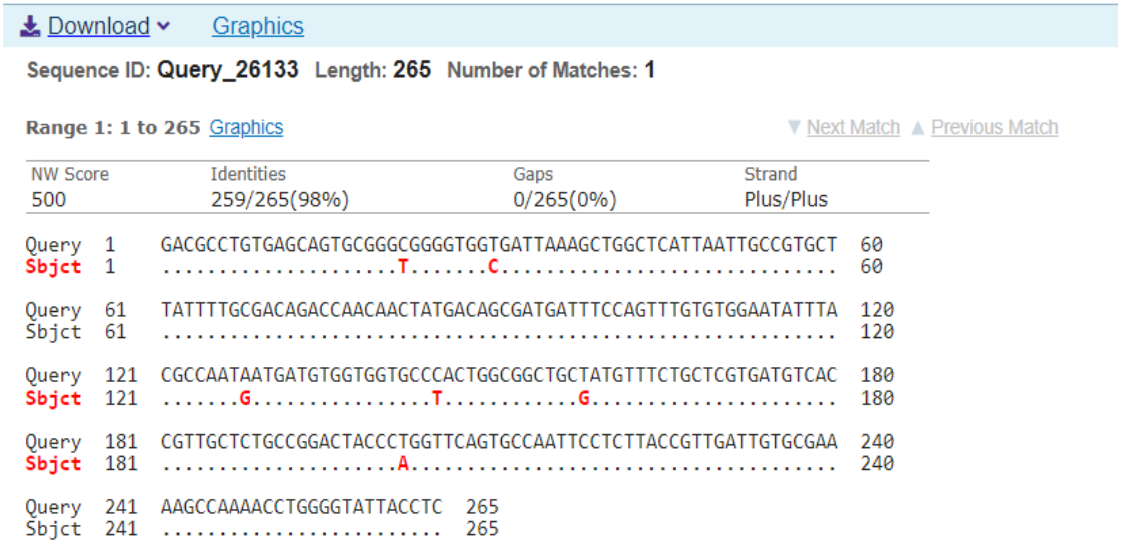
Sequence ID: **Query_61323** Length: **264** Number of Matches: **1**

Range 1: 1 to 264 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
466	253/265(95%)	1/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    .....................C..G.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................T...........CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G..C.............A...........-.............G..........  239

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  240  .........T................  264
```

*fimH2*and 9



Download Graphics

Sequence ID: **Query_26133** Length: **265** Number of Matches: **1**

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
500	259/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGTGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    .....................T.......C.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCCACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G..............T............G.....................  180

Query  181  CGTTGCTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  .....................A.....................................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH3*and 4

Appendix

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Sequence ID: Query_57081 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
500	259/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................T.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGTGATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ..................................C.......................  180

Query  181  CGTTACTCTGCCGGACTATCCAGGTTCGGTGCCGATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ....G............C........A.....A.........................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH3*and 5

Download Graphics

Sequence ID: Query_15383 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
480	255/265(96%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................T..G.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ......................T...........C....T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G..C............A...........A..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH3*and 6

Appendix

Download Graphics

Sequence ID: Query_51229 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
500	259/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................T.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G.............................G.....................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G.............A.........................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH3*and 7

Download Graphics

Sequence ID: Query_11465 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
505	260/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................T.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ....................................G.....................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G.............A.........................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH3*and 8

Appendix

Download Graphics

Sequence ID: Query_38123 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
480	255/265(96%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................T..G.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................T...........C....T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G..C.............A...........A..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH3*and 9

Download Graphics

Sequence ID: Query_261401 Length: 265 Number of Matches: 1

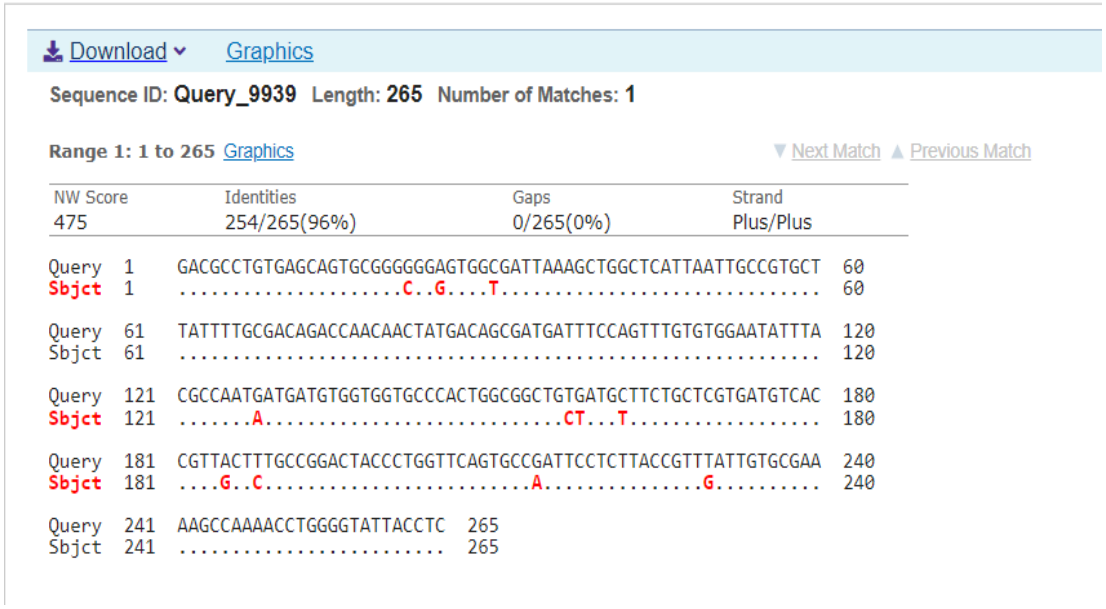
Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
480	255/265(96%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T...............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGTGATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......A.................C...........CT......................  180

Query  181  CGTTACTCTGCCGGACTATCCAGGTTCGGTGCCGATTCCTCTTACCGTTGATTGTGCGAA  240
Sbjct  181  ....G.............C..T.....A.....A..........................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH4*and 5

Appendix



Download Graphics

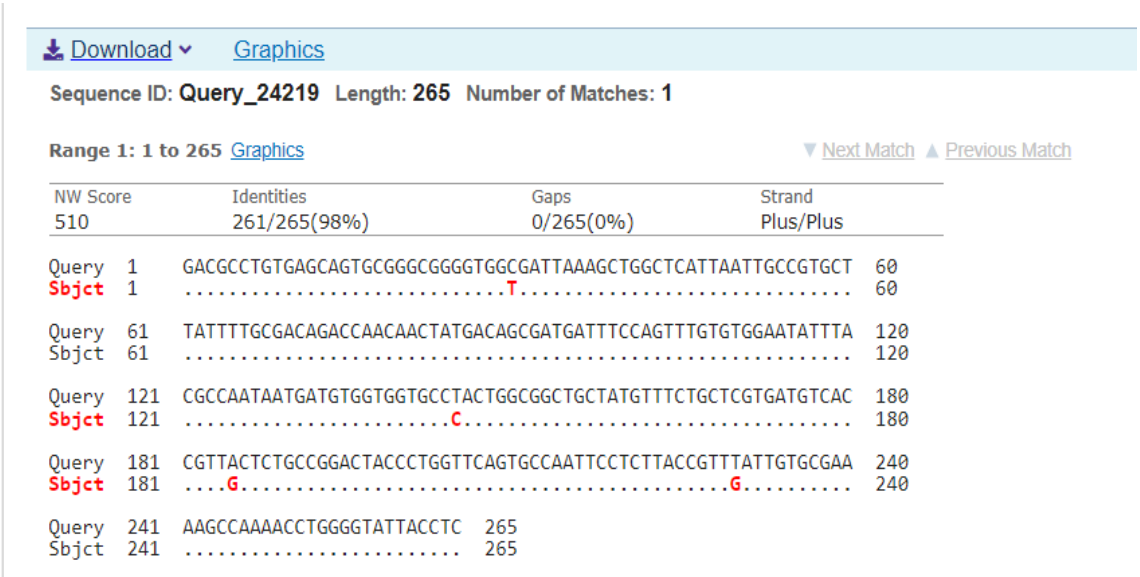
Sequence ID: Query_9939 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
475	254/265(96%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................C..G....T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......A.............................CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G..C.........................A..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH4*and 6



Download Graphics

Sequence ID: Query_24219 Length: 265 Number of Matches: 1

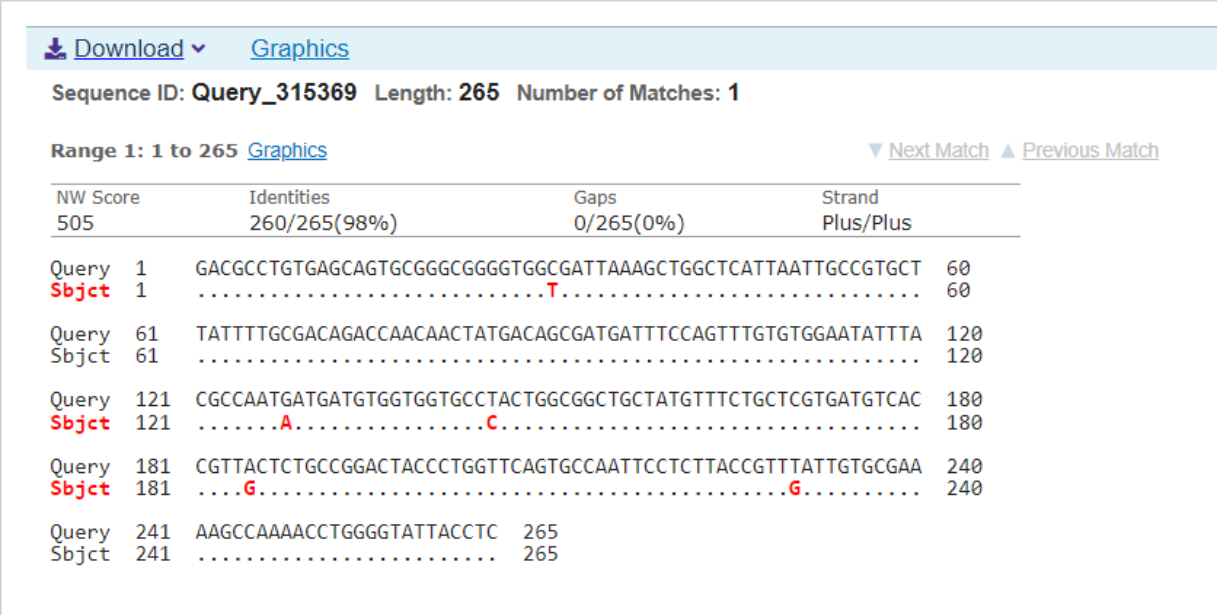
Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
510	261/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................C...................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G.............................................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH4*and 7

Appendix



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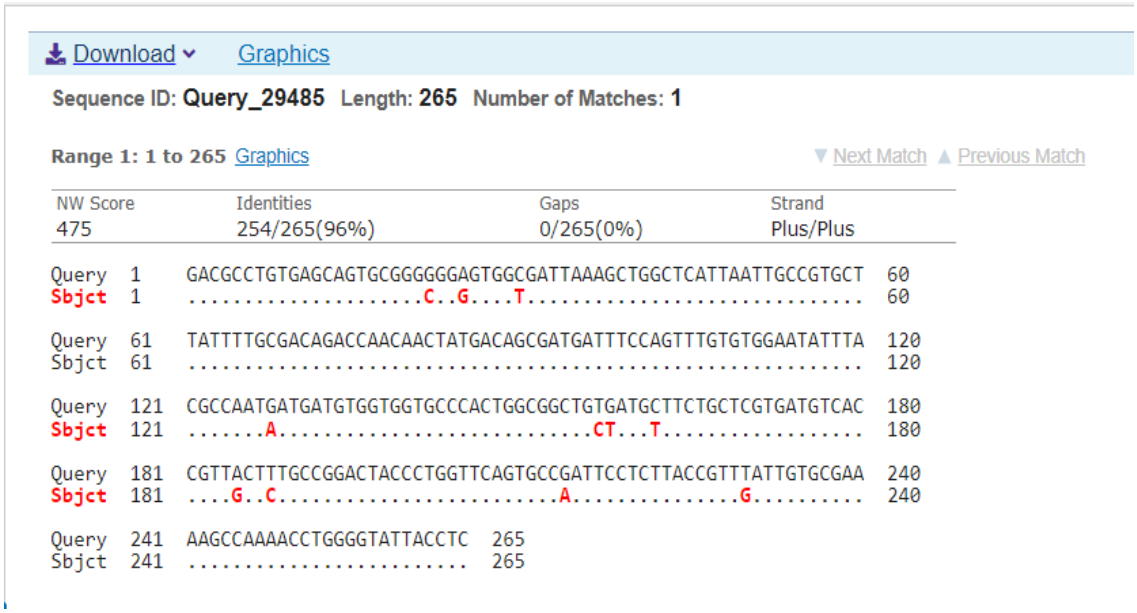
Sequence ID: Query_315369 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
505	260/265(98%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......A.............C..................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G.........................................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH4*and 8



Download Graphics

Sequence ID: Query_29485 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
475	254/265(96%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ....................C..G....T.............................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......A............................CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ....G..C.........................A..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH4*and 9

Appendix

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Sequence ID: Query_3133 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
485	256/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................C..G.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................T.................T...................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......C..........T..A.....G.....................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH5*and 6

Download Graphics

Sequence ID: Query_43057 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
490	257/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G............................TG......................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ..................T..A.....G.....G..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH5*and 7

Appendix

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Sequence ID: Query_60757 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
495	258/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ..................................TG......................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ..................T..A.....G.....G..............G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH5*and 8

Download Graphics

Sequence ID: Query_22435 Length: 265 Number of Matches: 1

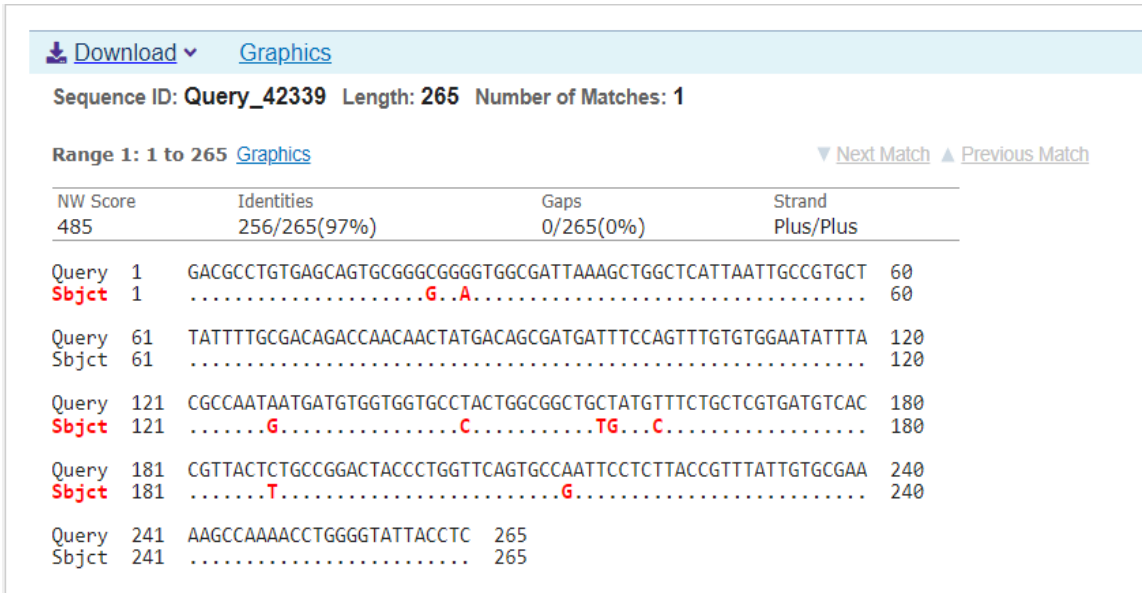
Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
485	256/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................C..G.....................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................T.................T...................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......C..........T..A.....G.....................G..........  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH5*and 9

Appendix



Download Graphics

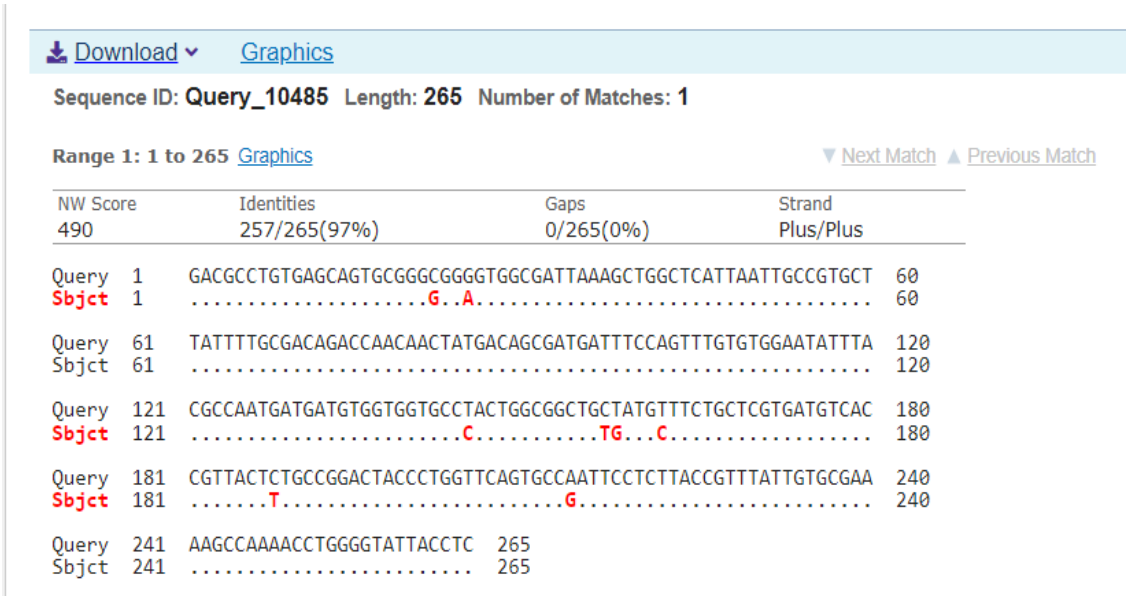
Sequence ID: Query_42339 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
485	256/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................G..A.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......G.................C...........TG...C..................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......T.........................G............................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH6*and 7



Download Graphics

Sequence ID: Query_10485 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
490	257/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ......................G..A.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................C...........TG...C..................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......T.........................G............................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH6*and 8

Appendix

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Sequence ID: Query_50403 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
530	265/265(100%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ............................................................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ............................................................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH6*and 9

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Sequence ID: Query_1615 Length: 265 Number of Matches: 1

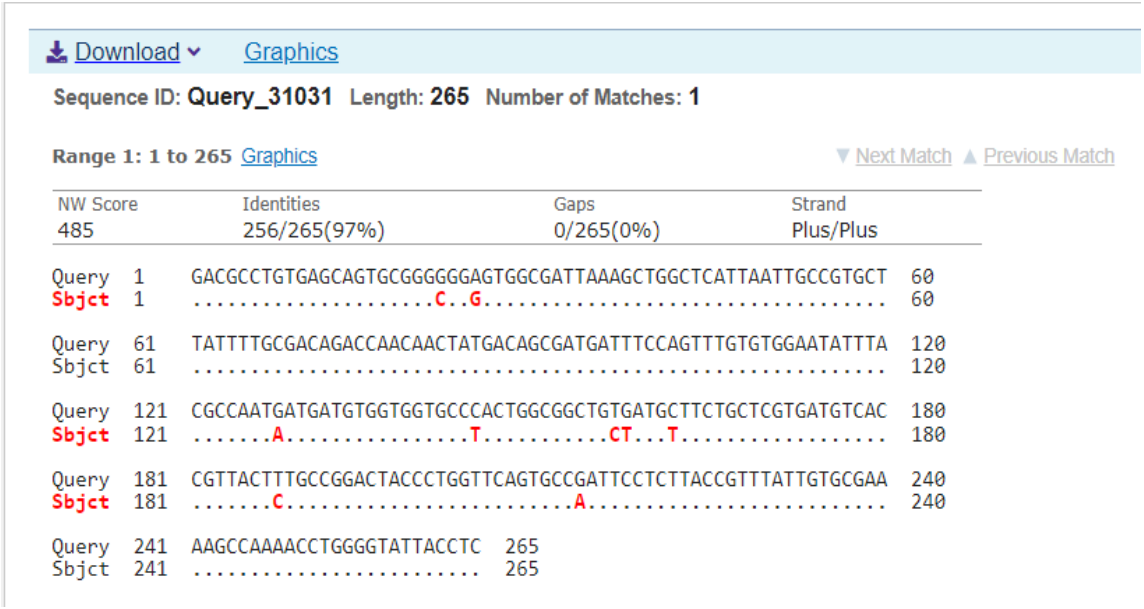
Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
525	264/265(99%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    ............................................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCTACTGGCGGCTGCTATGTTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......A....................................................  180

Query  181  CGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  ............................................................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH7*and 8

Appendix



Download Graphics

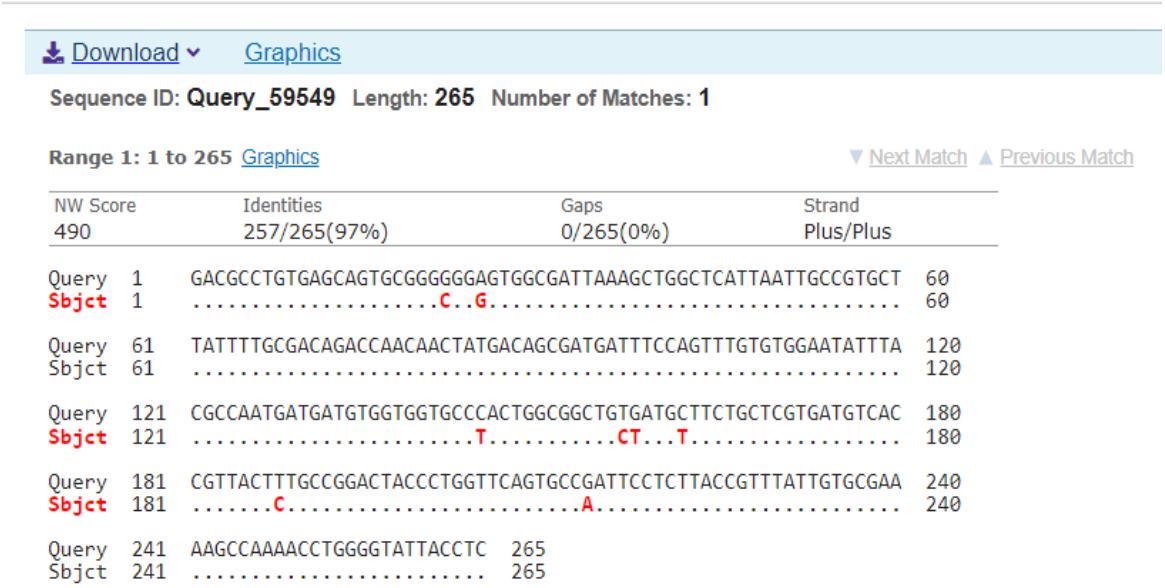
Sequence ID: Query_31031 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
485	256/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    .....................C..G.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  .......A.............T...........CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......C.........................A...........................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH7*and 9



Download Graphics

Sequence ID: Query_59549 Length: 265 Number of Matches: 1

Range 1: 1 to 265 Graphics ▼ Next Match ▲ Previous Match

NW Score	Identities	Gaps	Strand
490	257/265(97%)	0/265(0%)	Plus/Plus

```
Query  1    GACGCCTGTGAGCAGTGCGGGGGGAGTGGCGATTAAAGCTGGCTCATTAATTGCCGTGCT  60
Sbjct  1    .....................C..G.................................  60

Query  61   TATTTTGCGACAGACCAACAACTATGACAGCGATGATTTCCAGTTTGTGTGGAATATTTA  120
Sbjct  61   ............................................................  120

Query  121  CGCCAATGATGATGTGGTGGTGCCCACTGGCGGCTGTGATGCTTCTGCTCGTGATGTCAC  180
Sbjct  121  ........................T...........CT...T..................  180

Query  181  CGTTACTTTGCCGGACTACCCTGGTTCAGTGCCGATTCCTCTTACCGTTTATTGTGCGAA  240
Sbjct  181  .......C.........................A...........................  240

Query  241  AAGCCAAAACCTGGGGTATTACCTC  265
Sbjct  241  .........................  265
```

*fimH8*and 9

الخلاصة

تعد عدوى المسالك البولية (UTI) من أكثر أنواع العدوى البكتيرية شيوعًا ، حيث تصيب 150 مليون شخص كل عام في جميع أنحاء العالم. تم جمع (١١٧) عينة ادرار من مرضى التهابات المسالك البولية من مستشفى الحلة التعليمي ومستشفى النسائية والاطفال في مدينة الحلة / العراق ، وذلك خلال الفترة من شهر تشرين الثاني ٢٠٢١ الى شباط ٢٠٢٢ .

من خلال دراستنا هذه، تم الحصول على ٥٠ عزلة شخصت كبكتيريا القولون بواسطة الوسط التفريقي EMB ونظام Vitek2 ، وتم تأكيد تشخيص العزلات البكتيرية باستخدام الجين التشخيصي 16S rRNA باستخدام تقنية تفاعل البلمرة المتسلسل . أظهرت الدراسة الحالية أن نسبة الإصابة عند الإناث هي (٨٢٪) كانت أعلى من الذكور (١٨٪)، و كان المعدل الاعلى للإصابة ضمن الفئة العمرية (٢١-٣٠) .

كانت نتائج المقاومة للمضادات الحياتية كالتالي Ampicillin ٥٠ ٪ ، Pipercillin ٦٢ ٪، Amoxicillin-clavulanate ٩٨٪ ، Cefepime ٣٦ ٪ Ceftazidime ٧٢٪ ، Cefixime ٤٨٪ ، Cefotaxime ٦٨ ٪ ، Aztreonam ٢٦ ٪ Imipenem ٤٪، Meropenem ٠ ٪ ، Amikacin ٢٢ ٪ ، Gentimicin ٤٨ ٪، Tobramicin ٤٠٪ ، Levofloxacin ٣٤ ٪ ، Ciprofloxacin٣٠ ٪ و Trimethoprim ٥٢٪ . أظهرت عزلات بكتريا القولون نسبة عالية من المقاومة المتعددة للمضادات الحيوية المختلفة (MDR) حيث كانت (٤٤) عزلة من أصل (٥٠) عزلة MDR ، وبنسبة (٨٨ ٪).

قدرت انتاجية تكوين الغشاء الحيوي بطريقة الأنابيب التي شملت جميع العزلات وطبقا للنتائج تم تصنيفها كعزلات شديدة الالتصاق ٥ (١٠٪) و عزلات متوسطة الالتصاق ٣٦ (٧٢٪) بينما كانت العزلات ضعيفة الالتصاق ٩ (١٨٪) .

تم إجراء تفاعل البلمرة المتسلسل لتحديد وجود جينات عوامل الالتصاق لجميع عزلات بكتريا القولون المسببة لالتهاب المسالك البولية، وكانت النتائج كالاتي: الجين *fimH* ٤٦(٩٢٪) ، والجين*CsgA* ٤٩ (٩٨)٪ ، والجين *flu* ٤٦ (٩٢٪) . في الدراسة الحالية أجري تحليل التسلسل لناتج PCR لجين *fimH* للتسع عزلات المحلية من بكتيريا القولون مثلت ثلاث مجاميع لتكوين الاغشية الحيوية مظهريا (ضعيف ومتوسط وقوي) .أنجز تحليل التسلسل لتحديد قواعد النيوكلوتيدات المتشابه مع العزلات العالمية الاخرى خلال تطبيق NCBI- Blast-query nucleotide –online. اظهرت النتائج

نسب التشابه لهذه التسع عزلات المحلية مع السلالات المسجلة في البنك الجيني الاخرى كانت بين(٩٦٪ - ٩٨٪). أنجزت المحاذاة المتعددة لتسلسلات النيوكلوتيدات لمعرفة التغاير بين هذه العزلات المحلية باستخدام برنامج BioEdit جنبا الى جنب مع محاذاة الاحماض الامينية . في المواقع الثلاثة (٢٠٢و ٢١٤و٢٣٠) المتغايرة حدد تكرارا بين العزلات المنتجة للأغشية الحبوبة القوية *FimH* (٧و٨و٩) التي قادت الى التغير في انواع الحامض الاميني من التسلسل ٦٨و٧٢و٧٧ ، بعد التغيير مقارنة الكودونات مع العزلات المحلية الاخرى . بالرغم من ذلك ، العزلات المنتجة للاغشية الحيوية الضعيقة *FimH* (٢ و٣) اظهرت تغايرا وحيدا في النيوكلوتيدة في التسلسل ١٨٥ مع عزلة واحدة من المنتجة للاغشية الحيوية المتوسطة ، والتي كانت ناتجة من تغير في نوع الحامض الاميني عند التسلسل ٦٢.

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم / قسم علوم الحياة

دراسة جزيئية لجينات الغشاء الحيوي في بكتيريا *Escherichia coli* المعزولة من اصابات المسالك البولية

رسالة

مقدمة إلى مجلس كلية العلوم / جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير في العلوم/ علوم الحياة

من قبل

دينا حسن عبد عبود

بكالوريوس علوم حياة - احياء مجهرية
جامعة بابل/ ٢٠١٨م

إشراف

أ. د. زينة هادي عبيد علوان

١٤٤٤ هـ ٢٠٢٣ م