University of Karbala
College of Applied Medical Sciences
Department of Clinical Laboratories

Study of Some Biofilm Genes and Quinolone Resistance Genes in Uropathogenic *Escherichia coli* Isolated from Patients with Urinary tract infection

A thesis

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ
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We certify that this thesis which is entitled **(Study of Some Biofilm Genes and Quinolone Resistance Genes in Uropathogenic *Escherichia coli* Isolated from Patients with Urinary tract infection)** has been prepared by (**Mazin Jaafar Mohammad Redha**) under his supervisors in the Department of Clinical Laboratories /College of Applied Medical Sciences/ University of Kerbala in a partial fulfillment of the requirement for the Degree of Master in Clinical Laboratories.

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Dedication

To....

My dear father

The kind heart my mother

My Lovely Brother and

Sister

MY Best Friend Aiz Saadon

I dedicate this work

MAZIN, 2025

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Summary

Despite the health education and live style was better, some health problem still has the high rate of infection one of them was urinary tract infection (UTI). Morbidity and mortality associated with UTI were shown to be 150 million cases globally per year.

Urinary tract infection (UTI) is commonly caused by bacterial pathogens that often affect the urinary system including the kidney, ureters, bladder, and urethra. The bacterial pathogens invade the urinary tract involving the lower and the upper urinary tract and may sometimes spread to the bloodstream resulting in several clinical syndromes such as fever, flank pain, dysuria and hematuria.

This cross-sectional study aimed to Investigate of Some Biofilm Genes and Quinolone Resistance Genes in Uropathogenic *Escherichia coli* in UTI Patients included a total of (968) mid-stream urine samples collected from symptomatic patients of UTI of both sex with age range (1-80 years) were enrolled the two primary governmental hospitals in holy Karbala province, namely Imam Al-Hussein Medical City and Kerbala Pediatric Teaching Hospital During the period started from October 2023 to April 2024.

The sample collected was investigated for general urine examination and urine culture to identify *E. coli* bacteria and antibiotic susceptibility test using Vitek 2 compact system.

The positive bacterial growth from all samples was 261 (26.96 %) samples with 34 (3.51%) fungi and the remaining ٦٧٣ (69.52%) being negative growth. After using Vitek 2 System implementation result, gram negative bacteria was the most common infectious agent which accounting for (64 %) of total positive culture and the *E. coli* was the highest

percentage (61 %) of total positive bacterial growth and female patients was the higher infectious rate with this bacteria 77.45% in comparison to male 22.55%.

The antibiotic susceptibility test using Vitek 2 System show Ticarcillin was the highest resistant rate ٨٨,٨٩% followed by Piperacillin and Ampicillin while the most effective antibiotic was Imipenem and Meropenem with resistant rate about 6%.

One of the most important issues associated with the therapy of UTIs is the emergence and spread of multidrug-resistant, Topoisomerase IV which is composed of two subunits encoded by parC and par E, the primary function of which is chromosomal segregation following DNA replication, acquired quinolone resistance were attributable to mutations in quinolone resistance-determining region (QRDR) of topoisomerase IV. The Qnr proteins (qnrB and qnrS), which are members of the pentapeptide-repeat family, protect DNA gyrase and DNA topoisomerase IV from fluoroquinolone inhibition.

An important factor contributing to UPEC pathogenesis is the ability to form biofilms, CsgD consider as a major control and integration unit for biofilm formation due to its regulation of the expression of biofilm-associated extracellular matrix substances. While OmpT is an outer membrane serine protease functions as a defence mechanism for the bacteria by cleaving antimicrobial peptides secreted by the host epithelial cells. It also acts as a virulence factor by aiding in the spread of the bacteria within the host cells.

PCR revealed the presence of qnrB gene in (7.89 %) of the isolates, qnrS gene was in (18.4 %), ompT gene was in (68.4 %), parE gene was in

100% and csgD gene was in (97.4 %) among 38 *E. coli* isolates that showed highly antibiotic resistance specially to Fluoroquinolones group.

20 XDR *E. coli* isolates of 38 samples that involved in gene detection process, that showed the highest antibiotic resistance especially to Fluoroquinolones group, were sent to gene sequencing for both par E and csgD genes. The result of gene sequencing analysis of both par E gene and csgD genes, shows multiple variations were observed in different sites of each sample in both forward and reverse strand.

The genetic tree of the gene was analyzed using a specific program and it was compared with global isolates of *E. coli* bacteria.

The results of the phylogenetic tree of the gene par E (forward strand) for all 20 *E. coli* isolates when compared with the site NCBI of global isolates showed that the local isolates it occupied various positions within the phylogenetic tree, and did not lie at the same level with the location of NCBI strain except for the strain of sample ID number (23).

The results of the phylogenetic tree of the gene csgD (forward strand) for all 20 *E. coli* isolates when compared with the site NCBI of global isolates showed that the local isolates it occupied various positions within the phylogenetic tree, and did not lie at the same level with the location of NCBI strain except for the strain of sample ID number (37).

This study conclude that XDR *E. coli* was highly prevalent bacteria among UTI patients and that have highly mutation rate within the target-region that lead to make this bacteria more prevalent and more difficult to treat.

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Terms

Key	Abbreviation
Uropathogenic *Escherichia coli*	UPEC
Curli subunit gene D	CsgD
Curli subunit gene B, A, C	CsgBAC
Outer membrane protease T	OmpT
Multiple drugs resistance	MDR
Extensively Drugs resistance	XDR
Topoisomerase IV	TOP IV
Quinolone resistance determining region	QRDR
Gyrase A and B gene	gyrA and gyrB
Partition gene E and C	parE and parC
Quinolone resistance gene (A,B,C,D,S,VC)	qnrA, qnrB, qnrC, qnrD, qnrS and qnrVC

Aminoglycoside Acetyltransferase-Ciprofloxacin Resistance gene	(aac(6`)-Ib-cr gene)
Oqx Efflux Pump AB gene	OqxAB
Quinolone Efflux Pump A gene	QepA
Gram stain smear	GSS
Non- gonococcal urethritis	NGU
Acute uncomplicated cystitis	AUC
National Institutes of Health	NIH
Acute bacterial prostatitis	ABP
Chronic bacterial prostatitis	CBP
Chronic non- bacterial prostatitis	CNP
chronic pelvic pain syndrome	CP/CPPS
Xanthogranulomatous pyelonephritis	XGP
Emphysematous pyelonephritis	EPN
Inferior vena cava	IVC
primary healthcare	PHC
group B Streptococcus	GBS
European Association of Urology	EAU
Asymptomatic bacteriuria	ASB , ABU
Capsular polysaccharide transport system	KpsMT
Uropathogenic specific protein	Usp
Iron-regulated outer membrane receptor	IroN
Iron-regulated adhesin	Iha
Secreted enterotoxin 1	Set 1
Aggregative adherence secreted toxin A	AstA
S fimbriae/ F1C fimbriae	Sfa/foc
Iron uptake transporter A	IutA

Transfer protein T	TraT
Fimbrial adhesion H	FimH
Global Burden of Disease	GBD
disability-adjusted life years	DALYs
Interferon Gamma	IFNγ
Interleukin	IL
T-helper cells	Th1 , Th2
Catheter-associated urinary tract infections	CAUTIs
type 1 and 2 diabetes mellitus	T1DM and T2DM
Haemoglobin A1c	HbA1c
Italian Society of Pediatric Nephrology	SiNePe
Vesicoureteral Reflux	VUR
Voiding Cystourethrogram	VCUG
coagulase-negative Staphylococci	CoNS
intestinal pathogenic *E. coli*	IPEC
extraintestinal pathogenic *E. coli*	ExPEC
enterotoxigenic *E. coli*	ETEC
enteroaggregative *E. coli*	EAEC
diffusely adherent *E. coli*	DAEC
enteroinvasive *E. coli*	EIEC
neonatal meningitis *E. coli*	NMEC
pathogenicity islands	PAI
Mobile genetic elements	MGE
nucleotide-binding oligomerization domain-like receptors	NLRs
Antibiotic Susceptibility Testing	AST
Eosin-methylene blue	EMB

Base Pair	Bp
Tris-Acetate-EDTA buffer	TAE buffer
Polymerase Chain Reaction	PCR
Statistical Package for The Social Sciences	SPSS

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

Urinary tract infections (UTIs) are one of continuous health problem in primary health care, the US Centers for Disease Control and Prevention defines UTIs as common infections occurring when bacteria, often from the skin or rectum, enter the urethra and infect the urinary tract (Carmona-Cartaya *et al .,* 2022) .

Bacterial infection was the most common cause on UTI, but Candida species may lead to fungal UTIs, while viral cystitis can be caused by viruses like adenovirus and herpes simplex, albeit less commonly than bacterial infections (Agrawal and Paunikar , 2024).

Escherichia coli are gram-negative bacteria within the family *Enterobacteriaceae* that can harmlessly colonize the human gut or cause intestinal or extraintestinal infections, including severe invasive disease such as bacteremia and sepsis. (Bonten *et al* ., 2021) . Uropathogenic *E. coli* strains possess an arsenal of virulence factors that contribute to their ability to overcome different defense mechanisms cause disease. (Dadi *et al* ., 2020). Flagellar motility enables the bacterium to ascend the urethra and colonize the bladder. Several toxins, such as hemolysin, are secreted during the infection to cause tissue damage and ensure nutrient release (Alshaikh *et al* ., 2024). An important factor contributing to UPEC pathogenesis is the ability to form biofilms (Torres-Puig *et al* ., 2022). Bacterial biofilms are structured clusters of bacteria coated in a polymeric matrix and attached to a surface. Similar or different bacterial species coexist within a biofilm and they often communicate between them. This makes the bacterial biofilm a favorable platform for intra and interspecific genetic material (virulence and drug resistance genes) exchange (Dossouvi *et al .,* 2023).

These structures are problematic due to their drug-resistant properties and their ability to evade the human defense mechanisms, thereby hindering the infection treatment. Individual bacterial cells bind one another forming a structured composition, thereby facilitating survival under various environmental conditions (Mirzaei *et al .,* 2020).

Escherichia coli produces proteinaceous surface structures called curli that are involved in adhesion and biofilm formation. CsgD is the transcriptional activator of curli genes (Holmqvist *et al .,* 2010). Curli production is directly activated by the binding of the transcription factor CsgD to the promoter of the csgBAC operon. So that CsgD consider as a major control and integration unit for biofilm formation due to its regulation of the expression of this biofilm-associated extracellular matrix substances (Liu *et al .,* 2014).

Curliated bacteria are resistant to damage from predation, desiccation, oxidative stress, and other antimicrobial agents. Curli fibers have also been implicated in pathogenesis process (Smith *et al .,* 2017).

OmpT is an outer membrane serine protease in Gram-negative bacteria as part of the omptin family (Goh *et al .,* 2024). functions as a defence mechanism for the bacteria by cleaving antimicrobial peptides secreted by the host epithelial cells. It also acts as a virulence factor by aiding in the spread of the bacteria within the host cells. Identifying peptide substrates that are efficiently cleaved by OmpT can thus help to improve both the sensitivity of peptide-based assays for *E. coli* detection in complex mixtures and in the design of D-amino acid based peptide inhibitors (Wood *et al .,* 2017).

One of the most important issue associated with the therapy of UTIs is the emergence and spread of multidrug-resistant (MDR) bacterial strains-owing to a plethora of different resistance mechanisms-severely limiting therapeutic alternatives for clinicians (Behzadi *et al .,* 2020). In the past 80 years, empirical use, overuse, and misuse of antimicrobial agents have been linked to outbreaks of antimicrobial resistance (Li *et al .,* 2022).

There is a capacity of *E. coli* germs to acquire resistance to multiple drugs (MDR). A high degree of antibiotic resistance is seen in these bacteria because they contain resistance enzymes such as β-lactamases, which provide resistance to β-lactams, as well as enzymes that give resistance to aminoglycosides and quinolones (Abbood , 2024) .

Antibiotic resistance mechanisms in UPEC can include chromosomal mutations in target genes and efflux pumps which allow for enhanced expulsion of antimicrobials from the cell interior (Whelan *et al .,* 2023).

Topoisomerase IV, an essential enzyme of *E. coli*, the primary function of which is chromosomal segregation following DNA replication, is composed of two subunits encoded by parC and parE, which are homologous to gyrA and gyrB of DNA gyrase (Breines *et al .,* 1997). Acquired quinolone resistance were attributable to mutations in quinolone resistance-determining region (QRDR) of topoisomerase IV, whose subunits are encoded by parC, and parE genes (Qian *et al .,* 2020).

Recently, plasmid mediated quinolone resistance (PMQR) was detected, raising concerns about transferability of this important resistance. In Enterobacteriaceae, PMQR is conferred by qnr (qnrA, qnrB, qnrC, qnrD, qnrS and qnrVC) genes. The Qnr proteins, which are members of the pentapeptide-repeat family, protect DNA gyrase and DNA topoisomerase IV from fluoroquinolone inhibition (Vázquez *et al .,* 2022).

Three main mechanisms of resistance to quinolones associated with PMQR are known, inducing low susceptibility to these compounds by protecting the binding site in DNA-gyrase (qnr gene), enzymatically modifying the drug (aac(6`)-Ib-cr gene), and expelling the agent from its site of action by coding for efflux pumps (oqxAB and qepA genes) (Vieira *et al .,* 2020).

Aim of the Study

This study aimed to Investigate of Some Biofilm Genes and Quinolone Resistance Genes in Uropathogenic *Escherichia coli* Isolated from Patients with UTI by the following objectives:

1- Isolation and Identification *E. coli* isolated from UTI patients VITEK®2 Compact system.

2- Evaluation of the antibiotic susceptibility pattern of *E. coli* by VITEK®2 Compact system.

3- Assessment of the spreading of virulence genes CsgD and ompT.

4- Investigate the dissemination of par E, qnrB and qnrS genes among isolates exhibit reduce susceptibility to quinolones group.

5- Explore the Sequencing of the two important genes csgD and parE to determine of the polymorphisms and phylogenetic tree.

Chapter two

Literature Review

Chapter two Literature Review

2.1. Urinary Tract Infections

2.1.1. Definition of Urinary Tract Infections

Urinary tract infections (UTIs) are considered to be the second most common bacterial diseases after pneumonia, mainly due to the high frequency of period recurrences and chronicity of infections. UTIs are also a leading cause of septicemia (Zalewska-Piątek and Piątek, 2020).

The UTIs is the inflammation of any part of the urinary system which consists of the kidney, bladder, ureter and urethra mediated by microorganisms (Ngong *et al .,* 2021). In both community and hospital settings are estimated to affect around 405 million people globally and nearly 0.23 million people died of UTIs, contributing to 5.2 million disability-adjusted life years (DALYs) in 2019 (Islam *et al .,* 2022).

The UTI can be asymptomatic and symptomatic. The asymptomatic UTI is defined as the presence of the organism; however, there are no signs and symptoms and it accounts for 17-51% of infections in the Kidney Transplant Recipients and risking the individuals for the subsequent UTI (Iqbal *et al .,* 2020).

Infections induced by bacteria that affect even a certain portion of the urinary tract are a major cause of UTI. These include increased urine frequency along with pain and the presence of cloudiness in the urine. Other symptoms comprise dysuria, cramps in the lower abdominal region, chills, fever, general weakness accompanied by nausea and vomiting (Asmat *et al .,* 2021), burning sensation during urination, loss of bladder control, increased frequency of urination especially in small amounts, low back pain and bloody or foul smelling urine (Fenta *et al .,* 2020).

2.1.2. Epidemiology

UTI represents one of the most prevalent bacterial infections globally and is increasingly becoming a significant health concern for patients worldwide, often accompanied by substantial healthcare expenses (Mengistu *et al* ., 2023). World Health Organization (WHO) reports the higher incidence of world's death rates due to this disease beside diarrheal infection (Mansoor and Salman , 2023).

The absolute number of cases of UTIs increased by 60.40% from 252.25 million (95%UI: 223.31–279.3) in 1990 to 404.61 million (95%UI: 359.43–446.55) in 2019. The global fatalities due to UTIs were 236,790 (95%UI: 198,430–259,030) in 2019, which increased by 140.18% from 98,590 (95%UI: 89,030–106,320) deaths in 1990. Worldwide, the number of DALYs caused by UTIs was 520,200 (95%UI: 445,400–570,500) in 2019, which had a 68.90% increase compared with 308,000 (95%UI: 2,652,000– 338,200) in 1990 (Yang *et al* ., 2022).

In the 7 GBD super-regions, South Asia had the highest death counts (20.07 thousand [95% UI: 14.56–27.12] attributed to resistance and 72.12 million [95% UI: 53.06–96.02] associated with resistance). However, Central Europe, eastern Europe, and central Asia had the highest death rates (1.45 per 100,000 [95% UI: 0.96–2.23] attributed to resistance and 5.72 per 100,000 [95% UI: 3.84–8.59] associated with resistance). The regions of sub-Saharan Africa, including southern sub-Saharan Africa, western sub-Saharan Africa, eastern sub-Saharan Africa, and central sub-Saharan Africa, had the lowest death rates (Li *et al* ., 2022).

In 2007 in the United States alone, there were 10.5 million ambulatory visits for UTIs, accounting for 0.9% of all ambulatory visits, and almost 2–3 million visits were to hospital emergency departments.

UTIs are also common among inpatients. In 2004, a study conducted in 49 Swiss hospitals showed that 1.5% of hospitalised patients developed symptomatic UTIs (Zeng *et al* ., 2022).

Uropathogenic *Escherichia coli* (UPEC) is estimated to responsible for approximately 80 percent of all UTIs (Kadhim and Rasheed , 2021).

2.1.2. Classification of urinary tract infection (UTI) according to the site of infection

2.1.2.1. Lower urinary tract infection

Urethritis is defined as inflammation of the urethra. This syndrome is usually caused by sexually transmitted pathogens, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and rarely pathogens such as *Herpes* simplex viruses 1 and 2, *Trichomonas vaginalis*, or *Adenovirus* among some others. Acute and persisting urethritis might result in significant morbidity, including arthritis, epididymo-orchitis, or prostatitis (Sadoghi *et al .,* 2022).

Urethritis is classified, based on Gram stain smear (GSS) testing of urethral secretions, into gonococcal urethritis (caused by *Neisseria gonorrhoeae* (NG)) and non-gonococcal urethritis (NGU) (Jordan *et al .,* 2020). Though urethritis can occur in either males or females, the majority of the literature focuses on afflicted males. Urethral discharge is the most common symptom of urethritis, but other non-specific symptoms can occur, including dysuria and frequency.

The presence of leukocytes (or leukocyte esterase), indicative of inflammation in the urethra, is considered relatively sensitive for the detection of urethritis, and when combined with clinical presentation, will trigger treatment (Dickson *et al .,* 2024).

Cystitis : Acute uncomplicated cystitis (AUC) is one of the most common and widespread pathological conditions among women of all ages, with a relevant impact on social costs and quality of life. Although AUC is a benign disease, recurrent episodes are associated with reduction in quality of life, everyday activities, such as social and familiar relationships, leisure time and physical activities, working ability, and psychosexual disorders (Di Vico *et al .,* 2020).

The Cystitis can be divided into infectious and non-infectious. Patients with infectious Cystitis often complain of irritating emptying symptoms, difficulty urinating, frequency, urgency, and pain in the pubic hair, with severe bleeding occurring only in rare cases. Another major category of Cystitis is sterile or non-infectious Cystitis, which can be caused by radiation and chemical irritations. Unlike infection-induced Cystitis, non-infectious Cystitis is more clinically severe and can cause extreme pain, hematuria, and irritating emptying symptoms (Shih *et al .,* 2023).

Prostatitis ; There are many classifications of prostatitis in Western medicine. At present, the classification method of National Institutes of Health (NIH) in the United States in 1995 is used internationally. It was divided into four types: acute bacterial prostatitis type I (ABP), chronic bacterial prostatitis type II (CBP), chronic non-bacterial prostatitis type III (CNP)/chronic pelvic pain syndrome (CP/CPPS). Chronic prostatitis is divided into chronic bacterial prostatitis and chronic non-bacterial prostatitis (Feng *et al* ., 2023).

2.1.2.2. Upper urinary tract infection

Pyelonephritis : Acute pyelonephritis, known in clinical practice as an upper urinary tract infection (UTI), is defined as a tubulointerstitial disorder characterised by inflammation of the renal parenchyma caused by bacteriuria ascending from the bladder up the ureters to the kidneys (Hudson and Mortimore , 2020).

The Pyelonephritis is diagnosed when symptoms such as fever, chills, flank pain, nausea, vomiting, and costovertebral angle tenderness are present together with bacteriuria and pyuria (Greve *et al* ., 2020).

Pyelonephritis in pregnancy is associated with perinatal complications including septicemia, respiratory distress, low birth weight, and preterm birth , making it one of the most common non-obstetrical reasons for antepartum hospitalization (El Taha *et al* ., 2021) . There are many types of pyelonephritis including :

1- **Xanthogranulomatous pyelonephritis** (XGP)

Xanthogranulomatous pyelonephritis is a chronic and destructive granulomatous process of renal parenchyma which is associated with long-term urinary tract obstruction and infection. It is uncommon and often presents as asymptomatic renal mass. It can present as an acute urinary tract infection. Mostly caused by Proteus and other gram-negative bacteria and may lead to poorly functioning kidney unit (Abushamma *et al* ., 2020). (XGP) is a rare and potentially harmful diagnosis as it may lead to serious complications such as sepsis, renal failure, and potentially life-threatening conditions (Abushamma *et al* ., 2021).

2- **Emphysematous pyelonephritis (EPN)**

Emphysematous pyelonephritis (EPN) is an acute necrotizing infection of the renal parenchyma and perirenal tissue. It occurs more commonly in diabetics. The high tissue glucose levels provide the substrate for fermentation, which results in carbon dioxide production. The mortality rate has been reported to be around 19%. The extension of gas into Inferior vena cava (IVC) in EPN is quite a rare phenomenon (Vaddi *et al* ., 2020).

(EPN) is classified into four classes: Class 1 indicates gas confined to the collecting system; Class 2 indicates gas confined to the renal parenchyma without extension to extrarenal space; Class 3A indicates extension of gas or abscess to perinephric space-as in our patient; Class 3B pertains to extension of the gas or abscess to the pararenal space; and Class 4 refers to bilateral EPN or a solitary kidney with EPN (Dong *et al* ., 2022).

2.1.3. Types of urinary tract infection (UTI) according to the duration and recurrence of infection

2.1.3.1. Uncomplicated UTI:

Uncomplicated UTI is one of the most common infections treated in primary healthcare (PHC). Almost half of all women will experience at least one episode of uncomplicated UTI during their life time. Women attending PHC with symptoms that indicate uncomplicated UTI are, to a high extent, prescribed antibiotics. The gold standard for the diagnosis of uncomplicated UTI is the presence of clinical symptoms and the detection of the pathogen via urine culture. The clinical diagnosis is essentially based on a patient's medical history (Kornfält Isberg *et al* ., 2021).

Uncomplicated UTI occur in female individuals who are not immunocompromised, have no foreign bodies in the urinary tract and have not undergone urinary tract manipulation recently, and have no anatomical or neurologic abnormalities in the urinary tract (Werneburg, 2022). Uncomplicated UTI pathogenesis also steeps progressively due to pathogenic *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B Streptococcus (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida spp* (Hozzari *et al* ., 2020).

2.1.3.2. Complicated UTI

Complicated UTI is defined as an infection associated with hazards to the urinary tract or host immune factors, including urinary obstruction, urinary retention due to neurological diseases, immunosuppression, renal failure, kidney transplantation, pregnancy, and presence of foreign bodies such as stones, indwelling catheters, or other drainage devices (Yuan *et al* ., 2021).

It is possible that many cases of urinary abnormalities are not identified after the first episode of a UTI but are detected later in the event of a recurrence, Furthermore, the relationship between blood infections and the presence of underlying urinary abnormalities is controversial. In fact, complicated UTIs can at the same time be more frequent in case of alternations of the anatomy of the urinary tract (as in the case of patients with dilations from vesicoureteral reflux), but themselves can also cause kidney alterations following the inflammation process (renal scars, abscesses and long-term complications such as hypertension or kidney failure) (Buonsenso *et al* ., 2023).

2.1.3.3. Relapses

The recurrence of the same bacterial strain in urine shortly after the completion of an effective antibiotic course. This suggests either incomplete eradication of the initial infection or bacterial persistence, leading to reinfection. (Ibraheem *et al* ., 2022).

2.1.3.4. Reinfection (Recurrence of bacteria)

Recurrent UTI, usually defined as 3 or 4 UTI during a 12-month period, has a substantial impact on day-to-day functioning due to moderate or severe symptoms and persistent recurrence at episodic intervals. Severe symptoms include pain and/or burning during urination, frequent urination, suprapubic pressure, fever, and nausea. More serious long-term outcomes include treatment failures, hospitalizations including prolonged stays and readmissions, bacteremia, severe sepsis, septic shock, in-hospital mortality, and the spread of antibiotic resistance (Eldridge *et al* ., 2021). Recurrent UTIs differently from persistent infections in which the pathogen is not eradicated but instead persists in some of the infected people's cells (Dominoni *et al* ., 2023).

The European Association of Urology (EAU) guidelines define recurrent urinary tract infections (UTI) as recurrences of uncomplicated and/or complicated UTI with a frequency of at least three UTI in the past year, or two UTI in the last 6 months (Naber et al ., 2022).

2.1.3.5. Asymptomatic bacteriuria (ASB):

Asymptomatic bacteriuria (ASB) occurs in 1–5% of healthy premenopausal women. It appears to be associated with adverse outcomes in certain populations, but observational studies in healthy nonpregnant women suggest that it is a benign condition (Hooton *et al* ., 2021). Although asymptomatic bacteriuria is uncommon in children (less than 1% in boys and 1% to 2% in girls) (Colgan *et al* ., 2020). ASB is also more frequent in diabetic patients than in healthy controls (17% vs. 10%), and it can progress to symptomatic UTI in up to 20% of them within 6 months, especially if glycaemic control is suboptimal (Hernández-Hernández *et al* ., 2021).

The asymptomatic carrier state allows bacteria to persist without evoking a destructive host response and at least four different adaptation strategies have been identified. First, most ABU strains have a virulence-attenuated phenotype and successfully avoid triggering the innate immune response. Second, ABU strains actively suppress host gene expression, for example, by inhibiting RNA polymerase II, including genes crucial for the innate host defence against UTI. Third, attenuating Toll-like receptor 4 promoter polymorphisms reduce innate immune activation and downstream signalling in patients with ABU. Fourth, the mucosal TLR4 response is attenuated in the human urinary tract mucosa, owing to a lack of pattern recognition co-receptors for lipopolysaccharides such as CD14 (Ambite *et al* ., 2021).

2.2. Pathogenicity

The pathogenicity of Urinary tract infections (UTIs) associated bacteria is based on their ability to attach, colonize, and survive in the urinary tract environment (Ala-Jaakkola *et al* ., 2022). Its begin when gut-resident uropathogens colonize the urethra and subsequently the bladder through the action of specific adhesins. If the host's inflammatory response fails to eliminate all bacteria, they begin to multiply, producing toxins and enzymes that promote their survival. Subsequent colonization of the kidneys can evolve into bacteremia if the pathogen crosses the kidney epithelial barrier (Mancuso *et al* ., 2023).

Uropathogenic *E. coli* produce toxins that induce host cell damage, releasing essential nutrients that promote bacterial survival and ascension to the kidneys. Kidney colonization further leads to bacterial toxin production and host tissue damage. In severe cases, it can lead to the occurrence of diseases such as bacteremia (Zhou *et al* ., 2023).

Currently, two main routes of uropathogenic invasion are considered: an ascending route, which is realized through bacterial entry to the urothelium from colonies in the periurethral area, vagina, and/or rectum, and a hematogenous route (Stepanova, 2022). Some unusual cases like severe bowel infection or retroperitoneal abscesses may cause the direct spread of bacteria from adjacent organs via the lymphatic route (Khan *et al* ., 2020).

In the mechanism of infection ascending route, bacteriuria is considered to be required as an initial step towards kidney infection. Progression of ascending infection to the kidneys in cases of *E. coli* UTI may be enabled by flagella-mediated motility that propels the bacteria through the ureters. In rare cases, acute pyelonephritis can develop via a ‘descending’ hematogenous route without bladder infection (Sullivan and Ulett , 2020) . While the hematogenous pathway is more difficult to find in cases of urinary tract infection. Usually caused by prolonged bacteraemia or preceded by infectious endocarditis (Santoso and Ali , 2023). This mode of contamination has been demonstrated in animal models. One of the first studies on the subject, carried out by De Navasquez (1950), showed that the intravenous injection of *S. aureus* can cause pyelonephritis. However, a similar result was more difficult to achieve with Gram-negative bacteria, suggesting that this is not the common route of infection for most UTIs, since most of these infections are caused by Gram-negative bacteria, in particular UPECs (Arsene *et al* ., 2021).

In severe UTIs that caused by UPEC several virulence factors such as P fimbriae (pap), type1 fimbriae, afimbrial adhesin I (afaI), hemolysin (hly), cytotoxic necrotizing factor 1 (cnf 1), aerobactin (aer), S fimbriae (sfa), adhesins and fimbriae are contributed. As well, other virulence genes including kpsMT, ompT, usp, iroN, iha, set 1, astA, group II capsule synthesis; sfa/foc, S and F1C fimbriae; iutA, traT, serum resistance; and fimH have the main role in its pathogenicity (Zhao *et al* ., 2021).

The ability of Uropathogenic *Escherichia coli* (UPEC) to persist and grow as biofilms makes them more prone to cause pyelonephritis and even chronic or recurrent infections, increasing antimicrobial resistance and severity of UTIs (Baldiris-Avila *et al* ., 2020).

2.3. Risk Factor

2.3.1. Age and Sex

Age has been associated with the incidence of UTIs and with treatment failure of UTIs in multiple observational studies, sometimes yielding conflicting results. Therefore, recognizing how UTI incidence varies across different age groups can aid in the development of targeted prevention strategies and tailor-made treatment approaches, considering the unique physiological and immunological characteristics of specific populations (Zhan *et al* ., 2024).

In newborns and infants, the prevalence is higher in boys than in girls. Subsequently, the ratio reverses more and more sharply until adulthood. However, the difference between sexes disappears in the elderly, due to the faster increase in the prevalence in men related to prostate diseases and the instruments they require. It is also worth mentioning that about 10–20% of women experience urinary tract infections at least once in their lifetime (Crintea *et al* ., 2023) .

Studies using data from the Global Burden of Disease (GBD) show that in 2019 the absolute number of UTI cases had increased by 60.50% since 1990, to a total of 404.61 million patients. The age-standardized incidence of UTIs was 3.6 times higher for females, and UTI incidences increased with age in teenagers, peaking around 35 years. Mortality and disability-adjusted life years (DALYs) were significantly higher in patients aged 65–75 years in both sexes (Kim and Lee , 2023).

The UTIs are identically harmful for men, especially reproductive function. chronic bacterial prostatitis (CBP), a specific type of UTIs, has negative effect on sperm motility and morphology. Compared with normal ejaculate, a higher leukocyte count could be observed in CBP patients, which is associated with the pathophysiological changes of sperm damage. Once spread to the accessory gland, UTIs could cause a great decline of total sperm number and bilateral infection is more detrimental (Cao *et al* ., 2021).

The Females are three times more likely to get UTI than males, due to women's shorter urethra which opens nearer to the anus, nature of sexual activity, pregnancy, easy contamination of the urinary tract with faecal flora and hormonal changes that occur very quickly (Belete and Saravanan , 2020).

2.3.2. Catheterization

Catheter-associated urinary tract infections (CAUTIs) continue to be among the most common health-care associated infections worldwide: CAUTIs account for 40% of all hospital-acquired infections and 80% of all nosocomial (UTIs). CAUTIs may progress to complications such as cystitis, pyelonephritis, Gram-negative bacteremia, endocarditis, vertebral osteomyelitis, septic arthritis, and meningitis in all patients (Oumer *et al* ., 2021).

The Foreign bodies in the urinary tract, most commonly indwelling urethral catheters, promote bacterial colonization by multiple mechanisms, an important one being biofilm formation (Wagenlehner *et al* ., 2020). Urinary catheters provide a direct conduit from the ambient environment to the urinary bladder. This conduit, while critical for urinary drainage in some patients, also provides a channel for rectal and periurethral microbe ascension to the bladder where they may establish a foothold for infection. catheters may also irritate and traumatize the uroepithelium, thereby disrupting the physiologic mucopolysaccharide coating, and rendering it susceptible to bacterial adhesion and entry (Werneburg , 2022).

2.3.3. Diabetes Mellitus

Diabetic subjects have an increased risk of UTIs due to weakening of white blood cells, poor blood supply, bladder dysfunction due to nephropathy and glycosuria, immunodeficiency and neurological and vascular complications caused by the disease, its impact on the cellular barrier and local defenses altering the activity of polynuclears and phagocytosis (Raherison *et al* ., 2023) and (Tegegne *et al* ., 2023).

Dysuria is a complication of urinary tract infection in diabetic patients due to organ damage and even death due to the complexity of pyelonephritis. Also, these patients experience urinary retention, urgency, and incontinence during the night due to increased urination to excrete excess glucose (Salari *et al* ., 2022). Also Due to severe neuropathy, people with diabetes mellitus have an elevated possibility of CAUTIs, which may result in insufficient bladder emptying and the colonization of microorganisms (Rubi *et al* ., 2022).

Both type 1 and 2 DM increase the risk of UTI and bacteriuria caused by *Enterobacteriaceae* especially in female patients. Bacteria may grow more rapidly in the urine of DM patients with higher glucose level. In an in vitro study evaluating the growth rate of *Escherichia coli* strains in human urine with and without added glucose, bacteria grew faster in urine with higher concentrations of glucose (Kamei and Yamamoto , 2021).

2.3.4. Stones

Urolithiasis is a widespread problem with a male preponderance, showing a lifetime prevalence of 10% in men and 5% in women. The worldwide prevalence of urolithiasis is also steadily increasing, partially owing to its strong association with metabolic syndrome conditions such as obesity, hypertension, and diabetes. Urolithiasis can cause secondary complications, such as obstruction or UTI. Acute UTI may cause sudden deterioration of renal function, especially in the presence of urinary tract obstruction (Hsiao *et al* ., 2020).

There is a bidirectional link between two common urological disorders: kidney stones, or nephrolithiasis, and (UTIs). Urine flow obstruction and stagnant urine pockets caused by kidney stones can provide an ideal environment for the growth of bacteria and the onset of UTIs. Evidence shows that the existence of kidney stones might operate as a nidus for recurrent UTIs, encouraging the surface of the stone exhibits bacterial adherence and colonisation. Additionally UTIs, may contribute to the creation and progression of kidney stones by modifying urine pH and producing an environment suitable for stone crystallization (Singh *et al* ., 2023).

A minority of patients with an obstructing kidney stone will have a concurrent UTIs with accumulation of infected urine behind the obstruction, which can develop into obstructive pyelonephritis with potential consequences of severe sepsis requiring intensive care and even death (Haas *et al* ., 2020).

2.3.5. Vesicoureteral Reflux

According to the Italian Society of Pediatric Nephrology (SiNePe), VUR represents a risk factor for UTI development. It consists of an abnormal backward flow of urine from the bladder up to the kidneys, secondary to a structural or functional abnormality of the vesicoureteral junction or pathological increased intravesical pressure, due to neurogenic bladder, posterior urethral valves, or other obstructive uropathies (Chirico *et al* ., 2023). VUR was not a single entity but 2 distinct conditions, the first being the rarer congenital “reflux disease” detected predominantly in males prenatally as collecting system and ureteric dilatation, often with abnormal renal parenchyma and dilating IV–V VUR on VCUG following birth. The second and more common form of VUR, the “reflux symptom”, is usually detected in investigations after UTI in young girls with lesser grade I–III VUR and normal kidneys (Hewitt and Montini , 2021).

2.3.6. Behavioral

Behavioral risk factors are associated with urinary tract infections in women. Sedentary lifestyle >6 h/day, delayed voiding and sanitary hygiene were associated with an increased risk of recurrent UTI in postmenopausal women (Jagtap *et al* ., 2022).

In various study, UTIs in female students was associated with factors such as the way of cleaning anus after faecal excretion, daily intake of water, previous history of dysuria, genital itching, frequent urination, and pain at the sides of the abdomen and in the lower abdomen. (Ahmadi *et al* ., 2020).

Menstrual hygiene has been hypothesised as a risk factor for urogenital infections, including reproductive tract infections (RTIs) and urinary tract infections (UTIs). So Menstrual hygiene, the hygienic management of menstrual bleeding, represents one key requirement for menstrual health, describing the use of sufficient, clean menstrual materials and access to supportive facilities for washing the body and laundering materials (Borg *et al* ., 2023).

Additionally, to life style diet can influence the predisposition to urinary infections, Foods especially functional ones, bring a supply of valuable nutrients and protective factors for the body (vitamins, mineral salts, antioxidants, fibers, probiotics). Most biocompounds with therapeutic potential are found in fruits, vegetables, and other therapeutic plants. Medicinal plants are known, but there is also a series of vegetables and fruits which are rich in phytonutrients that can prevent or improve urinary infections (Mititelu *et al* ., 2024). Vitamin D induces cathelicidin (a human antimicrobial peptide) of the urinary bladder epithelium. As it provides protection against both gram negative and positive bacteria, we can conclude that this compound has a broad spectrum of action. Moreover it also show actions against some protozoa and fungi (Ali *et al* ., 2020).

Obesity seems to be also associated with an increased risk of bacterial urinary tract infections. A study reported that subjects with obesity were up to 2.5 times more likely to be diagnosed with a urinary tract infection than people without obesity, mostly in females. Obesity has been reported to be a risk factor for urinary tract infections also in pregnant woman and during the postpartum period (Muscogiuri *et al* ., 2021).

2.3.7. Constipation

Constipation and UTIs have been linked; several studies have reported an association between constipation and the recurrence of UTI in children; some studies have also reported that treatment of constipation appears to reduce recurrences of UTI (Muniz *et al* ., 2021). In constipation, pressure from the loaded and distended rectum on the bladder neck causes obstruction to urine flow and thereby impair bladder emptying. This mechanical effect also inhibits bladder by stimulation of detrusor stretch receptors which may cause vesicoureteral reflux (VUR) and UTI. Voiding dysfunction such as urinary incontinence and urinary retention are significantly increased in children with constipation (Halder *et al* ., 2021).

2.3.8. Water intake

Water is fundamental to existence and has numerous roles in the human body. It constitutes 75% of body weight in infants and 50% of body weight in adults (Nakamura *et al* ., 2020).

An important modifiable determinant of UTI recurrence is water intake, as increased hydration may be beneficial in the dilution and flushing of bacteriuria. A 12-month open-label randomized controlled trial found that increasing habitual fluid intake by consuming an additional 1.5 L water per day was effective in reducing the risk of recurrence of a UTI by 48% among women with a low baseline fluid intake (Zemdegs *et al* ., 2023).

Increased temperatures during summer can make people susceptible to dehydration (Alrashid *et al* ., 2022). By increases fluid loss from perspiration, thus decreasing extracellular fluid and decreased urine output, which decreases the mechanical clearance of bacteria from the urinary tract, and concentrates bacteria in urine (Simmering *et al* ., 2021).

2.4. Causative agent of UTI

The predominant organism causing both complicated and uncomplicated UTI is uropathogenic *Escherichia coli* followed by *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus mirabilis*, and group B *Streptococcus* (GBS), *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and numerous other pathogenes as *Candida spp*. Further, multi-drug resistant *E. coli* and *K. pneumoniae* are increasingly recognized to cause both CA-UTIs and hospital-acquired UTIs (Islam et al ., 2022). In addition to bacteria and fungi, the parasite Schistosoma haematobium causes considerable urogenital disease, mostly in sub-Saharan Africa, where snails harbouring this parasite are endemic (Lacerda Mariano and Ingersoll , 2020).

2.4.1. Bacterial Causes

In 1884 Hans Christian Gram developed a method to distinguish between Gram-positive and Gram-negative bacteria by using a crystal violet-iodine complex and a safranin counter stain. Gram- positive bacteria stained violet or purple and Gram-negative bacteria don’t retain the complex stain and counter stain with safranin to give a pink color. This difference is due to the composition or the morphology of the cell wall in each bacterial type (Breijyeh *et al* ., 2020) .

2.4.1.1. Gram positive bacteria

Gram-positive facultative anaerobic cocci include several phenotypically heterogenous genera from the Firmicutes phylum: *Staphycoccus spp*. are members of the *Bacillales* order, while *Streptococcus* (Group A, B, C and G *streptococci*, based on Lancefeld classification) and *Enterococcus spp*. (Group D *streptococci*) are members of the *Lactobacillales* order. *S. aureus* and coagulase-negative *staphylococci* (CoNS) were previously considered to be uncommon etiological agents in ascending UTIs in outpatients, however, they may have a more pronounced role in hospitalized, immunocompromised patients. The isolation of *S. aureus* from urine may also be an indicator of a more severe condition (e.g., bacteremia or endocarditis), where the microorganisms reach the kidneys through hematogenous dissemination (Gajdács *et al* ., 2020).

The prevalence of Gram-positive cocci in UTIs ranges between 2–15% in outpatients and 5–25% in inpatient samples; the occurrence of S. saprophyticus is overwhelmingly seen in young, sexually-active females, while over time, the epidemiology shift towards enterococci. Other–

although much more rarely occurring (<0.1%)–urinary pathogens include strict anaerobic bacteria (e.g., *Actinotignum schaali*, *A. urinale*, *Lactobacillus delbrueckii*), *Aerococcus spp*. (e.g., *A. urinae*), *Mycobacterium spp*., *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, and *Trichomonas vaginalis* (Behzadi *et al* ., 2021).

2.4.1.٢. Gram negative bateria / *Escherichia coli*

Escherichia coli is named after its discoverer Theodor Escherich, a German microbiologist, who in 1885 reported its isolation from infant stool and named it Bacterium coli commune. In 1945, John Bray in London described cases of “summer diarrhea” in infants associated with *E. coli*, which, at the time, was called Bact. coli neapolitanum. This was the first time *E. coli* was epidemiologically linked to a human illness (Riley , 2020).

The genus *Escherichia* comprises six validly published species, namely, *Escherichia albertii, Escherichia coli, Escherichia fergusonii, Escherichia hermannii, Escherichia marmotae,* and *Escherichia ruysiae* (Dahal *et al* ., 2023). *Escherichia.coli* is a Gram–negative bacterium belonging to Enterobacteriaceae, catalase positive, oxidase negative, non-spore forming and facultative anaerobic, positive testes for indole, methyl red and produce acid and gas from glucose, mannitol, maltose, lactose ,and sucrose while it gives negative test for Vogues–Proskauer, urease, H2S production, phenylalanine deaminase test and gelatin liquefaction, and it is classified as part of the *Enterobacteriaceae* family of gamma-proteobacteria (Sabah and Hassan , 2022).

The *E. coli* contains surface antigens, O (lipopolysaccharide), H (flagellum), and in some cases K (capsule), contribute to serological typing. Variation in the structure of the O antigen makes O-serotyping an important component in the typing of *E. coli* strains in taxonomic and epidemiology fields and has made it an essential tool in prevalence and surveillance research (Mohammed *et al* ., 2022) .

2.4.1.3. Classification of *E. coli* in the *Enterobacteriaceae* family of bacteria, and as follows (Ahmed , 2021) :-

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacterales

Family: *Enterobacteriaceae*

Genus: Escherichia

Species: coli

The pathogenic *E. coli* can be classified as either intestinal pathogenic *E. coli* (IPEC) or extraintestinal pathogenic *E. coli* (ExPEC). The IPECs are major diarrhoeagenic pathogens that cause gastroenteritis with six intestinal pathotype subgroups (Bong *et al* ., 2022).

(1) Enteropathogenic *E. coli* (EPEC), which causes diarrhea in children and animals; (2) Enterohemorrhagic *E. coli* (EHEC), responsible for hemorrhagic colitis and hemolyticuremic syndrome; (3) Enterotoxigenic *E. coli* (ETEC), the main cause of traveler’s diarrhea and porcine and bovine diarrhea; (4*) Enteroaggregative E. coli* (EAEC), that can cause persistent diarrhea in humans, and (5) Diffusely adherent *E. coli* (DAEC), a subclass of *Enteroaggregative E. coli* which causes diarrhea in children; (6) Enteroinvasive *E. coli* (EIEC), which causes watery diarrhea and

dysentery. EIEC and EAEC strains were found only in humans and not in animals (Sora *et al* ., 2021).

The EXPEC is mainly Uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli (NMEC)* and *sepsis-associated E. coli* (Kaur *et al* ., 2022). ExPEC strains have a greater ability to cause infections outside the intestine, where they can adhere and form biofilms on the surface of host tissues and medical devices, allowing them to persist and evade the host immune system and antimicrobial treatments. The ability of ExPEC strains to cause infections is often associated with their expression of virulence factors (VFs) such as fimbriae, toxins, and siderophores, which can also contribute to their multidrug resistance (Montes-Robledo *et al* ., 2023) .

Uropathogenic *E. coli* as described belongs to the extraintestinal pathogenic *E. coli* group and is associated with a subset of serogroups and serotypes (O1:H4, O1:H6, O1:H7, O1:H− , O2:H1, O2:H4, O4:H5, O6:H1, O7:H4, O7:H6, O7:H− , O18ac:H7, O18ac:H− , O22:H1, O25:H1, O75:H5 & O75:H7) and with the B2 or D phylogenetic groups (Ballesteros-Monrreal *et al* ., 2023) .

2.4.2. Fungus

Fungal urinary tract infection usually means urinary tract infection (UTI) caused by *Candida* species with special attention to *Candida albicans* as the major fungal infectious agent (Gharanfoli et al ., 2019). Fungi are pathogenic agents that can also cause disseminated infections involving the kidneys. Besides *Candida*, other agents, such as *Cryptococcus spp*., can cause UTIs. Non-yeast fungi, like some members of the *Aspergillus*, *Mucorales*, *Blastomyces*, and *Histoplasma families*, can also cause infections, especially among immunocompromised patients (Poloni et al ., 2020).

Candida spp. can be colonized in the lower or upper urinary tract system and create pyelonephritis, cystitis, urethritis and prostatitis in men. They might even appear in the upper urinary tract from the bloodstream or raise the urinary tract from a focus of Candida colonization at near the urethra (Ismail *et al* ., 2020).

The *Candida.* is the only fungus among the nine major uropathogens and the bladder executes different mechanisms of immune responses to *Candida*. from those to bacteria (Li *et al* ., 2022).

2.4.3. Viruses

Patients with UTIs are usually infected with bacteria. Viral UTIs are less common, but they still occur, affecting immunocompromised patients after organ transplantation and causing hemorrhagic cystitis, prostatitis, seminal vesiculitis, and urethritis. The most common viral pathogens known to cause UTIs are adenovirus, human polyomavirus (BK virus), cytomegalovirus and herpes simplex virus type-1 and type-2 (Park *et al* ., 2021). Evidence has recently emerged that the lower urinary tract is a potential target for SARS-CoV-2 infection, due to a significant ACE2 expression in urothelial cells. ACE2 has also been found in the human testis where it regulates the physiology of Leydig cells, Sertoli cell, and spermatogonia (Creta *et al* ., 2021).

2.5. Virulence factors

Uropathogenic *E. coli* (UPEC) strains produce virulence factors encoded in pathogenicity islands (PAI), plasmids, and transposons that allow them to colonize the urinary tract and persist there despite the action of the host immune system. These are divided into (1) cell surface-associated virulence factors, such as adhesins and invasins, and (2) secreted virulence factors, such as toxins and siderophore systems (Fonseca-Martínez *et al* ., 2023). However, there is no general agreement regarding the definitive discriminatory virulence factors within this pathotype. UPEC isolates need VFs for colonizing or invading host cells, escaping or disrupting hosts’ immune systems, damaging host tissues, and/or stimulating inflammatory responses. Among variety of VFs, some are generally accepted to be more associated with UPEC (Rezatofighi *et al* ., 2021). include adhesins, toxins, siderophores, polysaccharide-based protective coatings, inosines, and serum resistance-related proteins. The genes expressing virulence factors are located on bacterial chromosomes, plasmids, and even bacteriophages and can be transferred horizontally or vertically between bacteria (Allami *et al* ., 2022).

Moreover, fimbriae, iron-regulated gene homologue adhesin Iha , ferric aerobactin receptor IutA , cytotoxic necrotizing factor 1 (Cnf1), hemolysin (HlyA), uropathogenic specific protein (Usp), and outer membrane protease T (OmpT), are also shown as important virulence factors of *E. coli* in murine uropathogenesis. Carriage of these urovirulence factors is thought to enhance UPEC pathogenicity and is used to measure and categorize clinical UPEC strains isolated from different patient populations (Lin *et al* ., 2021).

2.5.1. Adhesin

Uropathogenic *E. coli* (UPEC) can adhere to the urinary tract epithelial cells via multiple receptors, for example, the mannosylated residues of uroplakine-1a and α3β1 integrins which are present in uroepithelial cells facing the lumen, which are recognized by UPEC’s fimbrial adhesin FimH, promoting colonization, invasion, and internalization, forming intracellular bacterial communities (IBCs), which are responsible for persistent infections, immune system evasion, and poor response to treatment (Mendez-Pfeiffer *et al* ., 2024) .

Adhesins can be distinguished according to their molecular structure in adhesive appendages and adhesive molecules. Adhesive appendages are multimeric complexes formed by different proteins, resulting in visible bacterial organelle, such as pili or fimbriae (Sarshar *et al* ., 2020). Adherence plays an important role in the pathogenesis of UPEC by increasing bacterial adhesion, colonization, and facilitating bacterial interactions between this microorganism and the host cell matrix, and consequently biofilm formation. The common adhesions in UPECs are type 1 fimbriae, P fimbriae, S fimbriae, F1C fimbriae, Dr. adhesins, and afimbrial adhesins (Mirzahosseini *et al* ., 2023) .

2.5.2. Siderophores

Iron is an indispensable cofactor that is necessary for many biochemical processes of almost all microorganisms, plants and animals. Its assimilation and homeostasis are critical for survivability and efficient growth of microbes. to scavenge, uptake and control iron levels, microorganisms have developed various refined strategies. Most commonly, under iron scarcity they secrete organic compounds, named siderophores (Mular *et al* ., 2024).

Siderophores (‘sidirou foreas’ in Greek: iron carrier) (Swayambhu *et al* ., 2021) are low molecular weight compounds with the ability to chelate and solubilize Fe3+. They are produced by most bacteria in order to facilitate the microbial acquisition of iron for their growth due to the limited concentrations of free iron (de la Fuente *et al* ., 2020).

Microorganisms which are unable to produce siderophore use siderophore which are produced by other microorganisms, called xenosiderophores. (Ghosh *et al* ., 2020).

2.5.3. Toxins

Results from the past decades have highlighted the possibility that the biological function of bacterial toxins are key virulence factors in bacterial pathogenesis, may not exclusively be to cause damage to the host tissue, allowing bacteria to colonize, invade, and spread. Bacterial toxins also have a more subtle ability to weak the host's immune response towards a delicate balance, which prevents tissue damage and immunopathology. Concomitantly, this may limit pathogen clearance, allowing the establishment of a niche for successful colonization, which may lead to persistent infections (Lopez Chiloeches *et al* ., 2021).

2.5.3.1. Hemolysin A

Hemolysin A (HlyA), named after its ability to lyse, for example, erythrocytes. Hemolysin is a member of the family of repeat in toxins (RTX) toxins (Beer *et al* ., 2022). HlyA of UPEC is cytotoxic to a wide range of cells and causes serious tissue damage during UTIs. HlyA is reported to induce kidney inflammation and injury (Wang *et al* ., 2020).

2.5.3.2. Cytotoxic necrotizing factor 1 (CNF1)

Cytotoxic necrotizing factor 1 (CNF1) is a bacterial virulence factor associated with some pathogenic *Escherichia coli* strains causing urinary tract infection and meningitis. It belongs to the cytotoxic necrotizing factors family that includes proteins from *E. coli* (CNF1, CNF2, and CNF3) and *Yersinia pseudotuberculosis* (CNFY) (Carlini et al ., 2021).

CNF1 is a key UPEC toxin, promoted migration and invasion of prostate cancer cells and prostate cancer metastasis by activating the Cdc42-PAK1 Axis (Guo *et al* ., 2020).

2.5.3.3. Serine protease autotransporters (SPATEs)

SPATEs are secreted by all recognized *Escherichia coli* pathotypes; agents of enteric/diarrheal disease including (EPEC), (EHEC), (STEC), (EIEC), (DAEC), (AIEC). SPATEs are also produced by extraintestinal *E. coli* pathogens (ExPEC), such as uropathogenic *E. coli* (UPEC) and septicemic *E. coli*, which are responsible for urinary tract infections (UTIs). SPATEs have been divided into class-1 and class-2 based on structural differences and biological effects (Navarro-Garcia , 2023).

Numerous serine protease autotransporters of enterobacteria (SPATEs) from class-2 have been identified in pathogenic *E. coli* to degrade a variety of mucins, thus play critical roles during the bacterial infection (Pan *et al* ., 2023).

2.5.4. Uropathogenic specific protein (Usp)

The *E. coli* uropathogenic specific protein (Usp) is a genotoxin active against mammalian cells and is associated with strains that provoke prostatitis, pyelonephritis and bacteraemia. Genotoxins have been shown to provoke carcinogenesis by promoting DNA damage in host cells (Rihtar *et al* ., 2020). Additionally, due to its ability to endow non-pathogenic *E. coli* with infectious capability, Usp is thought to be a crucial factor for UPEC infection (Khalaf and Flayyih , 2024).

2.5.5. Biofilm

Biofilms are the predominant lifestyle microorganisms and an example of a successful physiological adaptation, as they thrive in most natural environments as well as in harsh conditions. They are also often associated with many pathogenic forms of human diseases and can negatively impact health (Pinto *et al* ., 2021). Biofilm is defined as microorganisms bound to a surface of each other with the existence of an extracellular matrix composed of secreted elements of the organisms and/or of components of the microorganisms themselves (Ramadan *et al* ., 2021). Biofilms are communities of microorganisms and their microbial products assisting bacteria to attach to uroepithelial cells (Zhao *et al* ., 2020).

During a biofilm infection, simultaneous activation of both innate and acquired host immune responses may occur; neither of which are able to eliminate the biofilm pathogen, but instead accelerate collateral tissue damage. Consequently, biofilm-related diseases are typically persistent infections that develop slowly, are rarely resolved by the immune system, and respond inconsistently to antimicrobial treatments (Vestby *et al* ., 2020).

Biofilm Formation of *E. coli* is a well-characterized bacterium that plays an essential role in the human microbiome. However, some strains can become pathogenic and cause infections not only in the intestinal tract but also in other parts of the human body where they could form a biofilm. Biofilm formation in *E. coli* is a complex developmental process that occurs in different phases: reversible and irreversible attachment, maturation, and dispersion (Ballén *et al* ., 2022).

1- The attachment phase

The attachment phase involving the structural support is called a reversible attachment. The binding is reversible as bacteria are only poorly bound to a surface and are able to leave the surface at this stage. The bacteria leaving a surface return to their planktonic lifestyle. For bacteria that stays on the surface, they undergo irreversible attachment (Samrot *et al* ., 2021).

In this stage, planktonic bacteria approach and attach to the surface by means of surface appendages such as flagella and pili (Fu *et al* ., 2021). This adhesion is mediated by properties of the cell surface, i.e. the cell wall, the ultimate interface between a cell and its environment, and which gives the cell its shape and protects it from external stresses (Beaussart *et al* ., 2020).

The mechanical cues experienced during surface sensing cause molecular alterations at the biochemical level (mechanotransduction), which gradually increase the attachment strength of the loosely adhered bacterial cells. The intracellular signalling driven by mechano-transduction contributes to the production/expression of more adhesins to reinforce adhesion (Guzman-Soto *et al* ., 2021).

2- Irreversible attachment

During this stage, planktonic microorganisms can stick to each other or different species of surface-bound organisms, forming aggregates on the substratum and the adhesion becomes irreversible in the absence of physical or chemical intervention, thus the bacterial cells become attached firmly to the surface (Alotaibi and Bukhari , 2021). As soon as the bacterial settle on the surface successfully, the bacteria begin to multiply and excrete all kinds of extracellular substances to envelop themselves, which marks the beginning of irreversible adhesion and opens the portal to biofilm formation (Ma *et al* ., 2022).

3- Proliferation: The multilayered bacterial cells are profoundly accumulated, and the enormous amounts of EPS are produced.

4- Maturation: The attached multilayered bacterial cells grown into the matured biofilm with the typical 3D biofilm structure (Srinivasan *et al* ., 2021).

5- The dispersion stage: The biofilm starts shedding daughter planktonic cells that seed adjacent sites for the formation of new biofilms **Figure (2.1)** (Lila *et al* ., 2023).

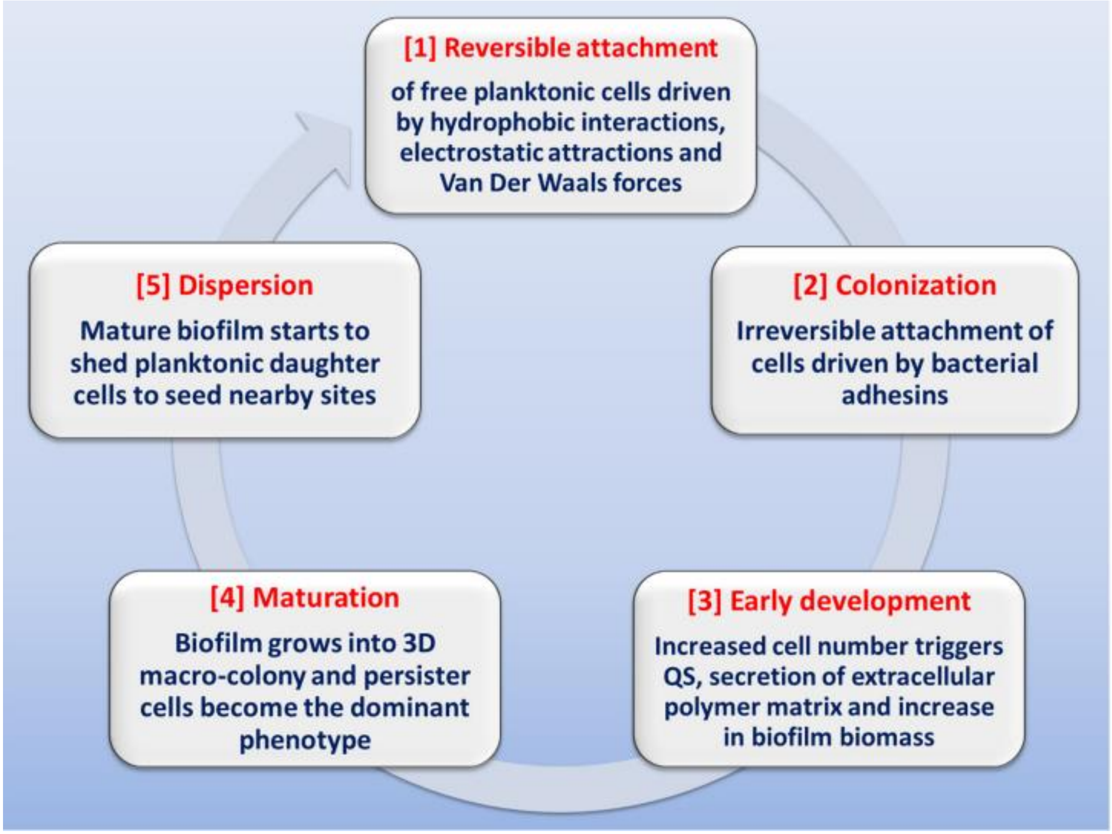


Figure (2.1) Schematic representation of the stages involved in biofilm formation.

2.5.6. Antimicrobial resistance (AMR)

There is an increasing tendency of antimicrobial resistance in urinary pathogens. In the past, these resistant microorganisms were mainly detected in comorbidities such as diabetes mellitus, reflux nephropathy or nosocomial infections. However, they are found in an important part of community-acquired UTIs (CA-UTIs) in nowadays. This trend is not limited to any country but a global problem and has been reported to be seen in all groups (Guclu *et al* ., 2021).

The *E. coli* typically acquires AMR genes through mobile genetic elements (MGE), such as plasmids, insertion sequences, transposons, and gene cassettes/integrons. a large number of resistance-encoding mobile elements, in particular plasmids, are shared between different members of the *Enterobacteriaceae* and thus further promote the spread of resistance genes. MGE can also encode for virulence factors, and there may be interplay between virulence and antimicrobial resistance (Bunduki *et al* ., 2021).

2.6. Immune response

Under normal circumstances, the Urinary Tract is resistant to long-term colonization and growth of microorganisms. This resistance emanates from various physiological processes, one of which is emptying out urine that flushes out harmful microbes that temporarily colonize urine in the bladder. Additionally, innate immunity such as cytokines, chemokines, secretory immunoglobulin, mucous production, prostatic secretion, barrier formation and high concentration of urea prevent persistent microbial colonization and infection of the UT (Yenehun Worku *et al* ., 2021).

The Epithelial cells lining the bladder and upper urinary tracts play key roles in defensive mechanisms against (UTIs). In addition to serving as physical barrier, urothelial cells (UCs) act as first responders upon recognition of several virulence factors, such as hemolysin and cytotoxic necrotizing factor, the siderophore aerobactin, bacterial capsules and lipopolysaccharide (LPS) (Alpizar *et al* ., 2020).

On the other hand, Lactobacillus in the vagina may prevent colonization by potential pathogens such as *E. coli*. Indeed, women with low levels of lactobacilli more commonly carry vaginal *E. coli* than do those with lactobacilli-dominated microbiomes (Lewis and Gilbert , 2020).

Attachment of bacteria to superficial bladder epithelial cells triggers an innate immune response mainly by signalling through Toll-like receptor 4 (TLR4), a member of the Toll/IL-1 receptor (TIR) domain super family. These activated uroepithelial cells secrete cytokines and chemokines, such as IL-6, IL-8 and antimicrobial peptides (Wagenlehner *et al* ., 2020).

The first step in initiating the host's response to the pathogen is the activation of innate immune cells (Guliciuc *et al* ., 2021). In general, the innate immune system is composed of (1) pattern recognition receptors like toll-like receptors (TLR); (2) plasma proteins, chemokines, and cytokines; (3) cellular components like epithelial cells, bone marrow-derived phagocytes, dendritic cells, and natural killer cells; (4) toxic molecules such as reactive oxygen and reactive nitrogen intermediates; and (5) antimicrobial peptides (AMPs) (Ching et al ., 2020).

Innate immunity is activated by pattern recognition receptors (PRRs). PRRs recognize pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) that are common to pathogens (Hirooka and Nozaki , 2021).

The local host inflammatory response in the urothelium is characterized by infiltration and transurothelial migration of neutrophils. Recent studies indicated that macrophages and urothelial cells recruit neutrophils into the infected urothelium by producing chemokines (Bottek *et al* ., 2020). Neutrophils represent the chief innate cellular responder to the infected urinary tract, recruited there by a rapid and robust burst of proinflammatory cytokines such as CXCL1 (KC in mice), IL-6, TFNα, IFNγ, and IL-1β (Kuhn et al ., 2023). Once at the infected site, neutrophils engage pathogenic bacteria through several parallel processes: phagocytosis (internalization and intracellular killing), degranulation (extracellular discharge of granules containing antibacterial compounds), and release of neutrophil extracellular traps (NETs) (Rosales , 2018).

Macrophages have important roles during UTIs, influencing host colonization, pathology, and disease outcomes. macrophages play multifaceted roles in both host defense and colonization during UTIs. These cells are sentinels, recognizing invading uropathogens through families of pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), C-type lectin receptors and inflammasome-forming nod-like receptors (NLRs), that detect both pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) (Wang *et al* ., 2022).

While Adaptive immunity is activated by infection when antigens from infecting bacteria reach antigen-presenting cells and activate local or distant lymphocyte populations. Plasma cells producing specific antibodies have been identified in the kidneys and bladder, especially in mice with a deficient neutrophil response. Infected patients secrete secretory IgA (sIgA) antibodies into the urine, which inhibit bacterial adherence. Circulating antibodies are detected in patients, with acute pyelonephritis and plasma cells detected in infected kidneys of susceptible mice. Lymphoid aggregates might form in chronically infected individuals and specific T cell populations regulate the efficiency of the host defence by affecting the crosstalk between innate and adaptive immunity (Butler *et al* ., 2022).

The adaptive immune system is capable of recognizing almost any antigenic structure; however, because antigen receptors are generated at random, they bind to antigens of any origin - bacterial, environmental, or self. In contrast, innate immune receptors are specific for structures found only in microbial pathogens (pathogen-associated molecular patterns), which is why they function to signal the presence of infection. The signals produced by the innate immune system, in turn, control the activation of adaptive immune responses; the adaptive immune system responds to a pathogen only after the innate immune system has recognized it (Khadim , 2023).

2.7. Avoidance of UTI

There are a few estimates that can be taken to lessen the danger of building up an UTI (Riaz , 2020) :

- Drink heaps of water and pee as often as possible.
- Avoid liquids, for example, liquor and caffeine that can disturb the bladder.
- Urinate soon after sex.
- Wipe from front to back subsequent to peeing and solid discharge.
- Keep the genital territory clean.
- Showers are liked to showers and abstain from utilizing oils.
- Avoid utilizing any perfumed items in the genital territory.
- Wear cotton clothing and baggy apparel to keep the region around the urethra dry.

2.8. Overview of antibiotic resistance genes

1- Par E

Most bacteria possess two type IIA topoisomerases, DNA gyrase and topo IV, that together help manage chromosome integrity and topology (Corbett *et al* ., 2005). Bacterial type II Topoisomerases Gyrase was discovered in 1976 and was the first type II topoisomerase to be described (Collins and Osheroff , 2024). In 1990, Kato *et al*. discovered a homolog of gyrase that they called topoisomerase IV (Kato *et al* ., 1990).

Topo IV is a bacterial type IIA enzyme that uses the hydrolysis of ATP to decatenate replication products, relax positive and negative (although less efficiently) supercoils, and knot and unknot DNA. However, topo IV does show some structural differences from DNA gyrase; unlike gyrase, it is unable to introduce negative supercoils into DNA. Topo IV is also around 100 times more active at decatenation in vivo in E. coli cells than is DNA gyrase (Bush *et al* ., 2015). In addition, they both act by creating a transient double-stranded break in one segment of DNA (the gate- or G-segment) and passing a second intact segment (the transport- or T-segment) through the break. In order to maintain the integrity of the genome during this process, gyrase and topoisomerase IV covalently attach to the 5 -terminus of each DNA strand. This ‘cleavage complex’ is a hallmark of enzyme activity (Ashley *et al* ., 2017).

Topoisomerase IV, which is highly homologous to gyrase, consists of two ParC subunits and two ParE subunits, encoded by the genes parC and parE (Lindbäck *et al* ., 2002). it was long known that ParC and ParE both were required for proper chromosome segregation in *Escherichia coli* (Anderson *et al* ., 1998).

Conditional mutations in the parC and parE genes cause a failure of proper nucleoid segregation, and of the decatenation of newly replicated small plasmids (Springer and Schimd , 1993).

The bacterial type II topoisomerases, (topoisomerase IV) is the target for the fluoroquinolone class of antibacterials . Members of this class include drugs such as ciprofloxacin, moxifloxacin, and levofloxacin (Collins and Osheroff , 2024).

2- Quinolone Resistance (QNR)

The first plasmid–mediated resistance gene in quinolones; qnrA1 was reported in 1998 from a clinical strain of *Klebsiella pneumoniae* and ever since then, more plasmids-transferable resistance mechanisms to quinolones have been identified (Adekanmbi *et al* ., 2022).

Subsequently, several classes of qnr genes (qnrB, qnrC, qnrD, qnrS and qnrVC) were identified that reduce susceptibility to fluoroquinolones. Qnr proteins bind to DNA gyrase and topoisomerase IV and protect the enzymes from inhibition by quinolones.

Qnr homologues exist on the chromosomes of many bacterial species but their spread into Gram-negative pathogens occurred by carriage on multidrug resistance plasmids (Garoff *et al* ., 2018).

QnrB-like determinants were identified in *Citrobacter koseri, Escherichia coli, Enterobacter cloacae,* and *Klebsiella pneumoniae* from the United States and India (Poirel *et al* ., 2006).

The first time that qnrS was identified in *P. rettgeri, P. guangdongensis* and in environmental *M. morganii***.** *P. guangdongensis* was described as a novel species by Yang et al. (2013) (Paiva *et al* ., 2017).

The Qnr proteins belong to the pentapeptide-repeat protein family and confer quinolone resistance by physically protecting DNA gyrase and topoisomerase IV from quinolone inhibition. A second mechanism for PMQR was later discovered in 2006 and consisted of a bifunctional variant of an aminoglycoside-modifying acetyltransferase, AAC(6')- Ib-cr, which harboured two specific amino acid substitutions, Trp102Arg and Asp179Tyr. This variant is able to acetylate the unsubstituted nitrogen of the C7 piperazine ring that is found in quinolones such as ciprofloxacin, thereby conferring quinolone resistance by decreasing the drugs activity (Correia *et al* ., 2017). The final mechanism is enhanced efflux pump activity mediated by quinolone efflux pump (qepA) and oqxAB associated with reduced susceptibility to fluoroquinolone and increased MICs (Kariuki *et al* ., 2023).

2.9. Overview of virulence genes

1- csgD

When *Escherichia coli* cells switch their growth mode from single planktonic cell growth to biofilm mode (Ogasawara *et al* ., 2011), Bacteria use a variety of extracellular fibres to mediate interactions with other cells and with their environment. Assembly of these fibres is complex and often includes chaperon proteins and outer membrane usher-like proteins that are dedicated to the secretion and proper localization of the thin (6–8 nm), highly aggregated surface fibre subunit proteins represented by Curli (Robinson *et al* ., 2006).

CsgD is a transcription factor belonging to FixJ/LuxR/UhpA family and is a master regulator of biofilm. CsgD transcriptionally activates csgBAC and csgDEFG operons for curli production. Besides, CsgD is involved in several cellular activities like it regulates several genes critical to biofilm formation, for example, cellulose biosynthesis genes, cell-surface structures, and stress response functions. (Ogasawara *et al* ., 2010) (Ogasawara *et al* ., 2020) (Azam and Khan , 2022) .

The csgD gene is expressed from a promoter that is divergent from the csgA promoter, The activated expression of the csgA gene results in the formation of curli fibers on the bacterial cell surface that form the major structural component of the biofilm, Curli fiber formation is induced by specific growth conditions, including temperature at 20◦C–25◦C, low osmolarity, and stationary phase (Kao *et al* ., 2023).

Curli are extracellular proteinaceous fibers made by Gram-negative bacteria. Curli-specific genes (csg) are primarily found in Proteobacteria and Bacteroidetes. The main function of curli fibers is associated with a sedimentary lifestyle and multicellular behavior in biofilms, as they form scaffolds that provide adhesive and structural support to the community. In certain pathogenic bacteria, curli have also been implicated in host colonization, innate response activation, and cell invasion (Bhoite *et al* ., 2019).

In previous study Changes in the csgD (agfD) promoter region can influence curli formation. Some isolates of the enteroinvasive *E. coli* O157 : H7 show a phase shift between curliated and non curliated cells that results from a single base pair change in the csgD promoter . A change in the agfD promoter in Salty enables the cells to make curli at 37° C (Chirwa and Herrington , 2003).

Indirect evidence that csgD not only controls the csgBA operon, but also other genes, comes from the observations that csgD overexpression induces activation of the glyA gene and that mutations in the csgD gene affect nutritional requirements and ability to grow on different carbon sources in environmental isolates of *E. coli* (Brombacher *et al* ., 2003).

2- OmpT

Gram-negative bacteria are a major class of organisms that have two cell membranes, a cytoplasmic or inner membrane (IM) and an outer membrane (OM). Proteins that are integrated into the OM mediate a variety of important physiological functions, including nutrient uptake, membrane homeostasis, and virulence (Wang *et al* ., 2021) .

The omptin family of outer membrane proteases, most commonly represented by outer membrane protease T (OmpT) of *Escherichia coli* targets the host immune response components and elicits varying pathogenesis (Kum *et al* ., 2021). OmpT is a proteolytic enzyme on the outer membrane of *E. coli* and acts as the key virulence factor of *E. coli* degrading host immune responsibility-related proteins, cell matrix proteins, and interfering with host hemostatic function.

OmpT is known to cleave short peptides preferentially at dibasic sites [such as -R (arginine) -R-, -K (lysine) -K-, -K-R-, and -R-K-]. Its pyrolysis efficiency was comparable to that of water soluble proteases such as chymotrypsin (Zhang *et al* ., 2021).

the proteolytic activity of OmpT mediates resistance against antimicrobial peptides such as protamine and cathelicidin LL-37 , and it is involved in the biogenesis of outer membrane vesicles (OMVs) . In addition, some studies have suggested that OmpT participates in the adhesion of ExPEC and APEC strains.

The OmpT protein identified as an antigen of Shiga toxin-producing *Escherichia coli* (STEC) (Torres *et al* ., 2021). OmpT owes its name to the fact that its expression is temperature dependent (Zhang and Baaden , 2023).

OmpT is a 33.5 kDa membrane protein that adopts a β-barrel structure consisting of 10 antiparallel β-strands spanning the outer membrane with an active site facing the extracellular environment. Previous work reports that OmpT requires lipopolysaccharide (LPS) as a co-factor for its proteolytic function. Once in the outer membrane, OmpT becomes active, most likely when it binds to the LPS that is found only in the outer leaflet of Gram-negative bacteria. An inactive OmpT during its transport from the cytosol to the outer membrane possibly serves as a safety mechanism evolved to prevent any accidental proteolysis of cytosolic proteins (Sinsinbar *et al* ., 2020).

Chapter three

materials and methods

3.1 Materials Used in the Study

3.1.1 Instruments and Apparatuses

The instruments and apparatuses used in this study are mentioned in **table (3.1)**

Table (3.1) Instruments and apparatuses and their corresponding manufacturers.

No	**Instrument or apparatus name**	**Company and Country**
1	Autoclave	Hirayama, Hiclave , Japan
2	Biological Cabinet	Cryste ,Korea
3	Burner	Amal ,Turkey
4	Cotton Swab	Hwashin ,Korea
5	Deep Freezer	Nenwell ,Japan
6	Densichek Plus	Biomerieux, France
7	Eppendorf tube	Sigma ,UK
8	Incubator	Genex ,USA
9	Latex Gloves	China,china
10	Loop	China ,china
11	VITEK® 2 Compact system	Biomerieux ,France
12	1.5ml, 0.5ml and 0.2ml Tube	JET BIOFIL, Singapore
13	Centrifuge	Fisher Scientific, USA
14	Gel Imaging System	Major Science, Taiwan
15	Micro spin Centrifuge	My Fugene, China
16	Micropipette	Human, Germany
17	Microwave Oven	GOSONIC, China
18	OWL Electrophoresis System	Thermo, USA
19	Quantus Fluorometer	Promega, USA
20	Refrigerator	TEKA, Spain
21	Thermal Cycler	Thermo Fisher Scientific, USA
22	Vortex	Quality Lab System, England
23	Dry Heat Block	OHAUS, USA
24	Biological Cabinet	Cryste, Korea
25	Burner	Amal, Turkey
26	Petridish	China, China
27	Plain tube 10 ml	Biozek, Netherlands

3.1.2 Stains and Reagents

The Gram stain is a differential stain was utilized to observe the morphology and cellular structure of organisms (Ondari , 2020).

3.1.3. The Culture Media

The following culture media were used, as explained in **table (3.2).** All of the culture media used in the study were prepared according to the manufacturer’s instructions.

Table (3.2) Cultures used in the study and their corresponding manufacturers.

No	Culture media	Purpose	company	country
1	MacConkey agar	Is a selective and differential culture medium for bacteria designed to selectively isolate G-ve and enteric rods and differentiate them based on lactose fermentation **(Odds, 1981)**	Himedia	India
2	Eosin Methylene Blue media	Is used for the isolation and differentiation of G-ve enteric bacteria from clinical and nonclinical specimens **(Odds, 1981).**	Himedia	India
3	Brain-heart infusion broth	Used as long-term storage after the addition of 15mL of glycerol to every 85mL of the broth	Himedia	India
4	Blood base agar	Enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties **(Odds, 1981)**	Himedia	India
5	Nutrient broth	Is a general purpose medium which can be used for cultivation of a non-highly nutritious bacteria **(Odds, 1981)**	Himedia	India

3.1.4 Laboratory Kits

The kits used in the current study are mentioned in table (3.3).

No.	Kit	Components	Manufacturer(origin)
1	Gram stain	1. Crystal Violet 2. Iodine 3. Acetone 4. Sufranin	Himedia, India
2	DNA Extraction kit	١.ABIOpureTM Total DNA	ABIOpure, USA
3	PCR Component	1. Agarose, Ethidium Bromide Solution (10mg/ml),	Promega, USA

		GoTag Green Master Mix, Nuclease Free Water, TAE 40X, Quantifluor dsDNA System, 100bp DNA Ladder. 2.Absolute Ethanol 3. Primers	ROMIL pure chemistry, UK Macrogen, Korea
4	Vitek 2 kit	VITEK® 2 Compact system	Biomerieux ,France

3.1.5. The Antibiotics

The antibiotics which were used for determining the sensitivity of isolated bacteria are listed in table (3.4).

Table (3.4) Antibiotics and their related information.

Type	Class of antibiotic	Types of antibiotics	Symbol	Manufacture /origin
Antibiotic of VITEK® 2 Compact system AST card	B-lactams/β-lactamase inhibitor combinations	Amoxicillin-clavulanic acid	AMC	India Himedia
	Monobactam	Aztreonam	AT	
	Cefeme	Cefotaxime	CTX	
		Ceftazidime	CAZ	
		Ceftriaxone	CTR	
	Penicillins	Piperacillin	PIP	
		Piperacillin/Tazobactam	P/TZP	
		Ticarcillin	TIC	
		Ticarcillin/clavulanic acid	TEC	
	Cephalosporin	Cefepime	FEP	
		Ceftazidime	CAZ	
	Carbapenem	Imipenem	IP	
		Meropenem	MEM	
	Aminoglyside	Tobramycin	TM	

		Gentamicin	GEN	
		Amikacin	AK	
	Quinolones	Ciprofloxacin	CRO	
	Tetracycline	Minocycline	MIN	
	Sulfonamides	Trimethoprim/sulfamethoxazole	TMP/SMX	
	Polymyxin	Colistin	Co	

3.1.6 Primer Sequences

The primers mentioned in **table (3.5)** were used in the current study for detection of some virulence factors and antibiotic resistance.

Table (3.5) Primers used in the study.

No.	Primer Name	Sequence	Annealing Temp. (°C)	Product size (bp)	Reference
1	qnrB-F qnrB-R	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	58	469	(Robicsek *et al* ., 2006)
2	qnrS-F qnrS-R	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	58	417	(Robicsek *et al* ., 2006)
3	OmpT-F OmpT-R	ATCTAGCCGAAGAAGGAGGC CCCGGGTCATAGTGTTCATC	60	559	(Ahmed , 2021)
4	ParE-F ParE-R	TACCGAGCTGTTCCTTGTGG GGCAATGTGCAGACCATCAG	56.2	265	(Iranzad and Hakemi 2017)
5	CsgD-F CsgD-R	TTACCGCCTGAGATTATCGT ATGTTTAATGAAGTCCATAG	50	651	(Hamad *et al* ., 2024)

3.2. Methods

3.2.1. Subjects and Study design

This study is a cross-sectional study that included a total of (968) mid-stream urine samples collected from symptomatic patients of urinary tract infections of both sex male and female with age range (1-80 years) were inrolled the two primary governmental hospitals in holy Karbala province, namely Imam Al-Hussein Medical City and Kerbala Pediatric Teaching Hospital. During the period started from October 2023 to April 2024.

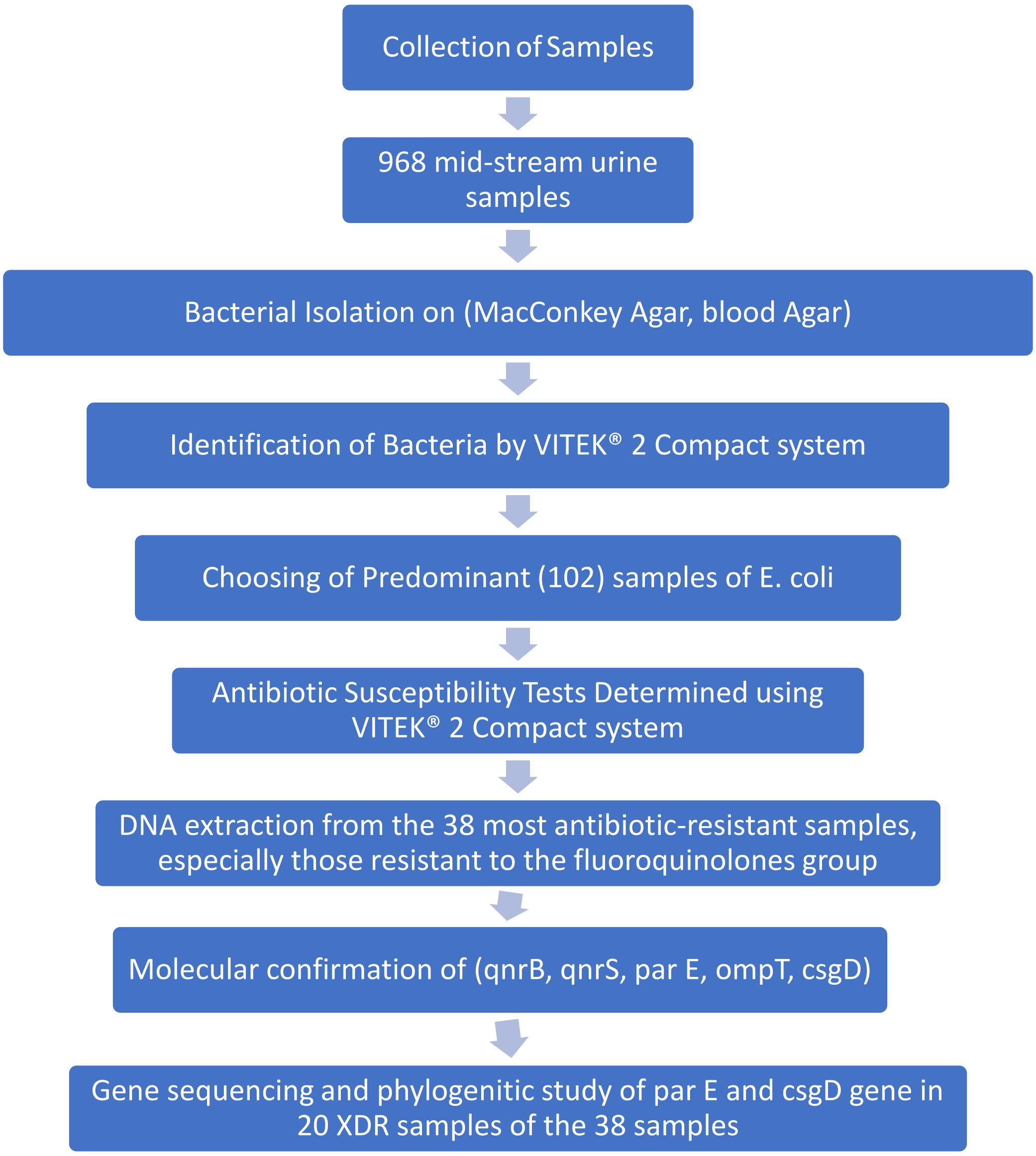


Figure (3.1) Study plane and diagnosis technique.

3.2.1.١ Inclusion criteria

Typical inclusion criteria for UTI patients included the following case history information include demographic, clinical and geographical characteristic such as age, sex and history of previous urinary tract infection (Appendix-1), samples from UTI patients were included in the study were as follow:

1) All symptomatic patients with urinary tract infections.

2) Both sexes male and female between 1-80 years.

3.2.1.٢ Exclusion criteria

1) Urine from patients under antibiotic therapy

2) Samples in non-sterile containers

3) Urine sample from catheter

4) Samples that are kept in ambient temperature for more than 30 minutes.

5) Urine sample from pregnant women.

3.2.1.٣ Ethical Considerations

The research received approval from the College of Applied Medical Science/University of Kerbala Ethical Committee and the Ethical Committee at Al-Imam Al-Hussein Medical City and Kerbala Pediatric Teaching Hospital. Prior to collecting the samples, all participants in this study were informed and verbally consented to participate.

3.2.2. Urine sample collection process

Mid-stream clean catch urine sample collection in sterile screw cup, used for general urine examination and culturing on blood and MacConkey agar.

3.2.3. Laboratory Diagnosis

3.2.3.1. Non-culture method

The second essential element for the diagnosis of UTIs is urine testing, including urine analysis or urinalysis (UA), which is a rapid diagnostic tool that provides significant clinical information about urine composition, and urine microbiological cultures. UA can be done via either dipstick testing or microscopic examination (Alateeq *et al* ., 2024).

1 . Urine dipstick method

In this method, multistrip is used, which is capable of detecting nitrite, a metabolic product of particular pathogens of the urinary tract, leucocyte esterase, protein, blood (as a sign of inflammation), erythrocytes, specific gravity (urine osmolality), nitrites, leukocyte esterase, glucose, ketones, bilirubin, and pH. If nitrite or leucocyte esterase is detected in the sample, it increases the possibility of having a UTI. However, dipstick method for blood and protein has poor sensitivity and specificity in the detection of UTI and may be misleading.

Standard urine test strips are also quite inexpensive; they can be used without any laboratory equipment or elaborative sample pretreatment, and they can be performed in the same way as point-of-care tests (Kaur and Kaur, 2021) (Pohanka and Zakova , 2024).

2 . Microscopically method

Firstly, centrifuge tubes were filled with 10 ml of mid-stream morning urine and then centrifuged for 5 min at 1500 rpm for manual microscopic examination. The supernatant was discarded until 0.5 mL of urine remained at the bottom of the tube, which contained a concentrated pellet of formed elements and was visible at the bottom of the tube. Then one drop of sediment was placed on a microscope slide, covered with a cover slip, and examined by a light compound microscope at 40X.

In a previous study microscopic urinalysis reveals that ≥10 white blood cells per cubic millimeter refer to pyuria. The best cutoff for detecting bacteriuria was discovered to be a urine white blood cell count of more than 25 cell/hpf, this refers to symptomatic UTI. While less than 25 cell/hpf, is considered asymptomatic UTI (IBRAHIM *et al* ., 2023).

3.2.3.2. Culturing method

All specimens were collected in aseptic containers, and a single loopful was promptly streaked over blood agar and MacConkey agar, followed by aerobic incubation for a duration of 18 hours at a temperature of 37 °C. A colony forming unit (CFU) count of ≥ 10s5 per ml was considered indicative of a substantial bacterial infection (Giuliano *et al*., 2019).

3.2.3.3 Isolation and Identification of the Bacterial Isolates

1- Characteristic of Cultural Isolation

The diagnosis of UTI mainly relies on standard urine culture, a method that involves plating urine onto agar plates containing 5% blood agar as well as MacConkey agar plates and incubating aerobically at 35°C for 24 h to obtain quantitative colony counts (Neugent *et al* ., 2020).

On Levine-EMBA selective media, Colonies were metallic green. This color change is caused by a reaction between *Escherichia coli* bacteria and Methylene blue. Agar Eosin Methylene Blue (EMB) was originally designed by Holt-Harris and Teague (1916) and subsequently modified by Levine (Sophian , 2022).

2- Preparation of culture media

The cultures; MacConkey agar, eosin methylene blue, nutrient agar, Brian-Heart Infusion broth, blood base agar was prepared each according to their manufacturer’s instructions. Sterilization was done using the autoclave at 121 °C and a pressure of 15 lbs, then poured into sterile petri dishes and tubes and incubated at 37 °C for 24 hours to make sure the media weren’t contaminated, then they were stored at 4 °C till needed.

1- Blood agar media

A liter of distilled water is used to suspend 39.5 g of the medium. For thorough dissolution of the medium, heat with regular agitation and boil for one minute, then autoclave as in (2.2.2.1). 5 to 7% blood agar can be made aseptically by mixing sterile agar medium with the required volume of fresh blood and cooling it to 40-45ºC. aseptically adding 5 ml of human blood for every 95 ml of the medium, mixing well to homogeneity, poured into sterile Petri-dishes. Finally cooled to 37ºC and left to solidify at room temperature (25ºC), then used for isolating bacteria and identifying the hemolysis caused by hemolysin-producing isolates and the type of hemolysis (Ibraheem *et al* ., 2022).

2- MacConkey agar media

MacConkey Agar is a type of agar used in laboratory settings. It is commonly used to differentiate between different types of bacteria based on their ability to ferment lactose. The agar contains specific indicators that change color depending on whether lactose fermentation has occurred. This allows researchers to analyze and identify different bacterial species. To prepare this medium, dissolve 40gm of agar in 1000 ml of D.W and sterilize it in an autoclave at 121C° for 20 minutes. Once cooled, the mixture was carefully poured onto the plates. These plates were specifically designed to selectively culture gram-negative bacteria (Haider , 2024).

3- Eosin methylene blue media

EMB was prepared by dissolving 36 g in 1000 ml of D.W, then autoclaved for 15 minutes at 121 degrees Celsius and distributed into plates after that The medium refrigerated at 4c until used, EMB medium used to distinguish *E. coli* from other members of the Enterobacteriaceae family (Ali , 2024).

4- Brain heart infusion broth-glycerol medium

This medium was made by autoclaving 15 mL glycerol with 85 mL brain heart infusion broth at 121°C for 20 minutes. It was utilized for long-term preservation of bacterial isolates as a stock (Ahmed , 2021).

3.2.3.4. Microscopic Identification

Gram stain

This investigation was used to segregate gram positive organisms from gram negative organisms. After bacterial culture smears on a clean slide. They were then placed on a staining rack; heat fixed then flooded with crystal violet and allowed to stand for 30 seconds. The slide was then rinsed with water for 5 seconds and then covered with iodine. They were allowed to stand for 1 minute and then rinsed with water. Decolourization was done using 95% ethanol for 15 seconds, followed by rinsing with water. Neutral red was then used as a counter stain. It was flooded for about 60 seconds and the slides rinsed with water and blot dried using a filter paper. Examination was done under a microscope at x100 under oil immersion (Ondari , 2020).

The organisms that when stained by gram stain become purple-brown under a microscope are named gram-positive organisms, the cell membrane of those organisms consists of higher peptidoglycan content, while the cell membrane of the gram-negative organisms consists of a higher lipid content and appear red or pink when examined under the microscope after staining by gram stain.

3.2.3.5. VITEK® 2 Compact System

The VITEK® 2 Compact system is used in confirming the diagnosis of isolates and sensitivity test for antibiotics that are used to diagnose most types through using kits which have specific diagnostic ability. Each sample needs two kits: one of them is used in diagnosis and the other is used for sensitivity test, the diagnosis kit contains 64 well, each well has dried substance and colored indicator. The VITEK® 2 Compact system is recording the color changing as a result of growth bacteria, the diagnosis kit of sensitivity test contains (18-20) antibiotics distributing on 64 well, so that each antibiotic has more than single concentration and the VITEK 2 system records the changing in turbidity after growth of bacteria.

The VITEK® 2 Compact system consists of an instrument and computer as described in **(Figure 3.٢)**, and an instrument that consists of 5 essential components:

A-Keypad.

B- Fill Door: where the transmission of the sample form tubes into the kit by transmission pipeline in the kit, this process continues for 70 seconds.
C- Load Door: cutting the transmission pipeline located on the kit and transfers it into the incubator; this process continues 3-5 minutes.

D- User Access door: where the incubation and measure changes; that occurs on kit due to growth of bacteria in order to get a result.

E- Waste Door: used to collect kits after finishing analysis and obtain the result (Alhadidi et al ., 2022).

Figure (3.٢) VITEK® 2 Compact system

The samples were performed according to the manufacturer's instructions (BioMerieux-France) as shown in the following steps

All the following steps were done according to the manufactures company (Biomerieux, France) **(**Naama , 2021**)**.

1- The suspension was prepared from a new culture at 24 hours at a temperature of 37˚C, then 3mL of physiological salt solution was placed in sterile tubes, then 3 colonies or less of bacteria were transferred to each tube.

2- The colonies were mixed well with the physiological saline solution inside the tube by the Auto Vortex device.

3- The turbidity of the suspension was standardized by Densichek at a rate of 0.5 - 0.65 with a McFarland standard solution.

4- Appropriate card was attached to the cassette.

5- the strip code of the card was read and matched with commentary card code.

6- Information has been recorded regarding the name and sequence of the sample.

7- The cassette was putted in the specified location of the device.

8- After the cards are filled out, the user records the cassette to the automatic reader incubator and the cards are checked automatically by the internal barcode reader, then plugged and loaded into the internal reader incubator. Then system automatically ejected the cards into a waste container.
9- The card was analyzed using the VITEC system after 4-8 hours, and the report was obtained, which is stored in the device's memory.

VITEK 2 GP and GN cards were used for bacterial identification. VITEK2 AST-N335 and AST-GN09 cards tested antimicrobial agents for aerobic Gram-negative bacilli were as follows: Meropenem, Imipenem, Amikacin, Piperacillin–tazobactam, Cefoperazone–sulbactam, Ampicillin–sulbactam, Nitrofurantoin, Cefoxitin, Tobramycin, Ceftazidime, Cefepime, Gentamicin, Ceftriaxone, Cefuroxime, Cefotaxime, Sulfamethoxazole–trimethoprim, levofloxacin, Ciprofloxacin, Cefozolin, and Ampicillin. VITEK 2 AST-P639 Card tested antimicrobial agents for Gram-positive cocci (*Staphylococcus* spp., *Enterococcus* spp., and *Streptococcus agalactiae*) were as follows: Linezolid, Vancomycin, Teicoplanin, Streptomycin, Gentamycin, Nitrofurantoin, Rifampicin, Moxifloxacin, Levofloxacin, Erythromycin, Ampicillin, Penicillin, Ciprofloxacin, and Clindamycin (Huang et al ., 2022).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing for *E. coli* isolates were conducted utilizing the VITEK® 2 Compact system, employing an AST (N222) card and following the identical identification technique as described before.

3.2.4. Preservation of Bacterial Isolates

1- Long-term Preservation

A media to preserve isolates for long-term was prepared by adding 15mL of glycerol to 85mL of brain-heart infusion broth which was then spread across multiple sterile tubes that were not affected by heat to then be sterilized by incubation. After being left to cool down to room temperature, the tubes were inoculated with colonies grown on nutrient agar and stored at 37 °C for 24 hours followed by storage at -20 °C, knowing that the isolates could survive for about 6 – 8 months (Ahmed, 2021).

3.2.5. Detection of Virulence and antibiotic resistance gene by Molecular Examination

3.2.5.1 DNA Extraction

Genomic DNA was isolated from bacterial growth according to the protocol of ABIOpure Extraction as the following steps:

- For pellet cells, 1ml of overnight culture for 2min at 13000 rpm. Supernatant was then discarded.
- For protein digestion and cell lysis, 20µl of Proteinase K solution (20 mg/ml) and 200µl of Buffer BL was added to sample then the tube was mixed vigorously using vortex and incubated at 56˚C for 30 min, for further lysis and it was incubated for 30min at 70oC.
- From absolute ethanol 200µl was added to the sample, pulse-vortex to mix the sample thoroughly.
- All of the mixtures were transferred to the mini column carefully, then centrifuged for 1 min at 6,000 x g above (>8,000 rpm), and the collection tube was replaced with a new one.

- From Buffer BW 500µl was Added to the mini column, then centrifuged for 1 min at 6,000 x g above (>8,000 rpm) and the collection tube was replaced with a new one.
- From Buffer TW 500µl was applied. Centrifuged for 1 min at 6,000 x g above (>8,000 rpm). The pass-through was discarded and the mini-column was reinserted back into the collection tube.
- The mini-column was Centrifuged at full speed (>13,000 x g) for 1 min to remove residual wash buffer, then the mini-column was placed into a fresh 1.5 ml tube.
- From Buffer AE 100µl was added and incubated for 1 min at room temperature, then centrifuged at 5,000 rpm for 5min.

3.2.5.2 Quantitation of extracted DNA

Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the quality of samples for downstream applications. For 1 µl of DNA, 200µl of diluted Quantifluor Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected.

3.2.5.3 Primers Preparation

Table (3.6) Primers Preparation

Primer Name	Vol. of nuclease free water (µl)	Concentration (pmol/µl)
qnrB-F	300	100
qnrB-R	300	100
qnrS-F	300	100
qnrS-R	300	100
ompT-F	300	100
ompT-R	300	100
parE-F	300	100
parE-R	300	100
csgD-F	300	100
csgD-R	300	100

These primers were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl.

3.2.5.4 Reaction Setup and Thermal Cycling Protocol

Table (3.7) Reaction Setup and Thermal Cycling Protocol

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
Master Mix	2	X	1	X	12.5
Forward primer	10	µM	0.5	µM	1
Reverse primer	10	µM	0.5	µM	1
Nuclease Free Water					8.5
DNA		ng/µl		ng/µl	2
Total volume					25
Aliquot per single rxn	23 µl of Master mix per tube and add 2 µl of Template				

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	50,53,56.2,or 60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

3.2.5.5. Agarose Gel Electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

3.2.5.6. Solutions

1 X TAE buffer, DNA ladder marker, Ethidium bromide (10mg / ml).

3.2.5.7. Preparation of agarose

- A volume of 100 ml of 1X TAE buffer was taken in a flask.
- Added 1.5 gm (for 1.5%) agarose to the buffer.
- The solution was heated to boiling (using Microwave) until all the gel particles were dissolved.
- Added 1µl of Ethidium Bromide (10mg/ml) to the agarose.
- The agarose was stirred in order to get mixed and to avoid bubbles.
- The solution was left to cool down at 50-60°C.

3.2.5.8. Casting of the Horizontal Agarose Gel

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was left to solidify at room temperature for 30 minutes. The comb was carefully removed, and the gel was placed in the gel tray. The tray was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel.

3.2.5.9. DNA Loading

PCR products were loaded directly. For PCR product, 5µl was directly loaded to well. Electrical power was turned on at 100v/mAmp for 60min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system.

3.2.5.10. DNA sequencing method

The DNA sequencing method was performed to study of confirmatory identification of bacterial isolates based on multiple sequence alignment analysis and phylogenetic tree relationship analysis of studied local bacterial isolates with NCBI-Gen bank Global isolates and determination of genetic changes based on single nucleotide substitution analysis, as well as submission the local isolates in NCBI-Gen Bank database. Multiple alignment of nucleotide and phylogenetic tree was done by https://mafft.cbrc.jp/alignment/server/index.html and sequences trimming using MEGA.

3.2.6. Statistical Analysis

IBM SPSS statistics packages version 23 has been employed in order to carry out the statistical analysis. In order to ensure that the results are presented in a manner that is both accurate and comprehensible, the results of the study have been summarized utilizing descriptive statistics including percentage values. Besides, the analysis of categorical variables and the determination of the proportion of responses that fall into each category were also carried out through the utilization of frequency and percentages. Nevertheless, chi-square analysis and the crosstabulation method were both employed in order to evaluate the probability of an association among categorical variables. Furthermore, graphical methods such as histograms, bar charts, stacked column and pie charts have been generated by Microsoft Excel 2019 in order to visually represent the distribution and relationships in the data.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Demographic distribution of the studied group.

A total of 968 urine sample of patients suffering from sign and symptoms of urinary tract infection had visit (Imam Al-Hussein Medical City and Kerbala Pediatric Teaching Hospital). The patients had an age range between (1-80) years. The positive bacterial growth from all samples was 261 (26.96 %) samples with 34 (3.51%) fungi and the remaining ٦٧٣ (69.52%) being negative growth. After morphological, microscopic and Vitek 2 System implementation was done, ١٠٢ (39.08 %) *E. coli* isolates were obtained from 261 total bacterial growth obtained **Table (4.1)**.

The result of negative growth may be as a result of other causative agents: virus, or anaerobic bacteria or may be diluted urine sample or may be antibiotic was taken.

Table (4.1) Distribution of microbial and nongrowth samples according to status, count, and percentage.

Status	Number		Percentage %	
Gram Negative	65	261	١٧,٢٥%	26.96 %
E. coli	102			
Gram Positive	94		9.71%	
Fungi	34		3.51%	
Nongrowth	673		69.52%	
Total	968		100.00%	

٤,٢. Identification of *E. coli* in studied samples

The *E. coli* isolates were identified depending on their physical characteristics. The isolate appeared as bright pink colonies when cultured on MacConkey agar and the colonies appeared green metallic sheen on EMB media. These results due to MacConkey agar containing crystal violet and bile salts which allows Gram-negative bacteria to grow while inhibiting Gram-positive bacteria growth. It was found that the bacteria were sugar lactose fermenters as shown in **Figure (4.1)**. on Eosin Methylene Blue (EMB), which it was differential medium for *E. coli* used to differentiate it from other *Enterobacteriaceae* members. metallic green sheen colonies were observed, which means that the colonies produced organic acids due to glucose and lactose fermentation which in the presence of eosin and methylene gives a metallic green sheen color as shown in **Figure (4.1)**.

To confirm the identification of the bacterial isolates, Vitek 2 System was utilized (Ahmed , 2021).

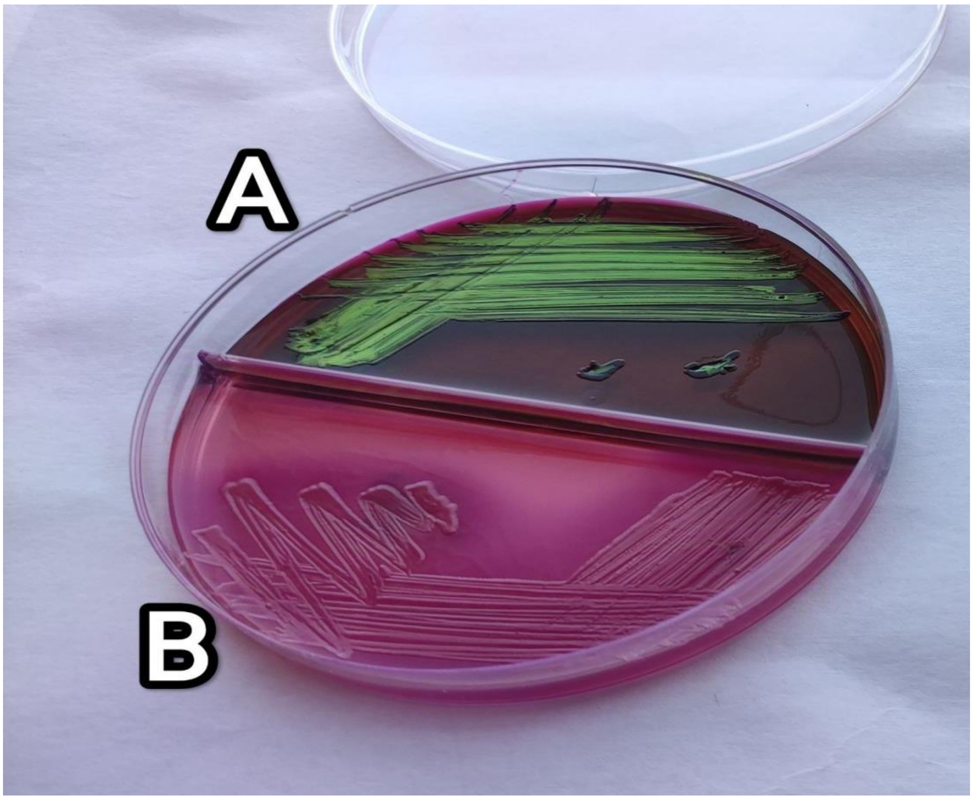


Figure (4.1) The culturing results of preserved *Escherichia coli* on (A) Eosin Methylene Blue (EMB) Agar and (B) MacConkey agar.

4.2.1. Distribution of gram-negative bacteria and *Escherichia coli* among total bacterial growth.

According to the result of VITEK® 2 Compact system, gram negative bacteria was the most common infectious agent which accounting for 167 (64 %) of total bacterial positive culture as in **Figure (4.2)** and the *E. coli* was the highest percentage 102 (61 %) of total gram-negative bacteria as it appears in **Figure (4.3)**.

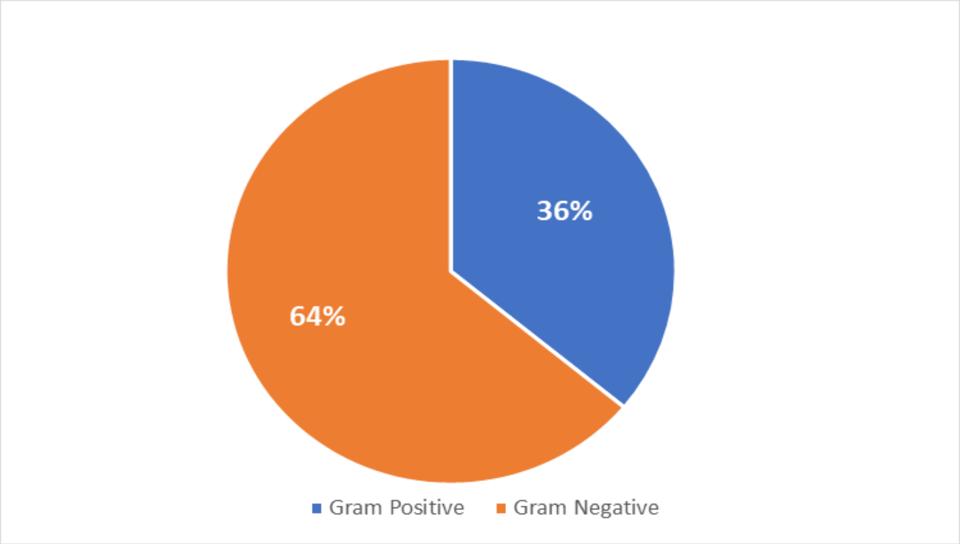


Figure (4.2) Distribution percentage of Gram-positive and Gram-negative samples.

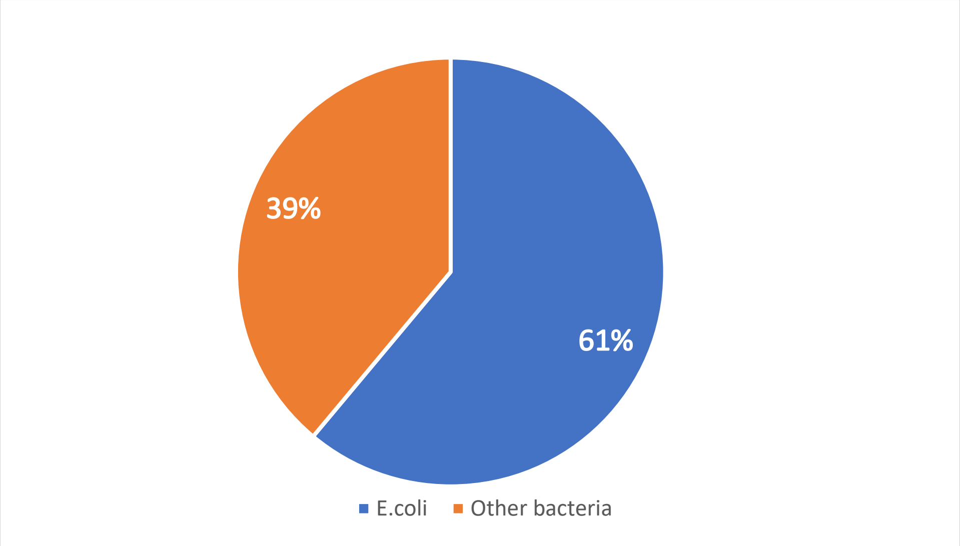


Figure (4.3) Percentage representation of *E. coli* and other Gram-negative organisms in the sample distribution.

which is in agreement with result of study conducted by (Saleh , 2021) which revealed that 63.3 % of total bacterial culture was gram negative and 36.7 % was gram positive and *Escherichia coli* was 70 % of total gram negative bacteria. several subsequent studies have provided evidence that gram negative bacteria and *E. coli* remains the most common cause of UTI Such as the one conducted by (Masaoodi *et al* ., 2023) which revealed that the gram negative bacteria the most common causative agent of UTI with 28.8% of *E. coli* .and another study in Egypt conducted by (Abou-Dobara *et al* ., 2023) show that Gram-negative bacilli were the most common with a 42.0% of *E. coli*.

4.3. Distribution of *Escherichia coli* isolates according to age group and sex

Table (٤،٢): Distribution of samples according to sex group

Sex	Number	Percentage%	P-Value
Female	79	77.45%	0.00003 **significant**
Male	23	22.55%	
Total	102	100.00%	

There were significantly differences between male and female in urine samples group with P value 0.00003.

The result as it appear in the **table (٤،٢)** show that the infection rate was higher in females with (77.45%) and this agrees with the results of (Naziri *et al* ., 2021) who observed that the percentage of females affected by *E. coli* were 77% to 23% for males, and also similar to the findings of (Staji *et al* ., 2019), whereby female participants were ٦٢.5% and male 37.5% of *E. coli* strains. Women experience urinary tract infections more frequently than males do; Female anatomy, sexual activity, and family history are risk factors (Faruk *et al* ., 2023) Furthermore, about 40-50% of females during their lives may have at least one symptomatic UTI and about 20-30% of adult females approximately can experience recurrence UTI within 6 months after primary infection by UTI, and about 3% will experience a third infection (Ibrahim *et al* ., 2021).

Table (٤،٣): Distribution of samples according to sex and age group

Age group	**Sex**		**Total**
	Female **N (%)**	**Male** **N (%)**	**N (%)**
1-20	25 (31.6)	4 (17.4)	29 (28.4)
21-40	26 (32.9)	3 (13.0)	29 (28.4)
41-60	16 (20.3)	11 (47.8)	27 (26.5)
61-80	12 (15.2)	5 (21.7)	17 (16.7)
Total%	79 (100)	23 (100)	102 (100)
P. value 0.025			

The association between Sex and age was significantly different in urine samples group with **P. value 0.025**

In female the age group of 21-40 years had the highest rate of UTI, accounting for 32.9% of all *E. coli* isolates, followed by the age group 1-20 years with 31.6%, and the age groups 41-60 years with 20.3%.

In male the age group of 41-60 years had the highest rate of UTI, accounting for 47.8% of all *E. coli* isolates, followed by the age group 61-80 years with 21.7%, and the age groups 1-20 years with 17.4%.

In this study the elevation of infection rate of the age group (21-40 years) in female listed in the **table (٤،٣)** agrees with the result of (FarajzadehSheikh *et al* ., 2019) who discovered that most of female patients sample belong to the age group (21-40 years). and disagrees with result of study conducted by (Ho *et al* ., 2019) which shows the lowest rate of infection for female at age group (21-40 years) and the higher rate of infection at the age group > 60 years.

The rate of infection in the age group 41-60 years demonstrates that males are most infected by *E. coli* at that age range, which is disagree with the results existed by (Ondari , 2020) was the higher rate of infection at age group (21-40 years).

4.4. Antimicrobial Resistance Pattern of *Escherichia coli*

The following antimicrobial agents were tested *Escherichia coli* isolates per the global Clinical and Laboratory Standards Institute (CLSI) by VITEK-2 system for Amikacin, Ampicillin, Aztreonam, Cefepime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Minocycline, Piperacillin, Piperacillin/Tazobactam, Ticarcillin/Clavulanic Acid, Ampicillin, Ticarcillin, Tobramycin, and Trimethoprim/Sulfamethoxazole. The outcomes of antimicrobial susceptibility are shown in **table (٤,٤)**

Table (4.4) antimicrobial susceptibility

Antibiotic name	resistance	sensitive	intermediate
Ticarcillin	88.89 %	7.41%	3.70%
Ticarcillin\Clavulanicacid	48.15 %	48.15%	3.70%
Piperacillin	85.19 %	11.11%	3.70%
Piperacillin\Tazobactam	29.17 %	70.83%	0.00%
Ampicillin	75.00 %	20.00%	5.00%
Ceftriaxone	53.13 %	46.88%	0.00%
Cefotaxime	67.92 %	32.08%	0.00%

Ceftazidime	28.30 %	66.04%	5.66%
Cefepime	28.57 %	70.00%	1.43%
Imipenem	6.02 %	93.98%	0.00%
Meropenem	6.38 %	93.62%	0.00%
Gentamicin	28.87 %	71.13%	0.00%
Amikacin	8.42 %	85.26%	6.32%
Ciprofloxacin	58.59 %	36.36%	5.05%
Levofloxacine	50.00%	46.15%	3.85%
Trimethoprim\Sulfamethoxazole	61.70%	38.30%	0.00%

Among 102 *E. coli* isolates interestingly, it was observed that the resistance rate was high towards Ticarcillin (88.89 %), follow by Piperacillin (85.19 %), Ampicillin (75 %), Cefotaxime (67.92 %), Trimethoprim/Sulfamethoxazole (61.70 %) and lower towards Ciprofloxacin 58.59%, Ceftriaxone 53.13 % and Levofloxacine 50.00%. the highest sensitivity rate was to Imipenem 93.98% follow by Meropenem 93.62% and Amikacin 85.26%.

According to the result of (Ali *et al* ., 2020) in Basrah south of Iraq which illustrated that the higher rate of resistance of *E. coli* clinical isolates was 95.3 % resistant to erythromycin , 92.9 % resistance to streptomycin and the lower resistant rate detected at Amikasin with 16.8 % . other study achieved in Al-Diwaniya, Iraq by (Alfetlawi and Jasim , 2022) show that resistance rates of *E. coli* strains were highly resistant to Ampicillin at 97.9% and Ceftriaxone at 81.3 %, however, relatively a lower resistance rate was detected in Meropenem at 2.1 % and Amikacin at 2.4 % . while other study like the one conducted by (AL-Nasrawi and AL-Hashimy,

2020) in AL Najaf found that antimicrobial resistance rates of *E. coli* were as follows Amoxicillin + Clavulanic acid 92.3 % and Trimethoprim – Sulfamethoxazol and Cefotaxime were 90.1 % all three of them were less effective against *E. coli* which was highly resistant to those antibiotics followed by Aztreonam 85,71 %, The lowest rate of resistance where to Imipenem 7.69 % and Meropenem 4.39 %. another study in Baghadad (Salman *et al* ., 2022) done on children reported that *E. coli* was at the highest resistance rate for Cefixime 100% followed by Cefotaxime 86.1 % and the lowest resistance rate at Amikacin 5.4 % and Nitrofurantoin 3.8 %.

4.4.1 Different patterns of antibiotic resistance

The distribution of antibiotic resistance Among collected samples of E. coli were as follows: ٢٠.59% of the isolates demonstrated NON MDR, 44.12% of the isolates demonstrated multidrug resistance (MDR), 27.45% of the isolates exhibited extensive drug resistance (XDR), Rate of ٧.84% of the isolated PDR exhibited resistance as shown in **Table (4.5)**.

Table (4. 5) Distribution of antibiotic resistance among different patterns

Category	**Total number**
NON MDR	٢٠.59%
MDR	٤٤.12%
XDR	٢٧.45%
PDR	7.84%
Total	١٠٠%
p-value 0.00004 significantly different	

*MDR: Multidrug-resistant, XDR: Extensive drug-resistant, PDR: Pan-drug-resistant

4.5. Distribution of antibiotic resistance and virulence factor genes of *E. coli* among the target 38 isolated samples.

The rate of antibiotic resistance and virulence factor genes distribution are shown in **Table (4.6)**

Table (4.6) Distribution of antibiotic resistance and virulence factor genes

Genes	Negative		Positive		Total	
	number	**Percentage%**	**number**	**Percentage%**	**Number**	**Percentage%**
ParE	0	0.0	38	100.0	38	100.0
csgD	1	2.6	37	97.4	38	100.0
qnrS	31	81.6	7	18.4	38	100.0
qnrB	3٥	92.11	٣	7.89	38	100.0
ompT	12	31.6	26	68.4	38	100.0

4.5.1. Identification of qnrB gene among target *E. coli* isolates

The presence of the qnrB gene in only four (**7.89**%) of *E. coli* isolates from 38 was detected using a PCR assay by using two targeting primers (**Table 3.5**) to cover the coding sequence of gene qnrB with 469 bp. isolate 4 ,13,25 and 32 carry qnrB gene but the rest of the target isolates do not carry qnrB as in **figure (4.4)**.

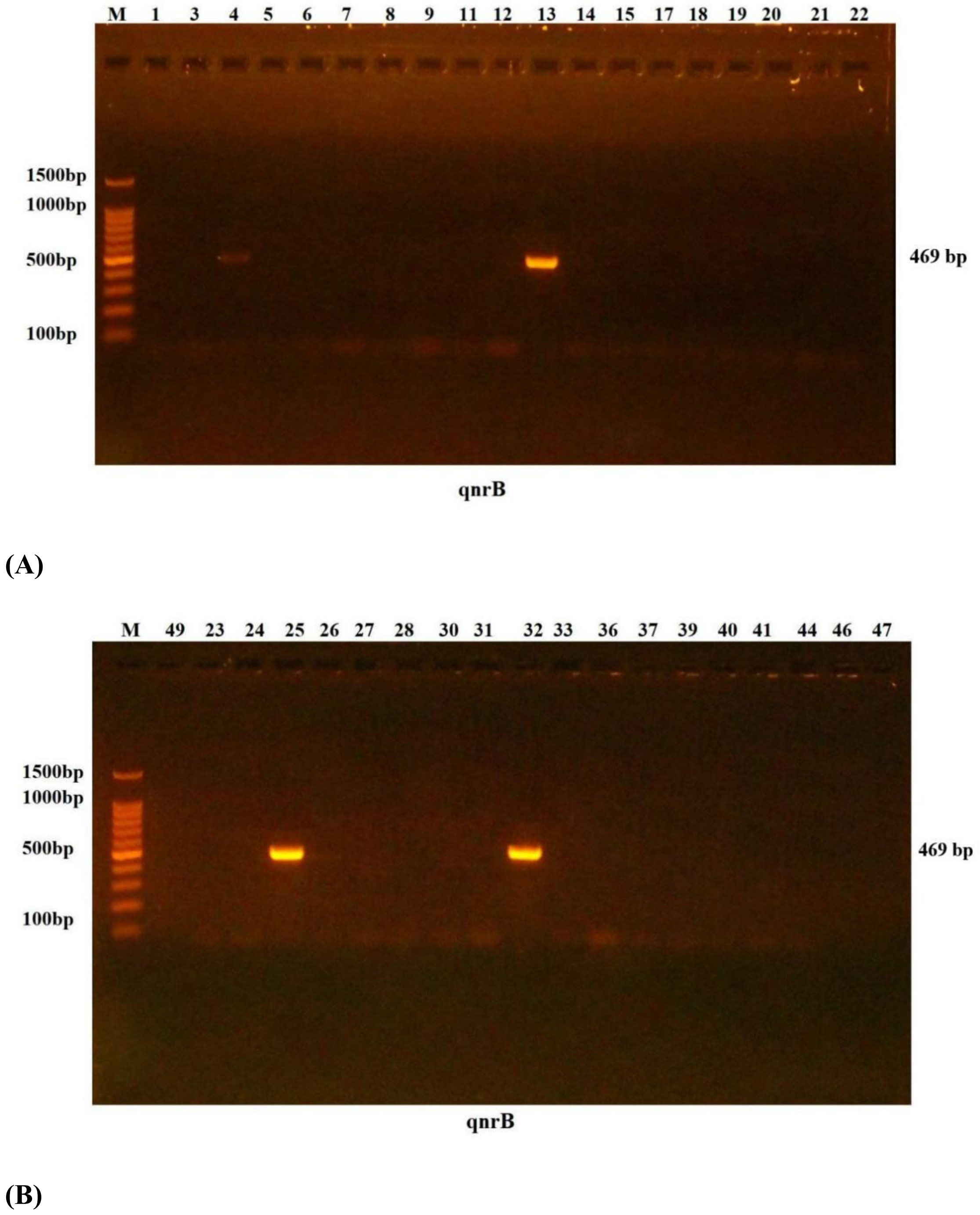
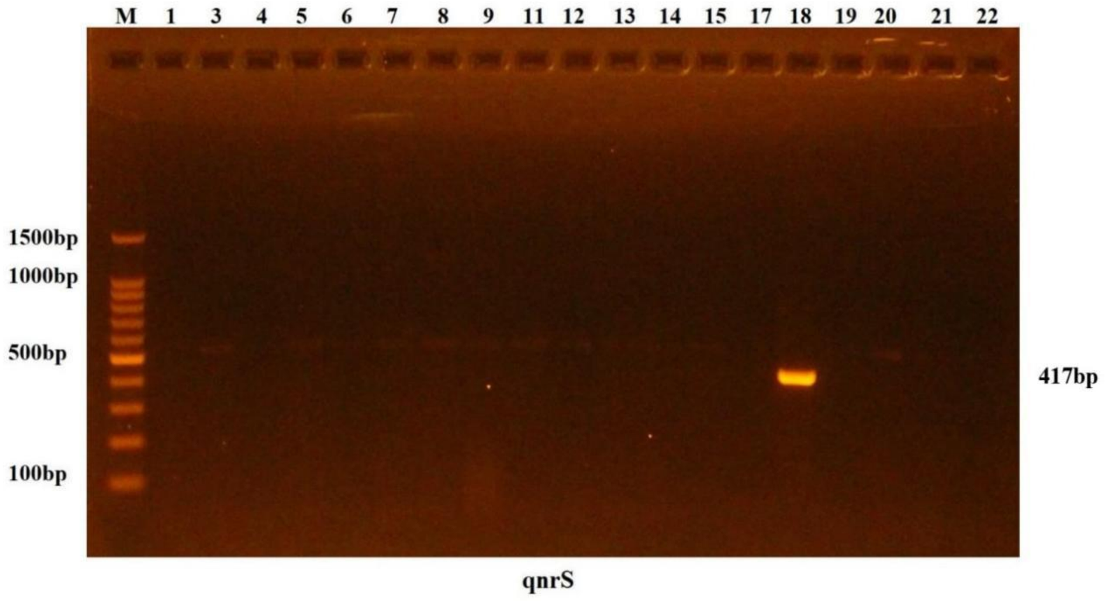


Figure (4.4) (A , B) PCR Results of the amplification of qnrB gene products of *Escherichia coli* with products size 469bp were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes (13,25 and 32) were positive with qnrB gene.

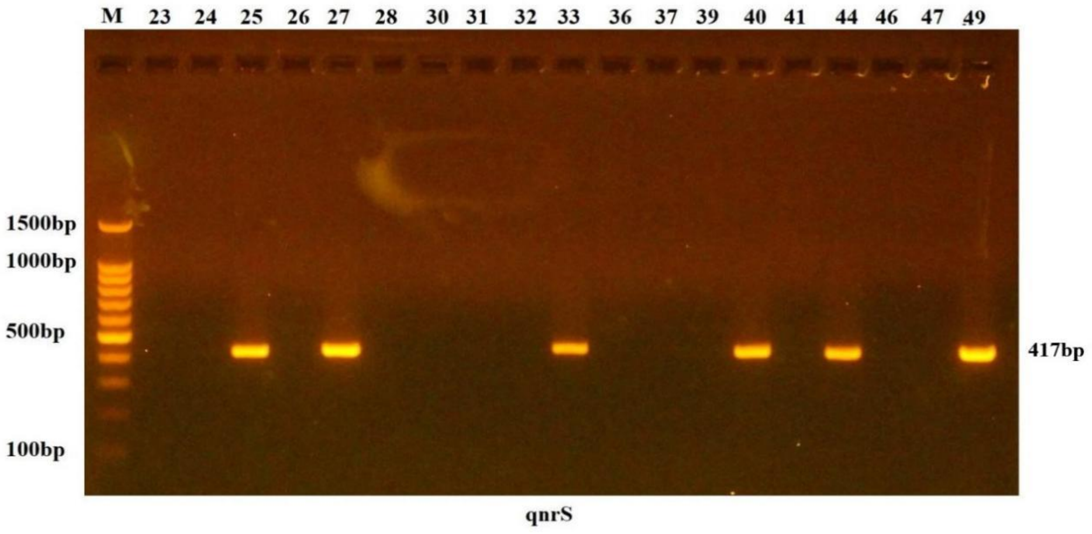
The present investigation agrees with the findings of Ibrahim**,** in 2023, who discovered the presence of qnrB in (7.89%) of isolates of *E. coli* (Ibrahim , 2023). and similar to study done in Tunisia by Kilani *et al.*, in 2020, who found that qnrB presence in (5.17%) isolates of *E. coli* (Kilani *et al* ., 2020). in other study like (An , 2024) found out that only (2.7%) isolates carried qnrB gene. While certain studies discovered a higher prevalence of qnrB gene in their target isolates as in the study conducted by (Nsofor *et al* ., 2021) was in (60%) of the isolates. and in (43.33%) of isolates in study done by (Abdul-Husin , 2021). in other study like the one conducted by (Amiri *et al* ., 2017) did not found a qnrB gene in the target isolates.

4.5.2. Identification of qnrS gene among target *E. coli* isolates

The presence of the qnrS gene in 7 (18.4 %) of *E. coli* isolates from 38 was detected using a PCR assay by using two targeting primers (**Table 3.5**) to cover the coding sequence of gene qnrS with 417 bp. isolate 18,25,27,33,40,44 and 49 carry qnrS gene but the rest of the target isolates do not carry qnrS as in **Figure (4.5)**.



(A)



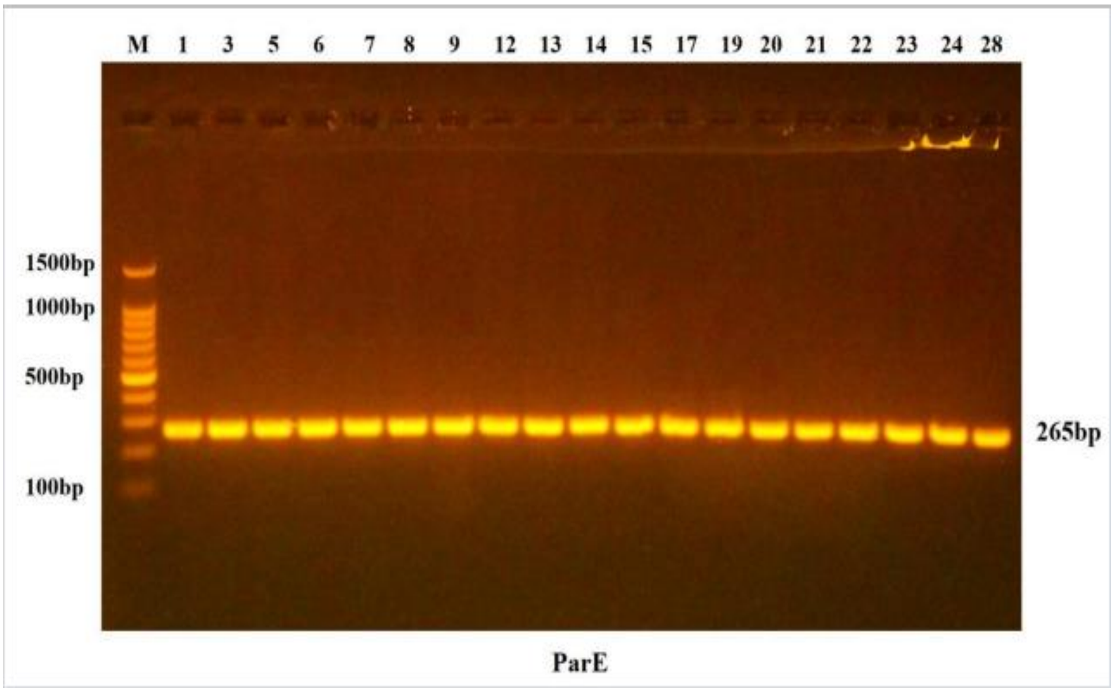
(B)

Figure (4.٥) (A , B) PCR Results of the amplification of qnrS gene products of *Escherichia coli* with products size 417 bp were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes (18,25,27,33,40,44 and 49) were positive with qnrS gene.

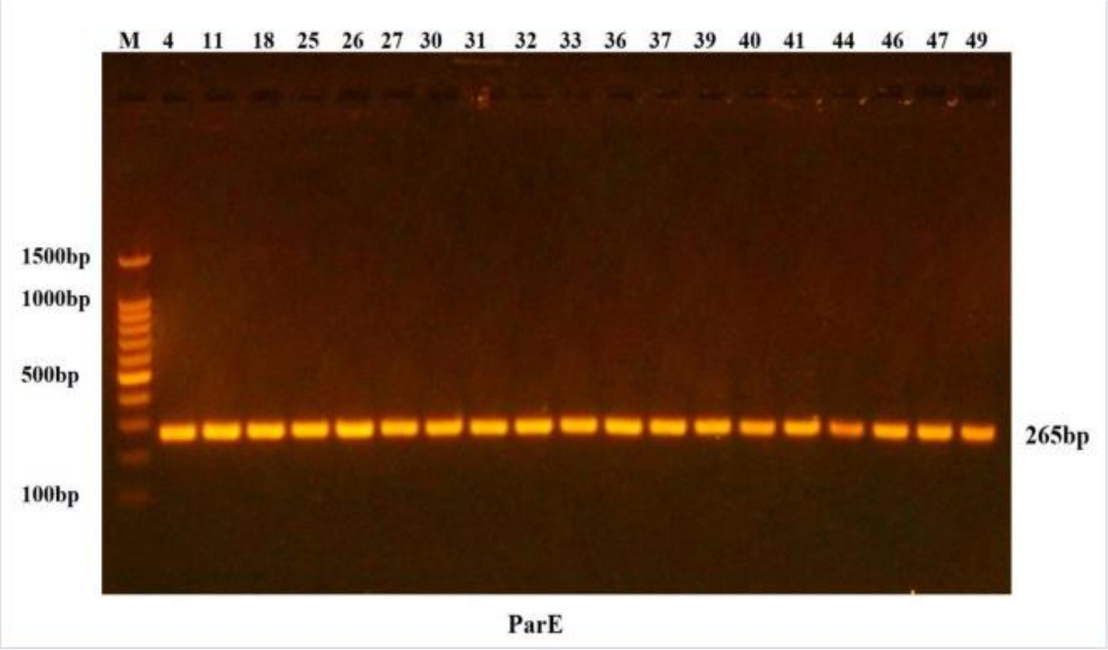
The present investigation is similar to the findings of (Kilani *et al* ., 2020) which showed that (6.90%) of isolates carried qnrS gene. several studies showed the presence of higher rate of qnrS gene in their isolates as in the study done by (Ibrahim , 2023) which found (35%) of isolates carried qnrS gene. and in the one that executed by (Abdul-Husin , 2021) revealed (53.33%) of isolates carried qnrS gene. while other studies discovered low number of isolates carry qnrS gene like the one achieved by (Jiang *et al* ., 2014) which discovered only (4.0%) of isolates carried qnrS gene. And the one achieved by (Amiri *et al* ., 2017) which found only (2.86%) of isolates carry qnrS gene.Some studies showed absence of qnrS gene in their isolates as reported in (Nsofor *et al* ., 2021).

4.5.3. Identification of parE gene among target *E. coli* isolates

The presence of the par E gene in all 38 *E. coli* isolates (100%) was detected using a PCR assay by using two targeting primers (**Table 3.5**) to cover the coding sequence of gene parE with 265 bp. The entire 38 isolates carry par E gene as in **Figure (4.6)**.



(A)



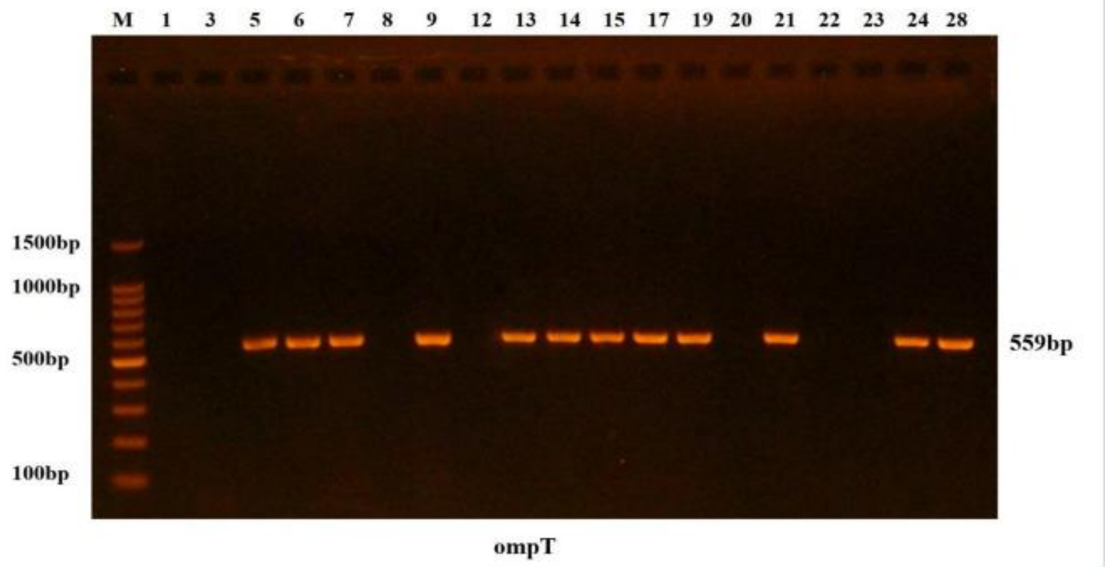
(B)

Figure (4.6) (A , B) PCR Results of the amplification of parE gene products of *Escherichia coli* with products size 265bp were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes (1-28) and (4-49) were positive with parE gene.

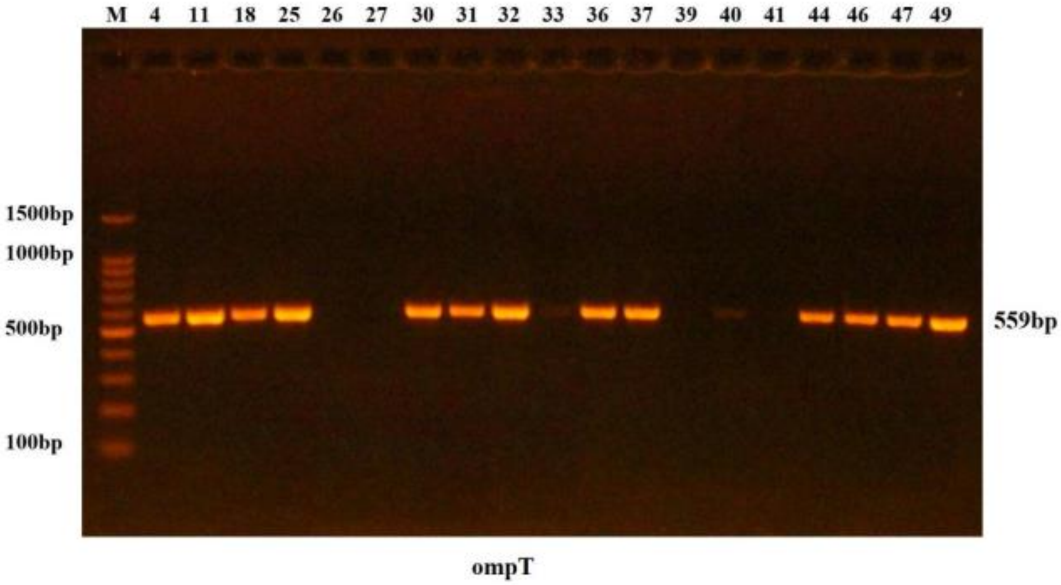
The present investigation agrees with result of studies achieved by (Khosravi *et al* ., 2020) in which parE gene found in the 100% of the target isolates of this study. and similar to the findings of study conducted by (Iranzad and Hakemi-Vala , 20١٧) in which the parE gene found in 91.3% of the target isolates.

4.5.4. Identification of ompT gene among target *E. coli* isolates

The presence of the ompT gene in 26 (68.4 %) *E. coli* isolates from 38 was detected using a PCR assay by using two targeting primers (**Table 3.5**) to cover the coding sequence of gene ompT with 559 bp. Of the 38 *E. coli* target isolates only 12 sample didn't carried ompT gene represented by (1,3,8,12,20,22,23,26,27,33,39,41) as in **figure (4.7)**.



(A)



(B)

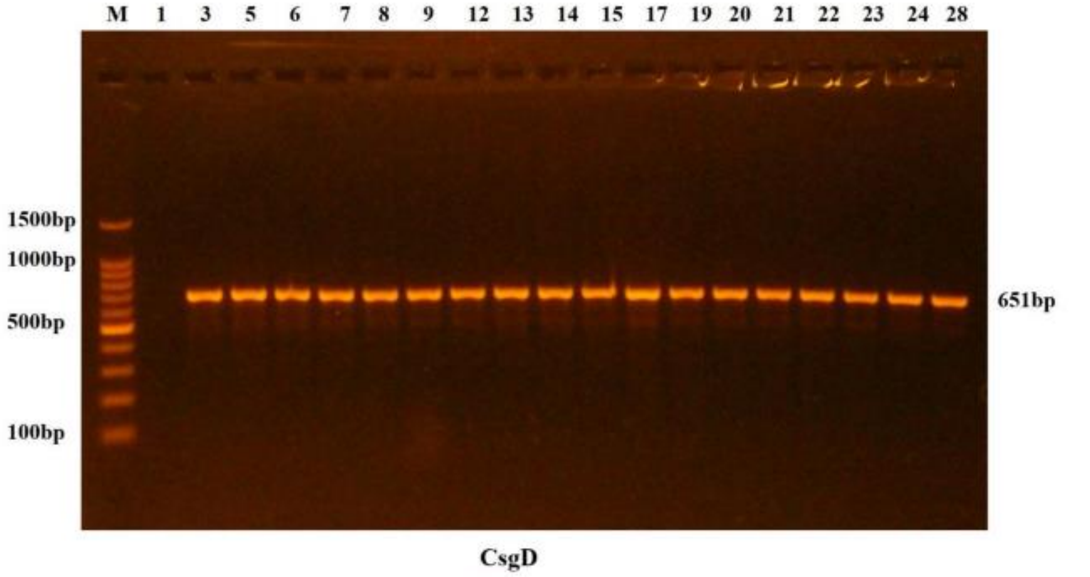
Figure (4.7) (A , B) PCR Results of the amplification of ompT gene products of *Escherichia coli* with products size 559bp were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes (1,3,8,12,20,22,23,26,27,33,39,41) were Negative with ompT gene.

The present investigation agrees with result of study achieved by (Baldiris-Avila *et al* ., 2020) which found that the ompT gene present in 66.8% of the target isolate. while in a study conducted by **(**Ali , 2024**)** ompT gene found in 50% of the target isolates. and this gene was in 42.9% of the target isolates in the study achieved by (Sun *et al* ., 2020). other studies like the one that executed by (Momtaz *et al* ., 2013) show lower proportions in the number of isolates carried ompT gene which was only six samples 4.88% of the total target isolate. while other study revealed higher percentage of this gene as the one done by (Desloges *et al* ., 2019) showed 91.67% of 24 UTI isolates carried ompT gene.

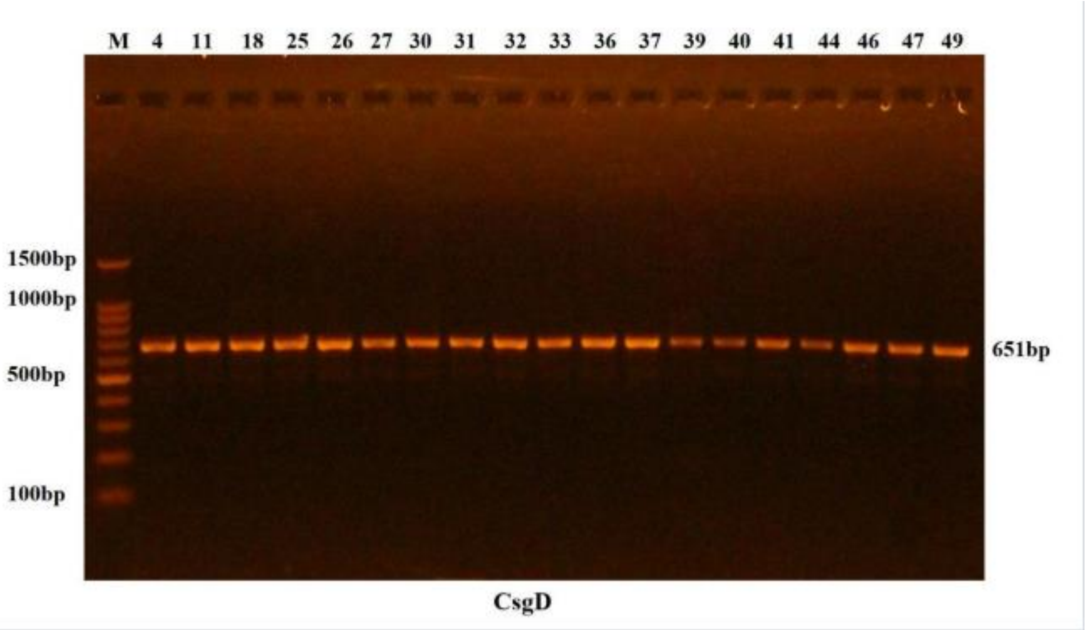
4.5.5. Identification of csgD gene among target *E. coli* isolates

The presence of the csgD gene in 37 (97.4 %) of *E. coli* isolates from 38 was detected using a PCR assay by using two targeting primers (**Table**

3.5) to cover the coding sequence of gene csgD with 651 bp. Of the 38 *E. coli* target isolates only one sample didn't carry csgD gene represented by number (1) isolate. as in **figure (4.8)**.



(A)



(B)

Figure (4.8) (A , B) PCR Results of the amplification of CsgD gene products of *Escherichia coli* with products size 651bp were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes (1) were Negative with CsgD gene.

The present investigation is similar to the result of studies achieved by (Zahran *et al* ., 2020) and (Hamad *et al* ., 2024) showed that 100% of isolates carried csgD gene.

4.6. Gene sequence analysis result and determine the type of mutations and percentage in (par E and csgD) genes

The highly detection rate of both par E and csgD genes suggests there might be a mutation within the target-region. Gene sequence analysis, performed to investigate this further.

20 XDR E. coli isolates of 38 samples that involved in gene detection process, that showed the highest antibiotic resistance especially to Fluoroquinolones group, were sent to gene sequencing for both par E and csgD genes. The base sequences were examined for two samples for each gene and according to their resistance to study the presence of new genotypes for resistance genes to the Fluoroquinolones antibiotic.

4.6.1. Par E gene sequence analysis

According to the result of gene sequencing analysis of par E gene which shows multiple variations in different sites of each sample in both forward and reverse strand as it appears in the **table (4.7)** and table **(4.8)** in which different types of mutation have been found.

Table (4.7) variations in forward strand of par E gene in compare with NCBI reference gene.

Number	NCBI > sample	Frequence	Number	NCBI > sample	Frequence
1338	C > T	(1) 5 %	1429	C > T	(10) 50%
1350	C > T	(1) 5 %	1446	T > C	(12) 50%
1371	T > C	(2) 5 %	1452	A > G	(10) 50 %
1372	G > T	(16)80%	1464	C > G	(10) 50%
1372	G > A	(1) 5 %	1473	G > T	(10) 50%

1386	C > T	(2) 10%	1486	C > T	(2) 10 %
1401	G > T	(10) 50%	1487	T > G	(15) 75 %

Table (4.8) variations in reverse strand of par E gene in compare with NCBI reference gene.

Number	NCBI > sample	Frequence	Number	NCBI > sample	Frequence
442	T > C	(10) 50%	469	T > C	(1) 5 %
448	A > G	(12) 50%	495	C > A	(10) 50%
450	A > DELETION	(3) 15 %	510	G > A	(2) 5 %
464	INSERTION (C)	(1) 5 %	524	C > A	(16) 80 %
465	INSERTION (A)	(1) 5 %	524	C>T	(1) 5 %
467	G > A	(10) 50%	525	A>G	(2) 5 %
468	A > T	(1) 5 %	633	G>A	(2) 5 %

In both table (**4.7**) and table (**4.8**) The first column represents the nucleotide site number on the strand and the second column represents the type of variations while the third column represents the frequency of the variation in each one of the 20 sample strand. (There was limited previous research on this gene for comparison).

4.6.2. csgD gene sequence analysis

According to the result of sequencing of csgD gene which shows multiple variations in different sites of each sample in both forward and reverse strand as it appear in the table **(4.9)** and table **(4.10).**

Table (4.9) variations in forward strand of csgD gene (F strand) in compare with NCBI reference gene.

Number	NCBI > sample	Frequence	Number	NCBI > sample	Frequence
١٢	C > T	(1) 5 %	١٢٣	T > A	(1) 5 %
١٣	T > C	(1) 5 %	١٦٧	G > A	(1) 5 %
١٥	G > T	(1) 5 %	١٧٣	A > T	(17) 85%

١٦	A >DELETION	(6) 30%	١٧٩	T > C	(17) 85%
١٦	A > T	(1) 5 %	١٩١	A > G	(4) 20 %
١٧	A > G	(1) 5 %	٢٤٢	T > C	(20)100%
٢٠	G > A	(1) 5 %	٢٥٤	G > A	(20)100%
٢١	A > G	(1) 5 %	٢٧٤	C > A	(4) 20%
٢٢	T > A	(1) 5 %	٤١٣	C > T	(3) 15%
٣٧	T > C	(1) 5 %	٤٥٢	G > T	(16) 80%
٤٦	INSERTION(T)	(1) 5 %	٤٨٣	A > G	(1) 5 %
٦٣	G > A	(20) 100%	٤٩٧	T > C	(9) 45%
١٠٥	INSERTION(C)	(1) 5 %	٥٣٦	C > T	(12) 60%
١١٢	G > A	(1) 5 %	٥٤٥	A > C	(3) 15%
١٢٠	T > C	(1) 5 %	٥٤٧	G > A	(2) 5 %
١٢١	T > C	(1) 5 %	٥٥١	A > G	(2) 5 %
١٢٢	T > C	(1) 5 %			

Table (4.10) variations in reverse strand of csgD gene (R strand) in compare with NCBI reference gene.

Number	NCBI > sample	Frequence	Number	NCBI > sample	Frequence
37	T > G	(1) 5 %	67	A > G	(8) 40%
38	T > A	(1) 5 %	76	INSERTION(T)	(3) 15%
39	A > T	(1) 5 %	91	INSERTION(T)	(2) 5 %
45	G > T	(1) 5 %	108	A > G	(8) 40%
46	A > G	(1) 5 %	121	T> DELETION	(1) 5 %
47	T > A	(1) 5 %	122	T > C	(1) 5 %
48	C > T	(1) 5 %	150	T > G	(1) 5 %
51	T > C	(2) 5 %	153	A > G	(4) 20%
52	A> DELETION	(2) 5 %	192	A > G	(3) 15%
55	C > T	(2) 5 %	331	G > T	(4) 20%
57	G > T	(16) 80%	414	T > C	(4) 20%
59	INSERTION(T)	(1) 5 %	426	A > G	(16) 80%
64	G > T	(1) 5 %	432	T > A	(16) 80%

65	C > G	(1) 5 %	438	C >T	(1) 5 %
66	A> DELETION	(4) 20%	635	A>DELETION	(1) 5 %
66	A > C	(1) 5 %			

In both table (**4.9**) and table (**4.10**) the first column represents the nucleotide site number on the strand and the second column represents the type of variations while the third column represents the frequency of the variation in each one of the 20 samples strand. (There was limited previous research on this gene for comparison).

4.7. phylogenetic tree analysis

The genetic relationships between species are fundamental to many studies in biology. Phylogenetic trees support our understanding of major transitions in evolution and are key to inferring the origin of genes, discovering molecular adaptation and chromosomal mutations, understanding morphological evolution, and reconstructing demographic changes in diverging specie (Kapli et al., 2020).

Drawing the comprehensive phylogenetic tree to know the evolutionary history of (par E and csgD) genes. The genetic tree of the gene was analyzed using the program https://mafft.cbrc.jp/alignment/server/index.html and it was compared with global isolates of bacteria E. coli.

4.7.1. phylogenetic tree analysis for par E gene forward and reverse strand

The results of the phylogenetic tree of the gene par E (**forward strand**) for all 20 *E. coli* isolates when compared with the site NCBI of global isolates showed that the sequences of local isolates it occupied various positions within the phylogenetic tree, and all the strains for (20) samples did not lie at the same level with the location of strain NCBI except for the strain for sample ID number (23) located at the same level with strain NCBI, **Figure (4.٩)**.

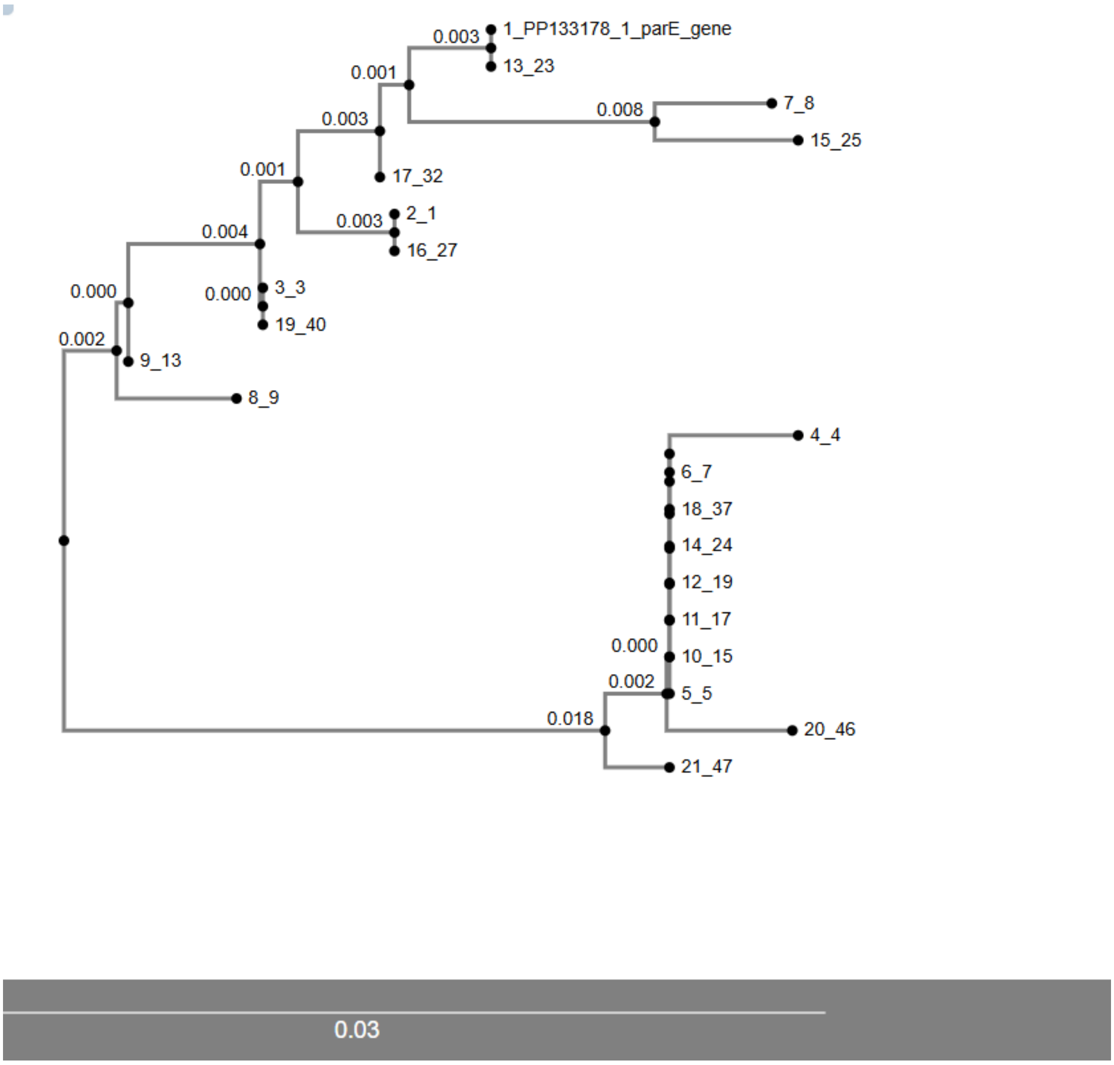


Figure (4.٩) phylogenetic tree of par E gene forward strand of 20 Escherichia coli isolates based on (https://mafft.cbrc.jp/alignment/server/index.html)

The results of the phylogenetic tree of the gene par E (**reverse strand**) for all 20 *E. coli* isolates when compared with the site NCBI of global isolates showed that the sequences of local isolates it occupied various positions within the phylogenetic tree, and all the strains for (20) samples did not lie at the same level with the location of strain NCBI except for the strain for sample ID number (1 , 23 , 27) located at the same level with strain NCBI,

Figure (4.١٠). (There was limited previous research on this gene for comparison).

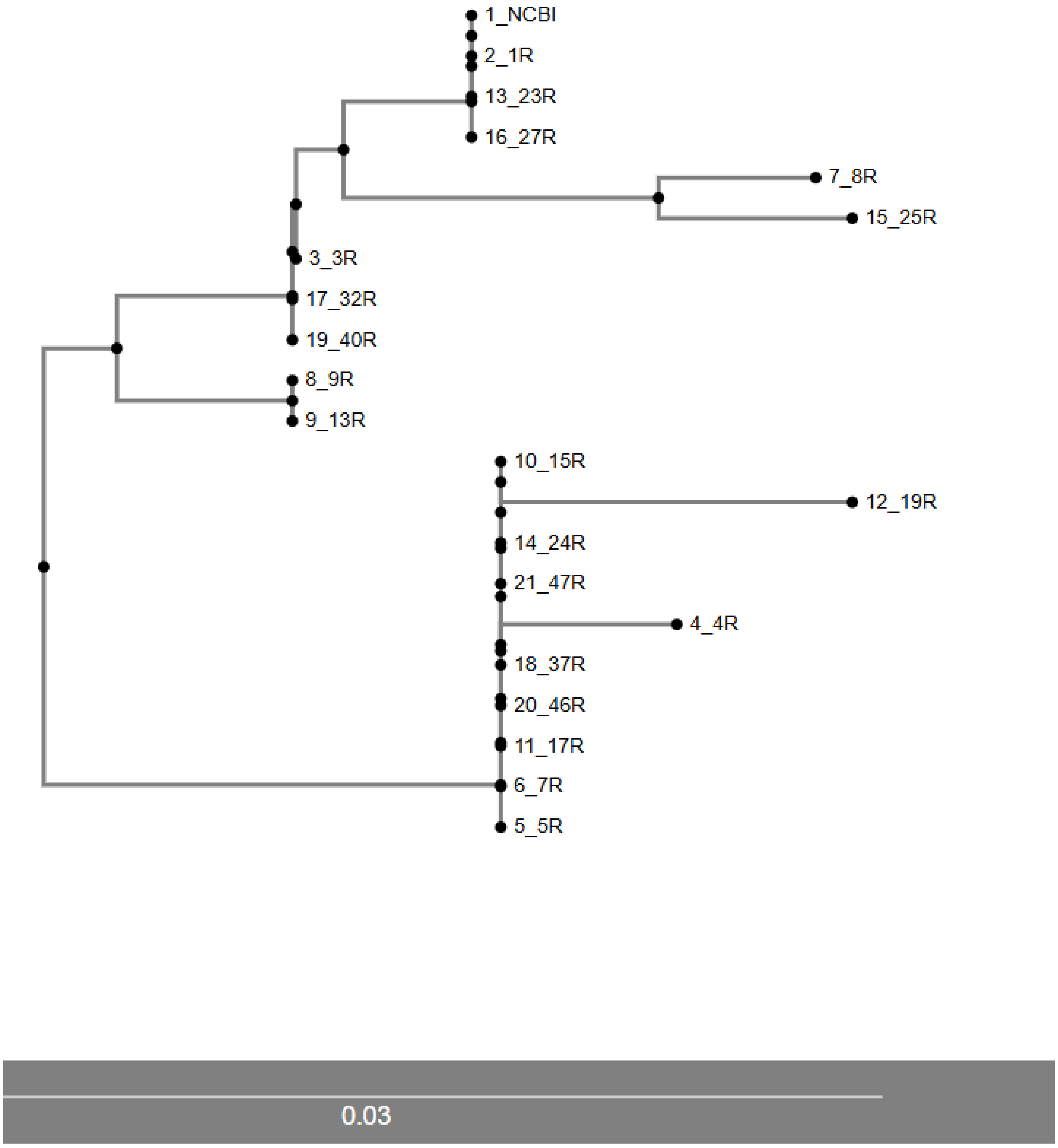


Figure (4.١٠) phylogenetic tree of par E gene reverse strand of 20 Escherichia coli isolates based on (https://mafft.cbrc.jp/alignment/server/index.html).

4.7.2. phylogenetic tree analysis for csgD gene forward and reverse strand

The results of the phylogenetic tree of the gene **csgD** (**forward strand**) for all 20 *E. coli* isolates when compared with the site NCBI of global isolates showed that the sequences of local isolates it occupied various positions within the phylogenetic tree, and all the strains for (20) samples did not lie at the same level with the location of strain NCBI except for the strain for sample ID number (37) located at the same level with strain NCBI, **Figure (4.١١)**. (There was limited previous research on this gene for comparison).

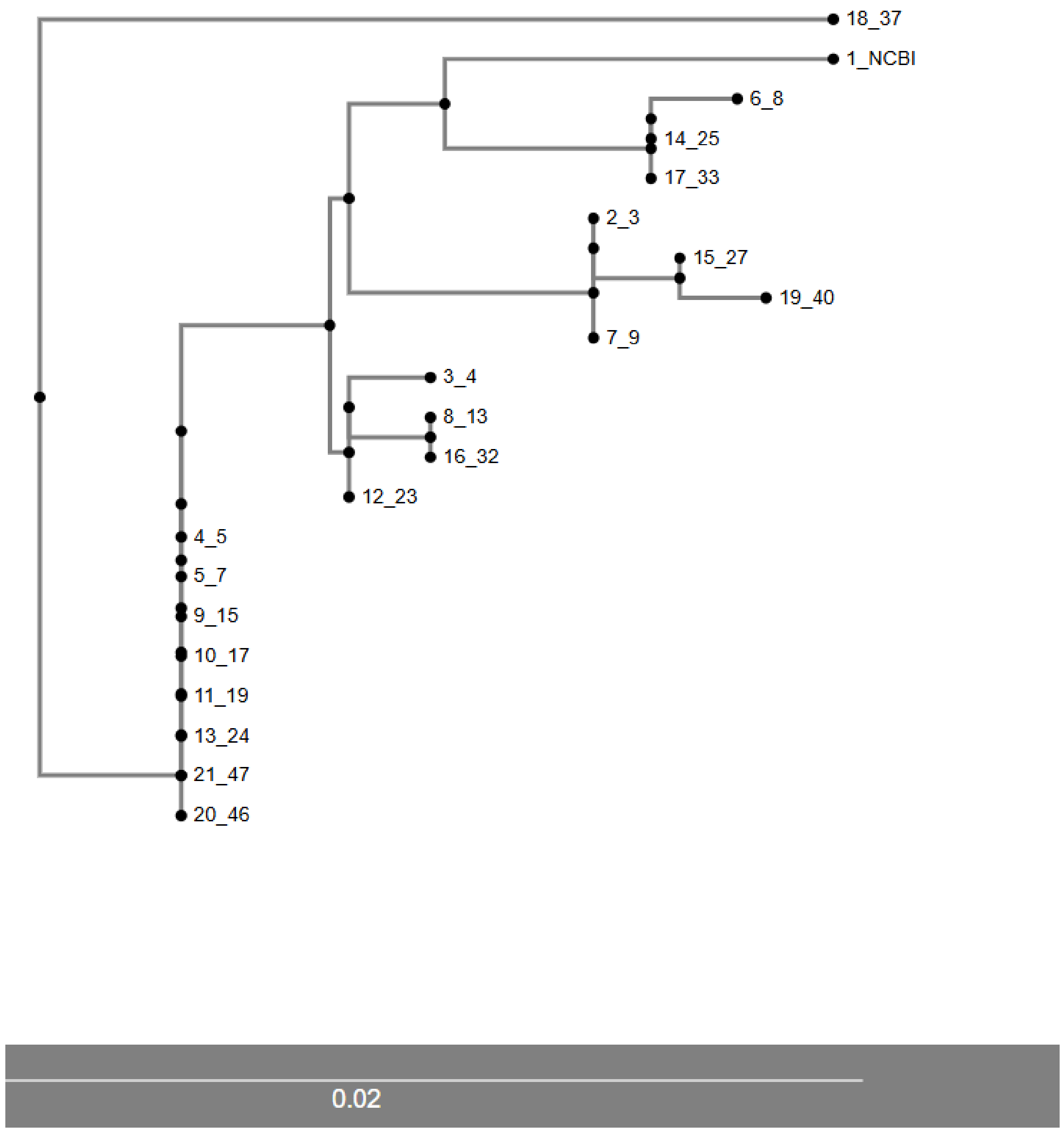


Figure (4.١١) phylogenetic tree of csgD gene forward strand of 20 Escherichia coli isolates based on (https://mafft.cbrc.jp/alignment/server/index.html).

The results of the phylogenetic tree of the gene **csgD** (**reverse strand**) for all 20 *E. coli* isolates when compared with the site NCBI of global isolates showed that the sequences of local isolates it occupied various positions within the phylogenetic tree, and all the strains for (20) samples did not lie at the same level with the location of strain NCBI except for the strain for sample ID number (25,33,3,9) located at the same level with strain NCBI, **Figure (4.۱۲)**. (There was limited previous research on this gene for comparison).

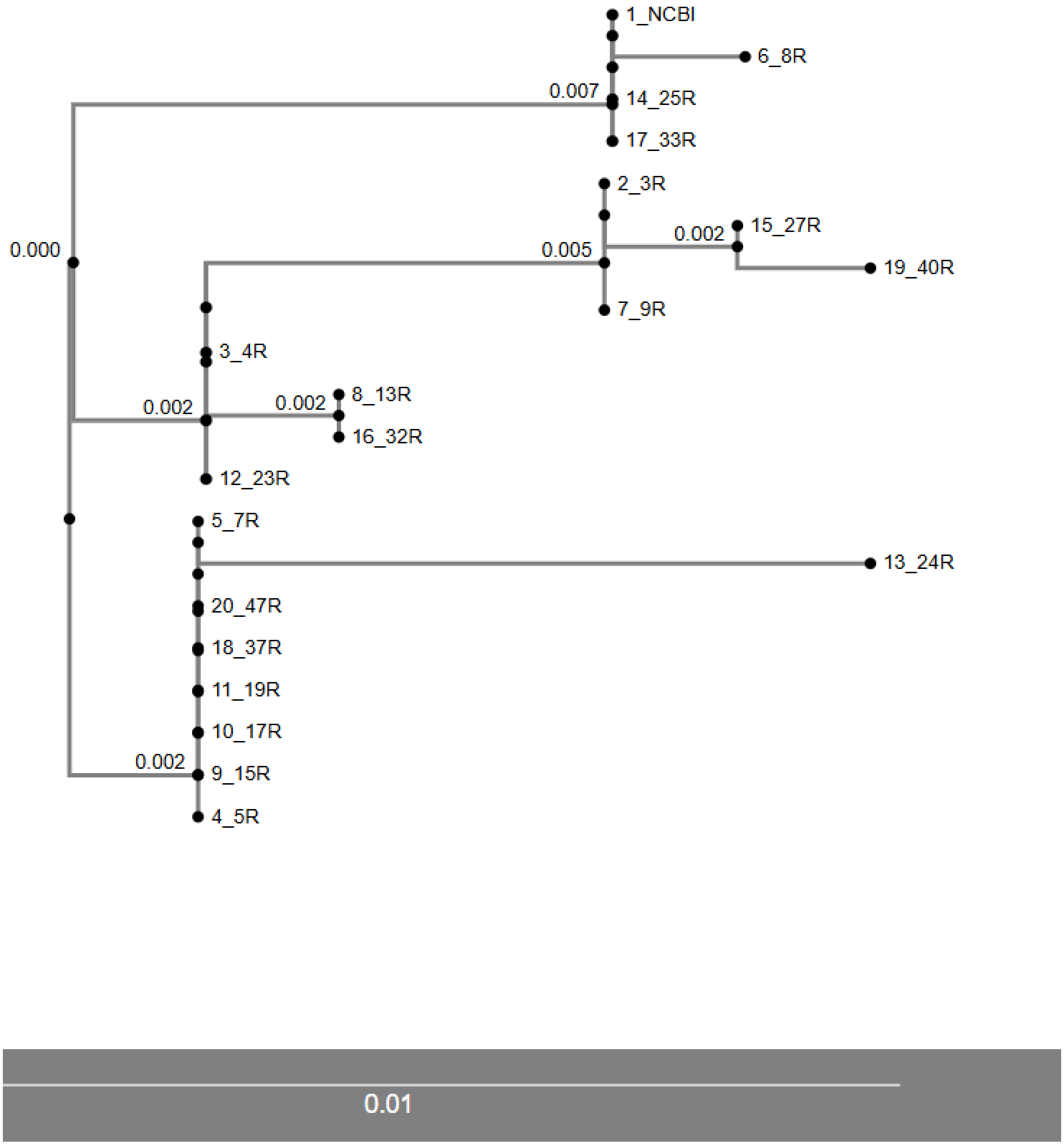


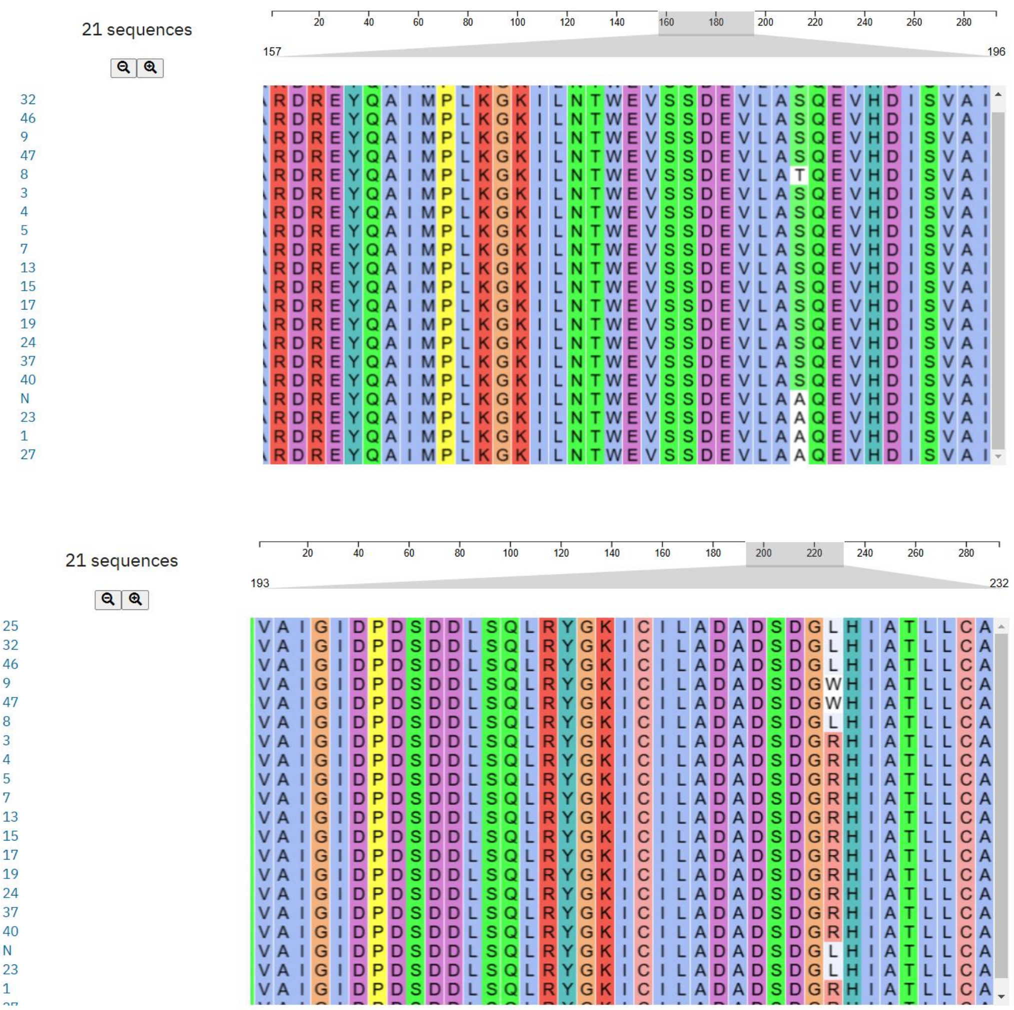
Figure (4.۱۲) phylogenetic tree of csgD gene reverse strand of 20 Escherichia coli isolates based on (https://mafft.cbrc.jp/alignment/server/index.html).

4.8. Multiple alignment of amino acide sequences of parE gene

According to **Figure (4.1٣)** multiple alignment of amino acid shows that there are some variations among certain samples as it indicated in **table (4.11)**.

Table (4.11) sample number, variations and amino acid site affected

Sample Number	NCBI>sample	Amino acid site
8	A>T	185
9	L>W	223
47	L>W	223



```
32  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
46  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
9   RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
47  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
8   RDREYQAIMPLKGKILNTWEVSSDEVLATQEVHDISVAI
3   RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
4   RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
5   RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
7   RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
13  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
15  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
17  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
19  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
24  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
37  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
40  RDREYQAIMPLKGKILNTWEVSSDEVLASQEVHDISVAI
N   RDREYQAIMPLKGKILNTWEVSSDEVLAAQEVHDISVAI
23  RDREYQAIMPLKGKILNTWEVSSDEVLAAQEVHDISVAI
1   RDREYQAIMPLKGKILNTWEVSSDEVLAAQEVHDISVAI
27  RDREYQAIMPLKGKILNTWEVSSDEVLAAQEVHDISVAI
```

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25  VAIGIDPDSDDLSQLRYGKICILADADSDGLHIATLLCA
32  VAIGIDPDSDDLSQLRYGKICILADADSDGLHIATLLCA
46  VAIGIDPDSDDLSQLRYGKICILADADSDGLHIATLLCA
9   VAIGIDPDSDDLSQLRYGKICILADADSDGWHIATLLCA
47  VAIGIDPDSDDLSQLRYGKICILADADSDGWHIATLLCA
8   VAIGIDPDSDDLSQLRYGKICILADADSDGLHIATLLCA
3   VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
4   VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
5   VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
7   VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
13  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
15  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
17  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
19  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
24  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
37  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
40  VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
N   VAIGIDPDSDDLSQLRYGKICILADADSDGLHIATLLCA
23  VAIGIDPDSDDLSQLRYGKICILADADSDGLHIATLLCA
1   VAIGIDPDSDDLSQLRYGKICILADADSDGRHIATLLCA
```

Figure (4.1٣) multiple alignment of amino acid sequences of parE gene with NCBI reference sequence.

The amino acid phylogenetic tree of ParE gene in **Figure (4.1٤)** shows that only sample 8 and sample 23 in same level NCBI strain while the rest of the samples occupied various positions within the genetic tree.

(There was limited previous research on this gene for comparison)

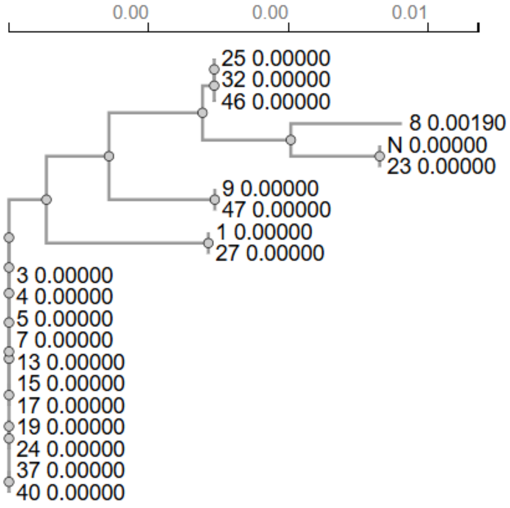

Figure (4.1٤) the phylogenetic tree of ParE gene according to amino acid seq of 20 Escherichia coli isolates based on (https://mafft.cbrc.jp/alignment/server/index.html).

4.9. Three dimensional models of the *E. coli* for par E, CsgD proteins

4.9.1. Three dimensional models of the *E. coli* for par E protein

Figure (4.1٥, 4.١٦ and 4.١٧,4.١٨) showed three dimensional models of the *E. coli* isolates (8, 32, 9, 47) for par E protein compared with the protein Data Bank (PDB), the results showed changes in DNA from sequencing that affected the quality and sequence of amino acids and thus reflected on the three-dimensional protein.

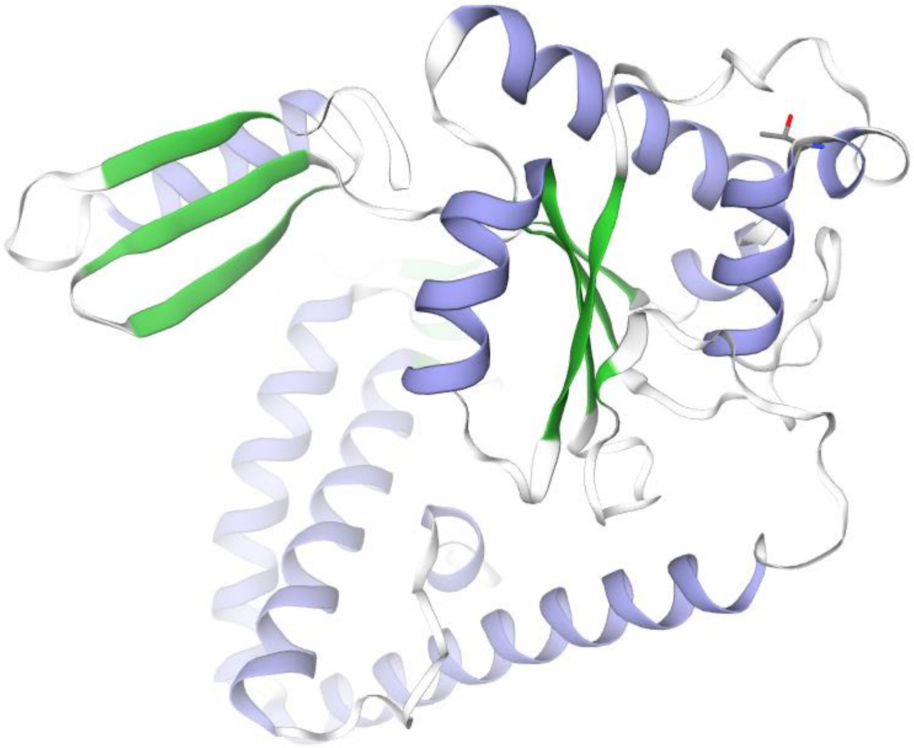

Figure (4.1٥) Three dimensional models of the *E. coli* of par E protein for (8) isolate.

Figure (4.١٦) Three dimensional models of the *E. coli* of par E protein for (9) isolate.

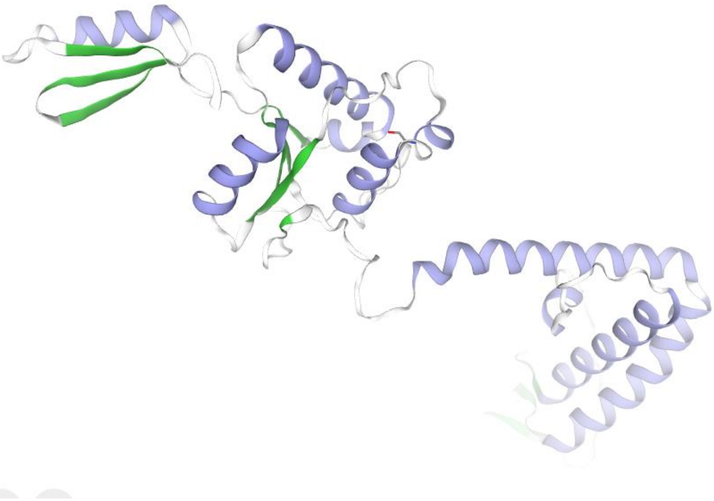

Figure (4.١٧) Three dimensional models of the *E. coli* of par E protein for (32) isolate.

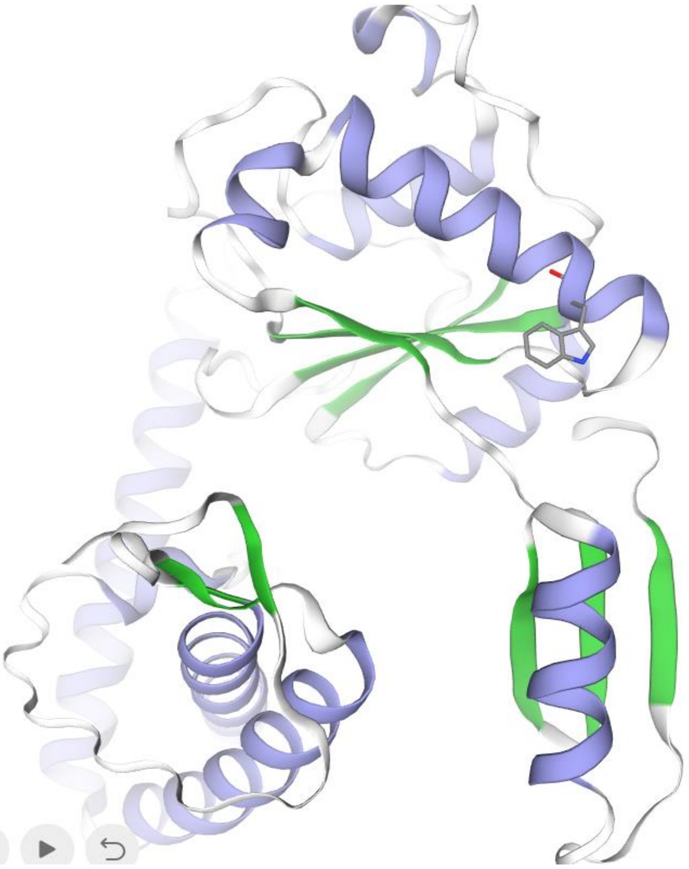

Figure (4.١٨) Three dimensional models of the *E. coli* of par E protein for (47) isolate.

4.9.۲. Three dimensional models of the *E. coli* for CsgD pretein

Because of multiple stop codon that has been found in the multiple alignment of amino acid sequences of **CsgD** gene it was difficult to forming a Three-dimensional model of the *E. coli* for CsgD protein.

Conclusions and recommendations

5. Conclusions and Recommendations

5.1. Conclusions

1- Most of *E. coli* isolated in this study were XDR bacteria.

2- Among antibiotic resistance genes ParE gene was the most prevalent gene among studied isolate followed by qnrS and qnrB genes

3- Among virulence genes csgD gene was the most prevalent gene among studied isolate followed by ompT gene.

4- Sequencing of the ParE and csgD genes revealed a high mutation rate among E. coli isolates compared to the NCBI reference sequences. This finding suggests a potential correlation between genetic mutations and the increased resistance and persistence of E. coli infections.

5- The phylogenetic analysis of the parE gene and csgD gene show multiple new strains that is different from strains that found in NCBI.

6- The new strains approved in this study was recorded in gene bank **Appendix 2**.

5.2. Recommendations

1- Study of antibiotic resistance of other types of bacteria that cause UTI.

2- Study of another virulence genes related to pathogensity of *E. coli*.

3- Study the combination of nanoparticles with antibiotics as a strategy to reduce resistance.

4- Investigate the qnrB and qnrS genes according to the serotype of *E. coli* isolates.

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Appendices

Apendix 1: Questionnaire

Name;

Age;

Sex;   Female   Male

Do you have any of the following symptoms? :

- Discomfort or pain passing urine
- Passing urine more frequently at night
- Urine that is more cloudy
- New discharge from the vagina
- New discharge from the penis
- None of the above

Do you have any of the following symptoms?:

- New pain in lower back
- Nausea
- Vomiting
- Fever
- Shivering
- Other symptoms

How long have you had these symptoms?:

- Less than 3 days
- 3 days to 1 week
- 1-2 weeks
- More than 2 weeks

What have you done to manage your symptoms?:

- Painkillers eg, Paracetamol
- Antibiotics
- Cranberry products
- Drinking more fluids
- Other remedies
- None

Have you had a Urinary tract infection (UTI) before, these are sometime called a bladder or water infection?:

- Yes- In the previous 6 months
- Yes- In the previous year
- Yes- In the previous 3 years
- Yes- more than 3 years ago
- No

Is there a possibility you may be pregnant?:

- Yes
- No

Do you have a urinary catheter (This is a tube that is inserted into your bladder, which is used to empty the bladder and collect urine) :

- Yes
- No

Do you take any medication at this period : if yes what is ? :

- Yes
- No

Do you have any family history for UTI :

- Yes
- No

Do you have any prostate issue :

- Yes
- No

Appendix 2 : *Escherichia coli* new strains

1- The GenBank accession numbers of Par E samples sequencing

BankIt2910277 BSeq#1	PQ838433
BankIt2910282 BSeq#1	PQ838434
BankIt2910284 BSeq#1	PQ838435
BankIt2910340 BSeq#1	PQ838436
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BankIt2910349 BSeq#1	PQ838438
BankIt2910352 BSeq#1	PQ838439
BankIt2910353 BSeq#1	PQ838440
BankIt2910357 BSeq#1	PQ838441
BankIt2910360 BSeq#1	PQ838442
BankIt2910386 BSeq#1	PQ838443
BankIt2910388 BSeq#1	PQ838444
BankIt2910391 BSeq#1	PQ838445

1- The GenBank accession numbers of csgD samples sequencing

BankIt2915342 BSeq#1	PV012308
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BankIt2915353 BSeq#1	PV012310
BankIt2915363 BSeq#1	PV012311
BankIt2915377 BSeq#1	PV012312
BankIt2915390 BSeq#1	PV012313
BankIt2915397 BSeq#1	PV012314
BankIt2915416 BSeq#1	PV012315
BankIt2915428 BSeq#1	PV012316
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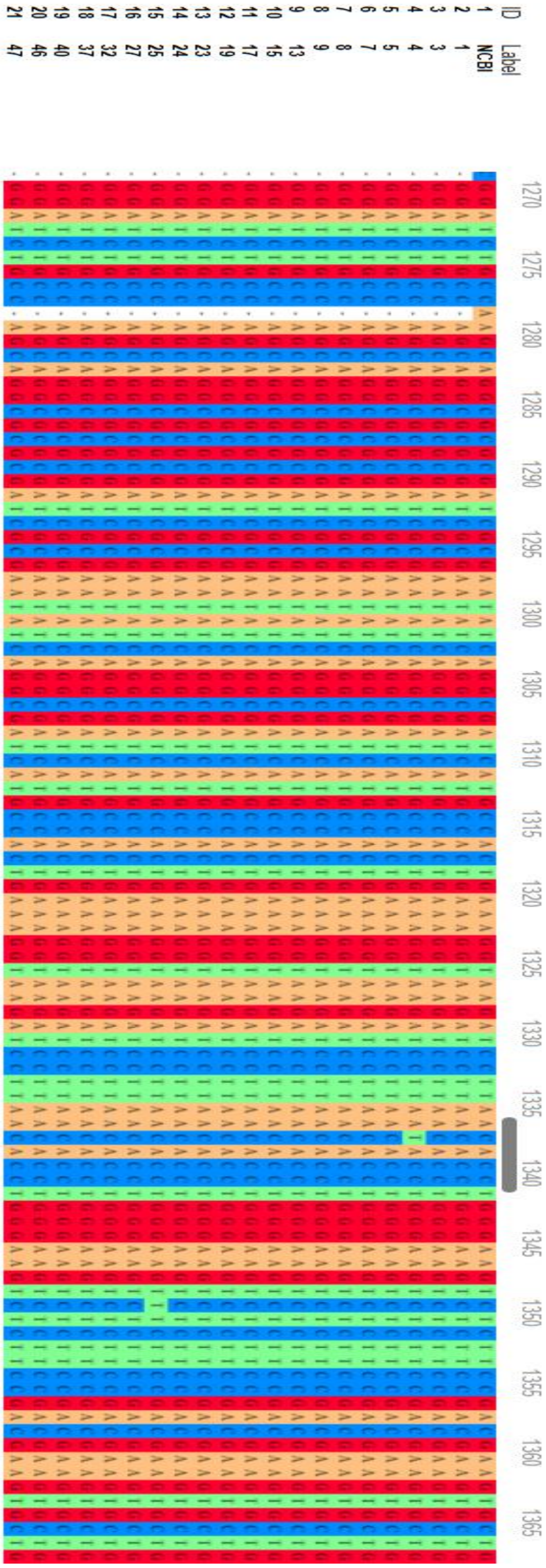
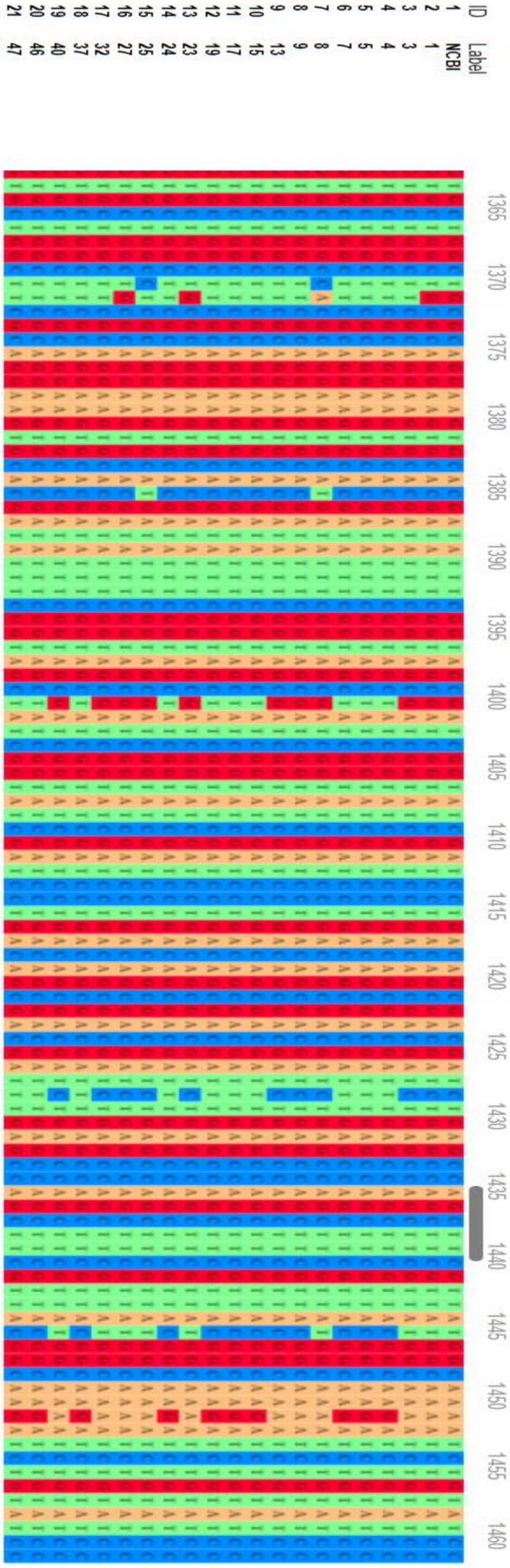
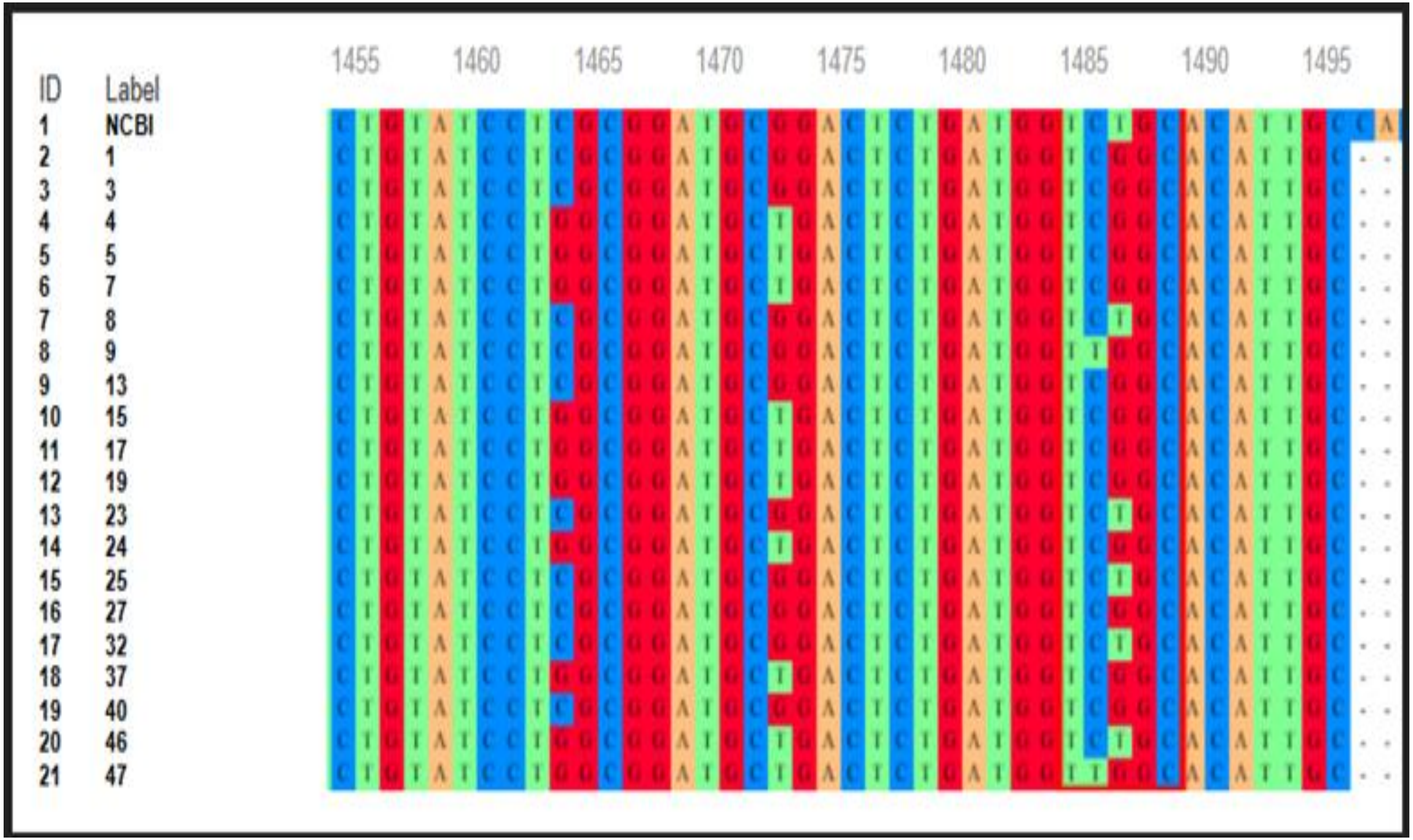
Appendix 3 par E sequencing forward strand







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3	3	CTGTATCCTCGCGGATGCGGACTCTGATGGTCGGCACATTGC..
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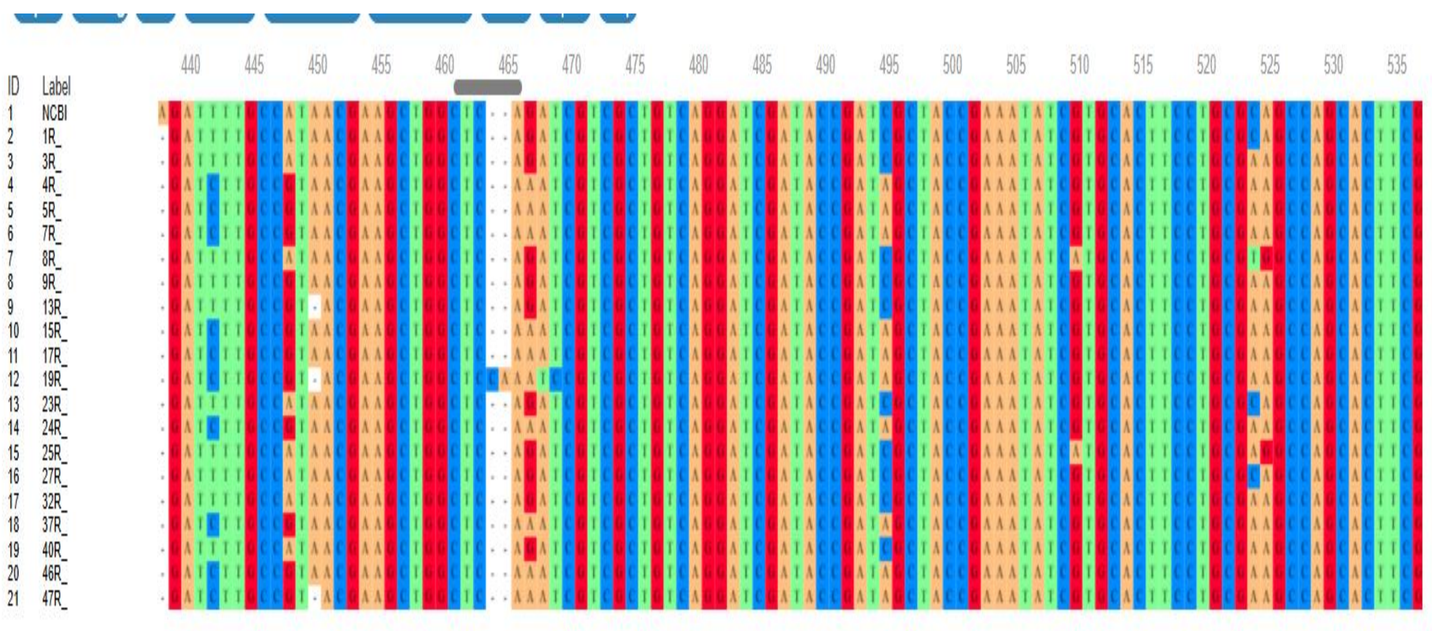
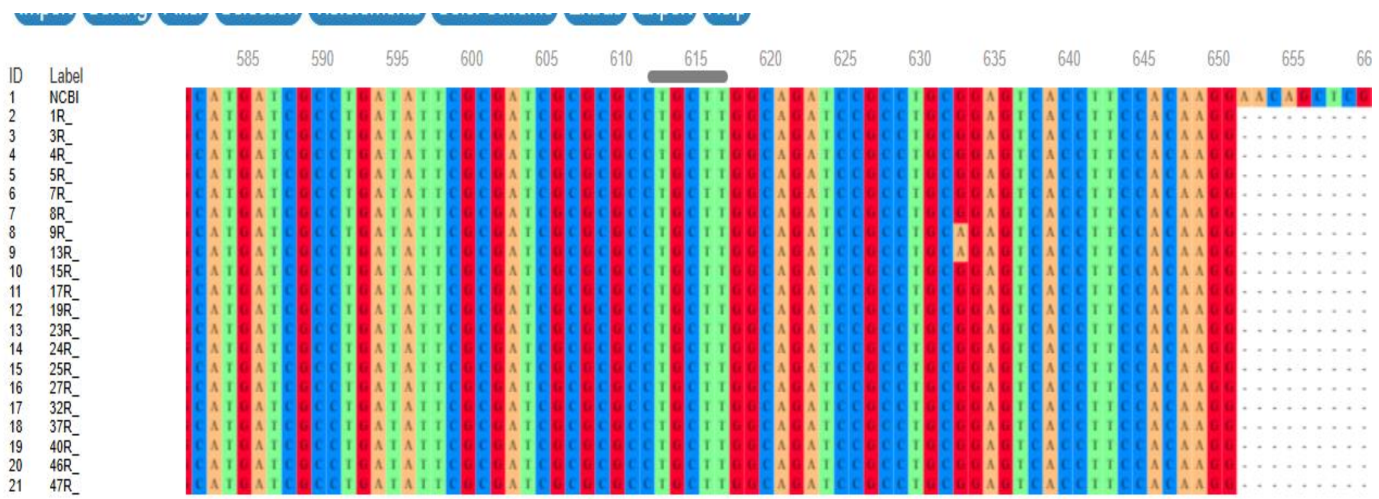
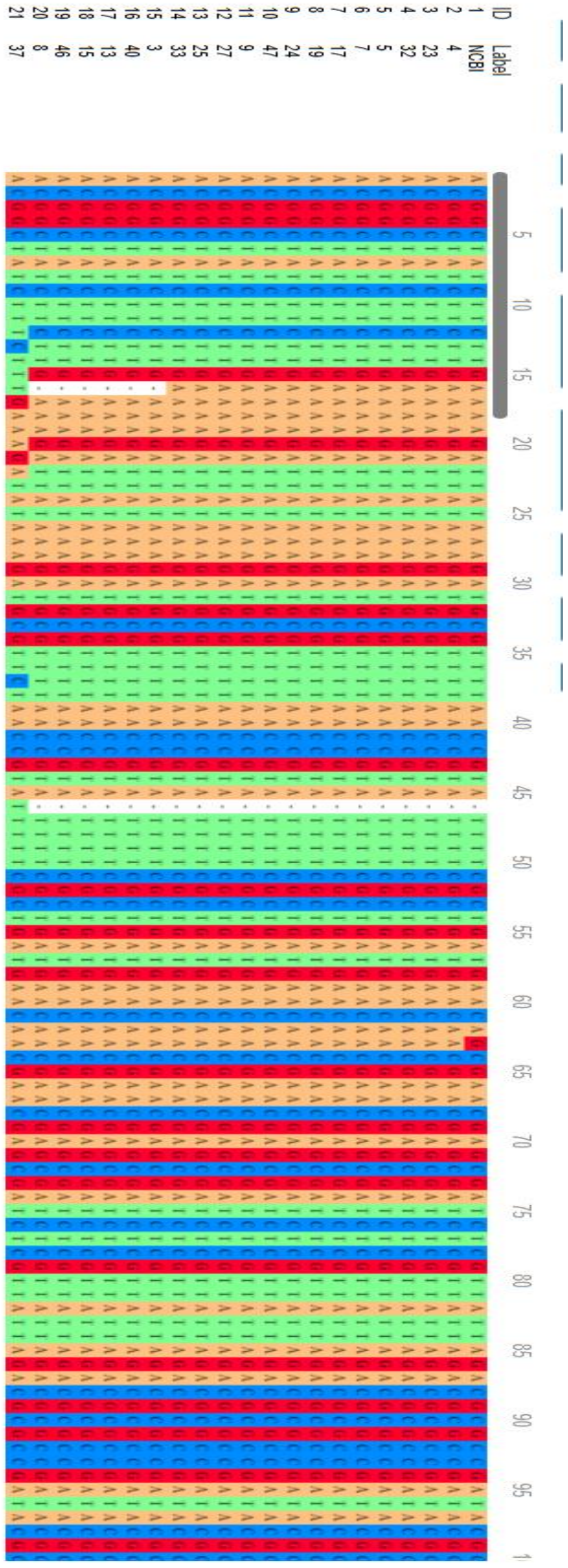
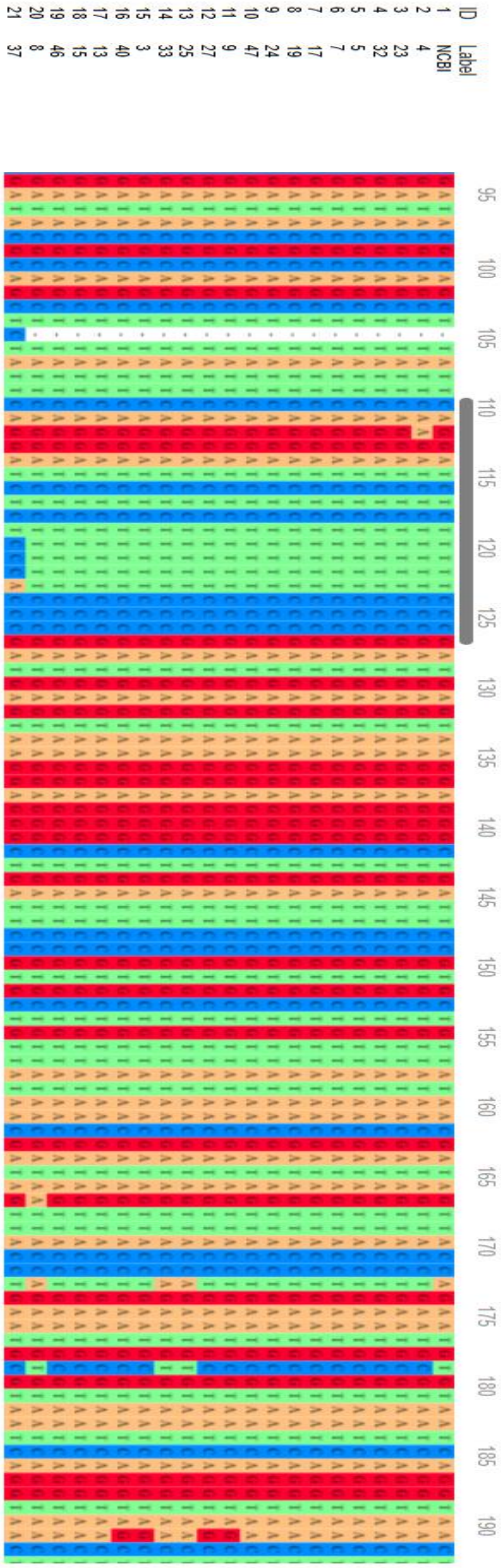
Appendix 4 par E sequencing revers strand





Appendix ٥ csgD sequencing forward strand



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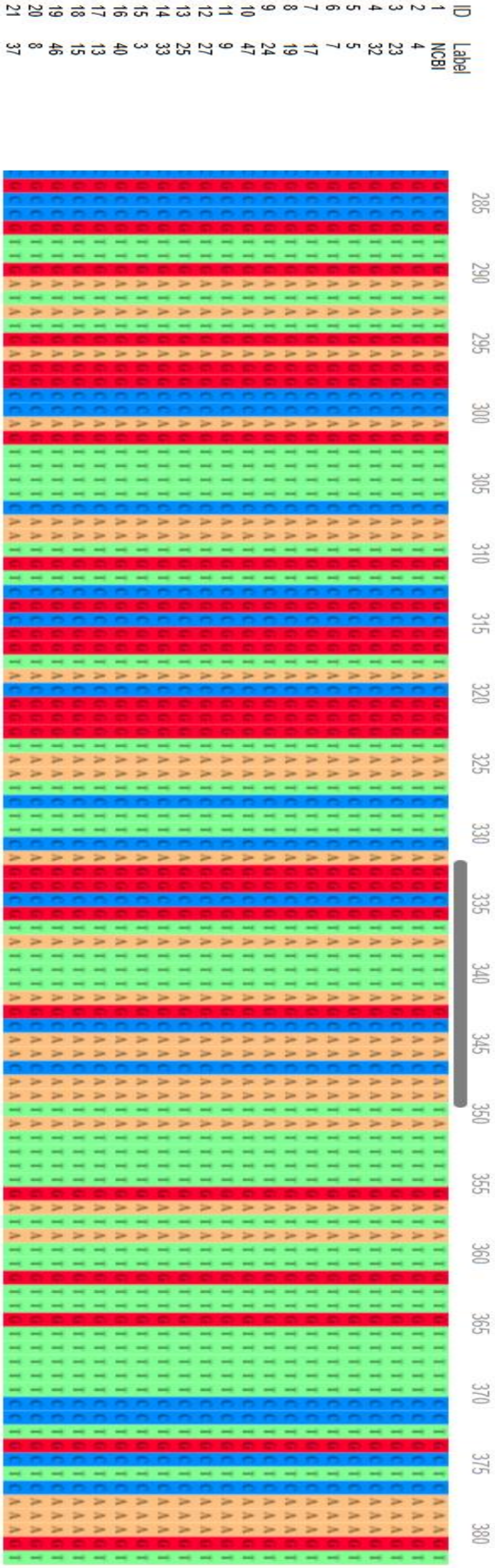
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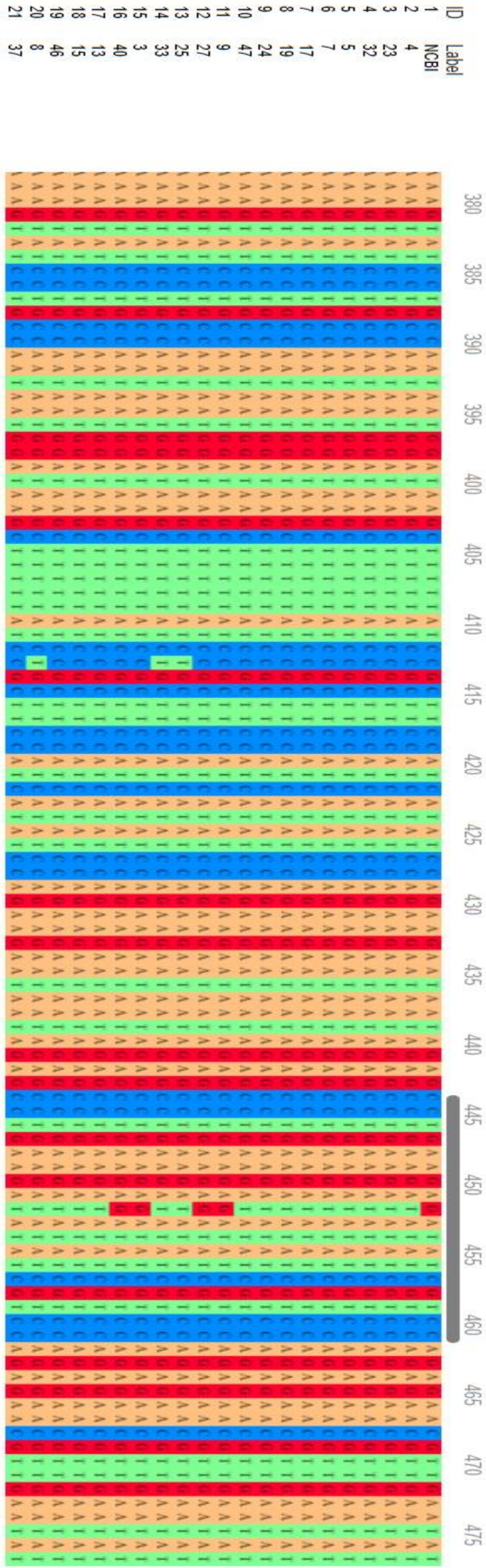
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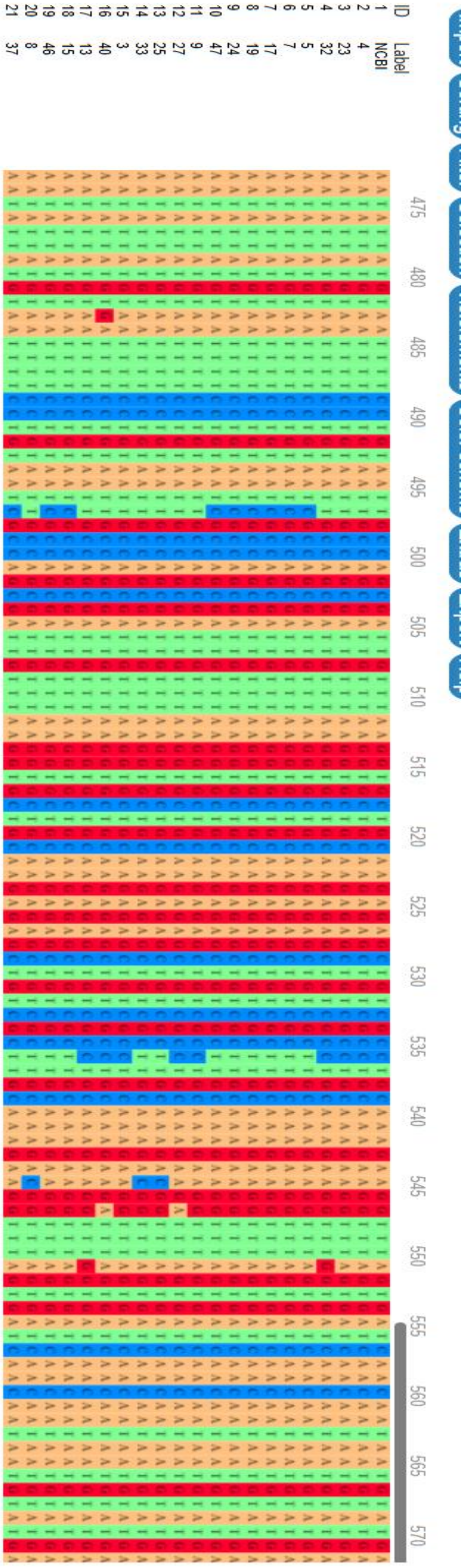
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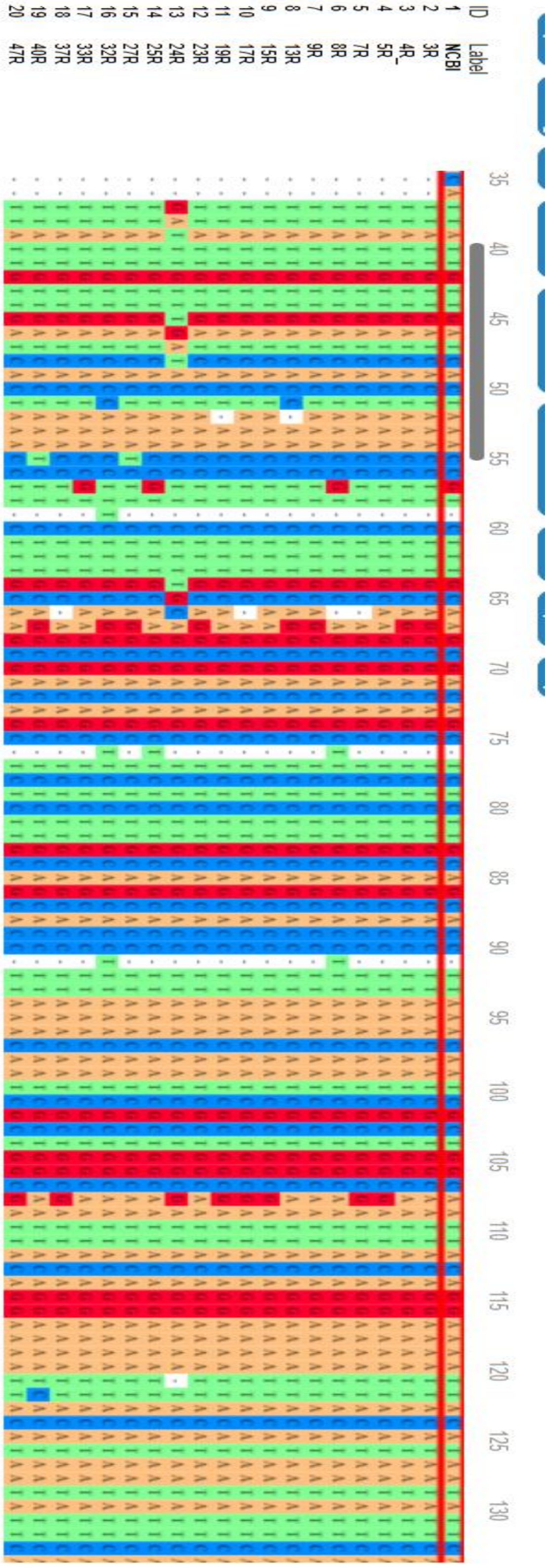
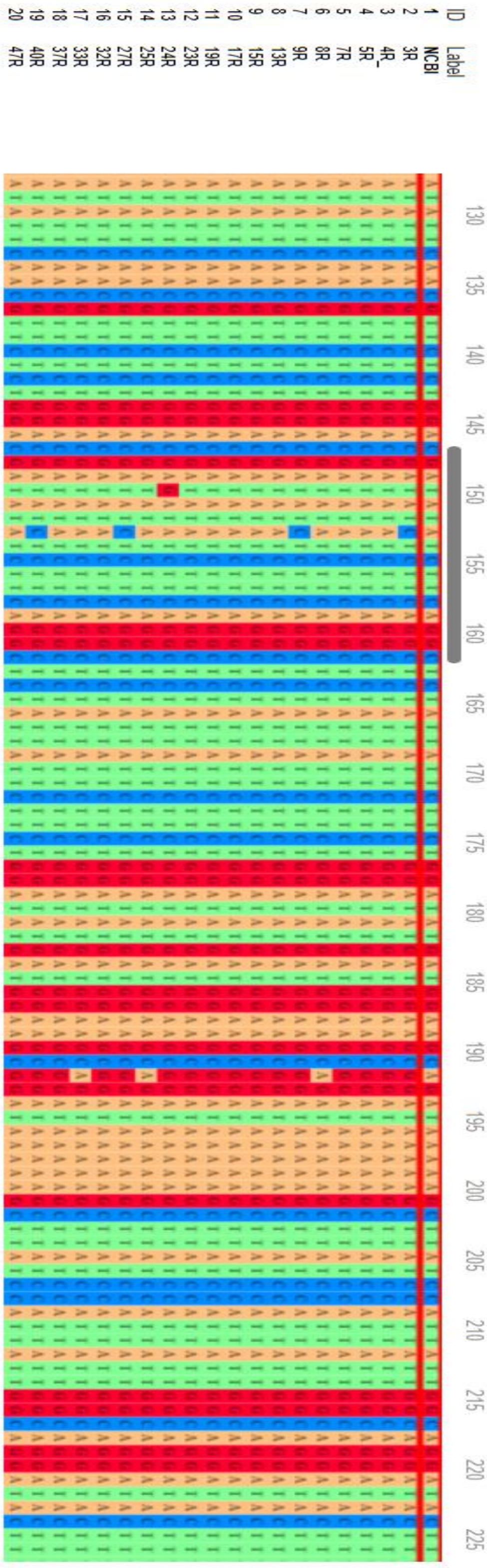
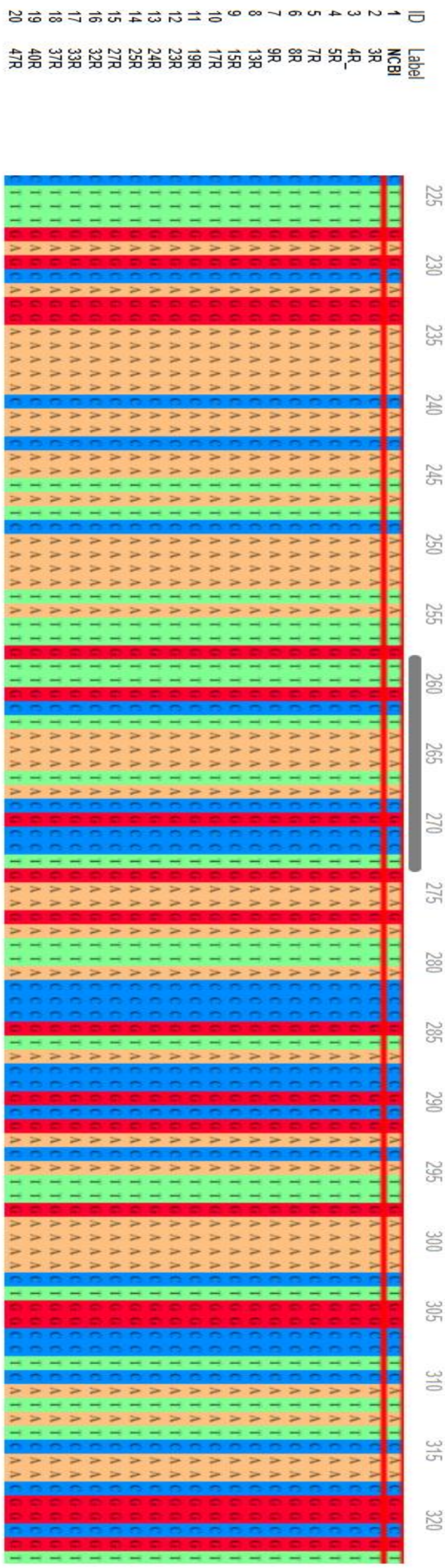
Appendix 6 csgD sequencing revers strand



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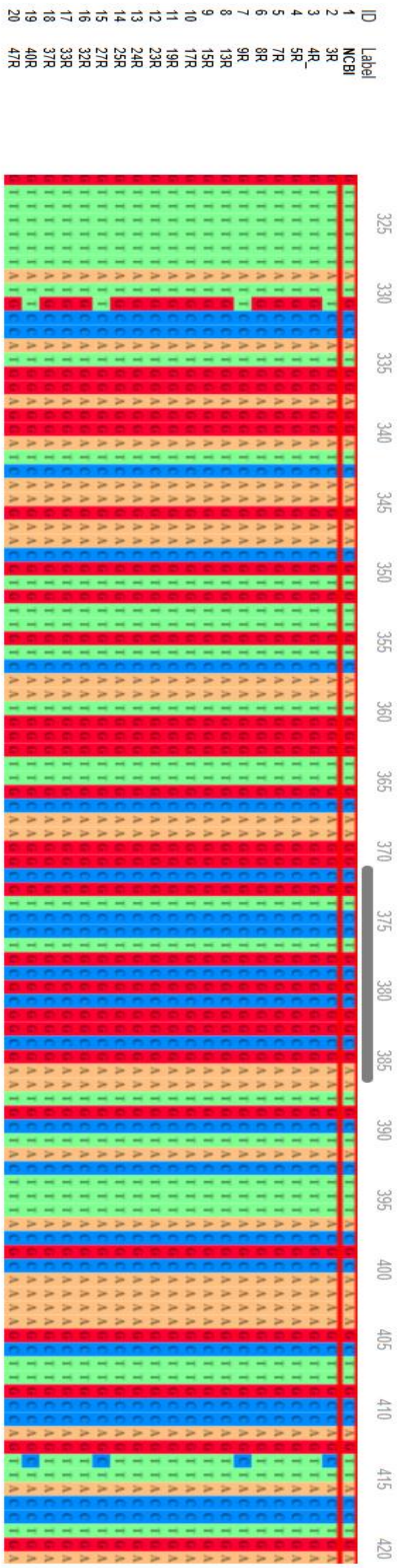
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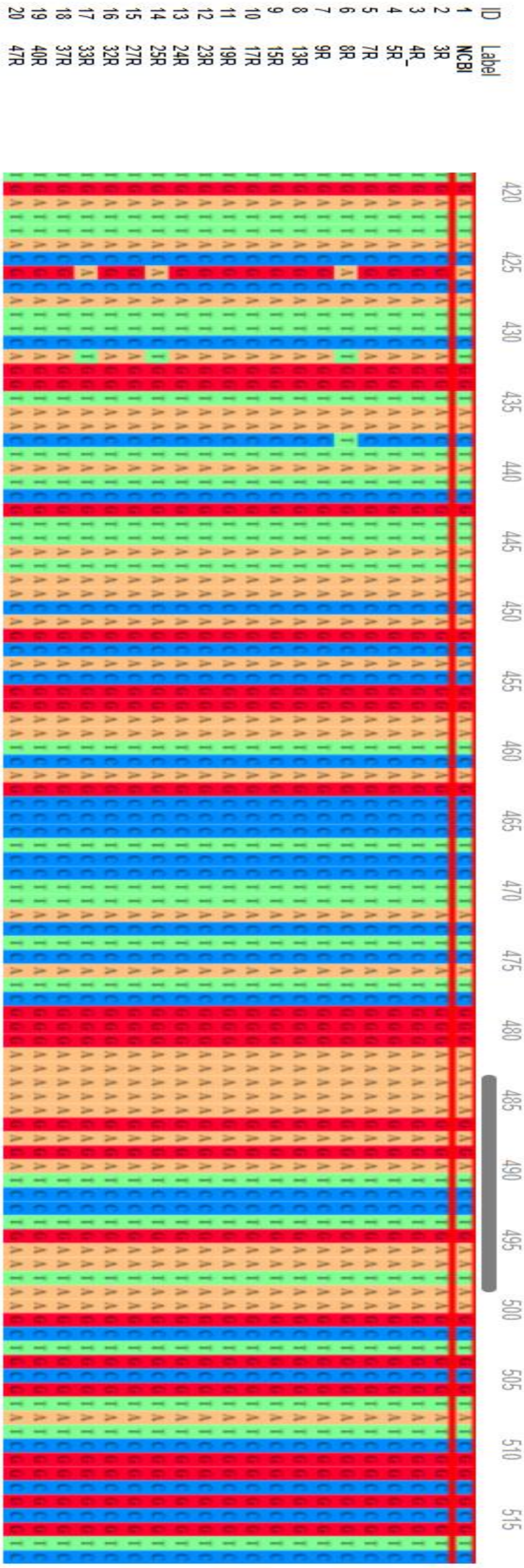
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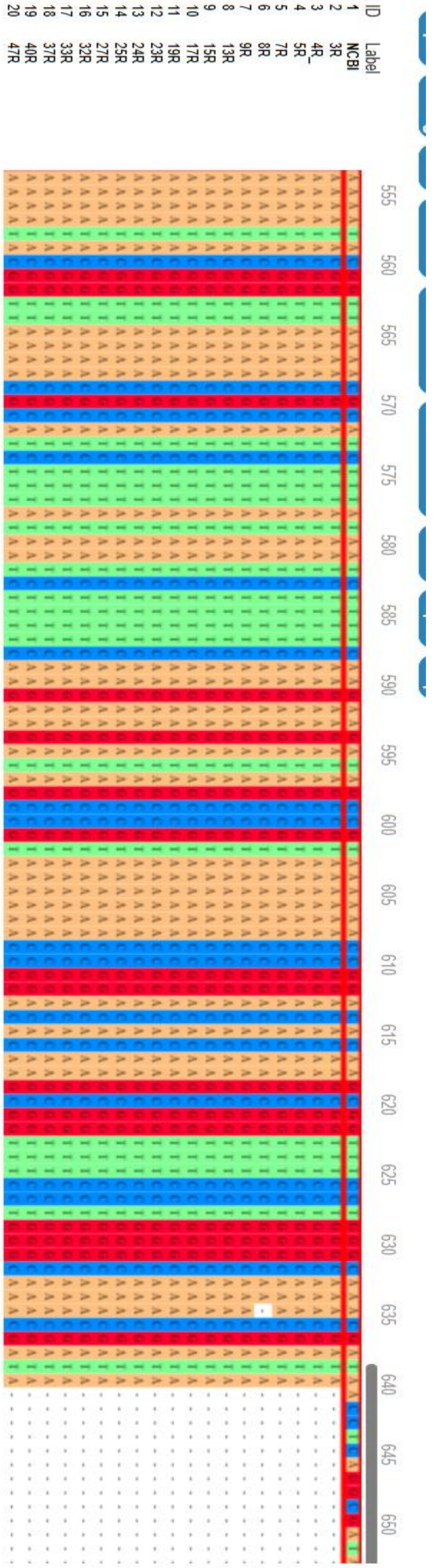
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الخلاصة

على الرغم من أن التثقيف الصحي ونمط الحياة كانا أفضل، لا تزال بعض المشاكل الصحية تتمتع بمعدل عالٍ من العدوى، ومن بينها التهاب المسالك البولية. المراضة والوفيات المرتبطة مع التهاب المسالك البولية تبين انها ١٥٠ مليون حالة في العالم سنويا.

التهاب المسالك البولية بشكل شائع يحدث بسبب مسببات الأمراض البكتيرية التي تؤثر غالبًا على الجهاز البولي، بما في ذلك الكلى والحالب والمثانة والإحليل. تغزو المسببات البكتيرية الجهاز البولي، مما يؤثر على الجهاز البولي السفلي والعلوي، وقد تنتشر أحيانًا إلى مجرى الدم، مما يؤدي إلى عدة متلازمات سريرية مثل الحمى وألم الخاصرة وعسر البول والبيلة الدموية.

هذه الدراسة المقطعية تهدف إلى التحقيق في بعض جينات البايوفلم وجينات مقاومة الكوينولون في الإشريكية القولونية المسببة لالتهابات المسالك البولية لدى المرضى. شملت الدراسة ما مجموعه (٩٦٨) عينة بول من منتصف التدفق تم جمعها من مرضى يعانون من أعراض التهاب المسالك البولية من كلا الجنسين وبفئة عمرية تتراوح بين (٨٠-١ سنة) في مستشفيين حكوميين رئيسيين في محافظة كربلاء المقدسة، وهما مدينة الإمام الحسين الطبية ومستشفى كربلاء التعليمي للأطفال، خلال الفترة من أكتوبر ٢٠٢٣ إلى أبريل ٢٠٢٤.

تم فحص العينات المأخوذة من خلال الفحص العام للبول وزراعة البول لتحديد بكتيريا الإشريكية القولونية واختبار الحساسية للمضادات الحيوية باستخدام نظام الفايتك ٢ المدمج.

كانت النتيجة الإيجابية للنمو البكتيري من جميع العينات ٢٦١ عينة (٢٦,٩٦٪)، مع وجود ٣٤ عينة (٣,٥١٪) من الفطريات، بينما كانت ٦٧٣ عينة (٦٩,٥٢٪) سلبية النمو. بعد استخدام نظام الفايتك كانت البكتيريا سالبة الجرام هي العامل المعدي الأكثر شيوعًا، حيث شكلت (٦٤٪) من إجمالي الزراعات الإيجابية، وكانت الإشريكية القولونية هي النسبة الأعلى (٦١٪) من إجمالي النمو البكتيري الإيجابي، وكانت الإناث أكثر عرضة للإصابة بهذه البكتيريا بنسبة ٧٧,٤٥ %مقارنة بالذكور بنسبة % ٢٢,٥٥.

أظهر اختبار الحساسية للمضادات الحيوية باستخدام نظام الفايتك أن التيكارسيلين كان الأعلى في معدل المقاومة بنسبة ٨٨,٨٩٪ يليه البيبيراسيلين والأمبيسيلين، بينما كانت أكثر المضادات الحيوية فعالية هي الإيميبينيم والميروبينيم بمعدل مقاومة حوالي ٦%

من أهم القضايا المرتبطة بعلاج التهابات المسالك البولية هي ظهور وانتشار البكتيريا المقاومة المتعددة للأدوية. يتكون التوبويزوميراز الرابع من وحدتين فرعيتين يتم (par E and par C), وتتمثل وظيفته الأساسية في فصل الكروموسومات بعد تضاعف الحامض النووي, مقاومة الكوينولون المكتسبة تساهم في الطفرات المنطقة المحددة لمقاومة الكينولون في التوبويزوميراز الرابع.

بروتين (الكيو ان آر) الي هي عضو من عائلة البنتابيتيد المتكرر تحمي الحامض النووي الجايريز والحامض النووي التوبسوميريز الرابع من تثبيط الفلوروكوينولون.

أحد العوامل المهمة التي تساهم في إمراضية الإشريكية القولونية المسببة لالتهابات المسالك البولية هي القدرة على تكوين البايوفلم, تعتبر (سي اس جي دي) وحدة التحكم وتكامل رئيسية لتكوين البايوفلم بسبب قدرتها على تنظيم تعبير المواد الخارجية المصاحبة للبايوفلم. بينما (او ام بي تي) يعمل كآلية دفاعية للبكتيريا من خلال تحلل الببتيدات المضادة للميكروبات التي تفرزها الخلايا الظهارية للمضيف. كما يلعب دورًا كعامل ضراوة، مما يُساهم في انتشار البكتيريا داخل خلايا المضيف.

البي سي آر كشف وجود جين qnrB في (٧,٨٩٪ من العزلات) وجين qnrS في (١٨,٤٪)

وجين ompT في (٦٨,٤٪) وجين Par E في (١٠٠٪) وجين csgD في (٩٧,٤٪)

من عزلات الايكولاي ال ٣٨ والتي أظهرت مقاومة علاجية عالية خصوصا لمجموعة الفلوروكوينلونات.

تم إرسال ٢٠ عزلة من الإشريكية القولونية المقاومة للأدوية (XDR) من اصل ٣٨ عينة

التي شركت في التحديد الجيني والتي أظهرت مقاومة علاجية عالية لمجموعة الفلوروكوينلونات بشكل خاص لسلسلة الجينات لكل من جيني parE و csgD.

أظهرت نتائج تحليل تسلسل الجينات لكلا الجينين وجود تباينات متعددة في مواقع مختلفة من كل عينة في كل من الشريط الأمامي والعكسي.

تم تحليل الشجرة الجينية للجينين باستخدام برنامج وتمت مقارنتها مع العزلات العالمية لبكتيريا الأشريكية القولونية.

جين (الشريط الأمامي من par E)أظهرت نتائج الشجرة التطورية له ان كل العينات ال(٢٠)

أظهرت مواقع مختلفه عند مقارنتها مع عينات المعهد الوطني للمعلومات الحيوية عدا عينة (٢٣).

جين (الشريط الأمامي من csgD) أظهرت نتائج الشجرة التطورية له ان كل العينات ال(٢٠)

أظهرت مواقع مختلفه عند مقارنتها مع عينات المعهد الوطني للمعلومات الحيوية عدا عينة (٣٧).

تخلص هذه الدراسة إلى أن الإشريكية القولونية المقاومة للأدوية بشكل واسع كانت البكتيريا الأكثر انتشارًا بين مرضى التهابات المسالك البولية، وأن لديها معدل طفرات عالي في المنطقة المستهدفة مما يجعل هذه البكتيريا أكثر انتشارًا وأصعب في العلاج

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم المختبرات الطبية

دراسة بعض جينات البايوفلم وجينات مقاومة الكوينولون في الإشريكية القولونية المسببة لألتهابات المسالك البولية المعزولة من مرضى التهاب المسالك البولية

رسالة مقدمة الى

مجلس كلية العلوم الطبية التطبيقية – جامعة كربلاء
وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

كتبت بواسطة
مازن جعفر محمد رضا
بكالوريوس تحليلات المرضية / كلية الصفوة الجامعة ٢٠١٨

بإشراف
أ.م .د. إسراء سعيد عباس
أ.م د. أمير كاظم حنون

١٤٤٦ هـ **2025**
م