MOLECULAR IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PROFILES OF *ESCHERICHIA COLI* ISOLATED IN URINE CULTURES FROM SHAMIYA STATE HOSPITAL (IRAQ)

MASTER THESIS

Ali Hameed Neamah ALHUSSEINI

Supervisor
Prof. Dr. Mehmet Oğuz ÖZTÜRK

DEPARTMENT OF
MOLECULAR BIOLOGY AND GENETICS
June 2024

For this study, ethics committee approval was received from Afyon Kocatepe University, Science and Engineering Sciences Scientific Research and Publication Ethics Board, dated 02.10.2023 and numbered 2023/32.

AFYON KOCATEPE UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

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ABSTRACT

M.Sc. Thesis

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Afyon Kocatepe University

Graduate School of Natural and Applied Sciences

Department of Molecular Biology and Genetic

Supervisor: Prof. Dr. Mehmet Oğuz ÖZTÜRK

In this study, *Escherichia coli* strains isolated from urine samples of patients admitted to Shamiya State Hospital (Iraq) were identified by conventional and automatic microbiological methods. *Escherichia coli* by percentage 29%, *Staphylococcus aureus* by percentage 21%, *Klebsiella pneumoniae* by percentage 18%, *Proteus mirabilis* by percentage 12%, *Enterococcus faecalis* by percentage 16% and *Pseudomonas aeruginosa* by percentage 4%. In our study, we used 11 Antibiotics (CFM, AM, AK, CN, CTX, ATM, CIP, LEV, IPM, TET, MRP) to show resistant, intermediate and susceptibility of *Escherichia coli*. The presence of Tet-A, Qnr-B, Ctx-M, TEM resistance genes in the identified *E. coli* strains was investigated by PCR. The CTX gene is a resistant gene compared to other genes in the total of bacterial isolates. The TET-A and TEM genes are a weakly resistant genes compared to the CTX gene. The QNRB gene is the weakest resistant gene compared to the CTX gene among the total isolates of bacteria. Demographic characteristics of individuals from whom *E. coli* strains were isolated were determined. The results obtained contributed to the development of clinical strategies for the prevention and management of *E. coli* infections causing UPEC in the Shamiya region.

2024, xiv + 107 page

Keywords: Ctx-M, *Escherichia coli*, Qnr-B, Shamiya-Iraq, Tet-A, Tem.

ÖZET

Yüksek Lisans Tezi

ŞAMİYE DEVLET HASTANESİ (IRAK) İDRAR KÜLTÜRLERİNDEN İZOLE EDİLEN ESCHERICHIA COLI'NİN MOLEKÜLER TANIMLANMASI VE ANTİMİKROBİYAL DİRENÇ PROFİLLERİ

Ali Hameed Neamah ALHUSSEINI
Afyon Kocatepe Üniversitesi
Fen Bilimleri Enstitüsü
Moleküler Biyoloji ve Genetik Anabilim Dalı
Danışman: Prof. Dr. Mehmet Oğuz ÖZTÜRK

Amaç: Bu çalışmada, Shamiya Devlet Hastanesi'ne (Irak) başvuran hastaların idrar örneklerinden izole edilen *Escherichia coli* suşlarının konvansiyonel ve otomatik mikrobiyolojik yöntemlerle tanımlanması yapılacaktır. Tanımlanan *E. coli* suşlarının antimikrobiyal duyarlılıkları otomatik mikrobiyolojik sistem ile belirlenecektir. Tanımlanan *E. coli* suşlarında Tet-A, Qnr-B, Ctx-M, TEM direnç genlerinin varlığı PCR ile araştırılacaktır. *E. coli* suşlarının izole edildiği bireylerin demografik özellikleri belirlenecektir. Elde edilen sonuçlar, Shamiya bölgesinde UPEC'e neden olan *E. coli* enfeksiyonlarının önlenmesi ve yönetimine yönelik klinik stratejilerin geliştirilmesine katkı sağlayacaktır.

Yöntemler: Eylül'den Aralık 2023'e kadar, Irak'ın Al-Qadisiya Valiliği'ndeki Al-Shamiya şehrinde bulunan AL-Shamiya Genel Hastanesi'nden rastgele 157 örnek toplandı. Numuneler sterilize edilmiş kaplarda toplandı. Orta akım idrar örnekleri Blood Agar, MacConkey Agar ve Sistein Laktoz üzerine inoküle edildi ve gece boyunca 37°C'de inkübe edildi. Bakteriyel büyüme, MacConkey Agar ve Chrome Agar üzerinde kolonilerin rengi ve morfolojisi temel alınarak tahminen tanımlandı. İzolatların kimliğini doğrulamak için Gram boyama, motilite testi, katalaz testi, oksidaz testi ve izolatlarla ilgili olduğu düşünülen diğer biyokimyasal analizleri içeren standart tanımlama protokollerine tabi tutuldu.

Mikroskobik test, kültür ortamının morfolojik özellikleri ve biyokimyasal testler dikkate alınarak *E. coli* izole edildi ve tanımlandı. Test izolatlarının önceden hazırlanmış tüm smearlarına gram boyama uygulandı ve bakteri hücrelerinin düzeni, şekli ve boyutu, 100X'e ayarlanmış bir ışık mikroskobu yardımıyla gözlendi.

Üreticinin talimatlarına göre tüm *Escherichia coli* izolatlarını tanımlamak için Vitek2® sistemi (BioMerieux-France) kullanıldı. Her test izolatının standart ortamı bir taşıyıcı istasyonda hazırlandı ve bir VITEK kasetine yerleştirildi.

Bir barkod kullanılarak numune ile VITEK kartı arasında bağlantı kuruldu. Ayrıca cihaza numunelerin inkübe edilmesini ve sonuçların okunmasını sağlayan bir kaset yüklendi. İlk sonuçlar ya doğrudan cihazdan ya da sonuçların okunması için özel olarak hazırlanmış tablodan okundu.

Klinik Laboratuvar Standartları Enstitüsü'nün 2021 yılında yaptığı kılavuz doğrultusunda *E. coli* için antibiyotik duyarlılık testi (AST), Kirby-Bauer disk difüzyon yöntemi kullanılarak yapıldı. Besleyici et suyunda AST için bir aşı oluşturmak üzere 0,5 McFarland standardına (1,5x CFU/ml) uyan 5/6 *E. coli* kolonisi kullanıldı. Steril bir pamuklu çubuk, inokulum süspansiyonuna daldırıldıktan sonra 37°C'de inoküle edildi ve 15 dakika içinde tüp duvarının sıvı seviyesinin üzerine konuldu.

Muller Hilton Agar'ı temsil eden MHA plakasını nemlendirildi. AST için Antibatc firmasının antibiyotik diskleri kullanıldı. CLSI 2021 yorumlama kriterleri ml cinsinden inhibisyon bölgesini tahmin etmek için kullanıldı ve izolatlar hassas, orta veya dirençli olarak sınıflandırıldı. 300 ul damıtılmış suyun üç ila beş taze ve saf *E. coli* kolonisi ile aşılanması, bir MacConkey Agar plakası kullanılarak yapıldı.

Hücreler 100^{o}C'lik su banyosunda 20 dakika süreyle ısıtıldı. Bundan sonra hücreler, yarım saat süreyle buz üzerinde hızlı bir şekilde soğutuldu ve ardından kalan hücre artıklarını çıkarmak için 8500 rpm'de on dakika süreyle santrifüjlendi. Sonunda süpernatandan DNA şablonu oluşturuldu.

Toplanan veriler üzerinde istatistiksel analizler yaptıktan sonra değişkenlerin mutlak ve bağıl (%) frekanslarına bakarak dağılımlar çıkarıldı. İdrar yolu enfeksiyonu olan bireylerden elde edilen UPEC suşlarının çeşitli faktörleri arasındaki anlamlı ilişkileri tanımlandı. Ki-kare ($\chi 2$) testleri IBM SPSS istatistik yazılımı (Sürüm 20, SPSS Inc, Amerika Birleşik Devletleri) kullanılarak yapıldı. Her iki test için de < 0,01 P değerleri istatistiksel olarak anlamlı değer kabul edildi.

Bulgular: Araştırmada Shamiya Devlet Hastanesi'ne (Irak) başvuran 157 hastaların idrar örneklerini içerdi. Örneklem bakteri üremesi olmayan 66 hastayı (%42) ve bakteri üremesi olan 91 (%58) hastayı içermektedir. P değerinden (0,005) sıfır hipotezi reddedilmiş ancak alternatif hipotez kabul edildi. Buna göre bakteri büyümesi ile büyüme olmaması arasındaki farkın anlamlı düzey değeri 0,01 ila 0,05 arasında oldu.

Toplam örneklem 31 erkek (%19,7) ve 126 (%80,3) kadın hastadan oluşmaktadır. Toplam örneklemde birinci yaş grubunda (14-29 yaş) 87 hasta (%55,4), ikinci yaş grubunda (30-49 yaş) 46 hasta (%29,3) ve üçüncü yaş grubunda (50-79 yaş) 24 hasta (%15,3) tanımlandı.

Çalışmada *Escherichia coli* suşlarının Dirençli, Orta ve Hassas seviyedeki özelliklerini göstermek için 11 Antibiyotik (CFM, AM, AK, CN, CTX, ATM, CIP, LEV, IPM, TET, MRP) kullanıldı. Antibiyotik-CFM'ye 22 *E. coli* suşunun (%84,6) dirençli olduğu görüldü. Antibiyotikt-AM'ye 25 *E. coli* suşunun (%96,2) dirençli olduğu görüldü. Antibiyotik-AK'ye 13 *E. coli* suşunun (%50,0) dirençli olduğu görüldü. Antibiyotik-CN'ye 5 *E. coli* suşunun (%19,2) dirençli olduğu görüldü.

Antibiyotik-CTX'ye 25 *E. coli* suşunun (%96,2) dirençli olduğu görüldü. Antibiyotik-ATM'ye 15 *E. coli* suşunun (%57,7) dirençli olduğu görüldü. Antibiyotik-CIP'ye 17 *E. coli* suşunun (%65,4) dirençli olduğu görüldü. Antibiyotik-LEV'ye 15 *E. coli* suşunun (%57,7) dirençli olduğu görüldü. Antibiyotik-IPM'ye 3 *E. coli* suşunun (%11,6) dirençli olduğu görüldü. Antibiyotik-TET'ye 26 *E. coli* suşunun (%100,0) dirençli olduğu görüldü. Antibiyotik-MRP'ye dirençli *E. coli* suşuna rastlanmadı (%0,000).

Moleküler datalara göre, CTX'in (21/26, %80,8) değer ile diğer direnç genleri arasında en dirençli gen olduğu görüldü. TETA geni diğer genlere göre ikinci sırada direnç geni oldu (9/26, %34,6). TEM geni diğer genlere göre üçüncü sırada konumlandı (7/26, %30,0). QNRB geni diğer genlere göre daha az dirençli olduğu görüldü (6/26, %23,1).

Sonuç: Shamiya Devlet Hastanesi'ne (Irak) başvuran 157 hastaların idrar örneklerinden izole edilen ve İYE etkeni olan *Escherichia coli* suşlarının konvansiyonel ve otomatik mikrobiyolojik yöntemlerle tanımlanması yapıldı. Tanımlanan *E. coli* suşlarının antimikrobiyal duyarlılıkları otomatik mikrobiyolojik sistem ile belirlendi. İzole edilen *E. coli* suşlarında Tet-A, Qnr-B, Ctx-M, TEM direnç genlerinin varlığı moleküler yöntemlerle araştırıldı. *E. coli* suşlarının izole edildiği bireyler yaş ve cinsiyet özelliklerine göre belirlendi. Elde edilen sonuçlar, Shamiya bölgesinde UPEC'e neden olan *E. coli* enfeksiyonların önlenmesi, yönetimi ve klinik stratejilerin geliştirilmesine katkıda bulundu.

2024, xiv + 107 sayfa

Anahtar Kelimeler: Ctx-M, *Escherichia coli*, Qnr-B, Shamiya-Iraq, Tet-A, Tem.

ACKNOWLEDGEMENTS

I would like to thank my thesis supervisor Prof. Dr. M. Oğuz ÖZTÜRK for his great contributions to the subject of this research, the guidance of the experimental studies, the evaluation of the results and the writing process, Prof. Dr. Ali Nafiz EKİZ and Prof. Dr. Ferruh AŞÇI for their help during the research and writing process, and my professors and friends who helped me with their suggestions and criticisms in every subject.

I would like to thank my family for their financial and moral support throughout this research.

Ali Hameed Neamah ALHUSSEINI

Afyonkarahisar 2024

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LIST OF SYMBOLS and ABBREVIATIONS

Symbols	
bp	base pairs
CaOx	calcium oxalate
CaPhos	calcium phosphate
H2O2	hydrogen peroxide
dH2O	Distilled water
HCl	Hydrochloric acid
mL	Milliliter
µM	Micromolar
µL	Microliter
$\chi 2$	chi-square

Abbreviations	
AST	Antimicrobial Susceptibility testing
bla	Beta lactamase
CDC	Disease Control Centers
cfu	Cell unite forming
CLSI	Clinical laboratory standards institute
CNF	Cytotoxic necrotizing factor
CTX-M	beta-lactamases hydrolytic activity against cefotaxime antibiotic
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EMB	Eosin methylene blue
FQs	Fluoroquinolones
ICD	international classification diseases
IMVC	Indole, Methyl Red, Voges-Proskauer, Citrate Utilization
MDR	Multi drug resist
MSU	Mid stream urine
MHT	Muller Hilton agar
PCR	Polymerase chain reaction
RBCs	Red blood cells
SB	symptomatic bacteriuria
TBE	Tris-borate-EDTA
TEM	beta-lactamases called after first patient isolated from Temarian
TSI	Triple Sugar Iron agar
UPEC	Uropathogenic *E.coli*
UTI	Urinary Tract Infection
WHO	World Health Organization
β-Lactamase	Beta-lactam

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1. INTRODUCTION

Urinary tract infection (UTI) is an inflammatory occurrence that occurs as a affect of the settlement of many microorganisms in the human urinary tract system. UTI describes infection occurring anywhere in the urinary system, from the urethra to the bladder sac, from the ureters to the kidneys. UTI cases are examined in two groups: upper and lower urinary tract infections. While lower urinary tract infection occurs in the bladder sac and urethra, upper urinary tract infection occurs in ureter, pelvis, kidneys (Lane and Takhar 2011).

UTI is mostly caused by the colonization of the uroepithelium by microorganisms belonging to the colon and perianal region flora. Bacteria, most of which are Gram negative, cause UTI. *Escherichia coli* ranks first among gram-negative bacteria. This is followed by other Gram-negative bacteria such as *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* etc. and Gram-positive bacteria such as *Enterococcus faecalis*, *Staphyloccoccus aureus* (Hooton and Stamm 1997).

Some *Escherichia coli* strains may exhibit pathogenic properties due to various virulence factors they possess. For this reason, pathogenic *E. coli* strains are the most broad pathogenic bacteria in infectious diseases. Pathogenic *E. coli* strains of the urinary system are defined as uropathogenic *E. coli* (Johnson and Russo 2005).

UPEC strains characteristically have virulence factors; adhesins, toxins, proteases, invasins, serum resistance proteins and polysaccharide sheaths. With these virulence factors, UPEC strains can colonize and invade the host cell, cause damage to the host tissues, and escape from the host's immune system (Rasmussen et al. 2012).

UPEC is associated with various risk factors such as unhygienic environmental, gender activity, vaginal infection, diabetes, obesity and genetic predisposition. UPEC is the second most common infection causing hospitalization after pneumonia. If not diagnosed and treated quickly, it can cause sepsis. In case of failure or delay in treatment, it can cause mortality between 20-50% (Umar et al. 2016).

Identification of resistance factors in pathogenic bacteria is important for the correct and effective fight against these pathogens. The resistance problem that has developed as a data of widespread antibiotic usage has become an important problem in the therapy of urinary tract infections. Inadequacy in infection control evaluates and increased use of antibiotics in animals are other reasons for the increase in resistance. It is necessary to know regional resistances and conduct studies for this purpose, especially for the correct selection of antibiotics to be used in empirical treatment (Chomarat 2000).

In this study, *Escherichia coli* strains isolated from urine samples of patients admitted to Shamiya State Hospital (Iraq) will be identified by conventional and automated microbiological procedures. Antimicrobial susceptibilities of identified *E. coli* strains will be determined by conventional microbiological procedures.. This study of Tet-A, Qnr-B, Ctx-M, TEM resistance genes in the identified *E. coli* strains will be investigated by PCR. Demographic characteristics of individuals from whom positive resistant *E. coli* strains were isolated using automated microbiological systems and PCR methods will be identified. The results obtained will contribute to the evolution of clinical strategies for the prevention and management of *E. coli* infections that cause UPEC in the Shamiya region.

2. LITERATURE REVIEW

2.1 Description of Urinary Tract Infections

The human urinary system consists of various components such as the kidneys, the ureter, urethra, urine bladder, and male and female genital organ. Typically, humans produce 1.4 liters of urine each day, falling within the normal range of 0.6 to 2.6 liters per day (Rose et al. 2015). Urine displays distinct biochemical traits, including a pH level of 5.8, a color ranges from pale yellow to deep amber, the lack of a substance called red blood cells, protein and pus cells. Changes in the biochemical properties of urine, like fluctuations in urination frequency or inflammation in the kidneys, could suggest problems with the ureter, urethra, urinary bladder, and genital organs. Urinary tract infections (UTIs) are frequently caused by various bacteria such as the following: "*Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Enterococcus faecalis*, *Staphylococcus aureus*, *Citrobacter* spp., *Morganella* sp., *Providencia* sp., *Serratia* sp., *Mycoplasma* sp., and others" (Zaha et al. 2019).

Escherichia coli is the more frequently encountered bacterial species among these germs, with *Klebsiella* spp. following closely behind. Urinary tract infections, are most frequently encountered by females because that the unique structure of their urinary tract and reproductive organs. There is a reason for the increased likelihood of bacteria reaching the urinary bladder in females - their urethra is shorter. In females, the close proximity of the urethral opening to sources of bacteria from the anus is a contributing factor to the frequency of urinary tract infections (Tamalli et al. 2013).

Possible causes of urinary tract infections can be attributed to unprotected sexual activity, insufficient hydration, and the use of unsanitary garments, which is particularly common in rural areas. Common signs of urinary tract infections (UTIs) include acute lower back pain, discomfort or burning sensation while urinating, cloudy or discolored urine, and presence of fever or chills. Pyelonephritis, an inflammatory condition affecting the kidney, arises when infections extend to the kidney (Chandra et al. 2020). Urinary tract infections (UTIs) are popular bacterial illnesses that impact approximately

150 million people worldwide each year (Stamm and Norrby 2001). In 2007, approximately 10.5 million office visits were recorded in the United States for symptoms associated with urinary tract infections (UTIs), making up 0.9% of total ambulatory visits. In addition, there were an estimated 2–33 million emergency department visits for urinary tract infection (UTI) symptoms during that time period, as reported by Schappert and Rechtsteiner in 2011 and Foxman in 2010.The current costs related to these infections, including healthcare spending and lost productivity, it amounts to approximately US$3.5 billion annually in the United States alone. Urinary tract infections have an important impact on the health of male infants, elderly males, and females of all age groups. Several significant effects can arise from urinary tract infections, including recurrent relapses, pyelonephritis leading to septicemia, renal damage in newborns, premature birth, and complications resulting from excessive use of antimicrobials. These complications include the emergence of high-standard antibiotic resistance, the development of Clostridium difficile colitis (Flores-Mireleset al. 2015).

UTIs are further prevalent in female compared to male, primarily because of anatomical and physiological factors. Female anatomy increases the risk of uropathogens reaching the bladder compared to males. The proximity of the vaginal cavity and rectal opening to the urethral opening, along with the smaller length of the female urethra, allows bacteria to more easily ascend to the female bladder (Foxman 2014; Geerlings 2016). There are two types of urinary tract infections: lower urinary tract infections, also renowned as cystitis, and upper urinary tract infections, also renowned as pyelonephritis. The severity to patient's condition increases as the microorganism's invasion of the urinary tract becomes more pronounced (Smelov et al. 2016). UTIs distributing into two categories: complicated and uncomplicated. Uncomplicated UTIs are commonly observed in individuals with a normal urinary tract system, often occurring in community-acquired infections. On the other hand, patients with a defective or obstructive urinary tract system, or those who using catheters, are classified as having complicated UTIs. Treating complicated UTIs is known to be more difficult (Karam et al. 2019). According to a 2010 study by Giesen etal., 7% of women of all ages experience acute uncomplicated UTIs. This incidence rate reaches its peak in

women aged 15 to 24 years and in those older than 65. According to Epp and Larochelle's 2010 research, 30% of women who contract a urinary tract infection (UTI) may experience a recurrence within a six- to 12-month period. UTIs that are complicated are characterized through workers that weaken the urinary tract or body's defense system. Certain things can make it hard to pee, neurological diseases that make you hold your urine, weakened immune systems, kidney failure, kidney transplants, pregnancy, and the presence of foreign bodies like stones, indwelling catheters, or other drainage devices (Levison and Kaye 2013; Lichtenberger and Hooton 2008). Microorganisms which cause urinary tract infections include both gram-positive and gram-negative bacteria, beside fungi. Uropathogenic *Escherichia coli* is the primary cause of both uncomplicated and complicated (65%) urinary tract infections respectively (Mohammed 2021). Uropathogens include common gram-negative bacteria like *K. pneumoniae*, *P. aeruginosa*, and various *Enterobacter* species. Gram-positive bacteria, including *Staphylococcus saprophyticus*, *Enterococcus* spp., and Group B *Streptococcus*, accounted for approximately 15% of urinary tract infection cases (Naqid et al. 2020). There is an increasing prevalence of antibiotic-resistant urinary bacteria. The main sources of antibiotic-resistant bacteria were comorbid conditions such as diabetes, reflux nephropathy, and nosocomial infections (Guclu et al. 2021).

2.2 Classification of Urinary Tract Infections

2.2.1 Asymptomatic Bacteriuria

Asymptomatic bacteriuria is prevalent in older individuals, particularly among those residing in long-term care facilities (Rowe and Juthani-Mehta 2013). Silent bacteriuria is more likely to happen if you are older, have diabetes, have cognitive impairment, have anatomical problems with your urinary system, or have an indwelling catheter (Ariathiant 2022). Nicolle (1999) a condition when urine samples contain fully 105 colony-forming units per millimeter (cfu/ml) of a uropathagen without any signs or symptoms of a urinary tract infection (Nicolle et al. 2005). The Infectious Diseases of America (IDSA) has outlined its management recommendations for asymptomatic bacteriuria in their 2019 guideline. Asymptomatic bacteriuria must only be tested and

treated in pregnant women or those who are about to undergo invasive urologic operations. Treating bacteriuria that doesn't cause any symptoms in people with diabetes, old age, or spinal cord injuries has not been shown to improve results (Nicolle et al. 2022).

2.2.2 Complex Urinary Tract Infections

The complex urinary tract infection (cUTI) was identified by the presence of at least two symptoms, including chills, rigidity, fever, dysuria, urine frequency, urgency, lower abdominal or pelvic discomfort, and vomiting or nausea. Furthermore, there must be at least one aggravating condition, such as a history of urine retention in men, the presence of a catheter for the urinary tract, restrictive uropathy, or any operational or anatomical anomaly. Urinary tract infections that occur during pregnancy, in patients who have received a renal transplant, in individuals with impaired renal function, and after prostatectomy or radiotherapy are also classified as complicated cases. Urinary tract infections that are complicated have a greater likelihood of treatment not being successful and typically necessitate longer periods of antimicrobial treatment. Urinary tract infections (UTIs), particularly those acquired in healthcare settings, are frequently caused by bacteria that are resistant to multiple drugs (Contreras-Alvarado et al. 2021). Urinary tract infections (UTIs) account for approximately 1.8% of hospitalizations in the United States, with average costs per hospitalization reaching around $10,000. In addition, cUTI has been found to have a significant rate of therapeutic failure (26.6%), hospital readmission (around 9%), and a 30-day mortality rate of 8.7% (Moreno et al. 2023). Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, and *Pseudomonas* species, are the most current pathogens responsible for cUTI. It is significant to know that gram-negative pathogens often become resistant to cephalosporins and β-lactamase/β-lactam inhibitor combinations, even though β-lactam antibiotics are usually prescribed to treatment infections caused by these bacteria. This resistance is primarily due to the production of different beta-lactamase (Ezure et al. 2022). Complicated urinary tract infections in the United States are commonly linked to indwelling catheters, specifically catheter-associated UTIs (CAUTIs) (Zalewska-Piątek et al. 2020).

2.2.3 Uncomplicated Urinary Tract Infections

Annually, almost 12% of women are affected by community-acquired uncomplicated urinary tract infections (uUTIs). *Escherichia coli* is the primary culprit responsible for most cases, and there is a growing prevalence of antibiotic resistance in this organism globally (Stapleton et al. 2020). Uncomplicated urinary tract infections often occur in individuals who are in good health and do not have any anatomical, neurological, or physiological problems in their urinary system. These infections are linked to both lower and upper urinary tract infections, including urethritis, cystitis, and pyelonephritis, respectively (Zalewska-Piątek et al. 2020). *Escherichia coli* (86% of urinary tract infections (UTIs) in females are acute, uncomplicated cystitis, with "*Klebsiella* spp. 5%, *Staphylococcus saprophyticus* 3%, *Proteus* spp. 3%, *Enterobacter* spp. 1.4%, *Citrobacter* spp. 0.8%, and *Enterococcus* spp. 0.5%", *Escherichia coli* is the main bacteria responsible for urinary tract infections, with *Klebsiella* and *Proteus* coming in second and third, respectively.) 5% to 10% of urinary tract infections acquired in healthcare settings are the result of genitourinary system manipulation, while urethral catheterization accounts for 80% of these infections. Engaging in sexual intercourse heightens the risk of bladder infection, as the act introduces germs into the bladder. Utilizing a diaphragm or spermicide, as well as engaging in sexual activity with new or numerous partners and experiencing the first urinary tract infection (UTI) prior to the age of 15, all contribute to an elevated chance of developing a UTI (Lala et al. 2023).

2.2.4 Lower / Upper Urinary Tract Infections

Cystitis is an inflammatory disorder of the mucous membrane of the bladder, which is part of the lower urinary system. The symptoms of the condition include painful urination, frequent urination, sudden need to urinate, bedwetting, blood in the urine, discomfort in the lower abdomen, and foul-smelling urine. Additionally, it may encompass epididymitis, an inflammatory ailment affecting the epididymis. The symptoms consist of discomfort and swelling in the hemiscrotum and might serve as the initial indication of a lower urinary tract infection. Pyelonephritis is a widespread

bacterial illness that affects the renal pelvis and parenchyma in the upper urinary system. The symptoms of this condition encompass fever, chills, and flank discomfort, and can potentially escalate to the point of septic shock/toxemia (A‘t Hoen et al. 2021).Cystitis is linked to several risk factors, such as being female, having had a previous urinary tract infection (UTI), engaging in sexual activity, having a vaginal infection, having diabetes, being obese, and having a genetic predisposition (Flores-Mireles et al. 2015).

2.2.5 Recurrent of Urinary Tract Infections

The European Association of Urology (EAU) indicates, a recurrent of urinary tract infection (rUTI) is defined as experiencing 3/more urinary tract infection in the past 12 months or 2/more urinary tract infection in the prior six months. There are two main mechanisms that contribute to the current understanding of the pathophysiology of recurrent urinary tract infections (rUTIs). These mechanisms involve either frequent, repeated, ascending infections or persistent infections. Many recurrent urinary tract infections (rUTIs) are caused by strains of *E. coli*, with the same pathogens present at both the first infection and any subsequent ones (Prattley et al. 2020). Recurrent UTIs need to be confirmed through urine culture, with at least three instances of uncomplicated infection documented by culture within a 12-month period. A colony count of $\geq 10^3$ CFU/mL of uropathogens was deemed diagnostic, and antibiotic treatment was typically advised. Understanding risk factors played a crucial role in making treatment decisions. For example, according to Grabe et al. (2015), it was no longer recommended to treat asymptomatic bacteriuria in women with recurrent symptomatic UTIs if no risk factors were identified. Urinary tract infections (UTIs) can have a significant impact on a person's health and quality of life, as well as create economic burdens on healthcare systems. More than 76,000 hospitalizations occur in Australia each year due to rUTIs, resulting in an annual cost of AUD$909 million. This cost is on the rise due to the growing issue of antimicrobial resistance. In the USA, annual costs are estimated to exceed $2 billion (Kwok et al. 2022). Urinary tract infections are the primary source of many hospital-acquired infections, which can have serious health consequences. When it comes to UTIs, the most common types are

urethral and bladder infections. Individuals who are hospitalized, have diabetes, or have anatomical or neurological abnormalities that affect urine flow are more susceptible to urinary tract infections (Rezia et al. 2020).

2.3 Pathogenesis of Urinary Tract Infections

In relation to urinary tract infections, organisms causing the illness exhibit a wide range of diversity. *Escherichia coli* is the microorganism that is most commonly associated with asymptomatic bacteriuria. Additional examples are "*Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Enterococcus* spp., and division B *streptococcus*". It is crucial to note that the patients' circumstances might have an impact on the organisms found in people with asymptomatic bacteriuria. Healthy people are prone to having *E. coli*, but those with comorbidities susceptible to being colonized by multidrug-resistant microorganisms such as *P. aeruginosa*. *Enterococcus* species are more prevalent in men (Luu and Albarillo 2022). Urinary tract infections (UTIs) can originate through two pathways: hematogenous and ascending. Newborns often experience the hematogenic pathway, whereas the ascending route typically develops following the neonatal period. In neonates, urinary tract infection (UTI) can present as sepsis, which is typically characterized by non-specific clinical symptoms like loss of appetite, vomiting, inadequate feeding, irritability, drowsiness, seizures, paleness, low body temperature, and occasionally, yellowing of the skin (jaundice). In this age range, there is a significant likelihood of bacteremia, which is the presence of bacteria in the bloodstream, and a high mortality risk of around 10%. This is because the infection can spread to other areas of the body, such as the meninges, resulting in meningitis. The ascending pathway involves the movement, establishment, and rapid increase in numbers of uropathogenic bacteria in the urinary system. Uropathogenic bacteria can persist in the gastrointestinal system for extended periods before migrating to the periurethral region. Following their spread from the perineum to the periurethral region, bacteria ascend the urinary system against the direction of urine flow and initiate infection through several processes. The main ways they adapt are through fimbriae that help them stick to urothelial cells, flagella that help them move, being resistant to antibacterial defenses, and a few other methods (Oliveira et al. 2020). In addition to

virulence mechanisms, uropathogenic bacteria may engage in competition with host cells for nutrients, such as iron. All strains of uropathogenic bacteria produce certain compounds that are responsible for acquiring iron. Almost all strains of *E. coli* express enterobactin. Acute pyelonephritis-causing *E. coli* strains, on the other hand, make aerobactin, a protein that binds iron very well. These bacteria also generate additional iron-sequestering proteins, such as yersiniabactin (Oliveira et al. 2020). Minimal damage or sexual contact has been seen to facilitate the ascent of uropathogenic bacteria to the urine bladder. Typically, the act of peeing effectively eradicates these bacteria. Infections were shown to be associated with factors that compromise bladder integrity, such as the use of catheters, or with obstructions in urine flow, such as an enlarged prostate (Ryan and Ray 2014). The introduction of a catheter into the urinary bladder of a patient can result in urinary tract infections (UTIs) in around one to two per cent of instances. When the catheter is used with an open drainage system, it consistently leads to the presence of bacteria in the urine (bacteriuria) in all cases within three to four days. Grabe et al. (2008) said they thought that uropathogenic bacteria move through the mucopurulent gap between the urethra and the implanted catheter. This is thought to be one of the reasons why almost all patients develop bacteriuria over the course of four weeks.

2.4 Etiologic agents and common uropathogens that Cause UTIs

Despite the fact that any pathogenic microorganism has the potential to produce a urinary tract infection (UTI), Bacteria, particularly *Escherichia coli* (*E. coli*), are the main cause of urinary tract infections (UTIs) in children, accounting for over 90% of cases. The Infectious Diseases Society of America has identified a division of antibiotic-resistant bacteria, including enteric bacteria faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., known as "the ESKAPE pathogens" because of their ability to resist the influences of antibiotics. The presence of this bacterial group poses a difficulty when it comes to treating complex urinary tract infections with empirical antibiotic therapy (Isac et al. 2021). The most often seen Gram-negative causative agents in urinary tract infection cases in Kenya, ranked in descending order of prevalence, are

Escherichia coli, *Klebsiella*, *Enterobacter*, and *Proteus*. Simultaneously, *Staphylococcus* is the predominant genus of gram-positive bacteria, with *Enterococcus* being the next most prevalent. Identifying bacteria and their susceptibility to antibiotics enables effective treatment, regulates the rise in antimicrobial prescriptions, and mitigates the global issue of antimicrobial resistance (Kiiru et al. 2023). *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus* are common bacteria that cause severe urinary tract infections because of blockages in the urinary tract or catheterization (Padmavathy et al. 2012). Uncommon bacteria such as *Candida* spp., *S. aureus*, *Salmonella* spp., and *Mycobacterium* TB are known to cause infections in the urinary system through the bloodstream. These infections can also originate from other parts of the body (Grabe et al. 2008).

2.5 Risk factor associated with UTIs

2.5.1 Sex Activity

The use of spermicides, which can modify the vaginal pH and impact its native bacteria (particularly *Lactobacilli*), frequent sexual intercourse (four or many times in week), or having a new sexual person within the last year all increase the chance of certain complications. Urinary tract infections (UTIs) with symptoms are common among sexually active young women, according to prospective research. Recent sexual activity, using a diaphragm with spermicide, and a history of recurrent UTIs were strongly and independently connected to this incidence. The postulated risk factors for postcoital urination, such as vaginal douches, usage of hot baths, restricting undergarments, and the cleanliness and circumcision status of man partners, lack sufficient data to support their association (Storme et al. 2019). An increase in the quantity of germs in the bladder following sexual intercourse indirectly corroborates this notion. The use of condoms was associated with a high prevalence of urinary tract infections. Moreover, the presence of a spermicide coating significantly augments (by a factor of 7.4–11.5) the probability of urinary tract infections (UTIs). Contraceptive diaphragms increase the likelihood of urinary tract infections through two-fold (Albert et al. 2004).

2.5.2 The Menopause

Having a history of urinary tract infections (UTIs) during perimenopause increases the risk of recurrent UTIs following menopause, as anybody would expect. Since estrogen, glycogen production, and colonization by *Lactobacilli* all decrease after the menopaus, atrophy of a risk factor in this group. *Lactobacilli* colonization lowers vaginal pH via reducing pathogen colonization and glucose metabolism, which in turn produces lactic acid. Additionally, a larger post-void residual urine volume, urinary incontinence, protrusion of the anterior vaginal wall, and the need for either a short- or long-term urinary catheterization might increase the likelihood of a severe UTI (Storme et al. 2019).

2.5.3 Urinary Tract System

Abnormalities in older adults, the primary modifiable risk factors for urinary tract infections are urinary tract abnormalities such prostatic hyperplasia, diabetes mellitus, urethral catheterization, and sexual intercourse (Akhtar et al. 2021). The variations in pelvic architecture may make certain females more susceptible to recurring urinary tract infections, especially in women without any additional risk factors. Urinary tract infection risk factors are heightened by conditions that hinder the proper evacuation of urine, such as prolapse, bladder cancer, or bladder stones (Scholes et al. 2005).

An essential concern in the field of managing urinary tract infections in children is that solitary occurrence may serve as an indicator for an underlying renal abnormality. In 30% of children with congenital abnormalities of the kidney and urinary tract (CAKUT), UTI might manifest as the initial indication. Consequently, starting in the 1960s, the approach to treating urinary tract infections (UTIs) in children has been centered around the idea that repeated occurrences, especially when accompanied by vesicoureteral Reflux "VUR", heighten the likelihood of Chronic Kidney Disease "CKD", hypertension, and ultimately End-Stage Renal Disease "ESRD" (Oliveira et al. 2020).

2.5.4 Habits and Clothing

There is no scientific evidence indicating that tampon usage, poor urine hygiene, douching, or the use of tubs predisposes women to urinary tract infections (Fihn et al. 1985).

2.5.5 Pregnancy

Bacteriuria, both symptomatic and asymptomatic, is quite prevalent among pregnant women, and a significant risk factor is a prior history of infection. Urinary tract infection arises due to inadequate diagnosis during pregnancy, which subsequently increases the likelihood of infection. Pregnant women in such situations are vulnerable to severe complications. According to their research, the infection was found in 56% of women during pregnancy, and the rate was as high as 50% during the second trimester (Vasudevan 2014). Pregnancy is characterized by several anatomical and physiological alterations in the urinary system, which increase the likelihood of developing pyelonephritis. The expansion of the renal pelvis and the ureter takes place during the eighth week of pregnancy, leading to the displacement of the bladder. Symptomatic bacteriuria is categorized as acute cystitis and acute pyelonephritis, with the former referring to bladder infections and the latter to kidney infections. Several research studies have documented a greater occurrence of pyelonephritis in the latter part of pregnancy, and the expansion of the uterus throughout pregnancy is a crucial element in the development of pyelonephritis. As the uterus expands, it puts pressure on the bladder, preventing it from fully emptying. This leads to urine retention in the bladder, which creates an environment conducive to the growth of pathogens and ultimately results in infection (Vasudevan 2014). Several factors, such as the widening of the ureter, an increase in bladder volume, and a decrease in bladder muscle tone, contribute to an increase in urinary stasis and ureterovesical reflux. This, in turn, allows urine to remain in the bladder for longer periods, creating an environment that is conducive to the growth of pathogens and leading to infection. The quantity of bacteria and WBC into the urine sample determines the severity of the infection (Jeyabalan and Lain 2007).

2.5.6 Diabetes

Urinary tract infections were deemed one of the top 10 comorbidities or complicating conditions associated with diabetes. The etiologic bacteria often associated with urinary tract infections (UTIs) in people with diabetes include *Klebsiella* spp., group B *Streptococci*, *Enterococcus*, and *E. coli*. *Klebsiella* and group B *Streptococci* are significantly more prevalent in people with diabetes compared to those without diabetes, with a prevalence that is 2–3 times higher. A recent study found that *E. coli* was the most prevalent causal agent in patients, both with diabetes (56.1%) and without diabetes (56.8%) (Bhat 2020). Diabetic patients frequently experience severe complications of urinary tract infections (UTIs), such as emphysematous cystitis, pyelonephritis, and fungal infections. Diabetes is linked to urinary tract abnormalities, which involve both anatomical and functional characteristics. This anomaly leads to a rise in the utilization of medical equipment such as the urinary catheter, hence expanding the potential for infection. Numerous factors affect the prognosis of urinary tract infections (UTIs) in diabetics, also the danger of infection rises with increased duration and severity of the condition. The presence of elevated levels of glucose in the urine and a weakened immune system contribute to an increased susceptibility to infection. Elevated blood glucose levels have a detrimental impact on the performance of neutrophils, causing them to malfunction. This malfunction, in turn, increases the amount of calcium inside the cells, ultimately leading to phagocytosis. Additionally, vaginal candidiasis and vascular dysfunction might lead to recurring infections (Vasudevan 2014). Women with diabetes who have asymptomatic bacteriuria are at an increased risk of developing symptoms. There is a proposal that organisms now present in asymptomatic women with diabetes have the potential to invade the renal parenchyma and induce infection. People with diabetes are more likely to develop urinary tract infections caused by non-albicane *Candida* species compared to people without diabetes. Finally, the frequent use of bladder catheterization in individuals with diabetes also affects the underlying causes (Bhat 2020). Humans diagnosed diabetics are also prone to developing illnesses such as cystopathy, nephropathy, and renal papillary necrosis, which increase their susceptibility to urinary tract infections (UTIs). Diabetic cystopathy leads to vesicourethral reflux, it is the backflow of urine from

bladder to ureters and kidneys, resulting to recurrent infections. Approximately 30% of women who are diagnosed with diabetes have an increased susceptibility to medical problems such as cystocele, cyst urethrocele, or rectocele. These abnormalities can subsequently lead to chronic urinary tract infections in diabetic wemen. Diabetic women with complex urinary tract infections commonly have medical conditions such as renal and perirenal abscesses, emphysematous pyelonephritis, and emphysematous cystitis. Furthermore, diabetic females commonly experience chronic fungal infections, xanthogranulomatous pyelonephritis, and papillary necrosis. Diabetes-induced blockage of the urinary system can result in emphysematous urinary tract infections, leading to tissue death and bleeding in the affected area. Emphysematous urinary tract infections (UTIs) occurring in the upper urinary tract are the cause of pyelonephritis, pyelitis, and emphysematous cystitis (Vasudevan 2014).

2.5.7 Vitamin D deficiency

Vitamin D shortage is characterized by a lack of vitamin D in the body. The urinary system is often targeted by many types of infections, which means quick defensive measures need to be taken. Bladder epithelial cells release and express a human antimicrobial peptide called cathelicidin, which provides protection to the lower urinary tract. Vitamin D stimulates the production of cathelicidin in the epithelial cells of the urine bladder. Given its ability to offer defense against both gram-negative and gram-positive bacteria, it may be inferred that this chemical possesses a wide range of effectiveness. In addition, it also demonstrates efficacy against some protozoa and fungus (Ali et al. 2020).

2.5.8 Stone

It is known that bacteria cause at some point the formation and development of struvite urinary tract stones. However, the impact of bacteria on the formation of the more prevalent (Calcium Oxalate CaOx) and (Calcium Phosphate CaPhos) stones has not been thoroughly studied. Nevertheless, other discoveries strongly suggest a potential correlation between urinary stones and bacteria. These include an elevated prevalence

of urinary tract infections in people who complain of urinary tract stones, as well as numerous instances of culture-positive urinary stones, including those made up of calcium oxalate or calcium phosphate (Schwaderer 2017).

2.5.9 Renal Transplant

Urinary tract infection (UTI) continues to be the predominant form of infection acquired by kidney transplant recipients. Possible factors contributing to the issue might include the overuse of immunosuppressive medicines and/or the presence of chronic illness, the presence of foreign objects in the urinary system, damage to transplanted kidneys due to ischemia-reperfusion injury, non-functioning native kidneys, and abnormalities in the lower urinary tracts. Ongoing research aims to elucidate the specific roles played by each of these contributing variables and explore strategies to minimize them, thereby reducing the occurrence of urinary tract infections (UTIs). Antimicrobials continue to be the primary means of treatment and prevention, which has contributed to the emergence of multi-drug resistance organisms (Ness and Olsburgh 2020).

The organisms responsible for urinary tract infections (UTIs) after kidney transplantation might be bacterial, fungal, viral, parasitic, or mycoplasmal in nature. In transplant patients, the sequence of bacterial UTI pathogens is similar to that in individuals who have not undergone transplantation. More than seventy percent of urinary tract infections are represented by Gram-negative bacteria. The predominant bacterial pathogens responsible for urinary tract infections (UTIs) are *Escherichia coli*, *K. pneumoniae*, *Enterococcus* species, *Pseudomonas aeruginosa*, *Enterobacter*, and *Proteus mirabilis* (Zhang et al. 2021).

2.6 *Escherichia coli* and Urinary Tract Infections (UTIs)

2.6.1 *Escherichia coli*

Escherichia coli is being defined. Upon its original isolation, *Escherichia coli* was named Bacillus coli communis, a term derived from Latin that highlights its notable

feature as a "widespread bacterium found in the colon" and its ability to be easily grown in many substances. The initial specimen, as initially documented in 1885, was characterized by its distinct colony and cellular structure, as well as its capacity to metabolize glucose, generate acid, and curdle milk. After being renamed in 1919 to honor its discoverer, the criteria for classifying this species were broadened during the following decades to include a range of distinctive characteristics that differentiate *E. coli* from other enteric species. *E. coli* is characterized by being negative for oxidase test, urease test, and citrate test, and positive for lactose test, catalase test, and indole test. However, there is a limited variation in these features across different strains of *E. coli* (Cobo-Simón et al. 2023). The genus *Escherichia*, named after Theodor Escherich, a German pediatrician credited with the discovery of *Escherichia coli*, consists of facultative anaerobic Gram-negative bacilli that belong to the family Enterobacteriaceae (Gomes et al. 2016). *Escherichia coli* is the most common kind of bacteria that can survive with or without oxygen in the human colon. The bacterium usually establishes itself in the digestive system of newborn humans shortly after birth and maintains a symbiotic relationship with its human host, resulting in reciprocal advantages for many years. Nevertheless, *E. coli* includes both commensal and deadly variants. While commensal *E. coli* typically does not cause illness, except in individuals with weakened immune systems or when the normal protective barriers in the gastrointestinal tract are compromised (Liu et al. 2020).

In a study by Russo and Johnson (2000), there are eight forms of *Escherichia coli* that may be pathogenic in humans. These include six types that mostly affect the intestines and two types that cause infections outside of the intestines, named (ExPEC) or extraintestinal pathogenic *E. coli*. According to Nataro and Kaper (1998), there are six types of bacteria that can cause infections in the intestines. These are enteropathogenic *Escherichia coli* (EPEC), enterohaemorrhagic *Escherichia coli* (EHEC), enterotoxigenic *Escherichia coli* (ETEC), enteroaggregative *Escherichia coli* (EAEC), enteroinvasive *Escherichia coli* (EIEC), and diffusely adherent *Escherichia coli* (DAEC). ExPEC refers to specific two kinds of *E. coli* bacteria: uropathogenic *Escherichia coli* (UPEC) and meningitis-associated *Escherichia coli* (NMEC). *E. coli* strains with pathogenic properties are the cause of several significant epidemics of conditions such as newborn

diarrhea, bloody diarrhea, cystitis, pyelonephritis, meningitis, and others (Liu et al. 2020). It has been shown that *E. coli* is accountable for eighty percent of urinary tract infections. Conversely, this uropathogen was accountable for seventy to ninety five percent of society-acquired urinary tract infections and was the most prevalent cause of nosocomial infections (50%). The recurrence of urinary tract infections reason by this microbe was a significant concern for many individuals (Poey et al. 2012).

2.6.2 Classification of *Escherichia coli*

The German scientist Theodore Escherich initially discovered the *Escherichia coli* bacterium in 1885 while studying the natural bacteria found in children's intestines. These bacteria, shortly after birth colonize the intestine and are known as commensal bacteria. Scientist Bray (1945) detected a particular kind of *E. coli* bacterium, known as Enteropathogenic *E. coli* (EPEC) in England, which the primary source of diarrhea in newborns. According to Olowe et al. (2017), the genus *Escherichia* is neatly affiliated with different genera of the family *Enterobacteriaceae*, especially the genus *Shigella*. This genus encompasses distinct five kinds (*Escherichia coli*, *Escherichia fergosonii*, *Escherichia hermanii*, *Escherichia blattae*, and *Escherichia vuneris*), which exhibit variations in some metabolic responses. The species *Escherichia coli* is highly significant and prevalent in causing diseases in humans.

In Bergey's Manual *Escherichia coli* is classified into the *Enterobacteriaceae* family as follows:

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: *Escherichia coli* (Garrity et al. 2005).

2.6.3 Characterization of *Escherichia coli*

They are bacilli gram-negative and do not form spores, and they motile by means of flagella that surround the entire body. Their colonies are smooth and slightly, convex and moist, not mucous or mucous when containing a capsule, sharp edge complete, they are a bright pink color on MacConkey agar medium, they are a metallic green sheen color on eosin methylene blue (EMB) agar medium, and pink colonies are also formed on Chrome Orientation agar, cellulobios sugar does not ferment, and more than 80% of it ferments to rhamnose sugar, and as for sorbetole sugar more than 90% of it ferments. It does not produce S2H gas in triple sugar iron agar medium (TSI) and also non-hydrolyzed gelatin. The optimum temperature for its growth is (36 – 37) and grows in a pH range between (9- 4.4), and does not grow in the presence of potassium cyanide (KCN), most of them are productive (GUD) enzyme β-glucoronidase (Wanger et al. 2017, Jawetz et al. 2016).

According to Hemraj et al. (2013), which is positive for the catalase test, positive for the methyl red test, and positive for the indole test, which is the best test that distinguishes it from the rest of the members of the *Enterobacteriaceae* family. It is also negative for the oxidase and urease tests, and negative for the Vogase-Proskauer test. In addition, it does not consume citrate as its sole carbon source.

2.6.4 *Escherichia coli* Types

2.6.4.1 Enterotoxigenic *Escherichia coli* (ETEC)

Diarrhea affects millions of people annually, particularly in places with poor resources, and enterotoxigenic *E. coli* (ETEC) is a main reason of this condition. Annually, it is affects approximately 220 million persons, with a special emphasis on children under the age of five (von Mentzer and Svennerholm 2023). Although not exclusively attributed to it, ETEC is a significant factor in the occurrence of traveler's diarrhea and can also impact adults. Envision the presence of defiled food and water, which serves as the perfect environment for the proliferation of Enterotoxigenic *E. coli* (ETEC). This

virulent disease was transmitted through the fecal-oral route, as evidenced by research conducted in Bolivia. Surprisingly, ETEC is not only present in afflicted youngsters, but it also exists in soil, crops, and even river water. It flourishes in aquatic environments, presenting a significant threat of spreading through the ecosystem. The exact cause of diseases in La Paz is currently unknown, however research indicates that the environment has a significant influence (Calderon Toledo et al. 2023). While, in what manner does ETEC cause widespread destruction? The organism utilizes specific protein "hooks" named as colonization factors to adhere to the lining of the gastrointestinal tract, resulting in disruption. The adhesion of the bacteria is crucial for its ability to cause disease (Gaastra and Svennerholm 1996).

2.6.4.2 Enterohemorrhagic *Escherichia coli* (EHEC)

Enterohemorrhagic *Escherichia coli* (EHEC) is a bacterium that infects the human gastrointestinal tract and leads to widespread occurrences of bloody diarrhea and hemolytic uremic syndrome (HUS) around worldwide. The primary characteristics of EHEC pathogenesis are the synthesis of Shiga toxins, which cause the clinical manifestations linked to HUS, and the capacity to inhabit the intestinal epithelium by creating attaching and effacing (AE) lesions. EHEC has the ability to generate mucin-degrading enzymes, including the zinc metalloprotease StcE. This enzyme aids in the penetration of the mucus layer into the colonic epithelium (Martins et al. 2022).

The toxins are distributing as STX1 and STX2, together with several subtypes and variations based on receptor affinities and toxin strength. Moreover, most of EHEC strains possess a plasmid that encodes a hemolysin and a chromosomally positioned locus enterocyte effacement (LEE) pathogenicity island. The genes enable bacteria to produce adhesion and effacing lesions in intestinal mucosa cells, hence enhancing the severity of infection in humans. Close association of bacteria with the host cell is facilitated by the binding of intimin, which is generated by the eaeA gene, to its particular receptor. STEC requires both intimin (eaeA) and hemolysin (hlyA) to establish, attach to, and damage the mucous membrane of the gastrointestinal tract (Amin et al. 2022).

EHEC infections are mostly contracted by direct contact with ruminant excrement or indirectly through the consumption of infected foods, including beef, milk, apple juice, fruits, leafy greens, and water (Gelalcha et al. 2022).

2.6.4.3 Enteropathogenic *Escherichia coli* (EPEC)

The first pathogenic type of *Escherichia coli* bacteria was enteropathogenic *E. coli*, which stands for EPEC, associated with causing diarrhea. It was responsible for a small number of outbreaks of infantile diarrhea between 1940 and 1950 (Croxen et al. 2013). EPEC in 1982, was characterized as "diarrheagenic *Escherichia coli* belonging to serogroups suspected to be pathogens, but it has not been confirmed that their pathogenic mechanisms are related to heat-labile enterotoxins, heat-stable enterotoxins, or *Shigella*-like invasion." (Scaletsky et al. 1985).

EPEC strains are classified as diarrheagenic strains of *Escherichia coli* that have the ability to cause adhesion and effacement (A/E) lesions on the intestinal epithelium. However, they don't generate Shiga toxins or heat-labile enterotoxins or heat-stable enterotoxins. A primary distinguishing feature of EPEC is its capacity for generate A/E lesions, allowing it to induce localised damage through firmly adhering for intestinal epithelial cell superficies, disturbing cellular integrity, and ultimately resulting in the elimination of microvilli. EPEC can be categorized into ideal and non-ideal isolates, depending on existence/lack of EAF which contains the Bfp (Mare et al. 2021).

The pathogenesis of EPEC requires the formation of an ultrastructural lesion where the bacteria directly interact with the apical enterocyte membrane of the host. EPEC has the ability to adhere with the cell of host in a localized manner, which is facilitated through the bundle-forming filaments, leading to the formation of this lesion. Within the host cell, at the site of the lesion, there is a disruption of host cell processes due to tyrosine phosphorylation and signal transduction activities, leading to the occurrence of diarrhea. The occurrence of these is attributed to tightly controlled EPEC-secreted proteins that are synthesized by a type III secretion system. A significant number of these proteins are encoded by genes located inside a pathogenicity island (Clarke et al. 2003).

2.6.4.4. Enteroaggregative *E. coli* (EAEC)

(EAEC) Enteroaggregative *E. coli*, are newly identified disease-causing microorganisms that are mostly linked to sudden and ongoing situations of diarrhea and stunted development in babies of underdeveloped nations. Furthermore, EAEC (Enteroaggregative *Escherichia coli*) plays a significant role in causing acute diarrhea in individuals traveling to underdeveloped nations and in causing chronic intestinal infections in people with HIV/AIDS. A hybrid strain known as O104:H4 EAEC that carries the enterohaemorrhagic *E. coli* Shiga toxin gene was responsible for the 2011 epidemic in Germany. This outbreak led to more than 4300 situations of diarrhea, 900 hospitalizations, and 50 deaths. The process of pathogenesis includes attachment to the cells lining the intestines, triggering the production of mucus, forming a layer of microorganisms, causing harmful effects to the cells, and resulting in inflammation of the intestinal lining (Ellis et al. 2020). Enteroaggregative *E. coli* is a form of *Escherichia coli* which doesn't produce the enterotoxins seen in enterotoxigenic *E. coli* (ETEC). Additionally, EAEC has the capacity to stick to HEP -2 epithelial cells during a way that causes them to clump together, known as an aggregative (AA) phenotype. The pathogenesis of EAEC is believed to include inflammation of the mucosa and damage to the epithelial cells. Different types of EAEC have different possible virulence factors that are stored either on the bacterial chromosome or the pAA plasmid that is only found in EAEC (Boisen et al. 2020).

2.6.4.5 Enteroinvasive *E. coli* (EIEC)

(EIEC) Enteroinvasive *E. coli* is a type of bacteria which ability to reason infections in the intestines. EIEC is a pathotype of diarrheagenic *Escherichia coli* (DEC) that can cause shigellosis-like symptoms in both children and adults. Infections caused by EIEC are prevalent in low-income countries with inadequate hygiene, although they can also be found in other parts of the world. EIEC, like to Shigella, which reason of bacillary dysentery. Symptoms of bacillary dysentery include fever, abdominal cramps, diarrhea, occasional vomiting, and the presence of blood and pus in the stool. EIEC is capable of invading and penetrating the epithelial cells of the colon. The EIEC bacteria penetrate

the colonic mucosa, multiply inside cells, and then spread to neighboring cells, causing damage to the intestinal epithelial barrier. The genes associated with the invasion of EIEC can be found on both a large virulence plasmid and the chromosome (Farajzadeh-Sheikh et al. 2020). A kind III excretion system "TTSS", respondent proteins, and the VIRF global arranger are just a few of the components that help bacteria cause disease and invade host cells. These components are all encrypted by a senior infestation plasmid (PINV) that spans over 200 kb. This paper discusses the TTSS, which is responsible for introducing effector proteins into host cells. This process leads to EIEC internalization and cell-to-cell spread through actin polymerization (Iqbal et al. 2022).

2.6.4.6 Diffusely Adherent *Escherichia coli* (DAEC)

DAEC, the diffusely adhering *Escherichia coli* are a diverse pathovar known for their association with both gastrointestinal and urinary tract infections. The available whole genome sequences of these strains suggest that DAEC is tightly attached to enteroaggregative *Escherichia coli*. While, their distinct set of adhesins and iron acquisition systems set them apart from them. The role of DAEC in the molecular pathogenesis of UC remains unclear, despite being isolated from patients with ulcerative colitis (UC) (Sheikh and Fleckenstein 2023). DAEC is linked to diarrhea in children aged 1.5 to 5 years, urinary tract infection (UTI) in adults, pregnancy complications, and is a normal part of the intestinal microflora in both adults and children. In younger children, DAEC-induced watery diarrhea can be more persistent. Adults who are asymptomatic carriers of DAEC strains can cause several inveterate inflammatory intestinal diseases, such as Crohn's, inflammatory bowel diseases and coeliac. The pathogenesis of Diffusely Adherent *Escherichia coli* begins with the binding of the pathogen to particular host cells. DAEC strains are able to attach to the urinary tract and enteric epithelial cells, which helps them evade clearance by micronutrition and peristalsis. The attachment facilitates interaction between DAEC and the host cell, enabling the release and delivery of toxins as well as the initiation of signaling events within the host cells. Afa/Dr adhesins facilitate the attachment and adherence of DAEC to host cells through non-classical patterns (Pakbin et al. 2021). The appearance of a diffuse adherence pattern "DA" on HELA and HEP-2 epithelial

cells identifies DAEC strains. The pattern " DA" is characterized by bacteria evenly coating surface of the cell (Javadi et al. 2020). Decay-Accelerating Factor (DAF) guides the DAEC pathotypes to the brush edges of epithelial cells, where they can attach to them. The cytoskeletal structures of the microvilli are rearranged or destroyed after cell adhesion (Govindarajan et al. 2020). This passage talks about how some Afa/Dr adhesins can connect with CEACAM on the surface of epithelial cells. This interaction leads to the activation of CDC42, the aggregation of CEACAM at the site of bacterial adhesion, the effacement of brush-border microvilli, and ultimately the internalization of the bacterial pathogen through a process that does not rely on microfilaments but instead depends on microtubules and lipid rafts, according to Pakbin et al. (2021).

2.6.4.7 Adherent-Invasive *Escherichia coli* (AIEC)

In humans, AIEC has a major effect on small bowel inflammatory diseases such inflammatory bowel disease (IBD) and Crohn's disease (CD). In healthy people, this pathotype is also present in the gut microbiota, but it does not contribute to illness. Intestinal epithelial cell layer adhesion and invasion, as well as macrophage and epithelial cell multiplication, are all hallmarks of the AIEC pathotype (Pakbin et al. 2021).

Adhesion, attack, and cell multiplication inside the epithelial host are the three steps that make up the pathophysiology of AIEC. AIEC infections develop when invading microbes bind to a particular protein on ileal epithelial host cells called CAECAM6. In order to facilitate invasion, infection, and replication within epithelial host cells, the AIEC strain employs a variety of virulence factors, including outer membrane vesicles, outer membrane proteins, and long polar fimbriae (Pakbin et al. 2021).

AIEC plays a significant role in Crohn's disease by negatively affecting the mitochondrial function of epithelial cells. In addition, various forms of intestinal inflammation have demonstrated mitochondrial dysfunction. Furthermore, research suggests that mutations in genes that regulate mitochondria may increase susceptibility to IBD (Kamali Dolatabadi et al. 2021).

2.7 The Invasion mechanism of UPEC to the Host Epithelium

UPEC, which stands for uropathogenic *E. coli*, is a specific subgroup of *E. coli* strains that have the ability to cause urinary tract infections (UTIs). These strains are classified as (ExPEC) extraintestinal pathogenic *E. coli*, meaning they can cause infections outside of the intestines. UPEC is primarily found in the human gut and typically does not lead to any complications. Nevertheless, the presence of various virulence factors, including adhesins, toxins, and iron acquisition systems, enables UPEC to spread and establish itself in the urinary tract system by evading the immune system and adapting to challenging conditions (García et al. 2023).

Many scientists agree that certain types of UPEC bacteria can get into host bladder epithelial cells and multiply, creating biofilm-like communities inside the cells. The UPEC bacteria undergo changes in their shape, transitioning from a rod-like form to a round coccoid shape and sometimes even transforming into a filamentous structure. During escape from the host bladder cells, the filamentous, elongated morphology is prevalent. However, it is believed that re-infection of host cells occurs initially after the filaments revert back to a rod-shaped morphology (Persson et al. 2022). UPEC can be transmitted between humans through close personal relationships, such as living together or sexual activity. The uropathogens make their way from the periurethral area, ascending the urethra to the bladder sac that establish the infection. Bacteria rise through the ureters and colonize the kidneys. This is called pyelonephritis, and it makes it more likely that the bloodstream will become infected. Prostate infection often occurs alongside cystitis in men, which suggests that bacterial prostatitis should be considered a urinary tract infection (Lupo et al. 2021).

Pathogenic bacteria, like a UPEC, have the ability to bind to specific glycoconjugates present in cells. This allows them to adhere to host cells and is crucial in determining the target of infection (Poole et al. 2018). Fimbriae, which are superficial bacterial growths that allow them to resist the flow of urine and colonize the bladder, facilitate the attachment of UPEC to the urinary tract. Adhesins are proteins at the tip of the pili that bind carbohydrates and make it easier for the cells to stick to certain host glycans

(Lewis et al. 2016). UPEC and host glycans interact with each other during the early stages of infection. The bacteria utilize type 1 pili, which are filamentous adhesive organelles, to adhere to urothelial cells in the bladder. The coiled polymeric structure in these pili ends with the adhesin FimH at the very end. This makes it easier for UPEC to stick to the urothelium. This finding suggests that the FmlH adhesin may help UPEC avoid being flushed out of the body through urine by sticking to the surface of the intermediate urothelium in the later stages of infection (Lupo et al. 2021). *E. coli* infection can cause damage to the surrounding epithelial cells, resulting in cystitis (Johnson et al. 2005).

The cystitis infection spread to the kidney by infecting the renal tubule and sticking to the epithelial cells in the renal tissue. The release of the toxins, such as hemolysin, occurs when the uropathogens infect the renal epithelial tissue (Abraham 2011). It has been observed that the uropathogenic *E. coli* has the ability to enter the bloodstream from a kidney infection, leading to the evolution of bacteremia (Wiles et al. 2008).

2.8 The Strategies of *Escherichia coli* in UTIs

UPEC strains possess several virulence characteristics that help them evade defensive systems and induce illness. Virulence genes include fimbriae, iron-acquisition systems, flagella, and poisons as virulence agents. These components are essential for bacterial attachment, penetration, viability in iron-depleted conditions, and spread. Virulence genes may be located on both plasmids and the chromosome, enabling non-pathogenic strains to get novel virulence components from other DNA sources (Dadi et al. 2020).

UPEC strains may exhibit decreased sensitivity to antibiotics and decreased immunogenicity due to their role as opportunistic pathogens. Catheters enable uropathogens to create biofilms, offering them protection from the immune system and medications (King-sun 2007). Microorganisms may produce a polysaccharide capsule that enhances their survival in the urinary tract by reducing their sensitivity to the host immune system, including phagocytosis (Abraham 2011).

2.9 Virulence Factors in *E. coli*

2.9.1 Adhesions

2.9.1.1 Type 1 Fimbriae

External protein formations known as fimbria adhesions allow UPEC to stick to bladder cells and resist being flushed out of the urinary system by urine flow. Type 1 fimbria (T1F) is the most clearly defined and common type of fimbria adhesions. Research on UPEC populations' genomics has revealed a high prevalence of T1F, ranging from 86% to 100%. Furthermore, T1F has been shown to enhance the invasion of bladder and uroepithelial cells, resulting in the development of IBCs. This can lead to the formation of long-lasting reservoirs of infection, show increased resistance to antibiotics, and are thought to play a part in recurrent infections (Whelan et al. 2023). This particular form of fimbriae was commonly produced, leading to infections limited to the urinary bladder by strains responsible for lower tract infection. An invertible component was found in upper urinary tract infections caused by strains, leading to inhibition and reduced production of type 1 fimbriae. This might enable the organism to go up via the ureters to the kidney, where it can adhere utilizing the fimbriae to specific receptors on the tissue or organ (King-sun 2007). The fimbriae attachment sites were mostly found in the urinary system, namely in the mucosal layer of the bladder, the distal tubules, and the connective tissue layers of arteries in the kidney (Totsika et al. 2011). Fimbriae assist in the colonization of vaginal tissue and enhance adherence to vaginal mucus, a process that is heightened in females experiencing recurrent urinary tract infections (Zhang and Foxman 2003).

2.9.1.2 P Fimbriae

P fimbriae, similar to T1F, are external protein structures that aid in the attachment of UPEC to uroepithelial cells (Lane and Mobley 2007). P fimbriae are strongly associated with upper urinary tract infections and pyelonephritis. They are considered a critical virulence factor in UPEC because they help in adhering to epithelial cells in the urinary

tract and renal tubules (Whelan et al. 2023). P fimbriae are genetically determined by the cervical operon. PapA encodes the main structure of the fimbriae, papEF encodes the adaptor subunits, and papG encodes the adhesion proteins (Lane and Mobley 2007). Epithelial cells in many human tissues such as the urinary tract, buccal cavity, intestine, vagina, and leukocytes (PMNLs) contain receptors for P fimbriae (Svanborg et al. 2006). P fimbriae function a vital part in the human body and are significant in the formation of urinary tract infections. They have been detected in 80% of pyelonephritis cases attributed to *E. coli* strains. The P fimbriae aid in attaching to certain receptors and triggering inflammation in the mucosa via specific signaling pathways, which may lead to the dissolution of nearby cells (Kucheria et al. 2005, Koreň et al. 2013).

2.9.1.3 S Fimbriae

Uropathogenic strains generate S-fimbrial adhesins that lead to urinary tract infections and even newborn meningitis. S fimbriae and FlC fimbriae are tightly linked since they are part of the same family of fimbrial attachment. This kind of fimbria is genetically, immunologically, and functionally linked. S fimbriae attach to specific receptors on epithelial and endothelial cells located in the proximal and distal tubules, collecting duct, and vascular endothelium of the urinary tract (King-sun 2007). S fimbriae are mannose-resistant adhesins and are produced by the Sfa gene. This operon comprises nine genes. The SfaA protein is the main subunit, with SfaG, SfaH, and SfaS serving as minor subunits, and SfaF acting as the membrane's outermost translocation assembling protein (Behzadi 2017, Balsalobre et al. 2000).

S fimbriae specifically target alpha-sialyl-2,3-alpha-galactose residues found on the glycoproteins of urothelial tissues in the urinary tract and kidneys. In addition to urinary tract infections, *E. coli* bacteria with S fimbriae have been identified as a cause of meningitis in newborns, possibly because of their ability to attach to sialo-glycoproteins present on brain microvascular endothelial cells. Research utilizing molecular analysis found that the prevalence of the sfa operon in *E. coli* ranges from 42% to 87% (Whelan et al. 2023**).**

2.9.1.4 The Afa/Dr Family of Fimbrial Surface Adhesins

The Afa/Dr family of adhesions is closely related to UPEC, which can lead to cystitis in children, as well as pyelonephritis and recurrent urinary tract infections in youth and pregnant (Whelan et al. 2023). It is frequently found in the genome of ST131, a highly aggressive UPEC clone (Alvarez-Fraga et al. 2022). Dr Fimbria (Diffuse Adherence Fimbria) is part of the Afa/Dr family of fimbrial surface adhesions and is distinguished by its wide binding specificity compared to other adhesions. They can bind to the Dr blood group antigen on decay-accelerating factor (DAF), a glycoprotein located on the surface of uroepithelial cells (Nowicki et al. 2001). Furthermore, Dr. Fimbria is able to identify carcinoembryonic antigen (CEA)-related cell adhesion molecules (CEACAMs) CEA, CEACAM1, CEACAM3, and CEACAM6 as binding receptors. Dr. Fimbria's interactions with these receptors have been demonstrated to facilitate the internalization and invasion of UPEC into the host epithelial cells (Whelan et al. 2023).

From the bladder to the kidney, the colonic gland and urinary system were lined with receptors of the Dr family of adhesins. To demonstrate a greater number of receptors, these receptors were primarily concentrated in the kidneys, urinary bladder tissue, and other urinary system components, such as Bowman's capsule, connective tissue of the urinary bladder, distal tubules, and proximal tubules. Additionally, it permitted the colonization of the colon, which resulted in a urinary tract infection. Recent research examining clinical isolates obtained from expectant women who were infected with urinary tract infections suggests that they were particularly vulnerable to infection by *Escherichia coli* that carried the virulence factor known as Dr. adhesins. Considered by Colpan et al. (2013).

2.9.1.5 non-Fimbrial Adhesins

RTX proteins are commonly conveyed to the cell surface through the action of the type I secretory system. In addition to non-fimbrial adhesions, the toxins that are detected consist of cytolysins and hemolysin (Whelan et al. 2023). Non-fimbrial adhesion constitutes one secretion A (tosA), which is encrypted through the tosRCBDAEF

operon. This adhesion, which is situated above surface of the cell, is capable of forming connections with epithelial cells in the upper urinary tract and human kidneys (Luterbach et al. 2018). TosR regulates the operon in conjunction with the global regulatory proteins H-NS and Lrp. Its interaction with the regulatory mechanisms of other fimbrial adhesions, such as Pap and Foc, facilitates the organization of both fimbrial and non-fimbrial adhesions on the cell surface. It has been shown that TosA overexpression increases biofilm formation in vitro, in both LB broth and human urine (Vigil et al. 2012).

2.9.2 Curli and Amyloid Fibers

Extracellular proteinaceous curli fibers are generated by a diverse array of *Enterobacterales* species, inclusive *Salmonella* spp. and *Escherichia coli.* This is the first instance of a functional amyloid identified in bacteria, and it functions as a crucial constituent of the extracellular matrix composed of proteins in *E. coli* biofilms. They play an essential role in numerous pathogenic and physiological processes of *E. coli* and *Salmonella* spp. They facilitate adhesion, surface colonization, cell aggregation, and biofilm formation. In addition to their vital functions in cell adhesion and invasion of host cells, Curli fibers interact with host factors and the host immune system. In addition, they are potent inflammatory response stimulants. Biogenesis and structure of curli distinguish it from all other known bacterial fibers. The Curli fibers have a thickness ranging from 4 to 7 nm, are insoluble in sodium dodecyl sulphate (SDS), and are characterized by a unique cross beta-strand structure that ensures the fibrils' structural integrity. According to biophysical and biochemical research, curli are a subset of filaments called amyloids. Multiple mammalian diseases, including Parkinson's, Huntington's, prion, and Alzheimer's, and Prion, have been linked to the formation of amyloid fibers. The production of functional amyloids in bacteria is achieved via biosynthetic processes that are meticulously regulated and coordinated (Azam et al. 2023).

Curli, an essential component of the biofilm matrix of numerous bacterial species, including *E. coli*, is a functional amyloid protein. Curli fibers are generated when the

major subunit protein CsgA, through self-assembly, polymerizes into fibrils upon interaction with the minor subunit protein CsgB on the surface of the bacterial cell. CsgD is a transcription factor that controls the synthesis of curli by stimulating the expression of genes involved in nucleotide biosynthesis, csgG, csgA, and csgB, in addition to genes associated with curli biosynthesis and other metabolic pathways. CsgE, CsgF, and CsgG are components of the secretion system of outer membrane curli. CsgG is responsible for generating a pore-like outer membrane structure, whereas CsgE and CsgF serve as chaperones for the curli fibers that are secreted (Whelan et al. 2023).

2.9.3 Toxin Production and Cytotoxic Effects

2.9.3.1 Hemolysin

α-hemolysin (HlyA) is a pore-forming toxin and virulence factor produced by numerous strains of UPEC. According to various studies, the incidence of hemolysin production by UPEC ranges from 21% to 47% of isolates (Whelan et al. 2023).

The most common important toxin is Hemolysin, which includes two types: alpha (α) and beta (β) hemolysins. α-hemolysin is typically produced by uropathogen strains found in individuals with urinary tract infections (Kucheria et al. 2005).

This text provides a detailed explanation of the α-hemolysin type, which belongs to the RTX group of bacterial toxins. It discusses its catalytic action on erythrocytes and eukaryotic cells, facilitating invasion through epithelial cell barriers (Koreň et al. 2013). Implanting hemolysin into the cytoplasmic membrane initiated the hemolysis process. The study by Kucheria et al. in 2005 found that erythrocytes showed increased permeability to calcium ion (Ca2+), potassium ion (K+), sucrose, and mannitol.

The disruption in ion potential and concentration impacts the balance and function of red blood cells (RBCs). According to King-sun (2007), the damage of RBCs occurred. Iron was released from the damaged cells to be utilized by the microorganism for

propagation via siderophore systems. The cell swells and ruptures due to the water transferring into it as an effect of boosted osmotic pressure. The study by Kucheria et al. (2005) found that B-hemolysin exhibited a hemolytic activity similar to α-hemolysin, indicating its cell-related nature.

The *E. coli* hemolysin targets a variety of mammalian cells including red blood cells, renal epithelial cells, endothelial tissue, and immune system cells like granulocytes and monocytes. Bacteria producing β-hemolysin are more common in extra-intestinal strains rather than fecal commensals. *Escherichia coli* that results hemolysin was more frequently found in pyelonephritis cases compared to cystitis cases (Schmidt and Hensel 2004).

2.9.3.2 Cytotoxic Necrotizing Factor Type 1 (CNF1)

Numerous strains of Uropathogenic *E. coli* generate cytotoxic necrotizing factor type 1 (CNF1), an endotoxin which regulates host cell efficiency through inhibiting activation of the Rho family of GTPases (Doye et al. 2002). cellular processes are regulated by these GTPases, including phagocytosis, cell cycle progression, actin structure formation, and cell motility (Whelan et al. 2023). The gene cnf1, which encodes CNF1, has been identified in a pathogenicity island alongside genes that produce α-hemolysin and P-fimbriae (Blum et al. 1995).

Additionally, *E. coli* strains that cause neonatal sepsis and diarrhea in children are linked to CNF1. In relation to meningitis, there is speculation that *E. coli* producing CNF1 could infect the brain more efficiently across the blood-brain barrier; in fact, a mouse model of meningitis demonstrated that this protein exacerbated the severity of the disease (Whelan et al. 2023).

2.9.3.3 Serine Protease Autotransporter

Serine Protease Autotransporters of Enterobacteriaceae (SPATE) is an autotransporter subset of secreted proteins, contributes to the virulence and survival of UPEC.

(Pokharel et al. 2019). The cytotoxic activity of the secreted autotransporter toxin, which is encoded by the sat gene, is of specific significance in relation to the virulence of UPEC against urinary tract epithelial cells. Initial identification was made for *E. coli* CFT073, a strain obtained from a patient diagnosed with acute pyelonephritis. This strain exhibits cytopathic activity in vitro against kidney and bladder cell lines, and its prevalence is considerably higher in *E. coli* strains associated with pyelonephritis compared to those found in feces (Whelan et al. 2023).

2.9.4 Systems for Acquiring Iron

Metal ions are vital nutrients that are necessary for numerous critical biological processes, such as the synthesis of enzymes that are engaged in cellular metabolism (UPEC) (Saha et al. 2013). These are metalloenzymes, which necessitate co-factors consisting of metals such as nickel, copper, iron, and zinc, with iron being the most frequently demanded. Acquisition of iron is a critical point of competition between the host and the pathogen, given that iron is a crucial factor in the proliferation of UPEC within the urinary tract and the retention of iron to deprive invading pathogens of the nutrient is part of the defensive strategy of the host (Whelan et al. 2023).

According to Henderson et al. (2009), in response to this competition, UPEC has developed a number of iron acquisition systems. The initial one is siderophores, which are iron-chelating molecules. The organization can synthesize and secrete four of these siderophores: aerobactin, yersiniabactin, enterobactin and salmochelin. Among clinical *E. coli* isolates, enterobactin is the most conserved of these, with the others occurring in a variety of combinations (Whelan et al. 2023). The UPEC immune evasion strategy seems to rely heavily on these variations in genetic carriage of siderophore systems, as host mechanisms can inhibit the iron acquisition activity of particular siderophores. For instance, the innate immune protein lipocalin-2 (Lcn2) hinders the activity of enterobactin by binding ferric and aferric enterobactin (Goetz et al. 2002). Aerobactin is encoded by iucABCD; enterobactin is encoded by the operon entABCDEF; salmochelin is determined by the genes iroB and iroE; yersiniabactin is determined by the genes

ybtSETU, irp1, and irp2. UPEC acquire iron from the host's haemoglobin via the outer membrane receptors ChuA and Hma, in addition to siderophores (Whelan et al. 2023).

2.9.5 Capsule

Escherichia coli capsules are classified into four main groups based on their biosynthesis, assembly, and organization of their gene clusters. Group 2 capsules, which include various k antigens like k1 and k2, have specific characteristics and functions. k1 is linked to meningitis, while k2 is a complex polysaccharide that offers protection against the immune system through its antiphagocytic and serum resistance properties. The genes accountable for the biosynthesis of group 2 capsules are situated in three different regions: regions 1 (kpsFEDUCS) and region 3 (kpsMT) and are contributory in the assembly and exordium of capsular polysaccharides, while region 2 encodes enzymes necessary for synthesizing the required polysaccharides found in capsular (Goh et al. 2017, Frankel and Ron 2018, Croxen and Finlay 2010).

2.9.6 Enzymes

Escherichia coli producing extended-spectrum β-lactamase (ESBL) exhibit resistance to a wide range of β-lactam antibiotics, such as Penicillin, Cephalosporins, and Monobactams. *Escherichia coli* is a significant organism globally known for making ESBL, which is encrypted through *bla*-TEM. β-lactamase functions through breaking the amide bond in the β-lactam ring, resulting in deactivation of those antibiotics. Carbapenem's is typically found on gens linked to resistance for other non-beta-lactam antibiotics, leading to multi-drug resistance (MDR) bacteria. Carbapenem's refers to particular plasmid-mediated β-lactams are effective contra Carbapenem's such as imipenem, ertapenem, doripenem and meropenem (Frankel and Ron 2018).

2.9.7 Outer Membrane Proteins (OMPs)

Gram-negative bacteria are enveloped by a pair of membranes: a membrane on the inside (IM) and a membrane on the outside (OM). The outer membrane's outer leaflet,

which faces outwardly, is made up of lipopolysaccharides (LPS), a molecule with a strong negative charge that extends into the bacteria's surroundings. The outer membrane effectively blocks molecules with hydrophobic characteristics from entering bacteria, providing protection in environments such as the intestine. However, its lipid nature also prevents hydrophilic substances from passing through. In order to address this issue, bacteria utilize specific protein channels known as pirons that facilitate the entry of small molecule hydrophilic compounds into cells. (Riedal etal. 2019).

Outer membrane proteins (OMPs) play crucial roles in the outer membrane, serving as attachment factors, mediators for nutrient uptake, siderophore receptors, and enzymes like proteases and lipases. LptD functions as a 26-stranded β-barrel that plays a crucial role in inserting LPS into the leaflet's outermost layer of the OM and preserving the bilayer's asymmetry. BAM component is accountable for inserting β-barrel proteins (OMPs) into the outer membrane (Rallouer etal. 2015).

OMPT protein consists of a 10-strand antiparallel β-barrel outer membrane protease that is incorporated into the outer membrane through the BAM component. It is involved in the fission of antimicrobial peptides (AMPs) such as colicin. AMPs are compounds released through living organisms that exhibit antimicrobial properties, specifically against bacteria (Urashima et al. 2017).

2.10 Antibiotic

Antibiotics are natural compounds primarily produced by living organisms like plant microflora. Due to their potent and targeted biological effects on microorganisms, along with their minimal toxicity, various antibiotics are effective in eliminating bacteria in living organisms (Korzybski et al. 2013).

There was a lack of understanding about microbes and infectious diseases before the discovery of antibiotics. Countless individuals lost their lives due to the inefficacy of the strategies employed to manage and contain these deadly illnesses, often reaching epidemic proportions (Uddin et al. 2021).

In 1899, Emmerich and Löw conducted research on the medical advantages of Pseudomonas aeruginosa extractions, marking an early scientific record of antibiotic use. Investigations were paused due to discrepancies in outcomes (Ribeiro da Cunha et al. 2019). Most antibiotics utilized in human medicine nowadays are naturally created by environmental bacteria or fungi. Truly, Streptomyces, found in soil samples, is responsible for the majority of the antibiotics currently in use. Microorganisms need to create antibiotic compounds to protect themselves from each other in their environment, and they also have to develop defenses against different antibiotics. Moreover, the species that naturally produce antibacterials are resistant to them (Durand et al. 2019). Modern medicine has evolved due to the use of antibiotics. They offer essential therapies and procedures that are vital for treating infections. However, despite their achievements, their continued use in the twenty-first century faces challenges from two separate issues. One issue is that over time, the bacteria targeted by these medications build up a resistance to them. Another point to consider is that using traditional payment methods to discover and develop antibiotics is no longer cost-effective (Cook and Wright 2022).

Antibiotics have developed resistance patterns over time. Several classes of antibiotics, including Cephalosporins, Aminoglycosides, Tetracyclines, Quinolones, Macrolides and others, have also developed over time. Example, Cephalosporins have progressed from the initial generation to the fifth generation. These antibiotic categories employ various methods to fight bacteria. These mechanisms involve deactivating the formulation of bacteria cell walls Penicillins and Cephalosporins, Fluoroquinolones interfere with the formation of bacterial DNA, Tetracycline inhibits bacterial protein formation, and finally Macrolides and Aminoglycosides destroy protein formation. (Adzitey 2015).

2.11 Resistance to Antibiotics in UPEC

Antimicrobial resistance has been on the rise in recent decades, particularly among uropathogenic *E. coli*. Studies have shown sudden trends of resistance to antimicrobial agents among *Escherichia coli* worldwide. (Woodford et al. 2006).

The resistance sequences in *E. coli* have been developed and spread through various biological mechanisms. In addition, mutations leading to antibiotic resistance often occur due to positive selection pressure (Martinez and Baquero 2000).

The horizontal genetic transmission was the primary mechanism for the enhancement of antibiotic resistance, which has significantly influenced the transfer of virulence variables and antibiotic resistance in UPEC (Blahna et al. 2006).

Plasmids have played a crucial role in the spread of antibiotic resistance, encompassing nearly every important class of antibiotics. According to recent studies, due to the increasing resistance to the primary antibiotic treatment, managing urinary tract infections has become more challenging (Cagnacci et al. 2008).

Managing infections with UPEC has become more complicated due to the development of antimicrobial resistance, especially since the late 1990s (Pitout 2012). Over the past 30 years, there has been a significant rise in antibiotic resistance between pathogenic bacterial species, with the emergence of multiple resistant pathogens causing concern (Venturini 2011). Due to the growing administration of treatment and the emergence of mutated resistant strains in the uropathogen, antibiotic resistance has become a significant concern (Koreň et al. 2013).

Addressing community and hospital-acquired diseases caused by UPEC infection involves the use of antibiotics. Resistance to these strains may develop due to delays in appropriate treatment, leading to higher rates of morbidity and mortality (Venturini 2011).

When antibiotic sensitivity testing is not conducted in clinical medicine, the misuse of treatment becomes a significant factor in the development of MDR. This results in the spread of antibiotic-resistant organisms. The bacteria that become resistant to a variety of antibiotics due to prolonged use of a single antibiotic for weeks or months has been discussed by Livermore (2007).

When a bacterial strain becomes resistant to the initial medication, healthcare providers may need to resort to using stronger antibiotics that can be more toxic to the patient. This can lead to various challenges such as increased hospital-acquired infections, limited treatment choices, higher morbidity and mortality rates, and increased healthcare costs due to longer hospitalizations (Livermore 2007).

Uropathogenic *E. coli* is the most frequently isolated pathogen and a major reservoir for genes encoding antibiotic resistance. Antimicrobial resistance is influenced by various factors including antibiotic usage, hygiene, antibiotic presence in animal feeds, and crowded living conditions, along with the virulence of the organisms (Erb et al. 2007; Lau et al. 2008; Moreno et al. 2009). In addition, UPEC may involve antibiotic-resistant *E. coli* in the bowel, which could become the main reservoir of resistant pathogenic strains (Erb et al. 2007).

2.11.1 Tetracycline Antibiotics

The tetracycline antibiotics (TCs) produced by Streptomyces contain four naphthacene cores, giving them broad-spectrum capabilities. Tetracycline is effective in treating gram-negative bacteria and gram-positive bacteria. (Cánovas et al. 2021). These treatments are commonly used for bacterial respiratory infections, UTI, periodontal, Lyme, and rickettsial diseases. The term "broad-spectrum" was first applied to tetracyclines, the initial prominent group of antibiotics to receive this designation. In 1947, chlortetracycline, oxytetracycline, and tetracycline were discovered. Minocycline and doxycycline were initially released to the public in the 1960s. This activity is popular globally due to its diverse range of options, affordability, and overall safety. Tetracyclines are second only to penicillins in terms of the amount that is used each year (Roberts and Schwarz 2016). Tetracyclines are effective in eliminating bacteria through bound to the 30S ribosomal subunit of infected bacteria. Following the ribosomal binding, tetracycline disrupts the ability of aminoacyl tRNA to bind to a ribosome or messenger RNA molecule. Bacterial protein production is influenced by this. Tetracycline can inhibit mitochondrial protein synthesis and bind with the 70S ribosomes found in mitochondria (Dowling et al. 2017).

2.11.2 Aminoglycoside Antibiotics

Discovered in 1944, it was the first aminoglycoside antibiotic to be effective against pulmonary tuberculosis. Various aminoglycosides, including kanamycin, gentamicin, sisomicin, and lividomycin, have been consistently present over a period of four centuries. Aminoglycosides are known to inhibit protein synthesis through forming bound interactions with the rRNA subunit of the bacterial 30S ribosomal (Zhu et al. 2019). Renal and auditory toxicity had negative impacts, resulting in decreased usage worldwide during the 1970s and 1980s. Despite this, aminoglycosides remain widely used globally because of their high effectiveness and relatively low cost (Forge and Schacht 2000).

One of the extreme widespread ways resistances to Aminoglycosides occurs is through aminoglycoside-modifying enzymes (AMEs). These enzymes can modify aminoglycosides in different ways, in addition to acetyltransferases, phosphotransferases, and nucleotidyltransferases. Efflux pumps overexpression is another mechanism that contributes to aminoglycoside resistance. and reduced absorption of medications by bacteria. Another important factor leading to resistance against nearly all clinically available aminoglycosides is the production of 16S rRNA methyltransferase (16S-RMTase) (Zhang et al. 2021). Resistance genes can be found in plasmids, but drug-modifying enzymes are not effective. The 16 rRNA methylase enzyme is accountable for the emergence of resistance to all aminoglycosides, such as plazomicin and newer aminoglycosides (Wang et al. 2020).

2.11.3 Fluoroquinolone Antibiotics

Fluoroquinolones are widely used in clinical and veterinary medicine as synthetic antibacterial drugs. They are highly effective in treating a variety of clinical infections and demonstrate excellent tissue penetration when targeting specific enzymes, as demonstrated in the study by Chattaway et al. (2016).

Resistance to fluoroquinolones is caused by a range of factors, such as reduced permeability, enhanced active efflux, modified target sites, and antibiotic inactivation. One of the key factors in its development is the acquisition of quinolone resistance genes through a plasmid-mediated process (PMQR). Other Enterobacteriaceae species also possess the PMQR determinant, which can be detected in PMQR. One hypothesis suggests that the PMQR determinant plays a part in fluoroquinolone resistance in *E. coli*. These genes are shielded from quinolone inhibition by qnr, a gene present in both of them. Discovered in plasmids from *E. coli* isolated in the United States in 1994, qnr was first identified. Due to the excessive use of FQs, there has been a significant increase in FQ resistance, leading to challenges in treatment (Zhan et al. 2021, Wang et al. 2020).

2.11.4 Beta-lactam Antibiotics

Beta-lactam antibiotics are extensively utilized in clinical medicine (Bush and Macielag 2010). Amoxicillin with or without clavulanic acid. First-generation cephalosporins have been utilized for uncomplicated urinary tract infections, whereas third-generation cephalosporins have been prescribed for complicated urinary tract infections (Anonymous 2006). Third generation cephalosporins, β-lactam antibiotics were crucial for treating severe community onset or hospital-acquired infections caused by *E. coli* infection (Pitout 2012).

E. coli's resistance to β-lactam antimicrobial agents is mainly due to β-lactamases, enzymes that break down the β-lactam ring and render the antibiotic ineffective (Briñas et al. 2005). Resistance to the β-lactam antibiotic often stems from bacteria producing a specific enzyme that deactivates the antibiotic by breaking down the β-lactam ring. (Bush and Macielag 2010).

The extended spectrum B-lactamase genes were transferred via a plasmid, enabling organisms to acquire them through various resistance mechanisms. In a study by Brooks et al. (2010), the emergence of B-lactamase and its global distribution have presented a significant challenge in combating diseases caused by the bacteria strains.

Within Gram-negative bacilli bacteria, such as *Escherichia coli*, the transfer of TEM was the predominant plasmid-mediated beta-lactamase. Moreover, the organism also possesses the less frequently found enzyme known as SHV. These enzymes have the ability to break down penicillins and narrow spectrum cephalosporins such as cephalothin. The higher generation cephalosporins, including ceftriaxone, cefotaxime, and ceftazidime, are not affected by these enzymes. Nevertheless, the efficacy of third-generation cephalosporins can be enhanced by lactamase enzyme inhibitors like clavulanic acid (Canton and Coque 2006).

2.12 Genes Associated with Antimicrobial Resistance of *E. coli*

2.12.1 CTX-M gene

CTX-M-type enzymes have been identified as one gene from ESBLs since the mid-2000s, which are a type of ESBL. CTX-M has been identified in various species of Enterobacteriaceae. Reference from Gajdács et al. (2019).

However, it has been revealed in recent decades that there has been a rise in speed and significant growth in microbes expressing CTX-M-type enzymes. Due to this, CTX-M—lactamases have become the most prevalent ESBLs globally. Due to the widespread dissemination of CTX-Ms globally, it has been referred to as the "CTX-M pandemic." This is because their numbers are on the rise in all locations. Analysis of CTX-M enzyme sequences discover five main categories of acquired enzymes distinguished by their amino acid sequences (Latifi et al. 2021). The CTX-M novel family of ESBLs differs from the third generation of cephalosporin ceftazidime by showing a greater resistance to the drug cefotaxime. The CTX-M gene is named after its ability to hydrolyze the drug cefotaxime (Palzkill 2018).

2.12.2 TEM gene

Discovered in a patient's *Escherichia coli* in Greece in the 1960s, the TEM-1 enzyme is noteworthy. Various Gram-negative bacterial species can be transferred using TEM

enzymes due to their plasmid-mediated nature. Antimicrobial medications become ineffective when enzymes attack and degrade the beta-lactam ring. The antimicrobial agent capable of breaking down ampicillin is blocked by clavulanic acid and demonstrates superior effectiveness compared to oxacillin or carbenicillin (Ocean 2017).

The isoelectric point of the TEM-1 and the TEM-2 was found to be different, ranging from 5.4 to 5.6. These two enzymes are responsible for the breakdown of cephalosporins, such as cephalothin and cefazolin. There is resistance to the action of these antibiotics in higher-generation cephalosporins like cefepime, cefotaxime, and ceftazidime, all of which contain an oxyimino side chain (Akpaka et al. 2021).

Upon its discovery, cefotaxime was initially labeled as CTX-1, with the TEM-3 being the first TEM type recognized as an extended spectrum beta lactamase due to its potent activity against the drug (ESBL). Understanding the prevalence and spread of these TEM genes necessitates a deep understanding of their epidemiological characteristics. Utilizing molecular methods can be beneficial in this regard (Schneider et al. 2007).

2.12.3 TET A gene

Genes related to tetracycline resistance are frequently present in plasmids and transposons, and they are transferred between organisms during sexual reproduction. Some isolates, however, possess significant genes on the chromosome too. There are several mechanisms that can lead to tetracycline resistance, with efflux pumps, ribosome protection, and enzyme inactivation being the most prevalent. Mutations worsen antibiotic resistance. tetA, tetB, tetC, tetD, and tetG are the extreme widespread tet genes establish in bacteria with gram-negative, responsible for encoding efflux pumps (Jahantigh et al. 2020). When examining antibiotic-resistant genes, tetracycline resistance can serve as a valuable test scenario. An epidemiological-molecular evaluation can assist in gaining a deeper understanding of the appropriate treatments for resistant strains of these drugs. It is essential to monitor antibiotic resistance and its producing genes to prevent their spread (Jahantigh et al. 2020).

2.12.4 QNR B gene

Fluoroquinolone antibiotics have gained popularity in recent decades, resulting in upgrowth of bacterial strains that are resistant to their effects. Plasmid-mediated quinolone resistance, or PMQR, is considered a mechanism that plays a significant role in resistance among Gram-negative bacilli. PMQR was initially discovered in 1998. So far, three PMQR-mediated pathways have been identified and described in the literature. The acetyltransferase aac(6′)-Ib-cr, a variation of an enzyme contributory in aminoglycoside modification and resistance, along with active efflux pumps like QepA and OqxAB, exemplify these processes in action. Seven main Qnr families have been identified as a result. Every single one of these letters represents a distinct letter of the alphabet: QnrA through QnrVC. Reference: (Saki et al. 2022).

It was previously believed that Enterobacteriaceae only developed resistance to quinolones through mutations in DNA gyrase, DNA topoisomerase IV, and genes encoding outer membrane proteins. Enterobacteriaceae, meanwhile, have been documented to possess plasmid-mediated quinolone resistance (PMQR). Resistant to quinolones, PMQRs exhibit only a restricted degree of resistance (Kareem et al. 2021).

3. MATERIALS and METHODS

3.1 Materials

3.1.1 Instruments and Equipment

A comprehensive list of the instruments and equipment, including details on the manufacturing firms and their respective countries of origin are provided in Table 3.1.

Table 3.1 Instruments and equipment.

No.	Instruments and equipment	Manufacturer (country)
1	Autoclave	Hirayana (Japan)
2	Blood culture system	bioMérieux (France)
3	Centrifuge	Kockusan (Japan)
4	Chamber Hood	Tellsbio (Germany)
5	Compound light microscope	Olympus (philppines)
6	Filter paper	Difco (United States of America)
7	Gel electrophoresis system	Bioneer (Korea)
8	Hot Plate	Thermolyne (United States of America)
9	Incubator	Binder (Germany)
10	Inoculating needle wire loop	Himedia (India)
11	Micropipette set (1-1000µl)	Slamed (Germany)
12	Petri dish (90*15 mm)	China
13	pH meter	WTW (Germany)
14	Refrigerator	Dairel (Germany)
15	Shaking	GFL/Germany
16	Sensitive balance	Cellule (Switzerland)
17	Slides	Himedia (India)
18	Thermal cycler	Analytic jena (Germany)
19	Vitek 2 Compact Auto-analyzer	BioMerieux (France)
20	Vortex	IKA (China)
21	Water distiller	NÜVE (Turkey)
22	Water bath	Memmert (Germany)
23	Wooden sticks	Himedia (India)
24	Deep freze	ALS (Italy)
25	Eppendorf tubes Centrifuge	Kockusan (Japan)
26	Polymerase chain reaction system	Biometra (Germany)

3.1.2 Biological and Chemical Materials

The biological and chemical components of the present investigation are displayed Table 3.2.

Table 3.2 The biological and chemical materials.

No.	Materials	Manufacturer (country)
1	Agarose	Condalab (Spain)
2	Ethanol (96%)	BDH (USA)
3	Ethidium bromide	Bio basic (Canada)
4	Ethylene diamine tetra acetic acid (EDTA)	Fluka (Switzerland)
5	Glycerol	Hi-media (India)
6	Hydrogen peroxide 3%	Mast Diagnostic (USA)
7	TBE-buffer	Promega (USA)
8	TE- buffer	Promega (USA)
9	Tetra-methyl-pphenylenediaminedihydrochloride	BDH (USA)
10	transporter swabs	AMIES (China)
11	Kovac's reagent	Hi-media (India)
12	Methyl red	Hi-media (India)
13	Hydrochloric acid (HCl)	Hi-media (India)
14	Sodium chloride (NaCl)	BDH(USA)

3.1.3 Kits Types

The kits that have been utilized in the present research (Table 3.3).

Table 3.3 The kits that utilized during present research.

No.	Kits	Manufacturer (country)
1	DNA Extraction kit	Favorgen Biotech (Taiwan)
2	Gram stain kit	Hi media (India)
3	ViteK-2 system cards	bioMérieux (France)

3.1.4 Culture Media Provided by the Manufacturer

The culture media used in this investigation are summarized in Table 3.4. All of the media utilized in this study were sterilized in autoclaves at 121 °C and 15 bar for 15 minutes, as per the guidelines provided by the manufacturers (Tille 2015).

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Table 3.4 Culture media provided by the manufacturer used.

No.	Culture media	Manufacturer (country)
1	Blood Agar	Hi-media (India)
2	MacConkey Agar	Hi-media (India)
3	Muller-Hinton Agar	Hi-media (India)
4	Eosin methylene blue (EMB) agar	Hi-media (India)
5	Triple sugar iron agar	Hi-media (India)
6	Urea agar	Hi-media (India)
7	Desoxycholate citrate agar	Oxoid (England)
8	Nutrient Broth	Hi-media (India)
9	Chrome agar	Condalal- Spain
10	Simmons citrate	Hi-media (India)
11	Methyl red-Voges-Proskauer broth	Hi-media (India)
12	Motility test medium	Hi-media (India)
13	Nutrient Agar	Hi-media (India)
14	Peptone water	Hi-media India

3.1.5 Antibiotics

All of the antibiotic discs that were employed in the current investigation (Table 3.5).

Table 3.5 Discs of antibiotics provided by Bioanalyse Company (Turkey).

Class	Subclass	Antibiotic	Symbol	Con. (µg/ disc)
Aminoglycosides		Gentamicin	CN	30
		Amikacin	AK	10
Cephems	Cephalosporin II	Cefixime	CFM	30
	Cephalosporin III	Cefotaxime	CTX	)
Tetracyclines		Tetracycline	TE	10
Penicillin's		Amoxicillin	AM	20
Penems	Carbapeneme	Imipenem	IPM	10
		Meropenem	MRP	10
Quinolones	Fluoroquinolones	Ciprofloxacin	CIP	10
		Levofloxacin	LEV	5
Monobactams		Aztreonam	ATM	10

3.1.6 Materials of Polymerase Chain Reaction

Polymerase chain reaction materials include GoTaq® G2 Green Master Mix, DNA ladder, primers (forward and reverse), DNA template, and Nuclease-free water.

3.1.6.1 GoTaq® G2 Green Master Mix and DNA Ladder

The reaction buffers and GoTaq® G2 DNA Polymerase at the optimal concentrations for efficient PCR amplification of diverse DNA templates were included in the GoTaq® G2 Green Master Mix, which was produced by Promega Company in the USA and utilized in this investigation. Two dyes—yellow and blue—in the GoTaq® G2 Green Master Mix make it possible to observe development during electrophoresis. The utilized 100bp DNA ladder is manufactured by Promega Company (USA) and consists of eleven double-stranded DNA morsels measuring (100,200,300,400,500, 600,700,800,900,1000,1500bp), the 100 Bp DNA ladder was provided in a final pH of 7 solution containing 10 mM Tris-HCl and 1 mM EDTA, morsels.

3.1.6.2 Primers

Table 3.6 presents the genes and primer sequences that were used.

Table 3.6. Primer sequences and their length used for PCR

Genes	Oligo-Sequence (3′→5′)	Product size (Bp)	Reference
blaCTX-M	F: ATGTGCAGYACCAGTAA	512	(Tabi et al. 2018)
	R: CCGCTGCCGGTYTTATC		
blaTEM	F: TCAACATTTTCGTGTCGCCC	766	(Yang et al. 2020)
	R: AACTACGATACGGGAGGGCT		
QNRB	F: GATCGTGAAAGCCAGAAAGG	469	(Herrera et al. 2021)
	R: ACGATGCCTGGTAGTTGTCC		
tet A	F: GGTTCACTCGAACGACGTCA	577	(Boroumand et al. 2021)
	R: CTGTCCGACAAGTTGCATGA		

3.2 Reagents and Solutions Preparation

3.2.1 Gram Stain Solution

The preparation of Gramme stain solutions was achieved in conformity with the instructions provided by Collee et al. (1996) and the manufacturer.

1. Crystal violet was prepared by mixing crystal violet A, which consisted of 2 gr crystal violet and 20 mL ethyl alcohol, with crystal violet B, which consisted of 0.8 gr ammonium oxalate monohydrate and 80 mL distilled water; the resulting stain was stable after 24 hours.
2. Iodine solution made up of 1 gr of iodine, 2 gr of potassium iodide, and 300 mL of distilled water.
3. A decolorizer made up of 50 mL of 95% ethyl alcohol and 50 mL of acetone.
4. Safranin, consisting of 0.5 gr safranin O and 100 mL of 95% ethyl alcohol.

3.2.2 McFarland Standard (0.5)

As is the case with Barry (1976), McFarland standard (0.5) was prepared as below:
1- Solution A: Dissolve 1.175 grams of BaCl2.2H2O in 100 ml of distilled water.
2- Solution B: A volume of 1 ml from H2SO4 was introduced into 100 ml of distilled water. Subsequently, a volume of 0.5 ml was transferred from solution A to a volume of 99.5 ml of solution B.

McFarland standard tubes were maintained for a maximum of six months at ambient temperature. Utilizing a spectrophotometer to determine the density of a 0.5 McFarland standard.

At a wavelength of 600 nanometers, the absorbance should range from 0.08 to 0.1. Adjust density determinations to get an optical density equal to 1.0H CFU/ml bacterial suspension turbidity (Andrews 2001).

3.2.3 Oxidase Reagent

In an opaque glass bottle, the reagent was utilized for determine whether bacteria were capable of producing the enzyme oxidase (Al-Dhabaan 2019). It was through dissolving 1 gr from tetramethyl-P-phenylene diamine dihydrochloride in 100 ml of distilled water.

3.2.4 Catalase Reagent

Preparation, it was from hydrogen peroxide at a concentration of 3% from the original solution at a concentration of 30% in an opaque glass bottle, catalase reagent using to detect susceptibility of the bacteria to the enzyme catalase (Tadesse and Alem 2006).

3.2.5 Urea Solution

Preparation of the solution through diluting 40 gr from the urea powder in 100 ml of distilled water than sterilising it using a 0.22mm filter paper (Collee et al. 1996).

3.2.6 Tris Borate EDTA Buffer (TBE)

By adding 10 milliliters of Biobasic buffer, prepare this solution according to the manufacturer's instruction buffer in vial x 10 to 90 milliliters of sterile distilled water to get 1x concentration of TBE Sterilized glass, then kept in the refrigerator at a 2°C till to using (Mehrafshanet al. 2015).

3.2.7 Primer's Preparation

1- Macrogen Company offered lyophilized primers (TetA, qnrB, blaCTX-M, blaTEM).
2- In order to create a concentrated solution, the dried primers were dissolved in a water-based solution that is free from enzymes, resulting in a final concentration of 100 picomoles per microliter.
3- To make a working primer solution (10 mol/µl), 10µl of primer stock solution (frozen at -20°^{C}) was mixed with 90µl of nuclease-free aquatic (Diancourt et al. 2005)

3.3. Cultural Media Preparation

3.3.1 Blood Agar Medium

Blood was added (5%) to the blood agar base medium after it had been produced according to the manufacturer's instructions, which included dissolving (40 gr) from powder of blood agar in 1000 ml from distilled water, sterilizing it using an autoclave, and cooling it to roughly 40-45°C. The medium was then put onto sterilized Petri dishes. Enriched blood agar can isolate, culture, and detect hemolysis in bacteria. Most bacterial species were grown in it (Benson 2002).

3.3.2 MacConkey Agar Medium

MacConkey Agar dissolved in one liter of distilled water (55 grams) was brought to a constant boil while agitating, and the resulting medium was autoclaved for 15 minutes to ensure sterilization. Following the cooling of the medium to 45°C, it is left to consolidate at room temperature. Subsequently transferred to sterile Petri dishes (Sultana et al. 2017).

3.3.3 Nutrient Agar Medium

The nutrient agar medium was created by dissolving (28 gr) from its powder in 1000 ml from distilled water, according to information supplied by the manufacturer. After sterlizing the solution, it was kept at a of (40-45°C). It was poured into Petri dishes. In order to activate, cultivate, and detect production in the isolates, the medium was used for a short time (Isenberg 2004).

3.3.4 Eosin Methylene Blue Agar

By dissolving 37.46 grams of the eosin methylene blue agar in 1000 ml of distilled water, this medium was prepared. Then, sterilized by employing autoclave. Next, it was kept in (40–45) degrees Celsius. In the end, it was added in the Petri dishes. The

researchers employed Eosin Methylene Blue agar to isolate, enumerate, and differentiate Enterobacteriaceae members, with a particular focus on Escherichia coli, as described by (Farmer 1999).

3.3.5 Triple Sugar Iron Medium

In order to prepare this medium, 64.42 grams of Triple Sugar Iron powder were dissolved in 1000 ml from distilled water, boiled to homogenize, then liquid medium was poured into test tubes, more than sterilized using an autoclave; the tubes were set at a tilted level to solidify to obtain a slant.Triple Sugar Iron medium was selected to test the efficiency of test isolates for the use of carbohydrates and hydrogen sulfide precipitation (MacFaddin 2000).

3.3.6 Urea Agar Medium

According to Collee et al. (1996), the manufacturer's instructions were followed in the formulation of the urea base powder: 24.51 grams from powder of urea agar was dissolved in 950 mL from distilled water; the suspension sterilizing in the autoclave and cooled to 45 to 50 degrees Celsius; each 95 ml of urea base agar was then mixed thoroughly with 5 ml of sterile filtrated 40% urea solution; the mixture was then dispensed into sterile tubes in a slanting manner. The ability of bacterial isolates (including *Proteus* spp. and *K. pneumonia*) to produce urease was evaluated using a urea agar medium.

3.3.7 Desoxycholate Citrate Agar Medium

According to MacFaddin (2000), preparation mentioned media by dissolving 52 gr from powder in 1000 ml from distilled water. The second step was sterilized through employing an autoclave. Later on, it was cooled at a temperature of 40-45°C. In the end, it was added to Petri plates. Desoxycholate Citrate agar was used to isolate and recover intestinal pathogens, particularly *Salmonella* and many *Shigella* species.

3.3.8 Nutrient Broth

Preparation mentioned media by solving thirteen grams from powder in 1000 ml from distilled water. The second step, 15% from glycerol adding and mixing well. Next step adding it in screw-top containers. Next, sterilized by autoclave, was employed for sterilizing it. Finally, it was stored in the refrigerator to be used in the future and preserve the bacteria for a long period (Isenberg 2004).

3.3.9 Chrome Agar

Preparation Chrome agar media through dissolving 45 gr from its powder in (1)L from distilled water. Continual stirring was employed for its heating, and an autoclave was used for its sterilizing for 15 minutes. Next step cooling to 45 degrees Celsius and distributed onto sterile Petri plates, where it solidifies at ambient temperature. The samples were then kept in a dark container at 4°C (Abdullah et al. 2009).

3.3.10 Motility Test Medium

The medium was prepared through mixing 20 gr from its powder in 1000 ml from distilled water and distributed in the laboratory tubes. The tubes were sterilized by autoclave and left to cool and solidify to in an upright position. This medium is usually used to detect motile bacteria (such as *Proteus* spp.).

3.4 Study Ethics

The study was carried out according to the guidelines obtained from the Graduate School of Natural and Applied at Afyon Kocatepe University. The study samples from different clinical sources were obtained from patients after obtaining approval from them and under the supervision of specialized medical staff. This study did not employ genetically engineered microorganisms, environmental isolates, or biological and chemical materials that may affect public health and the environment, nor were there any duplicate samples obtained from the same patient.

3.5 Methods

3.5.1 Study Design

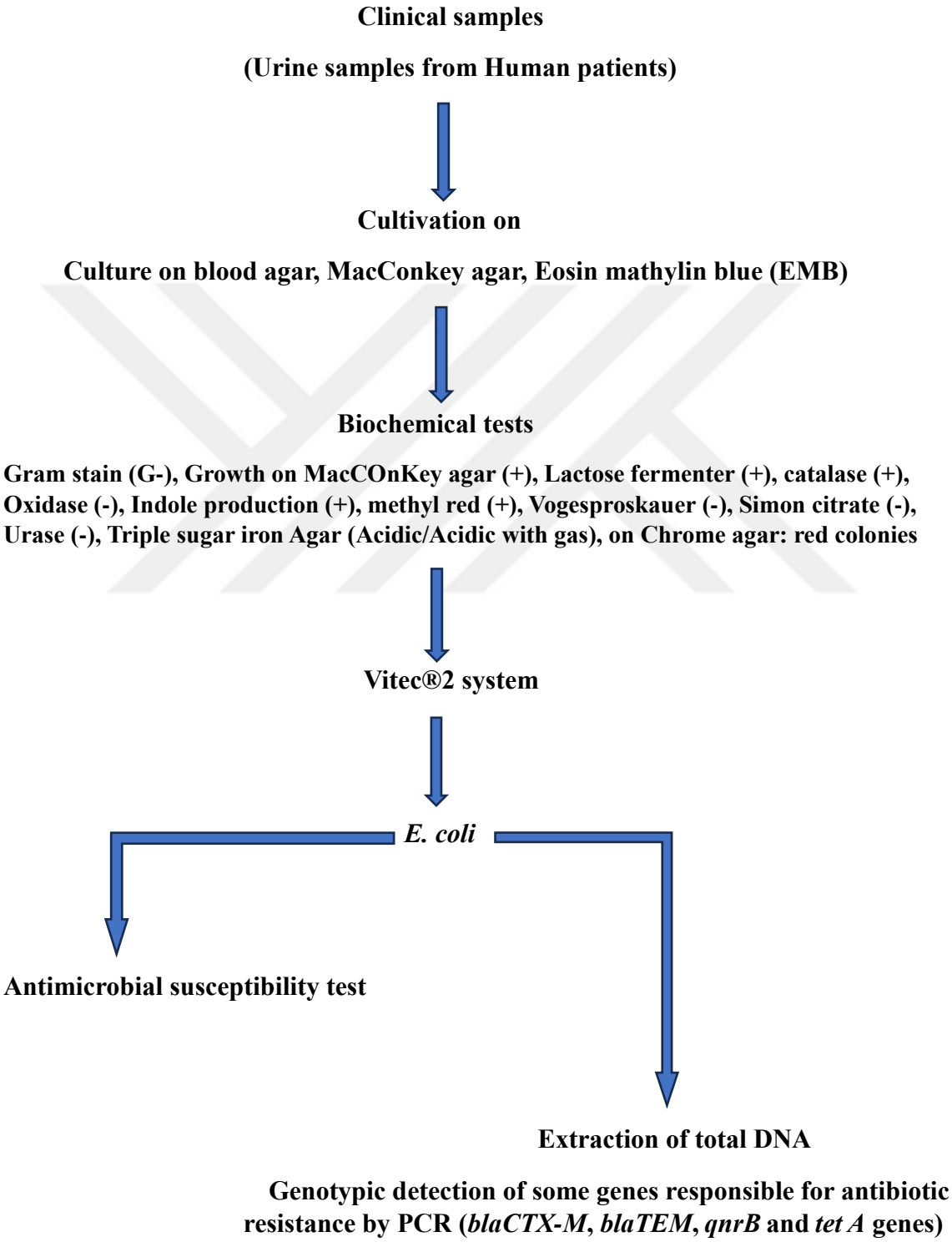

Figure 3.1 Study design

3.5.2 Specimens Collection

From September to December (2023), 157 samples were collected Randomly from the AL-Shamiya General Hospital in the city of Al-Shamiya in Al-Qadisiya Governorate/Iraq. The diagnostic microbiology textbook by Bailey and Scott's directions were followed to carry out the protocol for collecting the specimens (Tille 2015).

3.5.2.1 Urine Culture

The samples were gathered in sterilized containers. Midstream urine specimens were injected on blood agar, macConkey agar, and cysteine lactose and incubated overnight at 37ºC. The bacterial growth was presumptively identified on Chrome agar based on color and morphlogy of the colonies, as illustrated by Manufacturer Colonies on MacConkey agar.

To make sure the identity of the isolates, they were subjected to standard identification protocols, which included Gram staining, motility testing, catalase testing, oxidase testing, and other biochemical analyses deemed pertinent to the isolates (Biji et al. 2017).

3.5.3 Isolation and Identification of *Escherichia coli*

Taking into account the microscopic test, morphological properties of culture media, and biochemical tests, *E. coli* was identified and isolated. Moreover, it was done due to Bailey and Scott (Tille 2015).

3.5.3.1 Microscopic Characterization

Gram stain was applied to all previously prepared smears of test isolates, and the arrangement, shape, and size of bacterial cells were observed with an oil immersion lens mounted on a light microscope set at 100X (Bell and JA 2004).

3.5.3.2 Gram Stain

The Gram stain is a kind of differential stain that allows microbiologists to see the morphology and cell structure of organisms while also distinguishing between the two most common chemical cellular structures found in bacteria (Tille 2015).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 composed of a higher fat content and appears red or pink when examined under the microscope after staining by gram stain (Tripathi and Sapra 2020).

Figure 3.2 depicts the stages involved in gram staining, as well as the occurrence of gram- negative and gram-positive bacteria beneath the microscope. Gram stain was used to tint a smear obtained from a young, pure colony. Gram stain causes *Escherichia coli* to appear as pink rods. *Escherichia coli* is a gram-negative, straight, rod-shaped, non-sporing bacillus that occurs in single or pairs (Tille 2015).

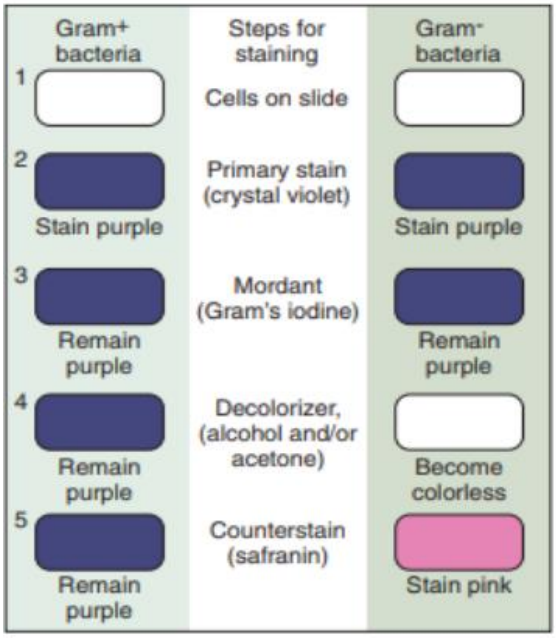

Figure 3.2 Principles and processes for Gramme staining. When Gram-positive bacteria are submerged in oil, they take on a purple hue. When gram-negative bacteria are submerged in oil, they become pink (Tille 2015).

3.5.3.3 Cultural Characterization

After gathering each specimen into a sterile container, one loopful was streaked with blood and MacConkey agar right away, and it was then incubated aerobically at 37 °C for a whole day. Colonies of the isolates were purified by subculture to identify their phenotypic characteristics such as edge, size, shape, color, pigment production, and hemolysis type.

Escherichia coli colonies are typically non-hemolytic, although several strains that have been isolated from infections are beta-hemolytic (Tille 2015).

Lactose fermenting bacteria make up the bulk of *E. coli* colonies, which often exhibit flat, dry, pink surfaces with darker pink regions around them from the precipitation of bile salts (Manishimwe et al. 2021). *E. coli* colonies have a green metallic shine on EMB agar (Tille 2015).

3.5.3.3.1 Growth on Blood Agar

Since this medium was enhanced, it was used to cultivate a wide variety of bacteria. After streaking on blood agar plates, all bacterial isolates were cultured to 24 hours at a temperature of 37 degrees Celsius. Once the incubation time is finish, the kind of hemolysis is identified (Collee et al. 1996).

3.5.3.3.2 Growth on MacCOnKey Agar

According to Collee et al. (1996). Members of the Enterobacteriaceae family were isolated using this medium, which was used as a selective and differential media. After being streaked on MacConkey agar plates, all bacterial isolates were cultured for 24 hours at 37 °C. The development of pink bacterial colonies is thought to be indicative of lactose-fermenting bacteria.

3.5.3.3.3 Growth on EMB Agar

On Eosin methylene blue (EMB) agar, *Escherichia coli* isolates were presumed to be recognized based on colonial morphology after being cultured for 24 hours at 37°C. Conventional biochemical assays were used to identify further and validate distinct greenish metallic sheen colonies on the EMB agar dishes (Farmer 1999).

3.5.3.3.4 Growth on Chrome Agar

According to the manufacturer's company protocol (Orientation Business – France), this medium was employed as a selective identification test for *E. coli* exclusively as follows:

On chrome agar medium plates, all putative bacterial isolates were streaked before being cultured for 24 hours at 37°C. Red colonies growing is regarded as a good indicator of *E. coli*.

3.5.3.4 Biochemical Test

The following biochemical tests were used to identify the bacterial isolates. *E. coli* is Oxidase, Catalase, hydrogen sulfide, and urea negative and indole and methyl red-positive bacteria.

3.5.3.4.1 Oxidase Test

On filter paper, a fresh bacterial colony was mixed with two or three drops of freshly prepared oxidase reagent (Tetra-methyl-p-phenylenediaminedihydrochloride). Within ten to fifteen seconds, the development of a violet or purple color indicates positive results (MacFaddin 2000).

3.5.3.4.2 Catalase Test

The test was conducted as follows: on a sterile slide, a mixture of 3% hydrogen peroxide and growth from a new (24-hour) and pure colony was added; the appearance of bubbles signifies a favorable outcome regarding the bacterial ability to manufacture the catalase enzyme (Reiner 2010).

3.5.3.4.3 Hemolysin Test

The pure isolates were inoculated on blood agar in the form of spots. The appearance of a clear zone around the colonies after incubating overnight at 37°C indicates the ability of the bacteria to production the hemolysin, enzymes that damage and lyse RBC (Benson 2002).

3.5.3.4.4 Triple Sugar Iron Test

The media was injected with a juvenile bacterial culture by piercing the lower of the tube and spreading it across the roof of the slanted medium. Next, incubating overnight a temperature of 37 degrees Celsius. Existence of a positive effect is shown through a red color, which indicates an alkaline environment with no acid and no generation of H2S (MacFaddin 2000).

3.5.3.4.5 Motility Test

The center of the motility test medium was inoculated with a young bacterial inoculum using a needle wire loop, and overnight incubation at 37 °C was carried out. The formation of cloudy growth outside the stab line is evidence that the bacteria are motile (MacFaddin 2000).

3.5.3.4.6 The Production of Urease

Overnight culture at 37°C was performed on a newly streaked bacterial colony on the urea agar slant. The change from yellow to pink in the medium color signified that the bacteria's ability to produce the urease enzyme was successful.

3.5.3.4.7 Indole Production Test

A novel pure bacterial isolate was introduced to peptone water and left to incubate overnight at 37°C. The next step was to add drops from Kovacs reagent to the experiment tube. Forming a pink ring means the bacterium's ability to producing indole from tryptophan was favorable.

3.5.3.4.8 MethylRed Test

Overnight at 37˚C, a fresh bacterial isolate was incubated after being injected into Methyl Red-Voges-Proskauer broth. Then, a little from methyl red reagent drops were placed in the experiment tube. Thus, the appearance of red color means the results are and the test is done successfully (MacFaddin 2000).

3.5.3.4.9 Voges-Proskauer Test

For inoculating a novel bacterial isolate, Methyl red-Voges-Proskauer broth was employed. Then, it was cultured a temperature of 37 degrees Celsius to (24 h).

A little from the Voges-Proskauer reagent drops were placed in the experiment tube. Consequently, when the red color appearance means that there is an appropriate result and the test was done correctly (MacFaddin 2000).

3.5.3.4.10 Simmons' Citrate Test

A novel isolated bacterium was seeded onto Simmons' citrate slanted agar. Then, left to incubate a temperature of 37 degrees Celsius for 24 hours. The positive outcome was indicated by the presence of the color blue (Collee et al. 1996).

3.5.3.4.11 Analytical Profile Index (API) 20E Test System

Analytical profile index (API) 20E test system verified the *E. Coli* isolates found by biochemical testing. As instructed by the manufacturer, this test was conducted. Biochemical test wells were infected with overnight bacterial suspensions and cultured at 37 °C for 24 hours, compared to a 0.5 McFarland reference tube. After the reagents were added, the findings were read as a seven-digit number that could be found using the API 20E analytical index.

3.5.3.4.12 Vitek2® COMPACT System Assay

Vitek2® system (BioMerieux-France) was used to identify all *Escherichia coli* isolates following the manufacturer's instructions. Each test isolate's standard inoculum was prepared at a carrier station and put in a VITEK cassette. An association between the sample and the VITEK card was then made using a barcode. Furthermore, a cassette was loaded onto the device, enabling it to incubate samples and read the outcomes. The initial results were read either directly from the device or from the chart that had been made specifically for reading the results.

3.5.4 Preservation of Bacterial Isolates

3.5.4.1 Short Time Preservation

Before being incubated a temperature of 37 degrees Celsius for eighteen to twenty-four hours and after that stored at 4 °C in the refrigerator, a single colony of pure bacteria was streaked over nutritional agar medium as a slant (Harley and Prescot 2002).

3.5.4.2 Long Time Preservation

Inoculating two fresh, pure colonies into a nutrient broth with 15-20% glycerol, they were then kept at -10 to -15 °C for 12 to 18 months (Harley and Prescot 2002).

3.5.5 Antibiotic Susceptibility Test

In line with the guidelines made by the Clinical Labortory Standrds Institute in 2021, the antibiotic susceptibility test (AST) on *E. coli* was performed using the Kirby-Bauer disc diffusion process (Kirby Bauer 1966). A 5/6 *E. coli* colony matching the 0.5 McFarland standard (1.5x CFU/ml) was used to create an inoculum for AST in nutritious broth. A sterile cotton swab was inoculated at 37°C after being dipped into the inoculum suspension and put into the tube wall above the fluid level within 15 minutes. Moisten the MHA plate, which stands for Muller Hilton agar. After waiting three to five minutes, the antibiotic disc was gently pressed down until it made complete contact with the agar. For AST, antibiotic discs from (Antibatc) were used. The CLSI 2021 interpretive criteria were used to estimate the zone of inhibition in ml, and the isolates were classified as sensitive, moderate, or resistant (Negi et al. 2021).

3.5.6 Molecular Detection of Resistance-associated Genes in *Escherichia coli*

3.5.6.1 Isolation of Total DNA

According to Omar et al. (2014), the following method was used: Inoculating 300 µl of distilled water with 3 to 5 fresh and pure colonies of *Escherichia coli* was done using a MacConkey agar plate. After that, for twenty minutes into a water bath of 100^{o}C, the cells were heated until they were lysed.

The cells were quickly chilled on ice for half an hour and then centrifuged at 8500 rpm for ten minutes to remove any leftover cell debris. In the end, the DNA template was created from the supernatant.

3.5.6.2 Polymerase Chain Reaction (PCR) Protocols

3.5.6.2.1 Mixture of PCR

In the present investigation, the total volume of the PCR mixture was twenty microliters. They are used as below:

A. Master mix 5 µl.

B. Primer forward and primer inverse (each alone) 2.5 µl.

C. DNA template 5 µl.

D- Deionizing water 5µl.

3.5.6.2.2 Thermocycling conditions of PCR

Table 3.9 The PCR cycle program settings and conditions employed in the present investigation.

Genes	Cycling condition oC/ Time					Reference
	Initial Denaturation oC Time	Denaturation	Annealing	Extension	Number of Cycles	Final extension / Time	
***bla*TEM**	94 C 5 min	94 C 30 sec	56 C 30 sec	72 C 1.5 min	35	72 C 7 min	(Yang et al. 2020)
***bla*CTX-M**	94 C 5 min	94 C 1 min	57 C 1min	72 C 1min	40	72 C 10 min	(Tabi et al. 2018)
qnrB	95 C 5 min	95 C 1min	55 C 1 min	72 C 1min	35	72 C˚ 10 min	(Badamchi et al. 2019)
Tet	95 C 3 min	95 C 1min	57 C 30sec	72 C 1 min	30	72 C 10 min	(Kurnia at el. 2018)

3.5.6.2.3 The Preparation of Agarose Gel and DNA Loading

According to Bartlett and Stirling (1998), the current procedure was carried out. This was done as follows:

1. A solution was prepared by combining one gram of agarose with 100 ml of Tris-borate-EDTA (TBE) at a final concentration of 10x (90 ml distilled water + 10 ml TBE).

2. We added half mg/mL of ethidium bromide stain after boiling the Tris-borate-EDTA solution and cooling it to 45°C.

3. After pouring the agarose into an equilibrated gel tray, it was allowed to settle and solidify further.

4. Following the loading of five microliters of PCR product into each well of the agarose gel, a DNA marker was added to one of the wells. Adding TBE buffer to the electrophoresis chamber followed the attachment of the gel sample to the chamber. An electric current of 70 volts powered the device for 80 minutes. Using a 1.5% agarose gel, the PCR products were detected.

5. Afterwards, a gel documentation system was used to identify the electrophoresis result. Confirmation of positive findings was achieved when the sample's number of DNA band base pairs matched the target product's dimensions.

3.5.6.2.4 Statistical Analysis

After doing statistical analysis on the composed information, we are able to deduce the distribution of the variables by looking at their absolute and relative (%) frequencies. To identify significant relationships between several factors of UPEC strains recovered from individuals with UTIs. The chi-square ($\chi 2$) tests were performed using IBM SPSS statistics software (Version 20, SPSS Inc, United States), p-values < 0.01 were considered highly statistically significant for both tests.

4. RESULT

4.1 Anthropometric Results

4.1.1 Growth and No Growth

From the results presented in the table below, we note that the number of patients in the test sample contained 157 patients. Our sample are containing (66) patients have no growth Bactria by percentage (42%) (66/157) and (91) patients have growth Bactria via six type Bactria by percentage (58%) (91/157). From the p-value (0.005), the null hypothesis has been unacceptable but Alternative hypothesis have been acceptable, this means difference between growth and no growth Bacteria is significant at significant level (0.01) (0.05). The following figure 4.1 show the results above Table 4.1.

Table 4.1 The growth and no growth in Bacteria

No growth	Percentage	growth	Percentage	t-test	p-value
66	42%	91	58%	7.563	0.005

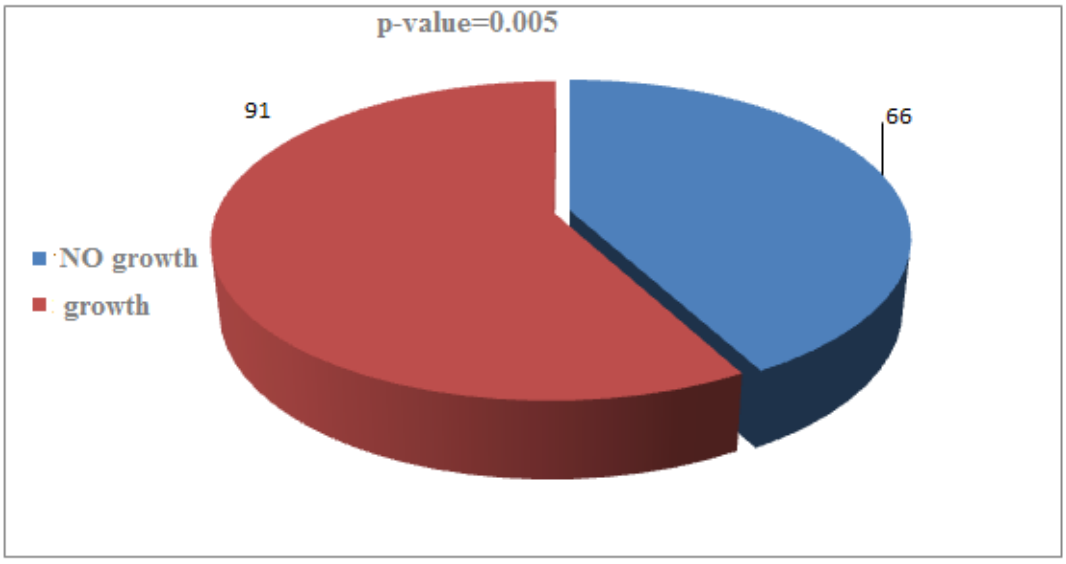

Figure 4.1 The growth and no growth in Bacteria

4.1.2 Types of Bacteria

Our current study recorded 91 bacterial growths are distributed on six different types of Bactria as following:

Escherichia coli by percentage (26/91) (29%), *Staphylococcus aureus* by percentage (19/91) (21%), *Klebsiella pneumoniae* by percentage (16/91) (18%), *Proteus mirabilis* by percentage (11/91) (12%), *Enterococcus faecalis* by percentage (15/91) (16%) and *Pseudomonas aeruginosa* by percentage (4/91) (4%). We see *Escherichia coli* Bactria is greater than infection compared with other type of Bactria. Where, *Escherichia coli* Bactria is comes in first infection through our sample under study. The *Staphylococcus aureus* is coming in second infection through our sample under study. The *Klebsiella pneumoniae* by is comes in third infection through our sample under study. The *Enterococcus faecalis* is comes in fourth infection through our sample under study. The *Proteus mirabilis* is comes in fifth infection through our sample under study. The *Pseudomonas aeruginosa* is comes in sixth infection through our sample under study. From the p-value (0.005), the null hypothesis has been unacceptable but Alternative hypothesis have been acceptable, this means difference between types of Bacteria infection is significant at significant level (0.01) (0.05). The following Figure 4.2 show the results above Table 4.2.

Table 4.2 Distribution of types Bacteria.

Types of Bacteria	Number	Percentage	Chi-square	p-value
Escherichia coli	26	29%	11.371	0.000
Staphylococcus aureus	19	21%		
Klebsiella pneumoniae	16	18%		
Proteus mirabilis	11	12%		
Enterococcus faecalis	15	16%		
Pseudomonas aeruginosa	4	4%		

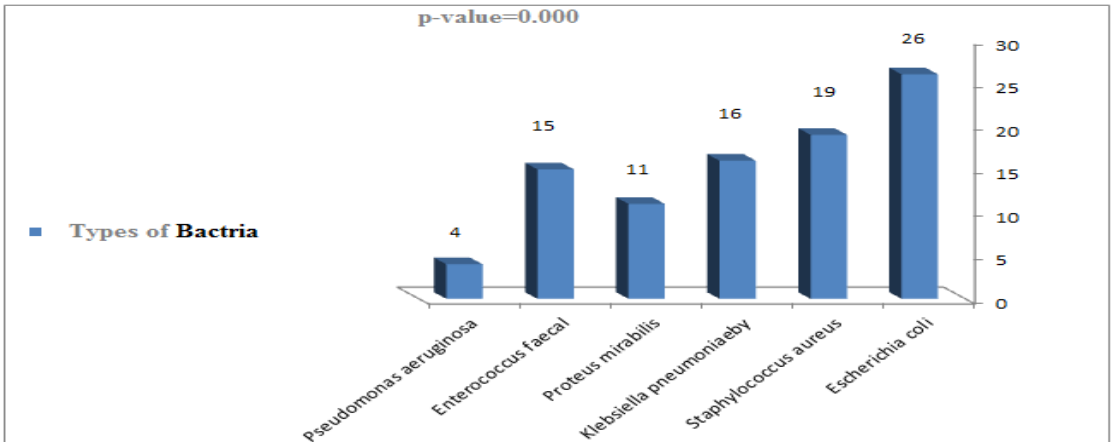

Figure 4.2 Distribution of types Bacteria

(A)

(B) **(C)**

Figure 4.3 *E. coli* colonies on (A) Blood agar plate, (B) MacConkey agar plate and (C) Chrome agar plate, after 24 hours of incubation under aerobic condition at 37 C˚**.**

4.1.3 Sex

From the results presented in the table below, we note in our total sample number of males are (31) by percentage (19.7%) (31/157). Also, our total sample is containing (126) female by percentage (80.3%) (126/157). From the p-value (0.000), the null hypothesis has been unacceptable but Alternative hypothesis have been acceptable, this means the difference between male and female are significant infection at significant level (0.01) (0.05). But in case no growth, there are 17 males by percentage 25.7% (17/66). But there are 49 females by percentage (74.3%) (49/66). From the p-value (0.098), the null hypothesis has been acceptable but Alternative hypothesis have been unacceptable, that is means the variance between male and female are not significant infection at significant level (0.01) (0.05) with no growth case. But in case of growth, we see the number of males is 14 by percentage (18.2%) (14/91). And the number of females is 77 by percentage (81.8%) (77/91). From the p-value (0.000), the null hypothesis has been unacceptable but Alternative hypothesis have been acceptable, that is means the variance between male and female are significant infection at significant level (0.01) (0.05) in growth case. But in *Escherichia coli* infection, there are 6 males by percentage (30.0%) (6/26), and there are 20 females by percentage (70.0%) (20/26). From the p-value (0.000), the null hypothesis have been unacceptable but Alternative hypothesis have been acceptable, this means the variance between male and female are significant infection at significant level (0.01) (0.05) in growth case of *Escherichia coli* infection. Via all cases study, we see the number of female greater than number of males Table 4.3.

Table 4.3 The total sample, no growth, growth and *E. coli* infection according to sex

Case study	Total sample		No growth		Growth		*Escherichia coli*	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Numbar	31	126	17	49	14	77	6	20
Percentage	19.7%	80.3%	25.7%	74.3%	18.2%	81.8%	0.30%	0.70%
t-test	1.342		2.671		11.902		8.482	
p-value	0.167		0.098		0.000		0.001	

4.1.4 Age

We study three age classes with for cases as following: total sample, no growth, growth and *Escherichia coli* infection. In total sample, we see in first class (14-29) there are 87 patients by percentage (55.4%) (87/157). But within second class (30-49) there are 46 patients by percentage (29.3%) (46/157). With third class (50-79) there are 24 patients by percentage (15.3%) (24/157).

From the p-value (0.000), the null hypothesis have been unacceptable but Alternative hypothesis have been acceptable, that is means the variance between three class age are significant infection at significant level (0.01) (0.05) with total sample. In no growth case, we see in first class (14-29) there are 39 patients by percentage (59.1%) (39/66). But within second class (30-49) there are 21 patients by percentage (31.8%) (21/66). With third class (50-79) there are 6 patients by percentage (9.1%) (9/66). From the p-value (0.0734), the null hypothesis has been acceptable but Alternative hypothesis have been unacceptable, that is means the variance between three class age are not significant infection at significant level (0.01) (0.05) with no growth situation. In growth case, we see in first class (14-29) there are 48 patients by percentage (52.7%) (48/91). But within second (30-49) there are 25 patients by percentage (27.5%) (27/91). With third class (50-79) there are 18 patients by percentage (19.8%) (18/91).

From the p-value (0.000), the null hypothesis has been unacceptable but Alternative hypothesis have been acceptable, that is means the variance between three class age are significant infection at significant level (0.01) (0.05) with growth case. But in *Escherichia coli* infection, we see in first class (14-29) there are 11 patients by percentage (42.3%) (11/26). But within second (30-49) there are 6 patients by percentage (23.1%) (6/26). With third (50-79) there are 9 patients by percentage (34.6%) (9/26). From the p-value (0.000), the null hypothesis have been unacceptable but Alternative hypothesis have been acceptable, that is means the variance between three class age are significant infection at significant level (0.01) (0.05) with *Escherichia coli* infection. The class age (14-29) is including majority patients via all cases Table 4.4.

Table 4.4 The total sample, no growth, growth and *Escherichia coli* infection according to Age.

Class	Total sample		No growth		Growth		*Escherichia coli*	
	Frequancy	Percentege	Frequancy	Percentege	Frequancy	Percentege	Frequancy	Percentege
(14-29)	87	55.4%	39	59.1%	48	52.7%	11	42.3%
(30-49)	46	29.3%	21	31.8%	25	27.5%	6	23.1%
(50-79)	24	15.3%	6	9.1%	18	19.8%	9	34.6%
Chi-square	11.543		2.542		16.782		8.324	
p-value	0.000		0.0734		0.000		0.000	

4.2 Antibiotics Resistance

In our study, we used 11 Antibiotics (CFM, AM, AK, CN, CTX, ATM, CIP, LEV, IPM, TET, MRP) to show the Resistant, Intermediate and Sensitive of *Escherichia coli* Bactra as following: In the antibiotic (CFM), we see 22 *Escherichia coli* are Resistant case by percentage (84.6%) (22/26), but there are 3 *Escherichia coli* are Intermediate to the antibiotic (CFM) by percentage (11.6%) (3/26). There is 1 *Escherichia coli* is Sensitive to the antibiotic (CFM) by percentage (3.8%) (1/26).

In the antibiotic (AM), we see 25 *Escherichia coli* are Resistant case by percentage (96.2%) (25/26), but there are zero Escherichia *coli* are Intermediate to the antibiotic (AM) by percentage (0.000%) (0/26). There is 1 *Escherichia coli* is Sensitive to the antibiotic (AM) by percentage (3.8%) (1/26).

In the antibiotic (AK), we see 13 *Escherichia coli* are Resistant case by percentage (50.0%) (13/26), but there are 9 Escherichia *coli* are Intermediate to the antibiotic (AK)

by percentage (34.6%) (9/26). There is 4 *Escherichia coli* is Sensitive to the antibiotic (AK) by percentage (14.4%) (4/26).

In the antibiotic (CN), we see 5 *Escherichia coli* are Resistant case by percentage (19.2%) (5/26), but there are 3 *Escherichia coli* are Intermediate to the antibiotic (CN) by percentage (11.6%) (3/26). There is 18 *Escherichia coli* is Sensitive to the antibiotic (CN) by percentage (69.2%) (18/26).

In the antibiotic (CTX), we see 25 *Escherichia coli* are Resistant case by percentage (96.2%) (25/26), but there are 0 *Escherichia coli* are Intermediate to the antibiotic (CTX) by percentage (0.000%) (0/26). There is 1 *Escherichia coli* is Sensitive to the antibiotic (CTX) by percentage (3.8%) (1/26).

In the antibiotic (ATM), we see 15 *Escherichia coli* are Resistant case by percentage (57.7%) (15/26), but there are 1 *Escherichia coli* are Intermediate to the antibiotic (ATM) by percentage (3.8%) (1/26). There is 10 *Escherichia coli* is Sensitive to the antibiotic (ATM) by percentage (38.5%) (10/26). In the antibiotic (CIP), we see 17 *Escherichia coli* are Resistant case by percentage (65.4%) (17/26), but there are 4 *Escherichia coli* are Intermediate to the antibiotic (CIP) by percentage (15.4%) (4/26). There is 5 *Escherichia coli* is Sensitive to the antibiotic (CIP) by percentage (19.2%) (5/26). In the antibiotic (LEV), we see 15 *Escherichia coli* are Resistant case by percentage (57.7%) (15/26), but there are 4 *Escherichia coli* are Intermediate to the antibiotic (LEV) by percentage (15.4%) (4/26).

There is 7 *Escherichia coli* is Sensitive to the antibiotic (LEV) by percentage (29.9%) (7/26). In the antibiotic (IPM), we see 3 *Escherichia col*i are Resistant case by percentage (11.6%) (3/26), but there are 1 *Escherichia coli* are Intermediate to the antibiotic (IPM) by percentage (3.8%) (1/26). There is 22 *Escherichia coli* is Sensitive to the antibiotic (IPM) by percentage (84.5%) (22/26).

In the antibiotic (TET), we see 26 *Escherichia coli* are Resistant case by percentage (100.0%) (26/26), but there are 0 *Escherichia coli* are Intermediate to the antibiotic

(TET) by percentage (0.000%) (0/26). There is 0 *Escherichia coli* is Sensitive to the antibiotic (TET) by percentage (0.000%) (0/26).

In the antibiotic (MRP), we see 0 *Escherichia coli* are Resistant case by percentage (0.000%) (0/26), but there are 0 *Escherichia coli* are Intermediate to the antibiotic (MRP) by percentage (0.000%) (0/26). There is 26 *Escherichia coli* is Sensitive to the antibiotic (MRP) by percentage (100.0%) (26/26).

From the p-value (0.000), the null hypothesis has been rejected but Alternative hypothesis have been accepted, that is means the variance there are relation between type of antibiotic and (Resistant, Intermediate and Sensitive) at significant level (0.01) (0.05) with *Escherichia coli* infection. The following Figure 4.4 summaries the results show in the above the Table 4.5.

Table 4.5 The kind of antibiotic with *Escherichia coli* Bacteria through Resistant, Intermediate and Sensitive.

Antibiotic	Resistant	Percentage	Intermediate	Percentage	Sensitive	Percentage	Sum
CFM	22	84.6%	3	11.6%	1	3.8%	26
AM	25	96.2%	0	0.00%	1	3.8%	26
AK	13	50.0%	9	34.6%	4	15.4%	26
CN	5	19.2%	3	11.6%	18	69.2%	26
CTX	25	96.2%	0	0.00%	1	3.8%	26
ATM	15	57.7%	1	3.8%	10	38.5%	26
CIP	17	65.4%	4	15.4%	5	19.2%	26
LEV	15	57.7%	4	15.4%	7	26.9%	26
IPM	3	11.6%	1	3.8%	22	84.5	26
TET	26	100.0%	0	0.00%	0	0.00%	26
MRP	0	0.00%	0	0.00%	26	100.0%	26
Chi-square=14.563				P-value= 0.000			

Abbreviations: (CFM) Cefixime, (AM) Amoxicillin, (AK) Amikacin, (CN) Gentamycin, (CTX) Cefotaxime, (ATM) Aztreonam, (CIP) Ciprofloxacin, (LEV) Levofloxacin, (IPM) Imipenem, (TET) Tetracycline, (MRP) Meropenem, (R) Resistance, (I) Intermediate, (S) Sensitive**.**

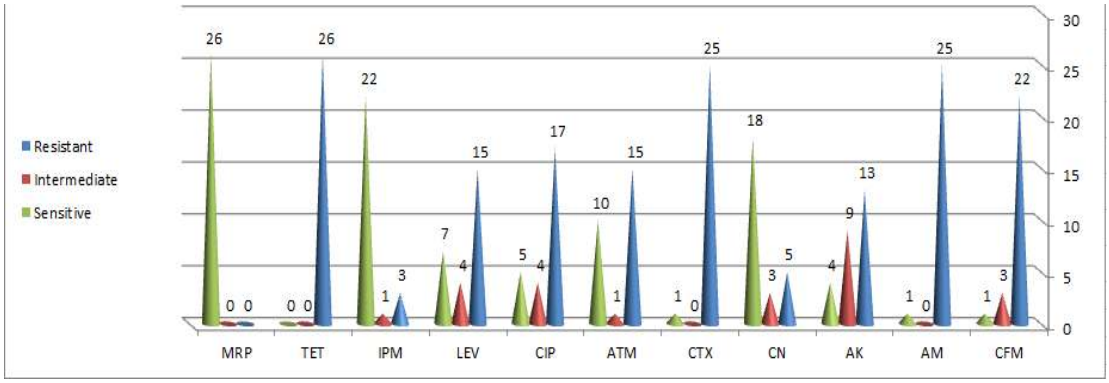

Figure 4.4 The type of antibiotic with *Escherichia coli* Bacetria through Resistant, Intermediate and Sensitive.

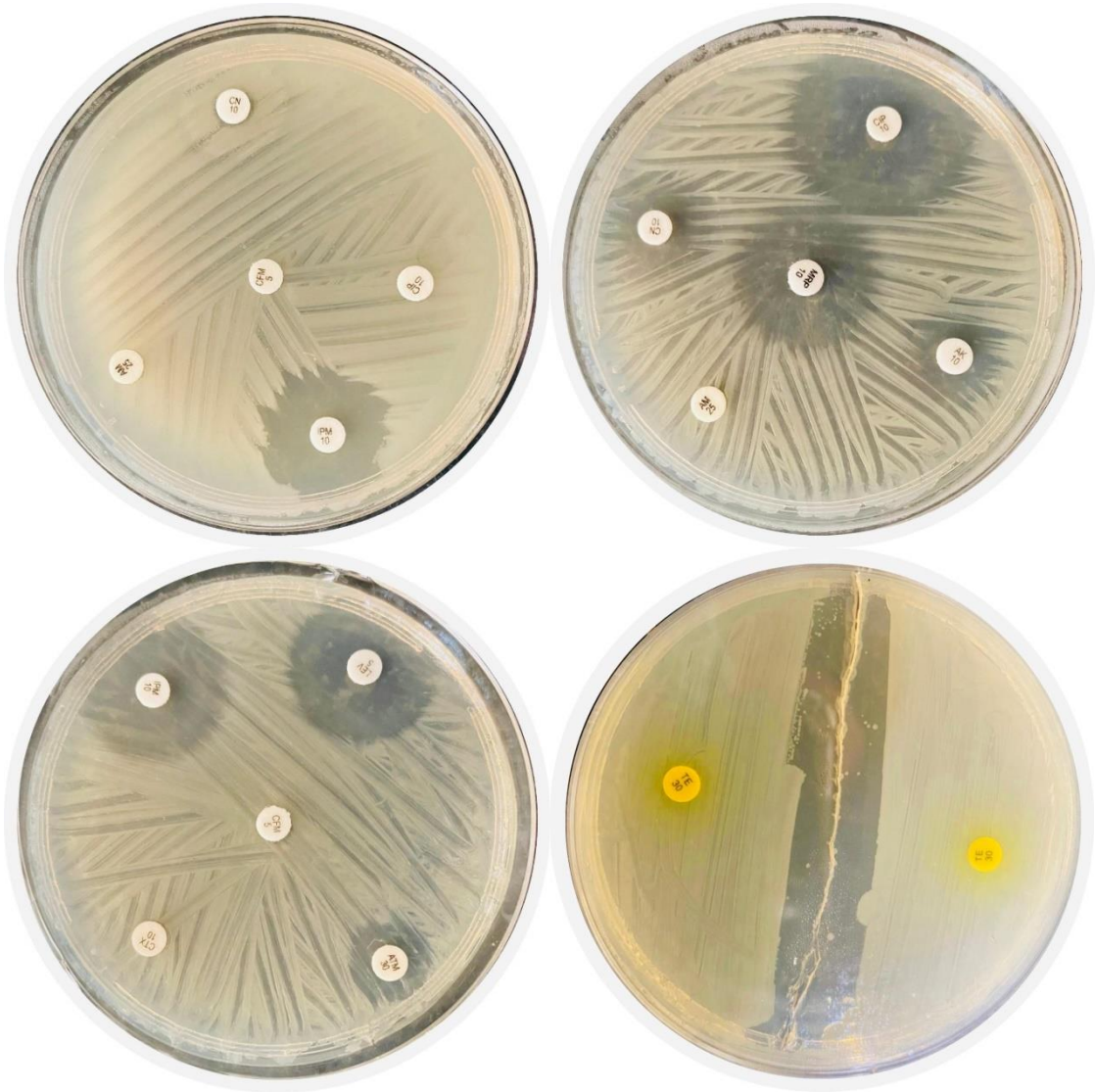

Figure 4.5 Kirby and Bauer's Antibiotic Susceptibility Test: Cefixime (R), Amoxicillin (R), Amikacin (S), Gentamycin (R), Cefixime (R), Aztreonam (S), Ciprofloxacin (S), Levofloxacin (S), Imipenem (S), Tetracycline (R), Meropenem (S).

4.3 Molecular Detection of Resistant Genes

From results listed in the below table, we see (CTX) has (80.8%) (21/26). Then (CTX) gen is Resistant gene compared with other gens in the total isolates of bacteria (Figures 4.6 and 4.7). The (TETA) gene is come in second order compared other gens, the (TETA) gene has (34.6%) (9/26), Then (TETA) gene is weak Resistant gene in the total isolates of Bactera compared with (CTX) gene (Figures 4.8 and 4.9). The (TEM) gen is come in third order compared other gens, the (TEM) gen has (30.0%) (7/26), Then (TEM) gen is weak Resistant gene in the total isolates of Bacteria compared with (CTX) gen (Figures 4.10 and 4.11). The (QNRB) gen is come in forth order compared other gens, the (QNRB) gene has (23.1%) (6/26), Then (QNRB) gene is weak Resistant gene in the total isolates of Bacteria compared with (CTX) gene (Figures 4.12 and 4.13). Therefore, the (CTX) gene is important gen in this our study Table 4.6.

Table 4.6 The Resistant and no Resistant of total isolates of Bacteria according different types of gene.

Gene name / Isolate Symbol	CTX	TEM	QNRB	TETA
E. coli (1)	(+) ve	(+) ve	(-) ve	(-) ve
E. coli (2)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (3)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (4)	(-) ve	(-) ve	(-) ve	(-) ve
E. coli (5)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (6)	(-) ve	(+) ve	(+) ve	(+) ve
E. coli (7)	(-) ve	(-) ve	(-) ve	(-) ve
E. coli (8)	(+) ve	(-) ve	(-) ve	(+) ve
E. coli (9)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (10)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (11)	(+) ve	(+) ve	(+) ve	(+) ve
E. coli (12)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (13)	(+) ve	(+) ve	(-) ve	(+) ve

Table 4.6 The Resistant and no Resistant of total isolates of Bacteria according different types of gene (continue).

E. coli (14)	(-) ve	(-) ve	(-) ve	(-) ve
E. coli (15)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (16)	(+) ve	(+) ve	(+) ve	(+) ve
E. coli (17)	(+) ve	(-) ve	(+) ve	(+) ve
E. coli (18)	(-) ve	(-) ve	(-) ve	(+) ve
E. coli (19)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (20)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (21)	(+) ve	(+) ve	(+) ve	(-) ve
E. coli (22)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (23)	(+) ve	(-) ve	(-) ve	(+) ve
E. coli (24)	(+) ve	(-) ve	(-) ve	(-) ve
E. coli (25)	(+) ve	(-) ve	(-) ve	(+) ve
E. coli (26)	(+) ve	(+) ve	(+) ve	(-) ve
Nu. +ve	21	7	6	9
Percentage	80.8%	30.0%	23.1%	34.6%
Nu. -ve	5	19	20	17
Percentage	19.2%	70.0%	76.9%	65.4%
Total	26	26	26	26
	Chi-square=12.093		P-value= 0.000	

CTX-M gene = β -lactamases refers to his potent in hydrolytic activity against cefotaxime.
TEM gene = β -lactamases called after first patient isolated from Temarian.
TETA gene (tetracyclin group).
QNRB gene (fluoroquinolone group).
(+ve) = posatve resulte , (-ve) = negative resulte.

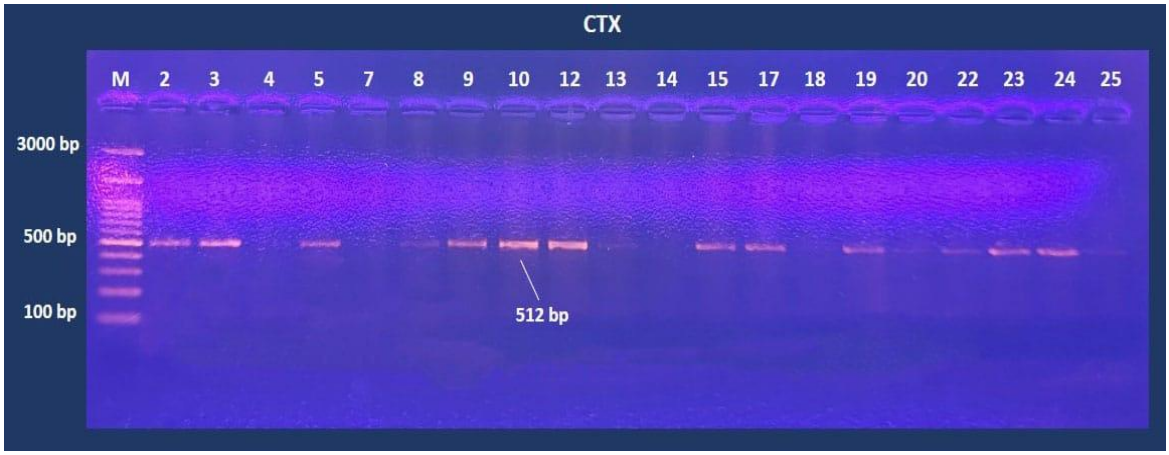

Figure 4.6 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of CTX-M gene 512 Bp from extracted total DNA of *Escherichia coli* (part 1).
Lane: L: 100 Bp Ladder Marker.
Lane: 2,3,5,8,9,10,12,13,15,17,19,20,22,23,24 and 25 positive results.
Lane: 4,7,14, and 18 negative outcomes.

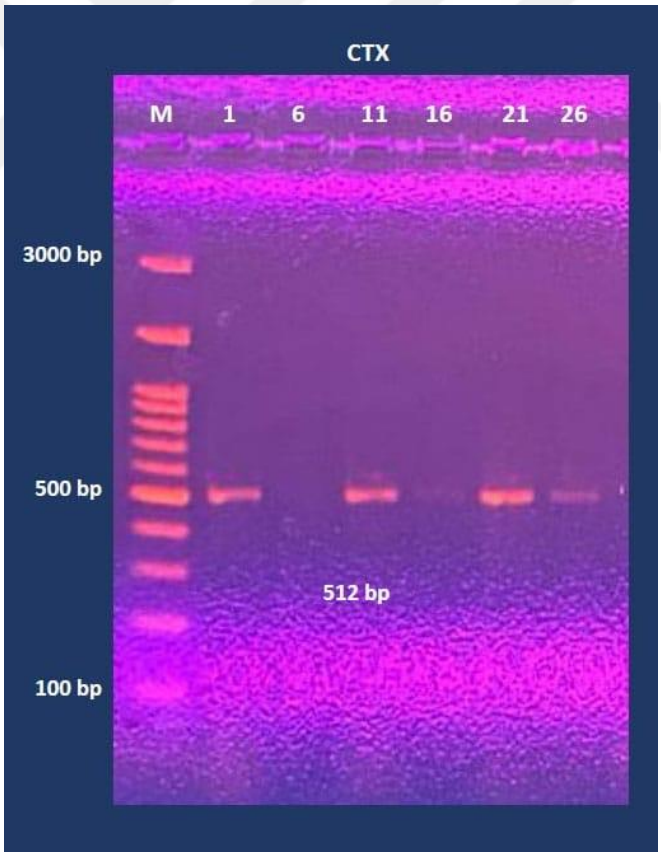

Figure 4.7 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of CTX-M gene 512 Bp from extracted total DNA of *Escherichia coli* (part 2).
Lane: L: 100 Bp Ladder Marker.
Lane: 1,11,16,21, 26 positive outcomes.
Lane: 6 negative outcomes.

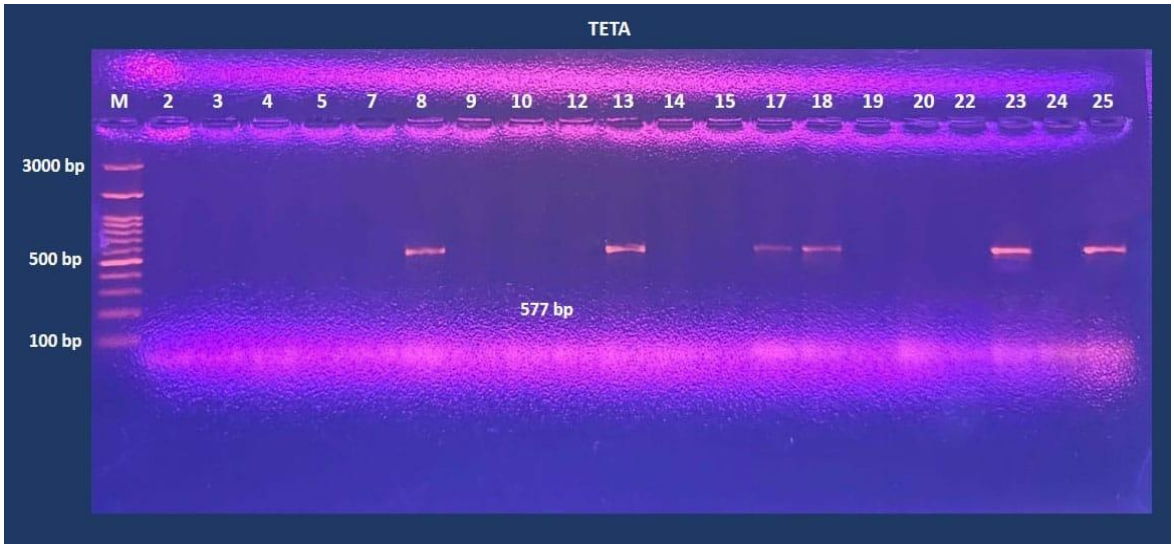

Figure 4.8 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of TET A gene 577 Bp from extracted total DNA of *Escherichia coli* (part 1).
Lane:L: 100 Bp Ladder Marker.
Lane: 8,13,17,18,23 and 25 positive results.
Lane: 2,3,4,5,7,9,10,12,14,15,19,20,22 and 24 negative results.

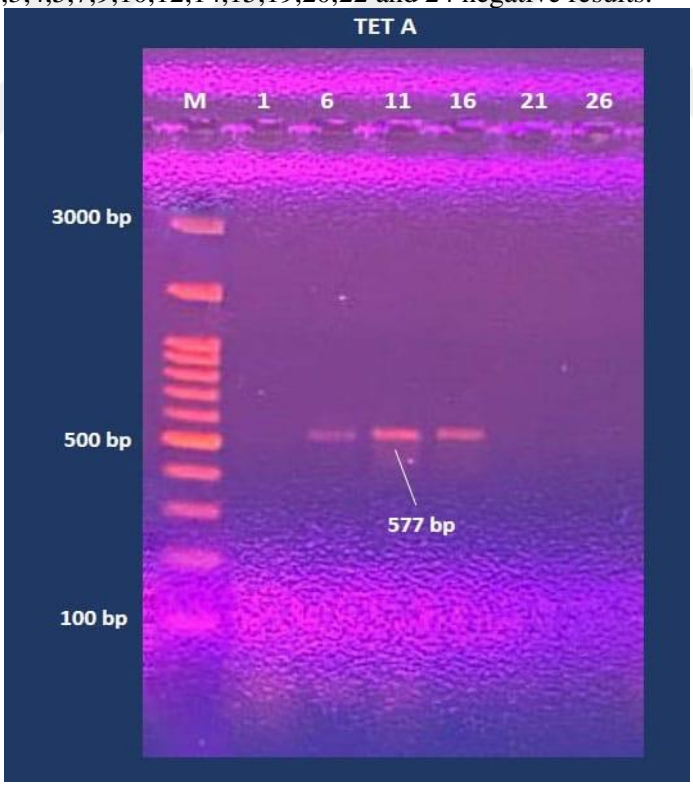

Figure 4.9 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of TET A gene 577 Bp from extracted total DNA of *Escherichia coli* (part 2).
Lane: L: 100 Bp Ladder Marker.
Lane: 6,11, 16 positive outcomes.
Lane: 1,21, 26 negative outcomes.

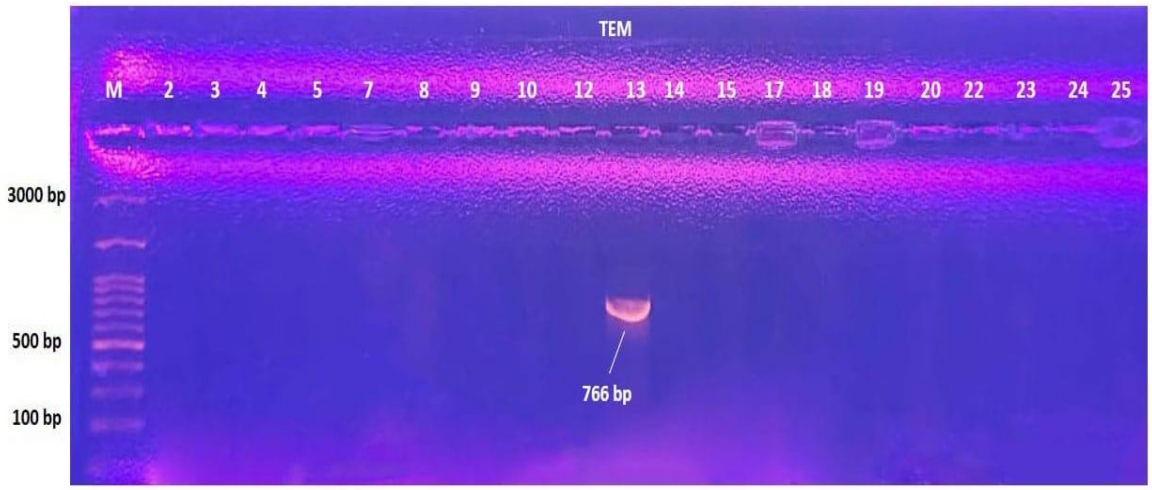

Figure 4.10 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of TEM gene 766 Bp from extracted total DNA of *Escherichia coli.* (part 1).
Lane :L: 100 bp Ladder Marker.
Lane: 13 positive results.
Lane: 2.3,4,5,7,8,9,10,12,14,15,17,18,19,20,22,23,24 and 25 negative outcome.

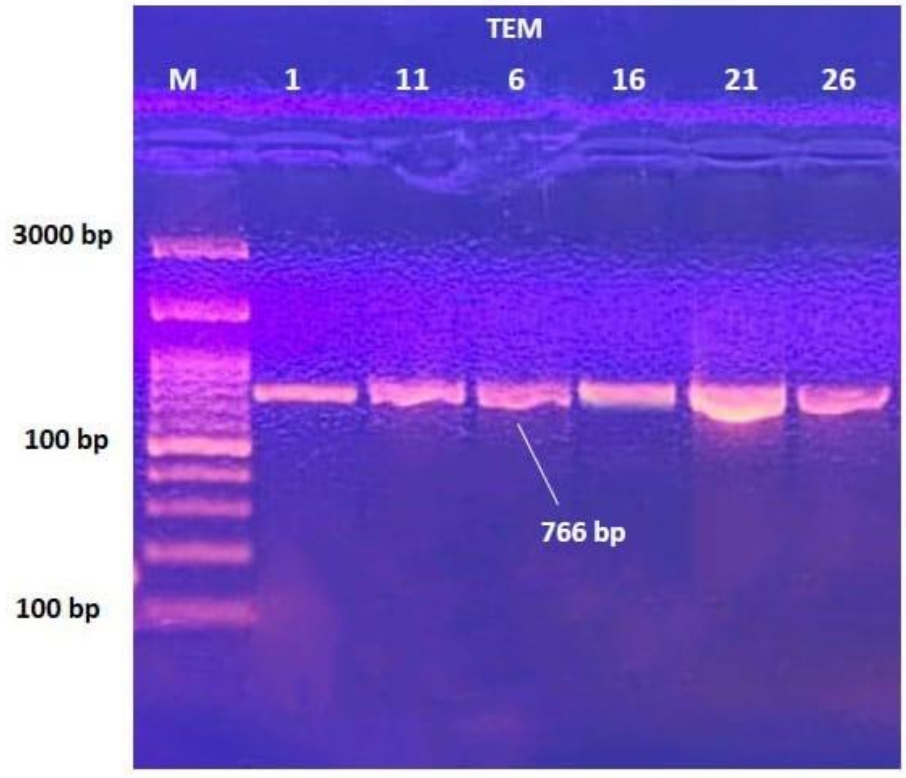

Figure 4.11 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of TEM gene 766 Bp from extracted total DNA of *Escherichia coli.* (part 2).
Lane: L: 100 Bp Ladder Marker.
Lane:1,11,6,16,21 and 26 positive results.

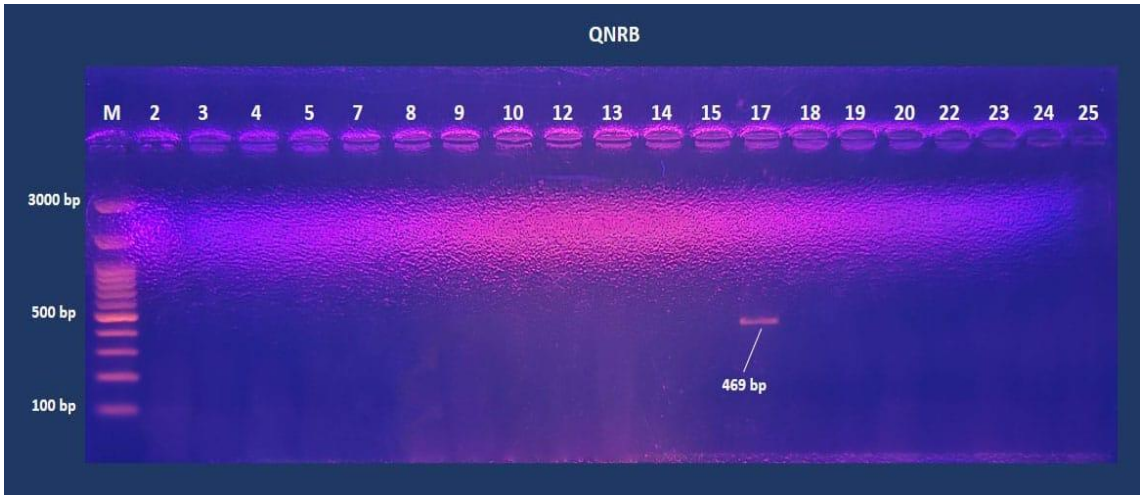

Figure 4.12 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of QNR B gene (469 bp) from extracted total DNA of *E. coli* (part 1).
Lane: L: 100 bp Ladder marker.
Lane: 17 positive outcomes.
Lane: 2.3,4,5,7,8,9,10,12,13,14,15,18,19,20,22,23,24 and 25 negative outcome.

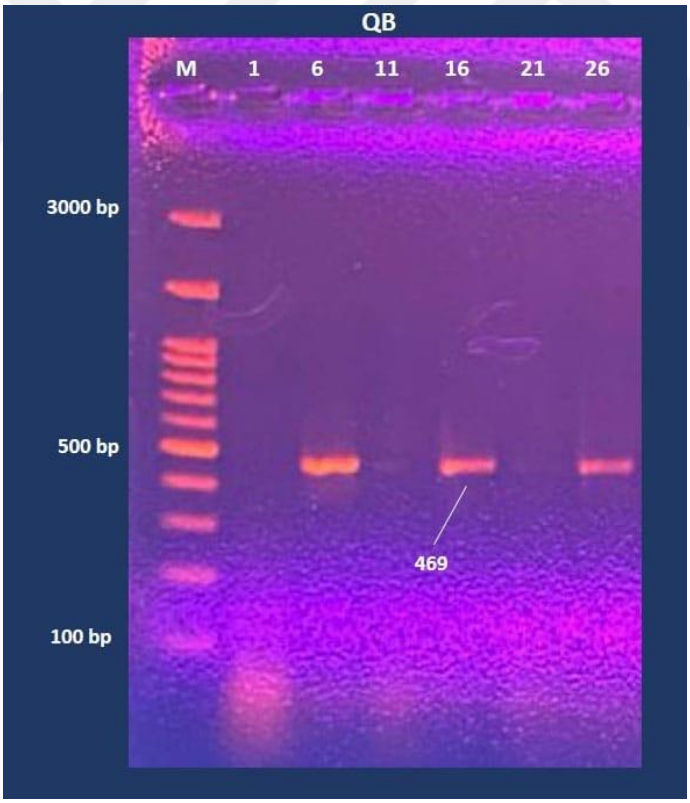

Figure 4.13 Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of QNR B gene 469 Bp from extracted total DNA of *Escherichia coli* (part 2).
Lane: L: 100 Bp Ladder Marker.
Lane: 6,11,16,21 and 26 positive results.
Lane: 1 negative results.

5. DISCUSSION and CONCLUSION

Urinary tract infections (UTIs) are one of the most popular bacterial infections, affecting millions human in worldwide latest years. Urinary tract infections, which can be seen in all age groups and genders. The UTI for women is more than 50 per cent. The spectrum of bacteria isolated from urinary tract infections is very broad and includes both Gram positive and Gram negative pathogens. *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Enterobacter* spp. and *Citrobacter* spp. are frequently isolated from UTIs. In recent studies conducted in many Asian countries, especially in Europe, and in the USA, it has been documented that there are similar epidemiological pathogen profiles for Gram-negative species in the epidemiology of UTI pathogens (Flores-Mireles et al. 2015).

Escherichia coli is the more frequently encountered bacterial species among these germs, with *Klebsiella* spp. following closely behind. Urinary tract infections, are most frequently encountered by females because that the unique structure of their urinary tract and reproductive organs. In females, the close proximity of the urethral opening to sources of bacteria from the anus is a contributing factor to the frequency of urinary tract infections (Tamalli et al. 2013).

In this study, 58% 91 of the 157 urine samples from patients suspected of UTIs yielded positive culture findings, while 42% 66 of the samples did not. *Escherichia coli* was found in just 29% of the samples (26/91), which is almost similar to the findings reported (28.09%) in the India (Thangavelu et al. 2022). On the other hand, contradict those previously when it comes to the isolation of (68.3%) of *Escherichia coli* reported in Iraq (Jalil et al. 2022).

Gram-negative bacteria *Klebsiella pneumoniae* by percentage (18%) (16/91), this present result is similar to that result (18%) in Pakistan previously described by Fatima et al. (2020), while it come to be different to those result (8.99 %) (8/89) in the India previously described by Thangavelu et al. (2022).

Proteus mirabilis was found in this study just (12%) of the samples (11/91), which is almost similar to the findings reported (12.8%) in Sudan (Ibrahim et al. 2022), while it come to be different to those results (7.7%) (7/91) in Saudi Arabia previously described by Alshareef et al. (2020).

Pseudomonas aeruginosa was found in the present just (4%) of the samples (4/91), which is almost agree to the findings reported (3.5 %) in Pakistan (Khan et al. 2022). Otherwise, this data refuses to those outcomes previously (15.7%) of *Pseudomonas aeruginosa* in Somalia described by Mohamed et al. (2020).

Gram-positive bacteria *Enterococcus faecalis* by percentage (16%) (15/91), in this present result is close to that data (14.61%) in India previously described by Thangavelu et al. (2022), while it come to be different to those result (47%) in Japan previously described by Uda et al. (2021).

The present analysis specimen the percent of *Staphylococcus aureus* isolates were (21%) (19/91), which is almost similar to the findings reported (21.4%) in Ethiopia (Tigabu et al. 2020), on the other hand, contradict those previously when it comes to the isolation of (1.1%) (1/91) of *S. aureus* reported in Saudi Arabia (Alshareef et al. 2020).

The total sample consisted of 31 male (19.7%) and 126 (80.3%) female patients. *Escherichia coli* infection almost agree to that data (72%) females, and (28%) males in Pakistan previously described by Khan et al. (2021), while it come disagree to those result (59.3%) females and (40.7%) males in Saudi Arabia previously described by Alshareef et al. (2020).

In the present study, total sample, 87 patients (55.4%) were identified in the first age group (14-29 years), 46 patients (29.3%) in the second age group (30-49 years), and 24 patients (15.3%) in the third age group (50-79 years). From the present analysis samples the percentage of *Escherichia coli* isolates are almost similar to the findings reported in Somalia (Mohamed et al. 2020), on the other hand, contradict those previously reported by Haque et al. (2023).

During the study, most of *E. coli* were isolates resistant to Cefotaxime (96.2%) and Amoxicillin (96.2%), these findings were almost similar with those released earlier by Valadbeigi et al. (2020) and Kadigi et al. (2020) respectively. The findings, on the other hand, differ with those reported previously by Aabed et al. (2021), the antibiotics were (20.3%) and (70.8%).

In the present study, the resistance to Tetracycline was found to be (100%). The findings almost matched those results published by Adekanmbi et al. (2021). The results contradict those that were achieved before by Haque et al.(2023), *E. coli* was a (46.67%) resistance rate drug which one.

Antibiotic resistance to Cefixime was discovered in this investigation (84.6%). The findings are almost comparable to those published by Emamghorashi et al. (2011). The results, on the other hand, disagree from those previously published by Raeispour and Ranjbar (2018), *E. coli* was a (50%) resistance rate drug which one.

Over the half of *E. coli* isolates were resistance to Aztreonam (57.7%) and Levofloxacin (57.7%) which almost agreement with Ali et al. (2016) and Mohamed et al. (2020) respectively. The results, on the other hand, disagree from those previously published by Iroha et al. (2019) and Haque et al. (2023) respectively, *E. coli* was a (85%) and (71.66%) resistance rate drug respectively. Antibiotic resistance to Ciprofloxacin was discovered in this study's findings (65.4%), which is similar findings published by Jafri et al. (2014). On the other hand, contradict those of Kadigi et al. (2020), and Ciprofloxacin resistance was found to be (29.4%).

In this study half of the isolated *E. coli* resistant to Amikacin (50.0%) almost agreed with these results Jafri et al. (2014) on the other hand, disagree from those previously published by Raeispour and Ranjbar (2018), and Amikacin resistance was found to be (8%). The resistance findings (19.2%) of this investigation of the drug Gentamycin were resemble to those previously published by Raeispour and Ranjbar (2018). On the other hand, contradict those reported by Kadigi et al. (2020), and Gentamycin resistance was found to be (50%).

Carbapenems were discovered to be the most efficient antibiotics for many illnesses caused by Gram-positive and Gram-negative bacteria, and our analysis demonstrated that (84.5%) to Imipenem and (100 %) to Meropenem of isolates were responsive to these antibiotics, and in the present results are (11.6%) and (0.00%) resistance rate respectively, these findings were comparable to those published by Ghadiri et al. (2014) and Aabed et al. (2021) respectively.

Imipenem and Meropenem resistance were (51.1%) found by Bahramian et al. (2021). The present study results showed the commonest gene was CTX-M (80.8%) (21/26) in the total isolates of bacteria. Thus, CTX gene is the most resistant gene compared to other genes in the total of bacterial isolates. This result which almost similar to (Youssef et al. 2019) who show prevalence of CTX-M gene in these isolates (84.6%), but disagree with Jaber (2015) which show CTX-M gene (0.0%) among *E. coli*.

The TETA gene is come in second order compared other gens, as shown has (34.6%) (9/26). These findings were similar to those published by Gharajalar and Sofiani (2017). Otherwise, differed from those previously reported by Sheykhsaran et al. (2018), who issued that the TETA gene rate was (14.4%).

The results of the current study also indicated that the TEM, came in third place with (30.0%) (7/26) of the total isolates that were subjected to the study. The results were almost similar to those results (37.9%) previously reported by Loyola et al. (2021). Alternatively, the findings are in conflict with those previously reported by Valadbeigi et al. (2020), who issued that the TEM gene rate was (100%).

In the present study, the frequency of the QNRB gene was (23.1%) (6/26), which is similar to the data previously reported by Abbasi and Ranjbar (2018), who show prevalence of QNRB gene in these isolates (25%). Instead, the findings of this study contradict those previously published by Aabed et al. (2021), who claimed that the gene expression rate was (0%). And so, the QNRB gene is the weakest resistant gene compared to the CTX gene among the total isolates of bacteria.

CONCLUSION

In this study, *Escherichia coli* strains isolated from urine samples of patients admitted to Shamiya State Hospital (Iraq) were identified by conventional and automatic microbiological methods. *Escherichia coli* by percentage 29%, *Staphylococcus aureus* by percentage 21%, *Klebsiella pneumoniae* by percentage 18%, *Proteus mirabilis* by percentage 12%, *Enterococcus faecalis* by percentage 16% and *Pseudomonas aeruginosa* by percentage 4%. Thus, E. coli ranked the first in terms of the incidence of the bacterial infections.

The total sample consisted of 31 male (19.7%) and 126 (80.3%) female patients. In the total sample, 87 patients (55.4%) were identified in the first age group (14-29 years), 46 patients (29.3%) in the second age group (30-49 years), and 24 patients (15.3%) in the third age group (50-79 years).

In the study, it aws used 11 Antibiotics (CFM, AM, AK, CN, CTX, ATM, CIP, LEV, IPM, TET, MRP) to show resistant, intermediate and susceptibility of *Escherichia coli*. The presence of Tet-A, Qnr-B, Ctx-M, TEM resistance genes in the identified *E. coli* strains was investigated by PCR. The CTX gene is a resistant gene compared to other genes in the total of bacterial isolates. The TET-A and TEM genes are a weakly resistant genes compared to the CTX gene. The QNRB gene is the weakest resistant gene compared to the CTX gene among the total isolates of bacteria.

As a result of the study, *Escherichia coli* strains, which are the causative agents of UTI and isolated from urine samples of 157 patients who applied to Shamiya State Hospital (Iraq), were identified by conventional and automated microbiological methods. Demographic characteristics such as age and gender characteristics of individuals from whom *E. coli* strains were isolated were determined. Antimicrobial susceptibilities of identified *E. coli* strains were determined. The presence of Tet-A, Qnr-B, Ctx-M, TEM resistance genes in isolated *E. coli* strains were investigated. The obtained results contributed to the development of prevention, management and clinical strategies for the bacteria infections causing UPEC in Shamiya region.

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