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GENOTYPING AND DETECTION OF VIRULENCE GENES FOR BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS IN SALAHUDDIN HOSPITAL (IRAQ)

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January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

GENOTYPING AND DETECTION OF VIRULENCE GENES FOR BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS IN SALAHUDDIN HOSPITAL (IRAQ)

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Master of Science in Biology

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This study is about the detection of microbial pathogens of urinary tract infection at Salah Al-Din General Hospital in Iraq in 2022. Urine samples were collected from 120 people with urinary tract infections aged between 2 and 70 years, after microscopic examinations and biochemical tests were performed to obtain (90) specimens (*E.coli*, *Staphylococcus aureus* and *P. mirabilis*) causing urinary tract infection and demographically has been distributed. An antibiotic susceptibility test was performed on 14 different antibiogram discs,90 specimens, *E.coli*, *S.aureus* and proteus *mirabilis* had the highest resistance to amikacin, nitrophenyl and imipenem, but were sensitive to amoxicillin, penicillin and cefixime. Polymerase chain reaction (PCR) was performed for four previously identified resistance genes (*coA, rsbA, fimH and cnf1*) for each of the following bacteria (*E.coli, S.aureus* and *P. mirabilis*) that were previously isolated.16S rRNA sequences were then studied using the DNA sequences method and all results were identical to those recorded in the NCBI program. From this study, we concluded that *E. coli* bacteria had the highest incidence of urinary tract infections as well as the highest rates of antibiotic resistance, it was for *E. coli* bacteria, followed by *S. aureus* and then *P. mirabilis*. For this reason, it is recommended that research to control these bacteria, which has increased recently in the hospital community, should focus on modern methods and control methods in hospitals.

2023, 67 pages

Keywords: UTI, *CoA* gene, *RsbA* gene, *FimH* gene, *Cnf1* gene, Antibiotic

ÖZET

SALAHUDDİN HASTANESİNDE (IRAK) İDRAR YOLU ENFEKSİYONLARINDAN İZOLE EDİLEN BAKTERİLER İÇİN GENOTİPLENDİRME VE DİRENÇ GENLERİNİN TESPİTİ

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Bu çalışma, Irak'ta Salahuddin Hastanesinde 2022 yılı içerisinde, idrar yolu enfeksiyonunun mikrobiyal patojenlerinin tespiti ile ilgilidir. Yaşları 2 ile 70 arasında değişen 120 idrar yolu enfeksiyonlu kişiden idrar örneklerinin toplandığı, mikroskobik incelemeler ve biyokimyasal testler yapılarak idrar yolu enfeksiyonuna neden olan (90) örneği (*E. coli*, *S. aureus* ve *P. mirabilis*) elde edilmiştir ve demografik olarak dağıtılmıştır.14 farklı antibiyogram diske 90 örnek için bir antibiyotik duyarlılık testi yapılmıştır. *E. coli*, *S. aureus* ve *P. mirabilis* amikasin, nitrofenil ve imipenem'e karşı en yüksek direnç gerçekleştirilmiştir, fakat amoksisilin, penisilin ve sefiksim'e duyarlıdır. Polimeraz zincir reaksiyonu (PCR), daha önce izole edilmiş olan aşağıdaki bakterilerin (*E. coli*, *S. aureus* ve *P. mirabilis*) her biri için önceden tanımlanmış olan dört direnç geni (*coA, rsbA, fimH ve cnf1*) için gerçekleştirilmiştir. Daha sonra DNA dizileri yöntemi kullanılarak 16S rRNA dizileri çalışıldı ve tüm sonuçlar NCBI programında kaydedilenlerle aynıdır. Bu çalışmadan, *E. coli* bakterilerinin en yüksek idrar yolu enfeksiyonu insidansına ve aynı zamanda en yüksek antibiyotik direnci oranlarına ulaştığı sonucuna varılmıştır. Bunu *S. aureus* ve *P. mirabilis* izlemiştir. Bu nedenle, hastane ortamlarındaki son zamanlarda artan bu bakterileri kontrol etmek için yapılacak araştırmaların modern yöntemlere ve kontrol yöntemlerine odaklanması önerilmektedir.

2023, 67 sayfa

Anahtar Kelimeler: İYE, *CoA* gen, *RsbA* gen, *FimH* gen, *Cnf1* gen, Antibiotic

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LIST OF SYMBOLS

µg	Microgram
µL	Microliter
℃	Degree celsius
cm	Centimeter
g	Gram
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter
ng	Nano-gram
nm	Nanometer
pg	Picogram

LIST OF ABBREVIATIONS

CLSI	Clinical and laboratory standards institute
coA	Coagulase gene
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
WHO	World health organization

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1. INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent bacterial infections in children and adults of both genders. It is considered one of the primary reasons for hospitalization in children and nosocomial infections (Kutasy *et al.* 2017). The incidence rate of UTIs can reach up to 7% in newborns (Wang *et al.* 2018).

Early treatment of a UTI reduces the rate of morbidity. However, the growing rates of antibiotic resistance to the antimicrobial agents that are commonly prescribed, such as cephalosporins, trimethoprim/sulfamethoxazole and fluoroquinolones, has become a major concern. Early treatment of a UTI also reduces the risk of developing complications. As a result, it is absolutely necessary to understand the prevalence of uropathogens and antibiotic resistance in pediatrics (Doyev *et al.* 2020). According to Koçak *et al.* (2016), effective antimicrobial treatment for urinary tract infections is essential for preventing long-term complications associated with renal scarring.

According to Awean *et al.* (2019), *Escherichia coli* (*E. coli*) is the most prevalent type of bacteria that causes UTIs, followed by *Enterococcus* spp., *Klebsiella pneumonia, Enterobacter* spp., *Proteus mirabilis, Staphylococcus* spp., and *Pseudomonas aeruginosa*. Before the discovery of antibiotics, urinary tract infections were responsible for approximately 20% of all deaths from infectious diseases (Pouladfar *et al.* 2017). It is usual practice to look for *E. coli* in the intestines of warm-blooded animals because this gram-negative bacillus, which belongs to the family Enterobacteriaceae and that it's can usually be discovered there. The vast majority of types of *E. coli* are not harmful, but some strains can cause food poisoning, diarrhea, and urinary system infections. There are a variety of other bacteria that have the potential to cause infections in the urinary system (Ardakani and Ranjbar 2016).

UTIs, are the most prevalent type of bacterial infection. They are distinguished by bacteria colonization of the urinary system and most frequently affect women of all ages and stages of life. Uropathogenic *E. coli* are the most often found pathogens isolated from

individuals diagnosed with UTIs. These strains of *E. coli* frequently originate from the gut flora. The most common symptom of a UTI is dysuria, which can range in severity from mild to severe (Amiri *et al.* 2017).

Pathogenic *E. coli* is typically classified into two major groups: extraintestinal pathogenic *E. coli* and diarrheagenic *E. coli,* both of which have two pathotypes: uropathogenic *E. coli* and neonatal meningitis caused by *E. coli*. These two major pathotypes are responsible for a variety of human diseases that are caused by *E. coli*. *E. coli* outside of the digestive tract, extraintestinal bacteria have the potential to colonize a variety of places and cause a wide range of diseases; nevertheless, the urinary tract is the organ that is most affected organ (Ranjbar *et al.* 2016).

In the realm of medical microbiology, having a solid understanding of the epidemiology of microbial populations is extremely valuable. Through the use of molecular typing, it is possible to recognize infection reservoirs, investigate the frequency of infections that occur in hospitals, and recognize the kinds of microorganisms that cause infectious diseases (Wu *et al.* 2018).

Molecular typing can be considered a key factor in identifying nosocomial infections and infectious agents and isolating certain strains of a specific bacteria. In addition, molecular typing can be utilized to isolate specific genotypes in the context of infection. In addition to this it expands our knowledge of the fundamentals of epidemiology, the evolution of bacterial diseases and the transmission of various bacterial infections (Ranjbar *et al.* 2016).

On technological advances, modern molecular biological techniques have emerged. Biology techniques, through which bacterial pathogens can be accurately identified and diagnosed, including Polymerase Chain Reaction (PCR). This gene is characterized by being present in all bacterial species and different sequences of nitrogen bases for each species. Through PCR interaction, the presence of harmful factors directly diagnosed and responsible bacteria can be investigated. Virulence factors can be detected by amplifying its encoded genes (Franchetti *et al.* 2020). The lateral transfer of resistance genes in bacteria is facilitated by mobile genetic elements such as transposons, plasmids and

integrons. *E. coli* has the potential to have an innate resistance to some antibiotics and also possesses genes that are responsible for its resistance to certain antibiotics including aminoglycosides, fluoroquinolones and beta-lactamases, among others (Blair *et al.* 2015).

The aim of the study:

1. Detection of bacterial types that cause urinary tract infections.
2. Detection of bacterial resistance to some antibiotics.
3. Detection of several genes responsible for antibiotic resistance in E. *coli, S. aureus* and *Proteus* Spp.
4. Molecular examination using the DNA sequencing method using the 16S RNA gene.
5. Performing sequence reading using the NCBI system and comparing the 16S RNA gene for the three studied samples compared with the standard gene

2. LITERATURE REVIEW

2.1 Urinary System

The urinary system often known as the renal system, refers to the organ system that is responsible for the production, storage, and elimination of urine, in humans it is composed of the two kidneys the bladder the two ureters and the urethra. The kidneys are bean-shaped organs that are located on either side of the vertebral column and lie retroperitoneally between the 12^{th} thoracic and 3^{rd} lumbar vertebrae, the kidneys filter blood and regulate fluid balance in the body (Standring *et al.* 2008).

Because the liver is located on the right side of the body the right kidney is situated a little bit lower in the belly than the left kidney, urinary systems serve multiple purposes, including blood filtration, removal of waste products from the body (primarily urea and uric acid) regulation of blood volume regulation of the pH of extracellular fluid and regulation of electrolyte balance (Standring *et al.* 2008). Urinary systems are located in the lower urinary tract (e.g. sodium, potassium and calcium) the control of the production of red blood cells, the production of vitamin D and the maintenance of normal blood pressure (Rod *et al.* 2004).

The kidneys secrete urine, which subsequently passes through the fibro-muscular ureters and accumulates in the bladder (Dasgupta *et al.* 2007). Because the muscle of the bladder, known as the detrusor muscle, is able to expand to make room for urine without raising the pressure inside it is possible to collect large volumes of urine (between 700 and 1000 milliliters) without endangering the renal system through exposure to high-pressure environments (Flores-Mireles *et al.* 2015). According to Williams *et al.* (1989), when a person urinates, the urethral sphincter, which is located at the bottom of the bladder, loosens its grip, and the detrusor muscle contracts, causing urine to be expelled through the urethra Urinary Tract Infection.

Bacteriuria is bacterial urine, and pyuria is pus, any point in the urinary tract, from the urethral meatus all the way up to the kidneys is susceptible to infection it is an exceedingly prevalent disorder and can affect people of any age in both males and females (Maceae *et al.* 1995).

The prevalence and incidence of UTIs are significantly higher in women than men for various reasons, including anatomical differences hormonal impacts, and behavioral patterns, UTIs are one of the leading causes of morbidity in children and are among the most common sources of infection in children younger than 5 years old (Flores-Mireles *et al.* 2015).

The first two years of a person's life are associated with the development of around 75 percent of all UTIs. The incidence of UTIs is at its highest during the first year of life, and it again reaches its highest point between the ages of 2 and 4 years, when children learn how to use the toilet, after the age of six years, UTIs are uncommon and are frequently linked to dysfunctional elimination (Flores-Mireles *et al.* 2015). A UTI can be asymptomatic or symptomatic which can be defined by a wide spectrum of symptoms ranging from minor irrelative voiding to bacteremia sepsis, or even death, UTI can be symptomatic or silent (Ranjbar *et al.* 2009).

2.2 Signs and Symptoms of UTI

There is a large amount of variation in signs and symptoms depending on the patient's age, with the symptoms becoming more specific as the child ages. When considering the possible causes of a high-grade fever, a UTI should always be considered, even in the absence of any specific symptoms. Around three percent of children aged preschool and younger have asymptomatic bacteriuria (Lane and Takhar 2011).

Jaundice with a delayed onset can be a sign of occult urinary tract infection in neonates, particularly if the conjugated fraction is also increased, in infants and young children aged 2 months to 2 years old who have a fever for no apparent reason, it is recommended by

many guidelines that the possibility of aUTI be examined, nonetheless the evidence on which this is founded appears to be lacking in scope (Shaikh *et al.* 2008). Patients who are older can be more difficult to diagnose because they may present with nonspecific symptoms such as malaise, fatigue, anorexia, or even fever or chills, in the absence of any of the more focal symptoms listed above that could point the clinician to the direction of the urinary tract as the cause of the issue in such patients, a UTI should be considered as part of a differential diagnosis that is fairly broad (Crain and Gershel 1990).

Pyelonephritis is characterized by a high temperature, chills, malaise, discomfort in the flank, nausea and vomiting, and/or abdominal pain, which may or may not be accompanied with indications related to the lower urinary system. When a patient has cystitis, there may be no abnormalities on a physical exam or only mild tenderness to palpation in the suprapubic region. On the other hand, a patient with pyelonephritis will typically look systemically ill in general and have tenderness to percussion in the costovertebral angle on a physical exam in most cases (Colgan and Williams 2011).

Additionally, some individuals could experience some minor soreness in the anterior upper quadrants of the abdomen, which are located above the kidneys. Although UTIs are more common in women than males, the symptoms and findings of a physical exam are typically the same in both sexes (Hooton *et al.* 2010).

2.3 Causative Organisms of UTI

Gram-negative bacteria are the most common cause of UTIs. These include *E. coli, Klebsiella* spp., *P. aureginosa, Proteus* spp., *Acinetobacter,* and *Serratia* spp. Gram-positive bacteria, such as *Enterococci*, *Staphylococcus*, and *Streptococcus agalactiae*, are responsible for a small percentage of UTI instances (Abraham and Miao 2015). *E. coli* is responsible for 60–80 percent of UTIs, followed by *Proteus* spp. (which is more common in patients with renal stones), *Klebsiella* spp., *Enterococci* and coagulase-negative *Staphylococci*. *E. coli* is the most frequent type of Gram-negative bacteria that causes UTIs. Infections caused by *Proteus* spp. and *Klebsiella* each account for 10% of the total, whereas other bacteria only account for 6% (Yüksel *et al.* 2006).

Certain organisms have adherence qualities that prevent them from being normally washed away by mucosal host defense mechanisms and by emptying of the bladder because they include P-fimbriae, which are organelles that can connect to or cling to certain receptors on uroepithelium cells which later interfere with the bacterial washout (Nguyen 2004).

Females are more likely to be exposed to *E. coli* than males, who are more likely to come into contact with *Proteus* spp. and *Klebsiella* spp. Infections in the urinary tract caused by *Proteus* spp., *Klebsiella* spp., and *Enterobacter* spp. are very common. Infections occur more frequently in children with recurrent UTIs and those who are treated with antibiotics for prophylaxis. About 65 % of hospital-acquired urinary tract infections are brought on by *E. coli* and other bacteria, such as *Pseudomonas* spp (Deepthi *et al.* 2017).

2.3.1 *Escherichia coli*

In individuals diagnosed with bacteremia, the opportunistic bacteria *E. coli* is the most commonly isolated organism. This is because it thrives in anaerobic environments. Infections caused by *E. coli* can sometimes be fatal (Owrangi *et al.* 2018). Extraintestinal pathogenic *E. coli* are *E. coli* strains that are capable of surviving in the circulation and causing infections that are not limited to the intestines (ExPEC). The urinary tract, a major entry point for bacteria that cause bloodstream infections, is the extraintestinal location that is most frequently colonized by these bacteria (Micenková *et al.* 2017).

Isolates of *E. coli* can be placed into one of seven groups, denoted by the letters A through F, according to the most recent phylogenetic classification. In general, ExPEC strains belong to groups B2 and D, whereas commensal strains that are able to persist in the intestines belong to either group A or B1 (Clermont *et al.* 2013).

Several virulence factors have been associated with bloodstream infections. These characteristics may facilitate bacterial cell invasion and colonization of the host. However, predictions of the initial severity of an infection dependent on the presence of

bacterial virulence factors are not completely accurate. *E. coli* is the most prevalent cause of bloodstream infections on a global scale. According to previous studies, *E. coli* is the most prevalent or second-most prevalent bacteria associated with bloodstream infections in Canada, Australia, Finland, Denmark, New Zealand, Iceland Sweden and the United States of America in contrast, it is the third most prevalent cause of bacterial infection in hospitals and other medical facilities in Lithuania (Kirtikliene *et al.* 2019).

2.3.2 *Proteus* spp

They are gram-negative, motile, aerobic, rod-shaped bacilli. It is a member of the Enterobacteriaceae family (Yatsunenko *et al.* 2012). A variety of Proteus species can be found in the human gut microbiota. They can be found just about wherever in the natural world, from animals to dirt to contaminated water (Drzewiecka 2016). They play a significant role as pathogens in both nosocomial and community-acquired UTI, in general, *Proteus* spp. are thought to be opportunistic pathogens in the old population and harmful for young people (Mondot *et al.* 2016).

Urinary tract infections that are difficult to treat are often caused by Proteus spp. Urolithiasis (kidney or bladder stone production), cystitis, and acute pyelonephritis are all illnesses that these organisms commonly cause in the upper urinary system. Other infections include septicemia and wound infections. Less than 5% of urinary tract infections are caused by *Proteus mirabilis*. Urine tract infections are caused in part by the organism's fast motility and its ability to adhere to the urinary epithelium (Schaffer *et al.* 2016).

Urine's pH increases and the bacteria are allowed to multiply unchecked because Proteus spp. can manufacture the urease enzyme, which catalyzes the breakdown of urea into ammonia and carbon dioxide. The increased acidity also makes it easier to develop kidney stones in the urine. Infections caused by *Proteus* can become chronic if the bacteria are able to survive inside the stones and continue to multiply despite medical treatment. The endotoxins produced by bacteria are also being investigated for their potential role in bacterial pathogenicity (Wright *et al.* 2017).

2.3.3 *Staphylococci* spp

They are gram-positive spherical bacteria usually arranged in grape-like irregular clusters, some single cells, pairs, short chains, and non-motile. They are normal human skin flora and mucous membranes spread endogenously or from infected skin. They included many species, but the main three species are *S. aureus*, *S. epidermidis* and *S. saprophyticus* (Munson and Carroll 2021).

Staphylococcus aureus is a Gram-positive, coagulase-positive cocci belonging to the Staphylococcaceae family. Carotenoids are responsible for the characteristic golden color of *S. aureus* colonies; this coloration has been interpreted as a virulence factor protecting the pathogen from oxidants produced by the immune system (Tong *et al.* 2015). *S. aureus* has a polysaccharide capsule to protect it from phagocytosis. The cell wall comprises peptidoglycan and teichoic acid moieties that protect it from lysis by osmotic conditions and aid in attaching the bacteria to mucosal surfaces (Schneewind *et al*. 1995).

Coagulase is an enzyme secreted by *S. aureus* that, when combined with prothrombin in the blood, causes fibrinogen to be converted into fibrin, therefore coagulating the plasma. Because of their inability to cause blood clots, the other species of the genus, which exclude *S. aureus* are known as coagulase-negative *staphylococci* (Rasigade and Vandenesch 2014).

2.4 Pathogenesis

Almost all UTIs are caused by bacteria that have inhabited the periurethral region and have an ascending etiology (Handley *et al.* 2002). Shortly after birth, both aerobic and anaerobic bacteria colonize the periurethral region, such as the distal urethra. These organisms play a role in establishing a defensive barrier against the colonialization of potential diseases. A change in the normal periurethral flora, such as that seen if upper respiratory tract infections are treated with a broad-spectrum antimicrobial, makes the periurethral area more vulnerable to colonization by potential uropathogens (Lidefelt *et*

al. 1991). An uropathogen colonization of the periurethral interior is an important element in the etiology of recurring infections in adults. The perineal flora is normally found in the distal urethra of a healthy person. Urine found in the proximal urethra, urinary bladder, and other more anterior regions within the urinary tract is sterile. Uropathogens must first access the urine bladder and multiply there before an infection can develop (Mysorekar *et al.* 2002).

2.5 Risk Factors

2.5.1 Age and sex

Across all age categories, the prevalence of UTI is greater in women than in males. Women's anatomy explains this since their urethra is shorter than men's, and the urethra and anus are close together. On the other hand, UTIs decrease in frequency around middle age but increase in older persons. Furthermore, menopause might raise the risk of recurring UTIs substantially (Chwa *et al.* 2019). Indeed, lower estrogen levels can cause vaginal atrophy, dryness, and a rise in pH, which changes the vaginal flora and lowers lactobacilli levels, resulting in the growth of potentially dangerous bacteria like those indicated above (Walsh and Collyns 2020).

2.5.2 Structural abnormalities

Certain renal diseases can make recurrent UTIs more likely. These are diseases that cause a leftover volume of urine after voiding. UTIs are also linked to kidney stones, which may provide bacteria with a surface on which to develop biofilms. Due to the biofilms generated, this disease makes it difficult to expel germs through the urinary bladder and even more difficult to destroy by the host's immune response (Walsh and Collyns 2020).

2.5.3 Genetic factors

Some people are more prone to UTIs due to a combination of hereditary and environmental factors. Women from families whose UTIs have been documented are more likely to get this infection, according to Walsh and Collyns (2020). Studies have shown that the genotypic aspects of the disease (such as uroplakins, uromodulin, neutrophils, and different gene polymorphisms) have been linked to recurrent UTI infections (Walsh and Collyns 2020).

2.5.4 Catheterization

The increased incidence of recurrent UTIs is often attributed to the use of catheters and other urinary drainage devices, which promote the development of bacterial biofilms and provide a reservoir of potential pathogens in contact with the bladder (Rowe and Juthani-Mehta 2013). Walsh and Collyns (2020) found that crystalline biofilms can impede urine flow, worsening the issue.

2.6 Virulence Factors

For a UTI to occur, the pathogens must be able to attach to the urinary epithelium, avoid removal by the host and then cause inflammation. Since they possess a variety of virulence factors that are involved in the sequence of events leading to infection (Raimondi *et al.* 2019).

2.6.1 Capsule of the bacteria

Some bacterial species, including *Streptococcus* and *Klebsiella*, exhibit a major virulence component in the form of a polysaccharide capsule that surrounds the bacterial wall in some strains of the bacteria. As well as promoting adhesion, it also inhibits phagocytosis. The majority of these antiphagocytic structures exhibit such high antigenic heterogeneity that the host is only protected from disease caused by bacteria of that type when antibodies

against that type of antiphagocytic factor are present. This is not the case for bacteria that produce other types of the same factor (Martin and Bachman 2018).

2.6.2 Hemolysin production

The metabolic virulence factor is what makes it possible for the colonizing bacteria to survive. Both gram-positive and gram-negative bacteria can synthesize cytotoxic hemolysin, and the creation of either alpha- or beta-hemolysin Causes transmembranous holes to form, lysing the host cells. Beta-hemolysin prevents neutrophil chemotaxis and phagocytosis. Only by actively expanding and multiplying cells in the presence of erythrocytes was it possible to verify that this hemolytic action is related with cells (Nagamatsu *et al*. 2015). The hemolytic factor appears to be substantially cell-associated and unstable, as no hemolytic activity was detected in cell-free supernatants and filtrates of bacterial cultures (Balachandran *et al.* 2018).

2.6.3 Extracellular protease production

Numerous bacterial species produce certain enzymes that are not inherently harmful and crucial to the infectious process. For example, collagenase enzymes, which encourage the spread of infection in the tissue, or elastase (also known as gelatinase) enzymes, which cleave collagen and cause IgG, IgA, and complement to lyse fibronectin to promote bacterial attachment on the surface. Alkaline protease, also known as metalloprotease, is an additional extracellular protease enzyme that prevents fibrin from forming, causing the fibrin to lyse. These extracellular protease enzymes allow the pathogens to deactivate the primary antibody present on the mucosal surface, remove the host's protection provided by the antibody, and affect numerous nutritional acquisition processes. Since they are regulatory enzymes (Bien *et al.* 2011).

2.6.4 Colonization factor antigens

The ability of bacteria to adhere to epithelial surfaces is regarded to be a major pathogenicity element that initiates UTIs. The presence of fimbriae on bacterial cells is most often linked to the ability of germs to adhere to surfaces. To connect to uroepithelial cells, bacteria use structures called fimbriae. Bacteria attached to the renal pelvic mucosa by fimbriae were observed using electron microscopy. In contrast, rod-shaped bacteria were hindered in their hematogenous infection of the renal parenchyma by the presence of fimbriae. Fimbriae improve bacterial cell adhesion to uroepithelial cells in vitro, however this makes the pathogen more vulnerable to phagocytosis (Sarowska *et al.* 2019).

2.6.5 Siderophores production

Whether prokaryotic or eukaryotic, iron is required for growth and metabolism. Many different types of animals employ the method of host defense known as "limiting the concentration of free extracellular iron" in order to combat harmful bacteria. Siderophores are iron chelating compounds secreted by many bacteria that are both extremely effective and low-molecular-weight (Riley 2014). The ability to produce siderophore has been linked to many bacterial species' pathogenic potential, including *E. coli*, *P. aeruginosa*, *Vibrio cholerae*, and othersn (Watts *et al*. 2012). The presence of an iron chelate induces the creation of the cognate receptor, allowing at least some bacteria to utilize exogenous siderophores found in the environment. Binding of ferric-citrate to an outer membrane receptor protein in *E. coli*, the best-characterized organism, triggers a signal-transduction cascade (Conceição *et al.* 2012).

2.7 Treatment of UTI

In every part of the world, antibiotic-resistant bacteria are rising among the germs that cause common diseases (Fair and Tor 2014). It is fascinating to see how the pattern of resistance shown differs from one hospital to another, from one town to another, from one huge hospital to another tiny hospital, from one state to another, and even from one

country to another. The development of antibiotic resistance demonstrates how important it is to treat patients with methods supported by evidence (Waglechner and Wright 2017).

In cases of UTIs, antibiotic treatment is frequently begun on an empirical basis before the outcomes of urine culture and susceptibility testing are available. The administration of appropriate antibiotics in patients with urinary tract infections appears to shorten the length of hospital stays, which benefits both patient outcomes and healthcare costs (Spoorenberg *et al.* 2014).

As a result, it is essential to perform routine surveillance on the antibiotic resistance or susceptibility patterns of uropathogenic (UPs). This will allow the guidelines for empirical antibiotic therapy to be modified to include antibiotics that have a low level of resistance, which will assist clinicians in the appropriate management of UTIs with a reduced risk of therapeutic failure (Sharma *et al.* 2016).

In most cases, the beginning of the treatment process takes place after the infection has been diagnosed; nevertheless, these preliminary efforts to cure the disease might lead to serious results, and the treatment itself must be determined once the etiological agent has been verified (Peyrani *et al.* 2019).

2.8 Antibiotic Resistance

Uropathogenic bacteria, are becoming increasingly resistant to antibiotics, which is causing significant new challenges in the treatment of UTIs (Sweileh *et al.* 2018). Numerous research are conducted each year to analyze the antibiotic resistance of UPs that have been isolated from individuals diagnosed with UTIs. The results of practically all of these investigations suggest that there has been a growth in the antibioresistance over the years (Magyar *et al.* 2017, Sweileh *et al.* 2018). In a study that was carried out in Hungary to evaluate the spectrum and antibiotic resistance of uropathogens between the years 2004 and 2015, Magyar *et al.* (2017) discovered that the five most frequently identified bacteria were *E. coli, K.pneumonia, E.faecalis, P.mirabilis* and *P.aeruginosa*.

Additionally, they discovered that during this time, the resistance of *E. coli* to ciprofloxacin significantly increased (Magyar *et al.* 2017).

In general, the development of antibiotic resistance in bacteria, regardless of whether or not they are UPs, is caused by several different mechanisms, such as changes in cell permeability and multiple efflux pumps, mutations of the antibiotic target, and horizontal transfer of resistance genes (Figure 2.1) (Mukherjee 2019, Palma *et al.* 2020).

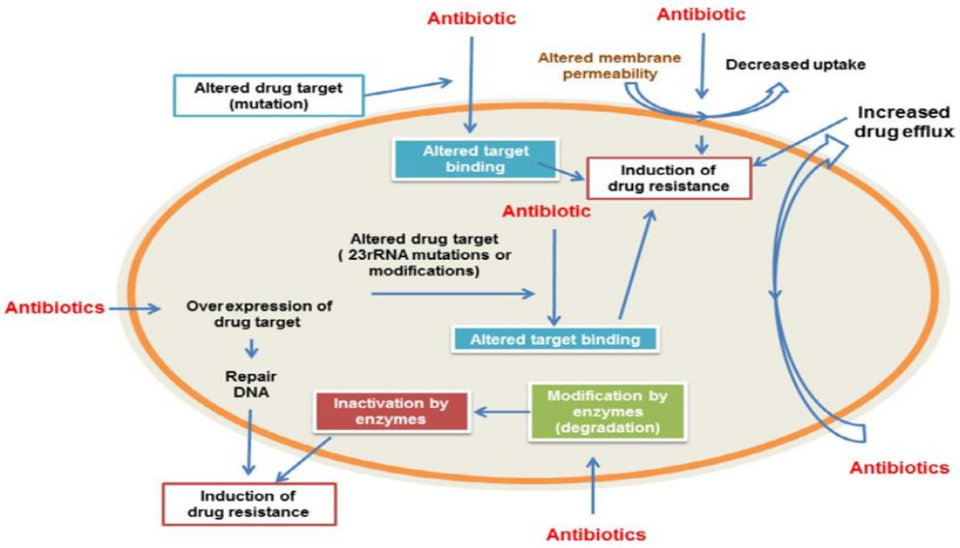


Figure 2.1 Mechanisms of antimicrobial resistance (Mukherjee 2019)

Nevertheless, despite the current knowledge on antibiotic resistance and the phenotypic observations, the mechanisms of the interference of virulence factors in antibiotic-resistant bacteria are not yet clearly identified. Although, there is a lot of information available on antibiotic resistance (Alabsi *et al.* 2014).

2.9 Identification Strategies of Resistant Bacteria

Establishing links between various bacterial isolates is an important step in the process of determining the origins of bacterial infections since such associations can shed light on how infections are spread. It helps determine the sources of the contamination and gain insights into pathogens' distribution, comprehending the degree to which infections have

changed over time, selecting the most effective therapy for diseases, and lowering the danger of antibiotic resistance (Rivas *et al.* 2015). Various typing systems have been developed, all of which have proven to be highly helpful in characterizing the epidemiology of infectious diseases. Phenotypic typing was the foundation for early methods, whereas components of bacterial genomes have provided the foundation for more recent approaches. In the field of microbial typing, methodologies based on DNA have developed into potentially strong methods (Rivas *et al.* 2015). These techniques include plasmid profile analysis (Ranjbar *et al.* 2007), RFLP (Restriction Fragment Length Polymorphism) (Arjomandzadegan *et al.* 2011), Ribotyping (Ranjbar *et al.* 2008), MLST (Multi Locus Sequence Typing) (Ranjbar *et al.* 2017), VNTR (Variable Number Tandem Repeat) (Pooideh *et al.* 2015).

The characteristics of different typing systems, like their discriminating power, reproducibility, easy performance, accurate interpretation of the results, and cost, are particularly significant when it comes to getting accurate results. A wide variety of approaches can be used to type *E. coli* and other types of bacteria. However, Pulsed-field Gel Electrophoresis (PFGE) is one of the most frequent approaches for producing DNA fingerprints (Ranjbar *et al.* 2017).

2.10 Genetic and Mechanistic Basis Antibiotic Resistance

Two traits that are unique to prokaryotic cells (a rapid growth rate and a haploid genome) are primary factors in the evolution of antibiotic-resistant mutations in cells that were previously susceptible to antibiotics: The quick growth rate results in a huge population in a relatively short period, and this raises the possibility that there will be one mutant cell within an average of 108 cells as a result of the fortunate acquisition of a resistance mutation (Matic *et al.* 1997).

Slow-growing bacteria such *as Mycobacterium tuberculosis* are able to overcome the slow growth by expressing certain mechanisms to evade immunity. These processes allow populations to endure in their natural habitats for long periods, allowing resistant variations to emerge. The haploid nature of the genome in bacteria allows for the quick

appearance of a mutant genotype. Transfer of genetic material expression antibiotic resistant among cells of mixed bacterial populations is another genetic approach for gaining antibiotic resistance. This can happen by naked DNA transformation, plasmid DNA conjugation via cell-cell interaction, or phage-mediated plasmid DNA transduction. One genetic approach for obtaining antibiotic resistance is mutations, which modify previously existent genetic material resulting in antibiotic resistance (Davies and Davies 2010).

These genetic changes can trigger one of three key biochemical pathways that lead to antibiotic resistance. These processes include a decrease in the target's affinity for the antibiotic, a drop in the drug's concentration at the target's location, or enzymatic inactivation of the medication. Bacterial cells have produced countless versions of these three essential processes due to selection forces imposed over the course of evolution. Each of these processes, alone or in combination, can result in clinically meaningful resistance to certain medicines or potentially all presently available antibiotics (Ebmeyer *et al.* 2021). Antibiotic resistance, on the other hand, is occasionally followed by a decrease in the fitness and pathogenicity of the resistant cell. This can be caused by a disruption in the affected target's normal function or by the overexpression of specific resistant genes (Moreno *et al.* 2006).

After the antibiotic that is being used for selection is present, the resistant mutant has a growth advantage. However, when the antibiotic is no longer present when the therapy has been completed, the mutant's reduced fitness might change this advantage into a disadvantage. Antibiotic resistance develops over time, yet, less fit mutants have the potential to acquire further genetic modifications that allow them to compensate for their decreased fitness. In conclusion, the presence of both resistant and compensatory mutations in a population can produce well-adapted clones that are easily spread between host populations (Erjavec *et al.* 2007).

2.11 Polymerase Chain Reaction (PCR)

A common technique for quickly producing millions to billions of copies of a given DNA sample is the polymerase chain reaction (PCR). Using this technique, scientists may quickly amplify a very little DNA sample to a quantity large enough to be studied in detail. Kary Mullis, an American scientist, developed PCR in 1983. Many of the techniques used in genetic testing and research, such as the study of ancient DNA samples and the detection of infectious organisms, are fundamentally based on PCR. In a series of temperature-changing cycles, copies of extremely tiny quantities of DNA sequences are exponentially amplified by PCR. Today, PCR is a widely used and frequently essential method in medical laboratory research for various purposes, including biological research and criminal forensics (Saiki *et al.* 1985).

The use of thermal cycling is essential to the majority of PCR methods. Thermal cycling facilitates a range of temperature-dependent activities, including as DNA melting and enzyme-driven DNA replication, by reversibly heating and cooling reactants. Primers, which are short, single-strand DNA fragments having sequences that are complementary to the target DNA region, and a DNA polymerase are the two primary reagents in PCR. In the first step of PCR, the DNA double helix is denatured by heating it in a process called nucleic acid denaturation. Primers attach to their corresponding DNA sequences as the temperature drops in the second step. DNA polymerase, using free nucleotide building blocks, uses the two DNA strands as templates to synthesize a new DNA strand. PCR generates DNA, which is then used as a template for self-replication, setting off a chain reaction that exponentially increases the amount of DNA present (Porta and Enners 2012).

Nearly all polymerase chain reaction (PCR) applications require a heat-stable DNA polymerase, such as Taq polymerase, which was originally isolated from the thermophilic bacteria *Thermus aquatics*. Denaturation happens at very high temperatures, which may denature a polymerase if it were being used. Before the introduction of Taq polymerase, adding DNA polymerase to each cycle was a laborious and costly process (Ninfa *et al.* 2009).

2.12 Antibiotic Resistance Genes

Researchers have compiled evidence of an extensive variety of organisms that have developed antibiotic resistance. However, only a small number of genes are shared among these strains that are responsible for the resistance qualities. In one study, for instance, the *tetK* gene, which confers tetracycline resistance, and the *blaZ* gene, which confers penicillin resistance, were found in one hundred percent of the multi-drug resistant *S. aureus* strains. Both of these genes are responsible for the bacteria's ability to withstand treatment with their respective antibiotics. Genes that allow bacteria to resist the majority of antibiotics currently in use have been found in the genomes of drug-resistant strains of bacteria. This finding is not unique (El Seedy *et al.* 2017).

This presents a significant opportunity for researchers since it enables them to determine, based on the presence or lack of particular antibiotic resistance genes, whether a particular bacterial strain is likely to be susceptible to the antimicrobial treatment that is being administered. PCR can screen hundreds of strains in a single day to determine whether they are resistant to a single drug or multiple drugs. The practice of searching for genes that confer resistance to antibiotics is referred to as genotyping. For the past few decades, polymerase chain reaction (PCR) has been utilized to genotype bacteria in terms of their antibiotic resistance (Rathore *et al.* 2018).

Variable sequences (81 bp tandem repeats) at the 3' terminus of the *coA* gene encoding coagulase protein allow various *S. aureus* species to be distinguished. Primers corresponding to a conserved region in the *coA* gene are used to perform typing, and this polymorphism is then used as an epidemiological marker. Varied strains can produce PCR results of different sizes due to the varying amounts of repetitive sequences within the *coA* gene (Cosandey *et al.* 2016). Sensory *rsbA* protein, encoded by the *rsbA* gene, may regulate swarming behavior by responding to changes in the surrounding environment. Formation of biofilms and extracellular polysaccharides were both prompted (Manos *et al.* 2006).

Pathogenic *E. coli* and other members of the Enterobacteriaceae family express the *fimH* gene, which encodes a mannose-binding adhesin shown at the tip of type 1 pil (Hase *et al.* 2009), that enables them to identify and bind terminal mannoses on glycoproteins expressed on epithelial cells (Ohno and Hase 2010), the Toll-like receptor 4 and the cell adhesion molecule 6 linked to carcinoembryonic antigen (CEACAM6) (TLR4). Biofilm formation and bacteria-specific mucosal immune responses may be enabled by other mannosylated abundant components like mucus, but this has not been reported (Barnich and Darfeuille-Michaud 2010).

CNF1 is a bacterial protein toxin produced by certain pathogenic strains of *E. coli* and is sometimes found in isolates from the feces of children with diarrhea. However, CNF1 is more commonly responsible for infections outside of the intestines, especially those of the urinary tract. Furthermore, these strains have been identified in neonatal meningitis and bacteremia (Michaud *et al.* 2017).

3. MATERIALS AND METHODS

This cross-sectional study was conducted from 3rd January 2022 to 1st June 2022 and included 120 patients attending Sallah Al-Deen Hospital in Tikrit city and Urology clinics. The patient's ages ranged between (2 months old to 70 years old). The study included 90 positive culture-proven UTI and 50 age and sex-matched healthy individuals as a control group. Questionnaire was obtained from the patients regarding their demographic information and medical history in adherence to the guidelines of the ethics committee of Tikrit University, Sallah Al-Deen, Iraq. Patients who had acute appendicitis, ulcerative colitis, chronic infectious disorders, chronic renal, cardiac, or hepatic conditions, or were receiving antibiotic therapy or other medications that could have influenced the outcomes were not included.

3.1 Antibiotics Used in the Study

Antibiotics used in the study are as shown in the Table 3.1.

Table 3.1 Antibiotics list and its concentrations and affectivity measures

Antibiotics	Symbol	Conc. (µg)	Sensitive (S)	Intermediate (I)	Resistant (R)
Amikacin	AK	30	17≤	16-15	14≥
Ampicillin/Cloxacillin	APX	30	25≤	24-18	17≥
Azithromycin	AZM	15	18≤	17-14	13≥
Cefixime	CFM	5	20≤	19-18	17≥
Cefotaxime	CTX	30	26≤	25-23	22≥
Ciprofloxacin	CIP	10	21≤	20-16	19≥
Erythromycin	E	15	18≤	17-14	15≥
Imipenem	IPE	10	16≤	-	-
Nalidixic acid	NA	30	19≤	18-14	13≥
Nitrofurantoin	F	100	17≤	16-15	14≥
Norfloxacine	NOR	30	17≤	16-13	12≥
Novobiocin	NV	30	22≤	21-17	16≥
Trimethoprim/Sulphamethozole	SXT	25	21≤	20-16	15≥
Vancomycin	VA	30	17≤	-	-

3.2 Indirect Examination

3.2.1 Urine culture

All samples from suspected cases were cultured regardless of the directed examination result. Each sample was cultured on Blood agar, MacConkey agar and Sabouraud Dextrose Agar to detect yeast and mold using the quantitative loop method. The plates were incubated for 18-24 hours at 35-37℃ aerobically. After the growth of the bacteria was established, a subculture was made on nutrients, blood and MacConkey`s agar for further identification. Other differential media were also used for specific bacteria, such as; Mannitol Salt Agar to differentiate between *Staphylococcus* species, as well as Sorbitol MacConkey agar and Eosin-methylene blue for Enterobacteriaceae (APPENDIX 3).

3.2.2 Identification of isolated bacteria

Bacteria were separated into cocci, bacilli, and Gram-negative and Gram-positive categories via microscopic analysis based on their gram staining (APPENDIX 4), size, shape, and cell organization. Colonial morphology on culture media was studied after 24 hours of incubation at 37°C. The Enterobacteriaceae (gram-negative bacilli) has some characteristic appearance. On Nutrient agar, *E. coli* produce circular, smooth, colorless colonies with entire edges. *Proteus spp.* produce small, circular, convex, smooth and translucent colonies. On MacConkey agar, *E. coli* showed pink colonies due to the ability of lactose fermentation. On blood agar, some bacteria showed hemolysis character (*E. coli*) produce white colonies around with clear zone of hemolysis resulting from completely lyse, or break down of red blood cells found in blood agar. This type of hemolysis is called beta-hemolysis. *P. mirabilis* showed swarming phenomena when grown on blood agar. The colonies of *P. aeruginosa* were flat and beta-hemolytic also. Gram-positive bacteria, such as *S. aureus* on Nutrient agar showed golden yellow, circular, convex, smooth, shiny, opaque and entire edge colonies. On blood agar *S. aureus* showed pigmentation and beta-hemolysis. On mannitol salt agar *S. aureus* grown changes the media color from orange to yellow at room temperature (APPENDIX 1).

3.3 VITEK Diagnosis

Confirm the diagnosis using the Vitek 2 compact system, which consists of two basic components, the instrument machine and the computer, and the machine consists of five basic components:

- The control panel. Keypad
- Fill the door, and the sample is transferred from the Kan tube to the inside of the kit by a conveyor pipe, which lasts 70 seconds.
- The loading door is cut in the conveyor pipe on the kit, and the kits are loaded into the incubator, and this process lasts from 3 to 5 minutes.
- The user access door was used to incubate and measure the changes in the kit as a result of bacterial growth to give the result.
- Waste door, where kits were collected after finishing the analyses and giving the result. This device was used to confirm the diagnosis of isolations.

The procedures for these tests were as follows:

- Preparation of the implant: the isolations under study were developed on the center of nutrient agar in a planning method and incubated with 37°C for 24 hours. Then a Kan tube was used for the purpose of diagnosis, and put 3 mL of normal saline solution by dispensation, then a Loop full was raked from a single colony.
- After being prepared, the sample was inserted into the filling door and transferred from the Kan tube into the Kit. From there, the samples were transported by hand to the loading door and placed in the incubator. Then the result was read after 4-6 hours.

3.4 Maintenance and Storage of Bacterial Isolates

Bacterial isolates were cultured on slants of nutrient agar after they were identified, and the slants were incubated at 37°C for 24 hours before being chilled to 4°C.

3.5 Antibiotic Susceptibility Test

The bacterial antibiotic sensitivity test under study was conducted using several antibiotics manufactured by the Turkish company (Bioanalyse) and is used globally as recommended (CLSI 2019). Then take the disk diffusion test according to Kerby-Bauer's modified method according to (Granato and Granato 2011). The test was carried out as follows:

- Moving 3-5 from developing bacterial colonies cultured within 18-24 hours to a tube test containing 500 milliliters of saline solution.
- The solution was seized with the macfarland solution 0.5, which is equivalent to 1.5x 108 cell/milliliter
- Using a sterile swab, bacterial trappers were deployed in various directions on the center of Muller - Hinton agar plate.
- Using sterile tongs, antibiotic discs were placed on the implant medium in about 5-7 tablets per dish, then incubated the dishes at 37°C for 24 hours.
- The diameter of the inhibition zone was measured by meter using a measuring ruler and compared to the standard to determine the extent to which the bacterial cell is resistant or sensitive to the antibiotics used.

3.6 Extracting DNA from the Bacteria Under Study

- The analysis kit prepared by (Geneaid Co.) was used to extract DNA from bacteria samples under study.
- The precipitate of the growing bacteria in the broth medium, equivalent to 1x109 cells, is taken into Eppendorf 1.5 mL tube.
- Lysozyme (0.8 mg/200 mL) is applied to an Eppendorf tube holding 200 L utilizing the Vortex mixing process.
- The tube is heated to 37°C for 30 minutes while being flipped every 3 minutes.
- Add 20 microliters of Proteinase K and mix using Vortex
- Incubate the mixture at 60°C for 10 minutes.

- Add 200 microliters of buffer solution, mix using Vortex, and incubate at 70°C.
- Add 200 microliters of absolute ethanol, mix manually, then transfer the mixture to the buffer solution tube installed in the collection tube, conduct a centrifuge with a power of 16.000g for 30 seconds, get rid of the leachate, then add 600 microliters of washing solution, centrifuge at the same speed and time before, get rid of the residue.
- Transfer the buffer solution column to a new 1.5 mL tube, add 100 microliters of the dissolved solution, leave for 3 minutes, then centrifuge at a speed of 16.000g for 30 seconds, and then save DNA until use (APPENDIX 2).

3.6.1 Agarose gel preparation and electrophoresis of DNA

- A 1% agarose gel is used for DNA migration and detection. To get this concentration, you'll need to dissolve 0.5 g of agarose powder in 50 mL of X1 TBE (Tris-Borate-EDTA) and then add 3 microliters of red safe tincture. This is achieved by subjecting the mixture to a thermal source and stirring it continuously until it boils, after which it is allowed to cool to between 50 and 60°C.
- After installing the specific comb to create the well's drill at the gel's edge and keeping in mind that the casting has to be done silently to prevent the production of bubbles, the gel solution is poured into the tray basin of the paging device and left there until it has hardened.
- The tray is then placed in the electric relay basin that contains the right amount of X1 TBE solution, and then the comb is quietly lifted.
- The migration samples are prepared by mixing 5 microliters of DNA sample with 3 microliters of loading solution. The relay device is then turned on by the flow of the electric current with a difference of voltage of 5 volts\cm, and the operation takes 2-1.5 hours. The gel is then photographed under ultraviolet radiation using the UV Trans-lumination gel imaging device to see the DNA bundles and the product of the PCR reaction.

3.6.2 DNA extraction from the gel

Nucleotide sequence testing was performed on the retrieved beams from the PCR reaction gel using multiple analyses pre-prepared by Geneaid and following and according to the steps:

- The package is cut from the agarose gel using a sterile scalpel while removing the largest amount of gel surrounding the package.
- Transfer approximately 300 mg of gel to the Eppendorf 1.5 mL tube, and 500 microliters of DF (Detergent-free) regulated solution is added and mixed using the forteke device.
- The tube is placed in an incubator at 55-60°C for 15 minutes, during which time a full piece of jelly is flipped every 3 minutes. Put the tube in a cool place to cool down naturally.
- The sample mixture is diluted to 800 microliters, placed in the DF column in the collection tube, centrifuged at 16.000g for 30 seconds, and the resulting leachate is discarded.
- After waiting a minute, replace the DF column in the collection prophet and flush it with 600 microliters of washing solution.
- Remove the leachate by centrifuging at 16.000g for 30 seconds.
- The previous process is repeated.
- Centrifuge for 3 minutes to ensure the DF shaft is dry.
- The collection tube is disposed of and the DF column is transferred to a new 1.5 mL tube.
- Add 20-50 microliters of Ellution buffer solution to the center of the column.
- Leave for two minutes to ensure the dissolved solution is absorbed.
- Centrifuge for two minutes 16.000g to obtain dissolved DNA (APPENDIX 5).

3.6.3 PCR reactions

- The concentration of DNA in all study samples is adjusted by dilution by TE (Tris-EDTA) solution to obtain the concentration required for PCR reactions, and was 50 µg/microliters per sample.
- Each PCR reaction's master reaction mixture was made by combining a DNA sample and the gene's specific initiator with the master-mix components in a 0.2 mL Eppendorf tube supplied by an English firm (Biolaps).
- The sample mixture is diluted to 800 microliters, placed in the DF column in the collection tube, centrifuged at 16.000g for 30 seconds, and the resulting leachate is discarded.
- The Thermo cycler device's reaction pipes were then placed into the device, and the multiplier reaction was carried out in accordance with the tailored program for each reaction.
- The volumetric manual Ladder DNA ladder was added to the sample before it was placed into the 2% agarose gel drill.
- The samples are retrieved from the Biolaps in one of the drills, run through the Electrophoresis device for 60-70 minutes, and then imaged in the gel using the UV Transillumination equipment.

3.6.4 Molecular diagnosis of the bacteria under study based on the 16srRNA region

Bacterial isolations were detected by amplifying the 16srRNA region in the bacterial genome, 4 microliters (100 nanograms) of molded DNA and 1 microliter (10 picomols) from each gene starter were added to the contents of the Master mix. The Thermocycler was then used to carry out the multiplier reaction, with the reaction pipes being introduced into the machine and a custom-made program being run to control the process (Table 3.2).

Table 3.2 Measurements the thermal cycler

No.	Stage	Temperature	Time	Cycle number
1	Initial denaturation	95	6 min.	1
2	Denaturation	95	45 sec.	35
3	Annealing	56	1 min.	
4	Extension	72	1 min.	
5	Final extension	72	5 min.	1

3.6.5 Diagnosis of bacterial virulence factors

Diagnosis of virulence genes for bacteria under study several virulence genes for the bacteria under study were molecularly diagnosed by adopting specific programs for each gene with primers designed to verify the presence of genes within the bacteria and as shown in the following Table 3.3:

Table 3.3 Sequence of primers products

No	Genes	Sequence of primers		Band size
1	*16S rRNA*	GACCTCGGTTTAGTTCACAGA	Forward	1200
		CACACGCTGACGCTGACCA	Reverse	
2	*coA*	ATAGAGATGCTGGTACAGG	Forward	400
		GCTTCCGATTGTTCGATGC	Reverse	
3	*rsbA*	TTGAAGGACGCGATCAGACC	Forward	467
		ACTCTGCTGTCCTGTGGGTA	Reverse	
4	*fimH*	TGCAGAACGGATAAGCCGTGG	Forward	508
		GCAGTCACCTGCCCTCCGGTA	Reverse	
5	*cnf1*	AAGATGGAGTTTCCTATGCAGGAG	Forward	498
		CATTCAGAGTCCTGCCCTCATTATT	Reverse	

3.7 Statistical Analysis and Data Management

- Clinical and demographic characteristics of patients are presented as means SDs and percentages.
- Comparison was carried out using Chi-square, Mann–Whitney U test and T-Test probability and analysis of variance (ANOVA) and interpreted as follows: P value> 0.01: Highly Significant (HS), 0.1≤(P value)≤0.5: Significant (S), P value> 0.05: Non-signifiant (NS).

4. RESULTS AND DISCUSSION

4.1 Epidemiological Study

4.1.1 Results of culture in isolated samples

Table 4.1 shows that the total number of 120 specimens that were subjected to culturing showed positive results for 90 (75%) of the total number. In contrast, the rest 30 (25%) of the samples showed negative results in culture media. It's important to mention that only a positive culture group will be included in this study.

Table 4.1 Culture results of isolated samples

Total number of samples	Results			
	Positive culture		Negative culture	
	No.	%	No.	%
120	90	75%	30	25%

4.1.2 Distribution of patients according to gender and age groups

Out of 120 samples, 90 of them were culture-proved UIT patients with positive results on culture media, 55 (61.1%) of patients were females, while the rest of the 35 (38.9%) patients were males. Study groups of 90 patients with urinary tract infections were divided into five age groups. Group (1) included 8 (8.89%) patients whose ages ranged from 1 to 5 years old, and their gender distribution was 6 (10.91%) females and 2 (5.71%) males. Group (2) included 18 (20%) patients whose ages ranged from 5 years old to 15, and their gender was distributed between 10 (18.18%) females and 8 (22.86%) males. Group (3) included 25 (27.78%) patients, which was the largest group of the study of 13 (23.64%) females and 12 (34.29%) males and their ages ranged between 15 and 30 years old. Group (4) included 30 (33.33) patients whose ages ranged between 30 and 50 years old, and females and males were 21 (3.18%) and 9 (25.71%), respectively. The last Group included 9 patients of 5 (9.09%) females, and 4 (11.43%) males of age ranged between 50 to 70 years old. Results are given in Table 4.2.

Table 4.2 Distribution of patients according to gender and age groups

No. of group	Age groups in years	Female		Male		Total	
		No.	%	No.	%	No.	%
1	**≤5 years**	6	10.91	2	5.71	8	8.89
2	**6-15**	10	18.18	8	22.86	18	20
3	**16-30**	13	23.64	12	34.29	25	27.78
4	**31-50**	21	38.18	9	25.71	30	33.33
5	**51-70**	5	9.09	4	11.43	9	10
	Total	**55**	**100**	**35**	**100**	**90**	**100**

While the incidence is similar between the sexes in the older population, urinary tract infections are more common in women than men. UTIs are more common in female patients, according to study by McLaughlin and Carson, and this is likely owing to anatomic and physiological differences between the sexes (proximity of the urethra with the vagina and the rectum) (Riccabona 2003). The incidence of pyelonephritis is drastically lower, by a factor of 20-30. They're responsible for about 40% of all UTIs and are the leading source of hospital-acquired infections. Females of childbearing age have between 2% and 7% of asymptomatic bacteria in their urine, while elderly women in nursing facilities may have as much as 50% (Salvatore *et al.* 2011).

Age-related hormonal shifts and a decline in uromucoid secretions are just two of the many anatomic and functional alterations that contribute to the increased prevalence of UTIs in elderly women. Increased rates of UTIs have been observed in women of advanced years, and this trend may be linked to hormonal shifts such declining estrogen levels. Bacterial colonization of the bladder may be exacerbated by a decrease in uromucoid secretions, which may lead to a drop in antibacterial activity and a reduction in the renal ability to eliminate acid and urea (Griebling 2005).

UTIs affect 3% to 5% of girls and 1% of boys by the time they reach puberty, with the first episode typically occurring in girls by age 5 and peaking during infancy and toilet training. In contrast, UTIs are common in boys during the first year of life, especially among those who are uncircumcised because they are more vulnerable to pathogens at this age. Age, gender, and sexual orientation were all significant predictors in generally

consistent data from other sources (Hunt *et al.* 2010), genitourinary tract abnormalities, diabetes (Amiri *et al.* 2015), married individuals (Souza *et al.* 2015), catheter, and duration of catheter bear statistically significant relationship with UTIs.

After the age of fifty, males are at an increased risk of developing UTIs. Prostate issues typically manifest during this time. The growth of the prostate gland, known as benign prostatic hyperplasia (BPH), is a common cause of urinary blockage and an increased risk of infection. Prostatitis is an infection of the prostate gland that is linked to recurrent UTIs in males. Only around 20% of UTIs are male, yet the complications these infections cause in males are more severe than in women. Hospitalization rates for men with UTIs are significantly higher than those for women (Griebling 2005).

4.1.3 Distribution of bacteria in urinary tract infection

E. coli was the most common bacterial species isolated from urine, with 48 (53.33%). *P. mirabilis* was the second most isolated bacteria, with 23 (25.56%) of samples. *S. aureus* was also found with 19 (21.1%) of total positive samples. Usually, the predominant pathogen responsible for UTI is *E. coli,* up to 80-85%. The occurrence of the infection due to viral or fungal agents is a rare phenomenon. In addition, the *Proteus* app. are also associated with UTI. Many studies have indicated that these bacteria are one of the most causes of tract infections. The results agreed with Wasmy Shahab *et al.* (2018) and researcher Khudhur Mohammad *et al.* (2018). The two researchers isolated *E. coli* bacteria during their studies by 40%, while the second researcher isolated it by 37%. Joni (2018) isolated *E.coli* bacteria by 50%, while, Oikonomou and Alhaddad (2017) isolated it by 29.3%.

The reason for the high incidence of *E. coli* is due to the factors that these bacteria possess. Virulence factors and mechanisms make it capable of causing infections such as its ability to adhere and release toxins. Movement and escape from the immune mechanisms of the host body (Sarowska *et al.* 2019). Researcher Wasmy Shahab *et al.* (2018) isolated these bacteria during their studies in a much lower percentage, as they reached 7.5%, while the results of researcher Taweel *et al.* (2018) showed the lowest percentage was only 2.9%.

S. aureus bacteria have been isolated as one of the pathogens of urinary tract infections. The results close to the results of Shankar *et al.* (2021) Isolated this bacteria by 0.76% in hemodialysis patients, and this percentage is much lower than the results of our study. The bladder receives the germs from the intestines. Bacteria adhere, then build a biofilm that blocks the body's immunological response and causes the infection. The incidence of UTI caused by *S. aureus* is typically viewed as a secondary infection, following only those that are spread by the blood. This study isn't the first to find a link between *S. aureus* and UTI prevalence. High prevalence of *S. aureus* was observed in previous investigations from Bushenyi (Uganda) and Awka (Nigeria), with respective rates of 43.7% and 22.5% (Ekwealor *et al.* 2016).

Previous studies have linked the increasing *Staphylococcal* UTIs to increased use of instrumentation such as bladder catheters (Iregbu and Nwajiobi-Princewill 2013). However, this study's high prevalence of Staphylococcus varied from other previous studies (Ochada *et al.* 2015). *P. mirabilis* was isolated with 25%; *P. mirabilis* is one of the causative bacteria of urinary tract infections due to a combination of virulence factors possessed by these bacteria. The most important of these are: its ability to form biofilms, the secretion of the urease enzyme, adhesion molecules, iron carriers siderophores and toxins production (De Oliveira *et al.* 2021).

Our results are not in line with those of the researchers Oikonomou and Alhaddad (2017) during their study of urinary tract infections in hemodialysis patients, as this isolation rate reached 6.7%, while researcher Khudhur Mohammad *et al.* (2018) isolated it by 7.4% (Table 4.3).

Table 4.3 Distribution of bacteria in urinary tract infection

Type of bacteria in the isolates	**The isolates**	
	No.	**%**
E. coli	48	53.33
P. mirabilis	23	25.56
S. aureus	19	21.1
Total	90	100

4.1.4 Antibiotic susceptibility test of isolated bacteria of study groups

E. coli was sensitive to Imipenem 100%, Ciprofloxacin 60%, Nitrofurantoin 100%, and Amikacin 100%, but it showed different resistance to the rest of the antibiotics. Our results were close to the search results of Kulkarni *et al.* (2017) if they showed their isolation from *E. coli* bacteria 96.71 and 90.89% sensitivity to Imipenem antagonists and Amikacin. Our results agreed with the researcher Granato and Granato (2011) if *E. coli* is sensitive to Imipenem and Ciprofloxacin, their isolates were also sensitive to the antagonist Nalidixic acid. Cefixime and Tetracycline showed resistance during our current study, as it shown in Table (4.4). In a study by Jayanthi and Shariff (2015), their isolations were sensitive to the antibiotics Ciprofloxacin and Amikacin and Imipenem by 33.3%, 77.5% and 60%, respectively.

Table 4.4 Antibiotic susceptibility test of the isolated organisms of study groups

Name of antibiotic	Percentage of sensitivity effect on isolated bacteria		
	E. coli **No.=48**	***S. aureus*** **No.=23**	***P. merabilis*** **No.=19**
Amikacin	100%	95%	100%
Ampicillin	0%	0%	0%
Amoxicillin	0%	0%	0%
Azithromycin	25%	15%	50%
Cefixime	0%	22%	0%
Erythromycin	0%	0%	0%
Imipenem	100%	100%	100%
Nalidixic acid	0%	0%	0%
Ciprofloxacin	60%	0%	100%
Cefotaxime	0%	0%	0%
Nitrofurantoin	100%	100%	100%
Novobiocin	0%	0%	0%
Trimethoprim /Sulfamethoxazole	0%	55%	0%
Vancomycin	50%	100%	66%

The bacteria of *P. mirabilis*, which was isolated in our current study, was 100% sensitive to the antibiotics; Amikacin, Ciprofloxacin, Imipenem and Nitrofurantoin. Our results agreed with Mohammed and Shehab (2019) during their studies on isolates of *P. mirabilis* bacteria where sensitivity to antibiotics Amikacin, Ciprofloxacin, Imipenem and Nitrofurantoin were 100%, 91.66%, 83.33 respectively.

These results are very consistent with our current results, and our results have not been consistent with those of Hussein *et al.* (2017) where the sensitivity to antibiotics; Amikacin Ciprofloxacin, Imipenem and Nitrofurantoin was 25%, 25% 10% and 80% respectively.

It was found through the results of the sensitivity test of gram-positive bacteria that *S. aureus* bacteria were 100% sensitive to the Imipenem, Nitrofurantoin, Amikacin and Vancomycin with 100%, 100%, 95% and 100 respectively. Our current results are close to the study of researcher Moosavian *et al.* (2016) during their study on how sensitive *S. aureus* bacteria are to antibiotics, including Imipenem and Vancomycin, as the sensitivity rate to these antibiotics reached 83.1 and 100%, respectively, these The percentage was close to our results. As well as the results of researcher Naimi *et al.* (2017), their isolations showed sensitivity to antibiotics Imipenem and Vancomycin by 90% and 100% in a row, and these results are consistent with our results.

In general, our current results have agreed with some studies and differed with others in sensitivity and resistance rates of antibiotics under the current study are due to many reasons, the most important of which are geographical distribution, source of isolations, method used, extent of antibiotic use and pressure has the result of excessive and indiscriminate use.

4.2 Molecular Studies

4.2.1 DNA extraction and purification

The DNA was extracted using the extraction kit supplied by Genaid, according to the steps included with the extraction kit. Subsequently, the DNA concentration was measured using a Nanodrop device. The results showed that the DNA concentrations of the isolates ranged between (15.3-30.4) ng/µL and its purity ranged between 1.6-1.9. As shown in Figure 4.1.

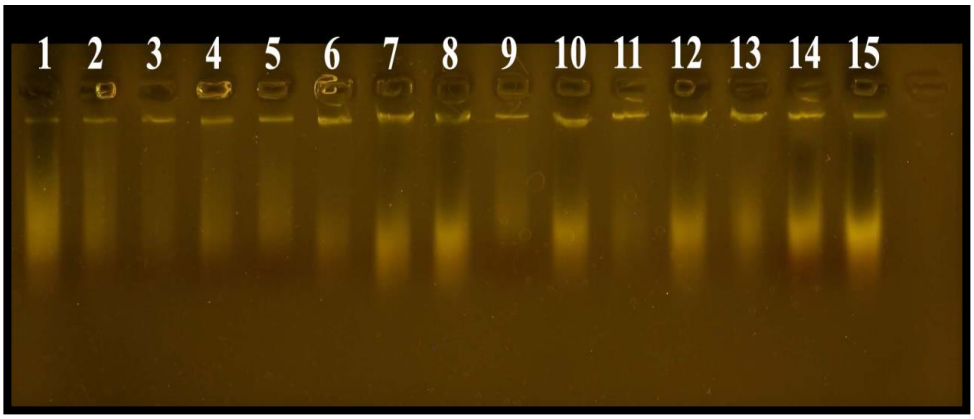


Figure 4.1 Agarose gel electrophoresis of the products of bacterial DNA extraction (Agarose 0.5%)

4.2.2 Molecular detection of virulence genes

Several virulence genes for the bacteria under study were investigated using a primer designed for this purpose. The study showed that the prevalence rate of *coA* gene was 86.7% in *Staphylococcus*. As for the *rsbA* gene, the prevalence rate was 93.3%. As for the *fimH* and *cnf1* genes' prevalence rates were 33.3 and 66.7%, respectively (Figure 4.2).

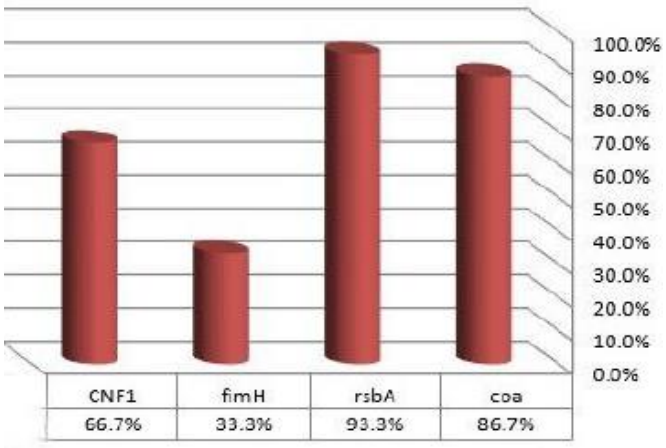


Figure 4.2 Prevalence of virulence genes in the studied bacteria 42

Molecular detection of *coA* gene: The product of the polymerase chain reaction on the agarose gel of *coA* gene showed the appearance of bands at the expected site (450) bp in 13 out of 15 samples (Figure 4.3) (Table 4.5).

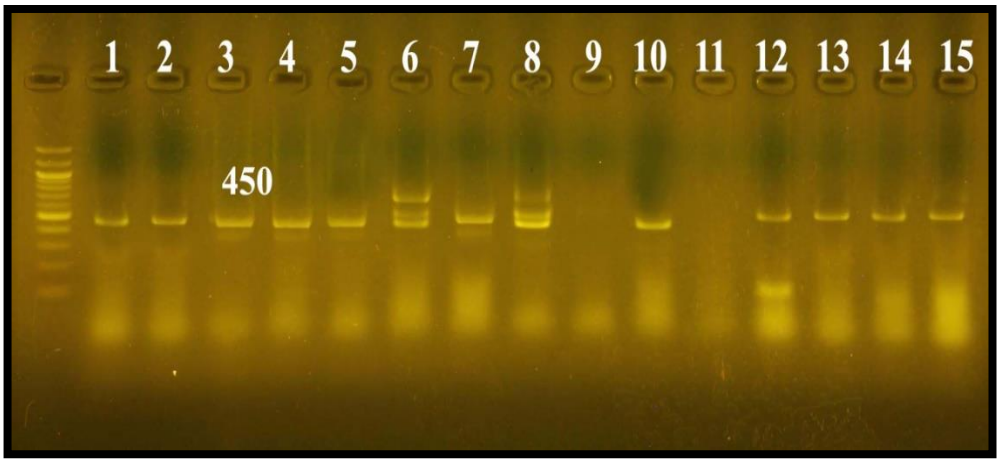


Figure 4.3 PCR product of the *coA* gene with a 450 bp reaction product that was migrated with a 2% agarose gel

Table 4.5 Distribution of *coA* gene

Isolate No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Coa Gene	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+

One of *S. aureus*'s most crucial virulence factors is the *coa* gene. When this gene is expressed, it is expected to counteract the effects of host defensive mechanisms such phagocytosis and boost bacterial proliferation and infection (El-Tawab *et al.* 2017). Scientists believe that *S. aureus'* coagulase gene contributes significantly to the pathogen's pathogenicity. To reliably detect virulent strains of *S. aureus*, amplification of the coagulase gene (*coA*) has been suggested (Morandi *et al.* 2010).

Molecular detection of *rsbA* gene: The product of the polymerase chain reaction on the agarose gel of *rsbA* gene showed the appearance of bands at the expected site (467) bp in 14 out of 15 samples (Figure 4.4) (Table 4.6).

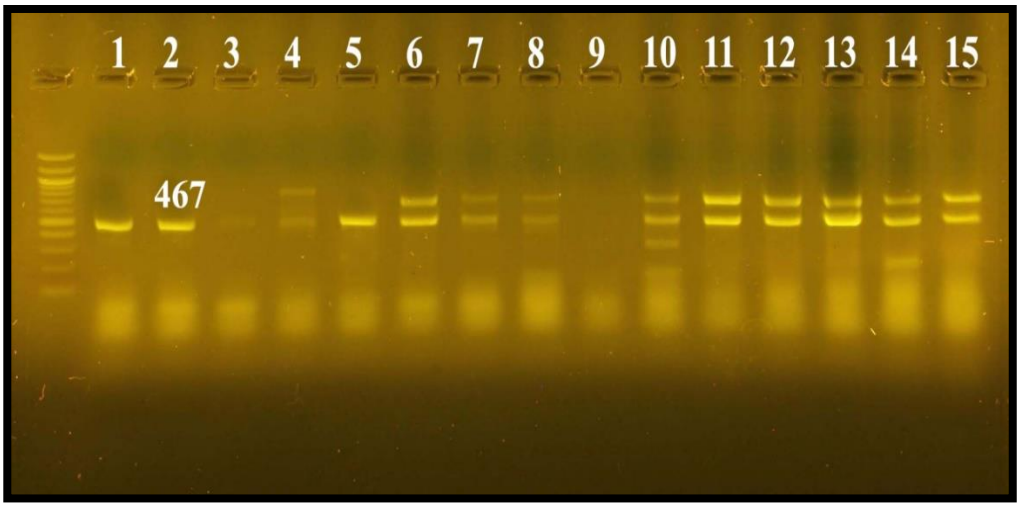


Figure 4.4 PCR product of the *rsbA* gene with a 467 bp reaction product that was migrated with a 2% agarose gel

Table 4.6 Distribution of *rsbA* gene

Isolate No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
rsbA Gene	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+

The membrane sensor involved in the induction of extracellular polysaccharides synthesis is expressed by the *rsbA* gene. Moreover, it controls swarming behaviors and promotes biofilm development in *P. mirabilis* (Algammal *et al.* 2021). Our results showed the gene prevalence in 93.3% of the isolates. This is consistent with the results of Algammal *et al.* (2021) who stated that the prevalence of the *rsbA* gene is 94.3%. While the results of Pathirana *et al.* (2018) indicated that the prevalence of the gene was 73.3%. In a study conducted by Badi *et al.* (2014) on patients with urinary tract infections, it was found that the prevalence of *rsbA* gene in *P. mirabilis* isolates was 70%. Lv *et al.* (2022) studied the prevalence of the gene in different types of animals and concluded that the prevalence of the gene was 68.1% in foxes, 61.1% in raccoons, and 92.3% in mink.

Molecular detection of *fimH* gene: The product of the polymerase chain reaction on the agarose gel of *fimH* gene showed the appearance of bands at the expected site (508) bp in 5 out of 15 samples (Figure 4.5) (Table 4.7).

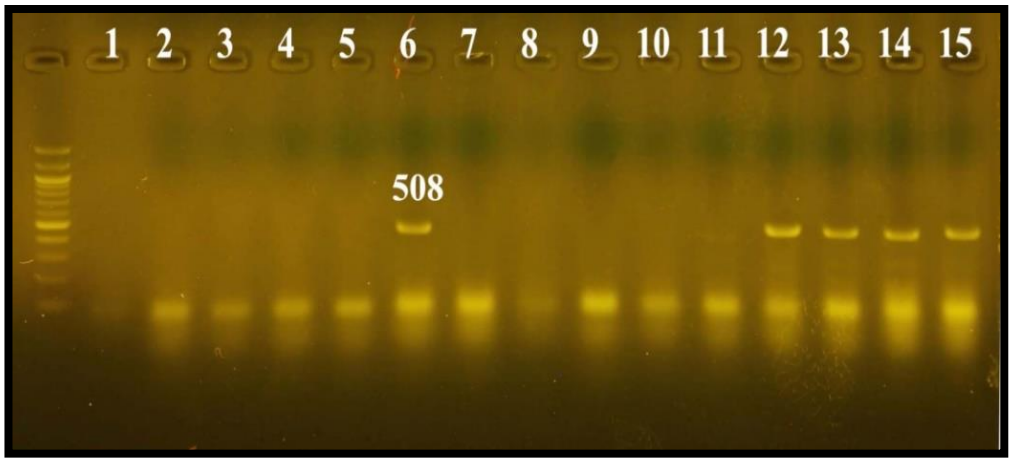


Figure 4.5 PCR product of the *fimH* gene with a 508 bp reaction product that was migrated with a 2% agarose gel

Table 4.7 Distribution of *fimH* gene

Isolate No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
fimH Gene	–	–	–	–	–	+	–	–	–	–	–	+	+	+	+

Urinary tract infections (UTIs) develop when UPEC connect to epithelial cells lining the urinary tract through a variety of specialized adhesions, the most important of which are type 1 fimbriae (Kaczmarek *et al.* 2012). Numerous virulence factors present in UPEC strains aid in colonization of the urogenital system. The *fimH* adhesion at the tip of the type 1 fimbriae mediates attachment to the urothelial cell surface, stopping the bacteria from being washed away by the urine and initiating the bacterial invasion process (Hojati *et al.* 2015).

PCR validated the presence of the *fimH* gene, and the results showed that it was present in 92.8% of UPEC isolates, 47.7% of hospitalized patient isolates, and 52.3% of outpatient isolates. Most UPEC strains tested positive for the presence of the *fimH* gene, confirming our findings and corroboration with those found in the literature. When comparing the virulence genes of UPEC strains, Tarchouna *et al.* (2013) discovered that the *fimH* gene was the most common, present in 68% (61/90) of the UTI isolates they examined.

When comparing the virulence genes of UPEC strains, Tarchouna *et al.* (2013) discovered that the *fimH* gene was the most common, present in 68% (61/90) of the UTI isolates they examined. In another study, Watts *et al.* (2010) found that 98% of *E. coli* strains collected from people with UTIs included the *fimH* virulence gene.

A study conducted by Mladin *et al.* (2009) examined the prevalence of virulence genes among the examined UPEC, and they discovered an 80% frequency for the *fimH* gene.

Molecular detection of *cnf1* gene: The product of the polymerase chain reaction on the agarose gel of *cnf1* gene showed the appearance of bands at the expected site (498) bp in 10 out of 15 samples (Figure 4.6) (Table 4.8).

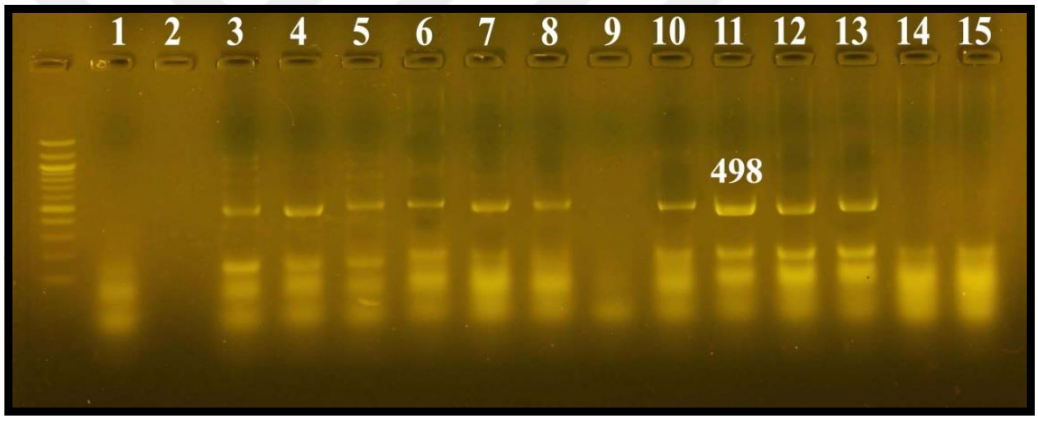


Figure 4.6 PCR product of the *cnf1* gene with a 498 bp reaction product that was migrated with a 2% agarose gel

Table 4.8 Distribution of *cnf1* Gene

Isolate No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
cnf1 Gene	–	–	+	+	+	+	+	+	–	+	+	+	+	–	–

Thirty-percent of the isolates have the *cnf1* gene region, which leads to pyelonephritis and kidney invasion. Specific areas of these genes play a crucial role in the inflammatory response to urinary tract infections (Bien *et al.* 2011). Previous studies indicated that *cnf1* genes were frequently found in (80-90%) of the isolates (Foxman *et al.* 1995, Blanco *et*

al. 1997). Birošová *et al.* (2004) studied the prevalence of the *cnf1* gene in *E. coli* isolated from different sources: urine, vagina, and rectum, his study found that the prevalence of the gene in *E. coli* isolated from urine was 80%, while the prevalence rate in *E. coli* isolated from the vagina was 95.7%, while the rectum the prevalence of the gene in bacteria isolated from it was 52%p. Aazam *et al.* (2012) showed that the presence of cnf virulence gene were 50.4 %. Oliveira *et al.* (2011) from Brazil reported that the prevalence of *cnf1* was 18%.

4.2.3 Molecular detection based on the 16S ribosomal RNA region

The 16srRNA gene was investigated for 12 samples (4 for each bacteria) to confirm the bacteria and send the products of the polymerase chain reaction of the gene to study the nucleotide sequence (Figure 4.7).

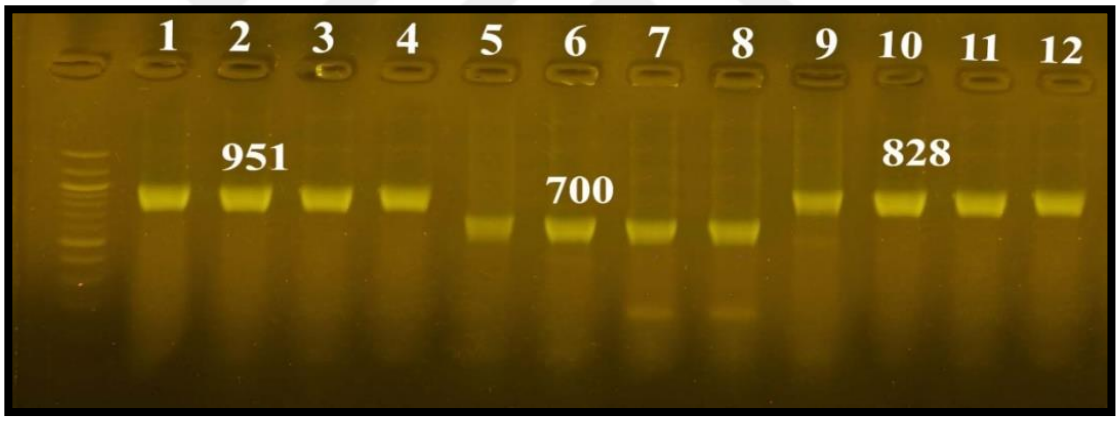


Figure 4.7 PCR product of the *cnf1* gene with a 951 bp reaction product for *S.aureus*, 700 bp for *P.mirabilis*, and 828 bp for *E. coli* that was migrated with a 2% agarose gel

4.2.4 Gene sequencing

The PCR products of 16S ribosomal RNA were sent for nucleotide sequencing, six samples were sent for nucleotide sequence analysis (two samples for each bacteria). Then the nucleotide sequencing results were analyzed by NCBI website using Optimize for highly similar sequences.

The nucleotide sequence products showed 902/934; (97%) and 924/951 (97%), respectively, identities with 16S ribosomal RNA of *S. aureus* strain WBG8287, sequence numbered CP070986.1, and the results show the presence of Gaps 4/934 and 7/951. The other strain showed identities 16S ribosomal RNA of *S. aureus* strain WBG8287 (Figure 4.8) (Figure 4.9).

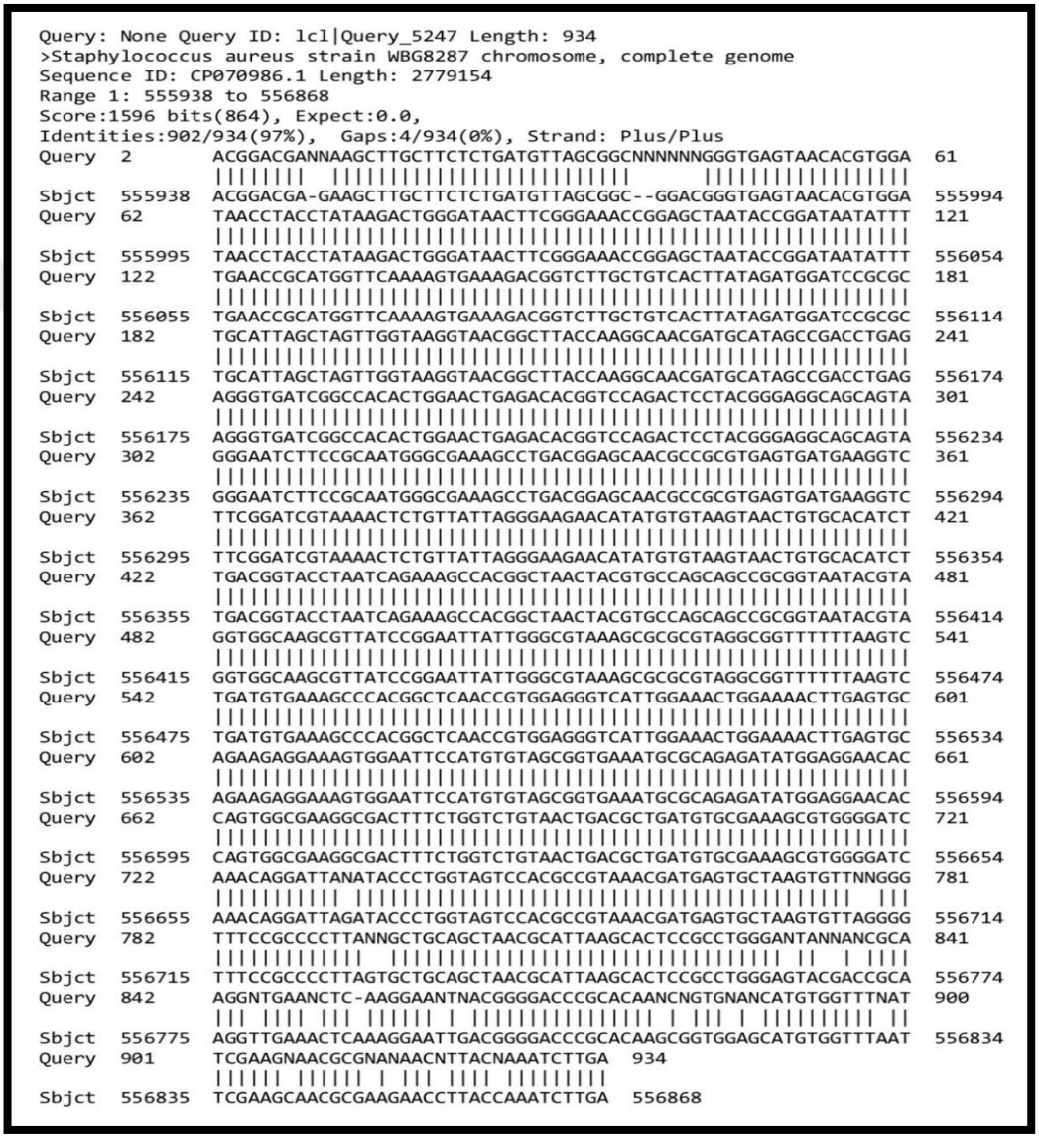
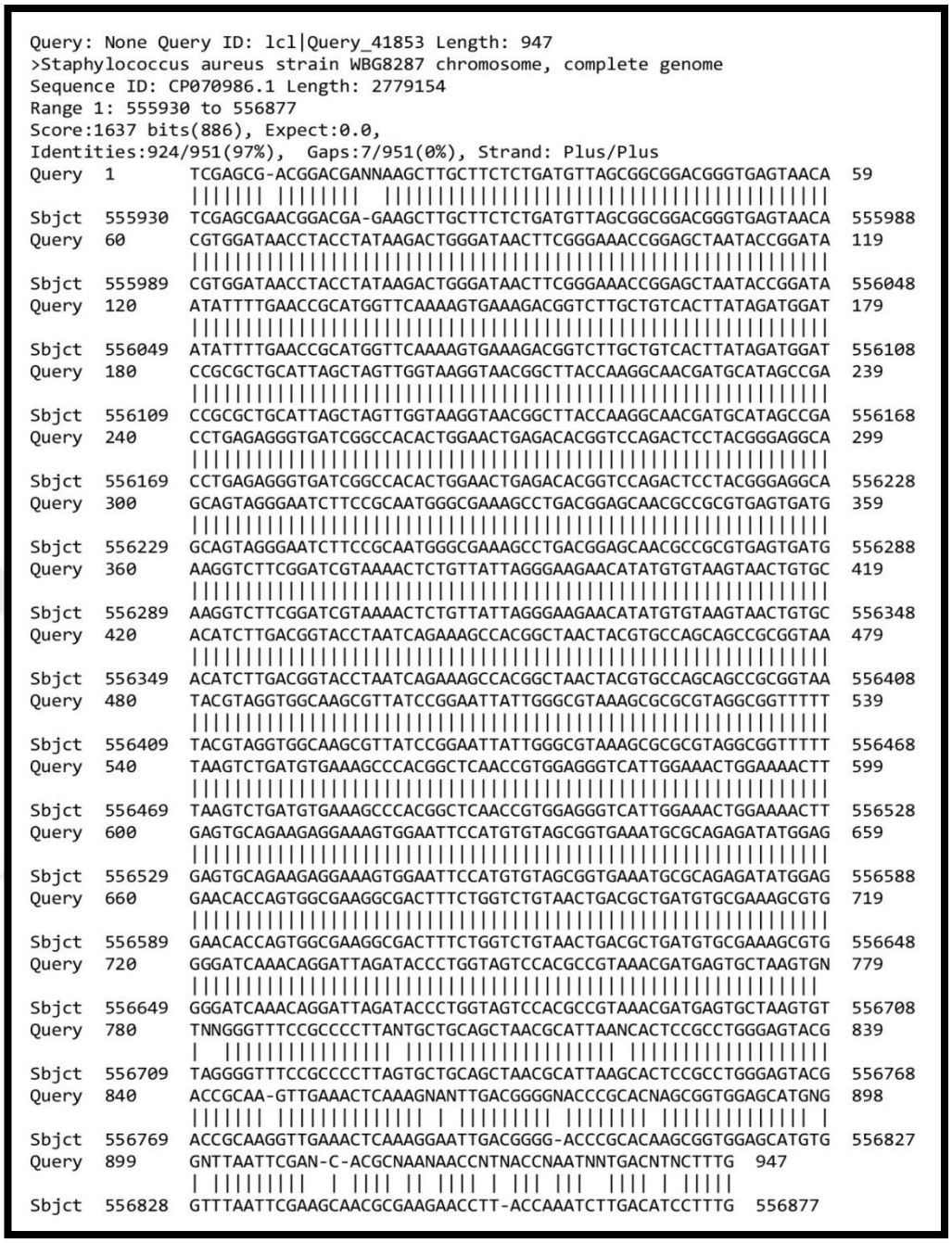


Figure 4.8 Sequence of PCR products of 16S ribosomal RNA of *S. aureus*



```
Query: None Query ID: lcl|Query_41853 Length: 947
>Staphylococcus aureus strain WBG8287 chromosome, complete genome
Sequence ID: CP070986.1 Length: 2779154
Range 1: 555930 to 556877
Score:1637 bits(886), Expect:0.0,
Identities:924/951(97%),  Gaps:7/951(0%), Strand: Plus/Plus
Query  1       TCGAGCG-ACGGACGANNAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACA  59
               ||||||| ||||||||  ||||||||||||||||||||||||||||||||||||||||||
Sbjct  555930  TCGAGCGAACGGACGA-GAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACA  555988
Query  60      CGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATA  119
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555989  CGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATA  556048
Query  120     ATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGAT  179
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556049  ATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGAT  556108
Query  180     CCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGA  239
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556109  CCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGA  556168
Query  240     CCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA  299
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556169  CCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA  556228
Query  300     GCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATG  359
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556229  GCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATG  556288
Query  360     AAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGC  419
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Sbjct  556289  AAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGC  556348
Query  420     ACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAA  479
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556349  ACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAA  556408
Query  480     TACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTT  539
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Sbjct  556409  TACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTT  556468
Query  540     TAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT  599
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Sbjct  556469  TAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT  556528
Query  600     GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAG  659
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556529  GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAG  556588
Query  660     GAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTG  719
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Query  720     GGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGN  779
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556649  GGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGT  556708
Query  780     TNNGGGTTTCCGCCCCTTANTGCTGCAGCTAACGCATTAANCACTCCGCCTGGGAGTACG  839
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Sbjct  556709  TAGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGAGTACG  556768
Query  840     ACCGCAA-GTTGAAACTCAAAGNANTTGACGGGGNACCCGCACNAGCGGTGGAGCATGNG  898
               ||||||| ||||||||||||| | ||||||||| |||||||| ||||||||||||||| |
Sbjct  556769  ACCGCAAGGTTGAAACTCAAAGGAATTGACGGGG-ACCCGCACAAGCGGTGGAGCATGTG  556827
Query  899     GNTTAATTCGAN-C-ACGCNAANAACCNTNACCNAATNNTGACNTNCTTTG  947
               | |||||||||  | |||| || |||| | ||| |||  |||| | |||||
Sbjct  556828  GTTTAATTCGAAGCAACGCGAAGAACCTT-ACCAAATCTTGACATCCTTTG  556877
```
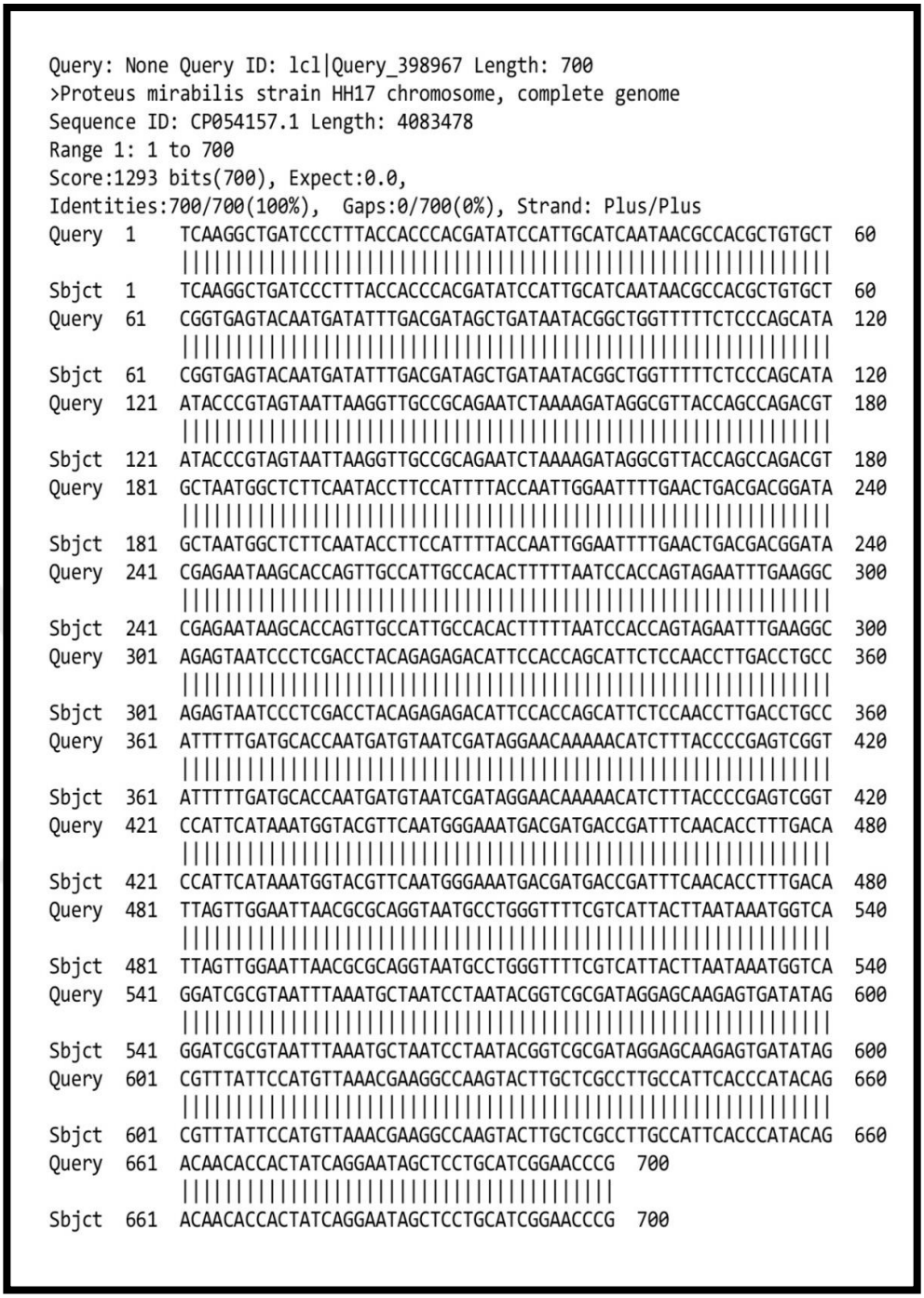

Figure 4.9 Sequence of PCR products of 16S ribosomal RNA of *S. aureus*

As for *Proteus* bacteria, the nucleotide sequence analysis found that it is 100% identical with *P. mirabilis* strain HH17. The other strain was identical 100% with *P. mirabilis* strain T60 (Figure 4.10) (Figure 4.11).



```
Query: None Query ID: lcl|Query_398967 Length: 700
>Proteus mirabilis strain HH17 chromosome, complete genome
Sequence ID: CP054157.1 Length: 4083478
Range 1: 1 to 700
Score:1293 bits(700), Expect:0.0,
Identities:700/700(100%),  Gaps:0/700(0%), Strand: Plus/Plus
Query  1    TCAAGGCTGATCCCTTTACCACCCACGATATCCATTGCATCAATAACGCCACGCTGTGCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCAAGGCTGATCCCTTTACCACCCACGATATCCATTGCATCAATAACGCCACGCTGTGCT  60
Query  61   CGGTGAGTACAATGATATTTGACGATAGCTGATAATACGGCTGGTTTTTCTCCCAGCATA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CGGTGAGTACAATGATATTTGACGATAGCTGATAATACGGCTGGTTTTTCTCCCAGCATA  120
Query  121  ATACCCGTAGTAATTAAGGTTGCCGCAGAATCTAAAAGATAGGCGTTACCAGCCAGACGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ATACCCGTAGTAATTAAGGTTGCCGCAGAATCTAAAAGATAGGCGTTACCAGCCAGACGT  180
Query  181  GCTAATGGCTCTTCAATACCTTCCATTTTACCAATTGGAATTTTGAACTGACGACGGATA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GCTAATGGCTCTTCAATACCTTCCATTTTACCAATTGGAATTTTGAACTGACGACGGATA  240
Query  241  CGAGAATAAGCACCAGTTGCCATTGCCACACTTTTTAATCCACCAGTAGAATTTGAAGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CGAGAATAAGCACCAGTTGCCATTGCCACACTTTTTAATCCACCAGTAGAATTTGAAGGC  300
Query  301  AGAGTAATCCCTCGACCTACAGAGAGACATTCCACCAGCATTCTCCAACCTTGACCTGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AGAGTAATCCCTCGACCTACAGAGAGACATTCCACCAGCATTCTCCAACCTTGACCTGCC  360
Query  361  ATTTTTGATGCACCAATGATGTAATCGATAGGAACAAAAACATCTTTACCCCGAGTCGGT  420
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Sbjct  361  ATTTTTGATGCACCAATGATGTAATCGATAGGAACAAAAACATCTTTACCCCGAGTCGGT  420
Query  421  CCATTCATAAATGGTACGTTCAATGGGAAATGACGATGACCGATTTCAACACCTTTGACA  480
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Sbjct  421  CCATTCATAAATGGTACGTTCAATGGGAAATGACGATGACCGATTTCAACACCTTTGACA  480
Query  481  TTAGTTGGAATTAACGCGCAGGTAATGCCTGGGTTTTCGTCATTACTTAATAAATGGTCA  540
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Query  541  GGATCGCGTAATTTAAATGCTAATCCTAATACGGTCGCGATAGGAGCAAGAGTGATATAG  600
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Query  601  CGTTTATTCCATGTTAAACGAAGGCCAAGTACTTGCTCGCCTTGCCATTCACCCATACAG  660
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Sbjct  601  CGTTTATTCCATGTTAAACGAAGGCCAAGTACTTGCTCGCCTTGCCATTCACCCATACAG  660
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```
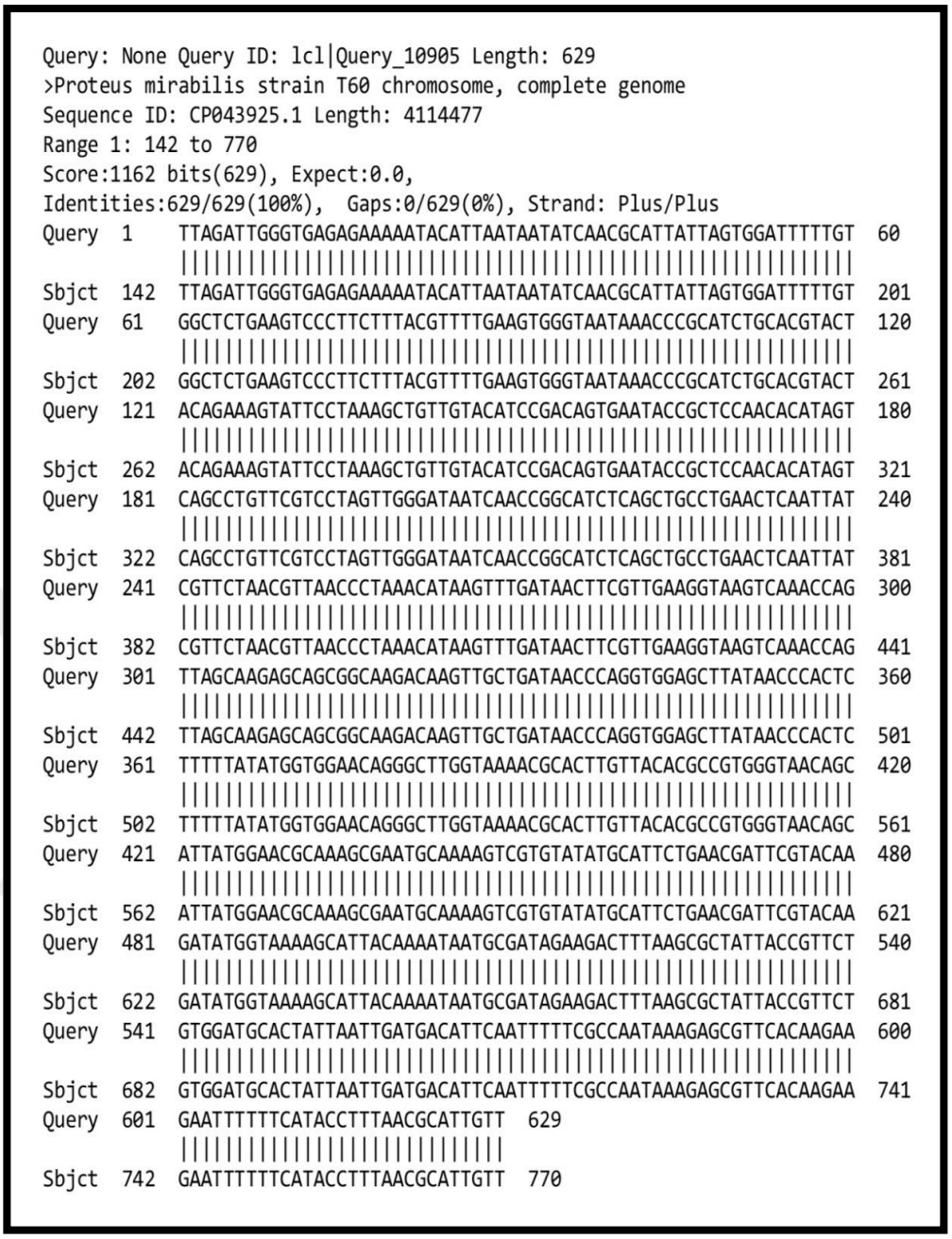

Figure 4.10 Sequence of PCR products of 16S ribosomal RNA of *P. mirabilis*



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>Proteus mirabilis strain T60 chromosome, complete genome
Sequence ID: CP043925.1 Length: 4114477
Range 1: 142 to 770
Score:1162 bits(629), Expect:0.0,
Identities:629/629(100%),  Gaps:0/629(0%), Strand: Plus/Plus
Query  1    TTAGATTGGGTGAGAGAAAAATACATTAATAATATCAACGCATTATTAGTGGATTTTTGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142  TTAGATTGGGTGAGAGAAAAATACATTAATAATATCAACGCATTATTAGTGGATTTTTGT  201
Query  61   GGCTCTGAAGTCCCTTCTTTACGTTTTGAAGTGGGTAATAAACCCGCATCTGCACGTACT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202  GGCTCTGAAGTCCCTTCTTTACGTTTTGAAGTGGGTAATAAACCCGCATCTGCACGTACT  261
Query  121  ACAGAAAGTATTCCTAAAGCTGTTGTACATCCGACAGTGAATACCGCTCCAACACATAGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  ACAGAAAGTATTCCTAAAGCTGTTGTACATCCGACAGTGAATACCGCTCCAACACATAGT  321
Query  181  CAGCCTGTTCGTCCTAGTTGGGATAATCAACCGGCATCTCAGCTGCCTGAACTCAATTAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  CAGCCTGTTCGTCCTAGTTGGGATAATCAACCGGCATCTCAGCTGCCTGAACTCAATTAT  381
Query  241  CGTTCTAACGTTAACCCTAAACATAAGTTTGATAACTTCGTTGAAGGTAAGTCAAACCAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  382  CGTTCTAACGTTAACCCTAAACATAAGTTTGATAACTTCGTTGAAGGTAAGTCAAACCAG  441
Query  301  TTAGCAAGAGCAGCGGCAAGACAAGTTGCTGATAACCCAGGTGGAGCTTATAACCCACTC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  442  TTAGCAAGAGCAGCGGCAAGACAAGTTGCTGATAACCCAGGTGGAGCTTATAACCCACTC  501
Query  361  TTTTTATATGGTGGAACAGGGCTTGGTAAAACGCACTTGTTACACGCCGTGGGTAACAGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  502  TTTTTATATGGTGGAACAGGGCTTGGTAAAACGCACTTGTTACACGCCGTGGGTAACAGC  561
Query  421  ATTATGGAACGCAAAGCGAATGCAAAAGTCGTGTATATGCATTCTGAACGATTCGTACAA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  562  ATTATGGAACGCAAAGCGAATGCAAAAGTCGTGTATATGCATTCTGAACGATTCGTACAA  621
Query  481  GATATGGTAAAAGCATTACAAAATAATGCGATAGAAGACTTTAAGCGCTATTACCGTTCT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  622  GATATGGTAAAAGCATTACAAAATAATGCGATAGAAGACTTTAAGCGCTATTACCGTTCT  681
Query  541  GTGGATGCACTATTAATTGATGACATTCAATTTTTCGCCAATAAAGAGCGTTCACAAGAA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  682  GTGGATGCACTATTAATTGATGACATTCAATTTTTCGCCAATAAAGAGCGTTCACAAGAA  741
Query  601  GAATTTTTTCATACCTTTAACGCATTGTT  629
            |||||||||||||||||||||||||||||
Sbjct  742  GAATTTTTTCATACCTTTAACGCATTGTT  770
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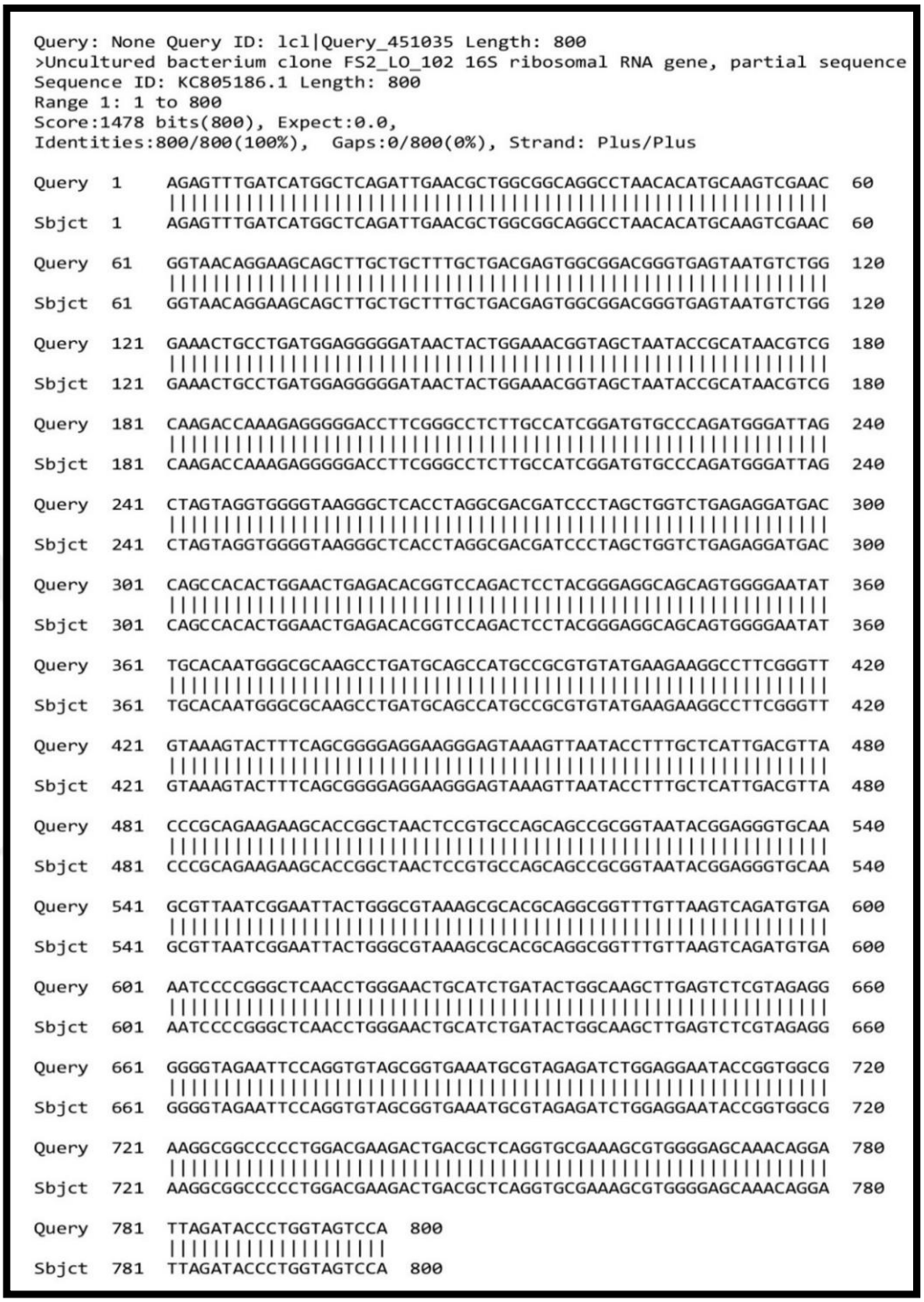
Figure 4.11 Sequence of PCR products of 16S ribosomal RNA of *P. mirabilis*

On the other hand, *E. coli* nucleotide sequence analysis found that it is 100 with 16S ribosomal RNA of *E. coli*. The other strain was 800/828 (97%) with *E. coli* strain LP50-1 with gaps of 2/828 (Figure 4.12) (Figure 4.13).



```
Query: None Query ID: lcl|Query_451035 Length: 800
>Uncultured bacterium clone FS2_LO_102 16S ribosomal RNA gene, partial sequence
Sequence ID: KC805186.1 Length: 800
Range 1: 1 to 800
Score:1478 bits(800), Expect:0.0,
Identities:800/800(100%),  Gaps:0/800(0%), Strand: Plus/Plus

Query  1    AGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAAC  60
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Sbjct  1    AGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAAC  60

Query  61   GGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGG  120
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Sbjct  61   GGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGG  120

Query  121  GAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCG  180
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Sbjct  121  GAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCG  180

Query  181  CAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAG  240

Query  241  CTAGTAGGTGGGGTAAGGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CTAGTAGGTGGGGTAAGGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGAC  300

Query  301  CAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT  360

Query  361  TGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTT  420

Query  421  GTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTA  480
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Sbjct  481  CCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA  540

Query  541  GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGA  600
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Sbjct  601  AATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGG  660

Query  661  GGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG  720
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Sbjct  661  GGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG  720

Query  721  AAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGA  780
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Query  781  TTAGATACCCTGGTAGTCCA  800
            ||||||||||||||||||||
Sbjct  781  TTAGATACCCTGGTAGTCCA  800
```

Figure 4.12 Sequence of PCR products of 16S ribosomal RNA of *E. coli*

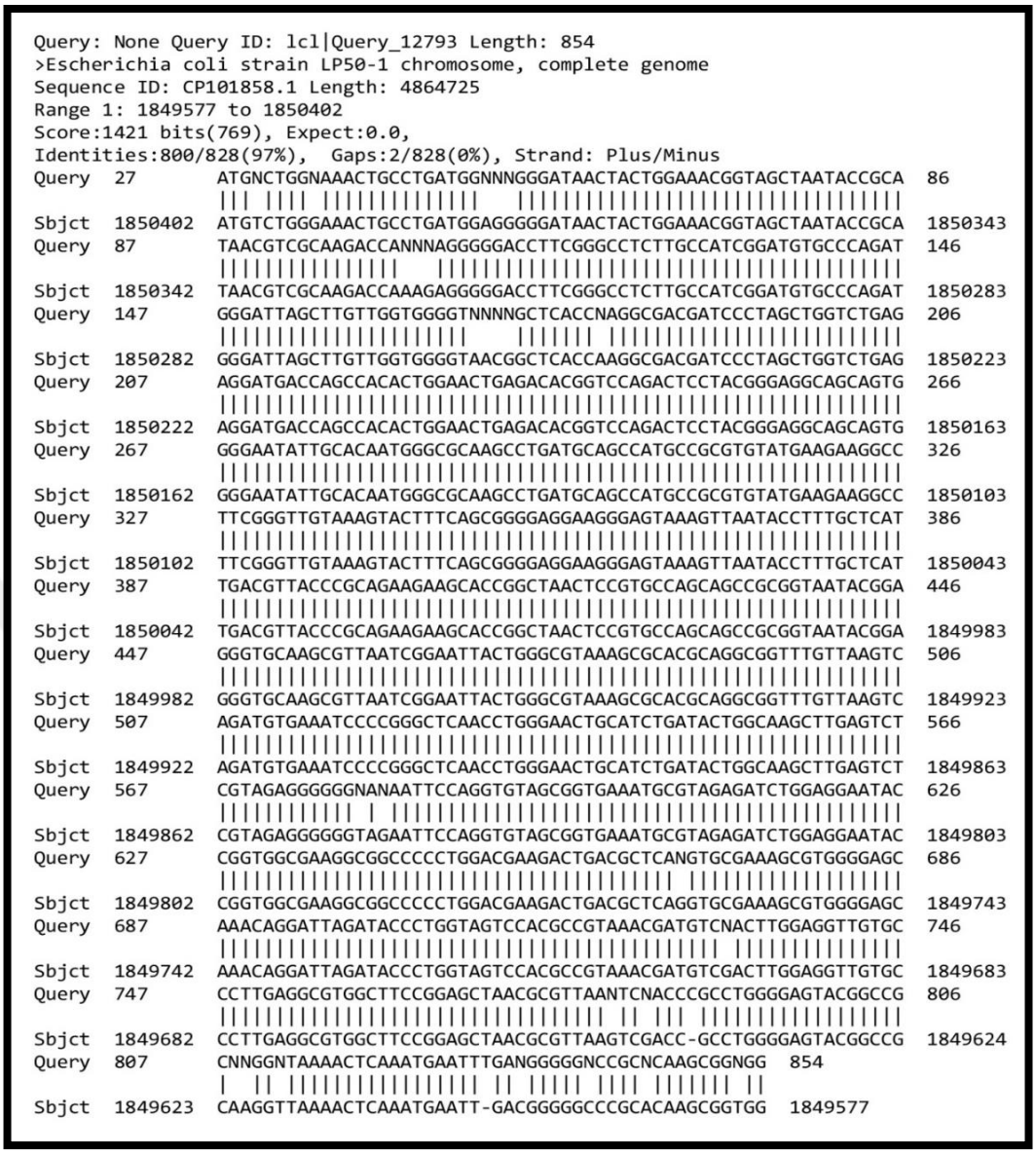

Figure 4.13 Sequence of PCR products of 16S ribosomal RNA of *E. coli*

Since the introduction of high-throughput sequencing, PCR-amplified 16S sequences have often been grouped according to similarity to produce operational taxonomic units (OTUs). Representative OTU sequences have been compared with reference databases to determine probable taxonomy. Although useful and effective, this use of 16S has required some assumptions, such as the now-historical notion that sequences with greater than 95% similarity indicate the same genus and greater than 97% identity represent the same species (Johnson *et al.* 2019) In our current study, the results of the nucleotide sequence analysis of the 16srRNA gene showed a match of +97%, which confirms that the bacteria belong to the same species.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- This study concludes that the bacteria *E. coli*, *S. aureus* and *P. mirabilis* mostly cause urinary tract infections.
- The highest rate of bacterial infection was among females.
- The infection was higher at the age of 30-40 years.
- We conclude that the antibiotics Amikacin, Imipenem and Nitrofurantoin are the most active role in eliminating urinary tract infections.
- Molecular studies are very important in detecting bacteria on a genetic level.
- The resistance possessed by bacteria was the highest percentage in rsbA gene.
- We conclude that molecular study is important in identifying the resistance genes possessed by studied bacteria after comparing the 16 S RNA sequence with what exists in the NCBI.

5.2 Recommendations

- Taking the right precautions might help lower UTI rates. Patients in the hospital, those with genitourinary tract anomalies, those with indwelling catheters, those with diabetes, women, and married couples are all prime candidates for routine UTI screening. The expense of preventing urinary tract infections can be reduced if these checks are implemented on a regular basis.
- The future adjustment of the use of antimicrobial medicines requires continuous monitoring of trends in resistance patterns of bacteria that cause UTIs.
- In the future, it will be important to recognize a person's individual susceptibility to UTIs due to their genes and provide care accordingly. Infection risk in urinary tract infections (UTIs) may be affected by heritable differences in genes involved in regulating the innate immune response.

REFERENCES

Aazam, K., Hassan, M. and Mahbobe, M. 2012. Detection of uropathogenic Escherichia coli virulence factors in patients with urinary tract infections in Iran. African Journal of Microbiology Research, 6(39): 6811-6816.

Abraham, S. N. and Miao, Y. 2015. The nature of immune responses to urinary tract infections. Nature Reviews Immunology, 15(10): 655-663.

Alabsi, M. S., Ghazal, A., Sabry, S. A. and Alasaly, M. M. 2014. Association of some virulence genes with antibiotic resistance among uropathogenic Escherichia coli isolated from urinary tract infection patients in Alexandria, Egypt: a hospital-based study. Journal of Global Antimicrobial Resistance, 2(2): 83-86.

Algammal, A. M., Hashem, H. R., Alfifi, K. J., Hetta, H. F., Sheraba, N. S., Ramadan, H. and El-Tarabili, R. M. 2021. atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-Proteus mirabilis. Scientific Reports, 11(1): 1-15.

Amiri, H. S., Ranjbar, R., Sohrabi, N. and Khamesipour, F. 2017. Molecular typing of uropathogenic Escherichia coli strains isolated from patients by REP-PCR. Modern Technologies in Medicine, 9(3): 71-75.

Amiri, M., Lavasani, Z., Norouzirad, R., Najibpour, R., Mohamadpour, M., Nikpoor, A. R. and Marzouni, H. Z. 2015. Prevalence of urinary tract infection among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful city, Iran, 2012-2013. Iranian Red Crescent Medical Journal, 17(8): 5-17.

Ardakani, M. A. and Ranjbar, R. 2016. Molecular typing of uropathogenic E. coli strains by the ERIC-PCR method. Electronic Physician, 8(4): 2291.

Arjomandzadegan, M., Owlia, P., Ranjbar, R., Farazi, A., Sofian, M., Sadrnia, M. and Titov, L. 2011. Prevalence of mutations at codon 463 of katG gene in MDR and XDR clinical isolates of Mycobacterium tuberculosis in Belarus and application of the method in rapid diagnosis. Acta Microbiologica Immunologica Hungarica, 58(1): 51-63.

Awean, G. Z., Salameh, K., Elmohamed, H., Alshmayt, H. and Omer, M. R. B. 2019. Prevalence of ESBL urinary tract infection in children. Journal Pediatr Child Health, 2(1): 004-7.

Badi, S. A., Nowroozi, J. and Sepahi, A. A. 2014. Detection luxS, qseC and rsbA genes' bands in Proteus mirabilis and Escherichia coli isolated from urinary tract infections. Pajoohandeh Journal, 19(3): 142-147.

Balachandran, M., Bemis, D. A. and Kania, S. A. 2018. Expression and function of protein A in Staphylococcus pseudintermedius. Virulence, 9(1): 390-401.

Barnich, N. and Darfeuille-Michaud, A. 2010. Abnormal CEACAM6 expression in Crohn disease patients favors gut colonization and inflammation by adherent-invasive E. coli. Virulence, 1(4): 281-282.

Bien, J., Sokolova, O. and Bozko, P. 2011. Characterization of virulence factors of Staphylococcus aureus: novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. Journal of Pathogens, 2011.

Birošová, E., Siegfried, L., Kmet'ova, M., Makara, A., Ostró, A., Grešová, A. and Valanský, L. 2004. Detection of virulence factors in α-haemolytic Escherichia coli strains isolated from various clinical materials. Clinical Microbiology and Infection, 10(6): 569-573.

Blair, J., Webber, M. A., Baylay, A. J., Ogbolu, D. O. and Piddock, L. J. 2015. Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology, 13(1): 42-51.

Blanco, M., Blanco, J. E., Rodrıguez, E., Abalia, I., Alonso, M. P. and Blanco, J. 1997. Detection of virulence genes in uropathogenic Escherichia coli by polymerase chain reaction (PCR): comparison with results obtained using phenotypic methods. Journal of Microbiological Methods, 31(1-2): 37-43.

Chwa, A., Kavanagh, K., Linnebur, S. A. and Fixen, D. R. 2019. Evaluation of methenamine for urinary tract infection prevention in older adults: a review of the evidence. Therapeutic Advances in Drug Safety, 10: 204.

Clermont, O., Christenson, J. K., Denamur, E. and Gordon, D. M. 2013. The C lermont E scherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental Microbiology Reports, 5(1): 58-65.

CLSI, Clinical and Laboratory Standards Institute. 2019. Performance standards for antimicrobial susceptibility testing.29th ed. CLSI Supplement, 39(1):42-45.

Colgan, R. and Williams, M. 2011. Diagnosis and treatment of acute uncomplicated cystitis. American Family Physician, 84(7): 771-776.

Conceição, R. A., Ludovico, M. S., Andrade, C. G. T. J. and Yano, T. 2012. Human sepsis-associated Escherichia coli (SEPEC) is able to adhere to and invade kidney epithelial cells in culture. Brazilian Journal of Medical and Biological Research, 45: 417-424.

Cosandey, A., Boss, R., Luini, M., Artursson, K., Bardiau, M., Breitenwieser, F. and Graber, H. U. 2016. Staphylococcus aureus genotype B and other genotypes isolated from cow milk in European countries. Journal of Dairy Science, 99(1): 529-540.

Crain, E. F. and Gershel, J. C. 1990. Urinary tract infections in febrile infants younger than 8 weeks of age. Pediatrics, 86(3): 363-367.

Dasgupta, R., Kavia, R. B. and Fowler, C. J. 2007. Cerebral mechanisms and voiding function. BJU International, 99(4): 731-734.

Davies, J. and Davies, D. 2010. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74(3): 417-433.

De Oliveira, W. D., Barboza, M. G. L., Faustino, G., Inagaki, W. T. Y., Sanches, M. S., Kobayashi, R. K. T. and Rocha, S. P. D. 2021. Virulence, resistance and clonality of Proteus mirabilis isolated from patients with community-acquired urinary tract infection (CA-UTI) in Brazil. Microbial Pathogenesis, 152: 104642.

Deepthi, B., Gopika, K. T. and Samyuktha, K. R. 2017. Nosocomial urinary tract infections. Skin Diseases & Skin Care, 2: 1.

Doyev, R., Ben-Shalom, E. and Megged, O. 2020. The predictive utility of prior positive urine culture in children with recurrent urinary tract infections. European Journal of Pediatrics, 179(3): 415-421.

Drzewiecka, D. 2016. Significance and roles of Proteus spp. bacteria in natural environments. Microbial Ecology, 72(4): 741-758.

Ebmeyer, S., Kristiansson, E. and Larsson, D. G. 2021. A framework for identifying the recent origins of mobile antibiotic resistance genes. Communications Biology, 4(1): 1-10.

Ekwealor, P. A., Ugwu, M. C., Ezeobi, I., Amalukwe, G., Ugwu, B. C., Okezie, U. and Esimone, C. 2016. Antimicrobial evaluation of bacterial isolates from urine specimen of patients with complaints of urinary tract infections in Awka, Nigeria. International Journal of Microbiology, 13.

El Seedy, F. R., Samy, A. A., Salam, H. S. H., Khairy, E. A. and Koraney, A. A. 2017. Polymerase chain reaction detection of genes responsible for multiple antibiotic resistance Staphylococcus aureus isolated from food of animal origin in Egypt. Veterinary World, 10 (10): 1205.

El-Tawab, A., Ammar, A. M., Hofy, F. I., Mohamed, S. R. and Abubakr, H. S. 2017. Studies on virulence genes of Staphylococcus aureus isolated from mastitic cows. Benha Journal of Applied Sciences, 2(3): 89-93.

Erjavec, M. S., Rijavec, M., Križan-Hergouth, V., Fruth, A. and Žgur-Bertok, D. 2007. Chloramphenicol-and tetracycline-resistant uropathogenic Escherichia coli (UPEC) exhibit reduced virulence potential. International Journal of Antimicrobial Agents, 30(5): 436-442.

Fair, R. J. and Tor, Y. 2014. Antibiotics and bacterial resistance in the 21st century. Perspectives in Medicinal Chemistry, 6: 14459.

Flores-Mireles, A. L., Walker, J. N., Caparon, M. and Hultgren, S. J. 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology, 13(5): 269-284.

Foxman, B., Zhang, L., Palin, K., Tallman, P. and Marrs, C. F. 1995. Bacterial virulence characteristics of Escherichia coli isolates from first-time urinary tract infection. Journal of Infectious Diseases, 171(6): 1514-1521.

Franchetti, L., Schumann, D. M., Tamm, M., Jahn, K. and Stolz, D. 2020. Multiplex bacterial polymerase chain reaction in a cohort of patients with pleural effusion. BMC Infectious Diseases, 20(1): 1-10.

Granato, P. A. and Granato, P. A. 2011. Laboratory Manual and Workbook in Microbiology: Applications to Patient Care. Belitung Nursing Journal, 7(1): 24-30.

Griebling, T. L. 2005. Urologic diseases in America project: trends in resource use for urinary tract infections in women. The Journal of Urology, 173(4): 1281-1287.

Handley, M. A., Reingold, A. L., Shiboski, S. and Padian, N. S. 2002. Incidence of acute urinary tract infection in young women and use of male condoms with and without nonoxynol-9 spermicides. Epidemiology, 431-436.

Hase, K., Kawano, K., Nochi, T., Pontes, G. S., Fukuda, S., Ebisawa, M. and Ohno, H. 2009. Uptake through glycoprotein 2 of fimH+ bacteria by M cells initiates mucosal immune response. Nature, 462(7270): 226-230.

Hojati, Z., Molaie, R. and Gholipour, A. 2015. The fimH gene in uropathogenic Escherichia coli strains isolated from patient with urinary tract infection. Jundishapur Journal of Microbiology, 8(2): S1.

Hooton, T. M., Bradley, S. F., Cardenas, D. D., Colgan, R., Geerlings, S. E., Rice, J. C. and Nicolle, L. E. 2010. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clinical Infectious Diseases, 50(5): 625-663.

Hunt, K. J., Walsh, B. M., Voegeli, D. and Roberts, H. C. 2010. Inflammation in aging part 2: implications for the health of older people and recommendations for nursing practice. Biological Research for Nursing, 11(3): 253-260.

Hussein, N. R., Daniel, S., Salim, K. and Assafi, M. S. 2017. Urinary tract infections and antibiotic sensitivity patterns among women referred to Azadi teaching hospital, Duhok, Iraq. Avicenna Journal of Clinical Microbiology and Infection, 5(2): 27-30.

Iregbu, K. C. and Nwajiobi-Princewill, P. I. 2013. Urinary tract infections in a tertiary hospital in Abuja, Nigeria. African Journal of Clinical and Experimental Microbiology, 14(3): 169-173.

Jayanthi, G. and Shariff, M. 2015. Phenotypic and molecular characterization of clinical isolated of drug resistant Pseudomonas aeruginosa. Journal of Medical Microbiology, 4(6):352-354.

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L. and Weinstock, G. M. 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nature Communications, 10(1): 1-11.

Joni, F. H. 2018. Isolation and diagnosis of bacteria causing urinary tract infection in pregnant and non pregnant females with diabetes mellitus type2. Al-Mustansiriyah Journal of Science, 29(4): 23-26.

Kaczmarek, A., Budzyńska, A. and Gospodarek, E. 2012. Prevalence of genes encoding virulence factors among Escherichia coli with K1 antigen and non-K1 E. coli strains. Journal of Medical Microbiology, 61(10): 1360-1365.

Khudhur Mohammad, T., Hamood, H. Q. and Naser, S. H. 2018. Studies on bacteriological and biochemical tests of urinary tract infections in some Iraqi

patients undergoing hemodialysis due to renal failure and chronic kidney diseases. Iraqi National Journal of Chemistry, 18(3)13-27.

Kirtikliene, T., Naugzemys, D., Steponkiene, A., Bogdevic, R., Vizuje, G., Zvingila, D. and Kuisiene, N. 2019. Evaluation of the inter-and intrahospital spread of multidrug resistant gram-negative bacteria in Lithuanian hospitals. Microbial Drug Resistance, 25(3): 326-335.

Koçak, M., Büyükkaragöz, B., Çelebi Tayfur, A., Çaltik, A., Köksoy, A. Y., Çizmeci, Z. and Günbey, S. 2016. Causative pathogens and antibiotic resistance in children hospitalized for urinary tract infection. Pediatrics International, 58(6): 467-471.

Kulkarni, S. R., Peerapur, B. V. and Sailesh, K. S. 2017. Isolation and antibiotic susceptibility pattern of Escherichia coli from urinary tract infections in a tertiary care hospital of North Eastern Karnataka. Journal of Natural Science Biology and Medicine, 8(2): 176.

Kutasy, B., Coyle, D. and Fossum, M. 2017. Urinary tract infection in children: management in the era of antibiotic resistance—a pediatric urologist's view. European Urology Focus, 3(2-3): 207-211.

Lane, D. R. and Takhar, S. S. 2011. Diagnosis and management of urinary tract infection and pyelonephritis. Emergency Medicine Clinics, 29(3): 539-552.

Lidefelt, K. J., Bollgren, I. and Nord, C. E. 1991. Changes in periurethral microflora after antimicrobial drugs. Archives of Disease in Childhood, 66(6): 683-685.

Lv, P., Hao, G., Cao, Y., Cui, L., Wang, G. and Sun, S. 2022. Detection of Carbapenem resistance of Proteus mirabilis strains isolated from Foxes, Raccoons and Minks in China. Biology, 11(2): 292.

Maceae, S., Shortliff, N. and Balkrial, M. 1995. Infection of the genitourinary tract. Smiths Grneral urology along medical book, 14th MC Grow. Middle East Edition, 237-246.

Magyar, A., Köves, B., Nagy, K., Dobák, A., Arthanareeswaran, V. K. A., Bálint, P. and Tenke, P. 2017. Spectrum and antibiotic resistance of uropathogens between 2004 and 2015 in a tertiary care hospital in Hungary. Journal of Medical Microbiology, 66(6): 788-797.

Manos, J. and Belas, R. 2006. The genera proteus, providencia, and morganella. Prokaryotes, 6: 245-269.

Martin, R. M. and Bachman, M. A. 2018. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Frontiers in Cellular and Infection Microbiology, 8: 4.

Matic, I., Radman, M., Taddei, F., Picard, B., Doit, C., Bingen, E. and Elion, J. 1997. Highly variable mutation rates in commensal and pathogenic Escherichia coli. Science, 277(5333): 1833-1834.

Micenková, L., Beňová, A., Frankovičová, L., Bosák, J., Vrba, M., Ševčíková, A. and Šmajs, D. 2017. Human Escherichia coli isolates from hemocultures: Septicemia linked to urogenital tract infections is caused by isolates harboring more virulence genes than bacteraemia linked to other conditions. International Journal of Medical Microbiology, 307(3): 182-189.

Michaud, J. E., Kim, K. S., Harty, W., Kasprenski, M. and Wang, M. H. 2017. Cytotoxic Necrotizing Factor-1 (CNF1) does not promote E. coli infection in a murine model of ascending pyelonephritis. BMC Microbiology, 17(1): 1-8.

Mladin, C., Usein, C. R., Chifiriuc, M. C., Palade, A., Slavu, C. L., Negut, M. and Damian, M. 2009. Genetic analysis of virulence and pathogenicity features of uropathogenic Escherichia coli isolated from patients with neurogenic bladder. Romanian Biotechnological Letters, 14(6): 4906-4911.

Mohammed, H. Q. and Shehab, A. T. 2019. Molecular study of bacterial infection from hemodialysis patients in Wasit centre, Journal of Pharmaceutical Sciences and Research, 12(3): 365-374.

Mondot, S., Lepage, P., Seksik, P., Allez, M., Tréton, X., Bouhnik, Y. and Marteau, P. 2016. Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. Gut, 65(6): 954-962.

Moosavian, M., Shoja, S., Rostami, S., Torabipour, M. and Farshadzadeh, Z. 2016. Identification of erm and msrA genes in inducible clindamycin resistance of clinical isolates of staphylococcus aureus by polymerase chain reaction and D-test in Iran. International Journal of Infectious Diseases, 45: 104.

Morandi, S., Brasca, M., Lodi, R., Brusetti, L., Andrighetto, C. and Lombardi, A. 2010. Biochemical profiles, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and multilocus variable number tandem

repeat analysis (MLVA) for typing Staphylococcus aureus isolated from dairy products. Research in Veterinary Science, 88(3): 427-435.

Moreno, E., Prats, G., Sabaté, M., Pérez, T., Johnson, J. R. and Andreu, A. 2006. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic Escherichia coli. Journal of Antimicrobial Chemotherapy, 57(2): 204-211.

Mukherjee, P. K. 2019. Quality control and evaluation of herbal drugs: Evaluating natural products and traditional medicine. Elsevier, 255-321.

Munson, E. and Carroll, K. C. 2021. Summary of novel bacterial isolates derived from human clinical specimens and nomenclature revisions published in 2018 and 2019. Journal of Clinical Microbiology, 59(2): e01309-20.

Mysorekar, I. U., Mulvey, M. A., Hultgren, S. J. and Gordon, J. I. 2002. Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic Escherichia coli* 210. Journal of Biological Chemistry, 277(9): 7412-7419.

Nagamatsu, K., Hannan, T. J., Guest, R. L., Kostakioti, M., Hadjifrangiskou, M., Binkley, J. and Hultgren, S. J. 2015. Dysregulation of Escherichia coli α-hemolysin expression alters the course of acute and persistent urinary tract infection. Proceedings of the National Academy of Sciences, 112(8): E871-E880.

Naimi, H. M., Rasekh, H., Noori, A. Z. and Bahaduri, M. A. 2017. Determination of antimicrobial susceptibility patterns in Staphylococcus aureus strains recovered from patients at two main health facilities in Kabul, Afghanistan. BMC Infectious Diseases, 17(1):1-7.

Nguyen, H. T. 2004. Bacterial infections of the genitourinary tract. Tanagho EA, McAninch JW. Smith's General Urology. Mcgraw-Hill, 16: 203-227.

Ninfa, A. J., Ballou, D. P. and Benore, M. 2009. Fundamental laboratory approaches for biochemistry and biotechnology. John Wiley & Sons, 480 page, New York.

Ochada, N. S., Nasiru, I. A., Thairu, Y., Okanlowan, M. B. and Abdulakeem, Y. O. 2015. Antimicrobial susceptibility pattern of urinary pathogens isolated from two tertiary hospitals in Southwestern Nigeria. African Journal of Clinical and Experimental Microbiology, 16(1): 12-22.

Ohno, H. and Hase, K. 2010. Glycoprotein 2 (GP2) grabbing the fimH+ bacteria into m cells for mucosal immunity. Gut Microbes, 1(6): 407-410.

Oikonomou, K. G. and Alhaddad, A. 2017. Isolation rate and clinical significance of uropathogens in positive urine cultures of hemodialysis patients. Journal of Global Infectious Diseases, 9(2): 56.

Oliveira, F. A., Paludo, K. S., Arend, L. N. V. S., Farah, S. M. S. S., Pedrosa, F. O., Souza, E. M. and Fadel-Picheth, C. M. 2011. Virulence characteristics and antimicrobial susceptibility of uropathogenic Escherichia coli strains. Genetics and Molecular Research, 10(4): 4114-25.

Owrangi, B., Masters, N., Kuballa, A., O'Dea, C., Vollmerhausen, T. L. and Katouli, M. 2018. Invasion and translocation of uropathogenic Escherichia coli isolated from urosepsis and patients with community-acquired urinary tract infection. European Journal of Clinical Microbiology and Infectious Diseases, 37(5): 833-839.

Palma, E., Tilocca, B. and Roncada, P. 2020. Antimicrobial resistance in veterinary medicine: An overview. International Journal of Molecular Sciences, 21(6): 1914.

Pathirana, H. N. K. S., De Silva, B. C. J., Wimalasena, S. H. M. P., Hossain, S. and Heo, G. J. 2018. Comparison of virulence genes in Proteus species isolated from human and pet turtle. Iranian journal of Veterinary Research, 19(1): 48.

Peyrani, P., Mandell, L., Torres, A. and Tillotson, G. S. 2019. The burden of community-acquired bacterial pneumonia in the era of antibiotic resistance. Expert Review of Respiratory Medicine, 13(2): 139-152.

Pooideh, M., Jabbarzadeh, I., Ranjbar, R. and Saifi, M. 2015. Molecular epidemiology of Mycobacterium tuberculosis isolates in 100 patients with tuberculosis using pulsed field gel electrophoresis. Jundishapur Journal of Microbiology, 8(7): 54.

Porta, A. R. and Enners, E. 2012. Determining annealing temperatures for polymerase chain reaction. The American Biology Teacher, 74(4): 256-260.

Pouladfar, G., Basiratnia, M., Anvarinejad, M., Abbasi, P., Amirmoezi, F. and Zare, S. 2017. The antibiotic susceptibility patterns of uropathogens among children with urinary tract infection in Shiraz. Medicine, 96(37) 22-45.

Raimondi, S., Righini, L., Candeliere, F., Musmeci, E., Bonvicini, F., Gentilomi, G. and Rossi, M. 2019. Antibiotic resistance, virulence factors, phenotyping, and

genotyping of E. coli isolated from the feces of healthy subjects. Microorganisms, 7(8): 251.

Ranjbar, R. E. Z. A., Pourshafie, M. R., Sadeghifard, N., Karami, A., Hamidian, M., Izadi, M. and Jonaidi, N. 2008. Molecular characterization of epidemic isolates of Vibrio cholerae O1 by arbitrarily primed PCR (AP-PCR). Iranian Journal of Public Health, 37(2): 83-87.

Ranjbar, R., Elhaghi, P. and Shokoohizadeh, L. 2017. Multilocus Sequence typing of the clinical isolates of Salmonella enterica serovar typhimurium in Tehran hospitals. Iranian. Journal of Medical Sciences, 1(2): 32.

Ranjbar, R., Haghi-Ashtiani, M., Jafari, N. J. and Abedini, M. 2009. The prevalence and antimicrobial susceptibility of bacterial uropathogens isolated from pediatric patients. Iranian Journal of Public Health, 38(2): 16.

Ranjbar, R., Hosseini, S., Zahraei-Salehi, T., Kheiri, R. and Khamesipur, F. 2016. Investigation on prevalence of Escherichia coli strains carrying virulence genes ipaH, estA, eaeA and bfpA isolated from different water sources. Asian Pacific Journal of Tropical Disease, 6: 278-283.

Ranjbar, R., Owlia, P., Saderi, H., Bameri, Z., Izadi, M., Jonaidi, N. and Morovvti, S. 2007. Isolation of clinical strains of Pseudomonas aeruginosa harboring different plasmids. Pakistan Journal of Biological Sciences, 17: 22.

Rasigade, J. P. and Vandenesch, F. 2014. Staphylococcus aureus: A pathogen with still unresolved issues. Infection, Genetics and Evolution, 21: 510-514.

Rathore, K., Joseph, B., Sharma, D. K., Gaurav, A., Sharma, S. K., Milind, M. and Singh, L. 2018. Evaluation of multiplex polymerase chain reaction as an alternative to conventional antibiotic sensitivity test. Veterinary World, 11(4): 474.

Riccabona, M. 2003. Urinary tract infections in children. Current Opinion in Urology, 13(1): 59-62.

Riley, L. W. 2014. Pandemic lineages of extraintestinal pathogenic Escherichia coli. Clinical Microbiology and Infection, 20(5): 380-390.

Rivas, L., Mellor, G. E., Gobius, K. and Fegan, N. 2015. Typing and subtyping methods for pathogenic Escherichia coli. Springer, 110 page, New York.

Rod, R. S., Trent, D. and Philip, T. 2004. Seeley's anatomy & physiology. McGraw-Hill Education, 1264 page, New York.

Rowe, T. A. and Juthani-Mehta, M. 2013. Urinary tract infection in older adults. Aging Health, 9(5): 519-528.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science, 230(4732): 1350-1354.

Salvatore, S., Salvatore, S., Cattoni, E., Siesto, G., Serati, M., Sorice, P. and Torella, M. 2011. Urinary tract infections in women. European Journal of Obstetrics and Gynecology and Reproductive Biology, 156(2): 131-136.

Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G. and Choroszy-Krol, I. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut Pathogens, 11(1): 1-16.

Schaffer, J. N., Norsworthy, A. N., Sun, T. T. and Pearson, M. M. 2016. Proteus mirabilis fimbriae-and urease-dependent clusters assemble in an extracellular niche to initiate bladder stone formation. Proceedings of the National Academy of Sciences, 113(16): 4494-4499.

Schneewind, O., Fowler, A. and Faull, K. F. 1995. Structure of the cell wall anchor of surface proteins in Staphylococcus aureus. Science, 268(5207): 103-106.

Shaikh, N., Morone, N. E., Bost, J. E. and Farrell, M. H. 2008. Prevalence of urinary tract infection in childhood: a meta-analysis. The Pediatric Infectious Disease Journal, 27(4): 302-308.

Shankar, M., Narasimhappa, S. and Madhura, N. S. 2021. Urinary tract infection in chronic kidney disease population: a clinical observational study. Cureus, 13:1.

Sharma, N., Gupta, A., Walia, G. and Bakhshi, R. 2016. Pattern of antimicrobial resistance of Escherichia coli isolates from urinary tract infection patients: A three year retrospective study. Journal of Applied Pharmaceutical Science, 6(1): 062-065.

Souza, R. B., Trevisol, D. J. and Schuelter-Trevisol, F. 2015. Bacterial sensitivity to fosfomycin in pregnant women with urinary infection. Brazilian Journal of Infectious Diseases, 19: 319-323.

Spoorenberg, V., Hulscher, M. E., Akkermans, R. P., Prins, J. M. and Geerlings, S. E. 2014. Appropriate antibiotic use for patients with urinary tract infections reduces length of hospital stay. Clinical İnfectious Diseases, 58(2):164-169.

Standring, S., Borley, N. and Collins, P. 2008. Functional anatomy of the musculoskeletal system. Gray's anatomy: the anatomical basis of clinical practice. 40th ed. Philadelphia. Churchill Livingstone Elsevier, 81-97.

Sweileh, W. M., Al-Jabi, S. W., Sa'ed, H. Z., Sawalha, A. F. and Abu-Taha, A. S. 2018. Global research output in antimicrobial resistance among uropathogens: A bibliometric analysis (2002–2016). Journal of Global Antimicrobial Resistance, 13:104-114.

Tarchouna, M., Ferjani, A., Ben-Selma, W. and Boukadida, J. 2013. Distribution of uropathogenic virulence genes in Escherichia coli isolated from patients with urinary tract infection. International Journal of Infectious Diseases, 17(6): e450-e453.

Taweel, I., Beatty, N., Duarte, A., Nix, D., Matthias, K. and Al Mohajer, M. 2018. Significance of bacteriuria in patients with end-stage renal disease on hemodialysis. Avicenna Journal of Medicine, 8(02): 51-54.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. and Fowler, V. G. 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3): 603-661.

Waglechner, N. and Wright, G. D. 2017. Antibiotic resistance: it's bad, but why isn't it worse. BMC Biology, 15(1): 1-8.

Walsh, C. and Collyns, T. 2020. Pathophysiology of urinary tract infections. Surgery Oxford, 38(4): 191-196.

Wang, J., He, L., Sha, J., Zhu, H., Huang, L., Zhu, X. and Guo, Y. 2018. Etiology and antimicrobial resistance patterns in pediatric urinary tract infection. Pediatrics International, 60(5): 418-422.

Wasmy Shahab, N., Ali, C. I. A. D. and Salih, S. M. 2018. Isolation and Identification of bacteria causing urinary tract infections in children in Kirkuk city. Tikrit Journal of Pure Science, 22(2): 8-12.

Watts, R. E., Hancock, V., Ong, C. L. Y., Vejborg, R. M., Mabbett, A. N., Totsika, M. and Schembri, M. A. 2010. Escherichia coli isolates causing asymptomatic bacteriuria in catheterized and noncatheterized individuals possess similar virulence properties. Journal of Clinical Microbiology, 48(7): 2449-2458.

Watts, R. E., Totsika, M., Challinor, V. L., Mabbett, A. N., Ulett, G. C., De Voss, J. J. and Schembri, M. A. 2012. Contribution of siderophore systems to growth and urinary tract colonization of asymptomatic bacteriuria Escherichia coli. Infection and Immunity, 80(1): 333-344.

Williams, P. L., Warwick, R. and Dyson, M. 1989. Bannister LH. Gray's Anatomy, 37: 756-8.

Wright, E. K., Kamm, M. A., Wagner, J., Teo, S. M., Cruz, P. and Hamilton, A. L. 2017. Microbial factors associated with postoperative Crohn's disease recurrence. Journal Crohns Colitis. 11: 191–203.

Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X. and Xue, L. 2018. Staphylococcus aureus isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Frontiers in Microbiology, 9: 2767

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M. and Gordon, J. I. 2012. Human gut microbiome viewed across age and geography. Nature, 486(7402): 222-227.

Yüksel, S., Öztürk, B., Kavaz, A., Özçakar, Z. B., Acar, B., Güriz, H. and Yalçınkaya, F. 2006. Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infections. International Journal of Antimicrobial Agents, 28(5): 413-416.

APPENDICES

APPENDIX 1. Laboratory devices and tools used in the study

APPENDIX 2. Chemicals used in the study

APPENDIX 3. Ready-to-use implantation circles used in the study

APPENDIX 4. Dyes used in the study

APPENDIX 5. Commercial kits for DNA assays

APPENDIX 1. Laboratory devices and tools used in the study

Apparatus	Company	Source
Autoclave	Gallankamp	England
Sensitive electronic Balance	Sartorius	Germany
Light microscope	Olympus	China
Incubator	Mennert	Germany
Centrifuge	Gallankamp	England
Vortex mixer	Griffin	Germany
Magnetic stirrer	Lab TECH	Korea south
Millipore filters (0.22)µm	Difco	USA
Hot plate	Gallankamp	England
Refrigerator	Kelon	Korea
PH-meter	Martini	USA
Eppendorf tubes	Sigma	England
Microfuge	Sony	Japan
Disposable syringes	Superestar	India
Conical flasks	BBL	USA
Disposable petri dishes	Al-Hani	USA
Test tubes	Superstar	India
Laminar flow cabinet	Labtech	Korea
Slides and caver slides	Superestar	India
Sterilized cotton swabs	SterellinLtd	England
Standard wire loop	Himedia	India
Electrophoresis system	Optima	Japan
AB DNA sequencing system DNA	Macrogen	Korea
U.V trans illuminator	San.Gabrill	USA
PCR thermal cycler	Thermo scient	USA
PCR tubes PCR	Ependorff	USA
Transport media swabs	SterellinLtd	England

APPENDIX 2. Chemicals used in the study

Chemical materials	Company	Source
H_2SO_4	BDH	England
NaCl	BDH	England
Ethanol 95%	Merck KgaA	Germany
H_2O_2	BDH	England
Urea	BDH	England
Gelatin	BB	USA
HCl	BDH	England
Absolute methanol	Merck KgaA	Germany
Isoamyl alcohol	BDH	England
Cetrimide (N-acetyl-N.N.N.trimethyl ammonium bromide)	BDH	England
α-naphthol	BDH	England
Glycerol	BDH	England
Glucose	BDH	England

APPENDIX 3. Ready-to-use implantation circles used in the study

Media	Company	Source
Blood agar base	Himedia	India
Eosin methylelen blue agar	Oxoid	England
Muller-Hinton agar	Oxoid	England
Simmon's citrate agar	Himedia	India
MacConkey agar	LAB M limited	U.K
Brain-heart infusion broth	Oxoid	England
Urea agar base	Himedia	India
Nutrient agar	LAB M limited	U.K
Nutrient broth	LAB M limited	U.K
Mannitol salt agar	Himedia	India
Nutrient gelatin medium	ACCUMIX	India
Chromogenic Coliform Agar (CCA)	Oxoid	England

APPENDIX 4. Dyes used in the study

Stains	Company	Source
Gram Stain Compounds: Crystal Violet Stain Safranin Stain Iodine Ethanol 95%	Ready to use Kit (Pro. Lab)	England
Methyl red	Pro.Lab	England

APPENDIX 5. Commercial kits for DNA assays

Kit	Contents	Company	Source
Prestotm Mini gDNA Bacteria Kit	Gt Buffer 30 mL GB Buffer 40 mL WI Buffer 45 mL Wash Buffer 100 mL with Ethanol Elution Buffer 30 mL GD Columns 100 pcs 2 mL Collection Tubes 100 pcs Proteinase K 1.1 Lysozyme Gram+Buffer 38 mL	Geneaid	USA
DNA ladder	Base pair (2000-100)	Bioneer	Korea
Master Mix + PCR	DNA polymerase dNTP (dATP, dCTP, dGTP, dTTP) $MgCl_2$ 3 mM Loading dye (Blue and Yellow) Mineral Oil Forward Primer Reverse Primer DNA template Deionized distilled water	Geneaid	USA

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