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Molecular Detection of some β-lactamase Genes Among *Proteus mirabilis*. Isolates from Patients with Urinary Tract Infections

A Thesis

Submitted to the Council of College of Science-University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿ يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ وَإِذَا قِيلَ انشُزُوا فَانشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

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We certify that the thesis titled **(Molecular Detection of some β-lactamase Genes Among *Proteus mirabilis* Isolates from Patients with Urinary Tract Infectionse)** was prepared by **(Kadhim Jawad Mohammed Jamin)** under our supervision at the Department of Biology / College of Science /University of Babylon as partial fulfillment of requirement for the degree of Master of Science in Biology.

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Dedication

To the cleanest two hearts in my life
To those of them who have gained the power of love without limits ..... My dear father & my mother;
To those who had a great impact on many obstacles and difficulties in my life ..... my dear brothers
To the homeland we are looking for, and we yearn to see it one day as we wish it safe and upright

To all those whose spring butterflies dance to those who open the anemones and yasin ..... to the martyrs

Kadhim

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Praise be to Allah and peace and blessings be upon his Messenger the Prophet Muhammad peace be upon him and his family and who followed him until the Day of religion. My Thanks are extended to the Deanship of the Faculty of Sciences and the presidency of the Department of Biology, professors for their scientific treatment for helping and guiding me.

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Kadhim

Summary:

Urinary tract infections (UTIs) are among the most common bacterial infections. The incidence increases with age, and a lifetime incidence of 50–60% in adult women. Urinary tract infections are a major burden on society and the healthcare system, as a result of increased antibiotic resistant among the bacteria species cause UTIs .

A total specimens 450 (325 male and 125 female) with urinary tract infections. These patients ranged in age from (17 to 70) years old from three Iraqi hospitals (Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, and Shomali General Hospital) from the period from July 2022 to the end of October 2022. Samples were grown on McConkey agar and Blood agar to separate Gram-negative, non-lactose-fermenting colonies exhibiting swarming phenomena. The isolated genetic detection by *atpD* gene. The result of culture, biochemical, Gram stain and genetic (*atpD* gene) present the percentage was 70 isolates (15.5 %) were found positive for *Proteus mirabilis*.

Antibiotic susceptibility test by disc diffusion method were performed for 12 antibiotics from different 7 classes to assign the isolated to Multi-drug resistant or non-Multi-drug resistance of *P. mirabilis* isolates study their resistance against 12 antibiotics belonging to different classes. The result of antibiotic susceptibility showed the all isolated resistant to Nitrofurantoin (92.8%), Trimethoprim –Sulfamethoxazole (85.7%), Cefotaxime (78.5%), Cefuroxime (68.5%), Cefatazidime (58.5%), Gentamycine (38.5%), Ciprofloxacin (37.1%) , Levoflaxacin (28.5%). While the lower resistant against Amikacin (10%) , Impenem (4.2%), Aztreonam (2.8%)and Meropenem (1.4%). The results revealed that 60/70

(85.7%) of *P. mirabilis* isolates were multidrug resistant (MDR) and 10/70(14.28%) non MDR.

The polymerase chain reaction used to detection beta-lactam genes (*bla-$_{TEM}$* , *bla-$_{CTX-MI}$*, *bla-$_{CTX-MIV}$* , *bla-$_{imp1}$*, *bla-$_{CTX-MII}$* ,and *bla-$_{OXA}$*) gene were achieved using specific primer pairs, The result showed 64(91.4%), 56(80%), 40(57.1%) ,40(57.1), 11(15.7%, 4(5.71%) where positive to the (*bla-$_{TEM}$*, , *bla*-CTX-MI, *bla*-CTX-MIV ,bla-$_{IMP-1}$, *bla*-CTX-MII.,and bla-$_{OXA}$) from 70 *P.mirabilis* isolates. The PCR products of 5 isolates for each *bla$_{CTX-MI}$*, *bla$_{CTX-MII,}$* *bla$_{CTX-MIV}$* and *bla$_{TEM}$* were sent for Macrogen/Korea for sequencing. All ABI file opend by BioEdit Sequence Alignment Editor. The incorrect sequences were trimmed and the correct sequences were submitted for NCBI BLASTN for similarity. The results revealed that, all 5 sequences (KJSJHD-1 to KJSJHD-5) of *bla$_{CTX-MI}$* amplicon give 100% similarity with *bla$_{CTX-M-15}$* . All 5 sequences (KJSJHD-6 to KJSJHD-10) of *bla$_{CTX-MII}$ amplicon give* 100% similarity with *bla$_{CTX-M-65}$* .All 5 sequences (KJSJHD-11 to KJSJHD-15) of *bla$_{CTX-MIV}$ amplicon give* 100% similarity with *bla$_{CTX-M-90}$* . All 5 sequences (KJSJHD-16 to KJSJHD-20) of *bla$_{TEM}$ amplicon give* 100% similarity with *bla$_{TEM-1}$* .

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List of Abbreviations

Abbreviation	Terms
UTIs	Urinary tract infection
MDR	Multidrug resistance

UPEC	Uropathogenic *Escherichia coli*
TSI	Triple sugar iron
LPS	Lipopolysaccharide
TBE	Tris- borat-EDTA
IE	Infective endocarditis
Pta	Proteus toxic agglutinin
EPS	Extracellular polymeric substances
MR/P	Mannose resistant/*Proteus*
UCA/NAF	Uroepithelial cell adhesion fimbria
ATF	Ambient temperature fimbria
PMF	*Proteus mirabilis* fimbria
Fla A	Flagellate A
Fla D	Flagellate D
OMP	Outer membrane proteins
CAUTI	Catheter–associated urinary tract infections
ESPL	Extended spectrum b-lactmase
MLST	Multilocus Sequence Typing

1.Introduction

A urinary tract infection (UTI) is an infection result from present and growing of bacteria in the urinary system. It is often caused by digestive tract bacteria that enter the urethral opening and start to reproduce, leading to an infection (Al-Shami *et al*. 2021). Around 150 million people worldwide experience urinary tract infections, one of the most prevalent bacterial infections (Yuan *et al.*, 2021). Male infants of all ages and elderly men and women frequently develop urinary tract infections, This might result in severe problems such as persistent infections, pyelonephritis with sepsis, and newborn kidney damage, preterm delivery, and side effects from repeated antibiotic use (Kranz *et al.*, 2018).

Uropathogenic *Escherichia coli* (UPEC), which accounts for more than 80% of community-acquired infections and causes the majority of urinary tract infections, while *Staphylococcus, Klebsiella, Enterobacter, Proteus,* and *Enterococcus* are linked to infections in healthcare settings (Flores-Mireles *et al*., 2015).

A member of the Morganellaceae family and the Enterobacteriales order, *Proteus spp.* is a gram-negative rod. These species are a typical component of the bacterial flora in both humans and animals' digestive tracts (Girlich *et al.*, 2020). *Proteus mirabilis*, a member of the Enterobacteriaceae family, can change its shape from a rod to one that is elongated and capable of movement in swarms. It is a bacterium with the potential to cause a variety of clinical illnesses (Abed *et al*, 2022) .

When compared to non-pathogenic bacteria, the bacteria that cause UTIs have more aggressive virulence traits, which enhance their ability to adhere to,

colonize, and invade host cells. Through the use of specific virulence factors, such as pili, capsules, lipopolysaccharides, and other cell surface structures, these bacteria are able to avoid evading the host's immune system(Yuan *et al.*, 2021). *P. mirabilis* on catheter development in the urinary system and bladder, which might be caused by isolates with a wide range of antibiotics resistance. Multidrug resistance (MDR) can be caused by resident gene mutations or resistance agents on chromosomes. It might, however, grow by acquiring resistance genes via horizontal transfer (Mirzaei *et al.*, 2021)

Numerous virulence factor, such as urease, hemolysin, fimbriae, and flageella, help *P. mirabilis* surrvive in the urinary system. The breadth and amounts of virulence genes that can influence bacterial growth and persistence in the urinary system vary amongst *P. mirabilis* strains(Hussein *et al.*, 2020). Microbial persistence is the specific capacity of microbes to be able to tolerate direct contact with lethal ranges of bactericidal antibiotics. The persistence phenomenon is abundant in a variety of bacterial species and was studied in *P. mirabilis* which is mostly known to cause recurrent urinary tract infections that are usually occurred in patients under longterm catheterization as well as increasing drug resistance was reported for *P. mirabilis* (Abokhalil *et al*., 2018).

Aim of the study:

Antibiotic resistance is a global health challenge, involving the transfer of bacteria and genes between humans, animals and the environment. The present study aim to investigation the prevalence antibiotics resistant genes among *proteus mirabilis*.Isolates from urinary tract infection paticnts. To reach this aims, the following objectives were achieved:

1- Isolation and identification of *Proteus mirabilis*. from urine sample of urinary tract infection patients (phenotypic and genotypic)
2- Antibiotic susceptibility test of *Proteus mirabilis*. isolates
3- Genetic investigation the resistance gene profile(*bla* -$_{TEM}$, *bla* -$_{CTX-MI}$, *bla* -$_{CTX-MII}$,*bla*-$_{CTX-MIV}$, *bla*-$_{IMP-1}$, *bla*-$_{OXA}$) among *proteus mirabilis* isolates.
4- Sequencing some β-lactamase genes (*bla* -$_{TEM}$, *bla* -$_{CTX-MI}$, *bla* -$_{CTX-MII}$,*bla*-*CTX*-$_{MIV}$).

2. Literature Review

2. 1 . Urinary tract infections

About 150 million people worldwide experience urinary tract infections (UTIs), which are among the most prevalent bacterial infections (Meštrović *et al.*, 2020). In adult women, the lifetime incidence ranges between 50 and 60 percent (Medina and Castillo-Pino, 2019).Urinary tract infections are a major burden on society and the healthcare system, in large part because of treatments that significantly increase antibiotic resistance (Ribić *et al.*, 2018). UTI caused by Escherichia coli and others has been extensively studied. However, complicated UTIs, especially those caused by Proteus mirabilis (PM), pose increasing medical challenges (Yuan, *et al.*, 2021).

Uropathogens living in the gut frequently cause the periurethral area to become contaminated during the pathogenesis of UTI. This is followed by colonization of the urethra and ascending migration to the bladder (Flores-Mireles *et al.*, 2015). Urinary tract infections are caused mostly by uropathogenic *Escherichia coli* (UPEC), which accounts for more than 80% of community-acquired infections, whereas healthcare-associated infections are caused by *Staphylococcus*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Enterococcus*. Uropathogenic *Escherichia coli* strains are found in abundance in the gut of patients with UTIs and are thus considered to originate from the gut (Nielsen *et al.*, 2014).

2.2. History of *Proteus spp.*

The germen scientist Hauser first defined the *Proteus* genus in 1885, recognizing the swarming activity of these peculiar organisms and naming two species *P. vulgaris* and *P. mirabilis* (Hauser, 1885).

Smith Theobald was the first scientist to study the activity of *P. vulgaris* in the consumption of glucose, lactose, and sucrose, and he came up with two essential characteristics of this organism in 1893. One of these is *P. vulgaris* ability to produce gas in addition to its fermentative capacity to produce gas and acid from sucrose, glucose and lactose (Hezamb and Salih, 2020).

In 1919, 73 *Proteus* strains were investigated for a wide range of biochemical reactions, all of these strains showed swarming behavior and were capable to digest casein and liquefying gelatin. Because of the similarity in biochemical properties, the *Proteus* genus is classified as an opportunistic Gram negative pathogen, motile, rod and belongs to the Enterobacteriaceae family. It can be found in the intestinal tracts of both humans and animals, as well as in the environment, manure, and sewage (Yang *et al.*, 2014)

Despite this, no strain of the genus produced any coloration on agar plates (Wenner and Rettger, 1919). Gustav Hauser described *Proteus spp.* bacteria in 1885, revealing its characteristic of vigorous swarming development. *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri*, and three unidentified genomic species make up the genus at this time, and there are 80 O-antigenic serogroups in all(Manos and Belas, 2006) .

At 36 °C, *Proteus spp.* have the ability to movment over solid and semisolid surfaces. They can proliferate and flourish in both aerobic and anaerobic conditions (Gross *et al*., 2018). *Proteus spp*. serves a significant role in the natural environment because they participate in the decomposition of organic material from animal sources, and they are frequently parasites or commensals (Drzewiecka, 2016).

Therefore, it shouldn't be surprising that *P. mirabilis* is simple to grow. It can be cultured on several common complicated and chemically specified minimal media, just like its related *E. coli*. The *P. mirabilis* is maltose-negative, urease-positive, lactose- and indole-negative, ornithine decarboxylase-positive, motile, and able to produce H_2S on triple sugar iron (TSI) agar (Pearson, 2008).

2.3. General characteristics of *Proteus spp*.

Proteus are Gram-negative rod-shaped bacteria in the Enterobacteriaceae family. *Proteus spp*. actually consists of four species: *P. mirabilis*, *P. vulgaris*, *P . hauseri*, and *P. penneri*, all of which are harmful to humans. *Proteus spp*. rods have cilia, which improves their pathogenicity (Zwayen, *et al*, 2020). The capacity to produce urease, which catalyzes the conversion of urea into ammonia and CO_2, as well as the phenomenon of swarming proliferation cause a reduction in urine pH, which may eventually culminate in the development of kidney or bladder stones(Kumar and Kumari, 2013).

MacConkey agar and Blood agar media were employed as selective media to explore the particular colony properties of *Proteus* isolates. Due

to the presence of bile salts, *Proteus* isolates produced pale-colored colonies on MacConkey agar medium (Bilal *et al.*, 2019) .

Virulence factors of *P.mirabilis* and *P.vulgaris* rods include lipopolysaccharide (LPS), flagella, fimbriiae, glycocalyxe, and the phenomenone of adhesion and hydrophobicitye of bacteria surface a principal modification of the bacteria *Proteus spp*.to causes infections (Ahmed, 2015).

Proteus spp. bacteria are basically present in food goods, water, soil, and natural fertilizers. Both humans and animals may have these bacteria, mostly in the gastrointestinal system. In addition to infections resulting from hernia surgery, acute otitis media, and skin and subcutaneous tissue infections, the species *P. mirabilis* can cause purulent meningitis in infants (Reśliński *et al.*, 2008).

The bacteria have been identified as human opportunistic pathogens and have been isolated from wounds ,urine, and other clinical sources. According to a theory, these proteolytic microbes are stored in the intestines. *Proteus spp.*, which are typically classified as parasites or commensals, may be hosts for a variety of wild and domestic animals. However, fascinating instances of their symbiotic relationships with higher organisms have also been recorded. *Proteus spp.* are commonly considered an indicator of fecal contamination in soil or water environments, which increases the risk of poisoning when contaminated seafood or water is consumed (Zhang *et al.*, 2021).

Drug-resistant bacteria that originate from the intestines might also pose a health danger. Autochthonic *Proteus spp.* strains found in these

habitats have special characteristics that are related to the positive properties of the bacteria found in soil and water. These rods contain a variety of metabolic capabilities that allow them to adapt to different environmental settings, such as high concentrations of heavy metals or toxic substances, which the bacteria may utilise as sources of energy and nutrition . The ability of *Proteus spp.* to develop plants and tolerate or use pollutants opens up the prospect of using these microbes in bioremediation and environmental protection (Drzewiecka, 2016).

2.4. Classification of *Proteus spp*.

The antigenic structure of *Proteus spp*. has been used to classify the bacteria (Belyavin *et al*., 1951; Krikler , 1953), phage sensitivity, biochemical characteristics (Kippax, 1957; Huang, 1966),Dienes personality type (Krikler, 1953; Story, 1954; De Louvois, 1969); bacteriocine production (Cradock-Watson, 1965) and resistotype (Kashbur *et al*., 1974) and this genus belongs to the tribe protease (It comprised three different genera *Proteus, Morganella,* and *Providencia*) (Mckell and Jones, 1976).

The two species *P.mirabilis* and *P.vulgaris* , were initial identified by Hauser in 1885. The strains were divided into two species by Hauser based on how quickly they liquefied gelatin: *P. vulgaris* liquefies gelatin "rapidly," whereas *P. mirabilis* liquefies gelatin "slowly." Hauser took note of the swarming behavior of the organisms.In 1965, Britten and Kohne developed a DNA hybridization method that would go on to be used as a tool for addressing various problems with bacterial taxonomy (Britten and Kohne, 1965), as a result of employing this method, new species were

described. The name *P. penneri* was given in honor of John Penneri, a Canadian microbiologist who made significant contributions to the understanding of the three *Proteus* species (Hickman *et al*., 1982).

According to Bergey's Manual of Systematic Bacteriology (Whitman *et al* ., 2012), *Proteus* is classified as following :

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Proteus*

Species*: P. mirabilis, P. vulgaris, P. penneri, P. myxofaciens, P.hauseri* .

2.5. Pathogenicity of *Proteus spp*

Proteus species are the most prevalent uropathogens in the general population and hospitals. Infection occurs only when the pathogenicity of the bacteria surpasses the host's regular defensive systems. We have natural defense systems such as urinary tract wall, phagocytic cells, and lymphocytes, as well as non-specific immunological responses (Dougnon *et al.*, 2020).

Proteus mirabilis is a common opportunistic pathogen causing severe illness in humans and animals(Algammal *et al.*, 2021). Acute pyelonephritis, urolithiasis (stone accumulation in the kidney or bladder), and cystitis are among the upper urinary tract infections (frequent locations of infection) Figure (1-1) (Mugita *et al.*, 2020).

Proteus species have received little attention as a possible cause of gastrointestinal illness despite being resilient, adaptable, and resident in the human gastrointestinal system. *Proteus spp*. host-microbe and microbe-microbe interactions as well as their potential pathogenicity due to population growth in response to environmental changes are now recognized as crucial components of illness caused by this genus. Somewhat neglected is *Proteus spp*. potential role in intestinal illnesses and infections (Hamilton *et al.*, 2018)

The majority of *Proteus spp.* bacteria are opportunistic human diseases. The virulence factors that enable the bacteria to colonize and survive in different host organism habitats as well as their functions in the pathogenesis of humans have both been extensively investigated. Most of the bacteria may be a source of nosocomial infections as well as severe urinary tract and wound infections, and they all tend to cause infections in persons who have weakened immune systems (Drzewiecka, 2016).

Proteus spp. have been associated with several other infections beyond UTIs, such as primary bacteremia and more rarely with other types of infections, such as respiratory tract, or wound infections (Kwiecińska-Piróg, *et al*., 2018). The ability of *Proteus* to form biofilms, in combination with its ability to cause bacteremia, represent the potential to cause

complicated bloodstream infections, such as infective endocarditis (IE), which is of particular importance, due to the notable morbidity and mortality that IE carries (Ioannou and Vougiouklakis, 2020) ..

2.6. Virulence Factors of *P. mirabilis*

To survive in higher organisms that are able to fight various bacterial infections, bacteria have had to develop different strategies These strategies include , in particular, virulence factors, which fall into three groups: virulence factors associated with transmissibility, invasiveness, and toxicity (Hu *et al.*, 2019,Sarowska *et al.*, 2019).

The *P. mirabilis* isolates showed encodes many virulence genes involved in infection (Abbas *et al* ., 2015). Many virulence genes, including urease, hemolysin, fimbriae, and flagella, help *P. mirabilis* survive in the urinary system (Burall *et al.*, 2004).

To the contrary, *P. mirabilis* strains differ in the kinds and levels of virulence genes that can affect bacterial development and persistence in the urinary tract. Numerous studies have looked at *P. mirabilis* virulence traits and the steps involved in the pathogenesis of UTIs in order to identify the variety of virulence genes and their prevalence among *P. mirabilis* isolates(Hussein *et al.*, 2020).

There are three potential poisons that are known to be very important in virulence. Examples include hemolysin, *Proteus* toxic agglutinin (pta), and the metalloprotease ZapA. Hemolysin may have a role in the development of acute pyelonephritis and the spread of infection to the kidneys. *Proteus* toxic agglutinin is an autotransporter that functions as a serine protease on the surface of bacteria(Adams-Sapper *et al.*, 2012). The colonization of the

kidney and bladder is helped by the pta protein. According to in *vitro* and in *vivo* UTI studies, hpmA and pta have additive effects, particularly in cystitis and maybe interstitial nephritis(Alamuri *et al.*, 2009). *P.mirabilis* possessese a diverse set of virulence factors, each of which is important in UTIs. These parameters are linked to the connection between bacteria and surfaces, invasion, damage to host tissues, evasion of the host immune system, and iron absorption .Many virulence factors in *P. mirabilis* are important in pathogenicity (Tabatabaei *et al.*, 2021).

2. 7. 1 . Biofilms

A biofilm is an aggregate of communicating bacteria attached to a solid surface or to each other and enclosed in an exopolysaccharide matrix. This is dissimilar to planktonic or free-living bacteria, in which microorganisms connections do not occur in the same way.Biofilms produce a slimy coat on solid surfaces and occur throughout nature also (Riedel *et al* .,2019).

The *P. mirabilis is* well recognized for its propensity to swarm, and growth on solid surfaces causes the organism to differentiate from its short, rod-shaped "swimmer cells" into very long, hyper-flagellated "swarmer cells," which can group together to form multicellular rafts. These cell rafts may travel over solid surfaces fast and cooperatively (Ali and Raheema, 2022).

Biofilm generation develops an alternate lifestyle in which bacteria accept multicellular behavior that allows them to survive in a variety of environmental niches for longer periods of time. The extracellular matrix formed by the bacterial cells is the most basic component of the biofilm.

Extracellular polymeric substances (EPS), extracellular DNA, proteins, water, and other macromolecules make up the extracellular polymer matrix. Tolerance to antimicrobial agents (antibiotics and disinfectants) as well as harsh environmental conditions is conferred by the biofilm matrix (declined pH, high CO_2 levels and decreased water availability. It is difficult to remove microorganisms using standard antibiotics in these cases (Iribarnegaray *et al.*, 2019).

Biofilm formation in the urinary system caused by *P. mirabilis* can prolong infection and limit antibiotic effect and immune response. The *P. mirabilis* stone formation in the urinary tract can obstruct urine flow through the catheter, bladder, or kidneys, resulting in serious consequences such as pyelonephritis, septicemia, and shock. Among the illnesses connected to biofilm development include endocarditis, cystic fibrosis, urinary tract infection, periodontitis, and chronic wound infection. Biofilm production might impair the immune system, allowing bacteria to readily evade the host (Zafar *et al.*, 2019).

Because biofilms act as a physical barrier to antibiotic entry, urethral pathogens become more resistant to antibiotics as they grow. Crystals build up in the catheter's biofilm and eventually block the lumen, preventing urine flow and resulting in complications like urinary incontinence, uncomfortable bladder distension brought on by urinary retention, vesicoureteral reflux, bacteriuria, increased infection, pyelonephritis, and potentially septicemia. According to one research, the majority (66–79%) of patients with blocked catheters had P. mirabilis infections, whereas 62% of patients with crystal catheters also had bladder stones. This shows that

bladder stones are more likely to occur when crystalline biofilms form (Yuan, Huang, Yang, Wang, Li and Yang, 2021).

2.7.2. Fimbriae and Adherence Ability

The *P. mirabilis* fimbriae are responsible for the bacterial adhesion, which is an important step in colonizing and establishing infections (Hasan *et al* ., 2020). There are five types of fimbriae that have been linked to infection such as Mannose-resistant / Proteus-like (MR/P) fimbriae are important because of their ability to adhere to epithelial cells, and are therefore important in adherence in the upper urinary tract. The MR/P fimbriae are also responsible for haemagglutination of human erythrocytes. This type of fimbriae exhibits strong immunogenic properties that could be important in vaccination (Miyashita *et al.*, 2018;Levine *et al*., 2020).

Fimbriae, which are responsible for *P. mirabilis* bacterial adherence, play a crucial role in colonizing and causing infections. There are five types of common fimbriae that are associated with infection: Mannose-resistant/*Proteus*-like (MR/P) fimbriae, Uroepithelial cell adhesion (UCA/NAF) fimbriae, Ambient-temperature fimbriae (ATF), *P. mirabilis,* and *P. mirabilis* P-like pili (PMP) fimbriae(Hasan *et al* , 2020).

2.7.3 Flagella and swarming motility

The most significant virulence factor in *P. mirabilis* that affects the invasion and dissemination of infection in urinary tract sections is motility(Kuan *et al.*, 2014). The infection starts in the periurethral area, invades the urethra, and then spreads to the bladder and other parts of the urinary system (Hickling *et al*., 2017).

Motility strongly promotes interaction with these sites. *P. mirabilis* is a flagellar peritrichous bacterium (Fattorini *et al* ., 2020) . This bacterium has a swarming motility, and when it swarms, the expression of pathogenicity is amplified (Kotian *et al.*, 2020). Once attached to the urethra, *P. mirabilis* must attach to the urethra and then travel via the urethra to reach the bladder. *P. mirabilis* is a motile organism with peripheral flagella that can differentiate into multicellular, elongated "colony cells" that are sequentially arranged to form cell rafts that can move swiftly and cooperatively across solid surfaces from a single, short, rod-like "swimming cell (Zare, *et al* 2022).

Swimming motility, which occurs on wet surfaces, is a type of group motility in which cells move across the surface in unison. Peritrichous flagella are seen in the majority of swarming bacteria. Many of them also make and secrete chemicals that aid in their movement across the substrate (Willey *et al*., 2017).

As swarms of cells have been observed in the renal parenchyma, "colony cells" are also believed to play a role in kidney colonization and the onset of pyelonephritis, especially during prolonged infection(Allison *et al*., 1994). Therefore, despite the fact that flagella undoubtedly play a role in the pathogenesis of *P. mirabilis*, it is still unclear how crucial swimming motion and colony cell differentiation are for the development and severity of the illness. The overproduction of flagella, which in swimming cells may play a significant part in the aggressive movement of bacteria from the urethra to the target tissue of the bladder and in close contact with the bladder epithelial cells, is one of the most conspicuous characteristics of population multicellularity. Unlike other bacteria that

move, *P. mirabilis* has a single locus on the chromosome that is roughly 54 kB long and encodes all of the flagellate components and chemotactic proteins(Pearson *et al.*, 2008) .

The two flagellin genes *flaA* and *flaB* were encoded by *P. mirabilis* in this area. Although *flaA* seems to be the main flagellin produced by *P. mirabilis*, there may occasionally be hybrids as a result of recombination between *flaA* and *flaB* (Manos and Belas, 2004). Due to the fact that the flagellum proteins can trigger an inflammatory response and be recognized by the host immune system, the antigenic variation *flab* and *flaAB* hybrids may be in the process of infection from immune, and *P. mirabilis* isolated from infected mice urine produced mostly hybrid flagella protein, demonstrating that the flagellum protein gene rearrangement may be a *P. mirabilis* immune escape mechanism (Nielubowicz *et al*, 2008; (Umpiérrez *et al.*, 2013).

2.7.4. Outer membrane proteins (OMP)

These proteins perform key roles in cell structure and morphology, nutrient acquisition, colonization and invasion, and protection against external toxic threats such as antibiotics(Horne, Brockwell and Radford, 2020). Gram-negative bacteria have a hydrophobic outer membrane bilayer, containing lipopolysaccharide (LPS) molecules, phospholipids and outer membrane proteins (OMPs), including pore-forming proteins .As such, this dynamic outer membrane acts as a barrier to several antibiotics that are typically effective against Gram-positive bacteria (Impey *et al.*, 2020).

In general, OM proteins (OMP) have mitogenic activity for B cells as well as immunogenic features. In addition, OM lipoproteins and their synthetic analogs serve as adjuvants and can stimulate macrophages to produce tumor necrosis factor. They can also mediate the passage of hydrophilic molecules through artificial model membranes and shield them from detergent-induced disaggregation (Jezi *et al.*, 2019).

There are three main proteins in the OM of *P. mirabilis* that measure 39.0, 36.0, and 17.0 kDa. These proteins match those that have previously been identified as *Proteus spp.* OMP. Through the detection of specific antibodies following an experimental UTI in mice, these proteins were highly immunogenic. An outer membrane heme receptor gene was disrupted by a transposon in a *P. mirabilis* mutant that was unable to utilise heme as the only source of iron. In the mouse model of UTI, the mutant was unable to colonize the urinary system as effectively as the wild type, highlighting the significance of the heme receptor in *P. mirabilis* pathogenicity(Lima *et al.*, 2007).

In instance, OmpA significantly raises the amount of O-specific IgG by acting as an immunomodulator of the immunological response to LPS. Additionally, this protein exhibits strong adjuvant activity and acts as a mitogen for murine B cells *in vitro*. The 39-kDa protein increases TNF secretion by LPS-stimulated macrophages and suppresses the generation of IL-1 and oxygen radicals(Weber *et al.*, 1993).

2.7.5. Lipopolysaccharide (LPS)

This is a Gram negative virulence factor that aids in the actions of bacterial antibodies found in serum, such as phagocytes. The LPS is made up of an O-specific sequence, a core zone, and lipid a (lipophilic zone), all of which act to anchor LPS to the outer membrane of *Proteus spp*.(Wang *et al.*, 2010).

The differences in the structure of O-antigens serve as a basis for the serological classification of *Proteus* strains. The serological classification scheme currentlyconsists of 78 serogroups (Drzewiecka and Sidorczyk, 2005).

Proteus LPS has so far discovered six different kinds of core regions. The presence of D-galacturonic acid separates the *Proteus* core area from the core regions of *E. coli* and *Salmonella*. Galacturonic acid was discovered to have a crucial role in the specificity of *P. mirabilis* LPS. According to the results of lipid A chemical analysis, *P. mirabilis* lipid A possesses some biological effects such as mitogenic activity, fatal toxicity, and the local Shwartzman reaction(Vinogradov, Sidorczyk and Knirel, 2002).

2.7.6. Toxins and Enzymes

Two toxins produced by *P. mirabilis*, hemolysin (HpmA) and *Proteus* toxigenin (Pta), have been associated to renal transmission and tissue damage, leading to acute pyelonephritis. The synthesis of urease (urease amylase), a 250-kDa polymeric nickel metalase that catalyzes the hydrolysis of its substrate urea into ammonia and carbon dioxide, by *P. mirabilis* is well recognized (Wang *et al*., 2021).

A . Hemolycin:

Hemolysin is a toxin that enters the eukaryotic cell's target membrane and produces pores, which trigger ion efflux and induce cell disruption. Hemolysin promotes the spread of bacterial infection in the kidney, causing pyelonephritis to proceed in ascending UTIs (Hasan *et al*.,2021).

The hemolysin genes (*hpm A* and *hpm B*) in *P. mirabilis* are a two-part secretion. The *hpmA* is seen to be located outside of membranes involved in the *hpm A* secretion cycle in the periplasm , where *hpm B* stores and activates it. Human renal proximal tubular epithelial cells generated by *hpm A* display cytotoxicity (Johnson *et al*.,2018)

The hpm A mutant *P. mirabilis* infection and the wild-type strain cannot colonize differently from one another Due to the presence of specific virulence factors, this hemolysin is likely less prevalent or masks its contribution during *in vivo* infection (Ostolaza *et al* ., 2019).

After 24 or 48 hours of incubation in 37°C, the β-hemolysis properties of P. mirabilis strains were tested by examining clear zones around bacterial Chapter One Literature Review 17 colonies on blood agar supplemented with 5% (v/v) bovine blood (Kang *et al.,* 2019).

B. *Proteus* Toxic agglutinin(Pta):

The outer membrane autotransporter protein known as *prototeus* toxic agglutinin is responsible for mediating cell aggregatione and has a catalytic -domain that can lyse kidney and bladder cells (Gupta *et al*., 2019). In addition to a severe colonization deficiency in the kidneys, urine

and spleen , the *P. mirabilis* negative *pta* gene exhibited decreased pathology (Engel *et al*., 2007).

C. Urease

Urease plays a crucial role in the pathophysiology of *Proteus mirabilis* as it catalyzes the production of kidney and bladder stones (Hasan *et al*., 2021).

This enzyme contributes to the development of kidney and bladder stones as well as the encrustation or obstruction of the urinary system (Armbruster *et al*., 2018). Animals create urea, a nitrogenous waste that is hydrolyzed by the enzyme urease and results in a considerable increase in urine pH due to the ammonia it produces. Guano (magnesium ammonium phosphate) and carbonate apatite (calcium phosphate), which are both precipitated by soluble polyvalent anions and cations, are produced at high pH levels. *In vitro* studies on urine inoculation of *P. mirabilis* showed that increasing the concentration of magnesium, calcium, and phosphate ions would enhance the strength of crystallization (Torzewska and Różalski, 2015) . These crystals are incorporated into developing biofilms in a process called urea demineralization (Jacobsen and Shirtliff, 2011) .

Proteues mirabilis infections are characterized by the development of stones, which provide a variety of advantages to microorganisms, including, but not limited to, shielding the bacteria from the host's immune system (because the bacteria are imprisoned in the pores of the stone and can reproduce). Ammonia is poisonous to host cells, urine is prevented from entering the ureter, immunoglobulin is unable to reach the bacteria hiding in the stone, and there is direct tissue injury. These events all

combine to create a microenvironment that is safe and nutrient-rich for bacteria (Hasan, Alasedi and Jaloob, 2021).

2.8.Antibiotic resistance

Antibiotics are a class of medicines used to treat infections. Antibiotics are sometimes known as antibacterial or antimicrobial medications. Antibiotics can be administered orally as liquids, tablets, or capsules, or injected intravenously (Willacy,2020).

Antibiotic resistance is the lack of bacterial susceptibility to an antibacterial agent's killing (bacteriocidal) or growth-inhibition (bacteriostatic) characteristics (Morier and Douglas,2022). Antibiotic resistance can emerge naturally in the strain or be transferred from one strain to another by mobile genetic components such as plasmids, transposons, and integrons. Inactivating the drug, changing the drug's target, reducing the drug's absorption, and active efflux of the drug are all ways to make bacteria resistant to antibiotics. The definitions for multidrug resistance (MDR), extensive drug resistance (XDR), and pan-drug resistance (PDR) were created in collaboration by the US Centers for Diseasee Control and Prevention (CDC) and the European Centre for Disease Control and Prevention (ECDC), and they are the main global agreement criteria for classifying the levels of drug resistance in bacteria.These resistance classes are useful for capturing epidemiological data and assessing the resistance situation in a specific geographic location (Gajdács et al., 2020). ive efflux of the drug are all ways to make bacteria resistant to antibiotics (Reygaert, 2018).

Clinical P. mirabilis has developed high antibiotic resistance, comparable to other Enterobacterales, which is a serious problem for hospitalized Chapter One Literature Review 28 patients. Strains with extended-spectrum β-lactamases (ESBLs) or AmpC-like cephalosporinases have proliferated in some areas, showing wide resistance to anti-infectives in addition to penicillins and cephalosporins (including oxyimino-compounds). More than 20% of P. mirabilis isolates producing nosocomial infections in Poland may be CMY-2-like AmpC producers (Filipiak et al .,2020)

2.9. Extended-spectrum β-lactamases gene (ESBLs)

A significant contributor to the resistance to expanded-spectrum b-lactam antibiotics is the production of ESBLs by Gram-negative bacteria. Since they were discovered in the early 1980s, they have spread around the world. In the past, TEM-type and SHV-type ESBLs were the two kinds of ESBLs that were most prevalent. The most common ESBL type nowadays is of the CTX- M type, with CTX- M-15 dominating the world, CTX-M-14 coming in second, and CTX- M-27 developing in some parts of the world. The frequent discovery of the genes producing ESBLs on plasmids and their location within transposons or insertion sequences have made it possible for them to proliferate. A variety of diagnostic methods, including as phenotypic and genotypic testing to detect b-lactamases, are available to the clinical microbiology laboratory. Global antibiotic resistance will continue to be strongly impacted by ESBL-producing gram-negative bacteria. They must be regularly watched out for in surveillance studies as well as patient isolates (Castanheira *et al* ., 2021).

1- TEM- β-lactamases gene

The initial plasmid -mediated b-lactamase , TEM -1, was first described in the early 1960 s (Datta and Kontomichalou,1965) . This enzyme comes in several forms called TEM-type ESBLS. Since it was initially identified in an isolate of Escherichia coli that was acquired from a blood culture from a Greek patient by the name of Temoneira (Medeiros *et al*., 1985), this enzyme has been known by that name.

The first TEM derivative, TEM- 2, differs from the original TEM -1 b -lactamase by a single amino acid change of Gln39 Lys (Sougakoff et al.,1988). This modification had little effect on the substrate profile of TEM-1, but TEM -2 served as the progenitor for many of the TEM- type ESBLs.TEM -3, the first TEM -type variation to exhibit the ESBL phenotype , was published in 1989 (Sougakoff *et al* ., 1988).

Amino acid modifications only occur at a small number of locations in the TEM enzyme.The amino acid residues (Ambler numbering) that most frequently contribute to the ESBL phenotype being conferred on TEM -type enzymes are Gly238 and Glu240 on the b3 b -pleated sheet, Arg164 on the neck of the X loop , and Glu104 situated directly across from Gly238 Glu240 at the opening of the active-site cavity (Raquet *et al*., 1994; Knox,1995).

Gly238Ser and Glu240Lys appear to have the most effects on ESBL phenotypic production among these alterations (Bradford *et al* ., 2001). The substrate profile has been subtly altered in several of the more recent TEM variations. For instance, TEM-184 (amino acid substitutions at Q6K,

E104K, I127V, R164S, and M182T) hydrolyzed aztreonam more effectively than ceftazidime or cefotaxime (Piccirilli *et al* ., 2018).

However, computer modeling and network analysis have made it possible to predict whether a specific sequence is likely to belong to functional groups 2 b (original broad spectrum), 2be (ESBL), or 2br (inhibitor resistant) (Zeil *et al* ., 2016). Some of the variants had a regional predominance at the height of the TEM-type ESBLs' popularity. For instance, TEM3 was rather frequent in France but uncommon in the USA (Soilleux *et al* ., 1996).

In contrast , TEM -10 was the most common TEM -type ESBL in the USA.21 It's interesting that TEM-26 was found in isolates from all over the world. TEM-type enzymes became less common as CTX-M-type b-lactamases overtook them as the most common ESBLs globally. Less than 1% of ESBL- producing *E.coli* and *Klebsiella pneumonia* were found to produce TEM-type ESBLs in a recent study of European isolates (Kazmierczak *et al* ., 2020).

2-CTX-M β-lactamases gene :

The CTX-M class of enzymes initially came to light in the late 1980s and concurrently emerged in several locations. The term CTX -M (cefotaximas from Munich) was first used in a German paper (Bauernfeind *et al* ., 1990).

Following these initial reports, outbreaks occurred in a number of nations. Later, the 'CTX-M pandemic' would be used to describe the global spread of isolates bearing these ESBLs (Celenza *et al* ., 2006; Picão *et al* .,2009).

Based on sequence homologies, the majority of CTX-M enzymes may be divided into five groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. The CTX-M15 group is by far the most prevalent CTX-M-1 subgroup, followed by CTX-M-3 and CTX-M-1.44 The most prevalent enzymes in the CTX-M-9 group were CTX-M-9 and CTX-M-14, although more recently, CTX-M-27 has been recorded often.Within their respective groups, CTX-M-2, CTX-M-8, and CTX-M-25 are the most prevalent variants. (Peirano *et al* ., 2020 ; Colmenarejo *et al* ., 2020).

The early CTX-M enzymes exhibited little activity against ceftazidime, in contrast to the TEM- and SHV-type ESBLs that had been previously discovered; nevertheless, subsequent CTX-M variants with increased ceftazidime hydrolytic activity were characterized. The CTX-M-15 and CTX-M-27 enzymes, which belong to the CTX-M-1 and CTX-M-9 groups, respectively, are significant instances of CTX-M enzymes that exhibit ceftazidime hydrolysis.When compared to its ancestor, CTX-M-3, CTX-M-15 exhibits a single amino acid change at position 240 (Asp to Gly) (Poirel *et al* ., 2002).

3. **OXa β-Lactamases**

Class D -lactamases, also known as oxacillinases or OXA-type -lactamases (OXAs), are active-serine-site enzymes that are comparable to Ambler class A and class C -lactamases. However, they differ from class A and C enzymes in amino acid structure, which can confer resistance to penicillins, cephalosporins, and, in some cases, carbapenems. Some OXA variants may be transmitted between pathogenic species because the OXA enzymes can be chromosomally or plasmid-mediated. The sensitivity

of OXA enzymes to inhibitors often varies greatly (Stojanoski *et al*., 2015).

4-**IMP β-lactamases gene:**

Carbapenemases in *Proteus spp*. Carbapenems (imipenem, ertapenem, meropenem, doripenem) remain in many countries the "last resort" antibiotics for the treatment of severe infection caused by ESBL-producing Enterobacterales. The recent emergence and rapid spread of carbapenemase-producing Enterobacterales (CPE) is a major public health issue as clinical therapeutic options are considerably limited. Acquired carbapenemases identified in *Proteus spp*. are (i) Ambler class A carbapenemases such as KPC-2, (ii) metallo-β-lactamases (MBLs, Ambler class B) VIM-1, IMP-type and NDM-1 (Naas *et al*., 2008; Ohno *et al*., 2017).

3. Materials and Methods

3.1 Materials

3.1.1. Apparatus and Equipment's

The instruments and equipment, which used throughout this study, are listed in Table (3-1)

Table (3-1): Instruments and equipment used with their Manufactures

Apparatus and Equipment's	Company / origin
Autoclave	Japan / Hirayama
Burner	Amal/ Turkey
Centrifuge	Hettich /Germany
Digital camera	Canon / Japan
Disposable petri dishes	Dean's gate/ China
Electric Sensitive balance	Precia / Swiss
Eppendorf tube (different size)	Eppendorf /Germany
Freezer	Media /China
Gel Imaging System	Major Science/ Taiwan
High-speed cold Centrifuge	Lab Net/ USA
Incubator	Fisher/ U.S.A
Light microscope	Nikon/ China
Microwave Ovenn	Gosonic / China
Multi-micropipettes Different size	Human /Germany
Plain tubes (Different size)	Afco/ Jordon
Refrigerator	Media /China
Safety cabinet (Hood)	card III / U.S.A
Thermal Cycler	Thermo Fisher Scientific/ USA

Vortex	Sturate / England
Water bath	China
Water distillatory	Gallenkamp / England

3.1.2. Disposable Materials

The following disposable material has been used in this study, as shown in Table (3-2).

Table (3-2): Disposable materials

Disposable Materials	Company/ Origin
Cotton swab	Afco / Jordan
Dropper	HAD/China
Eppendorf rack	
Eppendorf tube	Sigma /England
Glass slides	Himedia / India
Latex gloves	Broche /Malaysia
Mask	HAD/China
Parafilm	Afco /Jordan
PCR tubes	Eppendorf /Oxford
Petri dishes	Afco- Dispo /Jordan
Sterilizer needles(Different size)	
Test tube rack	HAD/China

3.1.3. Chemicals and Biological Materials

The chemicals and biological materials that were employed in this study were listed in Table (3-3).

Table (3-3): Biological and Chemical Materials

Material	Company / Origin
Agar – agar	Oxoid /England
Agarose	Promega / USA
Barium Chloride ($BaCl_2$), Sulfuric Acid (H_2SO_4),	CDH (India)
Deionized sterile D. W	Bioneer/South Korea
Ethanol 95%, 70%	BDH/England
Goldview dye	Biosharp / China
Free of Nuclease Water	Himedia/ India
Hydrogen peroxide(H_2O_2)	Al-Razi/Iraq
Kovacs regent	Himedia India
Loading Buffer Blue (6 x DNA)	Eurx (Poland)
Normal Saline	Rasan pharmaceutical Iraq
Sodium chloride(NaCl)	BHD /England
Standard Mcfarland solution 0.5	Biomerux/France
TBE BUFFE (Tris-Borate-EDTA Buffer)	Ambion /USA
TEMED (N,N,N,N-tetramethylene	BDH/England diamine
Urea solution (40%)	Flukachemika/ Switzerland

3.1.4. Culture Media

According to the instructions of the manufacturer Himedia (India) the cultivation media used for the current study were sterilized by autoclaving at 121 ° C for 15 min, Table (3-4) .

Table (3-4): Culture Media

Media	Purpose
Blood agar	It is useful for determining the hemolytic and Swarming phenomenon capabilities of an organism (Forbes,*et al*,2007;Brooks *et al* 2013)
MacConkey agar	It is a selective and differential media. It is used in the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria (Forbes,*et al*,2007;Brooks *et al* 2013)
Brain heart infusion agar	Enrichment media used for the cultivation, and maintenance of isolated bacteria for a short period (Forbes,*et al*,2007;Brooks *et al* 2013)
Brain heart infusion broth	Is a highly nutritious general-purpose growth medium recommended for preservation. It activates bacterial growth and keeps in frozen by adding 15% glycerol (Forbes,*et al*,2007;Brooks *et al* 2013)
Mueller Hinton agar	Used to determine the susceptibility of bacteria to antibiotics (CSLI, 2021)
Nutrient broth	It is a general-purpose medium that can cultivate non-highly nutritious bacteria; (Forbes,*et al*,2007;Brooks *et al* 2013)
Nutrient agar	A simple culture medium is widely used in

	microbiological testing to grow a variety of types of bacteria. It is one of the non-selective media used in the routine cultivation of microorganisms (Forbes,*et al*,2007;Brooks *et al* 2013)
Triple Sugar Iron Agar (TSI)	It's a differential medium that's used to identify bacteria based on the fermentation of glucose, lactose, sucrose, and hydrogen sulfide (H_2S gas). (Forbes,*et al*,2007;Brooks *et al* 2013)
Simmons citrate agar	Determining the ability of bacteria to utilized citrate as the sole of carbon source
Methyl Red (MR)/ Voges- Proskauer (VP) media	Used to determine whether the bacteria can ferment glucose and if it can produce acid (MacFaddin, 2000).
Urea agar medium	This medium was used to examine bacteria's capacity to manufacture the urease enzyme (Forbes,*et al*,2007;Brooks *et al* 2013)

3.1.5. Kits

The kits which were used in this study, are listed in Table (3-5).

Table (3-5): Study Kits

Kits	Company/origin
DNA ladder (100-1500) bp	Solgent/ Korea
Genomic DNA Mini Kit	FAVORGEN/Taiwan
GoTag Green Master Mix	Solgent/Korea
Gram stain Kit	Ward's Natural Science/ USA
Primers	Macrogen /Korea

3.1.6. Antibiotics Discs

The antibiotic discs were employed in the study are listed in the Table (3-6).

Table (3-6): Antibiotics Discs

Family	Antibiotics	abbreviations	Concentration	Company (Origin)
Quinolones	Ciprofloxacin	CIP	µg	Bioanalse/ Turkey
	Levofloxacin	LEV	5 µg	
Cephalosporin	Cefotaxime	CTX	30 µg	
	Cefuroxime (parenteral)	CXM	30 µg	
	Ceftazidime	CAZ	30µg	
Monobactams	Aztreonam	ATM	30 µg	
Carpenems	Impenem	IPM	10 µg	Biolab Zrt/Hungarian
	Meropenem	MEM	10 µg	
Ntrofurans	Nitrouranation	F	300 µg	
Aminoglycoside	Gentamycin	CN	10 µg	
	Amickcin	AK	30 µg	
Folate pathway antigonistic	Trimethoprim Sulfamethoxazole	SXT	1.25 /23.75 µg	

3.1.7. PCR Oligonucleotide Primers

The designed primer pairs were employed in the study are listed in the Table (3- 7).

Table (3-7): Oligonucleotide Primers used in this study

Groups	primers		5 – sequence-3	Amplicon size (bp)	Reference
Identification Gene	*atpD*	**F**	GGTGCGGGTGTTGGTAAAAC	**570**	**This Study**
		R	TGAATCCAGTGGGTCAACCG		
Antibiotics Resistant Genes	***bla*** $_{TEM}$ **(General)**	**F**	**CGTGTCGCCCTTATTCCCTT**	**723**	
		R	**CAGTGCTGCAATGATACCGC**		
	bla $_{CTTX-MI}$	**F**	**GAC GAT GTC ACT GGC TGA GC**	**499**	**(Kiiru *et al*.,2012)**
		R	**AGC CG C CGA CGC TAA TAC A**		
	bla $_{CTX-MII}$	**F**	**GCG ACC TGG TTA ACT ACA ATC C**	**351**	
		R	**CGG TAG TAT TGC CCT TAA GCC**		
	bla $_{CTX-MIV}$	**F**	**GCT GGA GAA AAG CAG CGG AG**	**474**	
		R	**GTA AGC TGA CGC AAC GTC TG**		
	bla-$_{OXA}$	**F**	**TTGGTGGCATCGATTATCGG**	**473**	**This Study**
		R	**GAGCACTTCTTTTGTGATGGC**		
	bla-$_{Imp1}$	**F**	**TGAGCAAGTTATCTGTATTC**	**740**	**This Study**
		R	**TTAGTTGCTTGTTTTGATG**		

3.1.8. Study Scheme

Scheme of study illustrated in Figure (3-1).

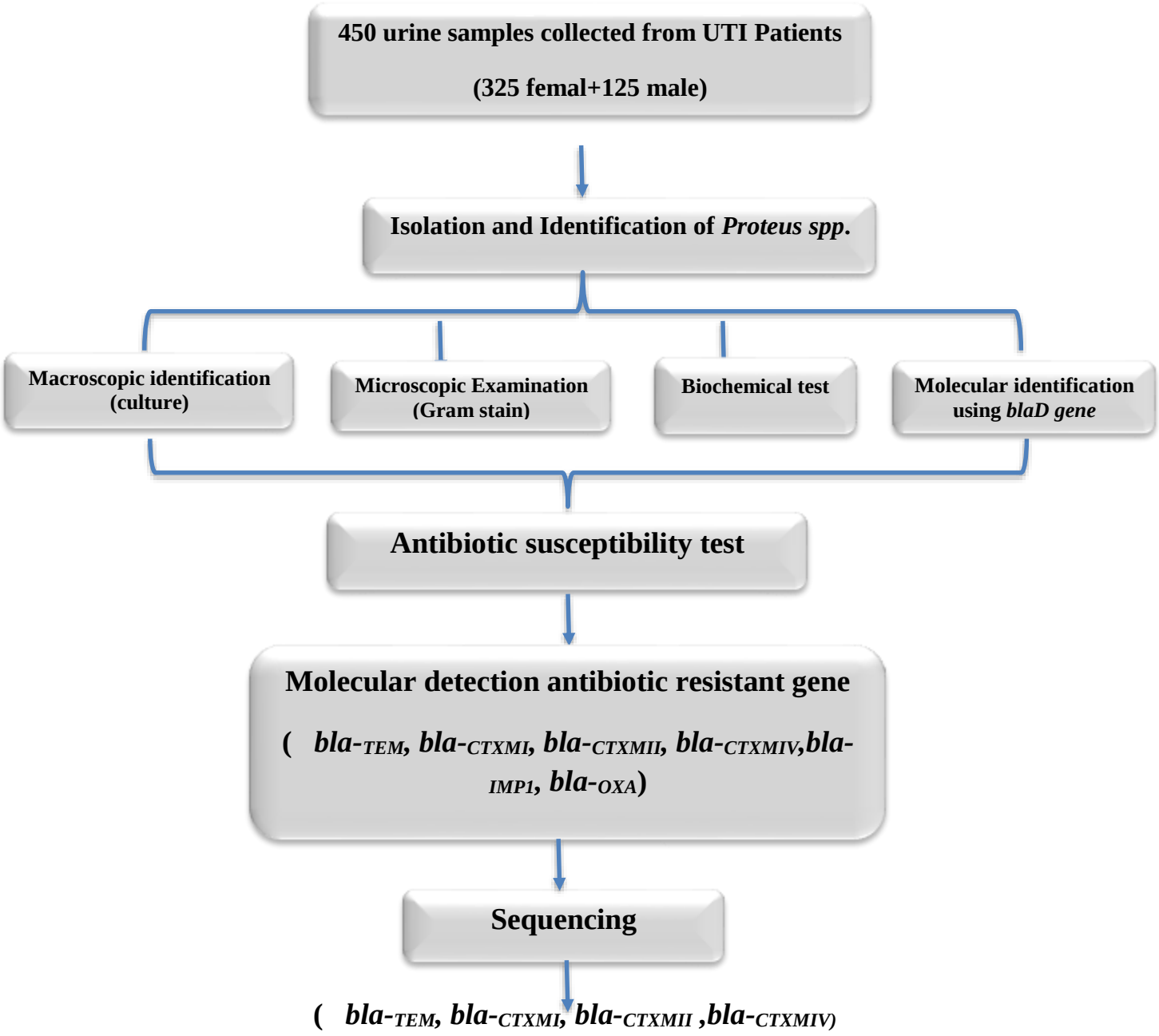


Figure (3-1): study Scheme

3.2. Methods

3.2.1. Specimens Collection

Collected 450 urine specimens (325 female and 125 male) with urinary tract infections. These patients ranged in age from (17 to 70) years old from three Iraqi hospitals (Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, and Shomali General Hospital) from the period from July 2021 to the end of October 2022. 1ml of these specimens were placed into brain heart infusion broth and transported to laboratory to culture on appropriate media for isolation bacterial species (Jordan *et al.*, 2017).

3.2.2. Identification of *Proteus mirabillis*.

The isolation and identification of bacterial were carried out using standard bacteriological procedures(Pathirana *et al.*, 2018). All colonies suspected as *Proteus* in primary cultures were purified by subculture on different media. Bacteria were isolated by spreading the samples on blood and MacConkey agar for the first isolation, then all plates were incubated at 37°C/24hrs. Bacterial colonies were firstly identified depending on their morphological shape, color, and size on MacConkey agar; Pale or colorless as non-lactose fermenters (NLF) colonies that can be easily distinguished from other Enterobacteriaceae by the swarming phenomenon on blood agar. These colonies were sub cultured on MacConkey agar plates several times to obtain pure cultures. Species were identified according to the morphological features on culture media, microscopic examination, and biochemical tests(Forbes, *et al*, 2007;Brooks *et al.*, 2013).

3.2.2.1 Culture Characteristics

From each primary positive culture, a single colony was obtained. The morphology characteristics (colony size, shape, color, swarming, odor, translucency, edge, and texture elevation) on blood agar were used to identify it. While for determining Enterobacteriaceae ability to grow and lactose sugar fermentation, colonies shape, bacterial species were cultured on MacConkey agar.

3.2.2.2. Microscopic Examination

A colony from each bacterial isolate was fixed on a slide and stained by Gram stain to examine their reaction and shape according to (MacFaddin, 2000).

3.2.2.3. Molecular Identification

DNA extraction from all isolates was performed using a high pure PCR template preparation kit (FavorPrep™ Cultured Cell Genomic DNA Extraction Mini Kit). molecular identification of *P.mirabilis* by using *atpD* gene , the nucleotide sequencing of the gene and the polymerase chain reaction described in Table (3.7).

3.2.3. Media Preparation

3.2.3.1. Blood Agar Medium

Blood agar medium was prepared by dissolving 40 g blood agar base in 1000 ml D.W. Heat to boiling and sterilize at 1.5 bar pressure (121°C) for 15 minutes by autoclaving, cool to 45ºC and 5% of fresh human blood was added, mix well then pouring into petri dishes. It was used as enrichment

medium for the bacterial isolates and to determine their ability to hemolysis Red blood cells (RBCs) (Forbes *et al*., 2007).

3.2.3.2. MacConkey Agar Medium

MacConkey agar medium was made by dissolving 49.53 g in1000 ml distilled water, Then heat medium to boiling. Sterilize at 1.5 bar pressure at 121°C for 15 minutes by autoclaving. Cool to 45-50°C, and distributed into petri dishes, this medium was used to isolate Gram-negative bacteria by testing their ability to ferment lactose (MacFaddin, 2000).

3.2.3.3. MR-VP Medium

Methyl Red-Voges.proskauer Medium/Broth (MR-VP medium) was mad by adding 17 g in in1000 ml distilled water, and distributed in to test tubes after boiling medium by heat, then sterilized by autoclave at 1.5 bar, 121°C for 15 minutes. This medium used to detect the partial and complete hydrolysis of glucose (MacFaddin, 2000).

3.2.3.4. Brain Heart Infusion (BHI) Broth

Brain Heart Infusion BHI broth was made according to the manufacturing company by dissolving 37 g in 1 liter of distilled water and autoclaved at 121ºC for 15 minutes (MacFaddin, 2000).

To prepared this media, 100 ml D.W was mixed with 1.3 gm nutrient broth and 0.7 gm agar-agar, pH was adjusted to 7.2, and the mixture was placed into test tubes, autoclaved, and left to set in the vertical position (Collee *et al*. , 1996)

3.2.3.6. Urea Agar Medium

This medium was prepared by combining 15 mL of urea solution sterilized by filtering (0.22μm in diameter) with 100 mL of urea agar base sterilized by autoclaving at 1.5 bar ,121°C for 15 minutes and cooled at 50°C. The medium was then distributed into sterile test tubes and allowed to harden in a slant shape after the pH was adjusted to 7.1(Mahon and Manuselis, 2007).

3.2.3.7. Triple Sugar Iron Agar (TSI)

It's a differential medium that's used to identify bacteria based on the fermentation of glucose, lactose, sucrose, and hydrogen sulfide (H_2S gas). To dissolve the TSI powder, it was suspended in 1000mL distilled water and brought to a boil. The medium was then sterilized by autoclaving for 1.5 bar,121°C 15 minutes, cool, and distributed into test tubes, where it solidified in a slant posture (Habibi *et al.*, 2015).

3.2.4. Sterilization

Glassware was sterilized at 160-180°C for 2-3 hrs. by using dry heat sterilization. Media and solutions (not affected by heat) were sterilized by using autoclaves under 15 bar/in pressure at 121°C for 15 minutes (Alagoa and Ozi, 2021).

3.2.5. Solutions and Buffers Preparation

3.2.5.1. Ethanol (70 %)

The 70 % ethanol solution was prepared by completed 70 ml of absolute ethanol (100%) with distilled water.

3.2.5.2 McFarland Standard Solution

The turbidity value of the standard of 0,5 MFU (McFarland Units) corresponds to approximately a culture density of 1,5 x 10^8 cells/ml. This cell density is specially needed as bacterial inoculum for the antibiotic susceptibility test.

3.2.6. Gel Electrophoresis Solution

3.2.6.1 Gold View Dye

Goldview is a novel cyanin nucleic acid dye for detecting DNA and RNA in agarose gel, an alternative to the ethidium bromide (EB). It is used with the same method and sensitivity as EB. It emits green fluorescence upon ultraviolet irradiation when bound to DNA or RNA.

3.2.6.2 Tris –Borat-EDDTA (TBE buffer).

This solution made by dilute 10X stock TBE buffer with distilled water producing 1 L by the addition of 50ml of 10X TBE buffer to950 ml of the D.W, this volume has been completed to 1L in 1L volumetric flask, the value of the pH has been adjusted to 8.3 (Dahwash *et al.*, 2021).

3.2.7. Reagents

3.2.7.1 Oxidase Reagent

One gm of N-N-N-N – tetramethyl – P phenylene diamine di hydrochloride (TMPPD, Wurster's reagent) was dissolved in 100 ml of D.W. and the final solution was stored in a dark bottle in the refrigerator to prepar

this reagent fresh. This solution was used to test the ability of different species to produce oxidase (Shields and Cathcart, 2013).

3.2.7.2 Catalase Reagent (3%)

According to Forbes e*t al.,* (2007), 5 mL of 6 % hydrogen peroxide was combined with 5 mL of distilled water and stored in a dark bottle in the refrigerator. This reagent was used to assess the ability of bacteria to produce catalase.

3.2.7.3 Gram Stain

A little quantity of bacteria from a colony was distributed with a drop of normal saline on a clean slide, fixed by heat, and then dyed with crystal violet, Iodine, alcohol, and safranine before being magnified 100 times and studied (Leboffe and Pierce, 2015).

3.2.8. Biochemical Tests

3.2.8.1.Oxidase Test

A single colony of bacteria isolates were transferred to a piece of filter paper by wooden applicator sticks, and then added 2-3 drops of oxidase reagent (1%) to the filter paper. Positive result was indicated by dark purple color presence within 10 seconds and negative results if no change in color happen. This test was used to identify the ability of bacteria to produce the enzyme oxidase (Li *et al.*, 2017).

3.2.8.2.Catalase Test

This test was performed to see if bacterial strains could produce catalase, an enzyme that could convert hydrogen peroxide to water and produce oxygen bubbles. A sterile loop was used to transfer a tested colony to a clean glass slide, and then a drop of 3 percent H_2O_2 was added. The presence of oxygen bubbles suggests a positive result (Atlas, Parks and Brown, 1995).

3.2.8.3.Urease Test

This test was performed to look for urease synthesis in the bacteria that were examined. The examined bacterial colony was inoculated on urea agar, which was previously produced and specified in section ,and incubated at 37°C for 24 hours. The change in color of the medium from yellow to pink denotes a positive result (MacFaddin, 2000).

3.2.8.4.Motility Test

In semi-solid motility media, bacterial isolates was tested for motility, , a pure culture was injected or stabbed in center of medium glass test tube by using needle tube then incubated overnight at 37ºC, movement away from the stab line or presence of cloudy around the stabbing line region indicated motility of bacteria (Chelius and Triplett, 2000).

3.2.8.5.Triple-Sugar Iron Agar Test (TSI test)

This test was used to detect the type of fermentation in bacteria as well as the type of sugar that they could ferment. Triple-Sugar Iron (TSI) agar slant medium previously prepared in section which contain 3 types of sugar (glucose, lactose, sucrose) was stabbed and streaked with the tested bacterial culture, then incubated overnight at 37˚C. The color of the slant and the

bottom of the media were used to determine the result after the incubation period .Sugar fermentation turns the bottom from red to yellow as a result to acid production, while the whole yellow medium detects the whole fermentation of sugars. Bubbles might appear in the medium due to gas (CO_2) formation from sugar aerobic fermentation while black residue might develop at the bottom due to the reaction of H_2S produced from anaerobic fermentation with ferrous sulfate in the medium (Atlas, 1995).

3.2.9. Maintenance of Bacterial Isolates

3.2.9.1 Maintenance for the Short Term

Bacterial isolates were kept on Brain heart infusion agar plates for a brief time and stored at 4 C° (Harley and Prescott, 2002).

3.2.9.2 Maintenance for the Medium Term

Culturing the bacteria on brain heart infusion agar slant using screw capped containers allowed for medium-term storage of up to four weeks. The tubes were incubated for 24 hours before being stored at 4C° (Harley and Prescott, 2002).

3.2.9.3 Maintenance for the Long Term

The isolates were cultured in a brain-heart infusion broth for 18 hours at 37°C before 20% sterile glycerol was applied and maintained at -20C° (Goldman and Green, 2015).

3.2.10. Antibiotics Susceptibility Test

The antibiotics susceptibility test was carried out by following KirbyBauer method described by CLSI ,(2022) (Table 3-5).

1 - A few colonies were transferred from a pure and fresh culture to a sterile test tube containing 5 mL of normal saline.

2 - Tube was compared to the 0.5 McFarland turbidity standard described in and the test suspension's density was modified to match the standard's by adding more bacteria or sterile saline.

3 - By dipping a sterile swab into the inoculum, the plates of sterile Mueller Hinton Agar were inoculated. Extra inoculum was eliminated by forcing and rotating the swab against the tube's wall just above the liquid's level.

4 - The swab was streaked three times across the medium's surface, each time rotating the plate at a 60^{o} angle after each application. Finally, the swab was crossed along the agar surface edge.

5 - The plates were left to dry at room temperature for a few minutes with the lid closed.

6- Using a pair of sterile forceps, the suitable antimicrobial – impregnated discs were located on the surface of the agar. On the Mueller Hinton Agar plates, discs should not be closer than 24 mm (center to center). Each disc was gently pressed down to ensure complete contact with the agar surface and that it did not slip out when the plate was inverted during the incubation process.

The diameter of each zone (including the diameter of the disc) was measured and recorded in mm after 18 hours of incubation. The antimicrobial susceptibility interpretation chart was used to record the data (CLSI,2022) . The measurements were taken with a ruler on the underside of the plate, and

the endpoint of inhibition was determined by looking at the edge of the plate where the growth began.

3.2.11. Molecular Identification

3.2.11.1. Extraction of Genomic DNA

Genomic DNA was extracted from bacterial growth using the ABIO pure extraction procedure, which included the following steps:

❖Two ml of an overnight culture was transferred to a 1.5ml micro centrifuge tube and centrifuged for 5 minutes at 13000 rpm to prepare pellet cells. The supernatant was thrown away.

❖ The cell pellet was re-suspended completely in 200 µl of Buffer CL.

❖ Buffer BL 200µl was added to sample after incubation, and then the tube was forcefully mixed by vortex and was incubated at 70˚C for 30 min.

❖ Absolute ethanol (200 µl) was added to the sample, which was then vortexed extensively.

❖ All of the mixtures were carefully transferred to a mini-column and centrifuged for 1 minute at 6,000 xg (>8,000 rpm). The collection tube was replaced with a fresh one.

❖ The mini-column was filled with 600 µl of Buffer BW, centrifuged for 1 minute at 8,000 rpm, and the collecting tube was replaced.

❖ Buffer TW was used in the amount of 700 µl. It was centrifuged at 6,000-8,000 rpm for 1 minute. The pass through was discarded and mini- column was re-inserted into the collection tube.

❖ To remove any remaining wash buffer, the mini-column was centrifuged at full speed for 1 minute. A new tube was used to contain the tiny column (1.5 ml).

❖ A 100 µl of Buffer AE was added and incubated for 1 minute at room temperature.

❖ Then it was centrifuged at 5,000 rpm for 5min.

The integrity of extracted DNA was examined by 1.5 % agarose gel electrophoresis and viewed using UV trans illuminator.

3.2.11.2 Quantitation of DNA

Concentration of the DNA of the isolates were estimated by use the Nano drop through putting 1µl of obtained DNA in instrument for the detection of the concentration in ng/µm and the purity has been obtained through the noticing of OD 260/280 ratio for checking DNA isolates contamination with the protein (Albadri, 2021)

3.2.11.3 Preparation of Primers Stock

Macrogen Company supplied all primers in a lyophilized form (Table 3-7). Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared as follow: 10µl of primer stock solution (stored at freezer -20˚C) was added to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl (Nasser *et al*., 2021).

3.2.11.4. Amplification Reaction

The genus and species were validated through molecular identification, which was carried out in 50 bacterial isolates using polymerase chain reaction (PCR) amplification of the(*bla*$_{TEM}$, , *bla* $_{CTX-MI}$, *bla* $_{CTX-MIV}$, *bla* $_{CTX-MII}$, *bla* $_{IMP1}$, bla $_{OXA}$) gene . PCR master was mixed and primers solutions were stirred by vortex, at room temperature. These solutions were added as described and the amplification program is shown in Table (3-8).

Table (3-8): Contents of the Reaction Mixture

Contents of reaction mixture	Volume (µl)
Master Mix	12.5
Doctor dye	2.5
Forward primer	1.5
Reverse primer	1.5
Template DNA	3
Nuclease free water	4
Total	25

The components of tubes were mixed by vortex and then placed in PCR apparatus.

Table (3-9) : PCR Thermal Cycling conditions

Genes	Thermocycling conditions										No. of Cycles
	Initial denaturatio		Denaturatio		Annealing		Extension		Final extension		
	Temp (C	Time min	Temp (C)	Time sec	Temp (C)	Time sec	Temp (C)	Time sec	Temp (C)	Time min	
atpD	95	5	95	30	55	30	72	30	72	5	30
bla$_{CTX-M-}$	94	2	94	15	55	30	72	30	72	3	30
bla$_{CTX-M-}$	94	2	94	15	55	30	72	30	72	3	30
bla$_{CTX-M-}$	94	2	94	30	62	30	72	30	72	5	30
bla$_{TEM}$	95	5	95	30	60	30	72	30	72	5	30
bla$_{Imp-1}$	95	2	95	30	51	30	72	100	72	5	35
bla$_{OXA}$	95	5	95	30	55	90	72	60	72	5	30

3.2.11.5 Agarose Gel Electrophoresis

After PCR amplification, the existence of amplification was confirmed using agarose gel electrophoresis.

3.2.11.5.1 Agarose preparation

❖ One hundred ml of 1X TBE taken in a flask.

❖ Agarose 1.5 gm (for 1.5%) was added to the buffer.

❖ The solution was heated until all of the particles were dissolved (in the microwave).

❖ Five µl of goldview (10mg/ml) was added to the agarose.

❖ The agarose was moved in order to get mixed and to avoid bubbles.

❖ At 50-60°C, the solution was allowed to cool (Sambrook and Russell, 2001).

3.2.11.5.2 DNA Loading

A total of five µl of PCR products were directly put into each well. For 60 minutes, electrical power was turned on at 100V/mAmp. DNA was transferred from the cathode to the anode poles. Gel imaging system UVtransilluminator scope was used to see the Goldview-stained bands in the gel(Bhattacharya and Van Meir, 2019).

3.2.12.DNA Sequencing of Amplified Product

The amplified DNA was re-extracted and purified by gel electrophoresis according to the protocol suggested by Macrogen sequencing corporation (Macrogen/Korea) and then submitted to this company for sequencing. The DNA sequence data of the studied genes were analyzed and aligned according to BioEdit and MEGA-X programs and compared with reference sequences available in the GenBank (NCBI) database for identification of polymorphisms and phylogenetic tree construction.

3.2.13. Ethical Approval

1-The study was done and the cases were collected after getting the agreement of the patients (verbal consent).

2- Approval of Babylon Science College Ethical committee.

3- Before starting the study, permission were taken from Babylon health presidency.

3.2.114. Statistical Analysis

The experimental results were expressed as percentage (%). Statistical analysis was carried out using chi-square. The value of $p \leq 0.05$ was considered to be statistically significant (Wayne, 2011).

4 . Results and Discussions

4 .1. Derwgraphic Study

In this study, a total of 450 specimens were taken from patients with UTI and divided into seven age groups: <20 years 76 (16.8%), 20–30 years 206 (45.7%), 31–40 years 89 (19.7%), 41–50 years 37(8.2%), 51–60 years 29 (6.4%), 61–70 years 12(2.6%), and >70 years 1(0.2%). Additionally, the specimens included 325 females and 125 males.

Majority of studies concluded the predominance of female UTI as compare to male UTI (Bilal *et al*.,2018).

It is worth noting that, there is a clear increase of UTI cases with age in female compare than in male as 72.2% of UTI cases were observed in women. This same trend was also observed for both males and females, despite the low number of males with UTI in this study. The majority of the bloodstream infections caused by *Proteus spp.* originate from a UTI and are often associated with urinary catheters. Despite the fact that a precise cause-effect relationship has not yet been established, multiple factors are suggested to be involved in the high occurrence of UTIs. These include but are not limited to glucosuria, increased bacterial adherence to uroepithelial cells due to hyperglycemia, and neurogenic bladder (Geerlings *et al*.,2008).

Table (4-1): Patient characters

Age / Sex	Male NO. (%)	Female NO. (%)	Total NO. (%)	p-value
< 20	23(5.1)	53(11.7)	76(16.8)	**0.001****
20-30	45((10)	161(35.7)	206(45.7)	**≤0.0001****
31-40	31(6.8)	58(12.8)	89(19.7)	**≤0.0001****
41-50	11(2.4)	26(5.7)	37(8.2)	**0.014***
51-60	9(2)	20(4.4)	29(6.4)	**0.041***
61-70	6(1.3)	6(1.3)	12(2.6)	**1.000**
>70	0(0)	1(0.2)	1(0.2)	**0.925**
Total	125(27.7)	325(72.2)	450(100%)	**≤0.0001****

* refer to significant difference at P≤0.05.

** refer to significant difference at P≤0.05.

The highest rate of bacterial infection was within the age group of 20-30 (10 %) years in male and in female (35.7) , followed by the age group of 31-40 (6.8%) years in male and in female (12.8), <20 (5.1%) years in male and in female (11.7), followed by 41-50 (2.4%) years in male and in female (5.7), 51-60 (2%) years in male and in female (4.4), 61-70 (1.3%) years in male and in female (1.3), and >70 (0%) years in male and in female (0.2). As shown in Table (4-1).

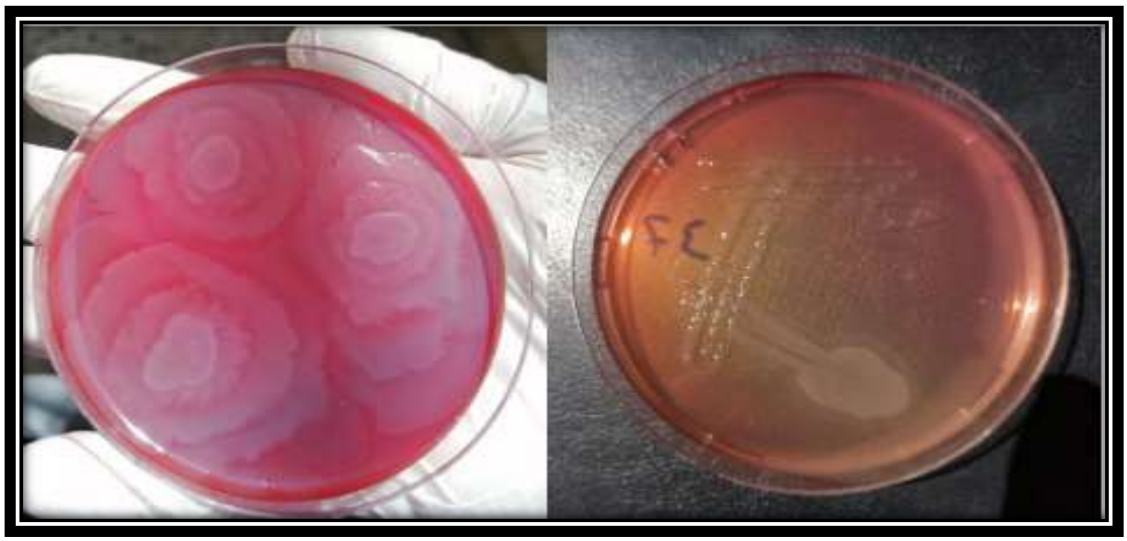
Depending on sex, the result show the UTIs was more prevalent among females compared to males. Females were thus 3.4 times more likely to develop UTI than males. This finding has been observed in other studies higher prevalence of UTI in females has been attributed to physiological and anatomical differences in both sexes. UTIs occur more frequently in women than men because the shorter, wider, female urethra appears to be less effective in preventing access of bacteria to the bladder (Vasudevan, 2014).

The results of the percentage distribution according to the sex of the patients agreed with the findings of (Al Benwan *et al*.,2010) showed that the majority of UTIs occurred in women (88.5%), in agreement with previous studies, and thereby confirming that adult women have a higher rate of UTI prevalence than men.

When culturing the samples on MacConkey agar, Because of the presence of bile salts, *Proteus* isolates produced pale-colored colonies on MacConkey agar medium which was used to grow *Proteus spp*. Isolates suspected of being *Proteus* emerged as tiny colonies, little convex and round with smooth edges do not swarm, pale or colorless non-lactose fermenter colonies, which distinguish it from other Gram negative organisms. The MacConkey agar ,a selective and differential medium that allows the growth of gram- negative bacteria while preventing the growth of gram-positive bacteria due to its content bile salt which inhibit the growth gram-positive bacteria (Al-Ansari *et al*.,2020).

Furthermore, *Proteus* culture has a distinct odor (fish smell). On blood agar *Proteus* isolates were shown swarming phenomenon on solid media (Figure 4-1).

Isolates are Gram-negative, rod-shaped, and facultative anaerobic. *Proteus* appeared as short pink Gram-negative rods under the microscope.



A **B**

Figure (4-1): Growth *Proteus mirbillis* on

A) Blood agar showing swarming phenomena

B) MacConkey agar showing non lactose fermenter

Several conventional biochemical tests were done to characterize the suspected *Proteus mirabillis* isolates and the results indicated that these isolates were primarily identified as *Proteus mirabillis*. . All the isolates have been shown positive results to catalase, urease, H_2S and motility tests, and negative to oxidase test and Indol production. *Proteus mirabillis.* isolates , unlike the coliforms, deaminates phenylalanine to phenyl pyruvic acid .All the isolated negative to indol test ,this biochemical test distinguish *proteus mirabillis* from *proteus vulgaris* positive to the indol . As shown in the (Table 4-2, and Figure 4-2).

These results are in agreement with the results of Jarjes, (2019) and Al-Jumaily and Zgaer, (2016) who were found (100%) of *Proteus* isolates have been showed strong production of urease, catalase, motility and H_2S while no production of oxidase was observed.

Catalases are enzymes that catalyze the breakdown of hydrogen peroxide (H_2O_2) into water and molecular oxygen (Gebicka and Krych-Madej, 2019). Urease is an important virulence factor in *Proteus* pathogenicity, and urease production is a distinguishing feature of *Proteus* isolates. The gene clusters encoding this enzyme have been cloned from a variety of bacteria, including *Proteus spp.*(Doshi *et al.*, 2022).

Swarming motility is a bacterial colony formation mechanism that has been linked to the pathogenicity of many microbes, allowing them to spread and survive in unfavorable environments like the presence of antibiotics. *Proteus mirabilis* colony expands outward in a bull's-eye pattern as measured by swarming motility test by culture on blood agar, generated by several waves of fast swarming following by consolidation to shorter cells (Doshi *et al.*, 2022).

Table (4-2) Properties of *Proteus mirabilis*

Characteristics	Result
Biochemical Tests	
Oxidase	**-**
Catalase	+
Urease production	+
Triple sugar iron agar) TSI	**alkaline / alkaline, H2S production**
Motility Test	+
Indol production	**-**
Vogeus Proskauer tests	**-**
Citrate utilization	**-**
Bacteriological Properties	
Swarming on Blood agar	**+**
Hemolytic activity	**-**
Lactose fermentation on MacConkey agar	**-**

The motility is the most essential virulence factor in *P. mirabilis* that stimulus the attacking and spreading of infection in urinary tract. *Proteus* isolates produce hydrogen sulfide (H_2S), a chemical substance. H_2S is involved in a variety of physiologic processes in humans, including inflammatory, immunological, endocrine, respiratory, neuromodulatory, and vascular functions. Sulfate-reducing bacteria, such as *Proteus*, are noteworthy H_2S producers in the gut lumen. H_2S generated by bacteria has

cytoprotective qualities at low concentrations by maintaining gut mucus integrity, but it is poisonous to the host at large doses (Buret *et al.*, 2022) .

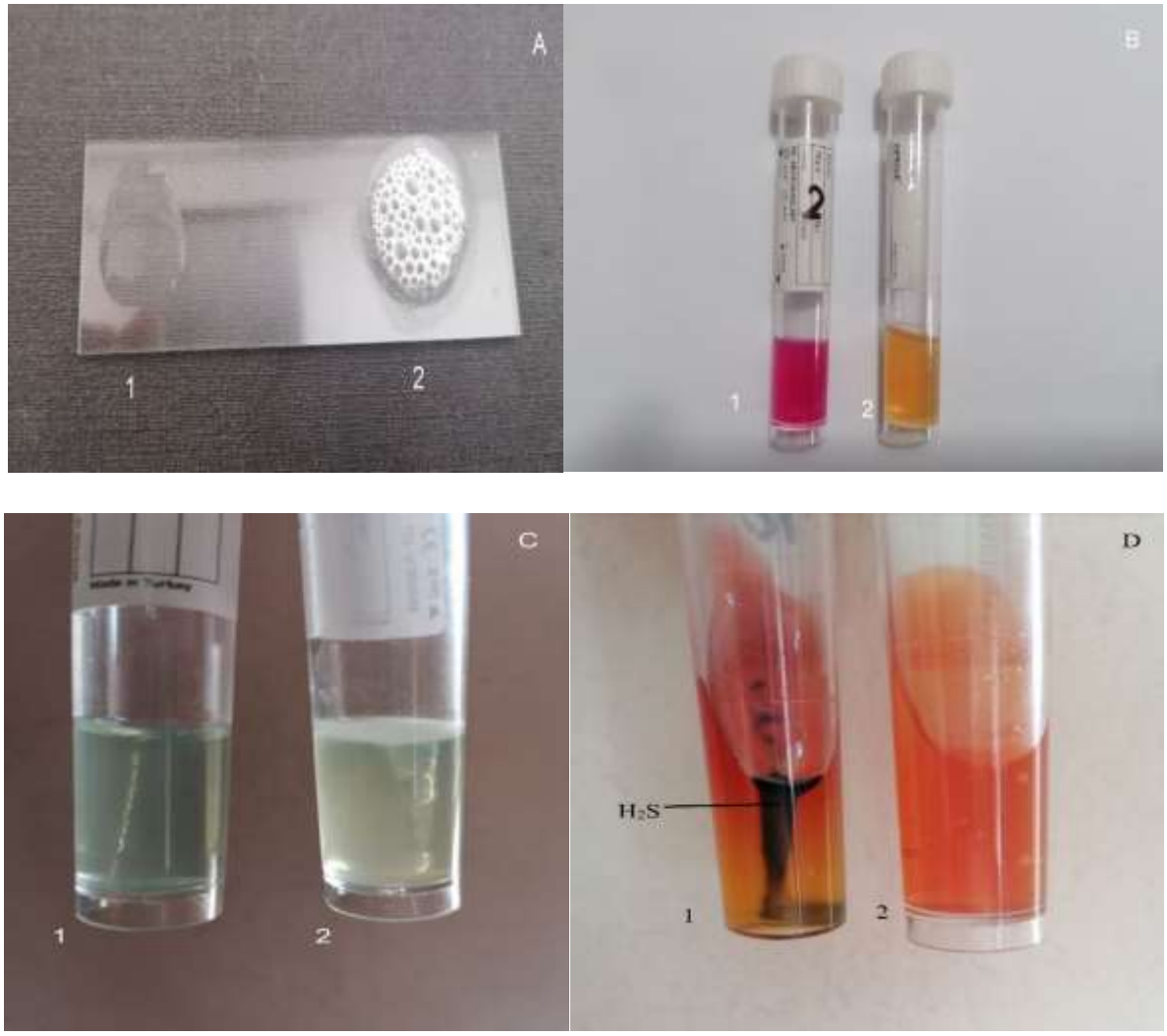


Figure (4-2) :Biochemical tests for *Proteus mirabillis*

A: Catalase test (1- Negative control , 2- Positive result

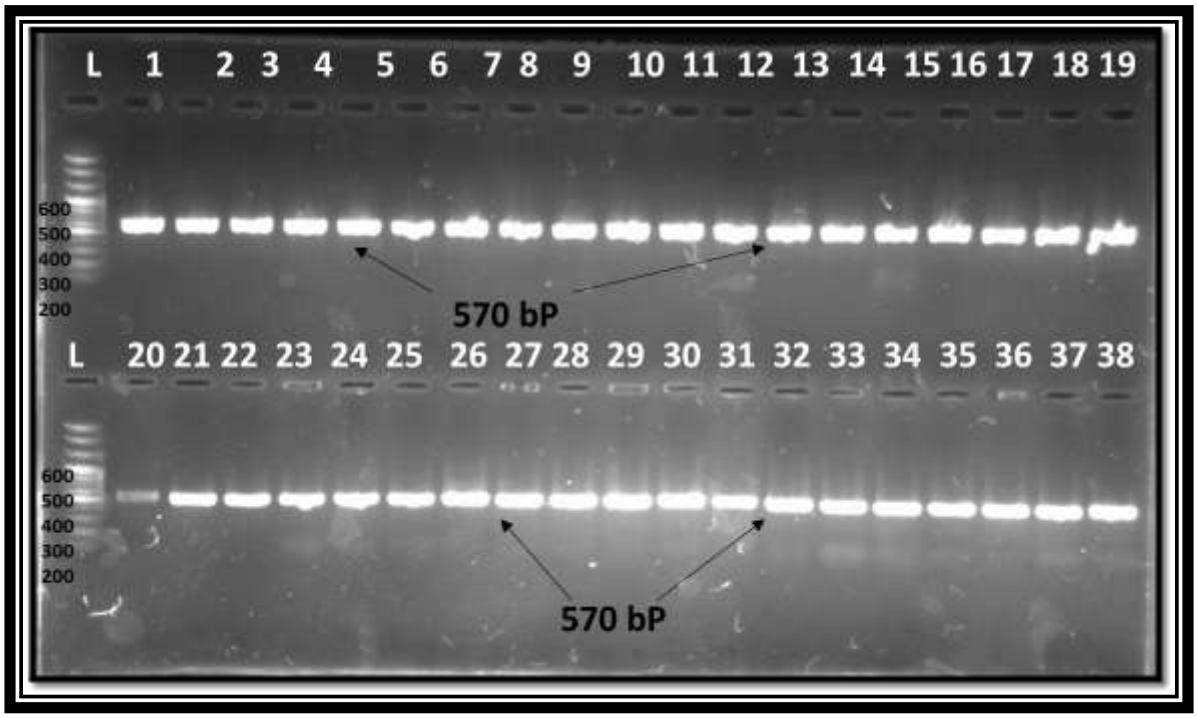
B: Urea test 1- positive result ,2- negative

C: Motility test : 1- negative control, 2 -positive result

D: H_2S test, 1-positive result ,2- Negative result.

The genetic detection of *Proteus mirabilis* isolated accrue by using *atpD* gene, it is specific beater species. The result show, this gene using by other researcher to detected the *Proteus* based on the finding that the *atpD* gene was more

conservative than the *16 S rRNA* gene in bacterial species, the *atpD* gene was chosen as the PCR target for *Proteus* species detection (Bi *et al* ., 2013). Using *atpD* PCR detection, identified 30 *P. mirabilis* strains from 541 samples (5.55%) (Qu *et al*., 2022).



Figure(4-3): 1.5% agarose gel electrophoresis was carried out at 70 volts for 60 minutes after goldview staining to find atpD PCR products. Lane (L) molecular size indicator for DNA (1500-bp ladder). exhibit encouraging outcomes, and the final product measures 570 bp.

The result of culture, biochemical, Gram stain and genetic (*atpD* gene) present the percentage of samples positive for *P.mirbillis* was 70 samples (15.5 %). Among 325(72.2%) isolates of *Proteus mirabilis* in female, 46(10.2%) isolates positive samples and 279(62%) isolates negative samples. Also among 125(27.7%) isolates of *P. mirabilis* in male, 24(5.3%) isolates positive samples and 101(22.4%) isolates negative samples. As shown in the Table (4-3).The

result showed the percentage of *P.mirabilis* isolated from male patients was 24 (34.2%), while from female patients was 46(65.7%) from the total 70 isolates Table(4-3), Table(4-4).

Table(4-3): Number and Percentage of *Proteus mirabillis* Isolates According to the Sex

Sex	**Positive Samples NO.(%)**	**Negative Samples NO.(%)**	**Total NO.(%)**
Female	**46(10.2)**	**279(62)**	**325(72.2)**
Male	**24(5.3)**	**101(22.4)**	**125((27.7)**
Total NO.(%)	**70(15.5)**	**380(84.4)**	**450(100)**
p-value	**≤0.0001****		**≤0.0001****

* refer to significant difference at P≤0.05.

** refer to significant difference at P≤0.05.

Table (4-4): Number and Percentage of *Proteus mirbillis* Isolates According to the age and sex

Age / Sex	**Male NO. (%)**	**Female NO. (%)**	**Total NO. (%)**	**p-value**
< 20	3(4.2)	8(11.4)	11(15.7)	**0.132**
20-30	8(11.4)	19(27.1)	27(38.5)	**0.034***
31-40	9(12.8)	9(12.8)	18(25.7)	**1.000**
41-50	3(4.2)	3(4.2)	6(8.5)	**1.000**
51-60	1(1.4)	2(2.8)	3(4.2)	**0.564**
61-70	0(0)	5(7.1)	5(7.1)	**0.102**
>70	0(0)	0(0)	0(0)	**1.000**
Total	24(34.2)	46(65.7)	70(100)	**0.009****

* refer to significant difference at P≤0.05.

** refer to significant difference at P≤0.05.

This result differed from that of other researchers, who discovered that a total of 38 patients with urinary tract infections at Al-Diwaniyah Teaching Hospital had a total of 15 isolates (or 39.4%) of *P. mirabilis* (Ghazay *et al* ., 2019).There were 250 samples collected from patients with urinary tract infections, and 60 of those samples (or 24%) had *P. mirabilis* that was positively proliferating(Al-Ezzy *et al* ., 2018), and a *P. mirabilis* was found in 53 percent of the 132 urine samples obtained from various Baghdad municipal hospitals (Al-Jumaily& Zgae, 2016) .While Ullah *et al* .,2018 found the percentage of *P. mirabilis* isolates was 8 (13.8 %) more than the result of this study.

Isolated were from urinary tract infections (UTI). The higher percentage of UTI infection than other infections may be due to the colonizing ability of Proteus mirabilis on the surface of urinary catheters or because it is part of normal flora of human gastrointestinal tract which increase the probability of UTI infection. This confirm that *P. mirabilis* is the third most common (after *E. coli* and *Klebsiella pneumoniae*) cause of complicated UTI causing 12% of infections (Zanichelli *et al.*, 2019).

In the current study of *P. mirbillis* isolates according to the age and sex , the results in male group were 3(4.2%), 8 (11.4%), 9 (12.8%), 3 (4.2%),1 (1.4%), 0 (0%), and 0 (0%) for the age group (<20, 20-30, 31-40, 41-50, 51-60, 61-70, and over 70 year respectively). On the other hand, the results in female group were 8(11.4%), 19 (27.1%), 9 (12.8%), 3 (4.2%), 2 (2.8%), 5 (7.1%), and 0(0%) for the age group (<20, 20-30, 31-40, 41-50, 51-60, 61-70, and over 70 year respectively). As shown in the table (4-4).

The results of the percentage distribution of the incidence according to the sex of the patients agreed with the findings of Shehab and Jassim (2019). In contrast, Shahraki *et al*., (2018) observed a percentage of 43 % males to 57 % females.

The possible reasons may be due to the types of populations studied, different geographic locations, types of hospitals. Furthermore, males may have routine outdoor work and are often at risk of infection from infected environments (Manandhar *et al.*,2017).

This variation may result from the geographic distribution, sample type, number of tested samples, uptake of the antibiotics, environmental and socio-economic factors, accurate an gynecologist takes a sample from a patient, the method of diagnosis, the number of tested samples, the type of samples, and cultural factors.

4.2. Antibiotic Susceptibility

The continuous increase in the antibacterial resistance of clinical bacterial strains has developed a main clinical problem. There is growing worry over the emergence and dissemination of diverse antibiotic resistance mechanisms among prevalent human pathogenic Enterobacteriaceae members, which reduces the number of treatment options available. However, the multidrug-resistant strains of *Proteus spp*. have also been reported worldwide. They have the ability to resist different types of antibiotics, that is what called by multi drug resistant (MDR) (Gajdács *et al.*, 2021).

The result of antibiotic susceptibility showed the high resistant against Nitrofurantoin (92.8%) , Trimethoprim –Sulfamethoxazole (85.7%) , Cefotaxime (78.5%), Cefuroxime (68.5%), Cefatazidime (58.5%), Gentamycine (38.5%), Ciprofloxacin (37.1%) , Levoflaxacin (28.5%). While the lower resistant against Amikacin (10%) , Impenem (4.2%), Aztreonam (2.8%)and Meropenem (1.4%) as shown in (Table 4-5, Figure4-4) .This results is similar to the result obtain by Mirzaei *et al* ., (2019) how found the bacterial isolates showed a high resistance to amoxicillin with 44.5% and sensitive to imipenem

and meropenem in percentage 11.8%, 4.5% respectively. Out of 150 isolates, 46 were confirmed as *P. mirabilis* while resistance towards levofloxacin and imipenem was noticed as 60% and 58% respectively (Khan *et al* ., 2020).

Several researchers have examined the ability of bacterial cells to resist different antimicrobial agents by disc diffusion method . The results of the present study were close to the results of (Sokhn *et al.*,(2020) who were illustrated that the resistance rate of *Proteus spp*. to antimicrobials was (0 %) towards both of Imipenem and Meropenem , Amikacin(18 %) and other study by Al-Shamarti,(2019) that found resistance against Cefatazidime(25 %) and Amikacin(16.7 %) .

According to the world health organization , 20 – 50 % of antibiotics are inappropriately used in community . WHO suggested participation of all people in the fighting of antibiotic resistance through improving admission to medical service , decreasing unnecessary and unreasonable use of antibiotics , taking a complete course of treatment , not sharing drug with other people , and not keeping part of course for another infection (Erku *et al* ., 2017).

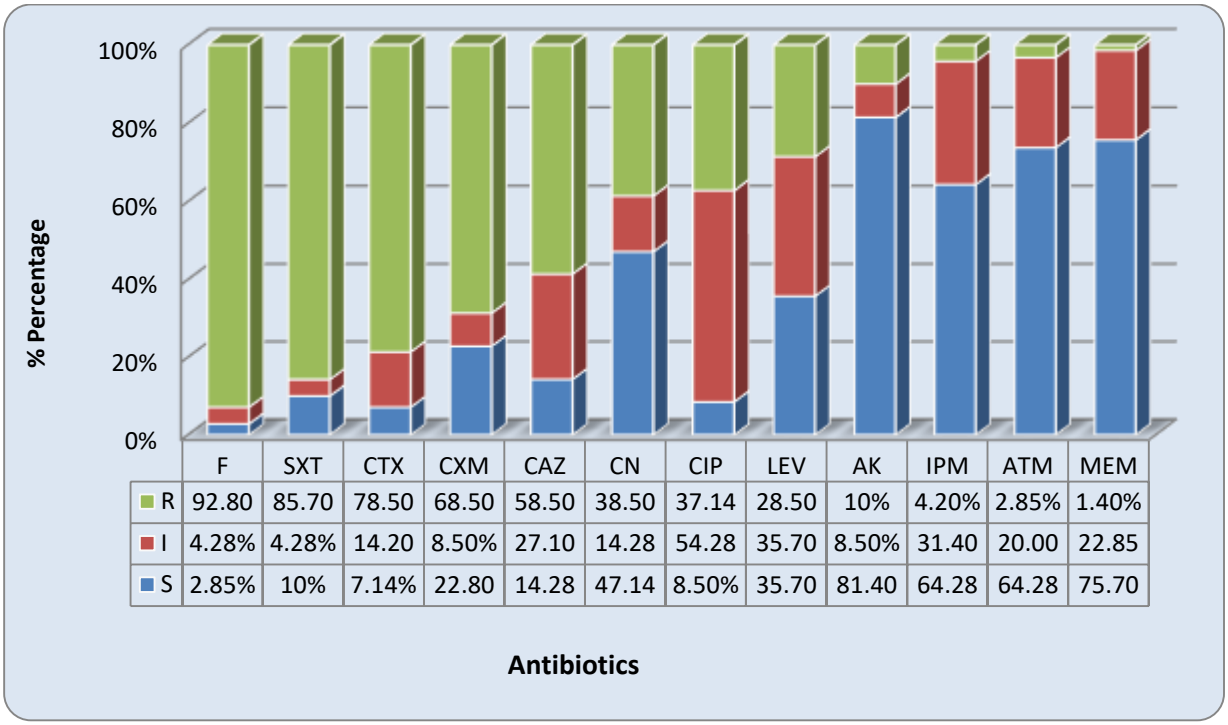
Compared to sensitive strains, which mostly displayed incompatible growth with aggressive swarming motion and weaker biofilm, multidrug-resistant *P. mirabilis* strains more frequently exhibited mutual growth amongst each other, weaker swarming motility, and stronger biofilm (Filipiak *et al.*, 2020).

The findings also showed that *P.mirabilis* is becoming more resistant to various medicines over time due to improper and haphazard administration of these drugs as well as a rise in *Proteus* infections. On the other hand, this bacteria had the capacity to produce β-lactamases, particularly extended spectrum β-lactamases (ESBLs), as well as the capacity to transfer genetic elements carrying the genes of these enzymes. A number of mutations occur with these type of enzymes, increasing resistance to antibiotics, especially β -lactam, in addition to

other mechanisms like altering the target site or altering the access to the target site by modification of penicillin binding proteins (PBPs) (Ahmed, 2015).

Table (4 -5) Antibitics susceptibility of *Proteus mirabillis* isolates (n=70)

Family	Antibiotic	Sensitive	Intermediate	Resistance
Quinolones	Ciprofloxacin	6 (8.5%)	38 (54.28%)	26 (37.14%)
	Levoflaxacin	25 (35.7%)	25 (35.7%)	20 (28.5%)
Cephems	Cefotaxime	5 (7.14%)	10 (14.2%)	55 (78.5%)
	Cefuroxime	16 (22.8%)	6 (8.5%)	48 (68.5%)
	Cefatazidime	10 (14.28%)	19 (27.1%)	41 (58.5%)
Monobactams	Aztreonam	45 (64.28%)	14 (20%)	11 (2.85%)
Carpenems	Impenem	45 (64.28%)	22 (31.4%)	3 (4.2%)
	Meropenem	53 (75.7%)	16 (22.85%)	1 (1.4%)
Ntrofurans	Nitrouranation	2 (2.85%)	3 (4.28%)	65 (92.8%)
Aminoglycoside	Gentamycine	33 (47.14%)	10 (14.28%)	27 (38.5%)
	Amikacin	57 (81.4%)	6 (8.5%)	7 (10%)
Folate pathway antigonistic	Trimethoprim Sulfamethoxazole	7 (10%)	3 (4.28%)	60 (85.7%)



	F	SXT	CTX	CXM	CAZ	CN	CIP	LEV	AK	IPM	ATM	MEM
R	92.80	85.70	78.50	68.50	58.50	38.50	37.14	28.50	10%	4.20%	2.85%	1.40%
I	4.28%	4.28%	14.20	8.50%	27.10	14.28	54.28	35.70	8.50%	31.40	20.00	22.85
S	2.85%	10%	7.14%	22.80	14.28	47.14	8.50%	35.70	81.40	64.28	64.28	75.70

Figure (4-4): Antibiotic Susceptibility of *Proteus mirabillis n*=(70)isolates Nitrouranation (F), Trimethoprim –Sulfamethoxazole (SXT), Cefotaxime (CTX), Cefuroxime (CXM), Cefatazidime (CAZ), Gentamycine (CN), Ciprofloxacin (CIP) , Levoflaxacin (LEV), Amikacin (AK) , Impenem (IPM), Aztreonam (ATM) and Meropenem (MEM).

4.3. Susceptibility Pattern of Multidrug Resistant Isolates

The study found 60/70 (85.7%) of *P. is mirabilis* isolates were multidrug resistant (MDR) and 10/70(14.28%) were non multidrug resistant MDR (Figure 4- 5).

P. mirabilis isolates as multidrug resistance in this study were resisted to at least three antibiotics from different class. Its resistant to four classes were the highest present with 27/70 (38.5%) followed by 3 classes MDR 22/70(31.4%), 5 classes MDR 5/70(7.1%), 6 classes MDR 5/70(7.1%)and 7classes 1/70 (1.4%) (Table 4-6).

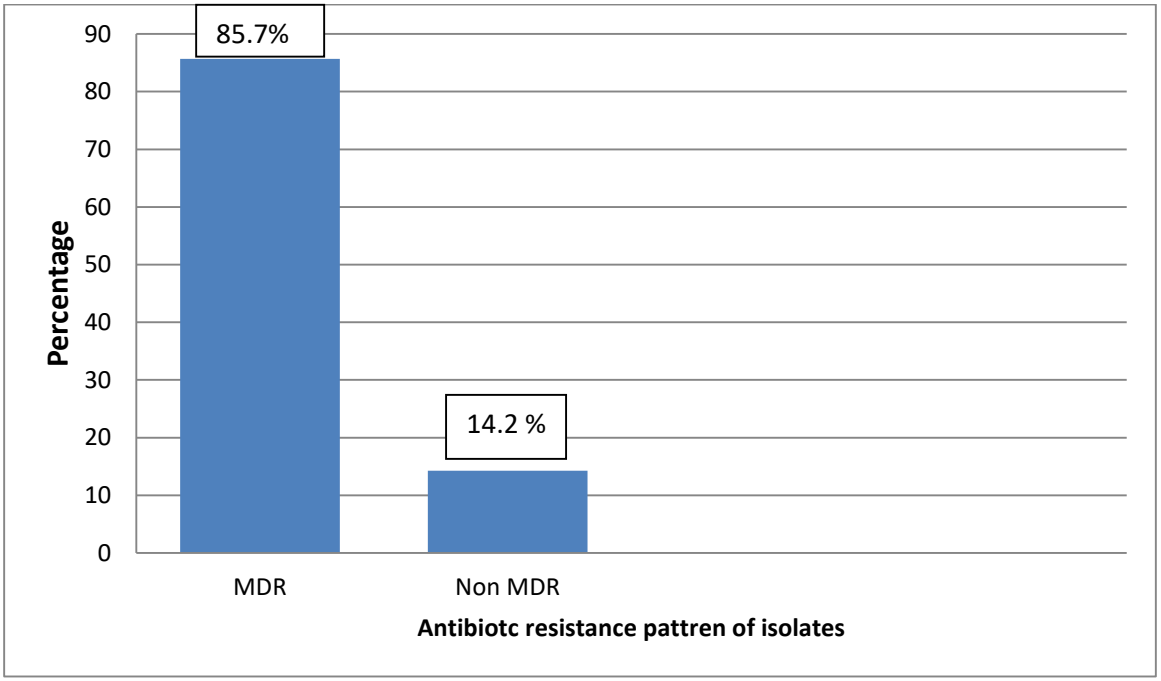


Figure (4-5): Percentage of multidrug and non - multidrug resistant among *proteus mirabillis* isolates (n=70)

Table (4- 6): Phenotypic resistant pattern of multidrug *Proteus mirabillis* isolates (n=70)

Class	Multidrug phenotype	No.	%
3	Cephems/ Ntrofurans/ Folate pathway antigonistic	15	31.4%
	Quinolones/ Ntrofurans/ Folate pathway antigonistic	7	
4	Quinolones/ Cephems/ Ntrofurans/ Folate pathway antigonistic	12	38.5%
	Cephems/ Monobactams / Ntrofurans/ Folate pathway antigonistic	10	
	Quinolones/ Monobactams / Ntrofurans/ Folate pathway antigonistic	2	
	Cephems/ Carpenems/ Ntrofurans/ Folate pathway antigonistic	3	
5	Quinolones/ Cephems/ Monobactams / Ntrofurans/ Folate pathway antigonistic	3	7.1%
	Quinolones/ Cephems/ Carpenems/ Ntrofurans/ Folate pathway antigonistic	2	

6	**Quinolones/ Cephems/ Monobactams / Ntrofurans/ Folate pathway antigonistic/ Aminoglycoside**	**4**	**7.1%**
	Quinolones/ Cephems/ Monobactams/ Ntrofurans/ Folate pathway antigonistic/ Carpenems	**1**	
7	**Quinolones/ Cephems/ Monobactams/ Ntrofurans/ Folate pathway antigonistic/ Carpenems/ Aminoglycoside**	**1**	**1.4%**
Total		**60**	**85.7**

4.4. Detection of β-lactams Genes among isolates

Beta_lactamase enzymes were examined by detecting β-lactams genes by PCR using specific primers for each β-lactams (*bla-$_{TEM}$,bla-$_{CTX-MI}$, bla-$_{CTX-MII}$, bla-C_{TX-MIV}, bla$_{-IMP-}$1, and bla-$_{OXA}$*) gene as shown in table 3.7 in chapter three. For 70 *P. mirabillis* isolates, all MBLs genes were amplified; the findings showed that 6/70 (8.5%) of the isolates lacked any MBLs genes, while 64/70 (91.4%) of the isolates had at least one MBLs gene. In *P. mirabillis* isolates, the proportion of MBLs genes was as follows: *bla-$_{TEM}$* (91.4%), *bla-$_{CTXMI}$* (80%), *bla-C_{TXMIV}* (57%) and bla-*IMP* (both 57%); *bla-$_{CTXMII}$* (12.8%) and *bla-$_{OXA}$* (5.7%). Table(4.7).[Figure(4-6,4-7,4-8,4-9,4-10.4-11)]

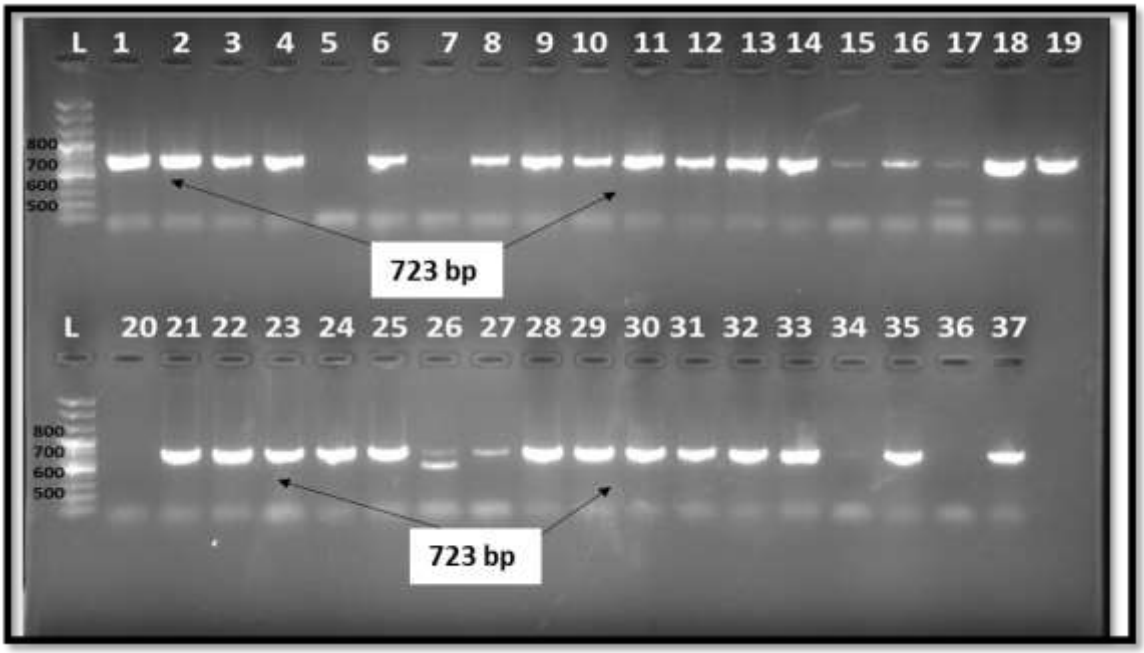


Figure (4-6): Electrophoresis of 1.5% Agarose gel at 70 volt for 60 minutes after staining with goldview for *bla*$_{TEM}$ (723 bp) PCR products. Lane (L) molecular size marker for DNA molecules (1500- bp ladder). show positive and negative results.

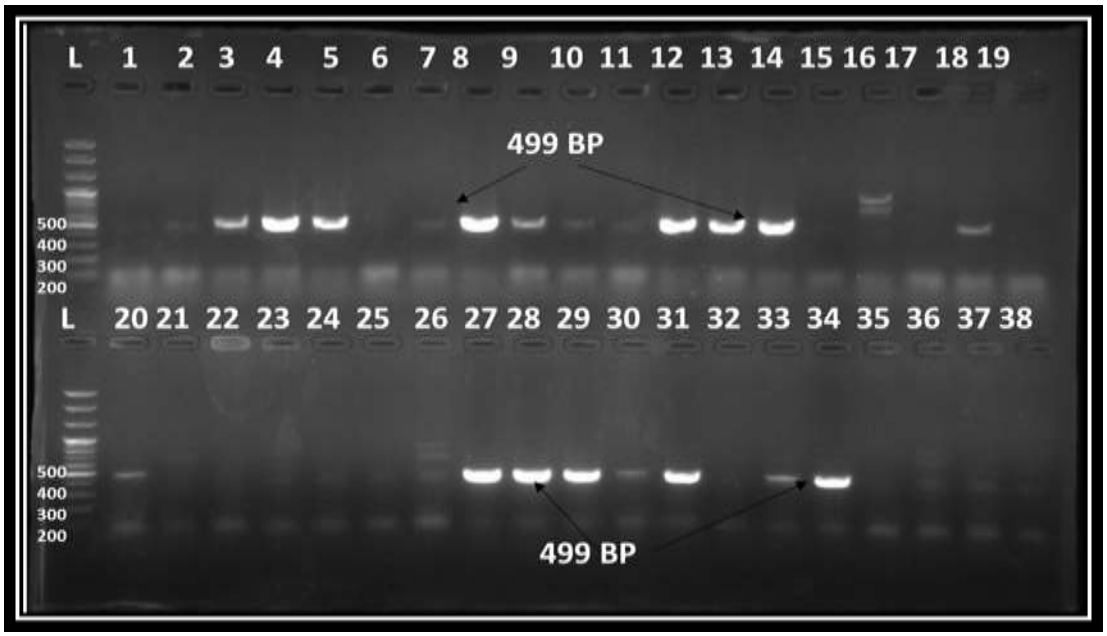
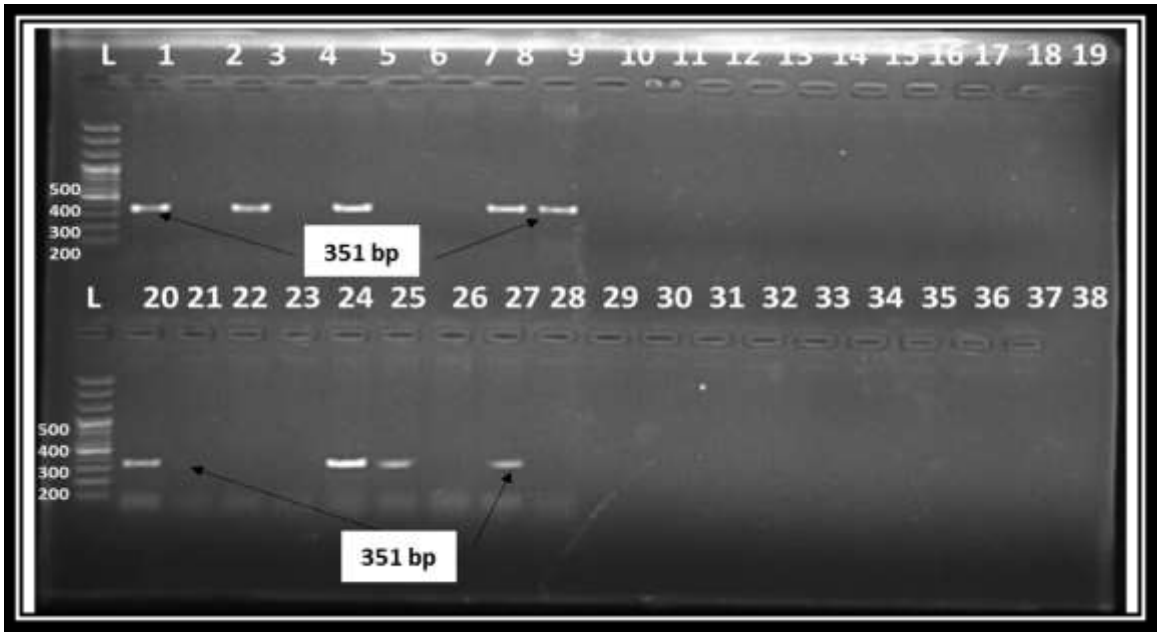
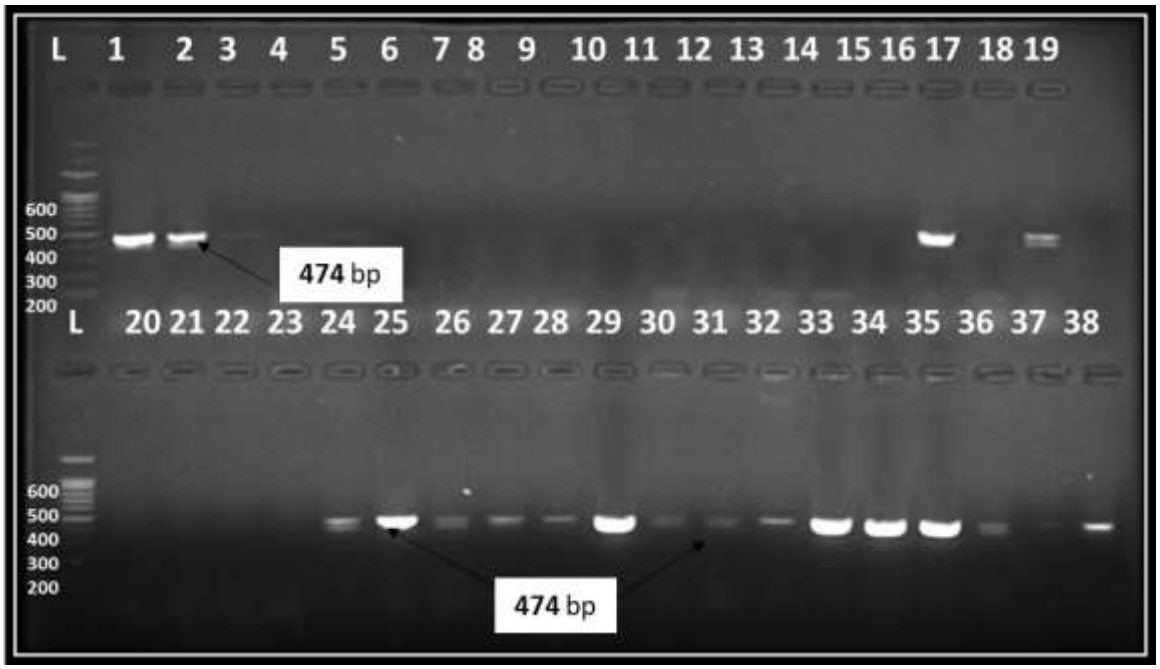


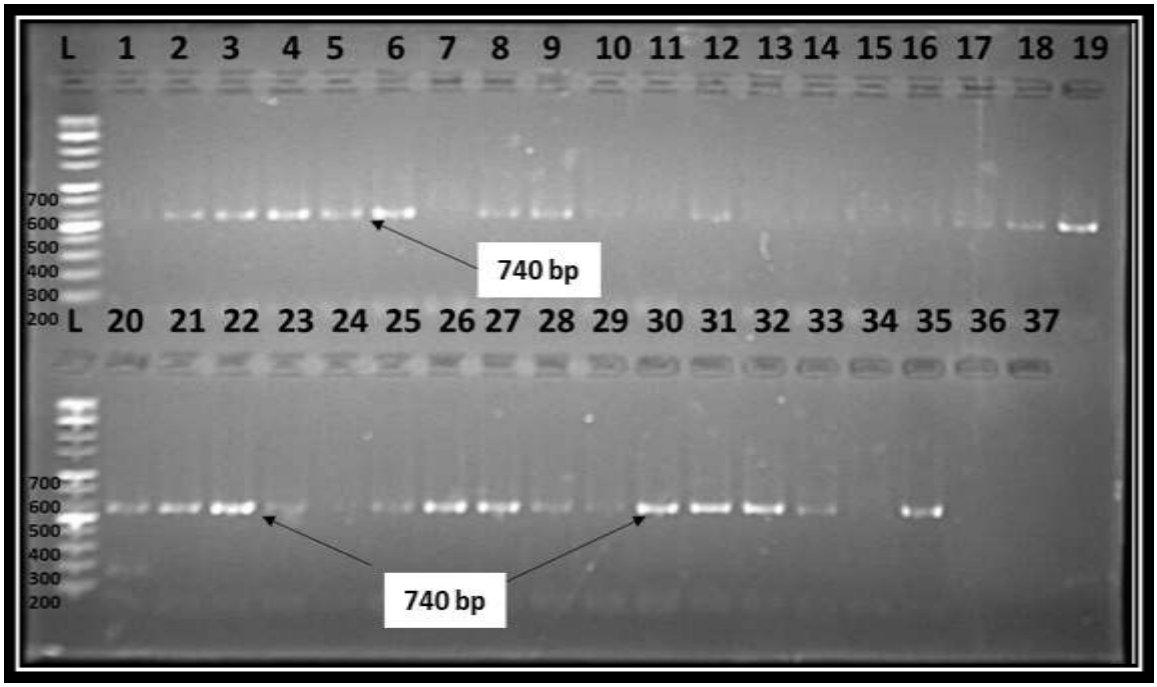
Figure (4-7): Electrophoresis of 1.5% Agarose gel at 70 volt for 60 min after staining with goldview for *bla*$_{CTX\text{-}MI}$ (499 bp) PCR products. Lane (L) molecular size marker for DNA molecules (1500-bp ladder). show positive and negative results.



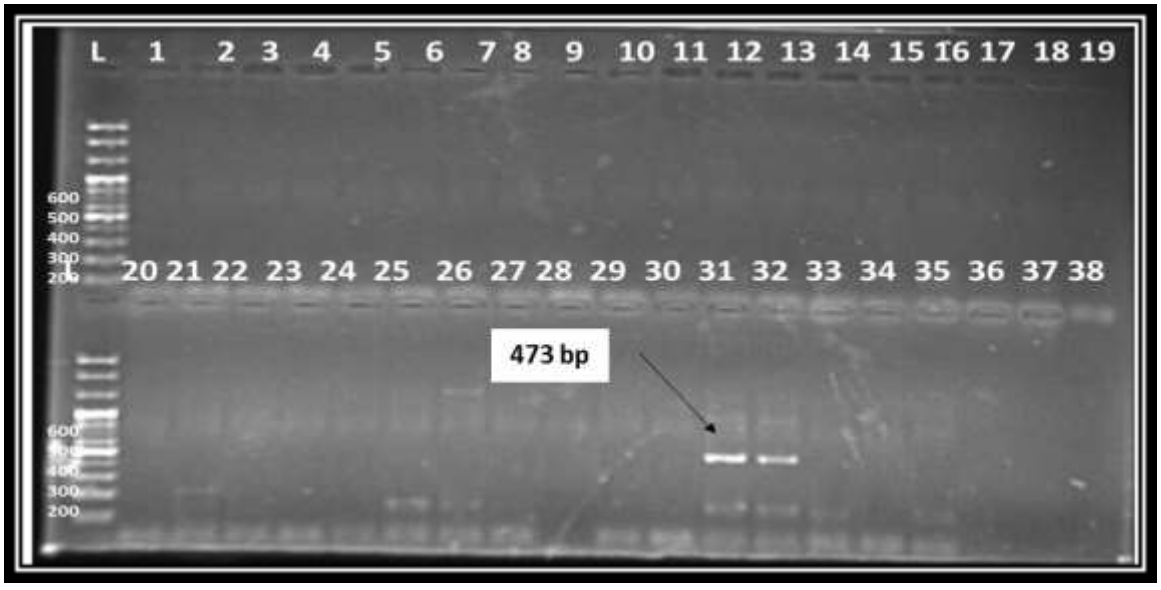
Figure(4-8): Electrophoresis of 1.5% agarose gel at 70 volt for 60 minutes after staining with goldview for *bla*$_{CTX\text{-}MII}$ (351bp)PCR products. Lane (L) molecular sizemarker for DNA molecules (1500-bp ladder).



Figure(4-9): Electrophoresis of 1.5% agarose gel at 70 volt for 60 minutes after staining with goldview for *bla*$_{CTX\text{-}MIV}$ (474bp)PCR products. Lane (L) molecular sizemarker for DNA molecules (1500-bp ladder). show positive and negative results.



Figure(4-10): Electrophoresis of 1.5% agarose gel at 70 volt for 60 minutes after staining with goldview for bla*IMP1* (740bp)PCR products. Lane (L) molecular sizemarker for DNA molecules (1500-bp ladder).



Figure(4-11): Electrophoresis of 1.5% agarose gel at 70 volt for 60 minutes after staining with Goldview dye for bla*OXA* (473bp)PCR products. Lane (L) molecular sizemarker for DNA molecules (1500-bp ladder). show positive and negative results

Table (4-7): Pattern of highest resistance isolates of *P. mirabillis* the different type of antibiotcs among these β-lactamases genes.

Gene / Antibiotic	*bla*$_{TEM}$		bla$_{CTXMI}$		bla$_{CTMIV}$		bla$_{IMP1}$		plaCTXMII		*bla*$_{OXA}$		*NON*	
	NO	*%*	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
Ciprofloxacin	**64**	**100**	49	87.5	34	85	35	87.5	8	88.8	3	75	0	0
Levofloxacin	**38**	**56.3**	33	58.9	22	55	24	60	7	77.7	3	75	0	0
Cefotaxime	**58**	**90.6**	54	96.4	38	95	40	100	9	100	4	100	0	0
Cefuroxime	**52**	**81.2**	46	82.1	32	80	33	82.5	7	77.7	3	75	0	0
Cefatazidime	**56**	**87.5**	49	87.5	33	82.5	33	82.5	7	77.7	1	25	0	0
Aztreonam	**19**	**29.8**	21	37.5	9	22.5	12	30	2	22.2	0	0	0	0
Imipenem	**21**	**32.1**	16	28.5	17	42.5	14	35	4	44.4	1	25	0	0
Meropenem	**15**	**2.4**	42	75	10	25	9	22.5	2	22.2	3	75	0	0
Nitrofurantoin	**64**	**100**	56	100	40	100	40	100	9	100	4	100	0	0
Gentamycine	**34**	**53.1**	34	60.7	12	30	17	42.5	5	55.5	3	75	0	0
Amikacin	**9**	**14.0**	8	14.2	4	10	6	15	0	0	0	0	0	0
Trimethoprim	**57**	**89.0**	49	87.5	33	82.5	34	85	1	88.8	4	100	0	0

Table (4-8) number and percentage antibiotic resistant gene among *Proteus mirabillis* isolates:

Gene	Positive samples NO.(%)
bla_{TEM}	64(91.4)
$bla_{CTX\text{-}MII}$	9(12.85)
$bla_{CTX\text{-}MI}$	56(80)
$bla_{CTX\text{-}MIV}$	40(57)
bla_{IMP}	40(57)
bla_{Ox4}	4(5.7)

The high prevalence gene was bla_{TEM} 64/70 (91%), this result agree with Fursova *et al.*, (2015)and Algammal *et al.*,(2021) who found the prevalence of bla_{TEM} was (90.6%) and (100%) respectively .Also the resulte obtain by other researcher similar the result by this study who found the lower The lower prevalence of bla_{OXA} *and* $_{blaCTX}$-MII with 4/70(7%) ,9(12.8) respectably for each one, figure 4.10 and figure 4.7, other studies showed 23.3% (Fursova *et al.*, 2015) for *bla –OXA*, 8(20.0%) (Ogeneh *et al.*, 2021) for *bla-*$_{CTX\text{-}MII}$.

The prevalence of $_{CTX\text{-}MI}$ was 56(80%), The $_{CTXM\text{-}I}$ type enzyme was detected in isolates P.mirabilis 100% (Auda, Al-Kakei and Hamed, 2013). The prevalence of bla $_{CTX\text{-}MIV}$ gene was 40/70 (57%), other studies showed (52.3%) from (Farhan, et al., 2019),(57.1%) from (Algammal *et al.*, 2021).The prevalence of *bla-IMP* gene was 40/70(57%),other studies showed (79.7%)(Dixon *et al.*, 2016).

The simplest classification for β -lactamases has been based on molecular structure ,there are four major classes. Classes A to C have been well documented as both chromosomally encoded and plasmid-mediated enzymes . The class D β -lactamases have been much more elusive and, for the most part, were identifiable only as plasmid-encoded β –lactamases in Gram-negative bacteria. These early enzymes were essentially penicillinases, which, unlike the class A β -lactamases, could hydrolyze and confer resistance to oxacillin as well as penicillin, hence the name oxacillinases and the prefix $_{OXA}$ (Gutkind *et al* ., 2013)

Different genetic elements have been found to be involved in the acquisition of *bla* $_{CTX-M}$ genes, including insertion sequences ISEcp1 and ISCR1(formerly CR1 element), and also phage-related elements. ISEcp1-like insertion sequence elements havebeen identified in association with genes encodingenzymes of the $_{CTX-M-1,\ CTX-M-2}$ and $_{CTXM-9}$ clusters. ISEcp1 belongs to the IS1380 familyand is able to mobilise bla_{CTX-M} genes by apeculiar transposition process (Poirel *et al* ., 2002).

It mobilises adjacent sequences by transposition, after recognition of a variety of DNA sequences as right inverted repeats (IRRs). Thus, a single copy of ISEcp1 located upstream of a bla_{CTX-M} gene is sufficient to mobilise the gene from the chromosome of a Kluyvera strain (Girlich *et al.*, 2020). The second main mechanism of acquisition of bla_{CTX-M} genes is linked to a peculiar insertion sequence-mediated transposition process called rolling-circle (RC) transposition (Lartigue *et al*., 2006).

The bla_{CTX-M} genes are not present as gene cassettes but are located downstream of the orf513 gene, between the two qacED1 / sul1 repeats . The orf513 gene was originally defined, together with a 33-bp recombination

crossover site located at its right-hand boundary, as part of the so-called common region CR1 (Mahrouki *et al.*, 2012).

In some cases, the carbapenem-resistant $_{OXA}$ β -lactamases ($_{OXA-48}$) have migrated into the *Enterobacteriaceae* and are becominga significant cause of carbapenem resistance. The emergence of $_{OXA}$ enzymes that can confer resistance to carbapenems, has transformed these β –lactamases from a minor hindrance into a major problem set to demote the clinical efficacy of the carbapenems (Evas *et al* ., 2014).

The recent worldwide emergence and dissemination of carbapenemase-producing Gram-negative rods (GNR) that are resistant to carbapenems is a significant concern with respect to patient care and infection control strategies (Queenan et al ., 2007).

The transmissible carbapenemases are divided into three different classes, class A (serine carbapenemases, such as *Klebsiella pneumonia* carbapenemase [KPC]), class B (metallo-B-lactamases [MBLs], such as $_{IMP,\ VIM}$, and $_{NDM}$), and class D (O_{XA} carbapenemases, suchas $_{OXA-23}$ and $_{OXA-48}$) (Miriagou *et al* ., 2010).

Rapid and adequate detection of carbapenemases is very important for appropriate antimicrobial chemotherapies and infection control measures. Various phenotypic confirmation tests for detecting carbapenemases have been performed, including inhibition tests of carbapenemase activity,the modified Hodge test (MHT), and detection of carbapenem hydrolysis (1–8).

However, there are no complete assays available to confirm and specify carbapenemases correctly because carbapenemase-producing bacteria, notably Enterobacteriaceae, show variable carbapenem MIC distributions (even under the breakpoint) and sometimes have carbapenemase-independent mechanisms, such as reduced permeability by porin alternations, active efflux pumping, and

hyperproduction of class C -lactamases (e.g., AmpC) or extended-spectrum -lactamases (ESBLs) that operate with or without carbapenemase activity. Moreover, phenotypic assays cannot specify types within each class of carbapenemases, such as IMP, VIM, NDM, SIM, and GIM in MBLs. Therefore, molecular confirmation of carbapenemases is recommended for suspected carbapenemase-producing strains (Cohen Stuart et al ., 2010; Nordmann et al., 2012).

However, although molecular detection methods such as PCR and sequencing of carbapenemase genes are reliable for confirmation of carbapenemases, it is difficult to perform such tests in routine clinical microbiology laboratories because of the skill levelrequired, the higher cost, and the special equipment required (Nordmann et al., 2012).

A simple and rapid alternative method is thus needed to confirm carbapenemase presence in bacteria. In Japan, IMP MBL is the most prevalent transmissible carbapenemase, particularly members of the IMP-1 group (Dixon *et al.*, 2016),while KPC is quite rare and OXA-48 has not been reported (Harada *et al.*, 2021). The first IMP MBL was described in *Pseudomonas aeruginosa* in Japan and is now found worldwide in non-glucose-fermenting Gram-negative rods (NFGNR) other than *P. aeruginosa* and Enterobacteriaceae (Cornaglia *et al* ., 2011).

Kitao *et al*. (2011) developed an immunochromatography (IC) assay for the production of IMPMBL in *P. aeruginosa* and *Acinetobacter*. This assay is easy to perform and rapid (20 min required), requires no special equipment, and detects the 24 established IMP types. In addition, it shows excellent correlation with PCR results. In countries like Japan, wherein IMP MBL is the most

prevalent mechanism of carbapenem resistance, this assay provides a useful alternative to PCR for classifying MBLs in clinical microbiology laboratories.

4.5. Sequence Analysis

All sequences of (5 isolates for each) *bla*$_{CTX-MI}$, *bla*$_{CTX-MII,}$ *bla*$_{CTX-MIV}$ and *bla*$_{TEM}$ were sent for Macrogen/Korea for Sanger sequencing. All ABI file opend by BioEdit Sequence Alignment Editor (Hall, 1999). The incorrect sequences were trimmed and the correct sequences were submitted for NCBI BLASTN for similarity. The results revealed that, all 5 sequences (KJSJHD-1 to KJSJHD-5) of *bla*$_{CTX-MI}$ *amplicon give* 100% similarity with *bla*$_{CTX-M-15}$ (Table 4-8). All 5 sequences (KJSJHD-6 to KJSJHD-10) of *bla*$_{CTX-MII}$ *amplicon give* 100% similarity with *bla*$_{CTX-M-65}$ (Table 4-18). All 5 sequences (KJSJHD-11 to KJSJHD-15) of *bla*$_{CTX-MIV}$ *amplicon give* 100% similarity with *bla*$_{CTX-M-90}$ (Table 4-23). All 5 sequences (KJSJHD-16 to KJSJHD-20) of *bla*$_{TEM}$ *amplicon give* 100% similarity with *bla*$_{TEM-1}$.

Table (4-9) Alignment of KJSJHD-1 *bla*$_{CTX-MI}$ amplicon sequence with *Proteus mirabilis* strain E96PMMO CTX-M-15 beta-lactamase (*bla*$_{CTX-M-15}$) gene, partial cds Sequence ID: KY640539.1

Score	Expect	Identities	Gaps	Strand
566 bits(306)	7e-160	306/306(100%)	0/306(0%)	Plus/Plus

```
Query  1    GTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  277  GTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCC  336

Query  61   CGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  337  CGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCC  396

Query  121  ATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  397  ATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAAT  456

Query  181  CTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457  CTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAA  516

Query  241  GGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  517  GGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGG  576

Query  301  GATAAA  306
            ||||||
Sbjct  577  GATAAA  582
```

Table (4-10) Alignment of **KJSJHD-2** *bla*$_{CTX-MI}$ amplicon sequence with *Proteus mirabilis* strain E96PMMO CTX-M-15 beta-lactamase (*bla*$_{CTX-M-15}$) gene, partial cds Sequence ID: KY640539.1

Score	Expect	Identities	Gaps	Strand
532 bits(288)	**6e-150**	**288/288(100%)**	**0/288(0%)**	**Plus/Plus**

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Query  1    ATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA  60
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Sbjct  295  ATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA  354

Query  61   ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGT  120
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Sbjct  355  ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGT  414

Query  121  GATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCA  180
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Sbjct  415  GATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCA  474

Query  181  TTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCA  240
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Sbjct  475  TTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCA  534

Query  241  GCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAA  288
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  535  GCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAA  582
```

Table (4-11) Alignment of **KJSJHD-3** blaCTX-MI amplicon sequence with *Proteus mirabilis* strain B-719/15 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (*bla*$_{CTX-M-15}$) gene, complete cds Sequence ID: KU510261.1

Score	Expect	Identities	Gaps	Strand
793 bits(429)	**0.0**	**429/429(100%)**	**0/429(0%)**	**Plus/Plus**

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Query  1    GCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGA  60
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Sbjct  533  GCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGA  592

Query  61   CAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  593  CAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATT  652

Query  121  CCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  653  CCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTG  712

Query  181  ACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  713  ACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGC  772

Query  241  AATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAT  300
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Sbjct  773  AATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAT  832

Query  301  AAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  833  AAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGAT  892

Query  361  CGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAGCCGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  893  CGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAGCCGT  952

Query  421  CGCGATGTA  429
            |||||||||
Sbjct  953  CGCGATGTA  961
```

Table (4-12) Alignment of **KJSJHD-4** *bla$_{CTX-MI}$* amplicon sequence with *Proteus mirabilis* strain E96PMMO CTX-M-15 beta-lactamase (*bla$_{CTX-M-15}$*) gene, partial cds Sequence ID: KY640539.1

Score	Expect	Identities	Gaps	Strand
532 bits(288)	**6e-150**	**288/288(100%)**	**0/288(0%)**	**Plus/Plus**

```
Query  1    ATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  ATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA  354

Query  61   ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGT  414

Query  121  GATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  GATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCA  474

Query  181  TTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  TTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCA  534

Query  241  GCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAA  288
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  535  GCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAA  582
```

Table (4-13) Alignment of **KJSJHD-5** *bla$_{CTX-MI}$* amplicon sequence with *Proteus mirabilis* strain B-719/15 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (*bla$_{CTX-M-15}$*) gene, complete cds Sequence ID: KU510261.1

Score	Expect	Identities	Gaps	Strand
798 bits(432)	**0.0**	**432/432(100%)**	**0/432(0%)**	**Plus/Plus**

```
Query  1    GCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  533  GCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGA  592

Query  61   CAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  593  CAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATT  652

Query  121  CCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  653  CCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTG  712

Query  181  ACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  713  ACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGC  772

Query  241  AATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  773  AATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAT  832

Query  301  AAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  833  AAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGAT  892

Query  361  CGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAGCCGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  893  CGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAGCCGT  952

Query  421  CGCGATGTATTA  432
            ||||||||||||
Sbjct  953  CGCGATGTATTA  964
```

Table (4-14) Alignment of **KJSJHD-6** *bla*$_{CTX-MII}$ amplicon sequence with *Proteus mirabilis* strain 84-4 class A extended spectrum beta-lactamase CTX-M-65 (*bla*$_{CTX-M}$) gene, *bla*$_{CTX-M-65}$ allele, partial cds Sequence ID: KY400920.1

Score	Expect	Identities	Gaps	Strand
383 bits(207)	**5e-105**	**207/207(100%)**	**0/207(0%)**	**Plus/Plus**

```
Query  1    ATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  180  ATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCG  239

Query  61   ATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  240  ATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCC  299

Query  121  GGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  300  GGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACG  359

Query  181  CTGGGTCATGCGCTGGGCGAAACCCAG  207
            |||||||||||||||||||||||||||
Sbjct  360  CTGGGTCATGCGCTGGGCGAAACCCAG  386
```

Table (4-15) Alignment of **KJSJHD-7** *bla*$_{CTX-MII}$ amplicon sequence with *Proteus mirabilis* strain 84-4 class A extended spectrum beta-lactamase CTX-M-65 (*bla*$_{CTX-M}$) gene, *bla*$_{CTX-M-65}$ allele, partial cds Sequence ID: KY400920.1

Score	Expect	Identities	Gaps	Strand
416 bits(225)	5e-115	225/225(100%)	0/225(0%)	Plus/Plus

```
Query  1    GCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  GCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGC  209

Query  61   CCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  210  CCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGC  269

Query  121  ACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  270  ACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGG  329

Query  181  GCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCATGCGCTG  225
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330  GCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCATGCGCTG  374
```

Table (4-16) Alignment of **KJSJHD-8** *bla*$_{CTX-MII}$ amplicon sequence with *Proteus mirabilis* strain 84-4 class A extended spectrum beta-lactamase CTX-M-65 (*bla*$_{CTX-M}$) gene, *bla*$_{CTX-M-65}$ allele, partial cds Sequence ID: KY400920.1

Score	Expect	Identities	Gaps	Strand
460 bits(249)	3e-128	249/249(100%)	0/249(0%)	Plus/Plus

```
Query  1    ATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126  ATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAAC  185

Query  61   AAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  AAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGC  245

Query  121  GATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  GATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGAC  305

Query  181  CCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306  CCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGT  365

Query  241  CATGCGCTG  249
            |||||||||
Sbjct  366  CATGCGCTG  374
```

Table (4-17) Alignment of **KJSJHD-9** $bla_{CTX\text{-}MII}$ amplicon sequence with Proteus mirabilis strain 84-4 class A extended spectrum *bla*$_{CTX\text{-}M\text{-}65}$ ($bla_{CTX\text{-}M}$) gene, *bla*$_{CTX\text{-}M\text{-}65}$ allele, partial cds Sequence ID: KY400920.1

Score	Expect	Identities	Gaps	Strand
483 bits(261)	6e-135	261/261(100%)	0/261(0%)	Plus/Plus

```
Query  1    ACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129  ACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAA  188

Query  61   TTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189  TTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGAT  248

Query  121  GAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249  GAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCG  308

Query  181  AGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309  AGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCAT  368

Query  241  GCGCTGGGCGAAACCCAGCGG  261
            |||||||||||||||||||||
Sbjct  369  GCGCTGGGCGAAACCCAGCGG  389
```

Table (4-18) Alignment of **KJSJHD-10** *bla*$_{CTX\text{-}MII}$ amplicon sequence with Proteus mirabilis strain 84-4 class A extended spectrum beta-lactamase CTX-M-65 (*bla*$_{CTX\text{-}M}$) gene, *bla*$_{CTX\text{-}M\text{-}65}$ allele, partial cds Sequence ID: KY400920.1

Score	Expect	Identities	Gaps	Strand
532 bits(288)	6e-150	288/288(100%)	0/288(0%)	Plus/Plus

```
Query  1    ACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129  ACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAA  188

Query  61   TTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189  TTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGAT  248

Query  121  GAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249  GAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCG  308

Query  181  AGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309  AGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCAT  368

Query  241  GCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGC  288
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369  GCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGC  416
```

Table (4-19) Alignment of **KJSJHD-11** *bla*$_{CTX-MIV}$ amplicon sequence with *Proteus mirabilis* strain SVP0812 insertion sequence ISEcp1 transposase (tnpA) gene, partial cds; and CTX-M-90 extended-spectrum beta-lactamase (*bla*$_{CTX-M-90}$) gene, partial cds Sequence ID: GU065288.1

Score	Expect	Identities	Gaps	Strand
752 bits(407)	0.0	407/407(100%)	0/407(0%)	Plus/Plus

```
Query  1     CGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAG  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  769   CGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAG  828

Query  61    TAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  829   TAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAA  888

Query  121   TCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  889   TCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACA  948

Query  181   CGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAA  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  949   CGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAA  1008

Query  241   TACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1009  TACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGC  1068

Query  301   CCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1069  CCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGC  1128
Query  361   CATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGC  407
             |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1129  CATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGC  1175
```

Table (4-20) Alignment of **KJSJHD-12** *bla*$_{CTX\text{-}MIV}$ amplicon sequence with *Proteus mirabilis* strain SVP0812 insertion sequence ISEcp1 transposase (tnpA) gene, partial cds; and CTX-M-90 extended-spectrum beta-lactamase (*bla*$_{CTX\text{-}M\text{-}90}$) gene, partial cds Sequence ID: GU065288.1

Score	Expect	Identities	Gaps	Strand
728 bits(394)	**0.0**	**394/394(100%)**	**0/394(0%)**	**Plus/Plus**

```
Query  1     TTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGTCGCGG  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  789   TTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGTCGCGG  848

Query  61    CGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  849   CGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGC  908

Query  121   CTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAATGACGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  909   CTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAATGACGC  968

Query  181   TGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGA  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  969   TGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGA  1028

Query  241   TTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1029  TTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGA  1088

Query  301   CGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCGAGAG  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1089  CGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCGAGAG  1148

Query  361   ACACCACCACGCCGCGGGCGATGGCGCAGACGTT  394
             |||||||||||||||||||||||||||||||||
Sbjct  1149  ACACCACCACGCCGCGGGCGATGGCGCAGACGTT  1182
```

Table (4-21) Alignment of **KJSJHD-13** *bla*$_{CTX\text{-}MIV}$ amplicon sequence with *Proteus mirabilis* strain SVP0812 insertion sequence ISEcp1 transposase (tnpA) gene, partial cds; and CTX-M-90 extended-spectrum beta-lactamase (*bla*$_{CTX\text{-}M\text{-}90}$) gene, partial cds Sequence ID: GU065288.1

Score	Expect	Identities	Gaps	Strand
752 bits(407)	**0.0**	**407/407(100%)**	**0/407(0%)**	**Plus/Plus**

```
Query  1     ACCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  767   ACCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACC  826

Query  61    AGTAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTT  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  827   AGTAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTT  886

Query  121   AATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887   AATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAA  946

Query  181   CACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGAC  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  947   CACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGAC  1006

Query  241   AATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTT  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1007  AATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTT  1066

Query  301   GCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1067  GCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACC  1126

Query  361   GCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTG  417
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1127  GCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTG  1183
```

Table (4-22) Alignment of **KJSJHD-14** *bla$_{CTX-MIV}$* amplicon sequence with *Proteus mirabilis* strain SVP0812 insertion sequence ISEcp1 transposase (tnpA) gene, partial cds; and CTX-M-90 extended-spectrum beta-lactamase (blaCTX-M-90) gene, partial cds Sequence ID: GU065288.1

Score	**Expect**	**Identities**	**Gaps**	**Strand**
769 bits(416)	**0.0**	**416/416(100%)**	**0/416(0%)**	**Plus/Plus**

```
Query  1     CCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  768   CCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCA  827

Query  61    GTAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  828   GTAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTA  887

Query  121   ATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAAC  180
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888  ATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAAC  947

Query  181  ACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  948  ACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACA  1007

Query  241  ATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1008 ATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTG  1067

Query  301  CCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1068 CCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCG  1127

Query  361  CCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTG  416
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1128 CCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTG  1183
```

Table (4-23) Alignment of **KJSJHD-15** *bla*$_{CTX-MIV}$ amplicon sequence with *Proteus mirabilis* strain SVP0812 insertion sequence ISEcp1 transposase (tnpA) gene, partial cds; and CTX-M-90 extended-spectrum beta-lactamase (*bla*$_{CTX-M-90}$) gene, partial cdsSequence ID: GU065288.1

Score	Expect	Identities	Gaps	Strand
765 bits(414)	**0.0**	**418/418(100%)**	**0/418(0%)**	**Plus/Plus**

```
Query  1    GCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  770  GCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGT  829

Query  61   AAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  830  AAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAAT  889

Query  121  CAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  890  CAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACAC  949

Query  181  GTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  950  GTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAAT  1009

Query  241  ACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCC  300
```

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                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1010  ACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCC  1069

Query  301   CGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCC  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1070  CGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCC  1129

Query  361   ATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTC  417
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1130  ATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTC  1187
```

Table (4-24) Alignment of KJSJHD-16 *bla*$_{TEM}$ amplicon sequence with *Proteus mirabilis* strain Pm 1A beta-lactamase TEM-1 (*bla*$_{TEM-1}$) gene, partial cds Sequence ID: KF811201.1

Score	Expect	Identities	Gaps	Strand
1218 bits(659)	**0.0**	**659/659(100%)**	**0/659(0%)**	**Plus/Plus**

```
Query  7     TGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGT  66
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  66    TGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGT  125

Query  67    GGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGA  126
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126   GGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGA  185

Query  127   ACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGT  186
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186   ACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGT  245

Query  187   TGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGA  246
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246   TGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGA  305

Query  247   GTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAG  306
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306   GTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAG  365

Query  307   TGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAACGATCGGAGG  366
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366   TGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAACGATCGGAGG  425

Query  367   ACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCG  426
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426   ACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCG  485

Query  427   TTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGC  486
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486   TTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGC  545

Query  487   AGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG  546
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  546   AGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG  605

Query  547   GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC  606
```

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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  606  GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC  665

Query  607  CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCG  665
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  666  CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCG  724
```

Table (4-25) Alignment of **KJSJHD-17** *bla$_{TAM}$* amplicon sequence with *Proteus mirabilis* strain Pm 1A beta-lactamase TEM-1 (*bla$_{TEM-1}$*) gene, partial cds Sequence ID: KF811201.1

Score	Expect	Identities	Gaps	Strand
1238 bits(670)	0.0	670/670(100%)	0/670(0%)	Plus/Plus

```
Query  1    TTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  56   TTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGG  115

Query  61   GTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  116  GTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTC  175

Query  121  GCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  176  GCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTAT  235

Query  181  TATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  236  TATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATG  295

Query  241  ACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  296  ACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAG  355

Query  301  AATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  356  AATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAA  415

Query  361  CGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  416  CGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTC  475

Query  421  GCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  476  GCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCA  535

Query  481  CGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  536  CGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTC  595

Query  541  TAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  596  TAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTC  655

Query  601  TGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTG  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  656  TGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTG  715

Query  661  GGTCTCGCGG  670
            ||||||||||
```

```
Sbjct  716  GGTCTCGCGG  725
```

Table (4-26) Alignment of **KJSJHD-18** *bla$_{TAM}$* amplicon sequence with *Proteus mirabilis* strain Pm 1A beta-lactamase TEM-1 (*bla$_{TEM-1}$*) gene, partial cds Sequence ID: KF811201.1

Score	Expect	Identities	Gaps	Strand
1219 bits(660)	0.0	660/660(100%)	0/660(0%)	Plus/Plus

```
Query  1    GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  126

Query  61   GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA  186

Query  121  CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTT  246

Query  181  GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG  306

Query  241  TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT  366

Query  301  GCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  GCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGA  426

Query  361  CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT  486

Query  421  TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  487  TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA  546

Query  481  GCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547  GCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGG  606

Query  541  CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  607  CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  666

Query  601  CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  667  CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT  726
```

Table (4-27) Alignment of **KJSJHD-19** *bla*$_{TAM}$ amplicon sequence with *Proteus mirabilis* strain Pm 1A beta-lactamase TEM-1 (*bla*$_{TEM-1}$) gene, partial cds Sequence ID: KF811201.1

Score	Expect	Identities	Gaps	Strand
1214 bits(657)	0.0	657/657(100%)	0/657(0%)	Plus/Plus

```
Query  1    GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  126

Query  61   GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA  186

Query  121  CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTT  246

Query  181  GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAG  306

Query  241  TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT  366

Query  301  GCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  GCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGA  426

Query  361  CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT  486

Query  421  TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  487  TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCA  546

Query  481  GCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547  GCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGG  606

Query  541  CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  607  CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  666

Query  601  CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGC  657
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  667  CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGC  723
```

Table (4-28) Alignment of **KJSJHD-20** *bla*$_{TAM}$ amplicon sequence with *Proteus mirabilis* strain Pm 1A beta-lactamase TEM-1 (*bla*$_{TEM-1}$) gene, partial cds Sequence ID: KF811201.1

Score	Expect	Identities	Gaps	Strand
1175 bits(636)	0.0	636/636(100%)	0/636(0%)	Plus/Plus

```
Query  1    AAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  88   AAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTC  147

Query  61   AACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  148  AACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACT  207

Query  121  TTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  208  TTTAAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTC  267

Query  181  GGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  268  GGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG  327

Query  241  CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT  387

Query  301  AACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  AACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTT  447

Query  361  TTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  TTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA  507

Query  421  GCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  GCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGC  567

Query  481  AAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  AAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATG  627

Query  541  GAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  628  GAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT  687

Query  601  GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGC  636
            |||||||||||||||||||||||||||||||||||
Sbjct  688  GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGC  723
```

4.6. Sequences Registration in Genbank

All 20 sequences were submitted for Genbank via BankIt tool. After confirmation by Genbank team the following 20 accession no. were received as mentioned in Table (4-27) bellow

Table (4-29) Genbank accession no.

bankit/file	Gene	Accession no.
BankIt2708348	*bla*$_{CTX\text{-}M\text{-}15}$	OR076411
BankIt2708348	*bla*$_{CTX\text{-}M\text{-}15}$	OR076412
BankIt2708348	*bla*$_{CTX\text{-}M\text{-}15}$	OR076413
BankIt2708348	*bla*$_{CTX\text{-}M\text{-}15}$	OR076414
BankIt2708348	*bla*$_{CTX\text{-}M\text{-}15}$	OR076415
BankIt2708396	*bla*$_{CTX\text{-}M\text{-}65}$	OR076416
BankIt2708396	*bla*$_{CTX\text{-}M\text{-}65}$	OR076417
BankIt2708396	*bla*$_{CTX\text{-}M\text{-}65}$	OR076418
BankIt2708396	*bla*$_{CTX\text{-}M\text{-}65}$	OR076419
BankIt2708396	*bla*$_{CTX\text{-}M\text{-}65}$	OR076420
BankIt2709153	*bla*$_{CTX\text{-}M\text{-}90}$	OR076421
BankIt2709153	*bla*$_{CTX\text{-}M\text{-}90}$	OR076422
BankIt2709153	*bla*$_{CTX\text{-}M\text{-}90}$	OR076423
BankIt2709153	*bla*$_{CTX\text{-}M\text{-}90}$	OR076424
BankIt2709153	*bla*$_{CTX\text{-}M\text{-}90}$	OR076425
BankIt2709511	*bla*$_{TEM\text{-}1}$	OR076426
BankIt2709511	*bla*$_{TEM\text{-}1}$	OR076427
BankIt2709511	*bla*$_{TEM\text{-}1}$	OR076428
BankIt2709511	*bla*$_{TEM\text{-}1}$	OR076429
BankIt2709511	*bla*$_{TEM\text{-}1}$	OR076430

Conclusions:

1- *Proteus mirabilis* was a major pathogen that caused a variety of illnesses, most notably urinary tract infections (UTIs).

2- Results indicate that *ptaD* gene seems to be an appropriate method for diagnosis *P.mirabillis* .

3.Most *P. mirabilis* isolates were multidrug resistance recovered from UTIs, the antibiotics that had activity against these isolates were Amikacin, Meropenem, Impenem, Aztreonam.

4.The high prevalent antibiotic resistant gene was $bla_{\text{-}TEM}$ among *P. mirabillis* isolate

5. All sequences Alignment for *(*$bla_{CTX\text{-}MI}$*,* $bla_{CTX\text{-}MII,}$ $bla_{CTX\text{-}MIV}$ and $bla_{TEM)}$ amplicon give100% simalarity with NCBI BlASTN sequnces.

Recommendations

1. These results show that hospitals should follow rigorous antimicrobial stewardship policies and robust microbiological surveillance techniques.

2. Study of other types of pathogens associated with urinary tract infections.

3. Molecular study of virulence factors of *P.mirabillis*.

4. The using of new antibiotics should be highly selective and is not used for long time to decrease the chance of the emergence of bacteria drug resistant.

5. The MLST test should be used to determine the evolution of antibiotics resistance.

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الخلاصة:

يعد أخماج السبل البولية (UTIs) من أكثر أنواع العدوى البكتيرية شيوعًا. يزيد معدل الإصابة مع تقدم العمر ، ويبلغ معدل الإصابة خلال الحياة مابين ٥٠-٦٠٪ عند النساء البالغات. تعد اخماج السبل البولية عبئًا كبيرًا على المجتمع ونظام الرعاية الصحية ، نتيجة لزيادة مقاومة المضادات الحيوية بين أنواع البكتيريا المسببة لأخماج السبل البولية.

جمعت (٤٥٠) عينة ادرار من المرضى الذين يعانون من أخماج السبل البولية من ثلاث مستشفيات (مستشفى الشوملي العام, مستشفى الاطفال والولادة, مستشفى الحلة الجراحي) خلال الفترة الممتدة من شهر(٧-١٠) /٢٠٢٢ . تمت زراعة العينات على أجار الماكونكي وأجار الدم لفصل المستعمرات سالبة الجرام ، والمستعمرات غير المخمرة للاكتوز التي تظهر ظاهرة التموج. كانت ٧٠ عينة (١٥,٥٪) موجبة لبكتريا *Proteus mirabilis*.

أجري اختبار الحساسية للمضادات الحيوية بطريقة الانتشار بالاقراص لـ ١٢ مضادًا حيويًا من ٧ اصناف مختلفة لتمييز العزلات المقاومة للأدوية المتعددة أو غير مقاومة للأدوية المتعددة. أظهرت نتيجة اختبار الحساسية للمضادات الحيوية مقاومة– Nitrofurantoin (٩٢,٨٪), Trimethoprim Sulfamethoxazole (٨٥,٧٪), Cefotaxime (٧٨,٥٪), Cefuroxime (٦٨,٥٪), Cefatazidime (٥٨,٥٪), Gentamycine (٣٨,٥٪), Ciprofloxacin (٣٧,١٪) , Levoflaxacin (٢٨,٥٪). While the lower resistant against Amikacin (١٠٪) , Impenem (٤,٢٪), Aztreonam and Meropenem (١,٤٪)(٢,٨٪) . أظهرت النتائج أن ٧٠/٦٠ (٨٥,٧٪) من عزلات *P. mirabilis* كانت تمتلك مقاومة للأدوية المتعددة و ٧٠/١٠ (١٤,٢٨٪) غير مقاومة للأدوية المتعددة.

استخدم تفاعل انزيم البلمرة المتسلسل للكشف عن جينات المقاومة لمضادات بيتا لاكتام (*bla-TEM* , *bla-CTX-MI*, *bla-CTX-MIV* , *bla-imp ١*, *bla-CTX-MII* ,and *bla-OXA*) باستخدام أزواج محددة من البادئات، أظهرت النتيجة ٦٤ (٩١,٤٪) ، ٥٦ (٨٠٪) ، ٤٠ (٥٧,١٪) ، ٤٠ (٥٧,١) ، (١٥,٧١١) ٪ ، ٤ (٥,٧١)٪ حيث كانت موجبة لجين (*bla-TEM*, , *bla*-CTX-MI, *bla*-CTX-MIV ,bla-IMP-١, *bla*-CTX-MII.,and bla-OXA) وعلى التوالي من ٧٠ عزلة *P.mirabilis* .

تم إرسال نواتج التضخيم ل ٥ عزلات بكتيرية لكل من جينات bla_{CTX-MI} , $bla_{CTX-MII}$, $bla_{CTX-MIV}$, bla_{IMP} bla_{TEM} إلى كوريا لدراسة تسلسل النيوكليوتيدات . فتحت كل ملفات ABI بواسطة محرر محاذاة التسلسل BioEdit , قطعت التسلسلات غير الصحيحة واعتمدت التسلسلات الصحيحة لـ NCBI BLASTN لدراسة مدى التطابق في التسلسلات مع العزلات القياسية المسجلة على موقع بنك الجينات. أظهرت النتائج أن جميع التسلسلات الخمسة (KJSJHD-١ - KJSJHD-٥) من نواتج تضخيم الجين bla_{CTX-MI} أعطت تشابهًا بنسبة ١٠٠ ٪ مع $bla_{CTX-M-١٥}$. جميع التسلسلات الخمسة (KJSJHD-٦ - KJSJHD-١٠) من نواتج تضخيم الجين $bla_{CTX-MII}$ تعطي تشابهًا بنسبة ١٠٠٪ مع *bla CTX-M-١٥* أعطت جميع التسلسلات الخمسة (KJSJHD-١١ - KJSJHD-١٥) من ناتج التضخيم $bla_{CTX-MIV}$ تشابهًا بنسبة ١٠٠٪ مع $bla_{CTX-M-١٠}$. جميع التسلسلات الخمسة (KJSJHD-١٦ -KJSJHD-٢٠) من نواتج تضخيم الجين bla_{TEM} أعطت تشابهًا بنسبة ١٠٠ ٪ مع *bla TEM-١*.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل / كلية العلوم

قسم علوم الحياة

التحري الجزيئي عن بعض جينات البيتالاكتاميز في بكتريا ***Proteus SPP*** المعزولة من مرضى أخماج السبيل البولي

رسالة مقدمة الى

مجلس كلية العلوم – جامعة بابل

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

من قبل

كاظم جواد محمد جامن العويدي

(جامعة بابل –بكالوريوس علوم الحياة– كلية العلوم ٢٠٠٨)

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