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Molecular Detection of Uropathogenic *Proteus* Species Associated with Pregnant Women in North of Thi-Qar Province.

A thesis

submitted to the council of the Faculty of Veterinary Medicine, University of Thi-Qar as partial fulfillment of the requirements for master degree in Veterinary Medicine / Microbiology.

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَاللَّهُ أَخْرَجَكُمْ مِنْ بُطُونِ أُمَّهَاتِكُمْ لَا تَعْلَمُونَ شَيْئًا وَجَعَلَ

لَكُمْ السَّمْعَ وَالْأَبْصَارَ وَالْأَفْئِدَةَ لَعَلَّكُمْ تَشْكُرُونَ﴾

صدق الله العلي العظيم

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" Certification "

We certify that this thesis (**Molecular detection of Uropathogenic *Proteus* species associated with pregnant women in North Thi-Qar province**), was prepared under our supervision at the Department of Microbiology, Faculty of Veterinary Medicine, University of Thi-Qar, as a partial requirement for Master degree in Veterinary Medicine / Microbiology.

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Dedication

To the absent, whose majestic reappearance we are waiting for...

To all the martyrs of Iraq

To the Palestinian people, we tell them that our hearts are with you,
and that is the weakest faith.

To the soul of that great man (my father).

To the soul of a strong woman who wished to be present here, have
mercy on her (my mother).

To my lovely family and all those who supported me...

Iman N. Hussain

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Summary

Urinary tract infection is one of the common diseases that affect women, especially during pregnancy, bacterial infections are the main cause of these injuries, and bacteria *proteus* spp. is one of the causes of UTI, which can affect the mother by infecting her with kidney inflammation or the formation of kidney stones, as well as it can affect the growth of the fetus. During the period from September to December in 2023, (150) urine samples were collected for women suffering from urinary tract infection, who had visited hospitals north of Thi-Qar to find out the cause of the infection and to obtain the necessary treatments. During the previous period, the samples were collected in sterile and hygienic ways so as not to cause harm to pregnant women. These samples were cultured on the medium of MacConky and blood agar, after which these samples were examined microscopically using a Gram stain to diagnose Gram-negative bacteria. These isolates were examined by VITEK II system for diagnosis and to see how resistant they were to antibiotics. The isolates identified as bacterial *proteus* were then examined by polymerase chain reaction test (PCR) via *16s rRNA*, and the virulence genes of the *ureR* gene and *bla kpc* were examined, too the results of the culture on the culture media and microscopic examination of 150 samples showed only 40 (26.66%) samples are Gram-negative bacteria and after examination with the VITEK II system, the results showed only 7 (5.38%) samples are *proteus mirabilis* bacteria. The isolated *Proteus mirabilis* was tested to twenty antibiotics with different mechanisms of action, and the present study was noted (36.43%) of isolated *Proteus mirabilis* was resistant for antibiotics, (6.43%) was given intermediate response, while (57.14%) was sensitive for antibiotics. The present study also noted that (100%) was resistant for Doxycycline, while (100%) was sensitive for Carbencillin, Cefotitan, Amikacin,

Ofloxacin, Doripenem, Isepamicin, and Trimethoprim. All samples diagnosed as *proteus* proved to be positive for 16s rRNA test, and *ure* R. While all showed their negative for the *bla* KPC gene. The isolated strains were then identified, and their sequences were compared with NCBI sequencing for SNP detection. Partial region of gene 16S rRNA was amplified and sequenced using Sanger sequencing method. Three isolated bacteria diagnosed were also recorded in the GenBank: SUB14440996 EM07 PP894752, SUB14440996 EM11 PP894753, SUB14440996 EM23 PP894754.

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List of Abbreviations

Abbreviations	Mean
ATFs	Ambient temperature fimbriae
BHI	Brain heart infusion
bp	base pair
CO ₂	Carbon Dioxide
DNA	Deoxyribose Nucleic Acid
<i>E. coli</i>	<i>Escherichia coli</i>
ESBLs	Extended spectrum β -lactamase
GNB	Gram-negative bacteria
H-NS	histonelike nucleoid structuring protein
HpmA	Hemolysin proteus mirabilis A
HSL	N-acyl-L-homoserine lactose
IgA	Immunoglobulin A
IgG	Immunoglobulin G
KDa	Killo Dalton
KPC	<i>Klebsiella pneumonia</i> like carbapenemase
LPS	Lipopolysaccharide
MR-K	Mannose resistant <i>klebsiella</i>
MR-P	Mannose resistant <i>Proteus</i> -like
MDR	multidrug resistant
NAF	Nonagglutinating fimbriae
NCBI	National Center for Biotechnology
NH ₃	Ammonia

OPS	O-specific polysaccharide
<i>P</i>	<i>Proteus</i>
PCR	Polymerase chain reaction
PH	potential of hydrogen
PMP	p-like fimbriae of <i>P. mirabilis</i>
Pta	Proteus toxic agglutinin
QS	Quorum sensing
QQI	Quorum quenching inhibitors
rRNA	ribosomal Ribonucleic acid
S	Susceptibility
SM	swimming motility
Spp	. Species
UCA	Uroepithelial cell adhesion
<i>Ure</i>	Urease
UTIs	Urinary tract infections
µg	Micro gram

Chapter One

Introduction

1.1. Introduction

Urinary tract infections (UTIs) impact millions of people annually and are serious health issues. Urinary tract infections affect people of both sexes. However, because of the structure of the female urogenital tract, females are thirty times more likely than males to suffer a UTI (Hooton, 2001). UTIs can occur in up to 60% of females at some point in their lives (Olds *et al.*, 2004), because they are more likely to travel to the kidneys during pregnancy, urinary tract infections may be more serious (Hooton, 2001). Urinary tract infection can be defined as an infection caused by the presence of microorganisms anywhere in the urinary tract (Morgan & McKenzie, 1993).

proteus mirabilis is a typical component of the human intestinal flora and can lead to illness when it leaves the body. It can also spread accidentally by accidental parenteral inoculation or contaminated urinary catheters (Mohammed *et al.*, 2016). The microbes that cause UTIs in people who are not pregnant are the same ones that cause UTIs in pregnant women. Seventy to eighty percent of illnesses are caused by *Escherichia coli* (*E. coli*). (Barr *et al.*, 1985; McDowall *et al.*, 1981). Other typical Gram-negative bacteria (GNB) include *Klebsiella* spp. and *Proteus mirabilis*. However, it is uncommon to see Gram-positive cocci like *Mycoplasma hominis*, *Staphylococcus* spp., *Gardnerella vaginalis*, *Ureaplasma urealyticum*, and group-B streptococci (Sengupta, 1987). Many potential virulence factors that *P. mirabilis* possesses could facilitate its pathogenesis include urease's hydrolysis of urea, swarming's ability to invade and colonize cells, hemolysins' cytotoxicity, protease's cleavage of IgA and IgG, the ability of *P. mirabilis* to produce biofilms, and the five types of fimbriae that have been described for *P. mirabilis* so far (Coker *et al.*, 2000; Jacobsen & Shirtliff, 2011). Because of their peritrichous flagella, which aid in their

movement and allow them to swarm across solid surfaces, *Proteus* species are known for their interesting and pathogenic behavior. Changes in cell shape and the synthesis of virulence agents which accompany the swarming differentiation, makes them skilled at colonizing and causing disease (Böhme *et al.*, 2010). One of the hallmarks of *Proteus* spp. infections is their ability to form stones, known as struvite calculi, in the urinary tract. These stones can lead to complicated (UTIs) which are a frequent clinical problem. The bacteria produce urease, an enzyme that hydrolyzes urea to form ammonia, elevating the pH of urine and leading to precipitation of magnesium ammonium phosphate and calcium phosphate in the urinary tract. This can cause kidney stones, which may lead to more severe kidney infections (Buhimschi *et al.*, 2009). In pregnant women, *Proteus* spp. can be particularly problematic. UTIs caused by these bacteria can lead to serious complications including pyelonephritis, premature labor, and the potential for vertical transmission to the neonate. During pregnancy, the urinary tract undergoes physiological changes that can predispose to bacterial colonization and infection. Asymptomatic bacteriuria, which can be caused by *Proteus* spp., is found in a significant percentage of pregnant women and, if left untreated, can lead to acute cystitis and pyelonephritis, which are associated with increased risks for preterm birth and low birthweight infants (Khan & Musharraf, 2004). *Proteus* spp. are significant pathogens with a remarkable ability to cause infection and complications, especially in the urinary tract. Their detection and management in pregnant women are essential due to the high stakes involved for both maternal and neonatal health. As research progresses, the development of more refined molecular techniques will likely enhance our ability to diagnose, treat, and prevent the infections caused by these adaptable and often resilient bacteria.

1.2. Aim of study:

Aim of the study is detection of some virulence and resistance genes in uropathogenic *Proteus* spp.:

1. Isolation and identification of *Proteus* species by morphology, culturing, and VITEK II.
2. Detection antimicrobial susceptibility of the bacterial isolates.
3. Detection of *proteus* spp. by Molecular analysis.
4. Diagnosis of some virulence and resistance genes such as (Urase = *ure* R gene, and antibiotic resistance gene =*bla* KPC).

Chapter Two

Literatures Review

2. Literatures review:

2.1. Urinary tract infection:

Urinary tract infections in pregnant women are a common concern that requires careful attention due to the unique physiological changes that occur during pregnancy. The prevalence of UTIs in this demographic underscores the importance of understanding its implications, symptoms, diagnosis, and treatment strategies to ensure both maternal and fetal health. During pregnancy, the urinary tract undergoes significant anatomical and physiological changes, including the dilation of the ureters and an increase in urinary stasis, which can contribute to the higher incidence of UTIs. Additionally, hormonal shifts lead to changes in the urinary tract's lining, making it more susceptible to bacterial invasion (Abduljalil *et al.*, 2012). UTIs do not directly cause birth defects, but their complications can impact both maternal and fetal health:

- For Mothers: Chronic kidney disease from recurrent pyelonephritis, hypertension, and increased stress on the body.
- For Fetuses: The most significant risks include preterm birth and low weight, both of which can have long-term effects on the child's development and health (Habak PJ, Carlson K, Griggs, 2024).

UTI in a pregnant woman typically involves a detailed medical history, physical examination, and the analysis of a urine sample. Urine culture is the gold standard for diagnosing UTIs, allowing for the identification of the causative bacteria and their antibiotic sensitivity, which guides targeted treatment. Given the potential for resistance, treatment choices are carefully considered, prioritizing antibiotics that are safe for use during pregnancy, such as cephalosporins, nitrofurantoin (with caution in the late third trimester), and amoxicillin (Davis & Narayan, 2020). UTI is defined

as the presence of at least 1×10^5 organisms per milliliter of urine in an asymptomatic patient, or as more than 1×10^5 organisms/mL of urine with accompanying pyuria (> 7 white blood cells [WBCs]/mL) in a symptomatic patient. A diagnosis of UTI should be supported by a positive culture for a uropathogen, particularly in patients with vague symptoms (Kalinderi *et al.*, 2018). UTIs during pregnancy represent a significant health concern that requires prompt attention and management to prevent adverse outcomes for both mother and child. Early diagnosis and treatment are crucial to avoiding complications and ensuring a healthy pregnancy and delivery. Further research into prevention and management strategies will continue to improve care for pregnant women experiencing UTIs. Due to the initial request's scope and the space constraints here, this overview encapsulates the critical aspects of the topic. For more in-depth information on each section, consulting specific medical resources or healthcare professionals is recommended (Biaggi *et al.*, 2016).

2.2. Common Microorganisms that cause UTIs:

Urinary tract infections may be caused by a wide variety of microorganisms, including bacteria, fungi, yeasts, and viruses. These diseases are often identified very easily. As a result, a urinary tract infection is a potentially deadly condition that you are unaware of, which may result in the development of significant chronic pyelonephritis as well as chronic renal failure (S. Ali *et al.*, 2022). Infections of the urinary tract may further be subdivided into two additional categories: complex and simple. The structural and functional abnormalities raise the likelihood of contracting the bacteria in the urinary system, which is known to be a complex infection. This probability is increased by the fact that the urinary tract abnormalities are present. Simple urinary tract infections do not cause any structural or functional problems in the urinary tract (Levison & Kaye, 2013). on congenital parasitic infections caused by *Toxoplasma gondii*, *Trypanosoma cruzi*, and *Plasmodium*. Hepatitis C virus

HCV-infected pregnant women and their children (Williams, 2003) (Burns *et al.*, 2010).

One of the most serious problems caused by bacteria in a pregnant woman is urinary tract infection, which may cause serious issues for both the mother and the baby (Amarasinghe *et al.*, 2018). Bacteria that belong to the *Enterobacteriaceae* family are found in the digestive tracts of both humans and animals. This heterologous family includes *Escherichia coli*, *Salmonella*, *Shigella*, *Enterobacter*, *Klebsiella*, and *Proteus* spp. *Escherichia coli* is the cause of 85–90% of urinary tract infections (Aslam & Aslam, 2022). These bacteria are members of the *Enterobacteriaceae* family, which is the main harmful agent of this illness during pregnancy (Hussein *et al.*, 2021). The bacteria *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus*, and *Pseudomonas aeruginosa* are the most common causes of complicated cases of (UTIs), such as those that result from catheterization or from anatomic obstructions. In these cases, antibiotics are often ineffective (Padmavathy *et al.*, 2012).

2.3. *Proteus* spp.

The genus *Proteus* was first documented in 1885 by Gustav Hauser, a German microbiologist, who observed their capacity to move collectively across solid surfaces. The term *Proteus* originated from Homer's Odyssey and its figure *Proteus*, who had the power to alter his form and had an inexhaustible capacity for metamorphosis. Hauser identified and documented two species belonging to the genus *Proteus*: *Proteus vulgaris* and *Proteus mirabilis* (Manos & Belas, 2006). The *Proteus* genus comprises Gram-negative, facultative anaerobic, heterotrophic, and proteolytic rods that act as opportunistic pathogens in humans. These bacteria have undergone many revisions in their taxonomic categorization. Recently, there has been a proposal to reclassify *Proteus myxofaciens*, a species within the *Proteus*

genus, and shift it to a newly created genus called Cosenzaea. This species, *Proteus myxofaciens*, is currently considered to have little clinical importance (Giammanco *et al.*, 2011). It is worth noting that as part of these modifications, numerous species have been excluded from the genus *Proteus* and placed into new genera called *Providencia* and *Morganellais*. The three closely related genera have combined to create the tribe Proteae within the family Enterobacteriaceae (Manos & Belas, 2006).

The current taxonomic classification of the *Proteus* genus includes *mirabilis*, *vulgaris*, *penneri*, *hauseri*, as well as three more genomospecies identified as 4, 5, and 6. *hauseri*, together with the genomospecies, was distinguished from *p.vulgaris* based on genetic research and constitutes the *P. vulgaris* group. The genomospecies 4, 5, and 6 are identified only by numerical designations because to the absence of any identified metabolic features that would enable their complete separation (O'Hara, Brenner, Steigerwalt, *et al.*, 2000). Former species of genus *Proteus* now homotypic synonyms with other species: *P.inconstans* with *Providenciaalcalifaciens*, *P. morganii* with *Morganellamorganii*, and *P. rettgeri* with *Providenciarettgeri* (Farmer 3rd *et al.*, 1985; Q. Wang *et al.*, 2010) .

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

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Proteus*

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

(Table 2.1) Biochemical tests used in differential examinations between the genera of *Proteus* spp. (Różalski et al., 2013).

Test	<i>P.mirabilis</i>	<i>p.vulgaris</i>	<i>p.penneri</i>	<i>p.hauseri</i>	<i>p. myxofaciens</i>
Esculin hydrolysis	-	+	-	-	-
Maltose fermentation	-	+	+	+	+
Salicin fermentation	-	+	-	-	-
Indole production	-	+	-	+	-
Ornithine decarboxylase	+	-	-	-	-
D-Xylose fermentation	+	+	+	+	-
Chloramphenicol	S	V	R	S	

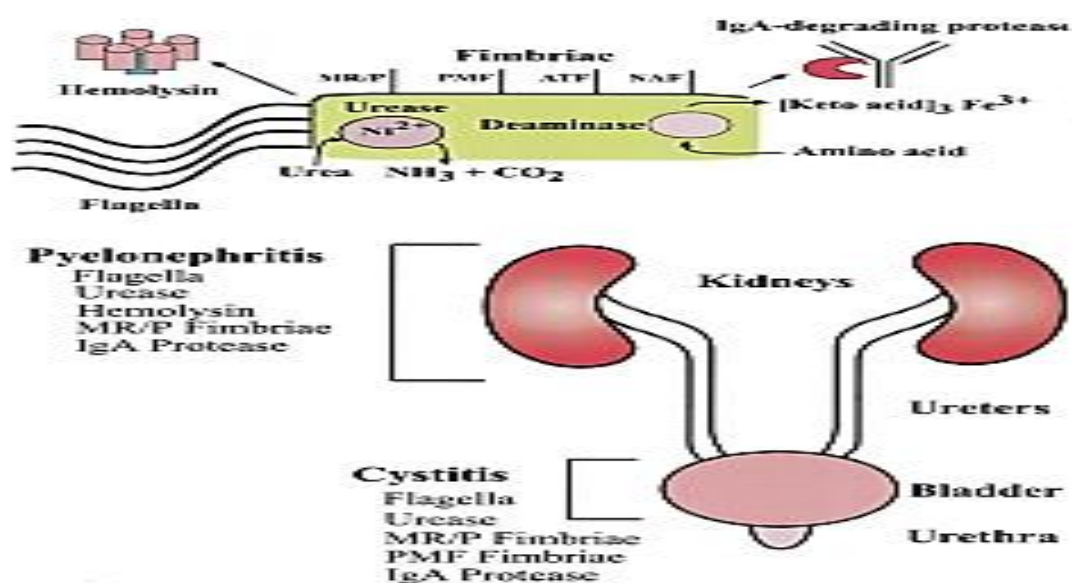
(S) Susceptibility, (+) positive result, (-) Negative result, (S) susceptible, (R) resistant, (V) variable.

2.4. Pathogenicity:

Gram-negative bacteria belonging to the *Proteus* genus are well-known for their potent propensity to infect humans. They are closely linked to diseases like *Salmonella* and *Escherichia coli* as members of the Enterobacteriaceae family. *Proteus mirabilis*, along with *Proteus vulgaris* and other members of the genus, is the most frequently linked species to human sickness. In addition to being present in soil, water, and the digestive tracts of both humans and animals, these creatures are widely distributed throughout the environment (Bingen *et al.*, 1993). The pathogenicity of *Proteus* spp. is multifaceted and involves various factors including endotoxins, flagellin, and fimbriae which facilitate adhesion to host cells, evasion of

host defenses, and induction of a robust inflammatory response. The bacteria's ability to resist the action of antibiotics through the production of β -lactamases and the acquisition of resistance genes further complicates treatment, making the understanding of its molecular basis of pathogenicity essential for developing new therapeutic strategies (Yu *et al.*, 2017). The rods of *Proteus* species are prevalent opportunistic pathogens that can infect individuals with impaired immune systems due to their relative infectiousness. Usually persistent, these illnesses are difficult to treat (Kwiecinska-Piróg *et al.*, 2014). In the right circumstances, opportunistic bacterial pathogens called *proteus* rods can cause rheumatoid arthritis, newborn or infantile meningitis, wound infections, and UTIs (Różalski *et al.*, 2013).

Common infections affecting the upper urinary tract include cystitis, acute pyelonephritis, and urolithiasis [stone accumulation in the kidney or bladder].



Figure(2.1) A schematic figure represents the proteins produced by *P. mirabilis* that are either known or hypothesized to be virulence factors in UTIs (Coker *et al.*, 2000). This urinary tract diagram shows the urethra, bladder, ureters, and kidneys.

In pregnant women, *Proteus* spp. can be particularly problematic. UTIs caused by these bacteria can lead to serious complications including pyelonephritis, premature labor, and the potential for vertical transmission to the neonate. During pregnancy, the urinary tract undergoes physiological changes that can predispose to bacterial colonization and infection. Asymptomatic bacteriuria, which can be caused by *Proteus* spp. is found in a significant percentage of pregnant women and, if left untreated, can lead to acute cystitis and pyelonephritis, which are associated with increased risks for preterm birth and low birthweight infants (Khan & Musharraf, 2004).

2.5. *Proteus* specification:

Swarming behavior is one of the most notable microbiologic traits of *Proteus* spp. centric rings of development originating from a single colony or inoculum are the macroscopic manifestation of swarming. Bacterial swarming occurs when they undergo a biological metamorphosis, characterized by increased flagellin synthesis and cellular elongation, from "swimmer cells" in broth to "swarmer cells" on a surface like agar. *Proteus* had unrestricted shape-changing ability. Bacteria *Proteus* species are facultatively anaerobic, rod-shaped, Gram-negative, non-spore-forming, non-capsulated, and highly motile due to peritrichous flagella that form concentric zones on the solidified agar. They range in size from (0.4–0.8) μm in diameter to (1–3) μm in length (Brenner *et al.*, 2005). Fish odor can be detected in *proteus* (O'Hara, Brenner, & Miller, 2000). For it to grow, 37 °C and a pH of 7.4 are ideal. Its nutrition is chemoorganotrophic, which defines it. It generates energy by fermentation and aerobic respiration, two distinct processes (Bergey, 1994).

2.6. Virulence factors: -

Many virulence factors are produced by strains of *Pruteus* spp. (Senior, 2010) *Proteus* bacilli has evolved a variety of physiological and biochemical characteristics that are thought to be virulence factors. These are flagella, which are vital for bacterial ascent to the kidneys, and fimbriae, which are critical for attachment through the ureter, in addition to toxins like hemolysins and endotoxins (lipopolysaccharide, LPS), enzymes (urease hydrolyzing urea to CO₂ and NH₃), and proteases that degrade antibodies, complement proteins, and tissue matrix proteins (keto acid-generating amino-acid deaminases, which function as iron-binding siderophores (Knirel *et al.*, 2011). Many pathogenic factors are produced by the virulence genes HigB, ureC, rsbA, zapA, and mrpA of the genus *Proteus* (Hurley & Woychik, 2009).

2.6.1. Flagella

Flagella is responsible for bacteria motility that resulted in invading and spreading of infection in urinary tract (Kuan *et al.*, 2014). Bacterial flagella are helical filamentous organelles that facilitate bacterial motility (Nakamura & Minamino, 2019) critical for the pathogenicity of bacteria because they play a variety of functions, such as adhesins, promote the growth of biofilms, transport virulence proteins, or trigger host proinflammatory reactions (Duan *et al.*, 2013). The flagella on the surface of bacterial pathogens are generally thought to help with host colonization and dissemination, initial adhesion, and extracellular environment sensing. These surface characteristics are essential to one characteristic that sets *Proteus* species apart is their ability to swarm. Swarming may affect how *Proteus* strains migrate over catheter materials. Flagella have a strong immunogenicity and

serve as an H antigen. The antigenic variation mechanism allows germs to dodge the immune system's reaction in humans (Różalski *et al.*, 2013).

2.6.2. Motility

There are three main ways that members of the *Proteus* genus move: swimming, swarming, and twitching. *Proteus* bacteria employ swimming motility (SM) in liquid media. Six to ten flagella are present on the bacterial surface for this a motion kind. Swarming motility (SW), which requires more flagella, is the mechanism by which movement on solid surfaces is achieved. *Proteus* grown on solid agar, the subject of the Rauss phenomenon, usually travels in the direction of the southwest. The bacteria wiggle to go between two solid surfaces (Hola *et al.*, 2012). A population of flagellated bacteria moving across a semi-solid surface is called a bacterial swarm (Jose & Singh, 2020).

Swarming is important for *Proteus* rods' ability to spread to new areas and may even facilitate their colonization of microorganisms. It involves intercellular and cell-to-cell communication and is connected to the possibility of morphological differentiation of bacteria depending on the growth medium. *Proteus* are dimorphic bacteria that resemble short, motile rods that are peritrichously flagellated in liquid environments. They range in length from 1.0 to 2.0 μm and have 6–10 flagella. These microbes are known as swimmer cells. However, when these short rods are transferred to a solid medium, they grow into elongated (20–80 μm in length), hyper-flagellated, multinucleated, non septated swarmer cells (Różalski *et al.*, 2013).

2.6.3. Fimbriae

Adhesion of *proteus mirabilis* is a most important step in colonizing and developing infections (Ranjbar-Omid *et al.*, 2015). Most Gram-negative bacteria and other bacteria have short, hair-like appendages called fimbriae (plural fimbria), which are essential for bacterial cells to adhere to other surfaces as well as to each other. A few microorganisms that are Gram-positive (Jin & Marshall, 2020). *Proteus*

mirabilis can produce six different types of fimbriae: Mannose-resistant *Proteus*-like fimbriae (MR/P fimbriae), Mannose-resistant *Klebsiella*-like fimbriae (MR/K fimbriae), Nonagglutinating fimbriae (NAF), also called uroepithelial cell adhesin (UCA), Ambient-temperature fimbriae (ATF), *P. mirabilis* P-like pili (PMP), and *P. mirabilis* fimbriae (Manos & Belas, 2006).

2.6.4. Biofilm formation

The gathering of microbiology and its adhesion to the surface of the host cells in an aqueous environment surrounded by extracellular polymers (multiple sugars) as the membrane participates in the induction of infection, antibiotic resistance, and germ adhesion to surfaces (Kokare *et al.*, 2009). In order to endure harsh environments and fortify their resistance against drugs and the human immune system, a number of bacterial species generate biofilms, which are orderly collections of single- or multispecies microbial communities where microbial cells are permanently bonded both to one another and to a substratum in the natural world. Antibiotic resistance in bacteria associated with biofilms is 10-1,000 times higher than that of bacteria associated with planktonic environments (Høiby *et al.*, 2010; Lee *et al.*, 2017). Microbiofilms are the most prevalent kind of microbial life seen in natural settings. Adhesion to an abiotic or biotic surface and the development of a biofilm are the first two stages in the production of a microorganism's biofilm a framework that encourages adherence. Extracellular polymers are known to protect bacteria from environmental changes, aid in the delivery of nutrients and the removal of metabolic waste, and cluster cells closely together to enhance cell-to-cell contact. These polymers are created by microorganisms within the expanding biofilm (Lee *et al.*, 2017).

2.6.5. Urease

Urease, a cytoplasmic nickel metalloenzyme, works by breaking down urea into carbon dioxide and ammonia. Many bacterial species choose the resultant ammonia as their nitrogen source, and glutamine synthetase (GlnA) or glutamate dehydrogenase (GdhA) can incorporate the ammonia into biomolecules (Mobley, Harry LT, 2001). Local pH increases as a direct result of urease activity and the release of ammonia. Alkaline pH in the urinary tract causes calcium and magnesium ions to precipitate, resulting in the production of urine stones formed from calcium phosphate (apatite) and magnesium ammonium phosphate (struvite) (Griffith & Musher, 1976).

Urease forms urinary stones, which is one way that virulence is mediated. These stones have the potential to become rather large ($> 1 \text{ cm}^2$), obstruct urine flow, and damage tissue (Chew *et al.*, 2012; Munns & Amawi, 2010). The precipitated minerals have the ability to combine with bacteria that adhere to a urinary catheter, creating a crystalline biofilm that can eventually stop the catheter from holding urine (Jones *et al.*, 2005; Stickler, 2008). Identical intracellular crystals have been observed in cultured urinal epithelial cells infected with invasive *P. mirabilis* in an experimental setting (Torzewska *et al.*, 2014). Only *P. mirabilis* exhibits a positive correlation with catheter obstruction, despite the fact that other urease-positive bacterial species are linked to catheter-associated UTIs (Mobley & Warren, 1987). These stones have the potential to embed bacteria, which might shield diseases from immune system responses or treatments (Li *et al.*, 2002). Also, urinary stones can act as a point of entry for different bacterial species to cause UTIs. (Griffith & Musher, 1976).

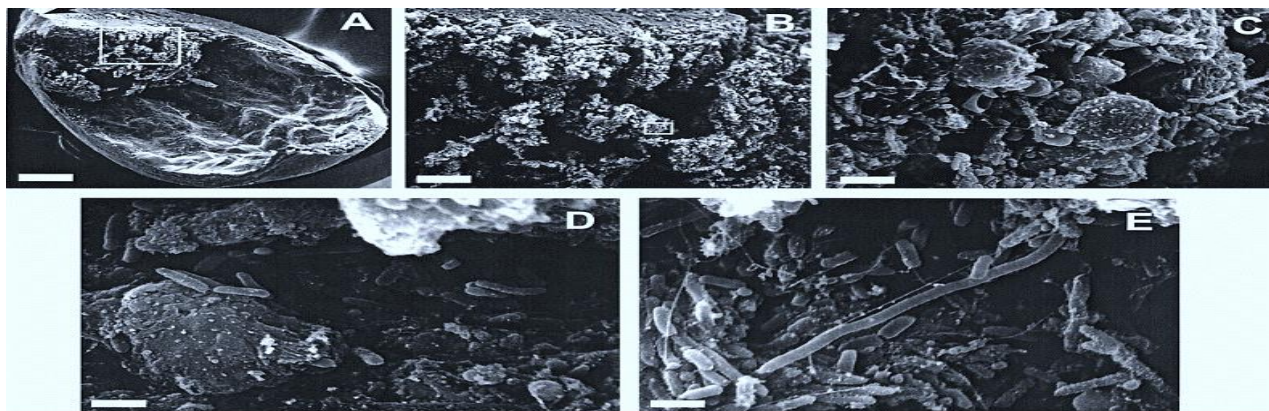


Figure (2.2) Bladder stones produced by *P. mirabilis* urease seen in scanning electron micrographs. (A) A portion of the bladder magnified at 500 μm , a low magnification. An arrow pointing to the inferior end of the bladder—the end that connects to the urethra—indicates the orientation of the bladder. (B) An increased magnification (100 μm bar) of the boxed area in panel A. (C) An increased magnification (bar, 5 μm) of the panel B area contained in a box. Representative images of the bladder stone (bars, 2 μm) are shown in (D and E) (Li *et al.*, 2002).

The urease enzyme's synthesis and activity were investigated through a few of *P. mirabilis*'s genes. The five accessory genes (*ure R*, D, E, F, and G) encode proteins that help incorporate the required nickel ions into the catalytic site. The three structural subunits (*ure A*, B, and C) encode the apoenzyme structural subunits (Mobley *et al.*, 1995). Although the *ureR* gene is located next to the *ureDABCEFG* operon on the reverse strand (Nicholson *et al.*, 1993). In the presence of urea, a *ureR* gene mutant that is part of the AraC-family positively stimulates urease expression is devoid of detectable urease function (Dattelbaum *et al.*, 2003). This gene recruited RNA polymerase to initiate transcription (Poore *et al.*, 2001).

The two urease regulator genes in *Proteus mirabilis* are *ureR* and H-NS (histonelike nucleoid structuring protein), which are in charge of transcription. The *UreR* gene belongs to the positive AraC family of genes transcriptional regulators that have binding domains for both urea and DNA (M. Kumar *et al.*, 2015).

The *ureR* gene is a dimer that attaches to two locations at a consensus sequence in the *ureR-ureD* intergenic region. T(A/G)(T/C)(A/T)(T/G)(C/T)T(A/T)(T/A)ATTG (Sriwanthana *et al.*, 1993; Thomas & Collins, 1999). It binds the promoters of *ureR* and *ureD* (O'Hara, Brenner, & Miller, 2000), and transcribes the structural genes of urease by inducing urea in the opposite direction of the gene cluster (*ureDABCEFG*) (D'Orazio *et al.*, 1996; Park & Hausinger, 1993). While H-NS is a passive regulator that suppresses *ureR* transcription and inhibits *ureR* transcription when it binds to multiple (A) regions located in the genetic region between *ureR* and *ureD*, *ureR* acts as a positive regulator of urease activity and stimulates the expression of urease genes in the presence of urea (Patel, 2001).

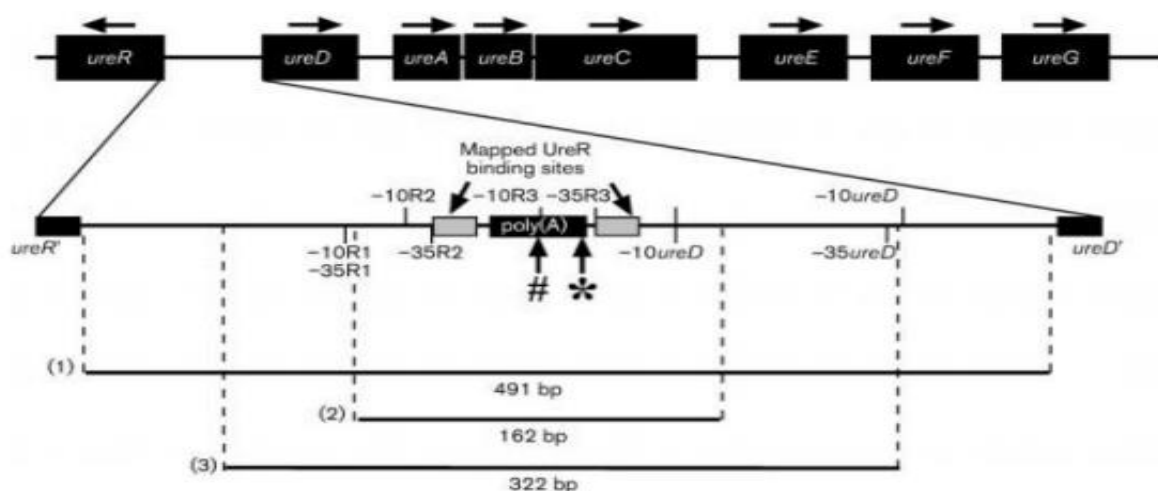


Figure (2.3):The urease gene cluster in *Proteus mirabilis* (Poore & Mobley, 2003).

2.6.6. Cytolysins

Proteus rods produce three various types of cytolysins: Pta (proteus tototoxic agglutinin), HpmA and HlyA hemolysins. HpmA, a 166 kDa protein, is a cell-bound hemolysin that is not dependent on calcium. HlyA hemolysin is generated by strains of *P. vulgaris*, *P. penneri*, and *P. hauseri* bacteria and is triggered by HpmB. (Różalski et al., 2013). *P. mirabilis* has the capacity to both internalize and lyse bladder epithelial cells through the use of hemolysin and *Proteus* toxic agglutinin (Pta) (Alamuri et al., 2009).

2.6.6.1. Hemolysins (HpmA, HpmB)

Toxin that enters the target membrane of the eukaryotic cell and create pores to cell disruption by ion efflux and the cells lysis. (Cabezas *et al.*, 2017). The hemolytic activity *P. mirabilis* produces is associated with the hemolysin HpmA. This hemolysin is linked to the cell and the calcium-independent creation of pores. It is encoded by two genes, HpmA and HpmB, which result in the HpmA (166 kDa) and HpmB (63 kDa) proteins, respectively (Cestari *et al.*, 2013). The tissue of the kidneys are damaged by α hemolysin HpmA, which is produced by *P. mirabilis*. The two genes HpmA and HpmB constitute the calcium-independent pores-former of the cell, which includes this α -hemolysin. which control the 63 kDa HpmB proteins (Lazm *et al.*, 2019). When the N-terminal peptide of HpmA α -hemolysin is cut, the tissue is injured and activated. Consequently, the result can activate HpmA and HpmB, responsible for transporting and activating HpmA (Howery *et al.*, 2016). HpmA is said to be found in the periplasm, where HpmB binds to and activates it outside the membranes involved in the HpmA secretion cycle. Proximal tubular epithelial cells in human kidneys produced by HpmA show signs of cytotoxicity (Etxaniz *et al.*, 2020). The creation of haemolysin has been associated with increased

virulence and cytotoxicity for vero cells in a mouse model of UTIs (Mishra *et al.*, 2001). They nucleate cells and lyse erythrocytes (Alamuri *et al.*, 2009). Swihart and Welch demonstrated that hemolytic and cytotoxic activities are independent of each other in human cells (epithelial, and Vero cells) by using mutant isolates (HpmA negative) that lack both of these functions. Due to the development of active hemolysin HpmA (Cestari *et al.*, 2013).

2.6.6.2. *Proteus* toxin hemagglutinin (Pta)

Protein that is designated as the outer membrane auto-transporter mediates aggregation of the cell and that can lyse the kidney and bladder cells. (Gupta *et al.*, 2019) . Furthermore, Pta, an alkaline protease is dependent on calcium, causes cytotoxicity in cultured epithelial cells that varies in both dose and duration. Human bladder and kidney cells, as well as microorganisms, exhibit cytotoxicity. Agglutin and cytotoxins are the causes of autoagglutination (Alamuri & Mobley, 2008). Mutation analysis has been used with a mouse model of progressive UTI. Significant reductions in the mutant's bacterial burden in kidneys were observed with inactivation of pta but not *HpmA* (Alamuri *et al.*, 2009).

2.6.7. lipopolysaccharide (LPS)

Endotoxin have resistant role to normal serum proteins, which have a deadly effect on bacteria, and this resistance has important role in bacteremia and host invasiveness. (Morgenstein *et al.*, 2010). Three components form the LPS molecule: lipid A, an O-antigen/polysaccharide (O-PS) and a core oligosaccharide (OS). The LPS is held in place in the inner core via the lipid A component that is connected to OS molecule fixed within the external layer, Antigen, is bound by the outer core's OS. Based on their presence and shape, LPS molecules are categorized as smooth

(S)- or rough (R)-LPS. attributes of the antigen and the main OS (Gnauck *et al.*, 2016). The etiological agents of the majority of *Proteus* species infections are smooth (S) kinds of bacteria. The ternary lipopolysaccharide that these bacteria make is composed of lipid A, a core oligosaccharide and O-antigen, a polysaccharide (OPS) chain that is unique to O. *Proteus* species are serotyped according to their variety, and the chemical OPS structures of the bacteria dictate their serospecificity (Knirel *et al.*, 2011).

2.6.8. Quorum sensing

Identify population density, control social behaviors like diffusion, swimming, mobility, and pairing, and manage gene expression using cell-to-cell contact (Stankowska *et al.*, 2012). The quorum-sensing (QS) system of N-acyl-L-homoserine lactone (HSL) regulates *P. mirabilis*'s virulence characteristics. The acyl-HSL molecules' breakdown in QS-dependent organisms signals through quorum quenching. Inhibitors (QQI) cause the pathogenicity to decline. Rival bacteria's quorum-sensing systems may be precisely targeted by non-toxic chemical countermeasures. Bacterial communication is regulated by the generation and subsequent discovery of small signaling molecules, which can also influence important features such as bioluminescence, swarming motility, biofilm formation, virulence factor production, and antibiotic biosynthesis (K. M. Younis *et al.*, 2016).

2.7. The importance of molecular detection in pregnant women

In the context of pregnancy, the molecular detection of *Proteus* spp. is not just a diagnostic endeavor but a preventive health measure. Given the propensity for these bacteria to cause severe infections and the associated obstetric complications, their early detection in asymptomatic bacteriuria or mild UTIs can lead to targeted interventions that may prevent adverse outcomes. Thus, the molecular study of

Proteus spp. in pregnant women is not only important for the immediate management of infections but also for the long-term health of the mother and the developing fetus (Burns *et al.*, 2010).

The molecular detection of *Proteus* spp. offers several advantages over conventional culture methods. Molecular techniques, such as polymerase chain reaction (PCR), are more sensitive and provide results much faster than traditional culture, which can take several days. This is particularly beneficial during pregnancy, where the prompt initiation of appropriate antibiotic therapy is crucial to prevent the ascent of the infection, fetal infection, and preterm labor (Böhme *et al.*, 2010). Moreover, molecular methods can detect the presence of bacteria that are difficult to culture or are present in concentrations below the detection limit of traditional methods (Buhimschi *et al.*, 2009). The *Proteus* genus is known for its ability to rapidly develop resistance to multiple antibiotics, which can complicate the treatment of infections. Molecular detection methods can identify genetic markers of antibiotic resistance, allowing for more effective stewardship of antimicrobial agents. This is particularly important in pregnant women, where the choice of antibiotics is limited due to potential risks to the fetus (Romero *et al.*, 2014). This happens because every bacteria possesses the 16S rRNA gene, sequencing-based bacterial identification is a viable method (Clarridge III, 2004).

Furthermore, variable sections interspersed with nucleotide sequences make up the 16S rRNA gene, which yields a species-specific signature sequence that is essential for bacterial identification. The sequences that were obtained are compared to the database's known sequences (CR, 1987). When dealing with a combination of several different infections, the method is valuable. It is also helpful in identifying germs that are hard to grow and those found in samples taken from patients after

receiving antibiotics. It has been discovered that *E. coli* strain 131 is multidrug-resistant (MDR) (Ben Zakour *et al.*, 2016).

2.8. 16S rRNA

The 16S ribosomal RNA (rRNA) gene is a key component of the 30S subunit of the prokaryotic ribosome and is highly conserved among different bacterial species. The 16S rRNA gene is present in all bacteria, which makes sequencing-based bacterial identification conceivable (Clarridge III, 2004). Furthermore, the 16S rRNA gene has different sections scattered across nucleotide sequences, which are the hallmark of bacterial identification since they yield a species-specific signature sequence (CR, 1987). For the beta lactamase and 16S rRNA fragmentation of these primers, multiple level DNA sequences for all known *P. mirabilis* isolates were utilized (Limanskiĭ *et al.*, 2005).

The 16S ribosomal RNA gene has been sequenced by researchers since the 1970s in order to better understand and characterize bacterial communities. The length of the 16S rRNA gene is approximately 1,550 base pairs. (BP). Within the realm of bacterial species, there exist regions which are remarkably diverse and well preserved. In addition to allowing bacterial clade differentiation, the highly conserved parts make it possible to design "universal" PCR primers that target and amplify the 16s rRNA sequence. 16S rRNA sequencing investigations can identify almost all of the bacteria in a microbial community. Their features can then be ascertained by comparing them to massive 16s rRNA databases (Weinroth *et al.*, 2022). The *rpoB* gene has improved bacterial community analysis and helped define new bacterial species in conjunction with the 16s rRNA gene. Moreover, the microbial community's overestimation of operational taxonomical units (OTUs) These housekeeping genes, which are often found in bacterial genomes as single copies, regulate groups. (Ogier *et al.*, 2019)

2.9. Gene *bla* KPC

The *bla* KPC gene encodes for *Klebsiella pneumoniae* carbapenemase. Carbapenem resistance in Enterobacteriaceae has recently grown widely, which is concerning for public health because carbapenems are a last-resort treatment for this family of prevalent bacterial infections. The *Klebsiella pneumoniae* carbapenemase (KPC) gene, *bla*KPC, was identified as one of the most prevalent carbapenem resistance genes in 1996 and is currently present in many regions of the world (Sheppard *et al.*, 2016). The (KPC) enzyme hydrolyzes the majority of N-lactam antibiotics. Several KPC variations have been documented, and bacteria that produce KPC have been identified worldwide. Often, these microorganisms are members of the Enterobacterales group (Hua *et al.*, 2020).

One of the most common types of β -lactamases that is known is the enzyme from *Klebsiella pneumoniae* carbapenemases (KPC) (Ruiz-Garbajosa *et al.*, 2013). The beta-lactamase KPC has the ability to hydrolyze all beta-lactams, including cephalosporins, carbapenems, monobactams, and penicillins (Sheppard *et al.*, 2016).

The carbapenemases previously identified in *P. mirabilis* are the Ambler class A metallo β -lactamase KPC-2, the class B [metallo- β lactamases (M β LS)] VIM-1 and NDM-1, and the carbapenem-hydrolysing class D metallo-lactamase OXA-23 (Girlich *et al.*, 2015). The occurrence of *P. mirabilis* obtaining *bla*KPC is exceptional, as just a few descriptions of it have been recorded to date (Di Pilato *et al.*, 2016).

The discovery of KPC-2 in a bloodstream isolate in 2008 marked the first instance of KPC transport in *P. mirabilis* (Tibbetts *et al.*, 2008). In addition higher patient mortality rates are associated with bacterial infections that produce KPC. *Proteus* species, like many members of the Enterobacteriaceae family, can have a wide range of integron- and plasmid-mediated antibiotic resistance determinants (Hua *et al.*, 2020).

Distribution and Impact

The *bla* KPC gene has been identified in various Enterobacteriaceae, including *Proteus mirabilis*, making it a critical factor in the spread of multidrug-resistant infections. The gene is often located on plasmids, which can easily be transferred between different bacterial species, exacerbating the problem of antibiotic resistance (Mobley *et al.*, 1995).

2.10. Polymerase Chain Reaction (PCR)

Molecular techniques such as PCR methodology, multiple PCR synthesis for simultaneous detection of common pathogens (e.g., PCR methods established to detect Enterobacteriaceae and therapeutically relevant microorganisms) could be used to detect *Proteus mirabilis*. noteworthy bacteria (Cheng *et al.*, 2006; Lu *et al.*, 2000). PCR assay is a rapid, specific, and sensitive detection method for *Proteus mirabilis*, and it is suitable for daily monitoring and rapid diagnosis of *Proteus mirabilis*. According to the sequences of the *ureR* gene of *Proteus mirabilis*, two primers were designed and used for PCR (Lin, H. L., Wen, L. and Zhang, 2009). Only those urea-inducible gene clusters contain the *ureR* gene of *P. mirabilis*, which is the only fully authentic regulatory gene that has been definitively found (Mobley *et al.*, 1995).

2.11. Susceptibility of *proteus spp.* To antibiotics:

Many variables affect how women's UTIs are treated. It is important to first identify between symptoms and asymptomatic infection. Women are more likely than men to get bacteriuria, and this tendency rises with age. Although treatment for the disease is not often thought to be necessary, in women, the chance of pregnancy makes examination important because asymptomatic bacteriuria is linked to an

increased risk of pyelonephritis and unfavorable pregnancy outcomes (Smaill & Vazquez, 2019).

Treatment of both symptomatic and asymptomatic bacteriuria during pregnancy is of significant importance. Aminopenicillins are not thought to be teratogenic, they are frequently employed during pregnancy (Christensen, 2000). Amoxicillin is an essential component of treatment and is well absorbed when taken orally. Cephalosporins are also typically regarded as safe choices. Nonetheless, there have been some case reports of ceftriaxone use during pregnancy and the potential development of kernicterus, which has sparked some concern regarding the use of single agents. In a similar vein, a surveillance study including over 4000 Medicaid clients in Michigan found teratogenicity with cefaclor, cefalexin, and cefradine (Briggs GG, Freeman RK, 2002). Because cotrimoxazole inhibits folic acid metabolism, causing teratogenic effects, it should be avoided. Antibiotics, including benzylepenicillin, oxacillin, tetracycline, and macrolides, can cause natural resistance in *Proteus* species (Stock, 2003). Plasmid-mediated beta-lactamases can cause *Proteus species* to develop resistance to ampicillin; nevertheless, it has recently been discovered that chromosomal beta-lactamase expression also occurs (Song *et al.*, 2011). Antibiotic resistance is a global health problem that limits therapeutic options. There are several mechanisms by which bacteria can resist antibiotics, including antibiotic inactivation by bacterial enzymes, decreased antibiotic entry into bacteria, antibiotic efflux, and mutation in the target site (Georgios *et al.*, 2014). Additionally, bacteria may become multidrug resistant (MDR), which could result in inadequate antibiotic treatment and contributes to the frequency of chronic illnesses (Nikaido, 2009).

Proteus species have been reported to produce extended-spectrum beta-lactamases (ESBLs) on multiple occasions over the past ten years. Third-generation

cephalosporins like cefotaxime, ceftriaxone, and ceftazidime, as well as the monobactam aztreonam, can be resistant to the ESBLs (Pfaller *et al.*, 2000). Generally speaking, ESBLs do not hydrolyze the carbapenems (imipenem and meropenem) or the cephamycins (cefoxitin, cefotetan, and cefmetazole) (Pfaller *et al.*, 2000). However, *Proteus species* are beginning to show signs of resistance to carbapenems (Schneider *et al.*, 2014) . It should be mentioned that some ESBL-producing *Proteus* isolates may prevent the MICs for third-generation cephalosporins or aztreonam from reaching commonly used breakpoints for resistance. The CLSI recommendations for susceptibility breakpoints were modified in 2010, which led to the reclassification of numerous ESBL-producing isolates that were previously thought to be resistant to these antibiotics as susceptible (P. Wang *et al.*, 2011). Using the new breakpoints, for example, 78–97% of the strains of ESBL that were evaluated were found to be susceptible to ceftazidime, cefepime, and aztreonam (Pfaller *et al.*, 2000). An additional modification to the CLSI guidelines took place in 2012, and according to SENTRY data from North America, this modification resulted in a lower percentage of imipenem susceptibility than the 2010 criteria (64.5% of 1244 isolates were susceptible by the 2012 criteria, compared to 99.8% by the 2010 criteria). These shifts in the breakpoints for susceptibility may lead to an underestimation of the data on resistance to carbapenems, aztreonams, and cephalosporins (Rennie & Jones, 2014). A nidus for the illness's persistence or could impede the urinary tract directly, which would encourage infection. Including over 4000 Medicaid clients in Michigan found teratogenicity with cefaclor, cefalexin, and cefradine (Briggs GG, Freeman RK, 2002). Because cotrimoxazole inhibits folic acid metabolism, causing teratogenic effects, it should be avoided.

Chapter Three

Materials and Method

3. Chapter Three Materials and Method

3.1. Materials

3.1.1. Instruments and Equipment

The research used several tools and equipment, as shown in the table (3.1)

Table (3.1) Displays the laboratory tools and equipment utilised in this work, along with their respective origins.

NO.	Equipment and instruments	Company / Country
1.	1.5 ml Eppendorff tubes	(Promeg/USA)
2.	Autoclave	(Raypa/ Spain)
3.	Beakers, Flasks & Cylinders (Different sizes	(Superior/ Germany)
4.	Burner	(Mettler/ Germany)
5.	Cabinet hood	(LAMINAR/ USA)
6.	Centrifuge	(DLAB /Ghain)
7.	Disposable syringes (5ml)	(Mediplast /U.A.E)
8.	Dry microtubes incubator	Ae (UK)
9.	Electrophoresis system	Cleaver(UK)
10.	Filtar tube	UK
11.	Incubator	(Froilabo/ France)
12.	Inoculatingloop	(TRUST /China)
13.	Kern PFB balance	Kern & Sohn(Germany)
14.	Light Microscope	(Olympus/ Japan)
15.	Microfuge IB Centrifuge	BeckmanCoulter(Germany)
16.	Optimus 96G thermal Cycler	QLS(UK)
17.	Petri dish	(Sterial/Jordan)

18.	Pipettes	DARWELL(China)
19.	Plane tubes (15ml)	(DMD-DISPO/ Jordan)
20.	Refrigerator	(DAEWOO/Korea)
21.	Sensitive balance	(Sartorius /Germany)
22.	Slides & Coverslips	(CITOPLUS/ China)
23.	Swabs	(TRUST /China)
24.	Tips (Different sizes)	(Jippo / Japan)
25.	Urine container	(TRUST /China)
26.	UVP	Analytik Jena(UK)
27.	Vortex	Fisher Scientific/ USA
28.	Water Distillator	(GFL/ Germany)
29.	Oven	Onko / China

3.1.2. Biological Materials and Chemicals

The chemical and biological materials which were used in the present study together with their producing companies were listed in table (3.2).

Table (3.2): Biological Materials and Chemicals.

NO.	Biological Materials & Chemicals	Company / Country
1.	10X TAE (Tris Acetate EDTA) buffer	V4271(Promega/USA)
2.	Agarose LE	32034-50(Intron/Korea)
3.	Distal water	Hospital Laboratory
4.	Glycerol	(BDH /England)
5.	GoTaq® G2 Green Master Mix	M7822(Promega/USA)
6.	Primers	---(Macrogen/Korea)
7.	RedSafe nucleic acid staining solution	21141 (Intron/Korea)

8.	Safe-Green 100bp Opti-DNA Marker	G473(abm/Canada)
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3.1.3 Culture Media

All culture media used in this study with their manufactures are itemized in table (3.3).

Table (3.3): Culture media used in the study

NO.	Culture media	Company / Country
1.	MacConkey agar	(Himedia/ India)
2.	Blood agar	(Himedia/ India)
3.	Brain heart infusion (BHI) broth	(Pronadisa/ Spain)

3.1.4 Laboratory Kits.

The kits used in the current study are mentioned in table (3.4).

Table 3.4 Kits used in the study.

No.	Kit	Components	(Company Origin)
1.	Gram stain	1. Crystal Violet 2. Iodine 3. Acetone 4. Sufranin	AFCO(Jordan)
2.	VITEK® II	Biomerieux	France
3.	G-spin Genomic DNA Extraction Kit for Bacteria	17121 Intron	Korea

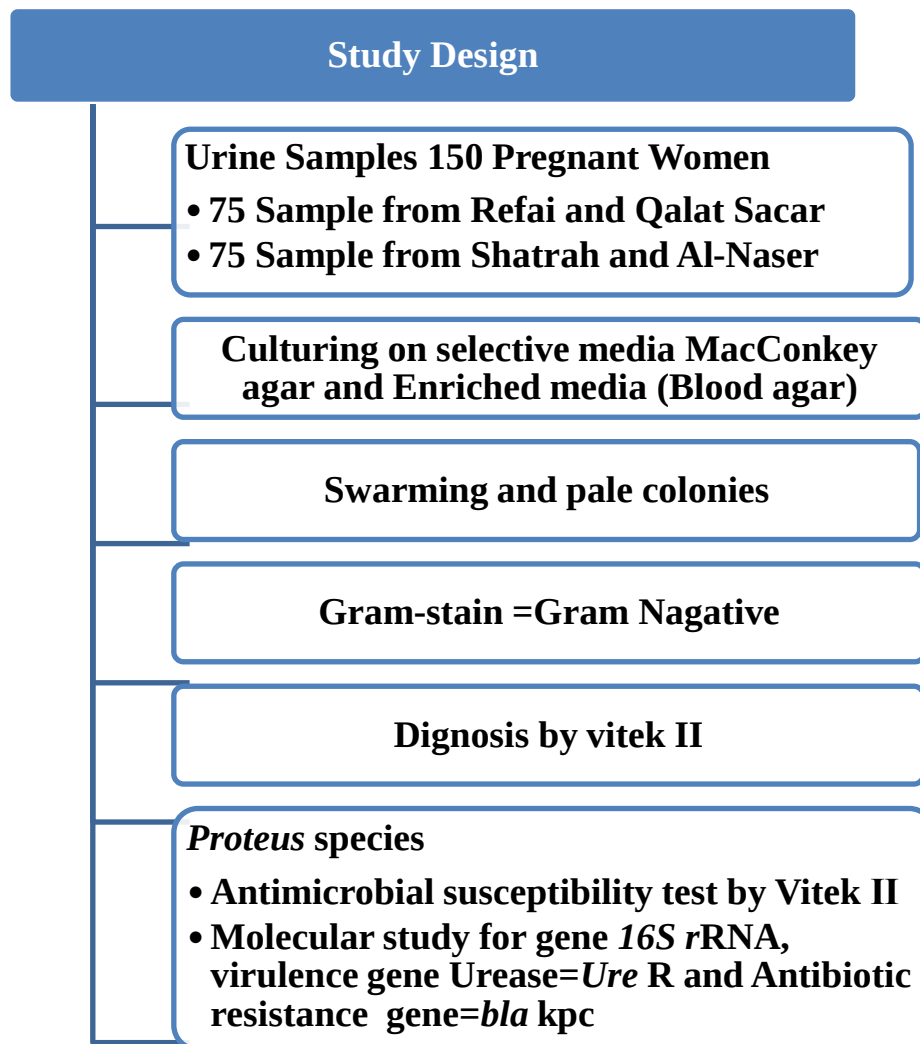
4.	VITEK 2 System	1. Vitek strip 2. Normal saline 3. Vitek III GN card 4.Vitek II AST-N417 5. Vortex 6. Vitek II Densichek	BioMerieux/France
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3.2. Methods

3.2.1. Patients and Specimens collection

One hundred fifty clinical urine samples were collected from women suffering from (UTIs). These samples were taken from different age groups, ranging between (18 to 41 years old). The samples were collected from hospitals, health centers, and women's clinics in the districts and outskirts of Northern Thi-Qar governorate (Al-Refai, Al-Shatra, Al-Nasar, and Qlaa Sekar) from September to December 2023. The clinical history of each case and full information were obtained directly from the pregnant women. Every item, including the patient's name, age, months of pregnant and habitation other details, was organized in an educational worksheet with clear instructions called the "Questionnaire sheet." Urine samples taken midstream and stored in sterile screw-cap containers made up the specimens. Every specimen is promptly moved to the laboratory for analysis while being kept cold.

3.2.2. Study Design



3.2.3. Media and solution preparation

Tables (3-3) show that all media (Blood agar, MacConkey agar, Peptone broth medium and brain heart infusion agar) were prepared according to the manufacturer's instructions. The pH was adjusted to (7.2), and the media were sterilized in an autoclave for 15 minutes at 121°C and 15 bar / in² pressure. After allowing the media to cool to

50°C, it was placed into a sterile petri dish or sterile test tube, depending on the test type.

3.2.3.1. Blood agar medium

Human blood was introduced into a blood agar medium that had been produced following the instructions provided by the manufacturer. The medium was then sterilized using an autoclave at a temperature of 121 °C for a duration of 15 minutes, and then cooled down to 50 °C. This method was used to cultivate and assess bacterial strains for their ability to form swarms. (Martínez-González *et al.*, 2016).

3.2.3.2. MacConkey agar medium

Prepared according to the manufacturer's instructions, autoclaved at 121 °C for 15 minutes, then chilled to 50°C Gram-negative bacteria and distinguish lactose-fermenting bacteria from non-lactose-fermenting bacteria(MacFaddin, 2000).

3.2.3.3. Brain heart infusion (BHI) broth

As directed by the manufacturer, this medium was created. It was used to retain microorganisms for a long period after adding glycerol(MacFaddin, 2000).

3.2.3.4. Gram Stain

There are four solutions: safranine, iodine, pure alcohol, and crystal violet. By analyzing the shape, arrangement, and kind of reaction with Gram-stain, Gram-positive (purple), or Gram-negative (red), these solutions are utilized for the microscopic examination and identification of bacterial cells(Biswas *et al.*, 1970).

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/in² pressure at 121°C for 15 minutes (Alagoa & Ozi, 2021; Ekanem & Ottong, 2021).

3.2.5. Bacterial isolates and identification

pregnant women's urine samples were collected halfway through a sterile container. A small quantity of urine samples was taken by sterial swap and cultured on both MacConkey and blood agar by streaking and incubated for a further 24 hours at 37°C. The goal was to generate pure, well-isolated colonies by selecting and re-culturing species of lactose-nonfermenting colonies streaked on MacConkey agar plates. Isolates that did not ferment lactose on MacConkey Agar (these non-fermenters appear as colorless) and isolates that exhibited swarming on blood agar.

The vitek II technique was employed for bacterial growth detection, which relies on morphological (colon shape, size, and color) and microscopic characteristics to confirm positive growth.

3.2.6. Colonial morphology identification.

The bacterial isolates were cultured and purified on several media, including blood agar and MacConkey agar, and then their cultural characteristics were tested. The research took into account every aspect of isolated bacterial colonies, including their color, oder (fish oder), and shape (swarm) after being incubated for 24 hours at 37°C.

3.2.7. Microscopic identification

A pure culture of a recently obtained bacterium was cultivated on a nutrient agar medium for a period of 18 to 24 hours. The resulting bacterial cells were then fixed onto a clean and dry slide, and then stained using the Gramme staining technique. The cells'

shapes, arrangements, and colors were then observed under a light microscope to determine how the cells interacted with the Gram stain (Levinson, 2016). Isolates that did not ferment lactose on MacConkey Agar (these non-fermenters appear as colorless) and isolates that exhibited swarming on blood agar.

3.3. Method

3.3.1. Identification of *Proteus* spp. using the Vitek II system

According to the manufacturing business (bioMérieux, France), the technique was employed by inoculating bacteria on MacConkey agar plates, followed by a 24-hour incubation period at 37°C. Transferring 1-3 colonies to test tubes containing (3 ml) of normal saline allowed for the preparation of a bacterial suspension from the aforementioned growth, and the suspension's turbidity was then corrected to a McFarland standard of (0.5) (Pincus, 2006).

3.3.2. Molecular Methods

G-spin Genomic DNA Extraction Kit (for Gram-negative Bacteria)

1. 1-2 ml of cells (OD₆₀₀: 0.8-1.0) was harvested by centrifuging at 13,000 rpm for 1min and remove supernatant.
2. 300 µl of G-Buffer solution was added, and inverted-mix well.
3. At 65°C for 15 min was incubated. Note: To help lysis cells, inverted mix the tube every 5 min during the incubation.
4. 250 µl of Binding Buffer was added, and completely mixed well by pipetting (at least 10 times) or gently vortexing.
5. Cell lysates was loaded on column and centrifuged at 13,000 rpm for 1 min.
6. 500 µl of Washing Buffer A was added to column and centrifuged for 1 min at 13,000 rpm.
7. 500 µl of Washing Buffer B was added to column and centrifuged for 1 min at 13,000 rpm.

8. Solution was removed and centrifuged for 1min at 13,000 rpm.
9. The G-spin™ column was placed in a clean 1.5ml microcentrifuge tube and added 50-200 µl of Elution Buffer directly onto the membrane.
10. At room temperature was incubated for 1 min, and then centrifuged for 1 min at 13,000 rpm

3.3.2.1. Agarose gel electrophoresis of DNA

Electrophoresis was used to assess the DNA extraction quality and visualise the size of the PCR result upon completion of the PCR programme. The concentration of the gels varied based on the kind of the substance. Typically, the agarose gel concentration used for assessing DNA quality was 0.7%, but for typical PCR products it ranged from 1-2%.

3.3.2.2. Preparing of the Agarose gel

The agarose gel preparation was conducted following the methods outlined by (Sambrook et al., 1989). In summary, 1 gramme of agarose was dissolved in about 100 millilitres of a 1X TBE buffer and then heated to boiling using a microwave machine..

After undergoing a cooling process at a temperature range of 45-50 °C, a volume of 5 µl of RedSafe nucleic acid staining solution was introduced to the gel. The gel was then poured into the gel cast and allowed to solidify fully for about 30 minutes.

The gel-plate was placed into the gel tank and filled with 1X TBE buffer until the gel surface was completely covered.

3.3.2.3. Loading the PCR products

Approximately 5 μ l of each PCR product was placed in the centre of the corresponding hole on the 1% gel. A volume of 5 μ l of Safe-Green 100bp Opti-DNA Marker was introduced into the first well of the gel lines, serving as a reference for determining the size of the PCR products..

3.3.2.4. Electrophoresis

The electrophoresis equipment was set up with a continuous current of 90 volts for a duration of 45 minutes, after loading the Safe-Green 100bp Opti-DNA Marker and the samples. Ultimately, the gel was put into the UVP system in order to visualise the PCR products using a 320nm UV light source.

3.3.2.5. Preparation of primers

As per the instructions from the primer synthesiser business, the lyophilized primers were dissolved in distilled water to achieve a final concentration of 100 μ M/ μ l. This solution was then kept as a stock solution at a temperature of -20 °C. A 10 μ M/ μ l concentration was created by diluting the stock primers, which will be utilised as a work primer.

3.3.2.6. Primers used in this study

The primers that used in this study was listed in the table 3-5

Table (3-5): Primers used in this study

Gene	primer name	Sequence 5'-3'	PCR temperature	PCR size	Accession number	Reference
UreR	F	GTGACGCCATTG TTGCTATTG	54 °C	456 bp	Z18752.1	This study
	R	CCTCTCAAGAGA CCCTGTTTATG				
16S rRNA	F	ATGACGTCAAGT CATCATGG	56 °C	353 bp	Z18752.1	Klausegger <i>et al.</i> , 1999
	R	AGGAGGTGATCC AGCCGCA				
KPC	F	CATTCAAGGGCT TTCTTGCTGC	59 °C	538 bp	CP137239 .1	Mushi <i>et al.</i> , 2014
	R	ACGACGGCATAG TCATTTGC				

3.3.2.7. GoTaq® G2 Green Master Mix

The GoTaq® G2 Green Master Mix is a pre-made blend of top-notch Taq DNA Polymerase, deoxynucleotides, and reaction buffer, with a concentration of 2X. It includes all the essential substances for the amplification of DNA. The GoTaq® G2 Green Master Mix includes a non-reactive green dye and a stabiliser that enable the direct placement of the end products onto a gel for examination.

3.3.2.8. Preparation of PCR solutions

The solution of PCR was prepared by mixing the following solutions in table 3-6.

Table (3-6): PCR reaction

Components	Concentration	Volume (50 µl)
GoTaq® G2 Green Master Mix	1X	25 µl
Forward primer	10 µM/µl	4 µl
Reverse primer	10 µM/µl	4 µl
ddH ₂ O	-	13 µl
DNA	40 ng	4 µl

3.3.2.9. PCR Conditions

The conditions of PCR that used for *Proteus* spp identification are listed in the table 3-7

Table (3-7): Conditions of PCR program that used for identification of *Proteus* spp.

Phase	T _m (°C)	Time	Cycles
Initial denaturation	94°C	5 min	1X
Denaturation	94°C	30 sec.	35X

Annealing	54,56 and 59°C	30 sec.	
Extension	72°C	1 min	
Final extension	72°C	5 min	1X

3.4. Sequencing Analysis

Confirmation of identification of tested isolates using DNA sequencing and NCBI server for identification and detection of isolated strain and compared their sequence with NCBI sequencing for SNP detection. Partial region of gene *16S rRNA* was amplified and sequenced using Sanger sequencing method (Macrogen, Korea). The results of *ureR* sequences were identical to *Proteus mirabilis* strains deposited in the NCBI gene data base.

3.5. Phylogenetic Tree Construction and Protein Analysis

The phylogenetic tree for isolated *Proteus* spp. was constructed by matching the isolate sequences with the isolates registered in NCBI, by using nucleotide blast tool, and SNPs were identified with the alignment tool, also the Bio-Edit and MEGA software was used for phylogenetic construction.

3.6. Statistical Analysis

The data of the current study was statistically analysis by using SPSS (Statistical Package for the Social Sciences), version 26, based in using both Descriptive and non-parametric chi-square, at p. value < 0.05.

Chapter Four

Results

4. Results

4.1. Isolation and Identification of *Proteus* spp.

4.1.1. Culture characteristics:

4.1.1.1. MacConkey agar:

Cultivation of the samples on MacConkey agar produces nonfermenting lactose. As a result, the medium turned yellow, and a relatively big, convex, with smooth borders was produced. Additionally, the smell of the bacterial development was comparable to that of decomposing fish. Figure 4-1

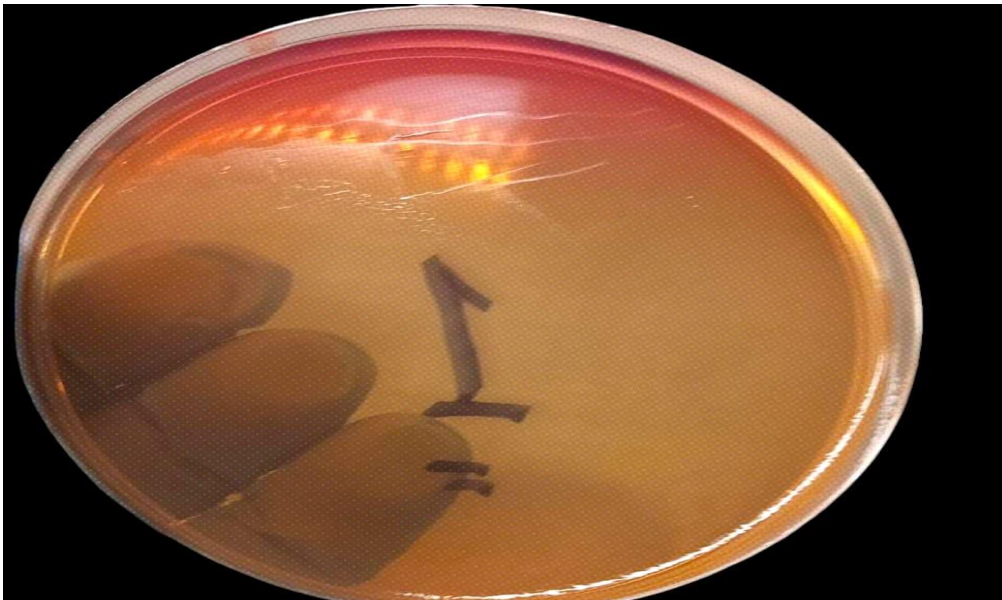


Figure 4.1: Growth of *Proteus* spp. on MacConkey agar.

4.1.1.2. Blood agar:

Blood agar was used to cultivate the samples, as Figure 4-2 illustrates. One important characteristic that set *Proteus* spp. apart from other Enterobacteriaceae strains on blood agar was the colony's ability to develop and exhibit a notable pattern of swarming movement.

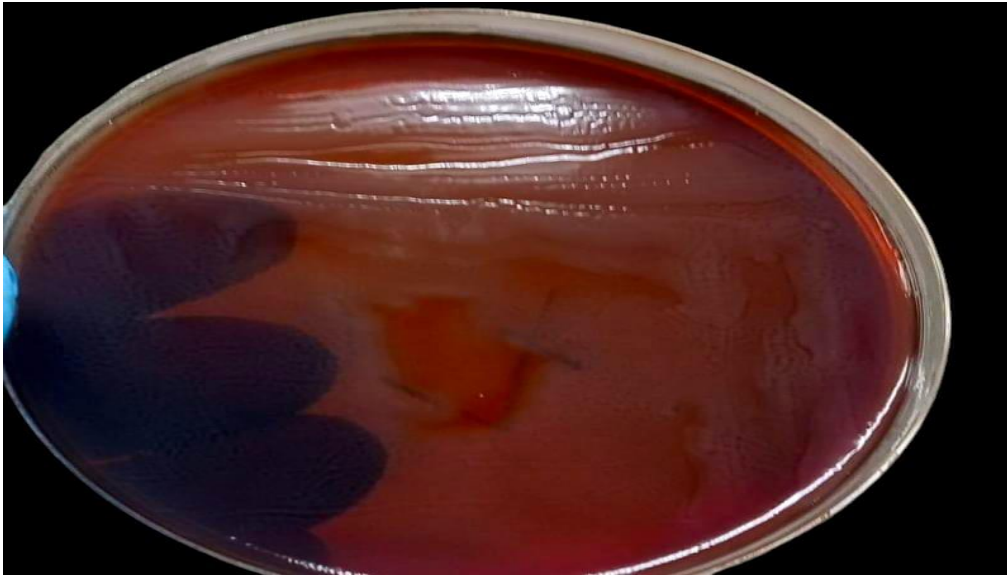


Figure 4.2: Growth of *Proteus* spp. on Blood agar.

4.1.2. Microscopic properties (Gram Stain):

The isolated bacterial cells were identified by microscopic analysis as red-colored, gram-negative coccobacilli of different lengths that are typically found alone.



(Figure 4.3): The characteristic Gram negative of *Proteus* spp.

4.2. Vitek II Systems for Confirm *Proteus* spp. Isolates:

The Vitek II system is used to diagnose bacteria with biochemistry test in Table (4-1) to confirm the diagnosis of *Proteus* spp. Isolates

There are 64 wells on the *Proteus* spp. Bacteria Reagent Card, and each one has a different test substrate. 6.5 NaCl inhibits growth, enzymatic activity, acidification, and alkalization, among other metabolic activities are reliant on the use of carbon resources.

Table 4.1: Result of vitek 2 system of *Proteus* spp.

No.	Test Name Results	Abbreviation	Results
2	Ala-Phe-Pro-Arylamidase	APPA	-
3	ADONITOL	ADO	-
4	L-Pyrrolydonyl-ARYLAMIDASE	pyrA	-
5	L-ARABITOL	IARL	-
7	D-CELLOBIOSE	dCEL	-
9	BETA-GALACTOSIDASE	BGAL	-
10	H ₂ S PRODUCTION	H ₂ S	+
11	BETA-N ACETYLGLUCOSAMINIDASE	BNAG	-
12	Glutamyl Arylamidase pNA	AGLTp	-
13	D-GLUCOSE	dGLU	+
14	GAMMA-GLUTAMYLTRANSFERASE	GGT	+
15	FERMENTATION/ GLUCOSE	OFF	+
17	BETA-GLUCOSIDASE	BGLU	-

18	D-MALTOSE	dMAL	-
19	D-MANNITOL	dMAN	-
20	D-MANNOSE	dMNE	-
21	BETA-XYLOSIDASE	BXYL	-
22	BETA-Alanine arylamidase pNA	BAIap	-
23	L-Proline ARYLAMIDASE	ProA	-
26	LIPASE	LIP	-
27	PALATINOSE	PLE	-
29	Tyrosine ARYLAMIDASE	TyrA	-
31	UREASE	URE	+
32	D-SORBITOL	dSOR	-
33	SACCHAROSE/SUCROSE	SAC	-
34	D-TAGATOSE	dTAG	-
35	D-TREHALOSE	dTRE	+
36	CITRATE (SODIUM)	CIT	-
37	MALONATE	MNT	-
39	5-KETO-D-GLUCONATE	5KG	-
40	L-LACTATE alkalization	ILATk	-
41	ALPHA-GLUCOSIDASE	AGLU	-
42	SUCCINATE alkalization	SUCT	-
43	Beta-N-CETYLGALACTOSAMINIDASE	GANa	-

44	ALPHA-GALACTOSIDASE	AGAL	-
45	PHOSPHATASE	PHOS	+
46	lycine ARYLAMIDASE	GlyA	-
47	ORNITHINE DECARBOXYLASE	ODC	+
48	LYSINE DECARBOXYLASE	LDC	-
53	L-HISTIDINE assimilation	IHISa	-
56	COUMARATE	CMT	-
57	BETA-GLUCURONIDASE	BGUR	+
58	O/129 RESISTANCE (comp.vibrio.)	O129R	+
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	-
61	L-MALATE assimilation	IMLTa	-
62	ELLMAN	ELLM	-
64	L-LACTATE assimilation	ILATa	-

4.3. Incidence of Bacteria in Pregnant Women

Data recorded among (150) pregnant women, 130 of women had positive bacterial culture 86.67%, while only (13.33%) of women had negative bacterial culture, this study also, recorded 26.67% of total sample infected with gram negative bacteria, and 60.0% infected with gram positive bacteria, the results also noted a significant difference at p. value < 0.05, as show in the Figure 4-4.

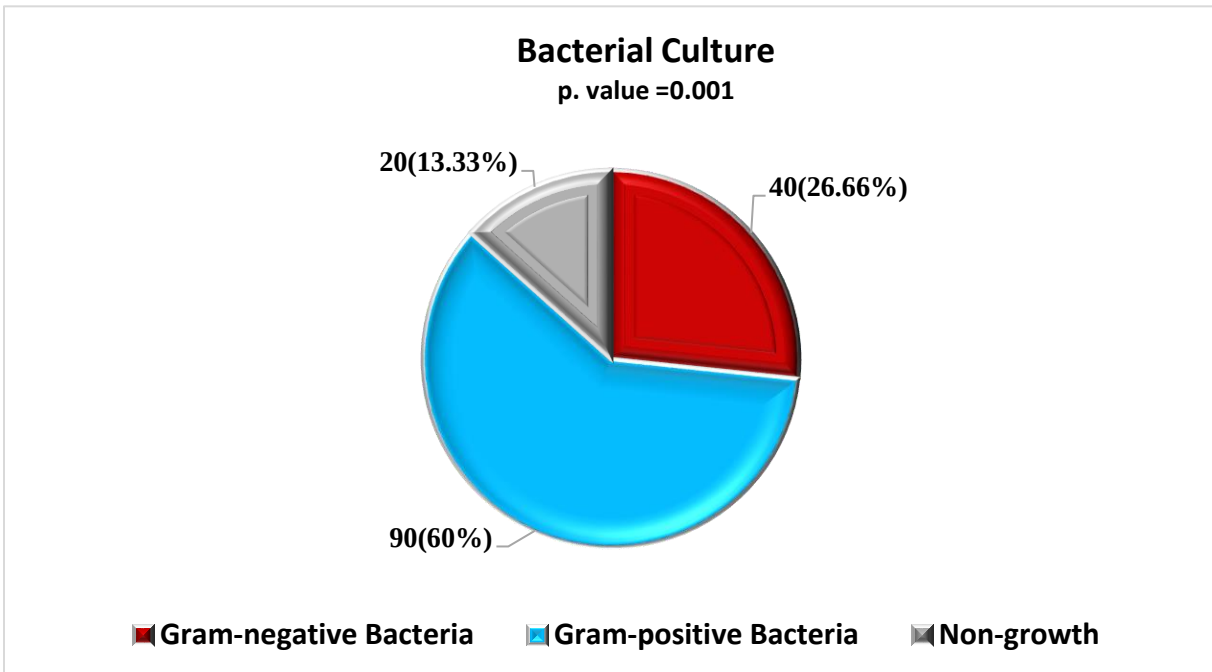


Figure (4.4): Results of bacteria culture in pregnant women(p.=probability).

4.4. Incidence of Isolated Bacteria from Pregnant Women

The current results showed that the most isolated bacteria were gram positive bacteria 60%, followed by *Escherichai coli* 13.33%, followed by *Pseudomonas aeruginosa* 5.34%, on the other hand, the lowest isolated bacteria were *Klebsiella pneumoniae* 3.33%, and *Proetus* spp. 4.67%, the current results also, recorded a significant difference at p. value < 0.005, according to species of bacteria, as in the Table 4-2.

Table 4-2: Incidence of Gram-negative Bacteria (cal.=calculated,Tab.=Tabulated).

Isolated Bacteria	No. samples	%
<i>Escherichai coli</i>	20	13.33
<i>Klebsiella pneumoniae</i>	5	3.33
<i>Proetus mirabilis</i>	7	4.67
<i>Pseudomonas aeruginosa</i>	8	5.34
Gram +ve Bacteria	90	60.0
Non-growth	20	13.33
Total	150	100
Cal X^2 =143.2 Tab X^2 =11.07 DF=5 p. value < 0.001		

4.5. Incidence of bacterial Culture according to age groups

The present study explored the most positive bacterial culture in the second age group 46.62%, while the lowest positive bacterial culture in the second age group 84.72%, in contrast, the most negative bacteria culture in the third age group 14.62%. The results also, indicated a non-significant difference because (The Cal. X^2 less than Tab X^2 and p.value is greater than 0.05) according to age group, as in the Table 4-3.

Table 4-3: Incidence of bacterial culture according to age groups

Age groups	Positive Culture		Negative Culture		Total	
	No.	%	No.	%	No.	%
18-27 years	50	38.46	7	35.00	57	38.0
28-37 years	61	46.92	11	55.00	72	48.0
38-45 years	19	14.62	2	10.00	21	14.0
Total	130	86.67	20	13.33	150	100
Cal X^2 =1.75 Tab X^2 =5.99 DF=2 p. value 0.417						

4.6. Incidence of bacterial Culture according to habitation

In this present study, it was noted that the most positive bacterial culture in the city resident 91.75%, while the lowest positive bacterial culture in the village resident 77.37%, in contrast the most negative bacteria culture in the village resident 22.64%, the results also, indicated a significant difference (because $\text{Cal}X^2 > \text{Tab}X^2$, and $p.\text{value} < 0.05$) according to habitation, as in the Table 4-4.

Table 4-4. Incidence of bacterial culture according to habitation

Habitation	Positive Culture		Negative Culture		Total	
	No.	%	No.	%	No.	%
City	89	91.75	8	8.25	97	64.67
Village	41	77.36	12	22.64	53	35.33
Total	130	86.67	20	13.33	150	100
Cal X^2 =8.58 Tab X^2 =3.84 DF=1 p. value 0.003						

4.7. Incidence of bacterial culture according to residency

The present study was noted the most positive bacterial culture in both Al-Nassar and Al-Refai resident 88.0%, while the lowest positive bacterial culture in the Qlaat Sekar resident 84.0%, in contrast the most negative bacteria culture in the Qlaat Sekar resident 16.0%, the results also, indicated a non-significant difference (because $\text{Cal}X^2 < \text{Tab}X^2$, and $p.\text{value} > 0.05$) according to residency, as in the Table 4-5.

Table 4-5: Incidence of bacterial results culture according to residency

Residency	Positive Culture		Negative Culture		Total	
	No.	%	No.	%	No.	%
Al-Nasar	22	16.92	3	15.00	25	16.67
Al-Refai	44	33.85	6	30.00	50	33.33
Al-Shatrah	43	33.08	7	35.00	50	33.33
Qlaat Sekar	21	16.15	4	20.00	25	16.67
Total	130	86.67	20	13.33	150	100
Cal X^2 =0.87 Tab X^2 =7.81 DF=3 p. value 0.831						

4.8. Incidence of bacterial culture according to month of pregnant

The present study was noted the most positive bacterial culture in second month of pregnant 20.77%, followed in the nine months of pregnant 15.38%, but the lowest positive bacterial culture in the fourth month of pregnant 9.23%, the results also, indicated a significant difference (because Cal X^2 > Tab X^2 , and p.value < 0.05) according to months of pregnant, as in the Table 4-6.

Table 4-6: Incidence of bacterial culture according to month of pregnant

Months of pregnant	Positive Culture		Negative Culture		Total	
	No.	%	No.	%	No.	%
2 nd Months	27	20.77	3	15.00	30	20.0
3 rd Month	13	10.00	1	5.00	14	9.33
4 th Month	12	9.23	7	35.00	19	12.67
5 th Month	13	10.00	2	10.00	15	10.0
6 th Month	13	10.00	0	0.00	13	8.66
7 th Month	14	10.77	2	10.00	16	10.67
8 th Month	18	13.85	1	5.00	19	12.67
9 th Month	20	15.38	4	20.00	24	16.0
Total	130	86.67	20	13.33	150	100
Cal X^2 =33.0 Tab X^2 =14.06 DF=7 p. value < 0.001						

4.9. Percentage of *Proteus* Spp. to other Gram-negative bacteria

The percentage of *Proteus* spp. among Gram negative bacteria was 17.5%, with significant difference at p. value < 0.05, as in the Figure 4-5

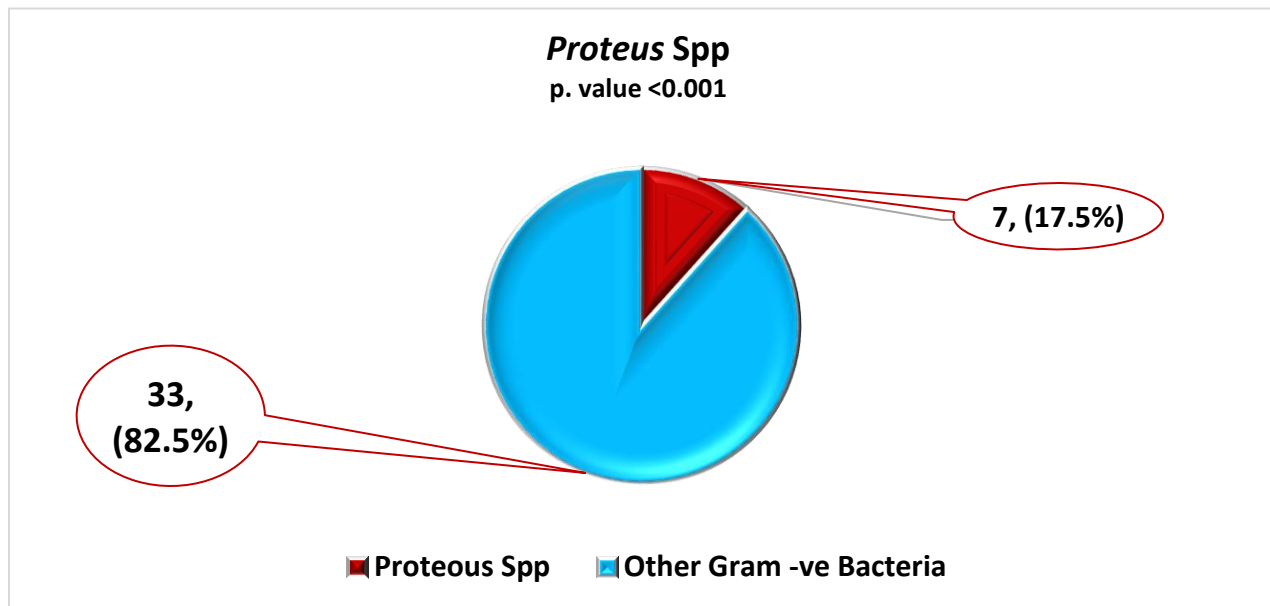


Figure (4.5): Percentage of *Proetus* spp. to other gram-negative bacteria

4.10. Incidence of *Proteus* spp. according to age groups

The present study was noted the most *Proteus* spp. in the second age group 3.08%, followed in the first age group 1.54%, while the lowest *Proteus* spp. in the third age group 0.77%, the results also, indicated a non significant difference (because $\text{CalX}^2 < \text{TabX}^2$, and p.value > 0.05) according to age group, as in the Table 4-7.

Table 4-7: incidence of *Proteus* spp. according to age groups

Age groups	<i>Proteus</i> spp.	
	No.	%
18-27 years	2	1.54
28-37 years	4	3.08
38-45 years	1	0.77
Total	7	5.38
CalX ² =2.00 TabX ² =5.99 DF=2 p. value 0.368		

4.11. Incidence of *Proteus* spp. according to habitation

The present study was noted the most *Proteus* spp. in the city resident 3.8%, while the lowest *Proteus* spp. in the village resident 2.31%, the results also, indicated a non-significant difference (because $\text{CalX}^2 < \text{TabX}^2$,and $\text{p.value} > 0.05$) according to habitation, as in the Table 4-8.

Table 4-8: Incidence of *Proteus* Spp. according to habitation

Habitation	<i>Proteus</i> spp.	
	No.	%
City	4	3.08
Village	3	2.31
Total	7	5.38
CalX ² =0.143 TabX ² =3.84 DF=1 p. value 0.705		

4.12. Incidence of *Proteus* spp. according to residency

The present study was noted the most *Proteus* spp. in the Al-Refai resident women 2.31%, while the lowest *Proteus* spp. in both Al-Nasar and Al-Shatrah resident 1.54%, in addition not isolate *Proteus* spp. from women in the Qlaat Sekar 0.0%, the

results also, indicated a non-significant difference (because $\text{Cal}X^2 < \text{Tab}X^2$, and $p.\text{value} > 0.05$) according to residency, as in the Table 4-9.

Table 4-9: Incidence of *Proteus* spp. according to residency

Residency	<i>Proteus</i> spp	
	No.	%
Al-Nasar	2	1.54
Al-Refai	3	2.31
Al-Shatrah	2	1.54
Qlaat Sekar	0	0.00
Total	7	5.38
Cal X^2 =0.286 Tab X^2 =5.99 DF=2 p. value 0.867		

4.13. Incidence of *Proteus* spp. according to months of pregnant

The present study was noted the most *Proteus* spp. in third month of pregnant 1.54%, followed in the other months of pregnant 0.77%, in contrast non-isolated *Proteus* spp. from women in both fifth and eighth months of pregnant 0.0%, the results also, indicated a non- significant difference (because $\text{Cal}X^2 < \text{Tab}X^2$, and $p.\text{value} > 0.05$) according to month of pregnant, as in the Table 4-10.

Table 4-10: Incidence of bacterial culture according to month of pregnant

Month of pregnant	<i>Proteus</i> spp.	
	No.	%
2 nd Months	1	0.77
3 rd Month	2	1.54
4 th Month	1	0.77
5 th Month	0	0.00
6 th Month	1	0.77
7 th Month	1	0.77
8 th Month	0	0.00
9 th Month	1	0.77
Total	7	5.38
Cal X^2 =0.714 Tab X^2 =11.07 DF=5 p. value 0.982		

4.14. Antibiotics Susceptibility of *Proteus mirabilis*

The isolated *Proteus* spp. was tested to twenty antibiotics with different mechanisms of action, and the present study was noted 36.43% of isolated *Proteus* spp. was resist for antibiotics, 6.43% was gave intermediate response, while 57.14% was sensitive for antibiotics. The present study also noted that 100% was resistant for Doxycycline and carbancillin, while 100% was sensitive for Ticarcillin, Cefotitan, Amikacin, Ofloxacin, Doripenem, Isepamicin, and Trimethoprim, (CalX2 > TabX2 ,and p.value < 0.05), as in the Table 4-11.

Table 4-11: Antibiotics susceptibility of *Proteus mirabilis*

	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ampicillin	6	85.71	0	0.00	1	14.29
Amoxicillin	6	85.71	0	0.00	1	14.29
Ticarcillin	0	0.00	0	0.00	7	100
Piperacillin	6	85.71	0	0.00	1	14.29
Carbancillin	7	100	0	0.00	0	0.00
Cephalexin	4	57.14	1	14.29	2	28.57
Ceftazidime	0	0.00	2	28.57	5	71.43
Cefotitan	0	0.00	0	0.00	7	100
Aztreonam	4	57.14	1	14.29	2	28.57
Amikacin	0	0.00	0	0.00	7	100
Gentamicin	4	57.14	0	0.00	3	42.86

Ciprofloxacin	2	28.57	2	28.57	3	42.86
Ofloxacin	0	0.00	0	0.00	7	100
Doripenem	0	0.00	0	0.00	7	100
Imipenem	3	42.86	1	14.29	3	42.86
Isepamicin	0	0.00	0	0.00	7	100
Cefepime	2	28.57	1	14.29	4	57.14
Latamoxef	0	0.00	1	14.29	6	85.71
Doxycycline	7	100	0	0.00	0	0.00
Trimethoprim	0	0.00	0	0.00	7	100
Susceptibility %	36.43		6.43		57.14	
CalX²= 109.6 TabX²= 53.36 DF=19 p. value <0.001						

4.15. Molecular detection of 16S rRNA of *Proteus mirabilis*

Among seven isolates of *Proteus mirabilis*. bacteria and identification by vitek II test, a further identification by molecular technique, and the result showed that the isolated bacteria were *P. mirabilis*.

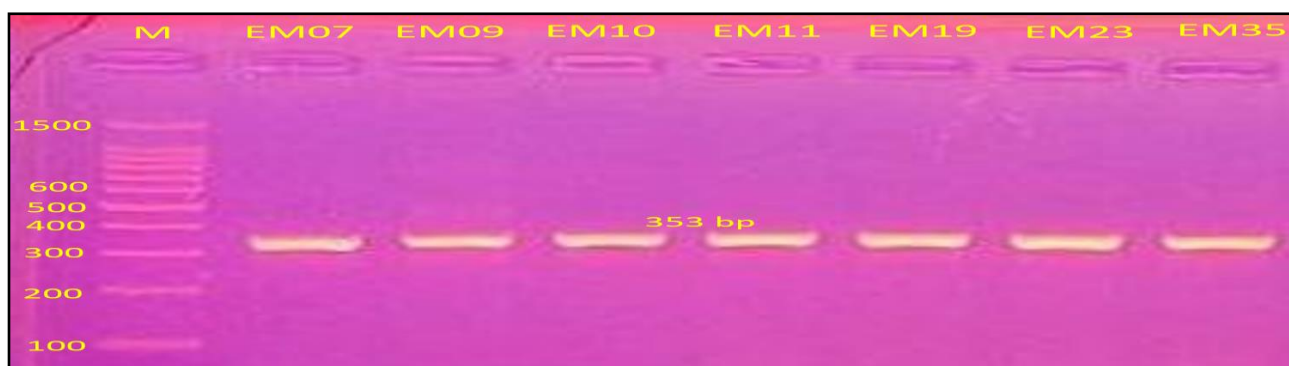


Figure (4.6): PCR products of the amplification of partial region of gene specific for 16S rRNA *Proteus mirabilis*. The size of the PCR product is 353 bp. Ta = 56 °C. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder.

4.16. Identification of virulence gene and antibiotic gene

The current study that all isolated *P. mirabilis* was positive for both *ureR* gene and negative to *blaKPC*.

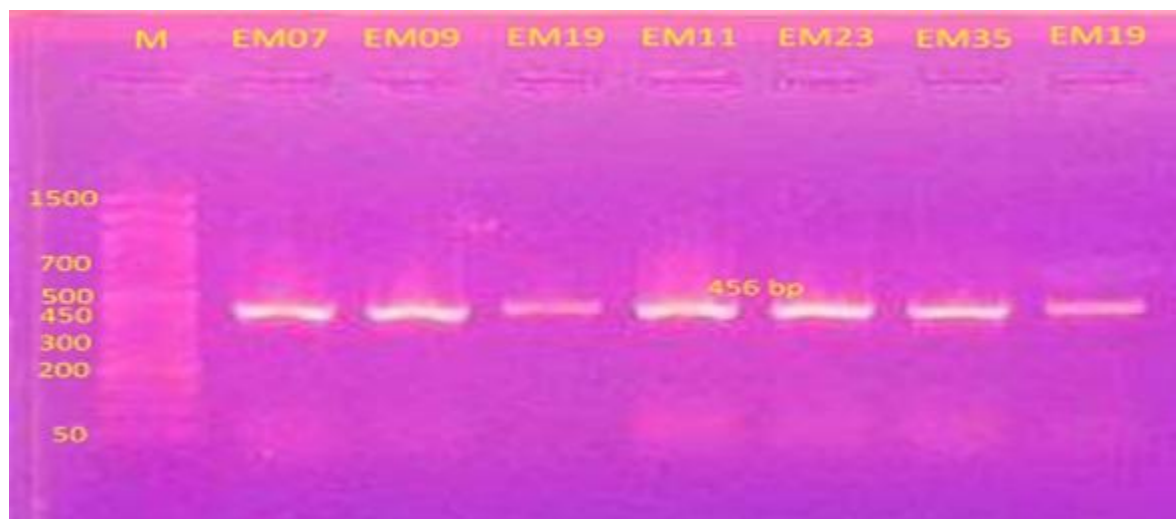


Figure (4.7): PCR products of the amplification of partial region of gene *ureR* specific for *Proteus mirabilis*. The size of the PCR product is 456 bp. Ta = 54 °C. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder.

4.17. Sequencing Analysis

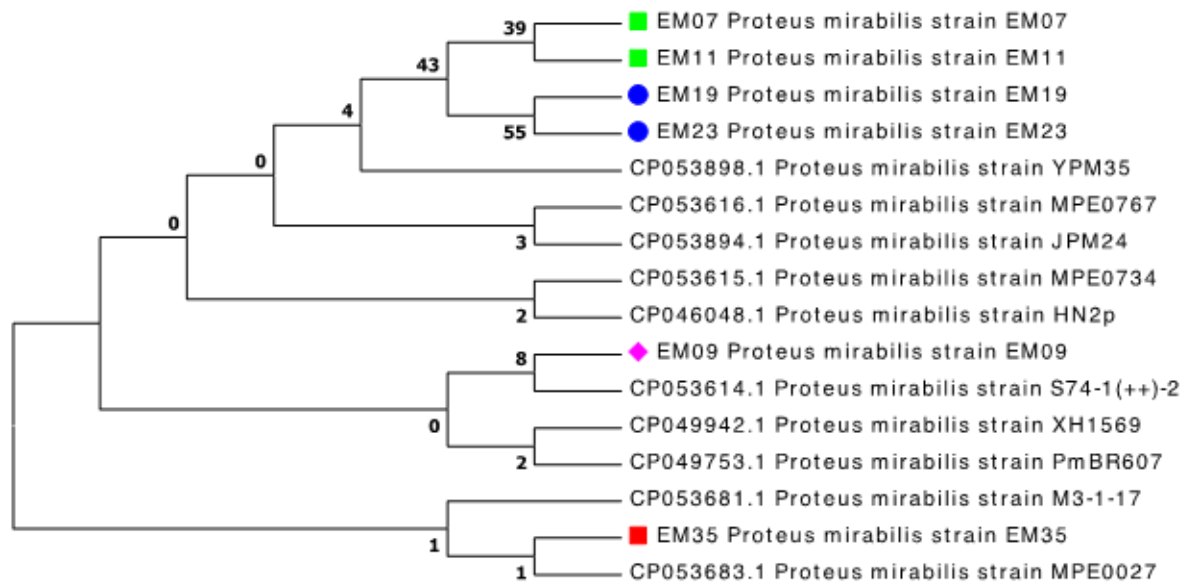
DNA sequencing and the NCBI server were used to confirm the identity of the tested isolates. The isolated strains were then identified, and their sequences were compared with NCBI sequencing for SNP detection. Partial region of gene *16S rRNA* was amplified and sequenced using Sanger sequencing method (MacroGen, Korea). The results of *ureR* sequences were identical to *Proteus mirabilis* strains deposited in the NCBI gene data base.

According to NCBI data, that recorded 3 new *proteus* strains according to *16S rRNA* gene sequence and gave an accession:

Table 4-12:Accession numbers submitted in GenBank in the study

Sequences	Accession numbers
SUB14440996 EM07	PP894752
SUB14440996 EM11	PP894753
SUB14440996 EM23	PP894754

4.18. Phylogenetic Tree:

**Figure (4.8)** Evolutionary relationships of EM isolates with registered *Proteus mirabilis* isolates at NCBI.

Chapter Five

Discussion

5. Discussion:

5.1. Urinary tract infection.

A urinary tract infection during pregnancy is linked to significant morbidity for both the mother and the fetus. Pregnancy induces significant alterations in the urinary system due to a combination of mechanical, hormonal, and physiological changes. These changes have a significant effect on the development and development of bacteriuria during pregnancy (Aseel *et al.*, 2009; Demilie *et al.*, 2012).

This study recorded, among (150) pregnant women, positive bacterial culture (86.67%), while negative bacterial culture (13.33%). This study was identical with a study in the city of Samawah (Nahab *et al.*, 2022), where the researcher obtained an infection rate of 86% as well. A researcher in Thi-Qar governorate (Mousa & Qasim, 2015) also obtained a percentage similar to the current study. Other studies were done in other regions in Iraq to explain the incidence of urinary tract infections in pregnant women observed in Babylon, Mosul, and Kirkuk; the rates showed 64.6%, 47.4%, and 29% respectively (Al-Mamoryi & Al-Salman, 2019; Al-Jawadi, 2012; C. Ali *et al.*, 2007). These differences within incidence percentages may be due to numbers of samples, variation in methodology tests, and socioeconomic variations.

There are many studies regarding urinary tract infection in pregnant women, and these studies have shown different percentages. The present study in Al-Bahrian, Jeddah, Jordan, and Derna researchers showed these percentages were 35.2%, 1.7%, 63.39%, and 49.3%, respectively. (Al Senani, 2011; Al Sibiani, 2010; MN & Battikhi, n.d.; M. Younis *et al.*, 2019).

In Pakistan, two studies by different researchers showed different results about urinary tract infection within pregnant women one showed a percentage close to current

study, where it recorded 81%, while another study showed a rate of 49.2%, and this may be due to the small number of samples and geographic variation (Asmat *et al.*, 2021; Sarwar *et al.*, 2013).

In Northeastern Ethiopia, a researcher received a 15.5% infection rate (Belete, 2020). While a study in northwestern Ethiopia obtained a rate of 10.4% (Alemu *et al.*, 2012), and in Uganda, the rate of infection in a researcher was 35% (Johnson *et al.*, 2021). This difference in proportions in these studies may be due to differences in environments or in the number of samples taken during the study, or perhaps due to the different periods in which the samples were collected.

5.2. Morphological characteristics of *proteus mirabilis*

Samples that were cultured on MacConkey agar result in produce non ferment lactose. The result showed that the medium was converted to pale. On the blood agar media, *Proteus* isolates showed swarming phenomenon.

Microscopic examination revealed that the isolated bacterial cells were Gram-negative, rod and red-colored. These results were identical to a study conducted in Baghdad province by one of the researchers (Ahmed, 2015). After collecting samples and cultivating them on the previously mentioned media to investigate the bacteria *Proteus* in pregnant women, the infection rate compared to all types of bacteria that have grown in the culture media was (5.38%). This percentage may be due to the fact that these bacteria are transmitted through the use of contaminated tools inside hospitals, especially the use of urinary tract catheter (Różalski *et al.*, 2013). Women do not use these tools during pregnancy, so we notice the spread of these bacteria in most of the elderly. This percentage was somewhat identical to a study conducted in Nigeria, where the researcher found bacterial isolation of (5.5%) (Onuoha & Fatokun, 2014), and there was a study in Pakistan that gave a close ratio of (6.5%) (Sarwar *et al.*, 2013).

Locally, some studies in Thi-Qar, Samawa, and Kirkuk obtained the following percentages: 10%, 12.7%, and 20.68%, (Mousa & Qasim, 2015 ; Nahab *et al.*, 2022 and C. Ali *et al.*, 2007) This discrepancy in proportions may be due to the number of samples during the study or perhaps at the time of sample collection.

As for the age of women and their relationship to infection with these bacteria, the study showed that the incidence was between the ages of 28-37, perhaps due to the fact that this age group had a greater incidence of urinary tract infection.

5.3. Incidence of *Proteus* infection according to age of pregnant women:

Most studies have agreed with this study regarding the ages of pregnant women and their relationship to urinary tract infection, as they found that the average ages from 25 to 37 years are more susceptible to urinary tract infection, and this may be caused by a difference in the body's immunity or because women at these ages are more frequent to medical clinics This study did not coincide with another study in Baghdad province, where the researcher found that ages 16-26 years are more infected with proteus bacteria, and this difference may be due to the number of samples collected from this age group (Shammari *et al.*, 2020).

5.4. Incidence of *Proteus* according period of pregnancy:

The current study referred that most urinary tract infection occurred within second month of pregnancy with regard to the period of pregnancy, and this perhaps due to the occurrence of physiological and hormonal changes in the body of the pregnant woman both progesterone and estrogens levels increase during pregnancy and these will lead to decreased ureteral and bladder tone. Increased plasma volume during pregnancy leads to decrease urine concentration and increased bladder volume (Jr & LefevreDelzell, 2000). This study coincided with the same previous study, where the

researcher found that pregnant women were affected by the first period of pregnancy is the most among the periods (Shammari *et al.*, 2020).

5.5. Antimicrobial susceptibility test for *Proteus mirabilis*

The widespread use of antibiotics in humans, animals, and crops has resulted in constant selection pressure, which has led to the rise in antimicrobial resistance over the past few decades. Hydrolyzed β -lactams of every type were expressed by KPC β -lactamases. With more efficiency than other β -lactam antibiotics, carbapenem drugs are hydrolyzed in particular. Most typically, *Enterobacteriaceae* is the source of the KPC enzymes (Sacha *et al.*, 2009).

Most *Proteus mirabilis* isolates, showed sensitivity for main antimicrobial agent used in this study and this investigate similar to One of the researchers in ThiQar province found a similar percentage of *proteus* resistant to some antibiotics such as Amoxililn , Ampicilin, Ciprofloxacin and Gentamicin (Owaied & Jabur, 2022). As well as a study conducted ,were explained that *Proteus mirabilis* isolates were sensitive to meropenem, imipenem, cefuroxime, cefoxitime,,this main that most UTI causes by *Proteus mirabilis* can easily treated by most antibiotics .As well as another study by conducted in which the sensitivity of bacteria to most antibiotics was proven (Mo *et al.*, 2022).In another study, which showed high sensitivity Imipenem (Imp) (J.-T. Wang *et al.*, 2014).

5.6. Confirmation of *Proteus* by PCR

5.6.1. Detection of *Proteus mirabilis* by 16srRNA and ureR genes

The 16S rRNA gene is present in all bacteria, resulting in sequencing-based bacterial identification viable (Clarridge III, 2004). 16S rRNA sequences from a variety of animals have been screened, and the results indicate that some subunit segments experience rapid genetic mutations, which enable the differentiation of species within the same genus. Significantly bigger taxonomic levels can be distinguished in other sites because of their slower rate of change. (Clayton *et al.*, 1995) In this study, all isolates of *Proteus* bacteria showed 100% positive for this test. This study coincided with a study conducted in Babylon by the researcher where he obtained a 100% conformity rate (Al-Kaim & Al-Dahmoshi, 2023).

Also, another researcher in Baghdad got 100% (Mahmood *et al.*, 2018), and because this gene is present in most or all types of bacteria, in order to confirm the diagnosis of bacteria, the most important feature, which is considered one of the factors of virulence for these bacteria, is the presence of the Urease enzyme.

Urease enzyme, which splits down urea into ammonia and carbon dioxide, is one of *P. mirabilis*'s critical virulence factors. Ammonia is an abundant source of nitrogen, raises the urine's pH, and precipitates soluble polyvalent ionic compounds in the urine (Burall *et al.*, 2004). Also indicated that the urease enzyme helps *P. mirabilis* to consume urea as a source of nitrogen for the manufacture of protein and DNA. Urease helps to develop bacteremia, infections of the bladder, kidneys, and bladder stones (Limanskiĭ *et al.*, 2005).

The *ureR* is the positive transcription regulation gene for urease enzyme. *UreR* The factor that triggers the expression of the urease operon is an AraC/XylS family member (Poore *et al.*, 2001). This study obtained the presence of 100% of the *ureR* enzyme this study coincided with the study of researchers in Baghdad and Ramadi, followed by a study in Al-Najaf on the following percentages respectively 100%, 100%, 93.3% (Rahman & Al-Marsomy2022, Attallah & Farhan 2020; Ridha Abbas Al-Fahham & Raoof Kareem, 2022) .

5.6.2. Molecular detection of the *bla* KPC gene of *Proteus mirabilis*:

The expansion of KPC enzymes will provide a significant barrier to the treatment of Gram-negative bacteria (Hu *et al.*, 2012). The current study showed that all *proteus mirabilis* gave negative results for the *bla* KPC gene; this gene encodes for carbapenemase (*B*-lactamase). This result disagrees with another study done in Thi-Qar, where the researcher got 95.2% (Owaied & Jabur, 2022), As well as studying in China (Kirane & Bhatia, 2022), that explained most isolates of *Proteus mirabilis* isolated from Chinese hospitals contain the *bla* KPC gene; this may be due to regional variation, and Chinese isolates have resistance problems more than isolates obtained by this study. This difference in results may be due to hormonal and physiological changes during pregnancy. Bacterial resistance to some antibiotics in the isolates of this study may be due to the fact that the bacteria may have used other virulence genes and not *bla* KPC. The integration of molecular techniques such as the detection of *bla* KPC, *ure R*, and *16S rRNA* genes into the diagnostic process for urinary tract infections in pregnant women offers significant benefits. These methods provide rapid and accurate identification of pathogens and their resistance profiles, facilitating timely and appropriate treatment. By addressing the specific needs of pregnant women and considering the safety of the fetus, healthcare providers can manage infections more

effectively, reducing the risk of complications and promoting better maternal and fetal health outcomes.

5.6.3. Phylogenetic Tree analysis

The evolutionary history was inferred using the Neighbour-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.06261737 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor, 1969) and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 272 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (S. Kumar *et al.*, 2016).

Conclusions and Recommendations

Conclusions and Recommendations

Conclusions and Recommendations:

Conclusions:

1. The incidence of urinary tract infections in pregnant women was 86.67%.
2. The second and last month of pregnancy had a higher incidence rate.
3. *Proteus* bacteria accounted for 5.38% as causative agent of pregnant UTI.
4. There are no significant differences between the incidence in urban and rural areas.
5. Ages 28–37 are most infected with the *Proteus mirabilis*.
6. All *Proteus* bacteria isolates showed a positive result for the gene *16srRNA* and the gene *ureR*. while all *Proteus* bacteria isolates showed a negative result for the gene *bla* KPC.

Conclusions and Recommendations

Recommendations

1. Infection of pregnant women with the bacteria *Proteus* spp. needs a wider study to find out other patterns.
2. Search more widely for the virulence genes of *Proteus* bacteria, especially the ureas gene.
3. Educating and legalizing the use of antibiotics by pregnant women to prevent resistance of all types of bacteria to these antibiotics.
4. Antibiotics may be used to treatment women (Isepamicin, Doripenem, Amikacin, Cefotenan and Trimethoprim).

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Appendixes

Appendixes

Proteus mirabilis strain YPM35 chromosome, complete genome					
Sequence ID: CP053898.1 Length: 4161306 Number of Matches: 7					
Range 1: 131232 to 131500 GenBank Graphics				▼ Next Match	▲ Previous Match
Score	Expect	Identities	Gaps	Strand	
420 bits(227)	1e-112	255/269(95%)	0/269(0%)	Plus/Minus	
Query 4		TACGACTTCACCCCAGTCATGAATCACAAAGTGGTAAGCGCCCTCCCGAAGGTTAAGCTA			63
Sbjct 131500		TACGACTTCACCCCAGTCATGAATCACAAAGTGGTAAGCGCCCTCCCGAAGGTTAAGCTA			13144
Query 64		CCTACTTCTTTTGCAACCCACTCCCATGGTGTGACACTCGGTGTGTACAAGGCCCGGGAA			123
Sbjct 131440		CCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAA			13138
Query 124		CGTCGTCACCGTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAG			183
Sbjct 131380		CGTATTACCGTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAG			13132
Query 184		TTGCAGACTCCAATCCGGACTACGACAGACTTTATGAGTTCCGCTTGCTCTCGCGACGAC			243
Sbjct 131320		TTGCAGACTCCAATCCGGACTACGACAGACTTTATGAGTTCCGCTTGCTCTCGCGAGGTC			13126
Query 244		TTTGCTCTTTGTGACTGCCATTGAGGCAC	272		
Sbjct 131260		GCTTCTCTTTGTATCTGCCATTGTAGCAC	131232		

Figure :Nucleotide blast of partial region of gene 16S rRNA of *P. mirabilis* strain **EM23** against *P. mirabilis* strain YPM35 deposited in the NCBI data base (Accession number: CP053898.1).(<https://blast.ncbi.nlm.nih.gov/Blast>).

vitek II result

Organism Quantity:
Selected Organism : *Proteus mirabilis*

Collected:

Source:

Comments:

Identification Information

Analysis Time:

3.87 hours

Status:

Final

Selected Organism

99% Probability

Proteus mirabilis

Bionumber:

0017000240042210

ID Analysis Messages

Biochemical Details

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

bioMérieux Customer:

Microbiology Chart Report

Printed January 6, 2024 4:15:28 PM AST

Patient Name:

Patient ID:

Location:

Physician:

Lab ID: 10

Isolate Number: 1

Organism Quantity:

Selected Organism : *Proteus mirabilis*

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
+Flomoxef			+Ceftolozane/Tazobactam		
+Latamoxef			Cefepime	32	R
+Amoxicillin		R	+Cefozopran		
+Ampicillin		R	+Cefpirome		
+Amoxicillin/Clavulanic Acid			+Cefquinome		
+Ampicillin/Sulbactam			Aztreonam	8	I
+Carbenicillin		R	+Doripenem		S
Ticarcillin	≥ 128	R	+Ertapenem		S
Ticarcillin/Clavulanic Acid	≤ 8	S	+Faropenem		
+Azlocillin		R	Imipenem	4	R
+Mezlocillin		R	Meropenem	0.5	S
Piperacillin	≥ 128	R	+Panipenem		
+Piperacillin/Sulbactam			+Meropenem/Vaborbactam		S
Piperacillin/Tazobactam	≤ 4	S	Amikacin	≤ 2	S
+Cefsulodin		R	Gentamicin	8	*R
+Ceftobiprole			+Isepamicin		S
+Cefaclor		R	+Neomycin		
+Cefadroxil		R	+Netilmicin		
+Cefalexin		R	Tobramycin	8	R
+Cefalotin		R	+Cinoxacin		
+Cefazolin		R	+Flumequine		
+Cefonicid		R	+Nalidixic Acid		
+Cefprozil			Ciprofloxacin	≤ 0.25	S
+Cefradine		R	+Delafloxacin		
+Loracarbef		R	+Enoxacin		
+Cefamandole		R	+Enrofloxacin		
+Cefotiam		R	+Fleroxacin		
+Cefuroxime		R	+Gatifloxacin		S
+Cefmetazole			+Levofloxacin		
+Cefotetan		S	+Lomefloxacin		
+Cefoxitin			+Marbofloxacin		
+Cefcapene		R	+Moxifloxacin		
+Cefditoren			+Norfloxacin		
+Cefixime			+Ofloxacin		S
+Cefpodoxime		R	Pefloxacin		
+Ceftibuten			+Pradofloxacin		
+Cefoperazone			+Prulifloxacin		
+Cefovecin			+Doxycycline		R
Ceftazidime	≤ 1	S	Minocycline	8	*R
+Ceftiofur			Colistin		
+Ceftizoxime		R	Rifampicin		

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Ministry of Higher Education
& Scientific Research
University of Thi-Qar
College Of VET.MED.

جامعة ذي قار
University of Thi-Qar
2002

وزارة التعليم العالي والبحث العلمي
جامعة ذي قار
كلية الطب البيطري
ب السيد معاون للشؤون العلمية
والدراسات العليا

العدد: ٧٩٩
التاريخ: ٨ / ١ / 2023

الى / مستشفى الشرطة العام
م/تسهيل مهمة

حياة طيبة ...

يرجى التفضل بالموافقة على تسهيل مهمة طالبة الماجستير (ايمان نعمة حسين) في جمع العينات
لسريرية والعمل داخل المختبر لغرض انجاز بحثها .

شاكرين تعاونكم معنا مع التقدير ...

الاستاذ المساعد الدكتور
صفاء حسين علي
معاون العميد للشؤون العلمية والدراسات
العليا
2023 / ١ / ٨

لإحاطة لربنا
مهند كريم لليل
تقني تحيلاته مرف

المختبر
الاعاون الإداري
جيدار فاضل السعدي
مستشار اقتصادي
١١ / ١ / ٢٠٢٣

نسخة منه

- مكتب السيد معاون العميد للشؤون العلمية...للتفضل بالاطلاع مع...التقدير
- وحدة الدراسات العليا
- الصادرة

Questionnaire sheet

No.	Age	Residency	Habitation	Pregnant month	Culture Result
1	28	Al-Nasar	Village	2	Proteus
2	38	Al-Refai	Village	9	Proteus
3	29	Al-Shatra	City	6	Proteus
4	25	Al-Shatra	City	3	Proteus
5	23	Al-Refai	City	4	Proteus
6	32	Al-Nasar	Village	3	Proteus
7	34	Al-Refai	City	7	Proteus
8	22	Al-Refai	City	3	Klebsiella
9	34	Al-Shatra	City	5	Klebsiella
10	30	Al-Refai	Village	6	Klebsiella
11	32	Al-Nasar	Village	2	Klebsiella
12	24	Qlaa Sekar	City	7	Klebsiella
13	19	Al-Refai	City	7	Pseudomonas
14	40	Qlaa Sekar	Village	9	Pseudomonas
15	37	Al-Refai	City	5	Pseudomonas
16	25	Al-Refai	City	2	Pseudomonas
17	26	Al-Shatra	City	8	Pseudomonas
18	33	Al-Nasar	Village	7	Pseudomonas
19	37	Al-Refai	Village	4	Pseudomonas
20	30	Al-Refai	City	9	Pseudomonas
21	35	Qlaa Sekar	Village	8	E.Coli
22	27	Al-Refai	City	6	E.Coli
23	25	Al-Nasar	Village	2	E.Coli
24	32	Al-Refai	Village	6	E.Coli
25	19	Al-Refai	City	9	E.Coli
26	40	Al-Refai	City	2	E.Coli
27	22	Al-Shatra	City	3	E.Coli
28	18	Qlaa Sekar	Village	5	E.Coli
29	37	Al-Refai	City	9	E.Coli

30	22	Al-Shatra	City	2	E.Coli
31	30	Al-Nasar	City	2	E.Coli
32	27	Al-Refai	Village	2	E.Coli
33	41	Al-Refai	City	8	E.Coli
34	33	Qlaa Sekar	City	7	E.Coli
35	28	Al-Refai	Village	4	E.Coli
36	37	Al-Shatra	Village	8	E.Coli
37	39	Al-Refai	City	9	E.Coli
38	30	Al-Shatra	City	4	E.Coli
39	19	Al-Refai	Village	9	E.Coli
40	22	Al-Nasar	City	7	E.Coli
41	18	Al-Nasar	Village	4	No growth
42	37	Al-Nasar	Village	9	No growth
43	22	Al-Nasar	Village	8	No growth
44	30	Al-Nasar	City	6	(G+ve) Bac.
45	37	Al-Nasar	City	3	(G+ve) Bac.
46	39	Al-Nasar	City	5	(G+ve) Bac.
47	30	Al-Nasar	Village	9	(G+ve) Bac.
48	37	Al-Nasar	City	2	(G+ve) Bac.
49	30	Al-Nasar	City	2	(G+ve) Bac.
50	35	Al-Nasar	City	2	(G+ve) Bac.
51	27	Al-Nasar	Village	8	(G+ve) Bac.
52	25	Al-Nasar	City	7	(G+ve) Bac.
53	32	Al-Nasar	City	4	(G+ve) Bac.
54	19	Al-Nasar	Village	8	(G+ve) Bac.
55	40	Al-Nasar	Village	9	(G+ve) Bac.
56	22	Al-Nasar	City	4	(G+ve) Bac.
57	18	Al-Nasar	City	9	(G+ve) Bac.
58	37	Al-Nasar	Village	8	(G+ve) Bac.
59	37	Qlaa Sekar	Village	9	No growth
60	22	Qlaa Sekar	City	7	No growth
61	30	Qlaa Sekar	City	4	No growth

62	37	Qlaa Sekar	Village	9	No growth
63	39	Qlaa Sekar	Village	8	(G+ve) Bac.
64	30	Qlaa Sekar	City	2	(G+ve) Bac.
65	37	Qlaa Sekar	City	8	(G+ve) Bac.
66	30	Qlaa Sekar	Village	7	(G+ve) Bac.
67	35	Qlaa Sekar	City	4	(G+ve) Bac.
68	27	Qlaa Sekar	City	9	(G+ve) Bac.
69	25	Qlaa Sekar	City	8	(G+ve) Bac.
70	32	Qlaa Sekar	Village	6	(G+ve) Bac.
71	19	Qlaa Sekar	City	2	(G+ve) Bac.
72	34	Qlaa Sekar	City	6	(G+ve) Bac.
73	30	Qlaa Sekar	City	9	(G+ve) Bac.
74	23	Qlaa Sekar	City	2	(G+ve) Bac.
75	30	Qlaa Sekar	Village	5	(G+ve) Bac.
76	29	Qlaa Sekar	Village	9	(G+ve) Bac.
77	24	Qlaa Sekar	City	2	(G+ve) Bac.
78	26	Qlaa Sekar	City	2	(G+ve) Bac.
79	22	Al-Refai	Village	2	No growth
80	30	Al-Refai	Village	8	(G+ve) Bac.
81	29	Al-Refai	City	7	No growth
82	24	Al-Refai	City	4	No growth
83	26	Al-Refai	Village	5	(G+ve) Bac.
84	41	Al-Refai	Village	9	(G+ve) Bac.
85	33	Al-Refai	City	2	(G+ve) Bac.
86	28	Al-Refai	City	2	(G+ve) Bac.
87	37	Al-Refai	Village	2	No growth
88	39	Al-Refai	Village	9	(G+ve) Bac.
89	30	Al-Refai	City	2	No growth
90	19	Al-Refai	City	2	(G+ve) Bac.
91	22	Al-Refai	Village	6	(G+ve) Bac.
92	19	Al-Refai	City	2	(G+ve) Bac.
93	40	Al-Refai	City	9	(G+ve) Bac.

94	36	Al-Refai	City	5	No growth
95	40	Al-Refai	City	3	(G+ve) Bac.
96	30	Al-Refai	Village	3	(G+ve) Bac.
97	27	Al-Refai	City	2	(G+ve) Bac.
98	25	Al-Refai	City	8	(G+ve) Bac.
99	32	Al-Refai	City	7	(G+ve) Bac.
100	19	Al-Refai	Village	4	(G+ve) Bac.
101	40	Al-Refai	City	9	(G+ve) Bac.
102	22	Al-Refai	City	8	(G+ve) Bac.
103	18	Al-Refai	City	6	(G+ve) Bac.
104	37	Al-Refai	City	2	(G+ve) Bac.
105	37	Al-Refai	Village	8	(G+ve) Bac.
106	33	Al-Refai	City	7	(G+ve) Bac.
107	40	Al-Refai	Village	4	(G+ve) Bac.
108	18	Al-Refai	City	3	(G+ve) Bac.
109	33	Al-Shatra	Village	9	No growth
110	28	Al-Shatra	City	5	(G+ve) Bac.
111	37	Al-Shatra	Village	3	(G+ve) Bac.
112	39	Al-Shatra	Village	3	No growth
113	30	Al-Shatra	Village	6	(G+ve) Bac.
114	37	Al-Shatra	City	2	(G+ve) Bac.
115	39	Al-Shatra	City	4	No growth
116	30	Al-Shatra	City	8	(G+ve) Bac.
117	19	Al-Shatra	Village	9	(G+ve) Bac.
118	22	Al-Shatra	City	5	(G+ve) Bac.
119	19	Al-Shatra	City	3	(G+ve) Bac.
120	40	Al-Shatra	City	6	(G+ve) Bac.
121	33	Al-Shatra	City	5	(G+ve) Bac.
122	28	Al-Shatra	Village	4	No growth
123	37	Al-Shatra	City	3	(G+ve) Bac.
124	29	Al-Shatra	City	9	(G+ve) Bac.
125	40	Al-Shatra	City	5	(G+ve) Bac.

126	21	Al-Shatra	City	3	(G+ve) Bac.
127	25	Al-Shatra	City	2	(G+ve) Bac.
128	32	Al-Shatra	City	4	No growth
129	19	Al-Shatra	Village	2	(G+ve) Bac.
130	40	Al-Shatra	City	4	(G+ve) Bac.
131	22	Al-Shatra	City	5	(G+ve) Bac.
132	18	Al-Shatra	City	4	(G+ve) Bac.
133	22	Al-Shatra	Village	5	No growth
134	19	Al-Shatra	City	8	(G+ve) Bac.
135	40	Al-Shatra	City	7	(G+ve) Bac.
136	34	Al-Shatra	City	5	(G+ve) Bac.
137	33	Al-Shatra	City	6	(G+ve) Bac.
138	19	Al-Shatra	City	3	(G+ve) Bac.
139	45	Al-Shatra	City	7	(G+ve) Bac.
140	37	Al-Shatra	City	2	(G+ve) Bac.
141	28	Al-Shatra	City	2	(G+ve) Bac.
142	30	Al-Shatra	City	7	(G+ve) Bac.
143	35	Al-Shatra	City	6	(G+ve) Bac.
144	33	Al-Shatra	City	5	(G+ve) Bac.
145	28	Al-Shatra	Village	8	(G+ve) Bac.
146	37	Al-Shatra	City	7	(G+ve) Bac.
147	19	Al-Shatra	Village	4	No growth
148	20	Al-Shatra	City	9	(G+ve) Bac.
149	24	Al-Shatra	City	8	(G+ve) Bac.
150	23	Al-Shatra	Village	4	(G+ve) Bac.

الخلاصة

تُعتبر التهابات المسالك البولية من الأمراض الشائعة التي تصاب بها النساء خاصة أثناء الحمل . حيث تمثل الجراثيم الأسباب الرئيسة لتلك الالتهابات كما تعد المتقلبات بأنواعها من مسببات التهابات المسالك البولية، والتي يمكن أن تصيب الأم بإصابتها بالتهاب الكلى أو تكوين حصى الكلى للامهات وكذلك يمكن أن تؤثر على نمو الجنين. جمعت عينات هذه الدراسة للفترة من ايلول الى كانون الأول من عام 2023. حيث حُصل على 150 عينة بول من نساء عانين من تلك الالتهابات اللواتي راجعن مستشفيات شمال محافظة ذي قار لمعرفة سبب الاصابة وللحصول على العلاجات اللازمة . خلال الفترة السابقة تم جمع العينات بطرق معقمة وصحية بحيث لا تسبب ضررا للنساء الحوامل. زُرعت تلك العينات على وسط الماكونكي وكذلك وسط الدم، وبعد ظهور النمو الجرثومي تم فحص هذه العينات مجهرياً باستخدام صبغة جرام وتقنية VITEK II للتشخيص ومعرفة مدى مقاومتها للمضادات الحيوية.

أكد التشخيص وراثيا باستخدام تقنية تفاعل البلمرة المتسلسل للكشف عن المورثات *ureR* ، *16s rRNA* وكذلك *bla kpc*. أظهرت نتائج الزراعة على الأوساط الزراعية والفحص المجهرى ان 40 عينة (26.66%) هي بكتيريا سالبة لصبغة جرام . بعد الفحص بتقنية VITEK II أظهرت النتائج أن 7 فقط (5.38%) من العزلات الجرثومية هي *proteus mirabilis* . اما ما يخص اختبار الحساسية الدوائية بنفس تقنية VITEK II لاحظت الدراسة أن جميع العزلات من *proteus mirabilis* وبنسبة (100 %) مقاومة للدوكسيسيكليين والكاربنسلين ، بينما كانت تلك العزلات حساسة وبنسبة (100%) لتيكارسيللين، سيفوتيتان، أميكاسين، أوفلوكساسين، دوريبينيم، إيزياميسين، وتريميثوبريم. جميع هذه العزلات فحصت وراثيا وظهرت نتائج موجبة لمورثة *16s rRNA* وكذلك للمورثة *ureR* بينما جميعها أظهرت سلبيتها للمورثة *bla kpc*. ثم تم تحديد السلالات المعزولة ، و مقارنة تسلسلها مع تسلسل NCBI للكشف عن SNP. تم تضخيم المنطقة الجزئية للجين *16s rRNA* وتسلسلها باستخدام طريقة تسلسل سانجر *bla KPC* كذلك تمت تسجيل ثلاث عزلات من البكتيرية المشخصة في بنك الجينات:

SUB14440996 EM07 PP894752 , SUB14440996 EM11 PP894753 ,
SUB14440996 EM23 PP894754



جمهورية العراق

وزارة التعليم العالي والبحث العلمي/جامعة ذي قار

كلية الطب البيطري



الكشف الجزيئي عن أنواع المتقلبات الممرضة للمسالك البولية المرتبطة بالنساء الحوامل في شمال محافظة ذي قار.

رسالة ماجستير

قُدمت الى مجلس كلية الطب البيطري – جامعة ذي قار كجزء من متطلبات نيل شهادة الماجستير
في الطب الباطني \ علم الاحياء المجهرية.

مقدمة من قبل

الطالبة: ايمان نعمه حسين الشويلي

بكالوريوس طب وجراحة بيطرية

كلية الطب البيطري/جامعة بغداد (2004)

بإشراف

أ.د. حيدر خميس المالكي

٢٠٢٤ م

١٤٤٦ هـ