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IMMUNOLOGICAL AND MICROBIOLOGICAL STUDY OF URINARY TRACT INFECTION PATIENTS

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MANAR MUTHANNA ABDULJABBAR ALTEKREETI

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IMMUNOLOGICAL AND MICROBIOLOGICAL STUDY OF URINARY TRACT INFECTION PATIENTS

By Manar Muthanna Abduljabbar ALTEKREETI

July 2022

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

Advisor : Asst. Prof. Dr. Yaşar KEMAL YAZGAN
Co-Advisor : Asst. Prof. Dr. Majeed Arsheed SABBAH

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Oral DÜZDEMİR
Department of Biology
Çankırı Karatekin University

Member : Asst. Prof. Dr. Yaşar Kemal YAZGAN
Faculty of Dentistry
Çankırı Karatekin University

Member : Assoc. Prof. Dr. Gökhan NUR
Faculty of Engineering and Natural Sciences
İskenderun Teknik University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

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Manar Muthanna Abduljabbar ALTEKREETI

ABSTRACT

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Manar Muthanna Abduljabbar ALTEKREETI

Master of Science in Biology

Advisor: Asst. Prof. Dr. Yaşar KEMAL YAZGAN

Co-Advisor: Asst. Prof. Dr. Majeed Arsheed SABBAH

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Urinary tract infection (UTI) is one of the widest bacterial infections in humans. The prediction of Urinary tract infection (UTI) and finding specific pathogens requires the identification of biomarkers that are more precise because their list has been extended. This study aims to identify common bacterial causative agents of urinary tract infection (UTI) and assess some biomarkers and clarify the relationship between bacterial pathogens and concentrations of these biomarkers. subjects in this research were (100) adult patients suffering from Urinary tract infection (UTI), In addition to (20) apparently healthy 10.33as a control group Midstream urine and blood samples were collected from patients and the control group. All included markers (Il-6, IL-8, and CRP) were notably higher in patients than in controls. The mean of IL-6 and IL-8 levels in patients was 413.7 ± 467 pg/mL and 160.2 ± 251.4 pg/m respectively compared with 23.11 ± 10 pg/mL and 11.1 ± 5.6 pg/mL respectively in the control group. Similarly, the mean levels of CRP in patients was 27.5 ± 12.7 mg/L which is extreme elvaed than of controls 10.7 ± 3.9 mg/L. with highly signifcant difference. The study showed no effect of sex on the high level of (IL-6 and IL-8) between males and females, as the study did not show a significant effect and that the immune response depends on the pathogen and the strength of the body's immune response against the same pathogen. The urinary tract infection (UTI) is one of the most wide spread location of bacterial infection in humans. The prediction of Urinary tract infection (UTI) and finding specific pathogens rquires the identification of a biomarkers which is more precise because their list have been extended. This study aims to identify common bacterial causative agents of urinary tract infection (UTI), to assess

some biomarkers and to clarify the reltionship between bacterial pathogens and concentrations of the biomarkers. Bacterial Profile in Patients *Escherichia coli* isolated from 34% of patients. *Streptococcus* fecalis was isolated from 23% of patients, *Klebsiella pneumonia* isolated from 14% of patients, *Staphylococcus saprophyticus* isolated from 12% of patients followed by *proteus mirabilis* 5%. *staphylococcus aureus* 7%. *Pseudomonas aeruginosa* was isolated from 5 % of patients.

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Keywords: Urinary tract infection, Microorganism, Immunity response

ÖZET

İDRAR YOLU ENFEKSİYONU HASTALARININ İMMÜNOLOJİK VE MİKROBİYOLOJİK ÇALIŞMASI

Manar Muthanna Abduljabbar ALTEKREETI
Biyoloji, Yüksek Lisans
Tez Danışmanı: Dr. Öğr. Üyesi Yaşar Kemal YAZGAN
Eş Danışman: Dr. Öğr. Üyesi Majeed Arsheed SABBAH
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İdrar yolu enfeksiyonunun (İYE), insanlarda en yaygın bakteriyel enfeksiyonlardan biridir. idrar yolu enfeksiyonunun (İYE) tahmini ve spesifik patojenlerin bulunması, listeleri uzatıldığı için daha kesin olan biyobelirteçlerin tanımlanmasını gerektirir. Bu çalışma, idrar yolu enfeksiyonunun (İYE) yaygın bakteriyel etkenlerini tanımlamayı ve bazı biyobelirteçleri değerlendirmeyi ve bakteriyel patojenler ile bu biyobelirteçlerin konsantrasyonları arasındaki ilişkiyi netleştirmeyi amaçlamaktadır. Bu araştırmadaki denekler, İYE'den muzdarip (100) yetişkin hastaydı, (20)'ye ek olarak, kontrol grubu olarak 10,33 Hastalardan ve kontrol grubundan orta akım idrar ve kan örnekleri toplandı. Dahil edilen tüm belirteçler (Il-6, IL-8 ve CRP), hastalarda kontrollere göre belirgin şekilde daha yüksekti. Kontrol grubunda sırasıyla 23,11 ± 10 pg/mL ve 11,1 ± 5,6 pg/mL ile karşılaştırıldığında, hastalarda IL-6 ve IL-8 düzeylerinin ortalaması sırasıyla 413,7 ± 467 pg/mL ve 160,2 ± 251,4 pg/m^2 idi. Benzer şekilde hastalarda CRP düzeylerinin ortalaması 27,5 ± 12,7 mg/L olup, kontrollere göre aşırı derecede yükselmiştir 10,7 ± 3,9 mg/L. son derece önemli bir farkla. Çalışma, önemli bir etki göstermediği ve bağışıklık tepkisinin patojene ve vücudun gücüne bağlı olduğu için, erkekler ve kadınlar arasındaki yüksek düzeyde (IL-6 ve IL-8) cinsiyetin hiçbir etkisi olmadığını gösterdi. Aynı patojene karşı bağışıklık tepkisi. İdrar yolu enfeksiyonu (İYE), insanlarda bakteriyel enfeksiyonun en yaygın yayılım yerlerinden biridir. UTI'nin tahmini ve spesifik patojenlerin bulunması, listeleri uzatıldığı için daha kesin olan bir biyobelirteçlerin tanımlanmasını gerektirir. Bu çalışma, idrar yolu enfeksiyonunun (İYE) yaygın bakteriyel nedensel ajanlarını tanımlamayı, bazı biyobelirteçleri değerlendirmeyi ve bakteriyel patojenler ile

biyobelirteçlerin konsantrasyonları arasındaki ilişkiyi netleştirmeyi amaçlamaktadır. Hastalarda Bakteriyel Profil Hastaların %34'ünden izole edilen *Escherichia coli*. Hastaların %23'ünden izole edilen *Streptococcus fecalis*, Hastaların %14'ünden izole edilen *Klebsiella pneumoniae,* Hastaların %12'sinden izole edilen *Staphylococcus saprophyticus* ve ardından %5 *proteus mirabilis* izole edilmiştir. *staphylococcus aureus* %7. Hastaların %5'inden izole edilen *Pseudomonas aeruginosa*.

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Anahtar Kelimeler: İdrar yolu enfeksiyonu, Mikroorganizma, Bağışıklık yanıtı

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Manar Muthanna Abduljabbar ALTEKREETI

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CONTENTS

LIST OF SYMBOLS

-	Minus
%	Percent
/	Divide
+	Plus
<	Greater than
=	Equel
>	Less than
±	Plus–minus
≤	Greater or equal to
≥	Less or equal to
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m^2	Square meter
mg	Milligram
mIU	Milli-international units
min	Minute
mL	Milliliter
mmol	Millimole
mol	Mole
ng	Nanogram
nm	Nanometer
rpm	Revolutions per minute
µL	Microliter

LIST OF ABBREVIATIONS

CRP	C - reactive protein
GN	Gram-negative
GP	Gram-positive
LPS	Lipopolysaccharide
TSIA	Triple sugar iron agar
UTI	Urinary tract infection

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1 INTRODUCTION

The second most frequent infectious presenting in community medical practice is urinary tract infection (UTI). Each year, around 150 million people are given a UTI diagnosis globally (Singh *et al.* 2018).

One of the infectious disorders affecting humans, urinary tract infections (UTIs). are brought on by bacterial infections that can affect any region of the urinary system. urinary tract infection (UTI) are classified according to whether they affect the upper urinary tract or the lower urinary tract. If they impact the lower urinary tract, they are referred to as simple cystitis (bladder infections), while they affect the upper urinary tract, they are referred to as pyelonephritis (a kidney infection) (Stamm 2006).

Local inflammation is the main component of non-specific innate immunity against infections of the bladder or kidneys, whereas specific immunity is followed by an adaptive immune response that includes both cell-mediated immune response and an antibody response to the pathogenic bacteria. The majority of the time, urinary tract infections will go away on their own. Immunoglobulin levels in the vaginal and urinary tracts of both kids and adults (women) are more likely to be normal in those who do not have urinary tract infections (Masajtis-Zagajewska and Nowicki 2017).

Among general practice, urinary tract infections (UTIs). are one of the most often encountered illnesses in female patients. Due to their shorter urethra, absence of prostatic secretion, pregnancy, and easier faecal flora infection of the urinary tract, women are more likely than men to acquire a UTI (Butler *et al*. 2006).

The infection may only cause bacteria to develop in the urine, which typically causes no symptoms, or it may cause a number of syndromes linked to an inflammatory reaction to the bacterial invasion. In reality, the term "UTI" covers a broad spectrum of illnesses, including symptomatic urinary tract infection (UTI), cystitis, urethritis, pyelonephritis, and acute pyelonephritis involving bacteremia or sepsis. (Joseph 2008).

Numerous cells start to create cytokines, which are considered small and soluble proteins, during infection and inflammation. Interleukin-6 is a pro-inflammatory cytokine that is produced during the early stages of infection. It takes corrective actions that, when neglected, would have sped up the immune system's development. Pro-inflammatory responses of IL-6 are facilitated by trans-signaling, and they are responsible for diseases that result in the production of C-Reactive Protein (CRP) . Pro-inflammatory cytokine IL-8 was elevated in response to IL-1 and TNF-. IL-8 is a chemokine that causes neutrophils to go to the area of irritation and cause pyuria in infected people. Levels of the cytokines mentioned above may increase during advanced stages of urinary tract infection (UTI) in both blood and urine (Al-Kaabi and Al-Khalidi 2020).

1.1 Aim of Study

The goal of this study was to evaluate the relationship between interleukin-6 (IL-6) and interleukin-8 (IL-8) levels in instances of human (UTI.), which is one of the most prevalent bacterial illnesses. The aim of this work is to study the humeral immune response in urinary tract infection (UTI) patients by measurement of IL6 and IL8.

2 LITERATURE REVIEW

2.1 Urinary Tract Infections

Millions of people are affected each year by the major health issue of urinary tract infections (Griebling 2005).

Despite the extensive use of medicines, it is still the second most prevalent bacterial infection in people, with more women than men experiencing UTI (Schaeffer and Nicolle 2016).

UTIs. can be categorized as either simple or complex. However, there are two different UTI presentations: lower UTI and upper UTI. Lower UTI is an infection of the bladder and upper UTI is an infection of the urethra, known as cystitis and urethritis, respectively. The other type of UTI is an upper UTI, which causes pyelonephritis and urethritis, infections of the kidneys and ureters, respectively. Because there is a chance of kidney damage, upper UTIs could be more serious than lower UTIs (Raynor and Carson 2011).

2.1.1 Routes of urinary tract infection

1. Ascending route: entry is normally by ascent from the urethra especially if vesicoureteral reflux is present. It is the most common route.
2. Hematogenous route: blood-borne bacteria infecting the kidney parenchyma, causing an abscess to develop.
3. Lymphatic route: occur when pressure is increased in the bladder, lymphatic flow to be directed toward the kidney (Montelin *et al.* 2019).

2.1.2 Pathophysiology

Lower UTIs, commonly known as cystitis, affect women much more frequently than men. This is mostly due to anatomical variations in women, like their condensed urethra and

moist periurethral environment. Normal progression of urinary tract infections includes colonization of the urethra, periurethral contamination by an intestinal uropathogen, and ultimately pathogenic movement to the bladder or kidney through the flagella and pili. (Bardsley 2018).

In the pathophysiology of urinary tract infection, The importance of bacterial adherence to the uroepithelium. When effective host defense mechanisms are defeated by bacterial virulence mechanisms, infections develop. Upper UTIs, sometimes referred to as pyelonephritis, happen when uropathogens pass through the ureters and reach the kidneys. Bacteria can become trapped in the urinary tract due to a physical obstruction or by adhering to a urinary catheter, kidney, or bladder stone. When pyelonephritis is severe, the damaged kidney may swell and have elevated abscesses on the surface (Nicolle 2016).

kidneys by hematogenous inoculation of bacteria Staphylococcus aureus bacteremia or endocarditis can result in suppurative necrosis or the development of an abscess within the renal parenchyma (Kaye and Sobel 2014).

Gram-negative bacilli, on the other hand, hardly ever result in kidney infection via the hematogenous route. The principal renal defect observed in an experimental model of pyelonephritis is the inability to maximally concentrate the urine (Holland *et al.* 2014).

Early in the infection, this concentration deficiency appears and can be quickly corrected with antibiotic therapy. A blockage may cause the damaged kidney to gradually degenerate, resulting in renal insufficiency (Flores-Mireles *et al.* 2015).

2.1.3 Predisposing factors

Bacteriuria rarely develops into symptomatic cystitis or pyelonephritis in adult women who are not pregnant and have a healthy urinary system. In Figure 2.1, common risk factors for UTIs are listed. Sexual activity can push bacteria into the female bladder since the urethra is typically infected by bacteria. Spermicides also promote *Escherichia coli*

adhesion to vaginal epithelial cells and uropathogen colonization of the vagina (Tony Mazzulli 2012, Shaikh *et al.* 2008).

Patients population	Risk factors
Premenopausal women of any age	• Diabetes • Diaphragm use, especially those with spermicide • History of UTI or UTI during childhood • Mother or female relatives with history of UTIs • Sexual intercourse
Postmenopausal and older adult women	• Estrogen deficiency • Functional or mental impairment • History of UTI before menopause • Urinary catheterization • Urinary incontinence
Men and women with structural abnormalities	• *Extrarenal obstruction* associated with congenital anomalies of the ureter or urethra, calculi, extrinsic ureteral compression, or benign prostate hypertrophy • *Intrarenal obstruction* associated with nephrocalcinosis, uric acid nephropathy, polycystic kidney disease, hypokalemic or analgesic nephropathy, renal lesions from sickle cell disease

Figure 2.1 Predisposing risk factors for urinary tract infection (UTI)

2.1.4 Clinical presentation

Age, infection stage, host reaction, and the kind of bacteria that causes the disease all affect the clinical signs of UTI. Young babies frequently exhibit vague symptoms, such as fever, agitation, vomiting, lethargy, or poor feeding. More obvious signs, like discomfort upon voiding and increasing frequency, appear in lower urinary tract infection as children and people get older. On the other hand, fever and flank pain are related to upper UTI. A recent meta-analysis assessed the effectiveness of pediatric UTI symptoms and indicators for diagnosis. The likelihood of UTI in babies was raised by a history of prior UTI, fever lasting over a 24-hour period, suprapubic discomfort, and lack of circumcision. The most dependable symptoms for older kids were dysuria, back discomfort, new-onset urine incontinence, and belly pain and fever (Arinzon *et al.* 2012). A proposed strategy for screening women with UTI symptoms attained a 90 percent likelihood of UTI without laboratory tests by combining a number of clinical symptoms (Griebling 2012). Compared to premenopausal women, postmenopausal women had

more severe clinical manifestations, including urine incontinence and more widespread nonspecific symptoms. (Ludwig *et al.* 2006).

2.1.5 Causative agent

Generally speaking, urine is thought to be sterile and germ-free. The urethra is any source of potential infection, and this is what causes the infection to first start (Tektook *et al.* 2017).

The most typical organisms isolated from the urinary system are enteric gram negative rods. E. coli is the most frequently found organism in charge of these. It is referred to be a "superbug" in urinary tract infections. *Klebsiella, morganella, and proteus* are a few more gram-negative microbes that were identified. *Staphylococcus saprophyticus, Staphylococcus aureus, Streptococcus,* and *enterococcus* are examples of gram-positive organisms that can cause UTI (Kline and Lewis 2016).

The causes of community- and hospital-acquired UTIs are different. (Tadesse *et al.* 2017). Only a little quantity of information about changes in the frequency of causative agents among outpatients has been reported. The majority of UTIs are still caused by enteric bacteria, particularly E. coli, while there is some indication that the proportion of UTIs caused by E. coli is declining (Kibret and Abera 2014).

E. coli, which makes up up to 80–85 percent of the pathogens responsible for UTIs, is the most common, followed by Staphylococcus saprophyticus, which makes up to 5-10 percent. Infections caused by fungi or viruses are a very uncommon phenomenon. Along with the bacterial species already mentioned, UTI is also linked to *Klebsiella, Proteus, Pseudomonas, and Enterobacter*. The urethra is when the germs enter the bladder, and blood and lymph can also become infected. It is believed that the microbiological etiology of UTIs is widely known and common (Kline and Lewis 2016).

Pathogens like *Klebsiella, Enterococcus, Proteus Species, and Enterobacter* are known to cause occasional uncomplicated cystitis and phylonephritis, *E.* coli is linked with population-acquired acute, uncomplicated illness (Kline *et al.* 2012).

The widespread bacteria that cause UTIs are well known for their tenacity and display the trait of antibiotic tolerance. They are also well known for the phenotypic and genotypic traits that make them top candidates for transmitting the illness. Host variables must be taken into account because they contribute equally to the infection's etiology and increase the victim's susceptibility. Age, diabetes, prolonged hospitalizations, and the type of medical equipment used by patients, such as catheters, are some of these (Hindi *et al.* 2013). The bacteria travel from the bowel to the bladder. After the bacteria attach themselves to surfaces, a biofilm forms that evades the immune system and is the primary source of infection (Tektook *et al.* 2017).

2.2 Immune System

A group of proinflammatory cytokines are released as part of the host's inflammatory response, which intensifies the inflammatory proces IL-6 and iL-8 are crucial mediators of inflammation that are generated in responses to bacterial infections (Tramma *et al.* 2012).

IL-6 is a multifunctional cytokine that controls a variety of bodily processes, including inflammation, the acute phase response, and organ development. By directly binding to the IL-6 receptor, This is demonstrated by neutrophils, T-helper cells, macrophages, hepatocytes, and podocytes, IL-6 is released. Additionally, there is a soluble IL-6 receptor, whose widespread expression enables IL-6 to influence a variety of target cells (Su *et al.* 2017).

The chemokine IL-8, on the other hand, is created by macrophages in response to IL-1, IL-2, and tumor necrosis factor (TNF). It is a significant neutrophil attractant. Healthy persons' urine contains trace quantities of both IL-6 and IL-8 (Gokce *et al.* 2010).

interleukin-6 and interleukin-8 levels in the urine are therefore considered to be indicators of urinary tract infection and most likely the location of infection in the UTI. When UTIs occur, the innate immune cells in the urinary system react quickly and strongly (Krzemień *et al. 2004*). These responses are strictly regulated, but occasionally the bladder's innate responses are prematurely shut down, which can harm the host, as was recently demonstrated for the development of insufficient adaptive immune responses to infection (Abraham and Miao 2015).

The bladder in particular tends to have weak adaptive immune responses, in contrast to the extensive innate immune responses of the urinary tract, which are highly susceptible to infections (Mora-Bau *et al.* 2015). Patients with UTIs that spread to the kidneys can produce antibodies that are specific for the pathogen, but those whose infections are limited to the bladder mysteriously fail to do so. An inflammatory reaction is triggered if this initial line of defense against infections entering the urinary system fails. Close interaction between the host and pathogen is made possible by the bacterial fimbriae's attachment to the bladder's uroepithelial cells (Benson *et al.* 1996).

2.2.1 Interleukin-6 (IL-6)

The cytokine interleukin-6 (IL-6) has pro- and anti-inflammatory. It is produced by the IL6 gene in humans. (Pedersen 2013), Several proinflammatory mediators, including cytokines and chemokines, are produced during this reaction. After UPEC infection, bladder and kidney epithelial cells seem to be a significant source of the cytokines interleukin (IL-6) and (IL-8), which are crucial for the emergence of local tissue damage (Sanchez-Carbayo *et al.* 2001), IL-6 performs a range of proinflammatory tasks, including as activating signals that lead to the recruitment of neutrophils and the synthesis of acute phase proteins (Sirota *et al.* 2013). It has been demonstrated that a high urine IL-6 concentration during the pyelonephritis (acute phase) is associated has a higher potential for persistent kidney scarring (Sheu *et al.* 2006). Children's vulnerability to UTIs has been linked to IL-6 gene variants, but not to scar formation (Al-Eisa *et al.* 2017).

2.2.2 Interleukin-8 (IL-8)

The renal tract's epithelial cells have the ability to manufacture interleukin 8 (IL-8) in response to various inflamatory triggers. High concentrations serum have been recorded in individuals with renal failure and the cytokine has been seen in inflamed renal tissue in patients with acute allograft rejection. Patients with a number of inflammatory renal illnesses, such as pyelonephritis, haemolytic uraemic syndrome, graft rejection after renal transplantation, and other types of glomerulonephritis, have been found to have IL-8 in their urine. Patients with uti have IL-8 in their urine. Interleukin 8 (IL-8) is a members of the family of cytokines and is essential for the formation of irritation at the site of infection, leukocyte activation, and chemotaxis. In order to determine the role of IL-8 in the diagnosis and the distinction between cystitis and pyelonephritis, a research was accordingly carried out. Upper urinary tract involvement and lower urinary tract involvement can be distinguished using IL-8 as a reliable marker. Furthermore, cytokine measurement is quick, easy, and non invasive, and it only takes a few hours (Remick 2005).

2.2.3 C-reactive protein (CRP)

The biomarker C-reactive protein (CRP) has been used to predict bacterial infections early on and to track the effectiveness of antibiotic treatment for these illnesses. According to recent studies, CRP blood levels can be used to distinguish between asymptomatic bacteriuria and acute pyelonephritis (upper UTIs) in children with UTIs (Sharif-Askari *et al.* 2020).

2.3 Types of Urinary Tract Infection (UTIs.)

The following categories are used to categorize UTIs:

A) The complicated or uncomplicated Urinary tract infection, depending on the diseases' risk factors.

B) Depending on whether the infection is recurring or recurrent for the first time, primary or recurrent UTIs.

2.3.1 Un-complicated urinary tract infection (UTI).

bacterial illnesses, most frequently E. coli, cause uncomplicated urinary tract infections. Typically, women are more impacted by this than males. The most typical type of uti that affects the lower part of the urinary system and is most prevalent in females is cystitis, sometimes known as a bladder infection. The bladder's surface is the sole part of this illness that typically is acute and transient. Only in cases when the urinary system develops anatomical abnormalities, the infection persists or becomes chronic, or both, can the deeper side of the bladder be impacted. When an infection is spread to the kidneys' and ureters' upper tracts, it is known as pyelonephritis or kidney infection (Bader *et al.* 2010).

2.3.2 Complicated urinary tract infection (UTI).

Bacteria are the usual cause of complicated urinary tract infections, which can affect both men and women of all ages. These infections are serious, frequently reoccurring, and more challenging to treat. These illnesses are almost always the structural or anatomical factor anomaly that can hinder the urinary tract's ability to assist in the elimination of bacteria and urine. Catheters can be used in hospitals or some outpatient settings for persistent indwelling catheters. In the first three months after an outpatient kidney transplant, renal and bladder dysfunction is particularly common. Patients who have complex UTI may experience recurrences if the underlying structural or anatomical issues are not successfully addressed (Bader *et al.* 2010).

2.3.3 Recurrent urinary tract infection (UTIs)

Most women with an uncomplicated UTI experience recurrences infrequently. Between 3 and 5 percent of Women have a persistent, recurrent uti that occurs after the resolution

of the untreated and treated previous episodes. Roughly a quarter of to fifty percent of these women are anticipated to get another infection within a year of the previous one. Relapse or reinfection are two different types of recurrence. Reinfection occurs when UTIs that reoccur are actually reinfections. Once the antibiotic has cleared the first episode, this happens several weeks following the treatment. It can be brought on by a different bacterial strain or one that is related to the original (Liu and Pop 2009).

The infection-causing organism typically enters the body through feces and travels up the urinary tract. Relapse, however, is a less common kind of recurring urine infection. When a urinary tract infection returns within two weeks of the previous episode's treatment and is typically the result of a failed therapy, it can be detected. Relapse typically happens during the kidney infection or is brought on by blockages like kidney stones, structural problems, or in men with chronic prostatitis (McArthur *et al.* 2013) (Figure 2.2).

Pathogens in uncomplicated UTIs	**Pathogens in complicated UTIs**
Escherichia coli *Staphylococcus saprophyticus* Klebsiella pneumonia *Enterococcus faecalis*	*Escherichia coli* *Staphylococcus saprophyticus* Klebsiella pneumonia *Enterococcus faecalis* *Proteus mirabilis* *Pseudomonas aeruginosa*

Figure 2.2 Pathogens in uccomplicated UTIs and in complicated UTIs

2.4 Cystitis

Cystitis, an infection of the bladder's urethra, is brought on by bacteria found in feces that colonize urethral and vaginal openings and then ascend to the bladder. Many germs in urine die because it is inherently acidic and contains a lot of urea. The bladder contains d-Mannose, a monosaccharide (sugar) that E. coli attaches to. 5%–10% of UTIs are caused by aerobic Gram-negative rods and enterococci, not alone E. coli (Gibson *et al.* 2015).

2.5 Pyelonephritis

The term "pyelonephritis" refers to an infection of the upper urinary system that has progressed to the kidneys. In comparison to lower UTIs, pyelonephritis has more complex clinical characteristics. Pyelonephritis is the medical term for an infection of the kidneys brought on by bladder bacteria that has moved up into the kidneys. The bacteria may occasionally enter the bloodstream and go to the kidney, although this is a rather uncommon cause of pyelonephritis (Ramakrishnan and Scheid 2005).

2.6 Bacteriuria without Symptoms

bacteria are found in two urine samples that were collected back to back without any obvious symptoms. Women of all ages can get it, although older women with diabetes are more likely to do so. For all catheterized patients, asymptomatic bacteriuria is present (Nicolle 2003).

2.7 Urethral Syndrome

Urinary syndrome is the term used when the medical symptoms of a urinary tract infection are present but the pee test does not show the presence of bacteria. A quarter to fifty percent of patients appear with lower urinary tract bacteria at the hospital infection concerns. It may be brought on by sexually transmitted germs, or it may be brought on by trauma, drugs, or long-term conditions like diabetes .(Costantini *et al.* 2006).

2.8 Interstitial Cystitis

UTI can result from interstitial cystitis. In this instance, the bladder wall is irritated and inflamed, and there has been frequent urgency for at least six months. Due to an unidentified cause, young to middle-aged women are more likely to experience it. Interstitial cystitis impacting more than a million people living in the US. The effects of interstitial cystitis 575 out of every 100.000 women, according to a government survey.

Compared to the general population of interstitial cystitis patients, children have a prevalence of bladder issues that is ten times higher (Ahmed *et al.* 2000).

2.9 Management of Urinary Tract Infection (UTI)

2.9.1 Prevention of urinary tract infection (UTI)

By determining the reasons and treating the UTI as soon as it occurs, recurrent UTI can be avoided. The National Institute for Health and Clinical Excellence recommends treating constipation, addressing faulty elimination syndromes, and encouraging patients to drink more fluids to keep their bladders free of bacteria in order to prevent recurring UTI (Wagenlehner *et al.* 2014).

2.9.2 Prophylaxis

The following methods can be used to avoid urinary tract infections

2.9.3 Post intercourse regimen

According to several research, using certain medications after sexual activity can help prevent UTIs in sexually active people when preventive antibiotics are insufficient to do so. To prevent infection, lotion with spermicidal properties nonoxynol-9 is administered in conjunction with antibiotics (Melekos *et al.* 1997).

2.9.4 Topical treatment

Some topical treatments, particularly those containing povidone and iodine, are used to prevent UTIs as well. These lotions have antibacterial properties and are applied locally to reduce inflammation. By altering the vaginal flora, topical vaginal estriol protects post-menopausal women from UTI (Wai Ngai *et al.* 2005).

2.9.5 Functional foods

Some foods can also help prevent UTIs, such as cranberry juice, which prevents E. coli from attaching to the bladder walls due to its anti-adhering properties. In females with recurrent UTI who used cranberry for a year, a meta-analysis revealed a 35% decrease in infection. According to Howell and his friends, cranberry extract (500 mg/kg) given for six months reduced UTI at a similar level as trimethoprim (100 mg). Cranberry extract contains sialic acid, which has an anti-inflammatory and analgesic effect. It can help to lessen the effects of a UTI. Although cranberry is useful in lowering the rate of recurring UTI, it is not advised for use in cases with active UTI (Howell 2007).

2.9.6 Immunoprophylaxis

Immunoprophylaxis, which is administered orally as an alternative to antibiotics, is crucial in the prevention of recurrent UTI. It was discovered that taking Uro-Vaxom Escherichia coli extract as an oral immunological prophylaxis for more than six months considerably avoids UTIs (Terra-Laba, Zagreb, Croatia). Additionally, The plant extracts are rapid, effective, and acceptable for treating UTIs, and they also lessen the need for antibiotics, according to research by Schulman and his team. which lowers the rate of recurring UTI. (Shaheen *et al.* 2019).

2.9.7 UTI delevopment risk factors

The likelihood of getting a UTI varies across individuals. These consist of:

1. Because the urethra is just four centimeters long and germs can only move this short distance from the outside to the inside of the bladder, females who are sexually active are particularly vulnerable. People who need urinary catheters, such as very sick individuals who are unable to empty their own bladder.
2. Diabetics; due to immune system abnormalities, people with diabetes are more susceptible to infection A

3. Man with a prostatic condition, such as an enlarging prostate gland that may prevent a full bladder.
4. Infants, particularly those with congenital anomalies of the urinary system that cause physical issues at birth (Chen 2009).

2.10 Type of Microorganism

UTIs are caused by a variety of bacterial species; Gram-negative (GN) bacteria account for 80 to 90% of causative bacteria, with *Escherichia coli*, (*Klebsiella, Enterobacter, Proteus,* and *Pseudomonas*) spp. accounting for 60 to 80% of cases. These microorganisms could, however, commonly be contaminants in urine culture. Additionally important UTI pathogens include Gram-positive (GP) bacteria such *Enterococcus, Staphylococcus,* and *Streptococcus agalactiae*. (Gross and Loper 2009).

2.10.1 *Escherichia coli*

Gram-negative, rod-shaped, oxidase-negative, facultatively anaerobic, catalase-positive, nonspore-forming, motile with peritrichous flagella or nonmotile, lactose fermenter, and able to grow either aerobically or anaerobically, Escherichia coli colonies are less than 3 mm in diameter and pink. After isolating the species, Dr. Theodor Escherich published the first description of it in 1885. One of the most prevalent causes of various typical bacterial infections in both humans and animals continues to be *E. coli*. It is a common contributor to enteritis, UTI, septicemia, and other clinical infections. The emergence of antibiotic resistance frequently presents difficulties for the therapeutic treatment of *E. coli* infections. Globally, the prevalence of strains of E. coli that are resistant to many drugs is rising, primarily as a result of the dissemination of mobile genetic elements like plasmids (Gomes *et al.* 2016).

2.10.2 *Klebsiella pneumonia*

A member of the genus Klebsiella that is nonmotile, facultatively anaerobic, gram-negative, and rod-shaped is *Klebsiella pneumonia*. After isolating the bacteria from the lungs in 1882, Carl Friedlander gave the first description of Klebsiella pneumoniae as an encapsulated bacillus. On regular culture media, *K. pneumonia* grows readily, and its colonies show up as pink mucoid blotches on MacConkey agar (Guilhen *et al.* 2016).

It is an acidic polysaccharide capsule-producing lactose-fermenting bacillus with at least 78 capsular (K antigen) serotypes. It is frequently categorized as an opportunistic nosocomial pathogen that may lead to a variety of illnesses, such as urinary tract infections and blood stream infections, and respiratory tract disorders including pneumonia (BSI). Additionally responsible for a significant number of community-acquired illnesses include endophthalmitis, meningitis, and pyogenic liver abscesses (Guilhen *et al*. 2017). There are essential virulence factors and contribute to its pathogenesis, include capsule, lipopolysaccharide (LPS), fimbriae, and siderophores (Paczosa and Mecsas 2016).

2.10.3 *Proteus mirabilis*

The most significant gram-negative facultative anaerobic rods are those of Proteus mirabilis. They are mobile, produce urease, and have swarming motility. widely distributed and primarily commensal in human and animal gastrointestinal tracts as well as in the environment's soil, water, and sewage (Adeolu *et al.* 2016).

Proteus was given that name by Homer's Odyssey and was first isolated by Hauser in 1885. Urinary tract infections are more frequently linked to Proteus mirabilis (UTIs). After E. coli and K. pneumonia, it is the third most typical cause of complex UTI. P. mirabilis has been discovered as an opportunist etiological factor for gastroenteritis brought on by eating contaminated meat and other food, as well as infection of the

respiratory system, epidermis, eyes, ears, nose, and throat. Additionally to UTI (Hussein *et al*. 2017).

Proteus mirabilis uses a variety of virulence elements, including urease and the production of stones, B lactamase, fimbriae and other contractures, the acquirement of iron and zinc, proteases and toxins, the formation of biofilms, the production of proticines, and the regulation of pathogenesis, to enter and colonize the host urinary tract (Schaffer and Pearson 2015).

2.10.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, facultatively anaerobic, rod-shaped, 0.5 to 0.8 m by 1.5 to 3.0 m, non-fermenter member of the Pseudomonadaceae family of bacteria. Carle Gessard, a French bacteriologist and chemist, made the discovery in 1882 (Cole *et al*. 2014, Rasamiravaka *et al*. 2015). It can be isolated from soil, plants, water, people, and animals things that are typically only experienced as temporary flora. Human colonization is widespread at moist locations like the axilla, perineum, and ear (Wisplinghoff *et al.* 2014). Immunocompromised patients are at risk from the opportunistic bacterium Pseudomonas aeruginosa. It is well known for being the primary cause of morbidity and death in people with cystic fibrosis (CF), as well as one of the leading sources of nosocomial infections. Pseudomonas aeruginosa strain infections can be fatal due to a variety of mechanisms for adaptation, survival, and resistance to numerous classes of antibiotics, and they are becoming an increasingly serious hazard to public health globally. The bacterium seldom ever affects tissues that are immune-sound, but it can target any tissue that is under stress from immunodeficiency. The urinary tract, respiratory system, bacteremia, dermis, soft tissue, bone and joint, blood, and gastrointestinal tract are all infected by P. aeruginosa. especially in individuals who have AIDS, cancer, TB, or serious burns (Buyck *et al.* 2007).

Multiple bacterial virulence factors that promote motility and adherence to host tissues are involved in P. aeruginosa pathogenesis. Some of these virulence factors include type

IV fimbriae, Lipopolysaccharide, flagellum, exotoxin A, enzyme proteases, alginate, signal transduction, and biofilms formation (Alhazmi 2015).

2.10.5 *Staphylococcus aureus*

A spherical, non-motile, Gram_positive, and non-spore-forming bacterium is Staphylococcus aureus. that can be found alone or in pairs, short chains, or clusters that resemble grapes. Its diameter ranges from 0.5 to 1.0 m. Microcapsules are produced by some strains of S. aureus. On solid medium, colonies are rounded, smooth, elevated, and gleaming. Most strains have colored colonies that range in color from rich golden yellow to gray. S. aureus colonies on blood agar or trypticase soy agar are opaque, 1 to 3 mm in diameter, and produce alpha toxin, which results in a large zone of clear (beta-type) hemolysis (Al-Kobaisi 2007).

These microorganisms are oxidase- and catalase-negative. The majority of S. aureus strains generate the enzyme coagulase. These facultative anaerobes multiply by aerobic fermentation, which mostly yields lactic acid., require carbohydrates as their main energy source. The temperature range where S. Aureus can grow is between 15 and 45°C (Fartyal and Kumar 2014).

It is drought-resistant, salt-tolerant, and grows well on substrate with 10% sodium chloride. It also tolerates pH levels between 4.8 and 9.8. The majority of other staphyloccocci are mannitol negative, and this one produces acid by aerobically fermenting mannitol. It is vulnerable to lysostaphin's lysis (Ando *et al.* 2004).

In the environment, it can be found in the air, dust, and water. It is typical component of human flora and can be found on the skin, mucous membranes, and nasal passages. Despite significant advancements, additional work has to be done to pinpoint the precise processes behind S. aureus pathogenicity in the urinary system. However, it is evident that adhesion factors and staphylococcal toxins play a crucial function in disease of S. aureus UTI. (Ando *et al.* 2004) discovered that adhesion molecules, leukocidin, and

enterotoxins were all highly expressed. Urease enzyme was also produced. The development of biofilms by *S. aureus* in the urinary system may be crucial (Ando *et al.* 2004).

2.10.6 *Enterococci faecalis*

A group of bacteria known as enterococci is frequently found in the mouth, vagina, and gastrointestinal system of people. The two enterococci that are most frequently isolated in clinical samples are Enterococci faecalis and Enterococci faecium. According to a report, Enterococci faecalis can be found in roughly 80% of human illnesses. It is widely acknowledged as one of the major causes of human UTI. Recent studies suggest that E. faecalis causes UTIs five times more frequently than E. faecium does. The patient in our situation also had kidney stones, which are prevalent in the urinary system. Theoretically, after the bacteria has entered the urinary tract and contributed to the development of urinary stones, it can easily cause UTI and progress to chronic pyelonephritis (Manshi *et al.* 2020, Li *et al.* 2020).

2.10.7 *Staphylococcus saprophyticus*

Staphylococcus saprophyticus, a common uropathogen that is gram-positive and coagulase negative, is responsible for up to 42% of simple UTIs in young girls. (Hur *et al.* 2016).

A uropathogen called Staphylococcus saprophyticus is responsible for 10–20 percent of urinary tract infections (UTI) in adult, world's sexual activity women. There have been reports of endocarditis, acute pyelonephritis, urethritis, and other potential side effects, particularly in immunocompromised patients. The human gastrointestinal tract, cervix, urethra, vagina, perineum, and rectum are all often colonized by S. saprophyticus (Lawal *et al.* 2021**)**.

3 MATERIALS AND METHODS

3.1 Componint

3.1.1 Tools and instruments

The tools and instruments utilized during the research are listed in Table 3.1

Table 3.1 Equipments and apparatus used in the present study

Apparatus	**Manufacture Company(origin)**
Autoclave	Harayma (Japan)
Burner	Amal (Turkey)
Centrifuge	Dlab Dm0408 (China)
Cover slide	
Deep Freezer	hitachi (Japan)
Disposable Petri dishes	Al-Hani (Lebanon)
Distillatory	GFL(Germany)
ELISA Reader	Biotek ELX800 (USA)
Eppendorf tube	--- (Chinese)
Gel tube	AFCO (Jordin)
Glass tubes flasks beakers	Marine field (Germany)
Hot Plate Magnetic Stirrer	Ikama Ret (India)
Incubator	Gallenkamp (England)
Light Microscope	Olympus (Japan)
Loop	Himedia (India)
Micropipette	Biobasic (canada)
Millipore filterpaper	Whatt mann (England
Multi channel pipettes	Premium (Germany)
Oscillatore frequency	KJ-201BS (China)
Parafilm	Parafilm –m (USA)
Plane tube	AFCO (Jordin)
Refrigerator	hitachi (Japan)
Sensitive Balance	Sartorius (Germany)
Slides	China))
Sterile disposable syringe (5ml)	Medeco (UAE)
Sterile swabe	Medsor Impex (India)
Tips different size	Promega (USA)
Urine specimen cup	AFCO (Jordin)
Vitek2-compact	Biomerieux (France)
Vortex	Buchi (Germany)
Water Bath	Memmert (Germany)

3.1.2 Kits

Kits utilized for this research show in Table 3.2

Table 3.2 Kits used in this study

No.	Kits	Company	Origin
1	GN Vitek 2 compact kit	Biomerieux	France
2	GP Vitek 2 compact kit	Biomerieux	France
3	Interleukin -8 human ELISA kit	Fine test	Wuhan/ China
4	Interleukin -6 human ELISA kit	Demeditec	Germany
5	C-Reactive protein Kit	Lansionbio	china

3.1.3 Media of culture

Culture media used in this study show in Table 3.3

Table 3.3 Culture media used in this study

No.	Media	Company	Origin
1	Blood agar base	Himedia	India
2	MacConkey agar		
3	Mannitol salt agar	Himedia	India
4	Eosin methylene blue (EMB) agar	Oxoid	England
5	Urea agar	Himedia	India
6	Brain heart infusion broth	Himedia	India
7	Triple sugar iron agar	Himedia	India
8	Peptone water medium	Himedia	India
9	MR-VP broth	Oxoid	England
10	Simmon-Citrate agar	Oxoid	England
11	Nutrient broth	Himedia	India

3.1.4 Both chemical and biological component

Chemical substances used in this study show in Table 3.4

Table 3.4 Chemical substances which used in the current work

No	Materials	Company
1	Crystal violate	SYRBIO (Syria)
2	Safranine	SYRBIO (Syria)
3	Iodine	SYRBIO (Syria)
4	Ethanol	Shanghai chemical
5	Glycerol	Shanghai chemical
6	Methyl red reagent	Barcelona (Espania)
7	(Tetra methyl-P-Phenylene Diamine Dihydrochloride	BDH (England)
8	Potassium hydroxide KOH	BDH (England)
9	Alpha-nephthol	BDH (England)
10	Kovac's reagent	Barcelona (Espania)
11	Oxidase Reagent	bioMerieux (france)

3.2 Technique

3.2.1 Sample gathering (Urine) and serum

Between February 2022 and the beginning of May 2022, a total of 120 clinical specimens containing urines and blood were taken from individuals who had urinary tract infections. The women's ages ranged from 15-55 years, while the men's ages ranged from 18-50 years and were collected from private laboratories. Clean midstream urine (MSU) was taken from each patient and put into a 20 mL calibrated, sterile, screw-capped, universal container that was first given to the patients. (APPENDIX 7)

The sample was properly labeled and transferred to the laboratory, where it was tested one hour after it was collected. Prior to collecting the urine sample, all patients were given detailed instructions on how to Aseptically collect the sample to prevent contamination. All urine samples were promptly inoculated via sterile loop (blood agar, MacConkey agar) plate. All agar plates underwent a 24-hour aerobic incubation period at 37°C.

Blood samples were collected, and allowed to clot at room temperature for at least 10 to 15 minutes, The tube is then centrifuged, and the liquid (serum) is collected and stored for use in immunological research. (level of IL-6, IL-8, crp).

3.2.2 Ready to use media

Ready-made media including: Nutrient agar, Eosin methylene blue (EMB) agar, Brain heart infusion broth, Urea agar, Simmon-Citrate agar Peptone water medium, MR-VP broth, Triple Sugar Iron agar, were created in accordance with the manufacturing firms' instructions, which are often fixed on the media container. They were autoclaved at 121 °C for 15 minutes under 15 bar/in2 of pressure to sterilize them, and they were then incubated at 37 °C for 24 hours to guarantee their sterility before being stored at 4°C and utilized.

3.2.3 Blood agar

40 g of commercially available Blood agar base (HiMedia) was dissolved in 1000 mL of distilled water to make the medium. The dissolved media was autoclaved for 15 minutes at 15 pound/inch^2 at 121°C. Cool the autoclaved medium to 45-50°C before mixing in 5% of sterile broth and incubated blood and spreading into sterile Petri plates. (Figure 3.1)

3.2.4 MacConkey agar

The medium was made by dissolving 49.53 g of MacConkey agar (HiMedia) in 1000 mL of distilled water. For 15 minutes, the dissolved medium was autoclaved. While still molten, the autoclaved media was thoroughly mixed and placed onto petridishes (25-30mL/ plate). (Figure 3.2)

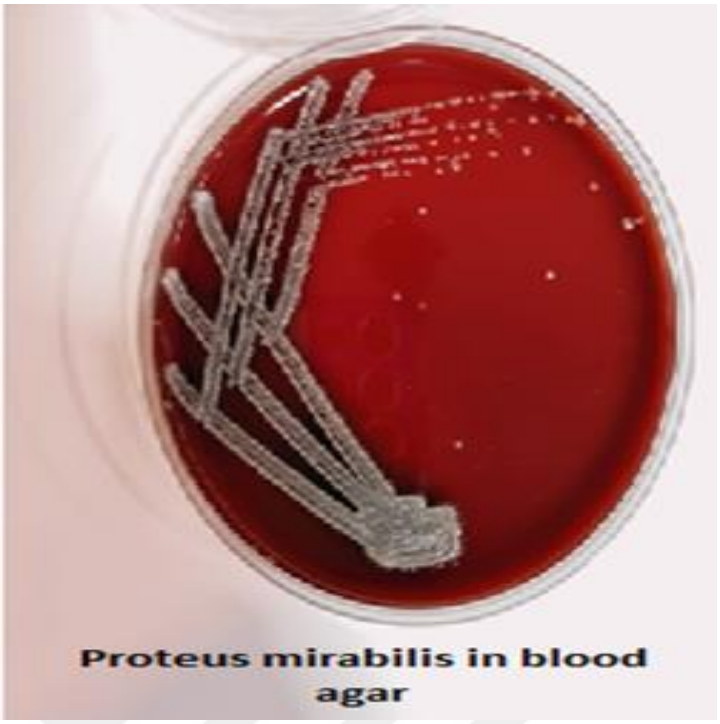

Figure 3.1 Bacteria growth in culture media 1

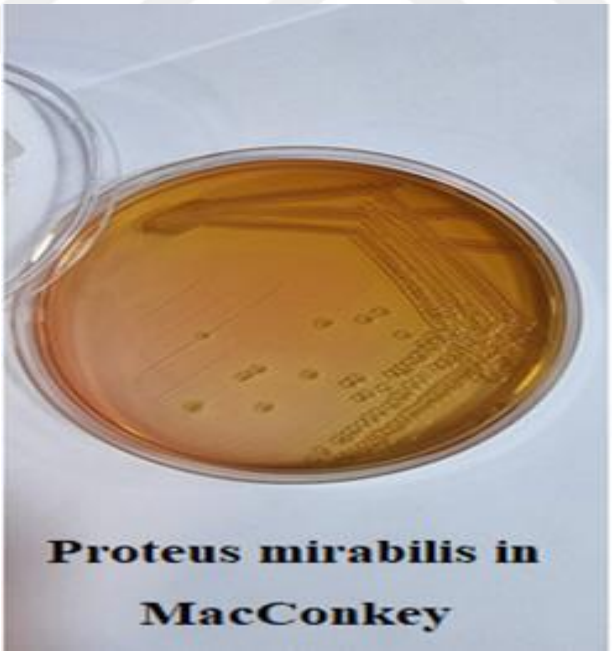

Figure 3.2 Bacteria growth in culture media 2

3.2.5 Maintenance of bacterial isolates

Temporary storage

A single colony of micro organism isolates was streaked on nutritional agar slants and cultured for 24 hours at 37°C, and they were kept in the refrigerator for one to three months at 4°C.

Long-term holding

Micro organism isolates were maintained for a long period in a media made by mixing 20 mL glycerol with 80 mL Brain heart broth, autoclaving for 15 minutes at 121°C, and allowing to cool at room temperature. The loop infected the broth with pure bacterial isolates, which were subsequently cultured at 37°C overnight. All isolates were maintained at a temperature of -18 ^{o}C.

3.2.6 Bacterial identification and separation

According to (Cheesebrough 1998), bacterial isolates are identified using automated vitek2 compact system and traditional microbiological techniques (colonial appearance, Gram staining, and biochemical assays). (APPENDIX 5, APPENDIX 6)

3.2.7 Examining the morphology

It was finished after the isolates were grown on blood agar and MacConky agar. The physical properties of bacterial development served as the basis for the earliest diagnostic tests. such as colony form, size, colors, odor, texture, edges, and lactose fermentation capabilities.

3.2.8 Microscopic examination

Gram stains

Cell morphology, such as form, size, and arrangement of cells, as well as their response to the dye, is a critical first step in identification. bacteria isolates are examined under an optical microscope, and fresh culture is used to make Gram stain smears (Leboffe and Pierce 2019)

Oxidase test

A pice of filter paper was moistened with some a drop of the oxidase reagent N,N,N,N-(Tetra methyl-P-Phenylene Diamine Dihydrochloride), and a single colony from a 24-hour culture was taken up with a stick and placed over the moistened filter paper. It takes between five and ten seconds for a violet hue to appear, signaling a successful outcome.

Catalase test

The purpose of this test was to see if bacteria could manufacture the catalase enzyme, through which water and safe oxygen are produced from hydrogen peroxide. After a tiny amount of growth from pure culture was applied to a microscope slide with a wooden stick, a drops of 3 percent hydrogen peroxide were added. The presence of gas bubbles indicates the presence of catalase.

Urease production test

After streaking with the sterile loop with the tested bacteria, the urea agar slant was inoculated and incubated at 37°C for 24 hours. Pink color represents a positive result for the test, whereas yellow color shows a negative result. This test was used to find out if P.mirabilis had the ability to analyze urea and produce ammonia and carbon dioxide.

IMViC test

This test was used to confirm the Gram negative identity and to diagnose Gram-negative bacteria. This test has four tests, which are as follows:

Indole Test

Each probable isolate was infected with a 24 hour culture of tryptophan broth and incubated at 37°C for 24 hours. If the culture generates tryptophanase, Kovac's reagent (0.5 mL) was applied immediately to the culture tube, and an indole ring appeared at the top of the broth.

Methyl Red Test

A 24-hour culture in MR-VP broth was injected into each possible isolate, which was then incubated for 24 hours at 37°C. Five drops of the methyl red reagent were added, the mixture was agitated, and the result was immediately read. A vivid red colour signals a positive response.

Voges-Proskauer Test

A 24-hour culture in MR-VP broth was injected into each possible isolate, which was then incubated for 24 hours at 37 °C. Three milliliters of a 5 percent naphthol solution and one milliliter of a 40% KOH solution were added. A positive response was indicated by the pink to crimson color that appeared within 2 to 5 minutes.

Simmon's Citrate Test

Each putative isolate was inoculated with a 24 hour culture on the Simmons citrate slant and incubated in 37°C for 24 hours. Bacterial growth on this medium generates a rise in

pH, which causes the pH indicator to change from green to blue, indicating a positive reaction.

TSI (Triple sugar- iron agar) test

The gram negative enteric bacilli are separated using triple sugar iron agar (TSI Agar) in order to prevent hydrogen sulfide production and carbohydrate fermentation. Dextrose, lactose, and sucrose are the three sugars used in TSI Agar. Phenol red is used to identify carbohydrate fermentation, while ferrous ammonium sulfate is used to identify hydrogen sulfide production (indicated by blackening). Gas generation and a switch in the pH marker's color from red to yellow indicate the fermentation of carbohydrates.

A considerable amount of acid turns both butt and slant yellow when 1.0 percent lactose/1.0 percent sucrose is added, showing the culture's ability to ferment either lactose or sucrose. H_2S production is indicated by the presence of ferrous sulfate. Indicators of acidification include phenol red (It is yellow in acidic condition and red under alkaline conditions).

Peptone, a source of nitrogen, is also present. For the TSI test, a sterile straight needle was used to take up a single colony of the testing bacterium and puncture through the medium to the tube's bottom. The surface of the agar slant was then streaked with the TSI that contained dextrose, lactose, and sucrose. The tube cap was removed after 24 hours and incubated at 37°C overnight to produce CO_2, H_2S, and carbohydrate fermentation.

1. **Confirmatory diagnosis using gram negative(GN I)**

The Gram Negative card is used to automate the identification of 135 taxa of Gram-negative bacilli that are both fermenting and non-fermenting. The GN card is based on newly developed substrates for measuring carbon source consumption, antibiotic resistance, and enzyme activity in addition to well-established biochemical techniques. (Rau *et al.* 2002). There are 43 biochemical assays in total, with one negative control In

about 10 hours or less, you'll get your final identification findings. The 64 wells on the reagent cards may each store a different substrate. Substrates measure a variety of metabolic processes such acidification, enzyme hydrolysis, alkalinization and growth in the presence of inhibiting substances (Figure 3.3).

W	Test	Mnemonic	Amount/Well
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	0.0384 mg
3	ADONITOL	ADO	0.1875 mg
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	0.018 mg
5	L-ARABITOL	IARL	0.3 mg
7	D-CELLOBIOSE	dCEL	0.3 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
10	H2S PRODUCTION	H2S	0.0024 mg
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	0.0408 mg
12	Glutamyl Arylamidase pNA	AGLTp	0.0324 mg
13	D-GLUCOSE	dGLU	0.3 mg
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT	0.0228 mg
15	FERMENTATION/ GLUCOSE	OFF	0.45 mg
17	BETA-GLUCOSIDASE	BGLU	0.036 mg
18	D-MALTOSE	dMAL	0.3 mg
19	D-MANNITOL	dMAN	0.1875 mg
20	D-MANNOSE	dMNE	0.3 mg
21	BETA-XYLOSIDASE	BXYL	0.0324 mg
22	BETA-Alanine arylamidase pNA	BAIap	0.0174 mg
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg
26	LIPASE	LIP	0.0192 mg
27	PALATINOSE	PLE	0.3 mg
29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
31	UREASE	URE	0.15 mg
32	D-SORBITOL	dSOR	0.1875 mg
33	SACCHAROSE/SUCROSE	SAC	0.3 mg
34	D-TAGATOSE	dTAG	0.3 mg
35	D-TREHALOSE	dTRE	0.3 mg
36	CITRATE (SODIUM)	CIT	0.054 mg
37	MALONATE	MNT	0.15 mg
39	5-KETO-D-GLUCONATE	5KG	0.3 mg
40	L-LACTATE alkalinization	ILATk	0.15 mg
41	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
42	SUCCINATE alkalinization	SUCT	0.15 mg
43	Beta-N-ACETYL-	NAGA	0.0306 mg
44	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
45	PHOSPHATASE	PHOS	0.0504 mg
46	Glycine ARYLAMIDASE	GlyA	0.012 mg
47	ORNITHINE DECARBOXYLASE	ODC	0.3 mg
48	LYSINE DECARBOXYLASE	LDC	0.15 mg
52	DECARBOXYLASE BASE	ODEC	N/A
53	L-HISTIDINE assimilation	IHISa	0.087 mg
56	COUMARATE	CMT	0.126 mg
57	BETA-GLUCURONIDASE	BGUR	0.0378 mg
58	O/129 RESISTANCE (comp.vibrio.)	O129R	0.0105 mg
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	0.0576 mg
61	L-MALATE assimilation	IMLTa	0.042 mg
62	ELLMAN	ELLM	0.03 mg

Figure 3.3 The well, test, Mnemonic, and amount /well, There are 43 biochemical assays in total, with one negative control

2. Confirmatory diagnosis using gram positive (GP I)

This card has the card (64) wells that can each contain a separate test substrate and is designed for the quick identification of non-sporeforming Gram-positive bacteria (mainly cocci), containing (47) biochemical tests (Figure 3.4).

Well	Test	Mnemonic	Amount/Well
2	D-AMYGDALIN	AMY	0.1875 mg
4	PHOSPHATIDYLINOSITOL PHOSPHOLIPASE C	PIPLC	0.015 mg
5	D-XYLOSE	dXYL	0.3 mg
8	ARGININE DIHYDROLASE 1	ADH1	0.111 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
11	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
13	Ala-Phe-Pro ARYLAMIDASE	APPA	0.0384 mg
14	CYCLODEXTRIN	CDEX	0.3 mg
15	L-Aspartate ARYLAMIDASE	AspA	0.024 mg
16	BETA GALACTOPYRANOSIDASE	BGAR	0.00204 mg
17	ALPHA-MANNOSIDASE	AMAN	0.036 mg
19	PHOSPHATASE	PHOS	0.0504 mg
20	Leucine ARYLAMIDASE	LeuA	0.0234 mg
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg
24	BETA GLUCURONIDASE	BGURr	0.0018 mg
25	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
26	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	0.018 mg
27	BETA-GLUCURONIDASE	BGUR	0.0378 mg
28	Alanine ARYLAMIDASE	AlaA	0.0216 mg
29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
30	D-SORBITOL	dSOR	0.1875 mg
31	UREASE	URE	0.15 mg
32	POLYMIXIN B RESISTANCE	POLYB	0.00093 mg
37	D-GALACTOSE	dGAL	0.3 mg
38	D-RIBOSE	dRIB	0.3 mg
39	L-LACTATE alkalinization	ILATk	0.15 mg
42	LACTOSE	LAC	0.96 mg
44	N-ACETYL-D-GLUCOSAMINE	NAG	0.3 mg
45	D-MALTOSE	dMAL	0.3 mg
46	BACITRACIN RESISTANCE	BACI	0.0006 mg
47	NOVOBIOCIN RESISTANCE	NOVO	0.000075 mg
50	GROWTH IN 6.5% NaCl	NC6.5	1.68 mg
52	D-MANNITOL	dMAN	0.1875 mg
53	D-MANNOSE	dMNE	0.3 mg
54	METHYL-B-D-GLUCOPYRANOSIDE	MBdG	0.3 mg
56	PULLULAN	PUL	0.3 mg
57	D-RAFFINOSE	dRAF	0.3 mg
58	O/129 RESISTANCE (comp.vibrio.)	O129R	0.0084 mg
59	SALICIN	SAL	0.3 mg
60	SACCHAROSE/SUCROSE	SAC	0.3 mg
62	D-TREHALOSE	dTRE	0.3 mg
63	ARGININE DIHYDROLASE 2	ADH2s	0.27 mg
64	OPTOCHIN RESISTANCE	OPTO	0.000399 mg

Figure 3.4 The well, test, Mnemonic, and amount /well, confirmatory diagnosis using gram positive (GP I) Card

This procedure of identification was carried out as follows

1. Place the test tubes on the tube carrying base of the device, where test tubes are assigned to each bacterial sample.
2. Adding the working saline solution with a concentration of 0.45 % NaCl and a volume of 3 mL to each tube.
3. Adding a bacterial colony to the tube of the first sample and reading the concentration of the bacterial suspension so that it is 0.5 on the McFarland scale, and so on for the rest of the samples.
4. Adding the sample type diagnostic cassette so that the diagnostic cassette is placed in the site of the tube with bacterial suspension
5. The tube carrying base is inserted into the device and we start loading the samples, then the data for each sample is entered into the device system and waiting for the work to be completed after 12-14 hours. (Table 3.5)

Table 3.5 Turbidity meter used for card inoculation

No.	Product	McFarland Standard Range
1.	GN	0.50 – 0.63 cell/ mL
2.	GP	0.50 – 0.63 cell/ mL

Inoculation

Using an integrated vacuum system, identification cards were injected with bacteria suspensions. While inserting the transfer tube into the relevant suspension tube, The identification card is inserted into the surrounding slot of a specific rack (cassette) carrying a test tube containing the microorganism suspension. Every 15 minutes, a card gets taken out of the incubator carousel (Figure 3.5).

The device works during the incubation period on the subjective analysis and storage of biochemical patterns, and after a period of incubation, the software device analyzes these

patterns and prints a diagnostic report for each card that exists within the Reader/ Incubator, as instructed by Biomerieux (Figure 3.6).

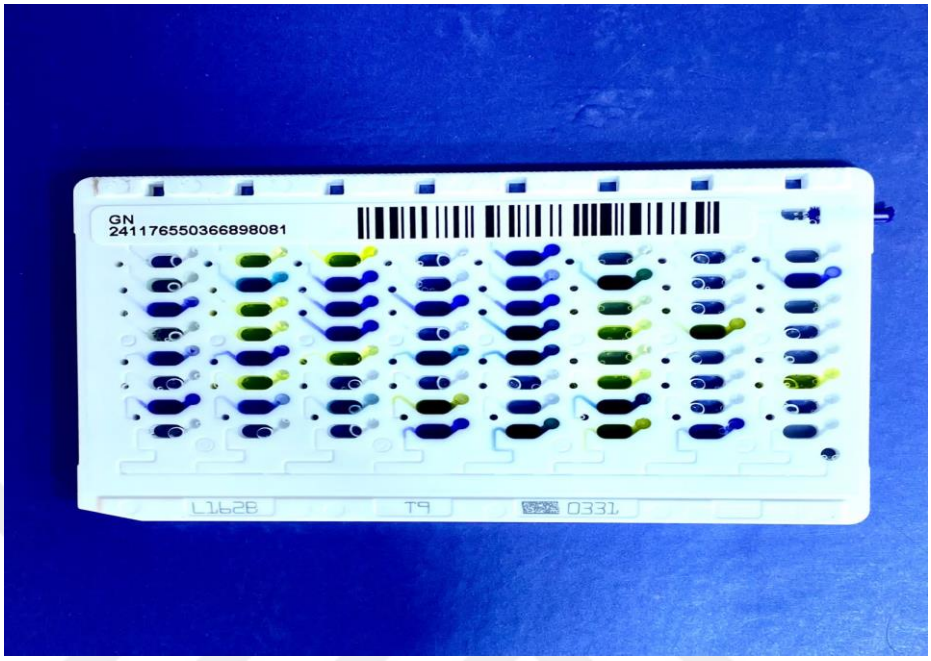

Figure 3.5 For Gram-negative bacilli that are clinically significant, VITEK® 2 ID cards deliver trustworthy, accurate findings

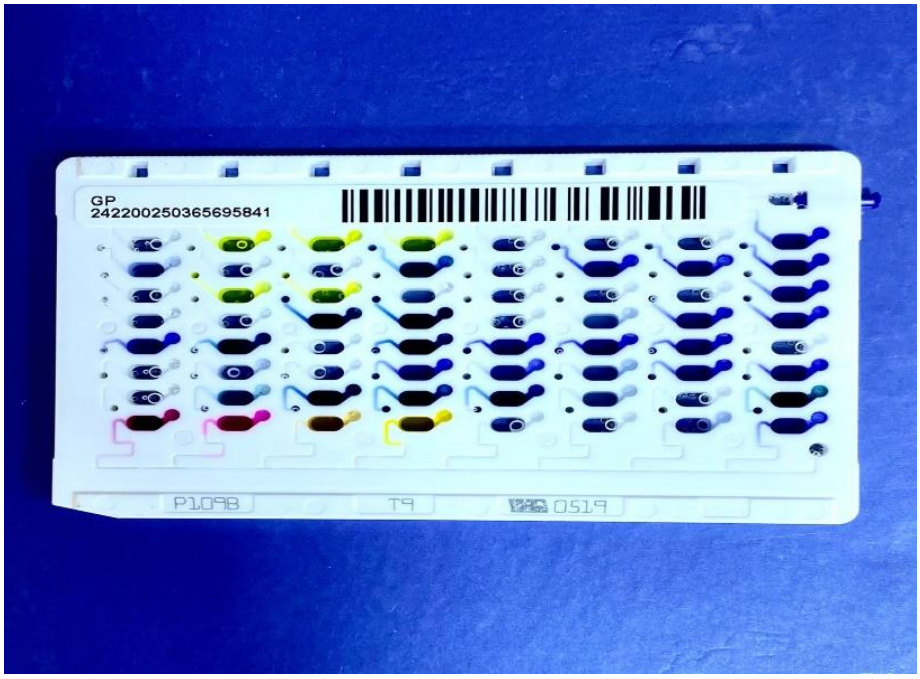

Figure 3.6 For clinically significant Gram-positive cocci, VITEK® 2 ID cards deliver trustworthy, accurate findings. Human IL-**8** (Interleukin **8**) ELISA Kit

(APPENDIX 10, APPENDIX 11, APPENDIX 12, APPENDIX 13, APPENDIX 14, APPENDIX 15, APPENDIX 16)

3.2.9 Human IL-8 (Interleukin 8) ELISA kit

Application

The in vitro quantitative measurement of IL-8 concentrations in serum, plasma, and other biological fluids is made possible by IL-8 ELISA Kit. (APPENDIX 1, APPENDIX 2, APPENDIX 4)

Guidelines for the approach

This product was made using a sandwich enzyme-linked immune-sorbent assay technology. The capture antibody was pre-coated onto 96-well plates. Biotin conjugated Ab were used for antibody detection. Following the addition of the standards, test samples, and biotin-conjugated detection antibody, the wells were rinsed with wash buffer. After HRP-Streptavidin was added, unbound conjugates were removed using wash buffer.

Utilizing TMB substrates, the HRP enzymatic process was observed. HRP accelerated the conversion of TMB to a blue product that became yellow when it was exposed to an stop solution. Calculate the target concentration after reading the O.D. absorbance at 450 nm using a microplate reader.

Preparation and storage of reagents

Before usage, give all samples and reagents 20 minutes to reach room temperature. Clean Buffer If crystals start to form in the concentrate, rewarm it in a water bath at 40°C while gently stirring it to completely dissolve the crystals. bring the solution to room temperature. To make 750 mL of wash buffer, dilute 30 mL of concentrated wash buffer

with distilled water or deionized. Return any unused solution to a temperature of 2-8°C. Standards: Add 1 mL of Sample Dilution Buffer to one Standard tube (designated as tube zero), let stand at room temperature for 10 minutes, and mix well. Labeling should include 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and blank for 7 EP tubes. Fill each tube with 0.3mL of Sample Dilution Buffer. Fill the 1st tube with 0.3 mL of the aforementioned solution Standard (from the zero tube) and thoroughly mix together. 0.3 mL from the first tube should be transferred to the second tube, and they should be properly mixed. After fully mixing, add 0.3 mL from the second tube to the third tube. Sample Dilution Buffer was used to complete the blank contro. (Biotin-labeled Antibody Preparation) Working solution: Set up the experiment an hour beforehand. Antibody Dilution Buffer and the Biotin-detection Antibody should be combined thoroughly at a 1:100 ratio. Prepare the HRP-Streptavidin Conjugate (SABC) within 30 minutes of commencing the experiment. SABC and SABC Dilution Buffer should be combined 1:100.

Assay Procedure

Reagents and Samples must be well mixed and distributed evenly before being diluted. TMB Substrate should be equilibrated at 37°C for 30 minutes before being added to the wells. A standard curve should be drawn for each test.

1. Place the standard and samples (at halve diluted with Sample Dilution Buffer), and control wells on the plate, and record their positions. Before inserting the sample, standard, and control – blank wells, wash two time.
2. Prepare Standards: add 100 μL of each of the following: zero, first, second, third, fourth, fifth, sixth (tube) and Dilution Buffer (blank) to standard wells.
3. Add Samples: fill the test sample wells with 100 μL of the correctly diluted sample.
4. 90 minutes at 37°C should be allotted for incubation once the plate is covered and sealed.
5. Wash: loss off the contents of the plate, then wash the plate twice with Wash Buffer

6. Biotin - Antibody: Fill each well with 100 µL of biotin- antibody solution working. Add the solution to each well without touch the sidewall, cover the plate, and incubate at 37°C for 60 minutes.
7. Wash: Remove the cover and use Wash Buffer to wash the plate three times, letting the Wash Buffer stay in the wells for a few seconds to a minute each time.
8. HRP-Streptavidin Conjugate (SABC): Spoon 100 µL of SABC Working Solution into each well, and incubate at 37°C for 30 minutes.
9. Wash: wash plate five times with Wash Buffer, and let each time the wash in the wells for 1-2 minutes
10. TMB: put 90 uL Substrate into each well, Within 10–20 minutes, cover the wells plate and incubate at 37°C in the dark.
11. Stop: put 50 µL Stop Solution into wells. The color will turn yellow immediately.
12. OD Measurement: After adding the stop solution, read the absorbance at 450 nm in the microplate reader right away. (Figure 3.7)

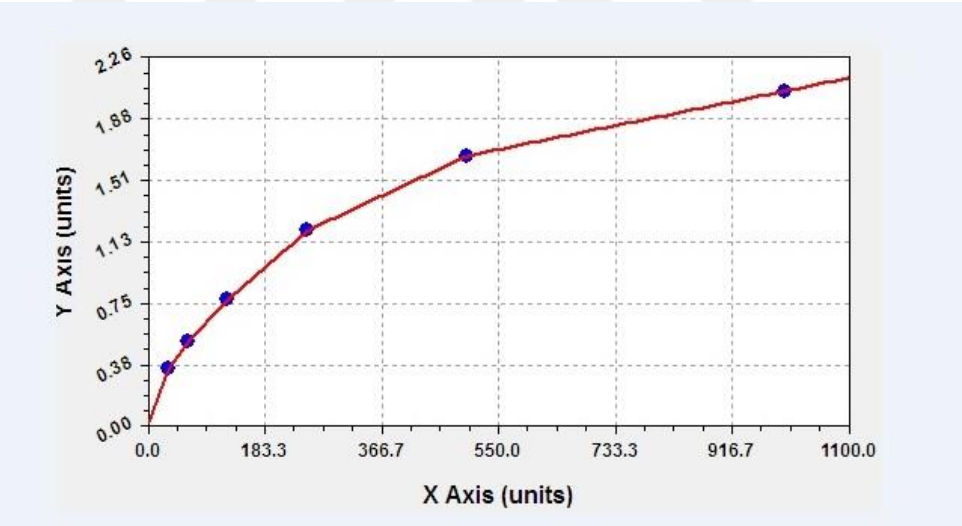

Figure 3.7 Standard curve of estimation Interleukins (IL8)

3.2.10 Human IL-6 (Interleukin 6) ELISA kit

Guidelines for the approach

The IL-6 Demeditec ELISA is a microtiterplate-based Enzyme Amplified Sensitivity Immunoassay in solid phase. The test utilizes monoclonal antibodies (MAbs) made against several IL6 epitopes. Samples and Calibrators deal with the capture monoclonal Ab (MAb 1) coated on well and with a monoclonal Ab (MAb 2) labelled with horseradish peroxidase (HRP). when an Sandwich creation is enabled by the incubation time. To get rid of enzyme-labelled antibody that isn't bound, the microtiterplate is washed. Enzyme-bound labels Through a chromogenic reaction, antibody is measured.

A chromogenic reaction is used to detect bound enzyme-labeled antibody. TMB is added to the chromogenic solution and incubated. The reaction is stopped by adding Stop Solution. using an absorbance measurement. Interpolation from the calibration curve is used to determine the amount of IL-6 in samples. (APPENDIX 1, APPENDIX 2, APPENDIX 3)

Reagent preparation

- Calibrators: prepare the calibrators with 1 mL distilled water.
- Controls: prepare the controls with 1 mL distilled water.
- Specimen Diluent: prepare liquid Diluent to the volume specified.
- Working Wash solution: Prepare an volume of Working Wash solution by adding 1 volume of Wash Solution (200x) to 199 volumes of distilled water

Procedure

- Estimate how many strips are required for the run.
- The strips should be put in the holding frame and fastened there.
- Fill each well with 50 µL of Incubation Buffer.
- Pipette 100 µL each of the Control, Calibrator and Sample into the wells.
- Incubate for 1 hour on a horizontal shaker set to 700 rpm and 100 rpm at room temperature (18 to 25°C).
- Remove it from each well.

- Wash the plate 3 times by:
- Put 0.4 mL of Wash Solution into each well
- removing the content of each well
- Pipette 100 µL of anti-IL- 6 HRP conjugate and 50 µL liquid diluent into the wells.
- Incubate for one hour on a horizontal shaker set to 700 rpm 100 rpm at room temperature (18 to 25°C).
- remove the liquid from each well.
- Wash the plate 3 times by:
- Put 0.4 mL of Wash Solution into each well
- Remove Aspirating the content of each well
- After the washing step, within 15 minutes, pipette 200 L of the chromogenic solution into each well.
- Place the microtiterplate on a shaker horizontal set to 700 rpm 100 rpm and incubate it for 15 minutes at temperature (18-25°C), avoiding direct sunshine.
- Pour 100 µL of Stop Solution into each well with a pipette.
- Read the absorbencies at 450 and 490 nm and calculate the results. (Figure 3.8)

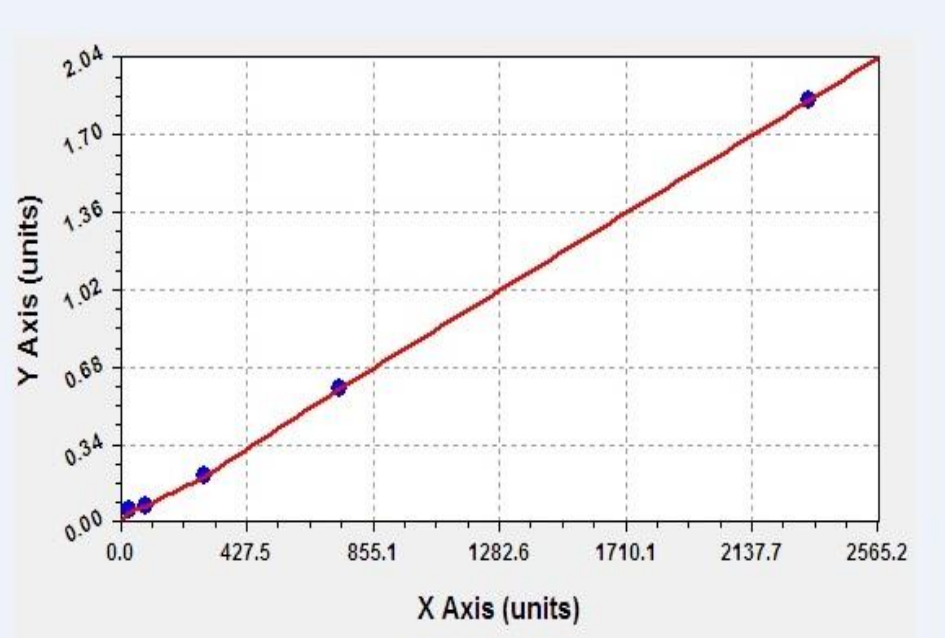

Figure 3.8 Standard curve of estimation interleukins (IL6)

3.2.11 C-Reactive protein

Test procedure

1. The sample and test strip should be brought to room temperature (15°C-30°C) before the test.
2. If necessary, perform QR code calibration.
3. Press the "Test" icon to enter the testing interface. When necessary, enter patient information, sample information, and doctor information.
4. Remove test strip from sealed pouch and put it on a clean table, horizontally placed
5. Transfer 5 L of material into one bottle of sample diluent with a pipette. Gently and completely combine. Then pour 100 L of combined fluid into the test strip's sample port.
6. Time to react: 3 minutes For the inside panel, immediately after dispensing the sample, place the test strip into the analyzer. Then select "Test." For the panel outside, put the test strip into the analyzer after the reaction time has elapsed of 3 minutes, and then select "Test."
7. The result will be shown on the screen and printed automatic. (APPENDIX 8, APPENDIX 9)

4 RESULTS AND DISCUSSION

4.1 Bacterial Profile in Patients

E. coli isolated from 34% of patients, *Streptococcus* fecalis isolated from 23% of patients, Klebsiella pneumoniae isolated from 14% of patients, *Staphylococcus saprophyticus* isolated from 12% of patients followed by proteus mirabilis 5%. *Staphylococcus auras* 7 %. *Pseudomonas aeruginosa* isolated from 5% of patients, these results are shown clearly in Figure 4.1

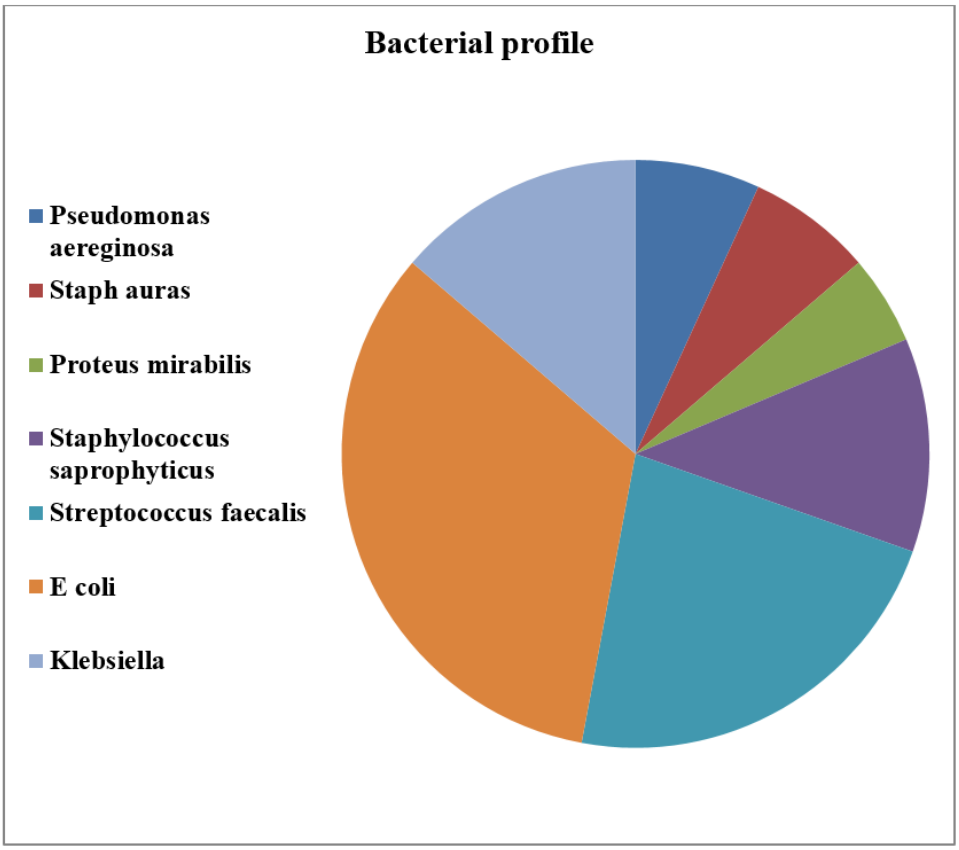

Figure 4.1 Bacterial profile in patients with UTI

Growth of *E. coli* and *S. aureus* accounted for the highist percentag of bacterial isolates as in many previous studies which chould be attributed to feces contamination (Faraj and Maarof 2017) *Uropathogenic E. coli* are distinct serotypes of extra intestinal *E. coli* that live as the normal flora of the GIT rarely cause problems in the intestine, but they can

cause serious illness if they go into the urine bladder, bloodstream, or wound, it accounts more than 1×105 per gram of stool (Nielubowicz and Mobley 2010). The results of this study agreed with dif-ferent studies that found that *Staphylococcus species* were the second bacterial pathogen isolated from urine samples (Eves 2017).

Other study, *Staphylococcus epidermidis* came in second as a bacterial pathogen (Lozano *et al*. 2015). *Klebsiella pneumonia* is the second most common bacteial infection the *K. pneumo-nia* was isolated from urine specimens, because these bacteria are a part of the intes-tinal flora similar to *E.coli*. The chance of UTI increases because it contains a protec-tive capsule which resists the immune defences especially phagocytosis. Another re-cent study in Baghdad city showed that the *K. pneumonia* was one of the isolated bacteria from urine (Mahdi *et al*. 2020) shown clearly in Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6 and Figure 4.7

Figure 4.2 Bacteria growth in culture media 3

Figure 4.3 Bacteria growth in culture media 4

Figure 4.4 Bacteria growth in culture media 5

Figure 4.5 Bacteria growth in culture media 6

Figure 4.6 Bacteria growth in culture media 7

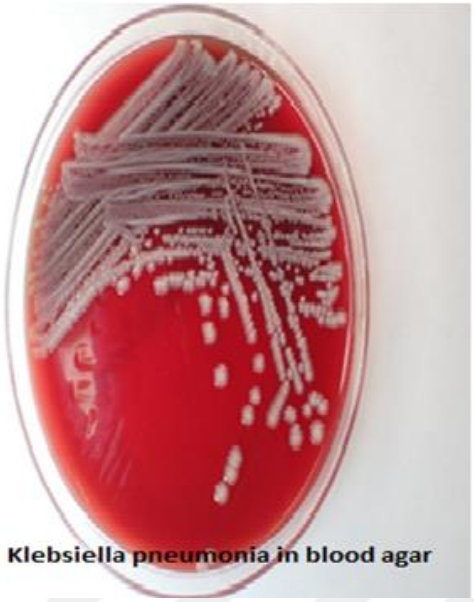

Figure 4.7 Bacteria growth in culture media 8

4.2 Levels of IL-6, IL-8, CRP and Assocition with UTI Patients

4.2.1 Level (IL-6)

Table 4.1 Mean of IL 6 in controls and UTI patients

IL-6	NO.	Mean ±SD	P value
Controls	20	23.11 ± 10	0.000000095
Patients	100	413.7 ± 467	

The mean value of IL-6 levels in patients sera was significant differences according to different bacterial pathogens. A significant differences between mean levels of IL-6 was observed between patients and control as shown in Table 4.1

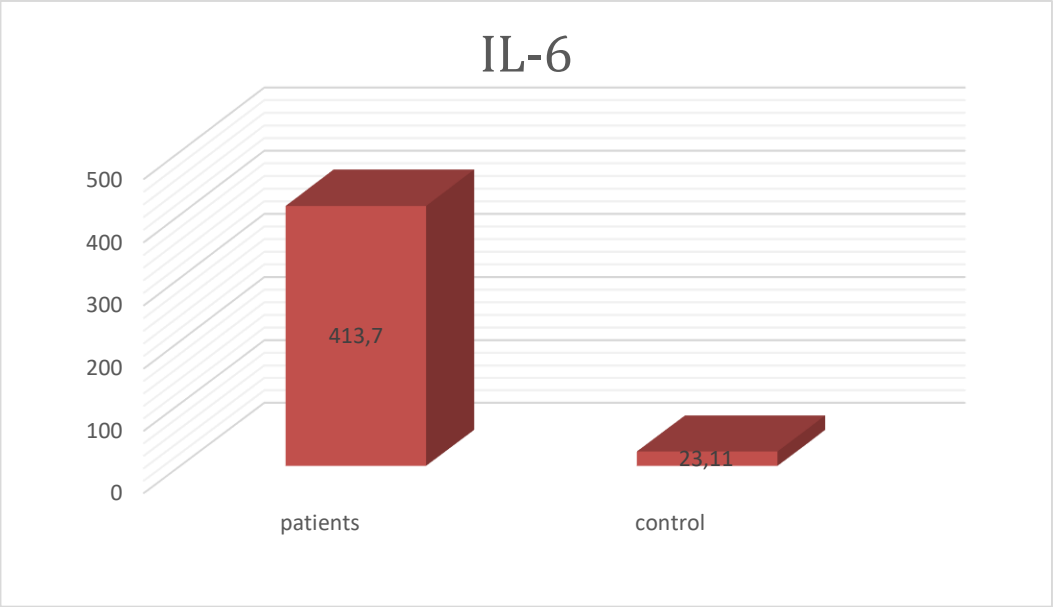

Figure 4.8 Level of IL-6 in patients and control

Given the importance of IL-6 in the response to *E. coli* infection and the fact that *E. coli* is the most common cause of UTI, an positive link between the severity of UPEC infection and IL-6 secretion can be predaction (Figure 4.8). The acute phase response, inflammation, and organ development are all regulated by IL-6, a multifunctional cytokine with proinflammatory and immunological regulatory roles, Interleukin 6 is a proinflammatory cytokine whose serum levels rise in the early stages of infection response (Darogha *et al*. 2021).

Interluken1 causes polymorphonuclear (PMN) neutrophils and macrophages to secrete IL-6, which then interacts with B-lymphocytes to produce antibodies.

Interleukin 6 is an inflammatory cytokine that regulates the immune system and plays a function in the acute phase response. Bacteria, for example, boosts IL-6 synthesis (Sundvall *et al*. 2014).

This result agree with other study (Watson *et al.* 2012). Despite the existence of continual bacterial colonization, there appeared to be no continuous IL-6 secretion.

This showed that a threshold concentration of >105 germs per milliliter is needed to completely trigger IL-6 release in the human urinary system. Given that >105 germs per mL is the threshold used to characterize serious bacteriuria, this quantitative relationship connection was rather noteworthy. The physiological justification for the medical definition of severe bacteriuria at this level may come from threshold IL-6 release at this level of bacterial concentration. according to search (Hedges *et al*. 1991).

The signaling pathways and transcriptional targets activated by IL-6 have not been evaluated during UTI. Interleukin 6 levels increase with UTI severity, but the specific contributions of IL-6 to host immunity against bacterial uropathogens are unknown, in comparison to an ABU, a symptomatic lower UTI is thought to be associated with more severe bladder inflammation. Interluken 6 levels in the urine can help distinguish between ABU and UTI in the patients (Nanda and Juthani-Mehta 2009).

The IL-6 receptor, to which IL-6 binds directly, is expressed by macrophages, neutrophils, T-helper cells, hepatocytes and podocytes. There is also a soluble IL-6 receptor, which, due to its ubiquitous expression, allows IL-6 to modulate a broad spectrum of target cells (Al Rushood and Amal 2020).

4.2.2 Interleukin 8 (IL-8)

The mean value of IL-6 levels in patients sera was significant differences according to different bacterial pathogens (Table 4.2).

Table 4.2 Mean of IL 8 in Controls and UTI patients

IL-8	NO.	Mean ± SD	P value
Controls	20	11.1 ± 5.6	0.0002
Patients	100	160.2 ± 251.4	

A significant differences between mean levels of IL-6 was observed between patients and control as shown in Figure 4.9

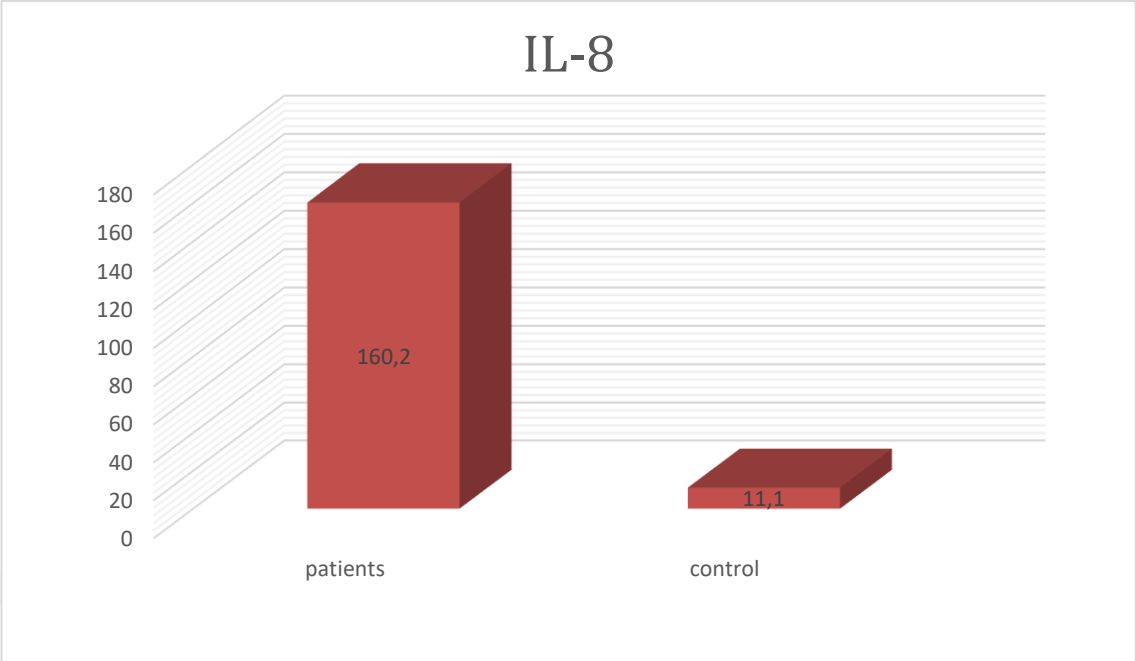

Figure 4.9 Level of IL-8 in patients and control

Interlukine 8 is involved in every inflamatory process, many studies shown that IL-8 can response systemicaly and localy to gram-negative bacteria and LPS. It is found on mucosal surface during bacterial infection and is thought the operate as a neutrophil chemoattractant (Schlievert *et al*. 2022).

An increase in IL-8 levels in the blood of a human volunteer during intravenous endotoxin or *E. coli* injection, respectively, indicates that IL-8 is involved in infection. As a result, it's fair to believe that IL-8 may play a role in UTI (Ko *et al*. 1993).

Interleukin 8 is involved in every inflammatory process. Its high concentration is seen in UTIs and can be used to predict acute pyelonephritis, but it has a limited specificity (Horváth *et al*. 2020). During gram-negative bacteremia after an intravenous infusion of E. coli, IL-8 was identified in the plasma of (Magnusson *et al*. 2019).

Interleukin 8 is found on mucosal surfaces during bacterial infection and is thought to operate as a neutrophil chemoattractant. Local synthesis of IL-8 and a fast influx of neutrophils into the urine are both stimulated by bacterial infections of the human urinary

system. The high connection between urine IL-8 levels and urinary neutrophil counts in individual patients suggested a function fo IL-8 neutrophil recruitment (Schlievert *et al*. 2022).

4.2.3 C-Reactive protein

There was asignificant difference between mean of CRP between patients and control as showen in Table 4.3 and was confirmed in Figure 4.10. The results showed that CRP is a non specific marker of etiology-related inflammation. (APPENDIX 9)

Table 4.3 Mean of CRP in controls and UTI patients

CRP	NO.	Mean ±SD	P value
Controls	20	10.7 ±3.9	0.000000000023
Patients	100	27.5 ±12.7	

Although high CRP levels are the most common in UTIs, CRP is a non specific marker of etiology-related inflammation. CRP is a sensitive acute-phase reactant. One of the most often used biomarkers in medical technology, it is a sign of inflammation, its fast synthesis in the liver after a tissue lesion reaches its peak (Shahkar *et al*. 2011). C-Reactive protein activates the complement system (Favalli 2020). (APPENDIX 8)

In contrast to CRP, PCT is raised by cytokines generated in response to bacterial infection and inhibited by interferon, which is released in viral infections, indicating a significant specificity for bacterial infections (Pfister 2014).

It's a sensitive systemic marker of inflammation and tissue damage, but it doesn't distinguish between infections (Oberhofer *et al.* 2012)

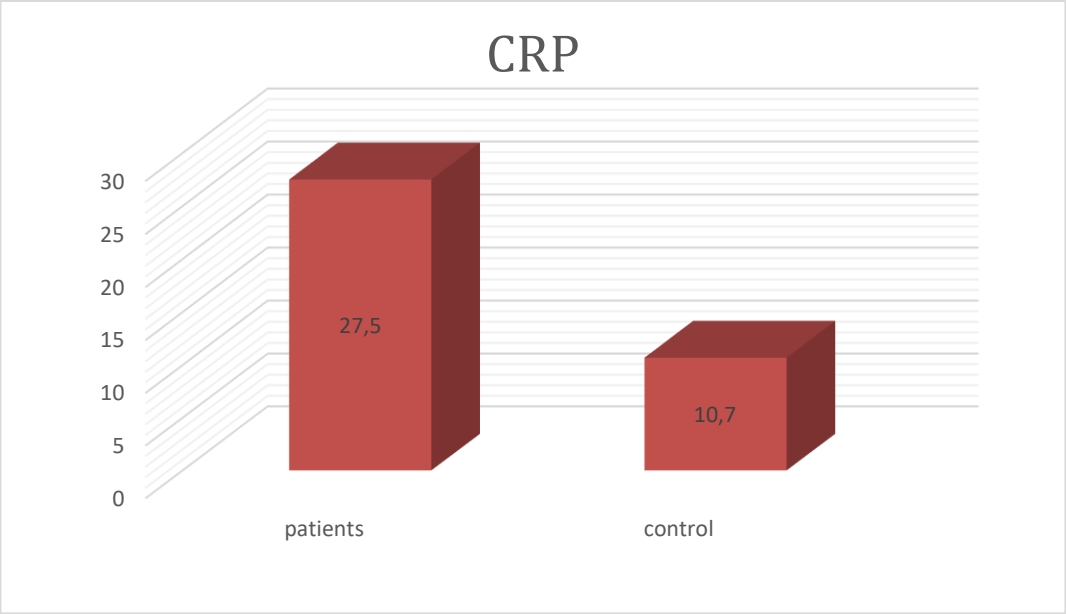

Figure 4.10 Mean level of CRP in controls and UTI patients

4.3 General Characteristics of the Study Populations Depending on Gender

The study showed no effect of sex and age on the high level of (IL-6 and IL-8) between males and females, as the study did not show a significant effect and that the immune response depends on the pathogen and the strength of the body's immune response against the same pathogen Table 4.4

Table 4.4 Mean of IL 6 and IL-8 in UTI patients males and females

IL-6	age	NO.	Mean ± SD	P value
Males	18-50	24	544.5 ± 652.9	0.19
Females	15-55	76	277.7 ± 362.1	
IL-8	age	NO.	Mean ± SD	P value
Males	18-50	24	183.3 ± 313.5	0.48
Females	15-55	76	115.3 ± 207.1	

5 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- The highest frequency of bacterial pathogen was for *E. coli* followed by *Streptococcus fecalis, Klebsiella pneumoniae* respectively.
- All tested biomarkers were higher in patients than control with high significant difference.
- The study showed no effect of gender on the high level of (IL-6 and IL-8) between males and females.

5.2 Recommendations

- Evaluation of other biomarkers in UTI patients to prediction of UTI and finding specific pathogens.
- Evalution of same biomarker in both serum and urine to compare between their levels.
- Assessment of biomarkers in a regular intervals to clarify the duration of biomarker's production
- Using moleculare methods in diagnosis of UTI pathogens to identify real pathogens in symptomatic patients that give no growth in culture.

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APPENDICES

APPENDIX 1. Enzyme-linked immunosorbent assay (ELISA) device for microplate Reader

APPENDIX 2. The tool has characteristics such as stability, quiet operation, suitable rotational speed, and compact design and is used for mixing sample testing

APPENDIX 3. Human interleukin-6 (IL-6) in serum is measured quantitatively in vitro using an immunoenzymetric assay

APPENDIX 4. Human interleukin-8 (IL-8) in serum is measured quantitatively in vitro using an immunoenzymetric assay.

APPENDIX 5. The VITEK 2 system offers rapid, precise microbiological identification and testing for antibiotic susceptibility

APPENDIX 6. Vitek kit for identification of (gram positive and negative) bacteria

APPENDIX 7. Laboratory centrifuges for separating blood samples

APPENDIX 8. CRP Kit (Dry Fluorescence Immunoassay) is used for in-vitro quantitative measurement of CRP in human serum, plasma and whole blood. This test is used for the detection and evaluation of infection, tissue injury and inflammation disorders

APPENDIX 9. Device from Lansion Biotechnology Co., Ltd. Use to measure c-reactive protein rate

APPENDIX 10. Microbiology Chart Report-1

APPENDIX 11. Microbiology Chart Report-2

APPENDIX 12. Microbiology Chart Report-3

APPENDIX 13. Microbiology Chart Report-4

APPENDIX 14. Microbiology Chart Report-5

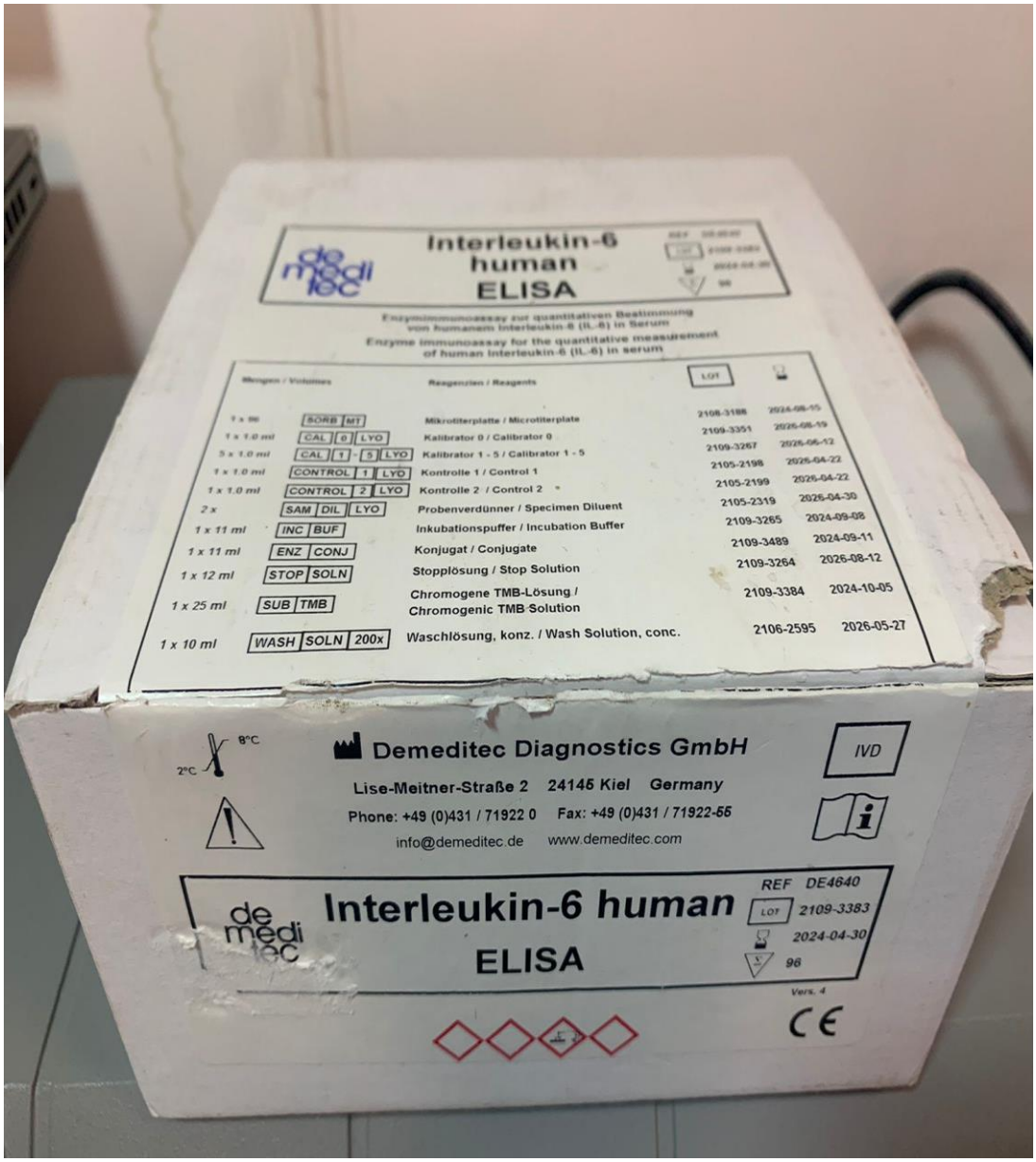
APPENDIX 15. Microbiology Chart Report-6

APPENDIX 16. Microbiology Chart Report-7

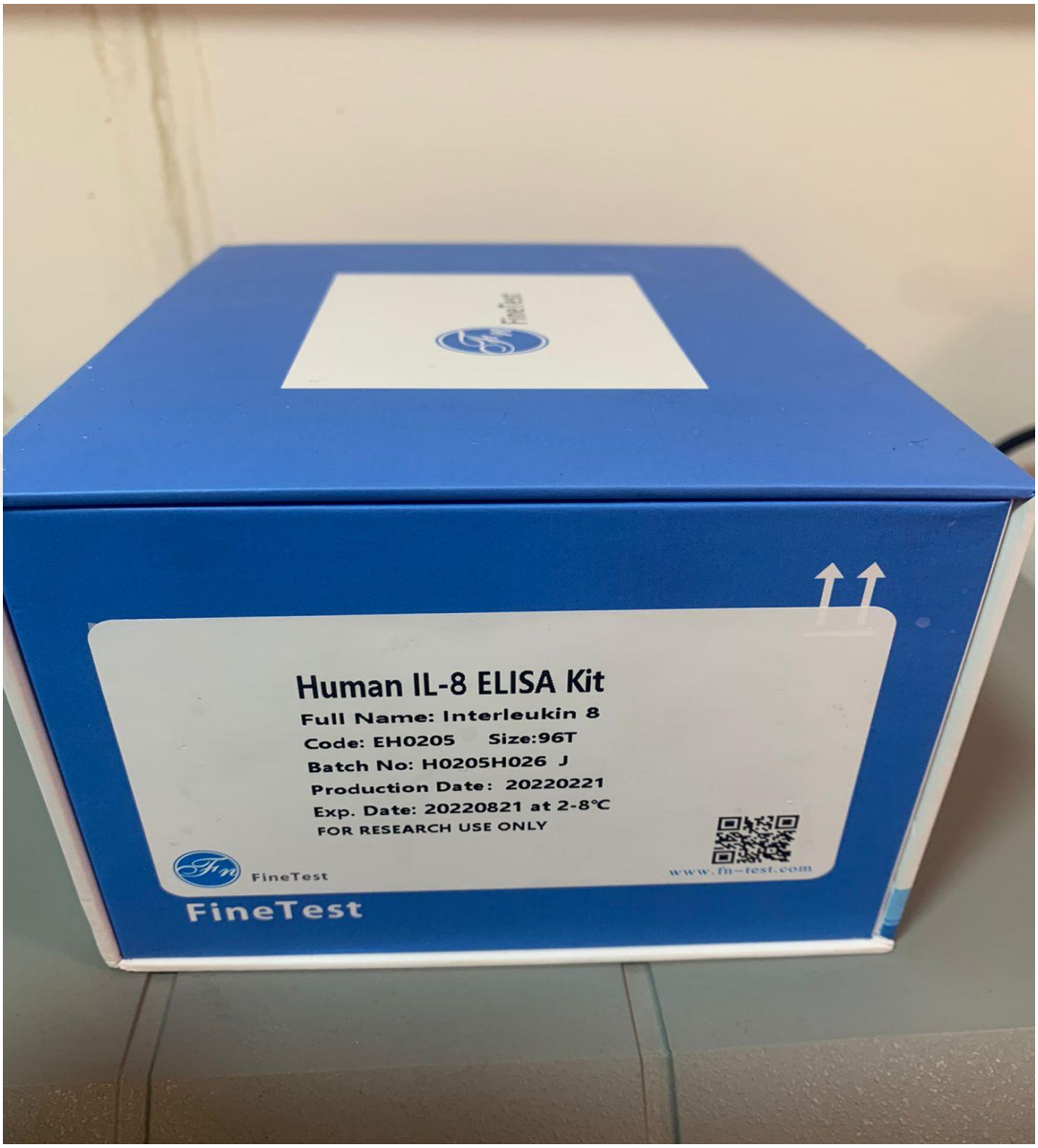
APPENDIX 1. Enzyme-linked immunosorbent assay (ELISA) device for microplate Reader

APPENDIX 2. The tool has characteristics such as stability, quiet operation, suitable rotational speed, and compact design and is used for mixing sample testing

APPENDIX 3. Human interleukin-6 (IL-6) in serum is measured quantitatively in vitro using an immunoenzymetric assay.



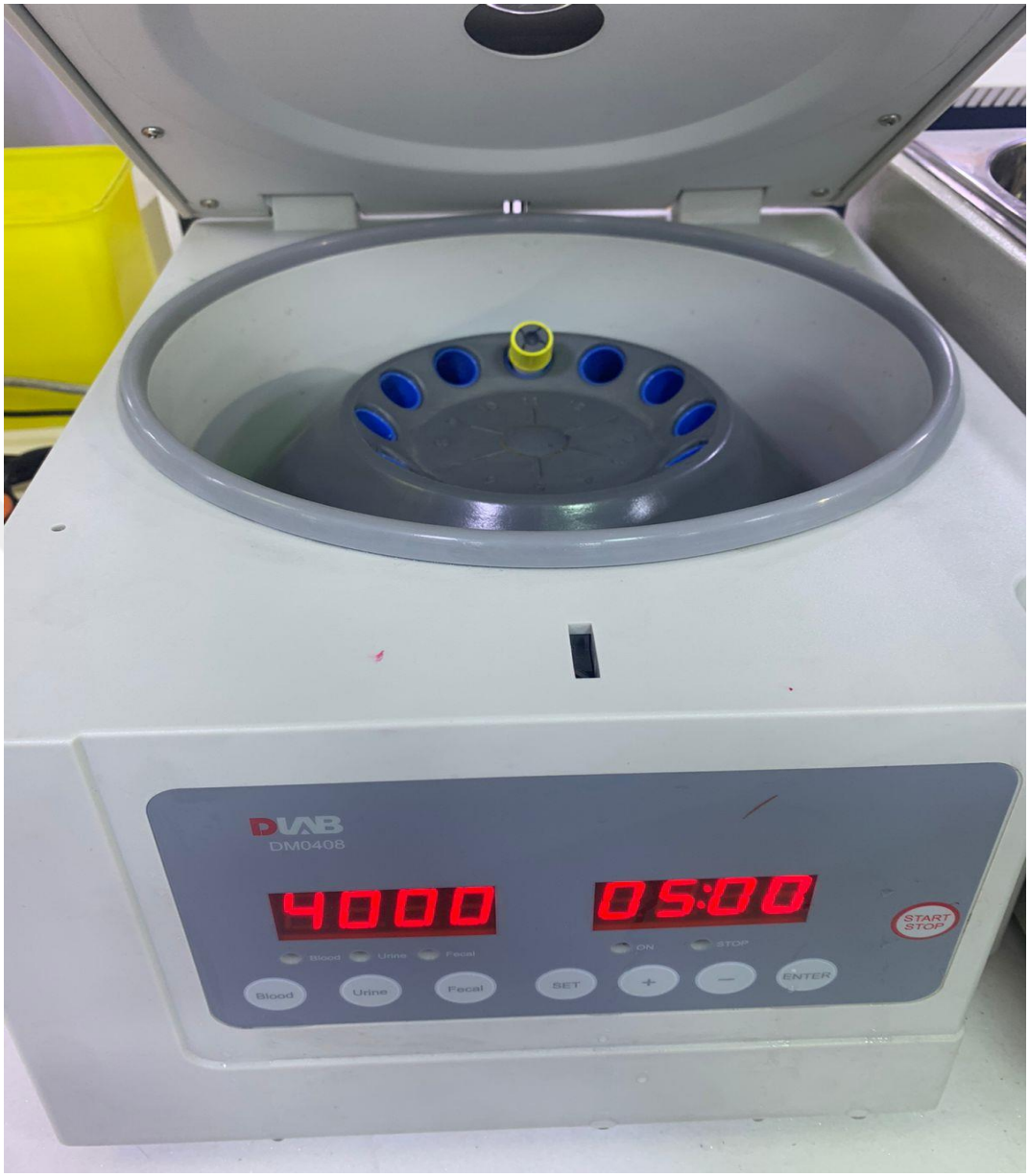
APPENDIX 4. Human interleukin-8 (IL-8) in serum is measured quantitatively in vitro using an immunoenzymetric assay.



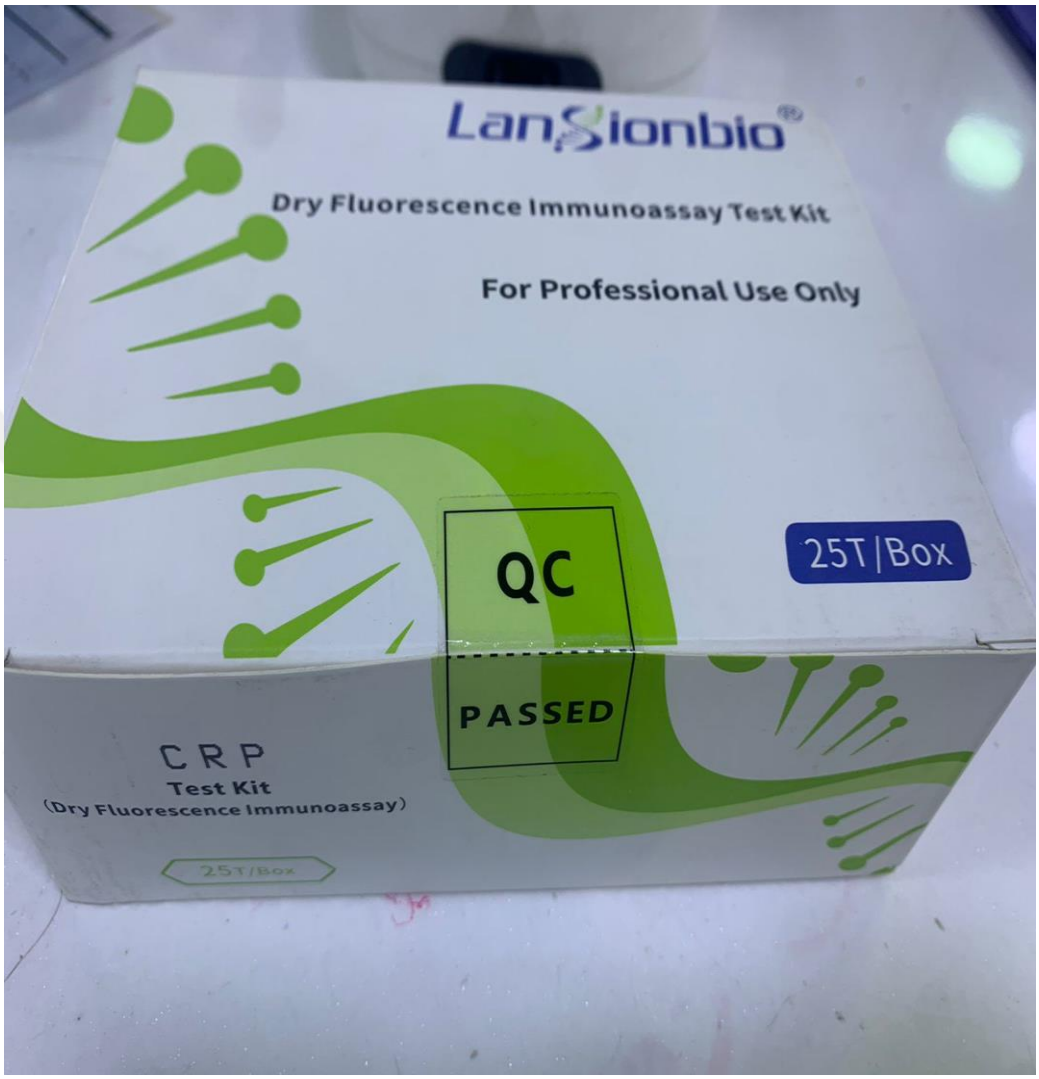
APPENDIX 5. The VITEK 2 system offers rapid, precise microbiological identification and testing for antibiotic susceptibility

APPENDIX 6. Vitek kit for identification of (gram positive and negative) bacteria

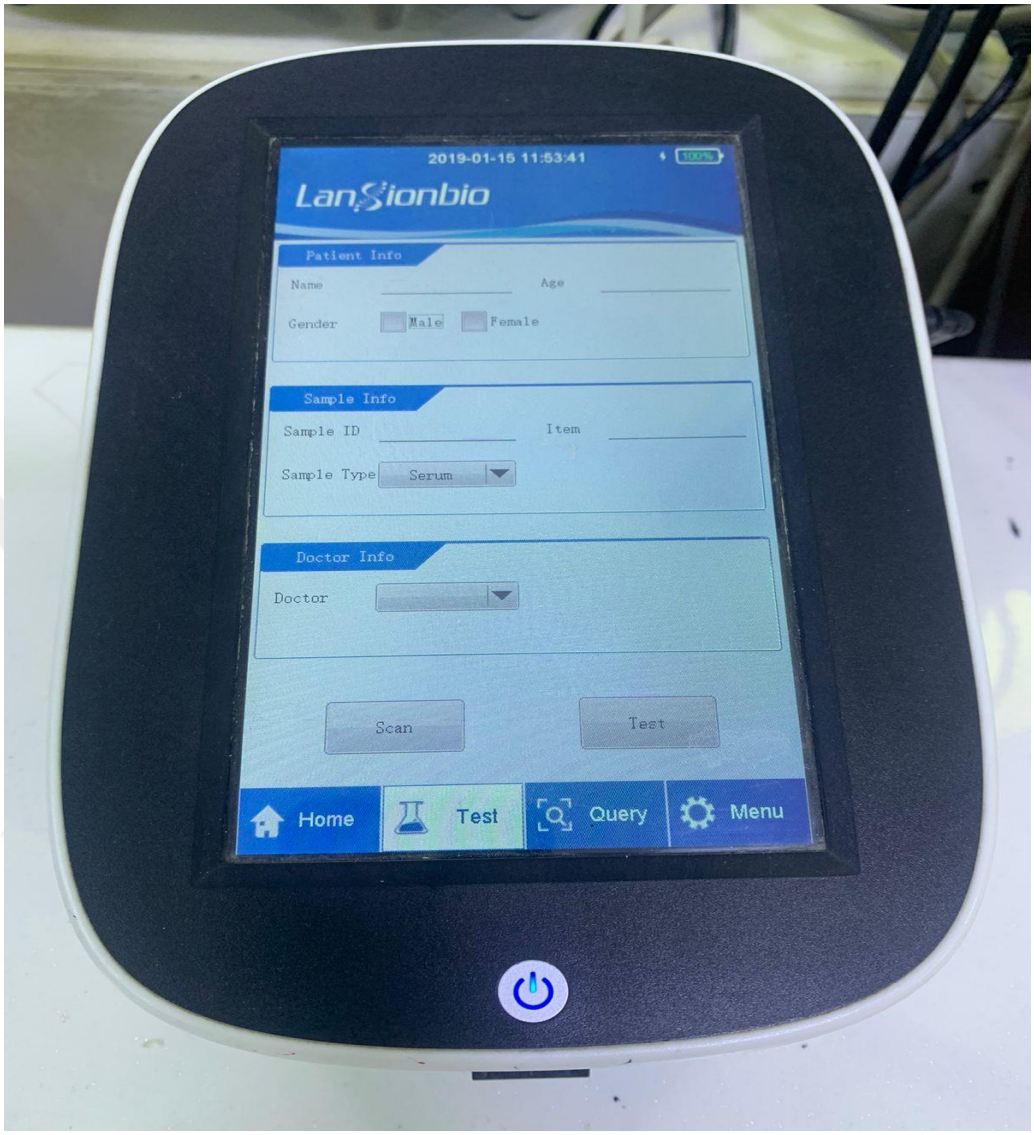
APPENDIX 7. Laboratory centrifuges for separating blood samples



APPENDIX 8. CRP Kit (Dry Fluorescence Immunoassay) is used for in-vitro quantitative measurement of CRP in human serum, plasma and whole blood. This test is used for the detection and evaluation of infection, tissue injury and inflammation disorders



APPENDIX 9. Device from Lansion Biotechnology Co., Ltd. Use to measure c-reactive protein rate



APPENDIX 10. Microbiology Chart Report-1

bioMérieux Customer:

مختبر تاج محل
Microbiology Chart Report

Printed May 28, 2022 2:07:27 PM CDT

Patient Name: تمارا ثامر .
Location: Urine
Lab ID: 31307

Patient ID: 31307
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: م. اوركيد

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.90 hours	**Status:**	Final
Selected Organism	96% Probability	**Escherichia coli**		
	Bionumber:	0405610554426610		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	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	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

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APPENDIX 11. Microbiology Chart Report-2

bioMérieux Customer:

مختبر تاج محل
Microbiology Chart Report

Printed May 28, 2022 2:10:28 PM CDT

Patient Name: مهند عطا الله , .
Location: Pus
Lab ID: 31409

Patient ID: 31409
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterococcus faecalis

Source: م سما الشفا

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.85 hours	**Status:**	Final
Selected Organism	91% Probability	**Enterococcus faecalis**		
	Bionumber:	156412777771671		
ID Analysis Messages				

Biochemical Details																
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	(+)	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

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APPENDIX 12. Microbiology Chart Report-3

bioMérieux Customer: مختبر تاج محل

Microbiology Chart Report

Printed May 30, 2022 2:49:31 PM CDT

Patient Name: غصون, Sample 2
Location:
Lab ID: 32285

Patient ID: 32285
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	7.98 hours	**Status:** Final
Selected Organism	96% Probability	**Klebsiella pneumoniae ssp pneumoniae**	
	Bionumber:	6607735753565153	
ID Analysis Messages			

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	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	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	lHISa	+	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	+	64	lLATa	+			

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APPENDIX 13. Microbiology Chart Report-4

bioMérieux Customer:

مختبر تاج محل
Microbiology Chart Report

Printed May 28, 2022 2:13:53 PM CDT

Patient Name: 33 محمد رضا غني
Location:
Lab ID: 31356

Patient ID: 31356
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus mirabilis

Source: MAHAMMAD

Collected:

Comments:	

Identification Information	**Analysis Time:** 4.05 hours	**Status:** Final
Selected Organism	95% Probability **Bionumber:**	**Proteus mirabilis** 0017100341543211
ID Analysis Messages		

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	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	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

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APPENDIX 14. Microbiology Chart Report-5

مختبر تاج محل

bioMérieux Customer: **Microbiology Chart Report** Printed May 28, 2022 2:12:49 PM CDT

Patient Name: 90, .
Location:
Lab ID: 31344

Patient ID: 31344
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: research **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.82 hours	**Status:** Final
Selected Organism	89% Probability	**Pseudomonas aeruginosa**	
	Bionumber:	1003453303500270	
ID Analysis Messages			

Biochemical Details																
2	APPA	+	3	ADO	-	4	PyrA	-	5	lARL	-	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	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	lMLTa	+	62	ELLM	-	64	lLATa	-			

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APPENDIX 15. Microbiology Chart Report-6

مختبر تاج محل

bioMérieux Customer: **Microbiology Chart Report** Printed May 28, 2022 2:11:55 PM CDT

Patient Name: هند سالم حميد , .
Location:
Lab ID: 31536

Patient ID: 31536
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 4.23 hours	**Status:** Final
Selected Organism	91% Probability **Staphylococcus aureus**	
	Bionumber: 070002477761271	
ID Analysis Messages		

Biochemical Details																
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	+	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

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APPENDIX 16. Microbiology Chart Report-7

bioMérieux Customer: مختبر تاج محل

Microbiology Chart Report

Printed May 28, 2022 2:17:12 PM CDT

Patient Name: 49 بان عادل
Location:
Lab ID: 32133

Patient ID: 32133
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus saprophyticus

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:** 7.98 hours	**Status:** Final
Selected Organism	**Staphylococcus saprophyticus** **Bionumber:** 030402017720231	
ID Analysis Messages		

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	(+)	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	(-)	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

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CURRICULUM VITAE

Personal Information

Name and Surname : Manar Muthanna Abduljabbar ALTEKREETI

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Biology	2020-Present
Undergraduate	University of Technology College of Science Department of Biology	2012-2016