Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Babylon
College of Medicine

Impact of sRNA (As1974) and HFq Binding Proteins on the Expression of Resistance Gene and Host micro RNA (miR-30C) in Iraqi UTI patients infected with *Pseudomonas aeroginosa*

A Thesis

Submitted to the Council of the College of Medicine, University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master in Science / Medical Microbiology

By

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2023 A.D 1444 A.H

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

((وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيماً))

صَدَقَ اللهُ العَلِيُّ العَظيم

سورة النساء: الآية ﴿١١٣﴾

Supervisor Certification

we certify that this thesis entitled “**Impact of sRNA (As1974) and HFq Binding Proteins on the Expression of Resistance Gene and Host micro RNA (miR-30C) in Iraqi UTI patients infected with** ***Pseudomonas aeroginosa***” was prepared by**" Bashaer Saed Jabbar Al-Lafta**" under our supervision at the college of Medicine, University of Babylon, as a partial requirement for the degree of Master of Science in Medical Microbiology.

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Dedication

To …..

The kind heart "My Father" and to the great Lady "My Mother" who always encourage me to reach the beach of Success.

My Husband, brothers, my sisters for their true feelings and to all friends.

Bashaer/ 2023

Acknowledgments

I would like to express my thanks to “Allah” the Most Gracious and Most Merciful, and to His prophet “Mohammad”.

I am deeply indebted to the bright candle “My Supervisor" Professor Dr. Ilham A. Bunyan Al-Saedi who faithfully worked hard to bring this work into big success. Allah will bless her for the guidance and great efforts she has done. This thesis wouldn't have been possible without her sincere help and assistance.

In fact, I feel grateful to Professor Dr. Hayam K. Al-Masudy Head of the Department of microbiology. My heartfelt thanks are due to the academians of the Department of Microbiology who have guided me through my year of study.

I am extremely grateful to Dr. MOHEND ABBASS NORI ALSHALAH Dean of the College of Medicine, University of Babylon for providing all the needed essential requirement for the completion of the present work.

Bashaer/ 2023

Summary

Two hundred clinical samples were collected from patients suffering from urinary tract infections (UTIs) of both gender, (129) urine samples from female patients and (71) from male patients, aged 17 to 53 years, they were diagnosed with UTIs whom attending to Baghdad Teaching Hospital, Ghazi Hariri Hospital, Central Laboratories in Medical City and Al-Yarmouk hospital in Baghdad /Iraq, from October (2022) to January (2023). Patients with urinary tract infections 129(64.5%) of those patients were female, while 71 (35.5%) were male. Urine samples were cultured for *Pseudomonas aeruginosa* isolation and identification, 148(74%) revealed positive bacterial cultures, while 52(26%) showed no growth. Only 56(37.84%) of the positive culture isolates belonged to *P. aeruginosa*, while 92/148 (62.16%) belonged to other genera of bacteria.

In this study, all bacterial isolates of *P. aeruginosa* were characterized by morphological, Microscopical, biochemical tests and VITIK 2 system. The susceptibility test of bacterial isolates against 12 selected antibiotics was conducted by VITIK 2. Resistance findings of the *P. aeruginosa* isolates during the current analysis against these antibiotics, which indicates that, the *P. aeruginosa* isolates are resistance 56(100%) to 7 of the antibiotics of interest during the current study, including Ticarcillin, Piperacillin, Imipenem, Meropenem, Gentamycin, Amikacin and Tobramycin. Colistin was the most effective antimicrobial agents against bacterial isolates because *P. aeruginosa* was showed a percentage of sensitivity about 22(39.29%) followed by Ciprofloxacin 17(30.36%), Ticarcillin/clavulanic acid 10(17.86%), Ceftazidime 7(12.5%) and Cefepime 4(7.14%) respectively.

In this study, the expression of *HFQ* gene in urine samples was investigated, and the results demonstrated a significant overexpression of HFQ in *P. aeruginosa* resistance compared to sensitive clinical samples, given by fold change after normalized with housekeeping gene *16sRNA* by folding (21.497±1.241 versus 1.92142± 0.04598) respectively.

In addition, the expression of *As1974* gene in urine samples were finding demonstrated down-regulation of As1974 in resistance after normalized with *16sRNA* with fold change (0.6622±0.0465) vs (2.012±1.0243) respectively. However, *MiR-30* gene in urine, and blood demonstrated down-regulation of *MiR-30* in urine and blood samples (1.36±0.34, 0.47821±0.03678), compared to healthy subjects (5.2463±1.213, 5.0432±1.7441) respectively.

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List of Abbreviation

Abbreviation	***Meaning***
CFU	Colony forming unit
cUTIs	Complex urinary tract infections
CCA	Chromogenic Coliforms Agar
CCR	Carbon Catabolite Repression
Crc	Catabolite repression control
Ct	Cycle threshold
cDNA	Complementary deoxyribonucleic acid
EDTA's	Ethylene Diamine Tetraacetic Acid
GAPDH	Glyceraldehyde-3-phosphate
GN	Gram negative
GSP	Gene Specific Primer
ICUs	Intensive care units
IFN-Ý	Gamma Interferon
LAB	Lactic acid bacteria
MBLs	Metallo -beta lactamase
MDR	Multiple drug resistance
miRNAs	MicroRNAs
ml	Milliliter
mRNAs	Messenger RNAs
NAD	Nicotinamide adenine dinucleotide
nt	nucleotide
P. aeruginosa	*Pseudomonas aeruginosa*
PAE	Post antibiotic effect
Pch	Pyochelin
pre-miRNAs	Precursor microRNAs
pri-miRNA	Primary miRNA
qPCR	Quantitative RT-PCR
RISC	RNA-induced silencing complex
SE	Standard error
sRNAs	Small noncoding RNAs
TLRs	Toll-like receptors
TNF	Tumor Necrosis Factor
UTR	Untranslated region
vfr	Virulence factor regulator
UTI	Urinary tract infection
µl	Microliter

Chapter One

Introduction and Literatures Review

1.1 Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative bacteria commonly found in water, soil, and even hot tubs. This bacterium is widespread and can infect mammals, causing a variety of illnesses. All around the planet, it is found it in the dirt, the water, the flora on skin, and even in most man-made settings (Spernovasilis *et al*., 2021).

Almost all strains of *P. aeruginosa* are motile by means of a single polar flagellum; it is a non-fermentative, aerobic Gram-negative rod measuring (0.5) to (0.8) µm by (1.5) to (3.0) µm in size. This organism thrives in damp places and can employ a wide variety of organic molecules for growth, giving it a remarkable capacity to colonize ecological niches where nutrients are scarce, from water and soil to plant and animal tissues (Bunyan *et al*., 2019).

Biochemically, *P. aeruginosa* isolates are often characterized by a positive oxidase test, growth at 42 °C, hydrolysis of arginine and gelatine, and nitrate reduction. Both pyoverdin and pyocyanin are soluble pigments made by *P. aeruginosa* strains. This latter blue pigment serves a vital role in the bacterium's iron metabolism and is produced in great quantities in low-iron conditions. Suppurative infections brought on by *P. aeruginosa* are characterized by the presence of a blue pus known as pyocyanin (from the Greek word pyocyaneus) (Sowndarya, 2018).

Symptoms of such infections include systemic inflammation and sepsis. Colonization of vital organs including the lungs, urinary tract, or kidneys can have devastating effects on the host. It is the most commonly isolated pseudomonad from medical samples. Patients with conditions like burns, wounds, cystic fibrosis, acute leukemia, organ transplants, and addiction to intravenous drugs are more likely to get a *P. aeruginosa* infection (Kumar and Bera, 2019). Patients with aberrant host defenses, such as those with weakened immune systems, the elderly, and those who have been hospitalized for extended periods of time, are particularly susceptible to infections caused by *P. aeruginosa* (Al-Bidhani, 2018). There are many processes by which this bacterium can evolve into a form that is resistant to practically all antibiotics now on the market, making it difficult, if not impossible, to treat a lung or bloodstream infection caused by this microorganism (Al-Bidhani, 2018).

P. aeruginosa can occasionally colonize human body locations, and it does so with a propensity for moist places like the perineum, axilla, ear, nasal mucosa, throat, and feces. Though colonization rates are modest in otherwise healthy people, they may increase after hospitalization, particularly in those who have been given broad-spectrum antibiotics (Adekunle *et al*., 2021). Infections only happen when the skin or mucous membranes are broken, when they are penetrated (via invasive devices), or when the immune defense mechanisms are weakened to begin with (Talapko *et al*., 2021). Patients with cystic fibrosis often suffer from persistent colonization and infection with *P. aeruginosa*, specifically the mucoid phenotype, which causes damage to lung tissue and a decline in pulmonary function. *P. aeruginosa* produces several toxic proteins that not only cause extensive tissue damage but also interfere with the immune system's defense mechanisms. These virulence factors include flagella, pili, lipopolysaccharides, alginate, alkaline protease, elastase, phospholipase C, exotoxin A, quorum sensing mechanisms, the type III secretion system, pyocianin, and (Rossi *et al*., 2021).

MicroRNAs (miRNAs) are a group of short RNAs encoded by the genome that function to control the expression of cellular messenger RNAs (mRNAs) that include partial or complete microRNA binding sites. A capped, polyadenylated primary miRNA (pri-miRNA) transcript is transcribed by RNA polymerase II as the first step in the conventional pathway of microRNA expression (Bitetti *et al*., 2018). The use of RNA-based technologies as a method of combating bacterial infections is gaining ground. Using siRNAs directed at tfgb1 has shown effective in combating pulmonary tuberculosis in preclinical models (Mourenza *et al*., 2022). However, several of the miRNAs mentioned above, as well as many others, present new prospects for the development of antimicrobial methods. Moreover, the pathogen's host colonization could be slowed or even halted by the development of anti-miRNAs that target particular miRNAs that aid bacterial infection. Anti-miRNAs are synthetic single-stranded RNAs designed to inhibit the activity of miRNA targets (Cai *et al*., 2021).

The present study only recognized the functions of some sRNAs, but little is known about the regulatory networks of these sRNAs and the functions of other uninvestigated sRNAs.

Aim of study

To evaluate the Impact of sRNA (As1974) and HFq Binding Proteins on the expression of resistance gene and host micro RNA (miR-30C) in Iraqi UTI patients infected with *Pseudomonas aeruginosa*.

Objectives

1. Isolation and Identification of *Pseudomonas aeruginosa* from UTI
2. Measurement of gene expression of small regulatory RNA As1974, HFq and the expression of microRNA-30C.
3. Data analysis (Fold Chang Method).

1.2 Literature review

1.2.1 Urinary tract infection

A urinary tract infection's likelihood can rise or fall depending on factors like the host's anatomical make-up, the immune system's response, and the aggressiveness of the invading organisms (Kline and Lewis, 2017). Cystitis is the first symptom of a urinary tract infection (UTI), which can develop into pyelonephritis, an acute infection of the kidneys, and ultimately scarring and renal failure. There are over 150 million cases of UTIs worldwide, with an estimated 10.5 million occurring each year in the United States alone (Olson *et al*., 2018). Urinary tract infections caused by bacteria are a worldwide public health issue. The uropathogenic *Escherichia coli* is the most frequent uropathogen, responsible for up to 95% of community-acquired UTIs and 50% of healthcare-associated UTIs (Zalewska-Pitek and Pitek, 2020).

Female are more prone to urinary tract infections than mele because of the shorter distance between the urethra and the anus in female and the longer urethra and antibacterial activity of prostatic fluid in males. Anatomical, hormonal, and other physiological changes during pregnancy put female at a higher risk for urinary tract infections (UTIs) (Gangakhedkar and Kulkarni, 2021).

Both the mother and the unborn child are at risk for serious injury if the female develops a urinary tract infection (UTI) while pregnant. About 30% of people with untreated UTI will develop cystitis, and up to 50% will develop pyelonephritis. Low birth weight and intrauterine development retardation are common in babies diagnosed with UTI. Up to 27% of premature births have been connected to a urinary tract infection in the mother (Dautt-Leyva *et al*., 2018).

If the infection stays in the urinary bladder, it is called cystitis; if it spreads to the pelvis and the kidney's parenchyma, we call it acute pyelonephritis. Acute ureteral infection (pyelonephritis) can lead to chronic kidney disease because scar tissue forms as a result of tissue remodeling (El-Ghar *et al*., 2021).

When infectious agents enter the bloodstream, septicemia can occur. This highlights the need of a prompt diagnosis and treatment. Focusing on early diagnosis and treatment is emphasized in the updated UTI guidelines (Alba-Patio *et al*., 2022).

Urinary tract infections (UTIs) can be either mild or severe. Complex urinary tract infections (cUTIs) are not limited to women of childbearing age and can affect people of any age and gender due to anatomical or functional abnormalities in the urinary system. Kidney issues can be brought on by a number of factors, including pregnancy, immunosuppression, kidney stones, blocked ureters, renal failure, and renal transplantation (Kwok *et al*., 2022).

Women and girls who are otherwise healthy have a high risk of getting a urinary tract infection (UTI), and *E. coli* is the most common cause of these infections. *S. aureus*, *Klebsiella*, *Enterococcus*, *Proteus*, *Pseudomonas*, and group B *Streptococcus*. Fifth on the list is *saprophyticus* (Kawalec and Zwoliska, 2022).

1.2.2 *Pseudomonas*

P. aeruginosa, *P. oryzihabitans*, and *P. plecoglossicida* are all examples of pathogenic bacteria that belong to these genera. As the second most common cause of nosocomial infections, *P. aeruginosa* is a persistent concern in healthcare settings. *P. aeruginosa's* released metabolites may contribute to its pathogenesis. Numerous proteins important to the pathogenesis of clinical strains are secreted by the bacterium's diverse secretion mechanisms (Bunyan *et al*., 2018).

The *Pseudomonas* went extinct hundreds of millions of years ago, like most bacterial families. The pseudomonads were among the earliest microbes to be observed because of their broad distribution in water and in the seeds of plants like dicots. Originally thought to be rod-shaped, Gram-negative, polar-flagella bacteria with some sporulating species, the genus *Pseudomonas* was later shown to be erroneous and the result of refractive granules of reserve resources. *P. aeruginosa*, the type species, was the best descriptor despite the hazy description (Camiade *et al.*, 2020).

Many strains have been reclassified as a result of new technologies and the breadth of approaches based on the investigations of conservative macromolecules. *P. aeruginosa* is an extremely widespread bacteria that can infect a wide variety of animal species, including humans. It permeates the planets aqueous and terrestial ecosystems, as well as the flora of human skin. It can survive in oxygenated and oxygen-depleted situations, allowing it to spread to a wide variety of natural and man-made settings. The flexibility of this organism allows it to infect injured tissues or persons with impaired immune systems in animals (Kivisaar, 2020).

Infections of this type typically cause fever, chills, and a general feeling of malaise. Infections in the lungs, urinary tract, or kidneys are particularly dangerous because of the severity of their effects on the body. This bacterium can survive and even thrive on a wide variety of surfaces, making it a common source of cross-infection in healthcare facilities (Camiade *et al*., 2020).

1.2.2.1 Identification

It is a rod-shaped, aerobic, Gram-negative bacterium that moves in a single direction (unipolarly). Besides being an opportunistic human pathogen, *P. aeruginosa* is also a plant pathogen. The species *P. aeruginosa* represents the genus *Pseudomonas* in its entirety (Rao *et al*., 2019).

Pigments such as pyoverdine (yellow green and fluorescent), pyocyanin (blue-green), and pyorubin (red) are secreted by *P. aeruginosa* (red-brown). The pearly appearance and grape- or tortilla-like odor *in vitro* are frequently used for a preliminary diagnosis of *P. aeruginosa* (Prabhurajeshwar, 2019).

Additionally, determining whether or not *P. aeruginosa* can grow at 42°C and the presence of pyocyanin and fluorescein are required for definitive clinical identification. *P. aeruginosa* is a hydrocarbon-using bacteria that can thrive in diesel and jet fuel, leading to microbial damage. It produces what look like dark, gellish mats, which are frequently incorrectly referred to as "algae" (Wiegand, 2019).

P. aeruginosa is technically an aerobic organism, but many biologists believe it to be a facultative anaerobe because of its ability to thrive in environments with low or no oxygen. Anaerobic growth can be attained by this organism using nitrate as a terminal electron acceptor, and in the absence of oxygen, arginine can be fermented via substrate-level phosphorylation. (André *et al*., 2021).

In cystic fibrosis patients, for instance, where thick layers of lung mucus and alginate surrounding mucoid bacterial cells might block the flow of oxygen, *P. aeruginosa* must adapt to anaerobic or micro aerobic settings in order to survive (Rossi *et al*., 2021).

1.2.3 Diseases caused by *P. aeruginosa*

P. aeruginosa has emerged as a major pathogen, particularly for those with weakened immune systems. Patients who have been hospitalized for longer than a week typically have this pathogen removed from their bodies. Nosocomial infections such UTIs, pneumonia, and bacteremia are common outcomes of this organism's spread in healthcare settings (Al-Dahmoshi *et al*., 2020).

It's a human opportunistic pathogen and the leading cause of nosocomial infections (infections acquired while in a hospital), and it's resistant to many antibiotics, making it very hard to get rid of. Because of its intrinsic and acquired resistance to antimicrobial drugs, *Pseudomonas* infections are notoriously difficult to cure and can spread to virtually any tissue in the body if the host's defenses are compromised. The majority of the time, it causes infections in the urinary tract, the skin, and the lungs (Fleiszig *et al*., 2020).

1.2.4 Epidemiology of *P. aeruginosa*

P. aeruginosa was first identified in 1882 when Gessard isolated it from a sample of green pus. *P. aeruginosa* ability to thrive in a wide variety of environments makes it a major contributor to chronic human infections. *P. aeruginosa* is a highly adaptable bacterium that can live both in soil and water. Its nutritional diversity allows for *P. aeruginosa* to tolerate toxic waste degradation (Pachori *et al*., 2019).

P. aeruginosa is a major plant pathogen that causes damage to lettuce, tomatoes, and tobacco. It is ubiquitous, showing up anywhere water is present (ponds, lakes, rivers, even distilled water is tainted with it) (Khanna *et al*., 2022).

Human individuals can swallow the *P. aeruginosa* from such sources; yet it does not attach effectively to typical undamaged epithelium. Therefore, *P. aeruginosa* can be a part of normal gut flora, and in people with functioning immune systems, it rarely if ever causes infection (Malet *et al*., 2022).

Because of the warm environment, hot tubs can harbor as many as 100 million *Pseudomonas* organisms per milliliter of water. *P. aeruginosa* is flexible; it finds the hospital and intensive care unit surroundings accommodating, with reservoirs of *P. aeruginosa* forming in the water in breathing apparatus. *P. zaeruginosa* can be cultured from hand creams, hand-washing sinks, and certain cleaning solutions due to its intrinsic and acquired resistance to many common antimicrobial agents (Fujitani *et al*., 2019).

P. aeruginosa thrives in moist, body-temperature environments and thus poses a significant threat to respiratory therapy and dialysis equipment. *P. aeruginosa* has been linked to the contamination of multi-use vials of respiratory medicines. Artificial fingernails or extenders are not recommended for use by healthcare workers due to the frequent finding of *P. aeruginosa* colonization of the fingernails. Pseudomonads can even survive in some antiseptic solutions used to disinfect endoscopes and surgical instruments (Yiek *et al*., 2021).

P. aeruginosa accounted for 4% of cases and was the third leading cause of gram-negative infection in a recent analysis of 24,179 adults with nosocomial bloodstream infections in the United States from 1995 to 2002. Nosocomial infection rates in pediatric intensive care units were 1.5 per 100 patient-days. Nosocomial infection rates were highest (2.3 per

100 patient-days) among cardiac surgery patients. The most common nosocomial infections were bacteremia (51.7%), respiratory infection (19.0%), and urinary tract infection (17.2%), all of which were linked to the use of invasive medical equipment. The most frequently isolated pathogens were coagulase-negative staphylococci (39%) and *P. aeruginosa* (24%). (Atay *et al*., 2019).

P. aeruginosa is involved in a number of human illnesses ranging from neonatal sepsis, to burn sepsis, to acute and chronic lung infections. Chronic neutropenias and deficiencies in neurtrophil function, hematologic malignancies, human immunodeficiency virus, acquired immunodeficiency syndrome, diabetes mellitus, and other defects in host defenses make this organism a prevalent opportunistic pathogen that causes infections in patients. In addition chronic pulmonary disease is common in patients with cystic fibrosis (Morin *et al*., 2021).

1.2.5 Pathophysiology

Opportunistic pathogens like *P. aeruginosa* can cause serious illness in otherwise healthy people. In healthy people, it almost never has any ill effects. Usually, an infection occurs when the immune system is compromised in some way, either because of a lack of defenses (such as neutropenia or immunosuppression) or because of a breakdown in the physical barrier that protects the body from germs (such as a mucous membrane or the skin) (Makhdoomi *et al*., 2019).

This bacteria is not only dangerous because of its presence, but also because of its low nutritional needs and high tolerance for a wide range of environmental circumstances. Infections caused by *Pseudomonas* involve a complicated and multifaceted etiology. *Pseudomonas* species spread rapidly and produce harmful toxins. First, bacteria attach and colonize an area, then an infection develops at that site, and finally the infection spreads throughout the body via the blood (Rossi *et al*., 2021).

In the setting of respiratory tract infection in patients with cystic fibrosis and those who affect mechanical ventilation, the relevance of adherence and colonization becomes most apparent. By aiding bacterial adhesion and invasion, extracellular proteases boost an organism's pathogenicity (Makhdoomi *et al*., 2019).

Ten percent of all hospital-acquired infections are caused by *P. aeruginosa*, making it one of the most frequently isolated nosocomial pathogens. Some healthy people carry it on their skin, and it has been isolated from the throat and feces of 5 percent and 3 percent, respectively, of people who were never hospitalized. Within seventy-two hours of admission, 20% of hospitalized patients already have GI carriage (Szabó *et al*., 2022).

Several illnesses can be brought on by *P. aeruginosa*. An initial, localized infection after surgery or burns frequently spreads to the rest of the body, causing a potentially lethal bacteremia. Infections of the urinary system caused by the bacterium *P. aeruginosa* are prevalent when it has been introduced by a catheter or irrigating solutions. Intriguingly, most people with cystic fibrosis are persistently colonized with *P. aeruginosa*, yet bacteremia with *P. aeruginosa* is uncommon in this population, perhaps because of high levels of circulating *P. aeruginosa* antibodies. Localized *P. aeruginosa* infections, however, are the leading cause of death for people with cystic fibrosis (Spernovasilis *et al*., 2021).

After using infected respirators, certain patients may develop necrotizing *P. aeruginosa* pneumonia. Post-operative or post-injury ocular infections caused by Pseudomonas aeruginosa can be quite dangerous. It occurs naturally in youngsters of pure culture who have middle ear infections. There have been rare reports of it causing meningitis after a lumbar puncture and endocarditis after heart surgery. Some outbreaks of diarrheal diseases have been linked to it. *P. aeruginosa* has been linked to bacteremia ever since the first case was described in 1890; today, it is responsible for fifteen percent of all Gram-negative bacteremia (Morin *et al*., 2021).

Bacteremia caused by *P. aeruginosa* is associated with a roughly 50% fatality rate. While some infections (such those affecting the eyes or ears) stay contained, others (like those caused by trauma or poor hygiene, or those affecting people with cancers like leukemia or lymphoma) can spread throughout the body and cause a life-threatening condition called sepsis. This discrepancy is probably attributable to host defensive mechanisms that have been manipulated (Kanj and Sexton, 2019).

1.2.6 Pathogenesis

Outside of healthcare settings, suppurative otitis is *P. aeruginosa* most prevalent infection. Hospitals are a potential breeding ground for both local and widespread illnesses. Catheterization patients frequently develop localized lesions such as wound infections, eye infections, and UTIs (Neu, 2020).

When it comes to burn infections, *P. aeruginosa* is by far the most prevalent and dangerous. Additionally, it is one of the causes of iatrogenic meningitis after a lumbar puncture. After a tracheostomy, lung infection is a common complication. Patients weakened by several infections are at increased risk for septicemia and endocarditis. Cancer or medication that suppresses the immune system. Patients with leukemia and other malignancies are particularly susceptible to developing ecthyma gangrenosum and other types of skin lesions, either on their own or as part of a systemic infection (Hocking *et al*., 2021).

After prolonged contact with water and cleaning agents, the nail bed might get infected. In infants, *P. aeruginosa* has been identified as a potential cause of diarrhea and sepsis (Andersen, 2019).

Diarrhea outbreak strains may be positive for the rabbit ileal loop reaction because to the presence of heat-labile enterotoxin NAD. In some tropical regions, P. aeruginosa is responsible for a self-limiting, febrile sickness (Shanghai fever) that is similar to typhoid fever (Nieder *et al*., 2018).

P. aeruginosa plays a crucial role in hospital infections because it is resistant to many antiseptics and medications and may spread easily throughout healthcare facilities. Because of its remarkable adaptability, it can live and grow in conditions where it receives just a small amount of food and water. Tools like respirators and endoscopes, household items like bedpans, pharmaceuticals like lotions, ointments, and eye drops, and even supplies like distilled water or plants and flowers are all at risk of contamination (Naher and Al-Sa'ady, 2020).

People can carry *P. aeruginosa* on their axial and perineal skin. Carrying feces around your digestive tract isn't typical, although it might become prevalent after taking oral antibiotics or being in a hospital (Solanki *et al*., 2018).

Not much is known about the pathogenesis mechanism. Toxic extracellular products including exotoxins A and S have been found in the culture filtrates; exotoxins A operates as NADase and is structurally similar to the diarrheas toxin. In cases of severe *P. aeruginosa* infections, an antibody response to exotoxin A is viewed as a positive indicator. Protease, elastase, hemolysin, and enterotoxin are some of the other hazardous byproducts. The slime coat functions as a capsule, amplifying pathogenicity (Sauvage and Hardouin, 2020).

1.2.7 Virulence factors

1.2.7.1 Extracellular slime layer substances

Glycolipoprotein mucoids surround the bacterial cell, protecting it from cell-degrading enzymes and playing a role in cell attachment to host epidermal surfaces; they are also largely responsible for *Pseudomonas aeruginosa* high level of antibiotic resistance (Mishra *et al*., 2020).

1.2.7.1.1 Capsule (Alginate)

They are glycolipoprotein mucoid substances that surround the bacterial cell, aid in cell attachment to the host epidermis, and shield the bacterial cell from degradation enzymes. Large quantities of alginate, an exopolysaccharide composed of mannuronic and guluronic acids, are produced by *P. aeruginosa* isolated from respiratory tracts. This exopolysaccharide gives birth to distinctively mucoid colonies (Bunyan *et al*., 2019).

After a long incubation on high-carbon, low-nitrogen media, all *P. aeruginosa* strains produce alginate, a heterogeneous mixture of hexoses. The mucoid polysaccharide alginate stands in for the capsule, which appears to aid the bacteria in evading innate defenses and providing protection from phagocytosis and antibiotics (Dolan *et al*., 2020). *P. aeruginosa* has a high level of antibiotic resistance because of this mutation, which occurs on faulty surfaces (Mishra *et al*., 2020).

1.2.7.1.2 Flagella

Thread-like flagella are made up of a single protein subunit called flagellin. The filament, hook, and basal body of a flagellum are all made up of various proteins. When it comes to movement, *P. aeruginosa* relies on its flagellum (LealMorales *et al*., 2022).

P. aeruginosa can be distinguished from enteric bacteria, which have peritrichous flagella (lateral flagella scattered across the entire cell surface), because it contains polar flagella (one or more flagella originating from one or both poles of the cell) (Bouteiller *et al*., 2021).

1.2.7.1.3 Pili (Fimbriae)

Thread-like flagella are made up of a single protein subunit called flagellin. The filament, hook, and basal body of a flagellum are all made up of various proteins. When it comes to movement, *P. aeruginosa* relies on its flagellum (LealMorales *et al*., 2022).

P. aeruginosa can be distinguished from enteric bacteria, which have peritrichous flagella (lateral flagella scattered across the entire cell surface), because it contains polar flagella (one or more flagella originating from one or both poles of the cell) (Bouteiller *et al*., 2021).

1.2.7.2 Enzymes and toxins

Toxic compounds released by *P. aeruginosa* include catalase, lipase, lecithinase, elastases, proteases, and two hemolysins, a heat-labile phospholipase C and a heat-stable glycolipid (Smith *et al*., 2022).

Furthermore, toxin A produces tissue necrosis and prevents protein synthesis by a method of action identical to that of *Corynebacterium diphtheriae* toxin, which affects the elongation of peptide chain, but with different structures (Stirpe and Battelli, 2020).

Some human sera, perhaps including those of patients who have made a full recovery from severe infections with *P. aeruginosa*, may have antitoxins against toxins A. These toxins can be broken down enzymes that permanently alter cell membranes and connective tissue in different organs, or they can be highly toxic toxins that penetrate host cells and kill them at or near the site of colonization (Baindara and Mandal, 2020).

Endotoxins are made up of lipopolysaccharide, a component of the cell wall that is released after autolysis or microbial death. Due to the presence of the lipid A found in lipopolysaccharide, these endotoxins are resistant to heat and can cause shock with hypotension, especially in the elderly. They can also lead to a decrease in white blood cell count, a rise in blood sugar levels, and problems in the kidneys, heart, and brain (Vanzolini *et al*., 2022).

1.2.7.3 The pyocyanin and other pigments

Most strains of *P. aeruginosa* create the blue phenazine pigment pyocyanin when grown in culture media between 28 and 30 degrees Celsius because it is soluble in water and is discharged from the cell (Morin *et al*., 2021).

When they discovered the old culture of *P. aeruginosa* inhibiting the growth of some Gram-negative and positive bacteria, they made the first reference to the activity of pyocyanin as an antibiotic (Sheng *et al*., 2022).

Since it is poisonous to all animals, pyocyanin broad-spectrum suppression of the microorganism is of no utility. The yellow pigment pyoverdin is produced by *P. aeruginosa* both within and outside of the cell, and it plays a role in the bacterium's resistance to antibiotics (El Feghali and Nawas, 2018).

1.2.7.4 Proteases

Proteases are pivotal in a wide variety of disease processes. Proteolytic enzymes have been connected to many different degenerative disorders, including arthritis, tumor invasion and metastasis, infection, and others (Patel *et al*., 2018).

Proteases produced by microbes have been hypothesized to play a role in the pathogenesis of a number of microbial illnesses. Exo-products, such as alkaline protease and elastase, are responsible for the destruction of tissues by degrading elastin, collagen, and proteoglycans, and hence contribute to *P. aeruginosa* pathogenicity. It has also been revealed that these enzymes break down proteins involved in host defense *in vivo* (Bunyan *et al*., 2019).

If we want to learn more about how these proteases contribute to the development of infectious diseases and how we can better use them in biotechnology, we need to first identify and characterize them. Therefore, it is highly important to have methods for the rapid and sensitive identification and characterisation of microbial proteases. Alkaline protease and elastase are two extracellular proteases that have been linked to Pseudomonas aeruginosa pathogenicity (Hirakawa *et al*., 2022).

1.2.7.4.1 Alkaline protease

A metalloproteinase, it effectively breaks down C1q and C3 of the complement system, as well as IFNG and TNF- (Axelrad *et al*., 2021). Several diseases, including keratitis, otitis media, cystic fibrosis, and bacteremia, trigger the production of alkaline protease (Al-Dahmoshi *et al*., 2020). The combination of elastase and alkaline protease is enough to eat away at the cornea's ground substance and any other supporting structures made of fibrin and elastin. Inactivation of Gamma Interferon (IFN) and Tumor Necrosis Factor (TNF) has also been linked to the combination of elastase and alkaline protease (Morin *et al*. 2021).

Gentamicin and Tobramycin were tested for their ability to stop *P. aeruginosa* from producing more of its own extracellular proteases, a process known as virulence. The medications prevent protease secretion by interfering with the outer membrane. One of the primary antimicrobial systems of polymorphonuclear leucocytes is myeloperoxidase-mediated chemiluminesccence, which was shown to be inhibited by alkaline protease and elastase by Rezzoagli *et al*., (2020). These results support the hypothesis that *P. aeruginosa* exo-proteases contribute to the pathophysiology of infections by acting as virulence factors.

The development of an extracellular alkaline protease is one of the traits thought to contribute to *P. aeruginosa* pathogenicity. Super inhibitory concentrations inhibited both elastase and protease activity (Husain *et al*., 2017).

Protease secretion in vitro can be blocked by using sub-inhibitory doses of aminoglycosides. Sub-MIC concentrations of ciprofloxacin, gentamicin, tobramycin, azlocillin, ceftazidime, and tetracycline inhibit protease secretion, including elastase, in vitro (Hussen Abdulla, 2012). Ciprofloxacin decreases protease and exotoxin A levels *in vitro* and in vivo, specifically in the rat granuloma-pouch model of chronic local *P. aeruginosa* infection (Mauch *et al*., 2018).

1.2.7.4.2 Elastase

P. aeruginosa produces the extracellular protease elastase, which is responsible for 88% of the proteolytic activity of *this bacteria*. It's a zinc-dependent metalloprotease that acts as a protease and elastase, breaking down a wide variety of human structural and functional proteins like collagen III and IV, fibronectin, and exposing lung elastin, laminin A and B chains, immunoglobulins A and G, gamma interferon, gastric mucin, complement components, fibrin, and transferrin as bacterial attachment receptors on the mucos (Draper, 2017).

It is produces two different elastases, designated elastase A and elastase B. *Pseudomonas* elastase A is an elastin-degrading metalloproteinase. Metalloprotease elastase B may break down elastin, collagen, fibrin, IgG, IgA, Interferon gamma, tumor necrosis factor gamma, and even complete basement membranes. *P. aeruginosa* proteases, especially elastase B and alkaline protease, have been found as destructors of host defense proteins; this finding suggests that elastase B plays a key role in virulence in burn and respiratory infections (Fox *et al*., 2020).

When looking at the post antibiotic effect (PAE) on the virulence factors of *P. aeruginosa* after treatment with supra - inhibitory concentrations of some antibiotics (Ciprofloxacin, Tobramycin, and Netlimicin), it has been reported that a higher suppression of elastase activity is associated with a longer PAE (Selvaraj *et al.*, 2022).

1.2.7.5 Lipase

Medical and industrial interest in *P. aeruginosa* has prompted extensive study of the organism in previous epochs of decline. It will take root in bioremediation methods for oil waste and other types of trash. The hydrolysis of long-chain triglycerides was a defining feature of lipase (Kumar and Ngueagni, 2021).

Castor oil degradation by lipases has been utilized to make other products and for technical lipases. The thermo stable enzymes were a subset of this category; these enzymes were superior for use in high-heat industrial settings and now account for over 65% of the worldwide market (Greco-Duarte *et al*., 2017). Paper, detergent, medicines, waste degradation, textile, food, pharmaceutical, leather, degumming of silk goods, liquid glue production, cosmetics, meat tenderization, cheese production, growth promoters, etc. are just few of the numerous industries that benefit from these enzymes (Sarmah *et al*., 2018).

P. aeruginosa produced numerous compounds, including alginate and the enzymes protease, lipase, urease, and asparaginase, all of which found extensive usage in the biotech industry. *Pseudomonas aeruginosa* lipase and protease have gained a lot of attention (Bunyan *et al*., 2019).

1.2.7.6 Urease

One of the virulence factors of *P. aeruginosa* is urease. As a corollary, the bacteria were determined to be a major contributor to the disease. Hydrolysis of urea to ammonia and carbonate is catalyzed by urease. The latter chemical naturally breaks down into ammonia and carbonic acid (Bertrand *et al*., 2018).

Numerous bacterial species exhibit the urease phenotype, and gene clusters encoding this enzyme have been cloned from these organisms. Nucleotide sequence in its whole, this enzyme's function is categorized by infection type (Madhavan *et al*., 2018).

Renal stones occur when the patient's urease breaks down urea into ammonia and carbon dioxide during a urinary tract infection, raising the pH to nine and causing the salts to precipitate out of the mucosal components. During a GI infection, *P. aeruginosa* is protected from the acidic environment of the stomach by urease (Chang *et al*., 2020).

1.2.7.7 Bacteriocin (pyocin)

Chemical substances called bacteriocins are made by bacteria; they have a protein moiety that is physiologically active and kills bacteria (Tumbarski *et al*., 2018). Gram-positive bacteriocins, such as those produced by lactic acid bacteria (LAB), have been the focus of much interest and study because of their widespread use as bio preservatives ingredients in model foods, most notably those produced by the dairy industry. They have also found applications in human therapeutics (Barcenilla *et al*., 2022). It has been found that, several bacterial strains, including serious pathogens like *Listeria monocytogenes* and *Pseudomonas* species, can create a bacteriocin with broad-spectrum action (Meade *et al*., 2020).

To enhance the food's microbiological quality and safety, bacteriocin can be employed as an antimicrobial agent to stop the growth of some pathogenic and food poilage bacteria. Proteins called bacteriocins are produced by many different types of bacteria, and they are very toxic to both Gram-positive and -negative bacteria. Additionally, producer strains exhibit a form of self-defense (Ibrahim *et al*., 2021).

Bacteriocin produced by *Pseudomonas* is stable in extreme temperatures, can withstand high pH, and is immune to several proteolytic enzymes. This bacteriocin is effective against a wide variety of bacteria. The most fatal effect was seen on invading pathogenic bacteria such as *Salmonella typhi*, *Salmonella typhimurium*, and *Shigella sonnei* (Selvam *et a*l., 2021).

Pseudomonas bacteriocin has also been shown to reduce the amount of enteric pathogenic bacteria in an engineered aquatic system (Pereira *et al*., 2022). Due to the potential future application of bacteriocin as an antibiotic agent or a food biopreservative, studies into its mode of action are crucial. The majority of bacteriocins are able to kill their targets by adhering to receptors on the surface of the bacterium. Once they reach the cytoplasm, they can interact with the membrane, ultimately killing the bacterium (Sen and Ray, 2019). Over (90%) of *P. aeruginosa* strains produce a protein called pyocin, which has an effect on other members of the same family, as well as the Enterobacteriaceae (Evans, 2022).

When compared to other antibiotics, pyocin has a far more restricted effect on other species within the same genus or that share a clostridium. Because of its potential to suppress both normal and malignant cells, pyocin production and sensitivity are considered dominant genetic traits (Heilbronner *et al*., 2021).

1.2.7.8 Hemolysin

Purified peptide hemolysin, blood and tissue culture cells are cytopathic to aeruginosa. Morphological alterations proved that the cell had undergone lysis and disintegration, which involved membrane and cytoplasm (Guha *et al*., 2021).

Normal sera and albumin both inhibit hemolysin's hemolytic action. Hemolytic and cytopathic effects are thought to result from a modification of the membrane's molecular structure. This discrepancy may be due to the fact that cells have varying numbers of reactive sites available, or it may be an indication that the two activities are linked to two separate enzymes (Bunyan *et al*., 2019).

P. aeruginosa, the production of hemolysin is what allows the bacteria to colonize the lung and other tissues. One is a phospholipase C and the other is a lecithinase; both are hemolysins. In degrading lipids and lecithin, they appear to work in tandem. Phospholipase C is encoded by an operon of genes located on the chromosome. The cytotoxic effects of the cytotoxin and the hemolysins on eukaryotic cells aid in the invasion process (Shahri *et al*., 2022).

1.2.7.9 Siderophores

More than (500) unique siderophores have been found from microbes; these are low-molecular-mass molecules with a high selectivity for chelating or binding iron (Pfister *et al*., 2021). Certain bacteria are capable of producing multiple kinds of siderophores. Aerobic bacteria and other organisms need iron for a wide range of cellular metabolic activities. Despite its presence as the fourth most common metal on Earth, iron has a (Abiraami *et al*., 2019).

As an insoluble precipitate that is a component of hydroxide polymers, iron is rarely encountered in its elemental form in nature, making it inaccessible to bacteria. In order to absorb iron from their natural environments, soil and water-dwelling bacteria need a way to dissolve the solid iron precipitates that are present there (Amor *et al*., 2020).

Humans and other mammals are similarly limited in their access to iron. Heme proteins and ferritin, an iron storage molecule, contain the vast majority of a cell's iron. Proteins bind iron firmly outside of cells. This means that pathogenic bacteria that establish infections in people or other animals must have some way to scavenge iron from these proteins and use it for their own metabolic processes (Ward and Cloonan, 2019).

Siderophores have a high affinity for iron, allowing them to bind to and transfer ferric iron ($Fe^{+}3$) from the environment, while also competing with mammalian proteins like tansferrin and lactoferrin for iron. Siderophores come in a wide variety of chemical structures, but the most common ones are hydroxamates and catecholate compounds. All eight of these molecules, despite their distinct structures, combine to create an octahedral complex with six binding locations for Fe+3 (Cain and Smith, 2021).

Some human diseases and infections might be treatable with the help of siderophores. To avoid iron toxicity, several siderophores are utilized therapeutically to treat chronic or acute iron overloaded disorders (Valkovi and Dami, 2022).

Iron overload treatment, however, must include the use of siderophores that are ineffective against infectious microorganisms. Secondly, siderophores are used in the clinic to transport antibiotics to microbes. Because they are too large to diffuse through the outer membrane porins, certain Gram-negative bacteria have developed resistance to antibiotics (Santos *et al*., 2020).

The siderophore receptor, however, allows for the entry of synthetic molecules containing both antibiotics and siderophores. One of the two main siderophores secreted by *Pseudomonas* is called pyochelin (Pch). Anaerobic bacteria like *P. aeruginosa* can absorb iron. Using a dedicated transporter in the outer membrane, it binds to iron in the extracellular media and brings it into the cell (Normant *et al*., 2021).

1.2.8 Antibiotic resistance

Traditional definitions of antibiotics refer to them as naturally occurring substances produced by microbes that have selective antibacterial activity but little impact on human cells. Their action is either bactericidal (killing bacteria) or bacteriostatic (preventing further bacterial growth) (Porras *et al*., 2020).

Until the development of synthetic antibacterial drugs, antimicrobial agents were used to refer to both natural and synthetic compounds used to combat infection. However, once the concept of antibiotics became well established, the latter term was adopted and is now commonly used to refer to all antibacterial agents (Jakubczyk and Dussart, 2020).

Opportunistic pathogen *P. aeruginosa* is of great importance. *P. aeruginosa* low antibiotic susceptibility is one of the most concerning features. A combination of factors, including the low permeability of bacterial cellular envelopes and multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (such as mexAB, mexXY etc.), contribute to the bacteria's low susceptibility to antibiotics (Qin *et al*., 2022).

P. aeruginosa also frequently acquires resistance to antibiotics through a combination of innate resistance and mutation of chromosomally encoded genes or horizontal gene transfer of antibiotic resistance determinants (Pachori *et al*., 2019).

For *P. aeruginosa* isolates to develop multidrug resistance, they must undergo a series of genetic changes, including the acquisition of mutations and/or the horizontal transfer of antibiotic resistance genes. In *P. aeruginosa* strains that cause persistent infections, hyperactive mutation aids in the selection of mutation-driven antibiotic resistance, but the clustering of several antibiotic resistance genes in integrons promotes the coordinated acquisition of antibiotic resistance determinants (Colque *et al*., 2020).

The response of *P. aeruginosa* populations to antibiotics has been studied extensively, and recent research have found that phenotypic resistance linked with biofilm development or the establishment of small-colony, variations may be significant (Xu *et al*., 2021).

First discovered in Japan in 1991, *P. aeruginosa* MBLs have since been described in other parts of Asia, Europe, Australia, South America, and even North America. Ambler class B MBLs include the IMP, VIM, SPM, GIM, and SIM enzyme families, and they hydrolyze a wide range of -lactam antibiotics. Metal chelators, such as EDTA and thiol-based compounds, can block the catalytic activity of these enzymes because of their need on zinc (Bunyan *et al*., 2018).

Genes for making MBLs are usually located on plasmids that can be transferred across organisms, but they can also be encoded in the DNA of the cell's own chromosomes. Therefore, *P. aeruginosa* isolates expressing MBL are commonly resistant to multiple categories of antimicrobial drugs, and this resistance can be passed to many types of bacteria due to the integron-associated gene cassettes (Karampatakis *et al*., 2018).

Since multi-resistant *Pseudomonas* isolates are becoming increasingly common, the use of carbapenems (such as Imipenem and Meropenem) as a last resort for treating infections is becoming increasingly common. Significant progress has been made in the treatment of life-threatening bacterial infections caused by beta-lactam-resistant bacteria since the introduction of carbapenems into clinical practice (Addis *et al*., 2021).

Reduced permeability, sustained suppression of chromosomal AmpC -lactamases, and over-expression of the up-regulating efflux system are all ways that, *P. aeruginosa* can acquire resistance to carbapenems. Carbapenem resistance among hospital-acquired *pseudomonas* has been occasionally attributed to the development of the MBLs throughout the past decade (Kunz Coyne *et al*., 2021).

The IMP and VIM kinds of acquired MBLs are by far the most prevalent and extensive, with a global distribution and multiple known allelic variations (Hussain *et al*., 2021). Nosocomial isolates of *P. aeruginosa* frequently exhibit multidrug resistance (Pachori *et al*., 2019).

The risks of death, illness, and medical bills all go up when people get infected with MBL-causing microorganisms. Because of insufficient screening recommendations, the global epidemiology of MBL-producing *P. aeruginosa* remains unclear in most countries. It has been estimated that in countries like South Korea (11.4%) and Brazil (20%), MBL-producers make up a significant part of imipenem-resistant *P. aeruginosa*. Multidrug-resistant *P. aeruginosa* is a major cause of nosocomial infections (Hawkins *et al*., 2021).

The timely isolation of MBLs is essential for effective patient care and prevention of further infection. Carbapenem-resistant *P. aeruginosa* MBL enzyme detection was accomplished by MBL screening assays, namely the imipenem/EDTA combination disc test (MBLCD), which is predicated on EDTA's capacity to sequester zinc ions and inactivate the metallo enzymes (Alsaadi *et al*., 2020).

Pathogen *P. aeruginosa* is responsible for a significant proportion of healthcare-associated illnesses. Patients in intensive care units (ICUs), burn units, and surgical wards have a higher mortality rate because it is typically multi resistant. It is a well-known pathogen in part because of its great intrinsic resistance to antibiotics; even the most modern medications can be rendered ineffective by even the slightest shift in susceptibility (Alhussain *et al.,* 2021).

Infections caused by *P. aeruginosa* are complicated by the fact that this bacteria is resistant to many commonly used medications. Extensive usage of the antimicrobial, especially Ciprofloxacin, has led to a growing occurrence of Ciprofloxacin-resistant isolates, despite the fact that Fluorquinolones had great activity against *P. aeruginosa* until the end of the 1980s (Motbainor *et al*., 2020).

It has been discovered that a significant number of *P. aeruginosa* nosocomial isolates in European hospitals are resistant to a newer generation of Fluorquinolone with both high potency and broad range antibacterial action (Qin *et al*., 2022).

Carbenicillin, Ticarcillin, Clavulanate, Piperacillin, Tazobactam, Mezlocillin, Ciprofloxacin, Gentamicin, Tobramycin, Netilmicin, Ceftazidime, and Cefeprime did not work well against *P. aeruginosa* isolates, and neither did any of the other antibiotics tested. The susceptibility profiles of strain populations isolated from different hospital wards or hospitals of varying sizes varied widely (Sun *et al*., 2019).

The incidence of Imipenem and Ciprofloxacin resistance was reported to be high in ICU isolates. Respiratory *P. aeruginosa* isolates have increased in their resistance to the antibiotic imipenem, according to a study conducted by the Centers for Disease Control and Prevention (Despotovic *et al*., 2020).

It is displays innate resistance, acquired resistance, and genetic resistance to antibiotics. Part of *P. aeruginosa* inherent resistance stems from the fact that its outer membrane is poorly permeable to many different antibiotics, allowing for a less number of those molecules to reach the bacterium's virulence factors. As a result of spending most of its time in the soil and mingling with soil-dwelling microorganisms like bacilli, actinomycetes, and molds, this organism has become resistant to many of the antibiotics produced by these microbes (Qiao *et al*., 2020).

Two new multidrug efflux mechanisms have been identified as possible contributors to the resistance. Furthermore, inducible beta-lactamase of *P. aeruginosa* is an example of an enzyme that preferentially inactivates antibiotics and hence contributes to antibiotic resistance. By exposing a strain to a set of inducing conditions, which can include antibiotic exposure, acquired resistance is induced, leading to unstable resistance but no discernible change in genotype. When the conditions that caused the resistance are no longer present, the resulting sensitivity will return to its original level (Baquero *et al*., 2021).

Mutation of a gene product or regulatory mechanism, acquisition of a drug-resistant plasmid, or the acquisition of transposable genes are all examples of genetic resistance (Partridge *et al*., 2018).

1.2.9 The function of small RNA in *P. aeruginosa*

Small regulation RNA (sRNA) is one of the important means for bacteria to adapt to environmental changes and is involved in post-transcriptional regulation, such as adaptation to stress, virulence, and biofilm formation (Wang *et al.*, 2022). Most sRNAs are between 70–140 nt in length, usually primary transcripts, and sometimes may come from the 30 terminal processing of longer mRNA precursors (Bossi *et al.*, 2020).

sRNA interacts with different target RNAs or proteins to affect their activity and function to regulate gene expression, which usually requires the participation of RNA chaperones such as Hfq and ProQ (Quendera *et al*., 2020).

The maturation and degradation of sRNAs are related to the action of ribonuclease. In another research, using high-throughput cDNA sequencing (RNA-seq), more than 500 new sRNAs were identified, significantly increasing the number of sRNAs found in *P. aeruginosa* (Chihara *et al*., 2019). The present study only recognized the functions of some sRNAs, but little is known about the regulatory networks of these sRNAs and the functions of other uninvestigated sRNAs.

1.2.10 Control of antibiotic resistance by using micro RNA

MicroRNAs (miRNAs) are a group of short RNAs encoded by the genome that function to control the expression of cellular messenger RNAs (mRNAs) that include partial or complete microRNA binding sites. A capped, polyadenylated primary miRNA (pri-miRNA) transcript is transcribed by RNA polymerase II as the first step in the conventional pathway of microRNA expression (Bitetti *et al*., 2018).

RNase III proteins play key roles in microRNA (miRNA) biogenesis. The nuclear RNase III Drosha cleaves primary miRNAs (pri-miRNAs) to release hairpin-shaped pre miRNAs that are subsequently cut by the cytoplasmic RNase III Dicer to generate mature miRNAs (Hajieghrari and Farrokhi, 2022).

Next, one strand of the unwinding microRNA duplex is incorporated into the RNA-induced silencing complex (RISC), which includes a protein called Argonaute and another protein called GW182 (also known as TNRC6a) (Tilley, 2020). Repression of translation and enhancement of target mRNA degradation follow from binding of microRNA-loaded RISC to the cellular mRNA target sites. It is unknown how many of the projected 600 miRNA targets are physiologically relevant, but considering that over 1,000 miRNAs have been annotated so far in the human genome, it is reasonable to assume that miRNAs regulate at least a significant portion of the human transcriptome (Makarova *et al*., 2021).

Because many mRNAs are targeted by more than one miRNA, regulatory complexity is multiplied. Development, cellular differentiation, proliferation, apoptosis, metabolism, and immunological response are only some of the processes that have been found to be under the regulatory influence of miRNAs (Tilley, 2020).

Consistent with the hypothesis that miRNAs play an important role in regulating gene expression, aberrant microRNA expression has been linked to numerous illnesses, such as cancer, neurodegenerative diseases, and cardiovascular disorders (Ruiz *et al*., 2021).

It is now becoming obvious that miRNAs play critical roles during microbial infections in addition to their well-established functions in physiological and pathological processes. Major threats to human health are posed by infectious diseases caused by a wide variety of microorganisms, including viruses, bacteria, parasites, and fungi (Catanesi *et a*l., 2020).

Despite significant progress in knowledge of microbial pathogenesis, significant obstacles remain in the way of effective prevention and treatment of infectious diseases (Klein and Hultgren, 2020). However, these same results imply that parasites change the expression of most miRNAs. The host microRNA response to bacterial pathogens has been less studied than its counterpart to viral and parasitic infections (Giri *et al*., 2019).

Bacterial pathogens, during the course of an infection, interfere with a wide variety of host cellular activities in order to ensure their own survival and proliferation. Bacterial effector proteins delivered into host cells via specialized protein secretion systems are responsible for a variety of manipulations, including favorable reorganization of the host's cytoskeleton as well as modulation of signal transduction pathways, membrane trafficking, and pro-inflammatory responses (Medina *et al*., 2022). But different pathogens use various molecular techniques to thwart the intricate circuits that control the host immune response. The discovery that Arabidopsis thaliana's FLS2 receptor senses flagellin from the external pathogen *Pseudomonas* and induces transcription of miR-393a laid the groundwork for the study of microRNA activities in the immune response to bacterial infection (Kim *et al*., 2020).

Pseudomonas, like virus-encoded suppressor proteins, secretes effector proteins into the host cell in order to counteract the host's short RNA-directed basal immunity. In this review, we focus on infections of mammalian host cells and give an overview of the current studies on the role of miRNAs in the response to bacterial pathogens (Muhammad *et al*., 2019).

1.2.11 Novel antimicrobial treatments based on miRNA-based technology

The use of RNA-based technologies as a method of combating bacterial infections is gaining ground. Using siRNAs directed at tfgb1 has shown effective in combating pulmonary tuberculosis in preclinical models (Mourenza *et al*., 2022).

However, several of the miRNAs mentioned above, as well as many others, present new prospects for the development of antimicrobial methods. Moreover, the pathogen's host colonization could be slowed or even halted by the development of anti-miRNAs that target particular miRNAs that aid bacterial infection. Anti-miRNAs are synthetic single-stranded RNAs designed to inhibit the activity of miRNA targets (Cai *et al*., 2021).

The similar method might be used to inhibit miRNAs needed by bacterial intracellular pathogens for their own replication. On the other hand, miRNA's anti-infective potential faces significant obstacles in the clinic (Streicher, 2021).

Off-target effects are the biggest problem with miRNA treatments. This may be because miRNAs have major off-target effects when they interact with partially complementary mRNAs. Furthermore, the presence of RNAses that can rapidly degrade miRNAs may hinder their distribution to infected cells. Enhancing the distribution strategy of microRNA stores to each specific targets at the cellular or even subcellular levels may help alleviate this problem. Several strategies have been developed to address this issue, such as the use of nanoparticles, viral delivery systems, high-density lipoproteins, liposomes, or exosomes to aid in their transport to and uptake by host cells (Mourenza *et al*., 2022).

When it comes to non-viral delivery techniques in the clinic, lipid nanoparticles are now the gold standard. A class of lipid particles called liposomes is often employed to direct RNA-based therapeutics (Aldosari *et al*., 2021).

However, liposomes' fundamental drawback is that their lipid bilayer is notoriously difficult to functionalize. As a result, extracellular vesicles made by the body are being looked at as a promising new way to transport miRNA (Wang *et al*., 2021).

Exosomes, in particular, are gaining popularity as a remedy because they are a normal aspect of intercellular communication and so neither toxic nor highly antigenic. Exosomes are vesicles that are produced in the endosomal system and subsequently released to the extracellular milieu. They play crucial roles in cell-to-cell communication. Possibly, exosomes effectively shield miRNA molecules from nucleases (Buratta *et al*., 2020). This has led to a surge in their application in the distribution of nucleic acid-based therapies. Additionally, exosomes have benefits over other delivery techniques, such as those based on adenoviruses, which may be neutralized by antibodies. Exosomes, like other RNA-delivery mechanisms, need to be tweaked to selectively kill infected cells (Saad *et al*., 2021).

Tetraspanins, integrins, lipids, lectins, heparan sulfate proteoglycans, and components of the extracellular matrix are the primary determinants of the interactions between exosomes and their target cells. Exosome isolation has been shown to enhance cellular fusion with the original parent cells. This means that the anti-cancer medications were delivered precisely where they were needed thanks to the isolation of exosomes produced by tumor cells (He *et al*., 2022). Modifications to the transmembrane proteins found on the surface of exosomes also improve their ability to adhere to their intended targets. Additionally, encouraging in vitro and in vivo outcomes were seen when rationally designed exosomes with varied membrane modifications were used to treat cancer (Salunkhe *et al*., 2020).

As a result of increased transport from endosomes to the cytosol of target cells, carbonate apatite or glycan polymers have been shown to improve target cell selectivity. Therefore, similar strategies, such as poly-L-lysine-lactose and carbonate apatite, could be used to target bacterial-infected cells in the liver. However, exosomes are still in the early phases of research as an efficient RNA-delivery mechanism to treat bacterial infections (Zhuang *et al*., 2020). There are two sources of exosomes during a bacterial infection: the invading pathogen and the host eukaryotic cells. In addition, exosomes produced by cells primed with bacterial lipopolysaccharide (LPS) may be able to more precisely target and activate a subset of macrophages. As a result, the lowest inhibitory concentration of exosomes needed to stop host cell infection induced by intracellular pathogens may be reduced, and the exosomal-delivery specificity of short RNAs may be improved (Salunkhe *et al*., 2020). However, further study is required to design a tiny RNA delivery system that is effective, scalable, easily produced, stable, and specific enough to be exploited in the setting of bacterial infection (Zhuang *et al.*, 2020).

1.2.12 As1974

Current studies found that at least six sRNAs are involved in the regulation of drug resistance in *P. aeruginosa*, and there are differences in the regulatory mechanisms of different sRNAs (Zhang *et al*., 2017). These mechanisms are as follows; the sRNA AS1974 is a major regulator to control the expression of multiple resistance pathways, including membrane transporters and biofilm-associated antibiotic resistance genes. The sRNA AS1974 can transform drug resistant strains into antibiotic sensitive ones (Law et al., 2019).

As1974 is the master regulator to moderate the expression of several drug resistance pathways, including membrane transporters and biofilm-associated antibiotic-resistant genes, and its expression is regulated As1974 expression is regulated by methylation in all MDR strains examined. It can down regulated in all drug- resistant strains compared with all drug-susceptible strains. Overexpression of As1974 was able to transform a MDR clinical strain to drug hyper susceptibility. Its bind to Hfq with high specify (Law, 2020).

1.2.13 Hfq

The Hfq protein is a global small RNA chaperone that interacts with regulatory bacterial small RNAs (sRNA) and plays a role in the post-transcriptional regulation of gene expression. The roles of Hfq in the virulence and pathogenicity of several infectious bacteria have been reported (Desnoyers *et al.*, 2013).

The Hfq RNA chaperone is a ubiquitous protein found in the majority of sequenced bacterial species. It was first identified as a bacterial host factor required for bacteriophage Qβ RNA replication in *Escherichia coli* (Altuvia *et al.*, 2018).

Hfq plays several roles, such as binding bacterial small RNA (sRNA) and messenger RNA (mRNAs), and facilitating RNA-RNA interactions. Moreover, it has been reported that Hfq can influence polyadenylation of mRNAs and act independently as a regulator of gene expression (Dos Santos *et al*., 2018).

In addition to RNAs binding, Hfq regulates the expression of various bacterial genes at the post-transcriptional level, and directly or indirectly affects a number of proteins and controls the ribo-regulation of the expression of multiple genes (Mediati *et al*., 2021).

A number of bacterial species, such as *Staphylococcus aureus* and *Bacillus subtilus,* have only one *hfq* gene, while some species, such as *Burkholderia* spp. and *Novosphingobium aromaticivorans* have two *hfq* genes, In *Bacillus anthracis*, three *hfq* gene sequences were identified, and their functional properties were investigated (Kim *et al*., 2018).

The construction of an *E. coli* mutant by *hfq* insertion mutations demonstrated the importance of Hfq. The mutant exhibited pleiotropic phenotypic changes, including reduced growth rates, altered cellular morphology, and improved cellular sensitivity to stress (Deng *et al*., 2016).

Furthermore, Hfq in *E. coli* was shown to be an essential element in the translation of *RpoS*, which encodes the σ_S subunit of the RNA polymerase, the master regulator for many stress-regulated genes. Hfq was also found to contribute greatly to the translational regulation of several other mRNAs, by assisting sRNA binding (Hetrick, 2013).

Moreover, Hfq has also been found to be responsible for the regulation of virulence in several animal-pathogenic bacterial species such as *Vibrio cholerae*, *Yersinia pestis,* and *Pseudomonas aeruginosa* (Kim *et al*., 2018).

Chapter Two

Materials and Methods

Chapter Two: Materials and Methods

2.1 Materials

2.1.1 Laboratory instruments and equipment

The main scientific apparatus, and technical instruments with disposable materials were listed in Table (2-1) and Table (2-2).

Table (2-1) Laboratory instruments and Equipment

NO.	Equipment & instruments	Company / Country
1.	Autoclave	HIRAYAMA / USR
2.	Burner	Memmert/ Germany
3.	Cabinet hood	BioLAB/ Korea
4.	Centrifuge	Hettich/Germany
5.	Incubator	Memmert GmbH/ Germany
6.	Light Microscope	Olympus/ Japan
7.	Refrigerator	Al Balsan / Turkey
8.	SaCycler-96 Real Time PCR SYSTEM	Sacace/ Italy
9.	Sensitive balance	Sartorius /Germany
10.	Shaker Water Bath	Julabo /Germany
11.	Vortex	Fisher Scientific/ USA

Table (2-2): Technical Instruments and Disposable Materials

Item	Company/ Country
Beakers, Flasks and Cylinders (Different sizes)	Superior/ Germany
Disposable syringes (5ml)	Mediplast /U.A.E
Eppendorf tubes (1.5 ml)	Sigma-Aldrich /USA
Micropipettes (Different sizes)	Eppendroff/ Germany
Millipore 0.22 µm	Bio-Hit/ Finland
Parafilm, Test tube & Eppendorff (12 ×8) Racks	ParafilmM/USA
Petri dish, Tips (Different sizes)	Sterial/Jordan
Plane tubes (10ml), Wooden stick	DMD-DISPO/ Jordan
Platinum wire loop, Platinum wire needle, forceps	Himedia/India
S wabs andTransport media Swabs	AFCOSWAB /Jordan
Slides and Coverslips, plastic and glass containers	OEM/ China

2.1.2 Chemical and biological materials

2.1.2.1 Chemical materials

The chemical material used in this study were listed in Table (2-3).

Table (2-3) Chemical Materials

Chemicals	Company/country
99%, 95% and 70% alcohol (Ethanol), Carbohydrates (glucose, maltose, lactose), Alpha-nephthol, P-Dimethyl aminobenzaldehyde, Peptone, Gelatin, Methyl red.	Flukachemika/ Switzerland
Gram Stain kit	Crescent/ KSA
Catalase reagent	Schuchariot/ Germany
Oxidase reagent, Phosphate buffer	Himedia / India
Normal saline	Pharmaline, Egypt

2.1.2.2 Biological materials

Several types of culture media are used in the current study listed in Table (2-4). All media were prepared according to the manufactures instructions, after sterilized by autoclaving (121C°-15 pound /inch² for 15 min). Culture media incubation was done for (24) hrs. After pouring it in sterile plates. It was done to avoid any contamination if present, and then stored at (4) C° until use.

Table (2-4) Culture media used in identification and isolation of *Pseudomonas aeruginosa.*

No.	Media	Purpose	Company/Origin
1.	Blood agar medium	Enrichment medium	Accumix™/India
2.	MacConkey agar medium	Differential medium	Accumix™/India
3.	Brain heart infusion agar broth	Activated medium	BIOMARK/India
4.	Nutrient agar medium	Cultivation medium	HIMEDIA/India
5.	Nutrient broth	Grow and preserve bacterial isolates	Accumix™/India
6.	Chromogenic agar	Selective medium	HIMEDIA/India
7.	Urea agar medium	Ability of bacteria to produce urase enzyme	HIMEDIA/India
8.	Peptone water agar medium	Ability of bacteria to decompose amino acid tryptophan to indole	Accumix™/India
9.	Motility agar medium	Ability of bacteria to motility	HIMEDIA/India
10.	Kligler iron agar medium	used for determining glucose and lactose fermenter isolates and possible hydrogen sulfide (H2S) production	AccumixTM/India

2.1.3 Molecular materials

Table (2-5) Molecular materials used in the present study

Biological Materials & Chemicals	Company\Country
EasyScript® First-Strand cDNA Synthesis SuperMix	Transgen/China
GoTaq® qPCR Master Mix	Promega, USA
FavorPrep Blood/ Cultured Cell Total RNA Mini Kit	FAVORGEN / Korea

2.2 Methods

2.2.1 Patients and Specimens collection

Two hundred (200**)** clinical samples (mid-stream urine samples) were collected in sterile screw-cap containers from patients suffering from urinary tract infections (UTIs) of both gender, (129**)** urine samples from female patients and (71**)** from male patients, aged 17 to 53 years, they were diagnosed with UTIs by the physicians on the basis of symptoms, whom attending to Baghdad Teaching Hospital, Ghazi Hariri Hospital, Central Laboratories in Medical City and Al-Yarmouk hospital in Baghdad /Iraq, from October (2022) to June (2023). Each specimen was immediately transferred under cooling conditions to the laboratory for analysis.

2.2.2 Collection of blood samples

A five millimeter of venous blood samples were down from 30 patients with severe UTI, the blood was used for study of microRNA in blood.

2.2.3 Ethical approval

All subjects involved in this work were informed and the agreement required for doing the experiments and publication of this work was obtained from each one prior the collection of samples. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee (at College of Medicine University of Babylon).

2.2.4 Study design

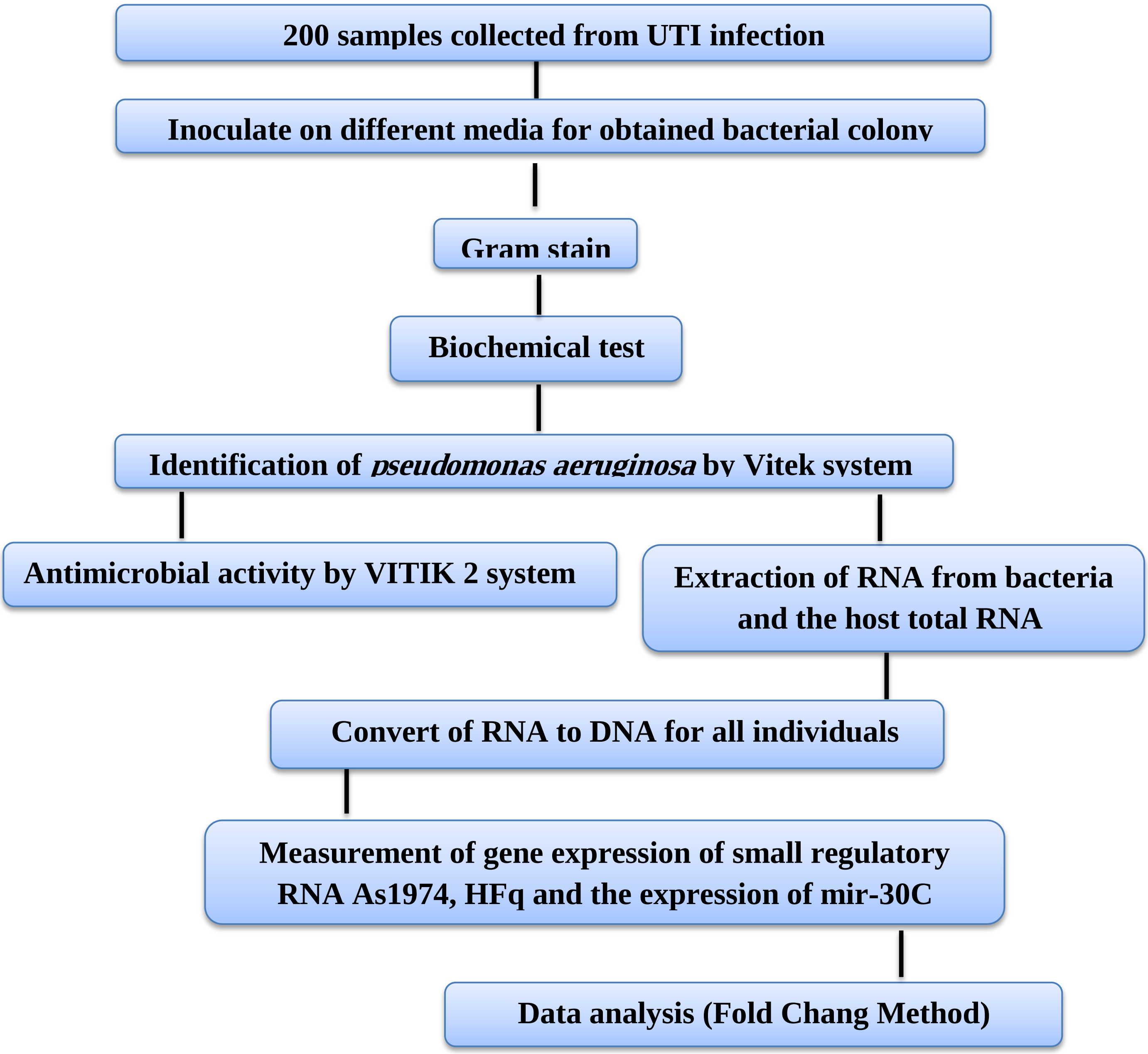


Figure (2-1): Scheme of study design

2.3 Preparation of stains, solutions, buffers and reagents

The Stains, Solutions, Buffers and Reagents were used in this study are listed in Table (2-6).

Table (2-6): The stains, solution and reagents used in this study

No.	Subject	Purpose	Reference
1.	Gram's stain solutions	Morphology and its arrangement of bacterial cells	Forbes *et al*., (2007)
2.	Hydrogen peroxide solution (H_2O_2)	Detection of ability of bacteria to produce catalase	Collee *et al*., (2006)
3.	McFarland Standard Solution	results in turbidity approximately equal to bacterial cells density of 1.5× 108 cell/ml	Baron *et al*., (1994)
4.	Oxidase reagent	used as an indicator in oxidase test	McFadden, (2000)
5.	Catalase reagent	used to identify bacterial ability to produce catalase enzyme	McFadden, (2000)
6.	Kovacs reagents	to detect the indole production	McFadden, (2000)
7.	Methyl red reagent	It was used to detect complete glucose hydrolysis	McFadden, (2000)
8.	Vogues – Proskauer reagent	It was used to detect partial glucose hydrolysis	Collee *et al*., (1996)

2.4 Isolation and identification of *Pseudomonas aeruginosa*

All urine samples firstly were centrifuged at 1,500 rpm for 5 minutes, then removing the supernatant, the pellet was cultured in a Brain heart infusion (B.H.I) broth medium and incubated at 37 ºC for 24 hours to promote bacterial growth on nutrient agar medium. After that they were streaked on the general and differential culture media, also incubated for 24 hours at 37 ºC. Lactose non- fermenting colonies that were streaked on MacConkey agar picked and re-cultured on fresh MacConkey agar plates to obtain pure well isolated colonies. The obtained colonies were streaked on cetrimide agar to investigate the produce of fluorescein and pyocyanin dyes and the plates were incubated for another 24hrs at 37ºC. The positive growth was diagnosed depending on morphological (Shape, size, margin, consistency, texture, edges, heights and color of colonies), microscopical features and biochemical tests; also, it was used vitek 2 system in the identification of bacterial growth.

2.5 Laboratory diagnosis

According to (Baron, 1994; Collee *et al*. 1996 and McFadden, 2000), the isolation and identification of *pseudomonas aeruginosa* were performed as follows:

2.5.1 Morphological tests

2.5.1.1Colonial morphology and microscopic examination

A single colony was taken from each primary positive culture. Its identification depended on the morphology properties (colony size, shape, colour, and translucency, edge, and elevation of texture). The colonies were then investigated by gram stain to observe bacterial cells. Specific biochemical tests and Vitek system were done to reach the final identification.

2.5.2 Biochemical tests

2.5.2.1 Oxidase test

A colony growing on nutrient agar medium for 24 hours was transferred onto sterilized filter paper by wooden chopsticks, then a drop of oxidase reagent is added to it, and the color change to purple in (30-60) seconds is evidence of the production of the oxidase enzyme (Hemraj *et al*., 2013).

2.5.2.2 Catalase test

A colony growing on nutrient agar medium for 24 hours was transferred onto a sterile, dry glass slide with wooden chopsticks, then one drop of 3% catalase reagent was added to it. The appearance of bubbles on the surface of the glass slide is evidence of the production of the enzyme catalase (Hemraj *et al*., 2013).

2.5.2.3 IMVIC tests

2.5.2.3.1 Indole test

This test was carried out by inoculating test tubes containing peptone water medium with bacterial colonies, and then incubating for 24 hours at a temperature of 37 ºC, then 1-2 drops of Kovacs reagent were added to the medium. The appearance of a red ring on the surface of the median indicates a positive test (Hemraj *et al*., 2013).

2.5.2.3.2 Methyl red test

This is a qualitative test of the acidity. The tubes of the (MR-VP broth) was inoculated with the selected bacterial colonies and incubated at 37^{o}C for 24 hrs. Then (5 drops) of methyl red reagent are added to it. The cultured MR-VP broth was tested for complete fermentation of glucose, which low pH down to 5, a bright red color is a positive test, and a yellow or orange color is a negative test (Hemraj *et al*., 2013).

2.5.2.3.3 Voges –Proskauer test

This test is used to detect bacterial ability to ferment glucose and produce acetoin. The tubes of (MR-VP broth) are inoculated with tested bacterial culture and are incubated at 37 ^{o}C for 24 hrs., then the result is read by adding 0.6 ml of VP1 reagent which is a (α- naphthol dissolved in Absolut ethanol) and 0.2 ml of VP2 reagent which is a (KOH dissolved in distilled water; appearance of red color after 15 min. due to partial analysis of glucose, which produce acetoin or acetyl Methyl–Carbinol (Hemraj *et al*., 2013).

2.5.2.3.4 Citrate utilization test

This test was used for the purpose of knowing the ability of bacterial isolates to consume citrate as the sole source of carbon and energy and on the consumption of ammonium salts as a source of nitrogen .This assay was performed by streaking a loopful of colony onto a petri dish with simmon citrate medium or simmon's citrate agar slant then incubating for 24-48 hours at 37°C.The change of the color of the medium from green to blue with the appearance of bacterial growth indication of a positive result while green color of the medium remains the same it is negative result (Hemraj *et al*., 2013).

2.5.2.4 Hemolysin production test

Bacterial isolates were cultured on plates containing blood agar medium, the plates were incubated at 37°C for 24 hours, then the results were read by noting the type of hemolysis (alpha, beta or gamma) (Hemraj *et al*., 2013).

2.5.2.5 Kliger's iron test

This test was used for the differentiation of members of the Enterobacteriaceae based on the fermentation of lactose, glucose, and the production of H_2S. Kligler iron agar (KIA) slant prepared was inoculated with tested bacterial culture by streaking on the surface and stabbing in bottom of the medium, incubated at 37°C for 24hrs., the positive result was noticed by changing the color from red to yellow as a result for acid production from sugar fermentation ,the results of sugar fermentation during this test are read as in Table (2.7), space at the bottom of the tube and the split in the agar in the middle of the tube indicates gas (CO_2) production from sugar aerobic fermentation, black residue appeared at the bottom of the tube indicates hydrogen sulfide (H_2S) production .Sometimes the butt portion will be entirely black, in such a case, it is assumed that the butt portion of the tube is acidic (yellow color is masked due to the H_2S production).

While the negative result observed by keeping the medium on its red color, bubbles and black residue doesn't form.

Table (2-7): Indicator results for sugar fermentation during Kliger's iron test

NO.	Slant Color	Bottom Color	Fermentation Result
1.	Red (Alkaline Slant)	Yellow (Acid Bottom)	(K/A) Only Glucose Fermentation (positive result)
2.	Yellow (Acid Slant)	Yellow (Acid Bottom)	(A/A) Glucose & lactose Fermentation (positive result)
3.	Red (Alkaline Slant)	Red (Alkaline Bottom)	(K/K) No Fermentation (negative result)

2.5.2.6 Urease Production Test

This test was used to detect bacterial capacity to produce urease enzyme which hydrolyze urea to ammonia and carbon dioxide. Urea agar slant was inoculated by streaking with a sterile loop with tested bacteria and incubated at 37ºC for 24hrs. Pink color indicates positive result for the test; while yellow color indicates negative result (MacFaddin *et al*., 2000).

2.5.2.7 Motility test

This test was done by using the tube that contained semisolid media (The media were inoculating with tested bacteria by a stabbing method and is incubated at 37ºC for 24hrs. the disseminating of growth out of the stab line was an indication of positive result (Aygan and Arikan, 2007).

2.6 Identification of *P. aeruginosa* by VITEK $_2$ – Compact system

The identification of *P. aeruginosa* was confirmed using of VITEK 2-Compact which represent an advanced colorimetric technology for bacterial identification, Gram negative (GN) card was used for this purpose for *ps. aeruginosa* Identification.

Procedure:

All the following steps were done according to the manufactures instructions (Biomerieux, France).

1. Three ml of normal saline was placed in plane test tube and inoculated with a lope full of isolated colony
2. The test tube was inserted into the Dens Check machine for standardization of the colony to McFarland standard solution ($1.5X10^{8}$cfu/ml).
3. The standardized inoculum was placed into the cassette and a sample identification number entered into the computer software via barcode.
4. The VITEK-$_2$ card type was then read from barcode placed on the card during manufacture and the card was thus connected to the sample identification port.
5. The cassette was placed in the filter module, when the card were filled, transferred the cassette to the reader/ incubator module.
6. All the subsequent steps were handled by the instruments, the instrument controls the incubation temperature, the optical reading of the cards and continuously monitors and transfers test data to the computer for analysis. When the test cycle was completed, the system automatically ejected the cards into a waste.

2.7 Detection of antimicrobial activity using theVitek 2system

This system was used according to manufacturing company (bioMérieux, France) by inoculation of bacteria on MacConkey agar plate, then incubation at (37ºC /24 hours). A bacterial suspension was prepared from the above growth by transferring 1-3 colonies to test tubes containing (3ml) from normal saline and the suspension turbidity was adjusted to a McFarland standard of (0.5) (Fritsche *et al*., 2011).

2.8 Molecular study

2.8.1 Primers used in the study

Table (2-8): The sequence of primers that used this study.

Primer	Sequence	Primer sequence 5'- - 3'	Tm (°C)	GC %
HFQ	F	GTCAAAAGGGCATTCGCTACA	59.7	48
	R	ATAGATGGAAACCGGGACGC	62.4	55
As1974	F	CTGCAAGGCCAGATCGAGT	62.2	58
	R	CTCGGTACCACGGTGGAGAT	64.8	60
mir-30	F	TGTAAACATCCTACACTCTCAGC	57.9	43
	R	ATGGCGGTAAGTCCAGATACG	61	52
Adapter	F	GCATAGACCTGAATGGCGGTAAGGGTGTGG	70	38
	R	TAGGCGAGACATTTTTTTTTTTTTTTTTTTT		
16s RNA	F	CAGCTCGTGTCGTGAGATGT	62	55.0
	R	G CGTAAGGGCCATGATGACTT	60	50
GAPDH	F	CACTAGGCGCTCACTGTTCTC	60	60
	R	AATCCGTTGACTCCGACCTT	59	**59**

2.8.2 FavorPrep Blood/ Cultured Cell Total RNA Mini Kit

1. Red blood cells lysis

1.1 200 ~ 300µl of anticoagulant-preserved fresh human whole blood was added to a micro centrifuge tube (1.5 ml tube).

1.2 1ml volume of RL Buffer was mixed with 200µl of the sample and mix well by inversion.

1.3 It was incubated on ice for 10min. Vortex briefly 2 times during incubation.

1.4 Then, it was centrifuged for 1 min at 4,500 rpm to form a cell pellet and discard the supernatant completely.

1.5 600µl of RL Buffer was added to re-suspend the cell pellet by briefly vortexing.

1.6 Then, it centrifuged for 1min at 4,500 rpm to form a cell pellet again and discard the supernatant completely.

2. 350µl of FARB Buffer and 3.5µl of ß-Mercaptoethanol were added to the cell pellet. Vortex vigorously for 1 min to re-suspend the cells completely.
3. A filter column was placed to a collection tube and transfer the sample mixture to the Filter Column. Centrifuge at full speed (18,000 rpm) for 2 min.
4. The clarified supernatant was transferred from the collection tube to a new micro centrifuge tube (not provided), and measure the volume of the supernatant.
5. 1 volume of 70 % RNase-free ethanol was added, and mixed well by vortexing.
6. A FARB mini column was placed to a collection tube, and transferred the ethanol added sample mixture (including any precipitate) to the FARB Mini Column. Then, it was centrifuge at full speed for 1 min, discard the flow-through and return the FARB mini column back to the collection tube.
7. 500 µl of Wash Buffer 1 was added to the FARB mini column, then, centrifuged at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
8. 750 µl of wash buffer 2 was added to the FARB mini column, then, centrifuge at full speed for 1 min and discard the flow-through and return the FARB mini column back to the collection tube. Note: Make sure that ethanol has been added into wash buffer 2 when first use.
9. Step 8 was repeated for one more washing.
10. The FARB Mini Column was centrifuged at full speed for an additional 3 min to dry the FARB mini column. -- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

11. The FARB mini column was placed to an elution tube (provided, 1.5 ml microcentrifuge tube).

12.40 ~ 100 µl of RNase-free ddH2O was added to the membrane center of the FARB mini column. The FARB Mini Column was Stand for 1 min. -- Important Step! For effective elution, it was make sure that, RNase-free ddH2O is dispensed on the membrane center and is absorbed completely. -- Important: it wasn't elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will be lower the RNA yield.

13. The FARB mini column was centrifuged at full speed for 1 min to elute RNA.

14. RNA was stored at -70C.

2.8.3 EasyScript® First-Strand cDNA Synthesis Super Mix Cat. No. AE301

2.8.3.1 Description

EasyScript® First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from total RNA or mRNA. The cDNA is efficiently synthesized by EasyScript® RT/RI Enzyme Mix and 2×ES Reaction Mix.

1. RNase H activity was deficiented to reduce RNA template degradation during the first-strand cDNA synthesis.
2. The product obtained from 15 minutes reaction was used for qPCR.
3. The product obtained from 30 minutes reaction wasused for PCR.
4. Anchored Oligo (dT), Primer was specifically designed to bind to the first base next to mRNA Poly(A)* on the 5' end with high specificity, ensuring high efficiency, and success rate of first-strand cDNA synthesis.

5. Random Primer (N9) or Gene Specific Primer (GSP) could be used to synthesize the first-strand cDNA.
6. cDNA up to 8 kb.

2.8.3.2 Protocol

1. A new sterile 0.2 tube have prepared for each sample and the following components were added.

Table (2-9): Components and volume of protocol of cDNA Synthesis Super Mix

Component	Volume
Random Primer(N9)	1 ul
2×ES Reaction Mix	10 ul
EasvScript®RT/RI Enzyme Mix	1 ul
RNase-free Water	Up to 20 ul
Eluted RNA	5 ul

Incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) then incubate at 85°C for 5 second**.** A Quantitative RT-PCR (qPCR) was performed using Luna Script RT Master Mix Kit (5X) (BioLabs, England), the RT-PCR mixture was contained 10 µl of the master mix, 0.5 µl of each forward Primers and reverse primers were added, 5 µl of cDNA from each sample was add, then the volume was completed to 20 µl by addition of 4 µl of nuclease free water. The GAPDH gene was used as the endogenous control. A qPCR was performed at 95°C for 1 minute for polymerase activation followed by 45 cycles of 95°C for 15 seconds for denaturation the double stranded cDNA and 60°C for 20 sec annealing steps with channel scanning, melting curve analysis was performed based of the separation characteristics of double stranded cDNA during cycles with increasing denaturing tm.

Ct value of target miRNAs was standardized to GAPDH reference gene and the expression of miRNAs was determined by the relative quantitative method using the comparative Ct formula: Folding $=2^{\wedge -\Delta\Delta CT}$

Table (2-10): Reagent and volume

Reagent	Volume 20 µl
GoTaq® qPCR Master Mix (2X)	10 µl
Forward Primer (20X)	0.5 µl
Reverse Primer (20X)	0.5 µl
Supplemental CXR Reference Dye	0.2µl per reaction
cDNA	5 µl
Nuclease-Free Water	to a final volume of 20µl

The reaction have been mixed thoroughly, and centrifuged briefly to collect solutions at the bottom of PCR tubes or plates, and then have been stored on ice protected from light. Then after the preparation have finished the tubes placed into the thermal cycler which have been programmed as follow.

Table (2-11): PCR Thermocycler Conditions

qPCR Steps	Temp.	Time	Cycle(s)
Initial activation	95℃	1min	1
Denaturation	95℃	15 sec	45
Annealing	60℃	20 sec	
Extension	90℃	15 sec	

The GAPDH gene was used as the endogenous control for urine and blood samples and 1*6SRNA* for bacteria. A qPCR was performed at 95°C for 1 minute for polymerase activation followed by 45 cycles of 95°C for 15 seconds for denaturation the double stranded cDNA and 60°C for20 min annealing steps with channel scanning, melting curve analysis was performed based of the separation characteristics of double stranded cDNA during cycles with increasing denaturing tm.

Ct value of target genes (HFQ, AS1974 and microRNA-30) were standardized to 16SRNA and GAPDH reference gene and the expression of target genes were determined by the relative quantitative method using the comparative Ct formula and folding =2^-ΔΔCT analysis.

2.9 Statistical analysis

Statistical analysis was carried out using SPSS version 25. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SE). Student t-test was used to compare means between two groups. A p-value of ≤ 0.05 was considered as significant (George and Mallery, 2019).

Chapter Three

Results and Discussion

Results and discussion

3.1 Data description of study population

Patients with urinary tract infections ranging in age from 17 to 53 had their urine sampled; as shown in Figure (3-1), 129/200 (64.5%) of those patients were female, while 71/200 (35.5%) were male.

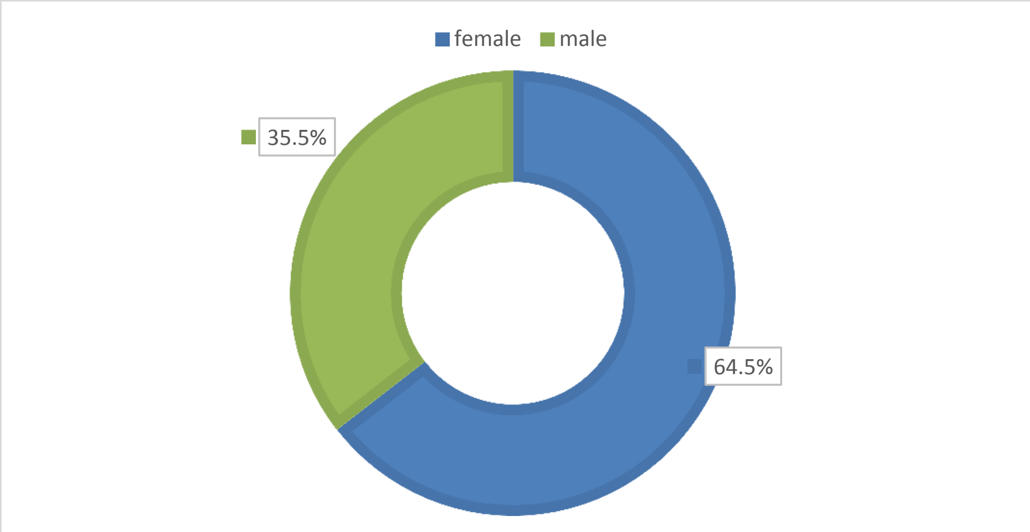


Figure (3-1): Frequency of patients according to gender

The current study's findings are consistent with those of AL-Khikani and Ayit (2019), who reported that female were more likely to experience UTI inflammation than male were (70% vs. 30%), and that the age range between 21 and 30 had the highest prevalence of UTIs. Researchers Almalki and Varghese, (2020) observed that, *P. aeruginosa* produced an infection rate of 53(36.05%) among those aged 11–20, the age group with the highest risk of infection with bacteria that caused UTI infection. In contrast, *P. aeruginosa* was isolated from UTIs by Alfa *et al*., (2022), who found that, females were more likely to be infected than males were (64.91% vs. 35.09%).

This study's findings were consistent with those of numerous other research, both locally and internationally, which have found that, *P.*

aeruginosa is the most often isolated bacterium from UTIs (in more than 30% of all samples).

Clinical sample isolation rates for *P. aeruginosa* have been reported in research to fluctuate between (20%) and (60%) from (2015) to (2020), Almangour *et al*., (2022). Considering that bacterial infections tend to spread more rapidly in people with weakened immune systems, it's possible that a lack of immunity is to blame for the broad variation in the severity of infections.

The patient may also contract *P. aeruginosa* due to the high prevalence of hospital-acquired bacterial infections. Antimicrobial resistance compounds the challenges of treating and eliminating this opportunistic infection because of the persistence of this bacterium (Choi *et al*., 2018). The number of patients sampled, where they were sampled from, and the method used to sterilize wounds and burns, and the number of times they were sterilized are all factors that could play a role (Pachori *et al*., 2019). Thus, since the patient's arrival at the hospital, the level of health care must be high, as the patient's stay in the hospital for several days for the purpose of receiving treatment may increase the rate of bacterial infection due to infection acquired from hospitals either directly from contact with patients or indirectly through the use of contaminated surgical tools (Zhou *et al*., 2018).

3.2 Isolation and identification of *P. aeruginosa*

3.2.1 Bacterial isolates

Two hundred urine samples were taken from patients of varying ages and sexes who had UTIs. Figure (3-2) shows that when urine samples were cultured for *P. aeruginosa* isolation and identification, 148/200

(74%) revealed positive bacterial cultures, while 52/200 (26%) showed no growth.

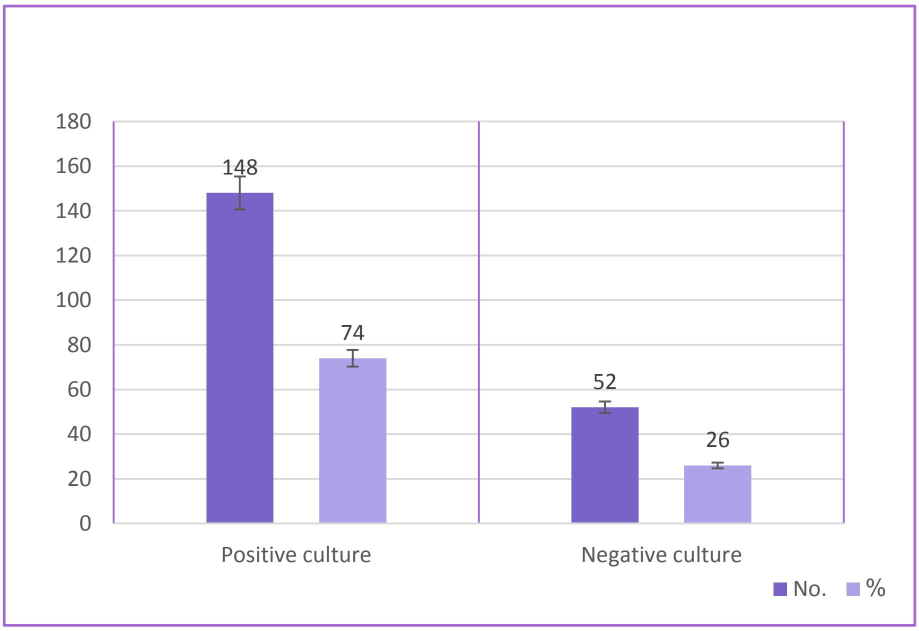


Figure (3-2): Distribution of samples according to positive & negative culture

As can be shown in Table (3-1), only 56/148 (37.84%) of the positive culture isolates belonged to *P. aeruginosa*, while 92/148 (62.16%) belonged to other genera of bacteria.

Table (3-1): Bacterial isolates recovered from the samples

Bacterial isolates	No.	%
Pseudomonas aeruginosa	56	37.84%
Others	92	62.16%
Total	148	100%

These findings corroborate a research by Lob *et al*., (2019) which found that, *P. aeruginosa* was isolated from 29% of UTI. Most *P. aeruginosa* isolates (36.9%) were determined to have originated from urinary tract infections, according to research by Garca-Fernández *et al*. (2020). Abdel Khalek *et al*. (2020) found, however, that *P. aeruginosa* isolates were obtained from UTI (36.9%). The majority of *P. aeruginosa* isolates (19.6%) were collected from urine. Kresken *et al*., (2020) research also shows that, the highest rate of obtaining *P. aeruginosa* from samples was from urine (95.7%).

In order to establish an infection, *P. aeruginosa* typically needs a significant rupture in the skin, which is the body's first line of protection against germs (Ladhani *et al*., 2021). When the skin or mucosal barriers are broken, bacteria and viruses can easily enter the body (e.g., trauma, surgery and serious burns), infections caused by *P. aeruginosa* after a burn were prevalent (Bunyan *et al*., 2019). Burn exudates, surgical wound exudates, and urine samples were the most common sources of *P. aeruginosa* infections, followed by ear discharges, making it one of the most commonly isolated nosocomial bacteria in hospital settings (Morin *et al*., 2021).

P. aeruginosa is one of the most widely distributed bacterial infections in healthcare facilities. *P. aeruginosa* remains a leading source of fatal infections, despite improvements in medical and surgical treatment and the widespread use of antimicrobial medicines (Jernigan *et al*., 2020).

3.2.2 Characterization and screening of *P. aeruginosa*

In this study, all bacterial isolates of *P. aeruginosa* were characterized by morphological, Microscopical, biochemical tests and Vitek 2 system.

3.2.2.1 Phenotypic characterization and microscopic identification

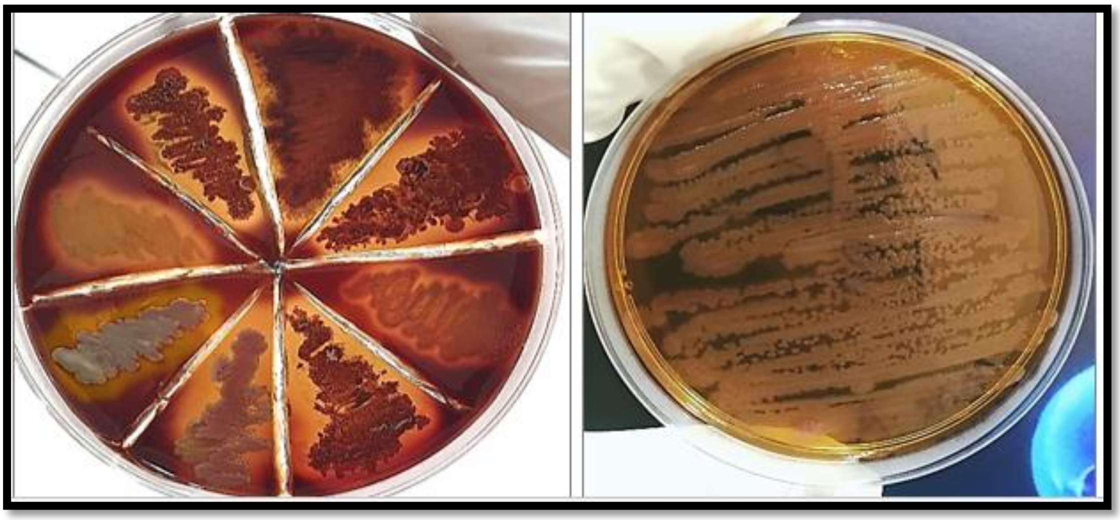
Bacteriological methods, such as colonial morphology (shape, color, and non-lactose fermentation on MacConkey agar) and microscopic examination (including morphology of bacterial cells by Gram stain to observe shape, arrangement of cells, and type of reaction with Gram-stain), were used to positively identify *P. aeruginosa*.

Cultures of suspected cases of *P. aeruginosa* were evaluated on a variety of selective media, including blood agar, MacConkey agar, and the mentioned Cetrimide and CCA agar, King A, and King B agars. Figure (3-3A) shows that colonies of bacteria grown in Blood agar medium were dark in color with a transparent halo, indicating their ability to blood hemolysis (beta hemolysis or complete hemolysis) while isolates grew pale colonies with smells like fermented grapes on MacConkey agar, indicating its inability to ferment lactose (Figure 2-3B).

P. aeruginosa are classified as Gram-negative bacteria because they grow well on MacConkey agar medium, which is selective because it contains the bile salts that impede the development of Gram-positive bacteria (Barman, 2021).

Colonies of *P. aeruginosa* growing on Cetrimide agar medium were a distinctive greenish-blue hue (Figure 3-4A), a phenotypic trait likely attributable to the bacteria's capacity to synthesize a water-soluble pyocyanin pigment. Similarities in pyocyanin pigment synthesis between the nutrient agar media and the nutrient broth (Figure 3-4 B and C).

However, due to their inability to produce glucuronidases, enzymes responsible for breaking down the chromogenic conjugate, these bacteria showed up as white or colorless colonies on the CCA media (Figure 3-5A). Isolates of *P. aeruginosa* grew greenish-blue colonies (Figure 3-5B) on King A agar because they produced the pyocyanin pigment, whereas colonies of *P. aeruginosa* grew greenish-yellow colors (Figure 3-5C) on King B agar because they produced the pyoverdin pigment. Gram staining revealed red, gram-negative bacteria when *P. aeruginosa* was examined under the microscope (Figure 3-5D).

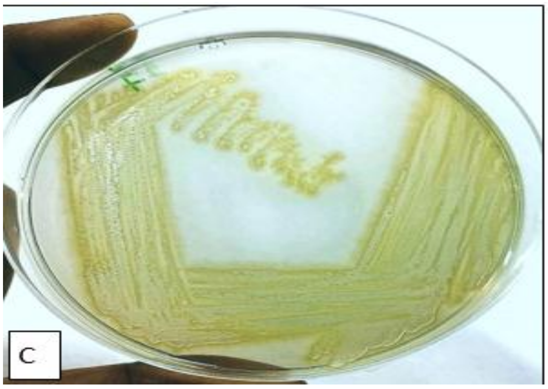


A **B**

Figure (3-3): (A) Colonies of *P. aeruginosa* (beta hemolytic) grown on Blood agar at 37°C for 24 hrs. (B) Colonies of *P. aeruginosa* (brown-pallid colonies) because it is non-lactose fermenter, had grown on MacConkey agar at 37°C.

Figure (3-4): (A) Colonies of *P. aeruginosa* (green) grown on Cetrimide agar at 37°C for 24 hrs. (B) Colonies of *P. aeruginosa* (greenish- blue) grown on nutrient agar at 37°C. (C) *P. aeruginosa* growth (green) on nutrient broth at 37°C.

A **B**



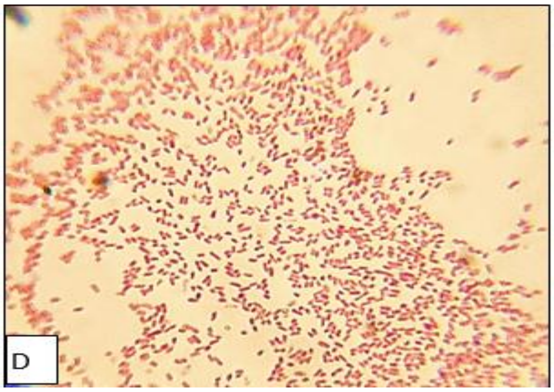


Figure (3-5): (A) shows morphological appearance of *P. aeruginosa* colonies on Chromogenic Coliforms Agar (CCA) medium, that appear in (pale white or colorless) colonies. (B): shows morphological appearance of *P. aeruginosa* colonies on King A Agar medium, that appear in (greenish-blue) colonies. (C): shows morphological appearance of *P. aeruginosa* colonies on King B Agar medium, that appear in (greenish-yellow) colonies, (D) Microscopic examination of *P. aeruginosa* after performing the dying process with a gram stain, that appears short rods, Gram-negative (red color) bacilli-bacterial cells.

P. aeruginosa colonies grown on Nutrient agar were identified by their greenish color and grape-like odor, per the work of Kamal *et al*., (2018). Yaaqoob *et al*., (2022) found that, on a blood agar-enriched culture medium, the bacterial colonies gave Beta-hemolysis, indicating the production of hemolysin.

Most *P. aeruginosa* colonies, according to research by Shaker and Al-musawi, (2018) produce pyocyanin, a greenish-blue dye, and a greenish-yellow pigment, Pyoverdine, which shines when exposed to ultraviolet (UV) radiation; both dyes are soluble in water. As a detergent that inhibits the development of most other organisms, (0.03%) Cetrimide is used to test *P. aeruginosa* capacity to grow on the selective and differential medium known as cetrimide agar.

Colonies of *P. aeruginosa*, which were a greenish blue tint when grown on King A medium, were mentioned by AL-Fatlawi and Muhsin (2021). In contrast, no isolates grown on King B agar generated the blue and green pigment (pyocyanin). This is because *P. aeruginosa*, unlike *P. fluorescens*, produces King B, and because the concentration of potassium and magnesium salt in the medium of King A is high enough to impede the production of fluorescein (Pyoverdin) that is formed on the medium of King B.

Microorganisms of the genus *Pseudomonas* are typically not picky. A protein hydrolysate, magnesium chloride, potassium sulfate, and agar are all that's needed to cultivate them. With the use of a microbe's distinct biochemistry, analytical microbiology may accurately identify the organism (Bonnet *et al*., 2020).

Chromogenic agar is used for selective *Pseudomonas* media. *Pseudomonas* species can be identified and differentiated in the medium through their metabolic reactions of proteolysis, lipolysis, fluorescent pigment production, nitrate consumption, glutamate utilization, hemolysis, and others (Hugh, 2020).

3.2.2.2 Biochemical identification

All isolates were subjected to biochemical testing, and the results were described in Table (3-2). The catalase test was conclusive for *P. aeruginosa* because the bacteria decomposed the hydrogen peroxide reagent into water and oxygen. Also, they were positive for the oxidase test by altering the color of the colony to purple when adding the reagent because *P. aeruginosa* possesses the enzyme cytochrome oxidase as a hydrogen acceptor.

Table (3-2): Biochemical tests for characterization of *P.aeruginosa*

Bacteria	Biochemical Tests							
	Catalase	Oxidase	Indole	Methyl red	VP	Citrate	KIA	Urease
P. aeruginosa	+	+	-	-	-	+	K/K, no gas, no H_2S	-

(+) positive result, (-) negative result, (VP) Voges –Proskauer test, (KIA) Kliger Iron Agar test, (K/K) Alkaline Slant/ Alkaline Bottom.

They also failed the Kligler iron test, as evidenced by the formation of an alkaline slant/alkaline bottom in the absence of H_2S and gas generation. These isolates failed the KIA test for their ability to ferment either glucose or lactose. Additionally, they were negative for the methyl red, Voges-Proskauer, and urease tests, but they were able to use citrate as their only carbon source, as evidenced by the blue shift in color observed on Simon citrate agar. In the presence of Kovacs reagent, a yellow ring formed on the surface of the peptone water medium, indicating that the Indole test had been negative for the *P. aeruginosa* isolates tested. This demonstrates that, *P. aeruginosa* is unable to manufacture the Tryptophanase enzyme, which is required for the conversion of tryptophan to Indole.

The Voges Proskauer, indole, and methyl red tests are all negative for *Pseudomonas*, whereas the catalase test is positive. Certain species, however, do not react positively to the oxidase test. Under iron-limiting conditions, *Pseudomonas* is known to secrete pyoverdin (fluorescein, a Siderophore), a brilliant yellow-green pigment (Chauhan and Jindal, 2020).

Pyocyanin (blue pigment, a siderophore), quinolobactin (yellow, dark green in presence of iron, a Siderophore), pyorubrin (a red pigment), and pyomelanin (a black pigment) are only some of the other hues that certain Pseudomonas may create (brown pigment). In the presence of blood agar, a hemolytic reaction becomes visible (Pattnaik *et al.*, 2019).

Pseudomonas uses carbohydrates for fuel via the Entner-Doudoroff route, using pyruvate as the final product (dissimilation). The enzymes involved in this process are distinct from those involved in glycolysis and the pentose phosphate pathway. *Pseudomonas* is unique in that it lacks the ability to catabolize its own fermentation products. Nitrate can serve as an electron acceptor for *P. aeruginosa* in place of oxygen. Using the denitrification pathway, growth can also proceed under anaerobic circumstances (Wirth *et al*., 2022).

To determine *P. aeruginosa* exact molecular make-up, the VITEK2 system was used (VITEK-2 GN Kit). With 56 samples, it was found 56(100%) *P. aeruginosa*. Figure (3-6) depicts these findings.

Selected Organism	99% Probability	Pseudomonas aeruginosa
	Bionumber: 0003053103500352	Confidence: Low discrimination

SRF Organism	

Analysis Organisms and Tests to Separate:
Pseudomonas aeruginosa 42C(99),PYOCYANIN(99),

Low Discrimination Organism

Analysis Messages:

Contraindicating Typical Biopattern(s)

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	+			

Figure (3-6) VITEK$_2$ system report for *P. aeruginosa* isolated from urine sample

3.3 Detection of antimicrobial activity by VITIK 2 for *P. aeruginosa*

The susceptibility test of bacterial isolates against 12 selected antibiotics was conducted by VITIK 2. Resistance findings of the *P. aeruginosa* isolates described in Table (3-3) during the current analysis against these antibiotics.

Table (3-3): Antibiotics susceptibility of *P. aeruginosa* isolates

Antibiotics	Resistant (No. &%)	Sensitive (No. &%)
Ticarcillin	56(100%)	0(0%)
Ticarcillin/clavulanic acid	46(82.14%)	10(17.86%)
Piperacillin	56(100%)	0(0%)
Ceftazidime	49(87.5%)	7(12.5%)
Cefepime	52(92.86%)	4(7.14%)
Tobramycin	56(100%)	0(0%)
Imipenem	56(100%)	0(0%)
Meropenem	56(100%)	0(0%)
Amikacin	56(100%)	0(0%)
Gentamycin	56(100%)	0(0%)
Ciprofloxacin	39(69.64%)	17(30.36%)
Colistin	34(60.71%)	22(39.29%)

The *P. aeruginosa* isolates were entirely resistance 56(100%) to 7 of the antibiotics of interest during the current investigation including Ticarcillin, Piperacillin, Imipenem, Meropenem, Gentamycin, Amikacin and Tobramycin as shown in Table (3-3). Colistin was the most effective antimicrobial agent against bacterial isolates because *P. aeruginosa* displayed a percentage of sensitivity of approximately 22/56 (39.29%) followed by Ciprofloxacin 17/56 (30.36%), Ticarcillin/clavulanic acid 10/56 (17.86%), Ceftazidime 7/56 (12.5%) and Cefepime 4/56 (7.14%) respectively.

This results were consistent with the findings of Glen and Lamont (2021), who also discovered that *P. aeruginosa* had developed a higher level of resistance to beta-lactam antibiotics. High levels of resistance were documented for *P. aeruginosa* against a class of antibiotics known as beta-lactams, according to research by Abd AL-Zwaid *et al*., (2022). While the hospital environment is a hotspot for the spread of antimicrobial-resistant bacteria.

The increased prevalence of *P. aeruginosa* strains resistant to cephalosporins may be attributable to the widespread adoption of these antibiotics for treatment. Synthesis of beta-lactamase enzymes, which deactivate these medicines, is one of them (Pachori *et al*., 2019).

Beta-lactamase production and PBP loss via mutation both contribute to Cephalosporin resistance in *P. aeruginosa* isolates (Bunyan *et al*., 2018). Despite the fact that, *P. aeruginosa* isolates tested for sensitivity to Gentamycin, the positive rate was (31.57%) according to Algammal *et al*., (2021), (71%) of isolates tested positive for resistance to Gentamycin according to Abdulhadi *et al*., (2021), the resent results showed high resistance to Gentamycin reach to (100%).

P. aeruginosa that has developed resistance to multiple antibiotics. It poses a significant treatment challenge because to its inherent resistance to numerous antimicrobial drugs, as well as its capacity to acquire multidrug resistance and mutate to become resistant to antibiotics (Chojnacki *et al.*, 2019).

Commonly, many resistance pathways contribute to multidrug resistance. Efflux pumps are frequently found in *P. aeruginosa* isolates that have developed resistance to multiple antibacterial treatments. These pumps work to expel antibacterial medications from the cell before they can accumulate to an effective concentration at the site of action, rendering the drugs ineffective (Eleftheriadou *et al*., 2021).

This study's findings indicating 69.64% of *P. aeruginosa* isolates were resistant to Ciprofloxacin are consistent with those of Odoi *et al*., (2022), who reported that (69%) of *P. aeruginosa* isolates are resistant to the antibiotic. Khan, (2021) also found that *P. aeruginosa* isolates showed resistance to Ciprofloxacin at a rather high rate (64%).

Nosocomial infections are commonly brought on by *P. aeruginosa*. There has been a rise in the prevalence of *P. aeruginosa* and the emergence of new resistant strains in both community-acquired pathogens and hospital-originated infections, despite improvements in sanitation facilities and the introduction of a wide variety of antimicrobial agents with anti-pseudomonal activities (Tümmler, 2019).

The results of this investigation confirmed that all *P. aeruginosa* strains tested were resistant to the antibiotic Meropenem. This study's findings on *P. aeruginosa* resistance to carbapenems were at odds with those of Acar *et al*., (2019), who reported a 46.7% resistance rate for Meropenem, but in line with those of, who reported that all *P. aeruginosa* isolates were resistant to Meropenem at a rate of (63%).

As a result of its high inherent resistance to numerous antibiotic families and its amazing propensity to develop novel mechanisms of resistance even during antibiotic therapy, *P. aeruginosa* is notoriously difficult to treat (Pang *et al*., 2019).

Macrolide and lincosamide antibiotic resistance can be broken down into three categories: target-site alteration (through methylation or mutation) that inhibits the antibiotic from binding to its ribosomal target; antibiotic efflux; and drug inactivation. Macrolide and lincosamide makers have been discovered to use such mechanisms, and they often employ a variety of strategies to counteract the antimicrobials they create (Abushaheen *et al*., 2020).

The results of this study showed that a significant proportion of the bacteria were resistant to the antibiotic colistin (60.71%), a finding that is nearly at odds with the findings of local and international researchers Shehab and Jassim, (2019), who found that, the proportion of *P.*

aeruginosa resistant to colistin was (6%); and in Baghdad. However, according to the findings of Arif *et al*., (2022), 100% of the *P. aeruginosa* bacterial isolates tested responded favorably to treatment with colistin.

3.4 The expression of *HfQ* gene in urine samples

The results of gene expression by using Real-time PCR demonstrated a significant overexpression of *Hfq* among *P. aeruginosa* resistance isolates (30 isolates) compared to sensitive clinical isolates (30) isolates, given by fold change after normalized with housekeeping gene *16sRNA* by folding (21.497±1.241 versus 1.92142± 0.04598) respectively as seen in Table (3-4) and Figure (3-7a, b). This could explain why HFQ role in virulence effect for *P. aeruginosa* resistance pointing to the deficiency or mutant HFQ in the sensitive strains which is revealed by Pusic *et al*., (2018). It has been demonstrated that the absence of HFQ in *P. aeruginosa* results in significantly reduced growth on no preferred carbon sources as well as a reduction in a number of virulence features, including motility and the generation of pyocyanin. HFQ have crucial role for many different bacterial species development or pathogenicity.

Table (3-4): The expression of *HFQ* gene in pathogenic resistance *Pseudomonas* and sensitive *Pseudomonas* in Urine samples

***HFQ* gene**	**ΔCT (mean±SE)**	**ΔΔCT (mean±SE)**	**Fold Change (mean±SE)**
Sensitive *P. aeruginosa*	20.9123± 2.84	0.0016±0.001	1.92142± 0.04598
Resistance *P. aeruginosa*	17.314± 4.07*	-2.287±0.88*	21.497±1.241**
P value	0.004	0.031	0.000

**** overexpressed, * significant differences p<0.05.**

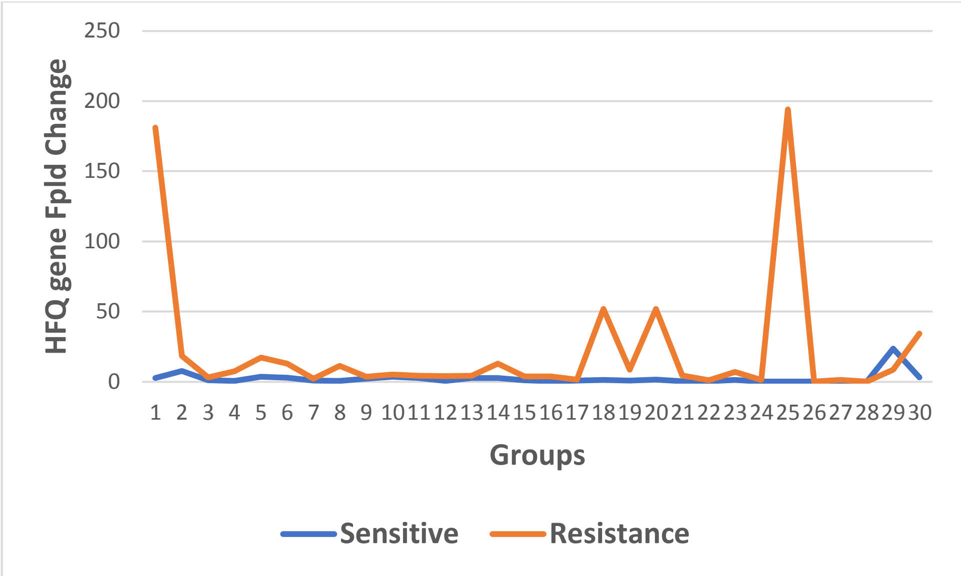


Figure (3-7a): The Expression of *HFQ* gene in sensitive and resistance *P. aeruginosa* in urine samples.

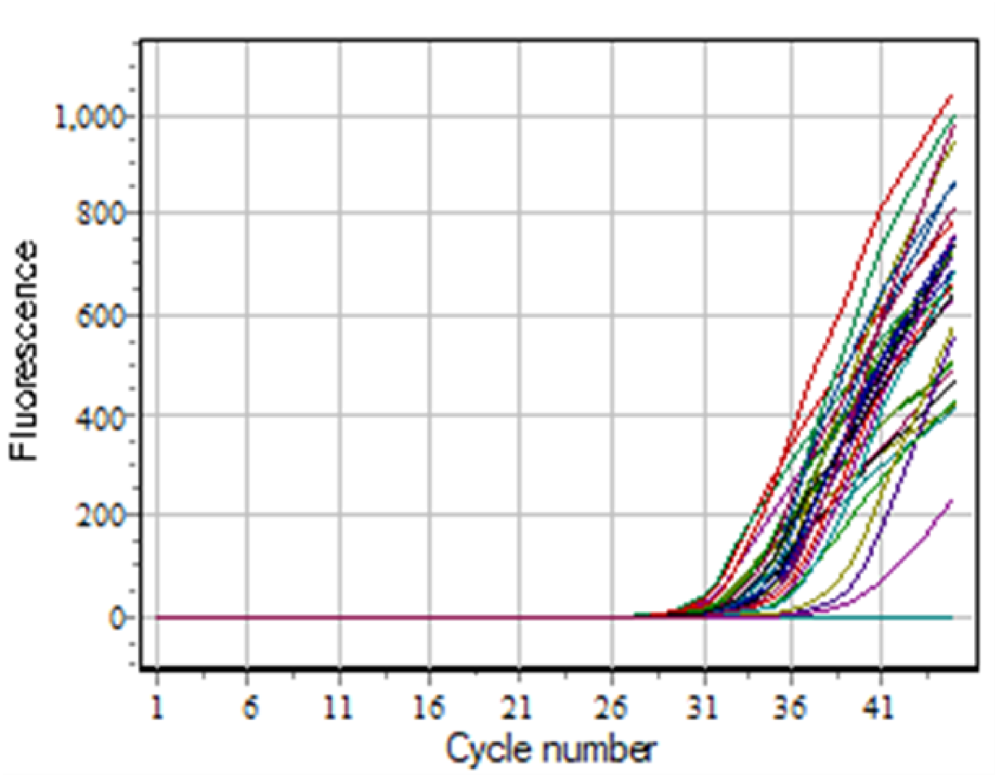


Figure (3-7b): *HFQ* gene urine dissociation curves by qPCR. The photograph was taken directly from qPCR machine

An increased susceptibility of *P. aeruginosa* hfq deletion strains to different classes of antibiotics. Transcriptome analyses indicated that Hfq impacts on different mechanisms known to be involved in antibiotic susceptibility, viz import and efflux, energy metabolism, cell wall and LPS composition as well as on the c-di-GMP levels. Furthermore, the sequestration of Hfq enhances the sensitivity toward antibiotics (Rensing and McDevitt, 2016)

In *P. aeruginosa*, Hfq was shown to bind directly to catabolic mRNAs to block translation during CCR. Hereby, the catabolite repression protein Crc acts as a co- repressor by stabilizing the RNA-Hfq complex. There is limited knowledge on the impact of Hfq on antibiotic susceptibility in bacteria (Pusic *et al*., 2018).

There are some examples in which functions involved in antibiotic susceptibility are directly or indirectly regulated by Hfq-mediated riboregulation via sRNA (Fantappiè *et al*., 2009). The increased sensitivity of an *E. coli hfq* mutant strains toward several toxic compounds coincided with reduced quantities of ArcB, which is a compenent of the Arc AB-ToIc efflux pump (Chao and Vogel, 2010).

Chevalier *et al*., (2017) mentioned that, the susceptibility of *P. aeruginosa* PAO1 and PA14 *hfq* deletion strains toward several classes of clinically relevant antibiotics. In general, the *hfq* deletion resulted in an increased sensitivity toward the antibiotics. Hfq impacts on energy metabolism and c-di-GMP levels. Thus, the absence of Hfq seems to impose a general fitness burden which is also apparent from the somewhat impaired growth of the hfq deletion strains. It was shown that dormant *P. aeruginosa* exhibit an impaired aminoglycoside uptake. This phenomenon called phenotypic tolerance is related to persistence of bacteria after antibiotic treatment (Pusic *et al*., 2018).

The transcriptome analysis revealed that Hfq governs the expression of many functions, which affect antibiotic susceptibility. Thus, physiological changes on the one hand and the regulation of specific antibiotic resistance determinants on the other hand seem to collectively contribute to the observed increased susceptibility to antibiotics (Bharwad and Rajkumar, 2019). The specific regulatory mechanisms exerted by Hfq on resistance genes/determinants being it via riboregulation or direct translational repression remain to be elucidated for the individual functions. However, the mutant hfq strains of *P. aeruginosa* increased sensitivity toward aminoglycosides, betalactams, fosfomycin and polymyxin B (Pusic *et al*., 2018). Hence, it is conceivable that Hfq regulates several functions conferring susceptibility at the posttranscriptional level.

The major RNA-binding protein Hfq interacts with mRNAs, either alone or together with regulatory small noncoding RNAs (sRNAs), affecting mRNA translation and degradation in bacteria. However, studies tend to focus on single reference strains and assume that the findings may apply to the entire species, despite the important intra-species genetic diversity known to exist use RIP-seq to identify Hfq-interacting RNAs in *P. aeruginosa* (Bharwad and Rajkumar, 2019).

The study conducted by Sobrero and Valverde, (2012) that most interactions are in fact not conserved among the different strains. Identify growth phase-specific and strain-specific Hfq targets, including previously undescribed sRNAs. Strain-specific interactions are due to different accessory gene sets, RNA abundances, or potential context- or sequence- dependent regulatory mechanisms.

The accessory Hfq interactome includes most mRNAs encoding Type III Secretion System (T3SS) components and secreted toxins in two strains, as well as a cluster of CRISPR guide RNAs in one strain. Conserved Hfq targets include the global virulence regulator Vfr and metabolic pathways involved in the transition from fast to slow growth. Furthermore, recent study use rGRIL-seq to show that RhlS, a quorum sensing sRNA, activates Vfr translation, thus revealing a link between quorum sensing and virulence regulation. Overall, our work highlights the important intra-species diversity in post-transcriptional regulatory networks in *Pseudomonas aeruginosa* (Vogel and Luisi, 2011).

Hfq regulates bacterial virulence through multiple direct and indirect mechanisms and the analysis identified overexpression of *HFQ* gene in many *pseudomonas* strain both known and novel virulence-related regulatory targets for Hfq in the different strains (Mann *et al.*, 2017).

The vfr mRNA, encoding for a global virulence regulator was strongly bound to Hfq in all three strains. Fascinatingly, while Vfr is known to regulate the T3SS, it has been shown to directly regulate the two-partner secretion system ExlBA, which is the main virulence factor in the T3SS-lacking PA7/IHMA87-like lineage (Han *et al.*, 2016).

Several investigations found many mechanisms could explain these observations. The 5’ leader sequence of vfr mRNA was proposed to form a stem-loop structure encompassing the Hfq-binding sites, RsmA-binding sites and ribosomal binding sites. RhlS could facilitate the binding of Hfq on the stem-loop by rearranging the mRNA structure, as shown for several positive sRNA regulators (DsrA for rpoS regulation and RyhB for shiA regulation. Alternatively, RhlS could protect the vfr mRNA against RNases (exo- or endo-nuclease), as shown for RydC, which protects cfa1 mRNA against RNase E67. This reveals an interesting feature of regulatory plasticity, as the conserved interactions between Hfq, vfr mRNA and RhlS result in different virulence-related regulatory outputs (T3SS or ExlBA) due to the diversity found in Vfr targets across *P. aeruginosa* lineages (Gebhardt *et al.*, 2020).

Additionally, the T3SS is directly regulated by Hfq through stabilization of the interaction between exsA mRNA, encoding the T3SS transcription regulator, and the sr0161 sRNA13. Here, we show that in addition, Hfq interacts with most of the T3SS-related mRNAs, including those of all four T3SS toxins. The role of Hfq in these interactions remains to be explored. Indeed, while the most studied function of Hfq is to facilitate regulatory mRNA-sRNA interactions, it can also play other sRNA-independent roles, including modulation of mRNA stability and translation. Altogether, our study reveals several new regulatory pathways through which Hfq regulates directly or indirectly the two major virulence factors, the T3SS and ExlBA, as well as many other virulence-related genes through Vfr regulation (Marsden *et al.*, 2016)**.** Biofilm formation is important to bacteria for colonization and stress resistance in their natural environments and is highly influenced and regulated by HFQ gene as may previous study reported (Berry *et al.*, 2018).

3.5 Expression of As1974 gene in urine samples

Resent study demonstrated down-regulation of As1974 in resistance after normalized with *16sRNA* with fold change (0.6622±0.0465) vs (2.012± 1.0243) respectively suggested that could be potential marker to transform the bacteria from resistance to sensitive as seen in Table (3-5) and Figure (3-8a, b). The methylation sites found in the genes 5' UTR control the expression of As1974, the master regulator that controls the expression of various drug resistance pathways, including membrane transporter and antibiotic resistance genes associated with biofilm. Our research reveals the sRNA that control the MDR pathways in *P. aeruginosa* clinical isolates (Law *et al*., 2019), in addition, utilizing sRNA to convert bacterial drug resistance to hyper susceptibility may be future strategy for combating MDR bacteria.

Table (3-5): The expression of As1974 gene in pathogenic resistance *P. aeruginosa* and sensitive *P. aeruginosa* in Urine samples

AS1974 gene	ΔCT (mean±SE)	ΔΔCT (mean±SE)	Fold Change (mean±SE)
Sensitive	19.93± 1.22	-0.06±0.001	2.012± 1.0243
Resistance	22.216± 3.15	2.014±0.673	0.6622±0.0465**
Pvalue	0.001	0.013	0.0001

**** Down regulated**

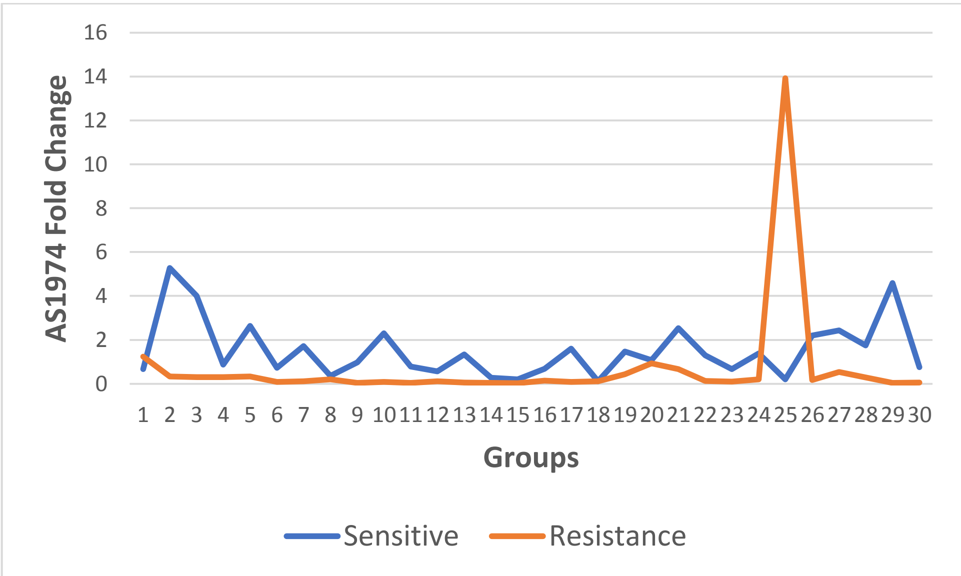


Figure (3-8a): The expression of *As1974* gene in sensitive and resistance *P. aeruginosa* in urine samples

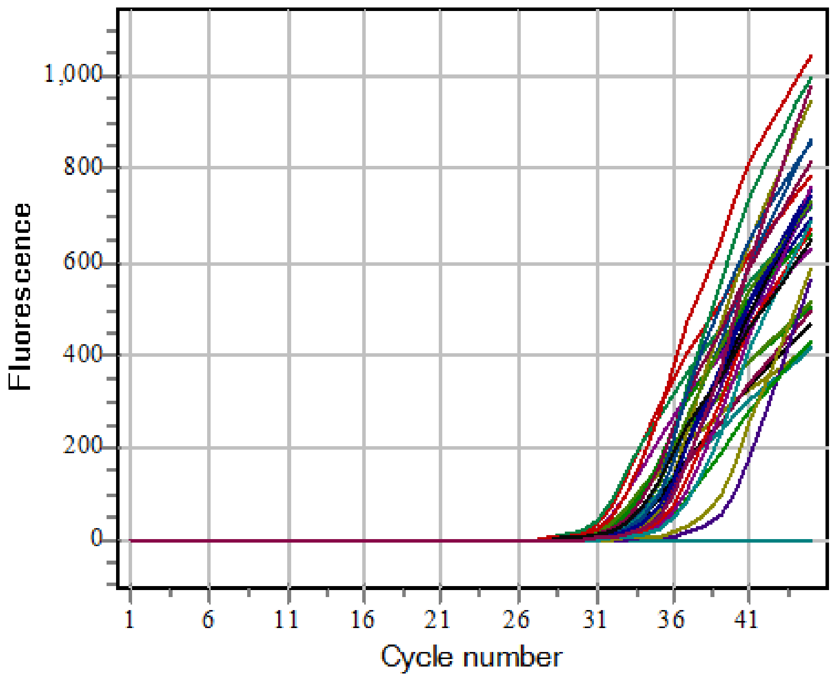


Figure (3-8b): As1974 in urine dissociation curves by qPCR. The photograph was taken directly from qPCR machine

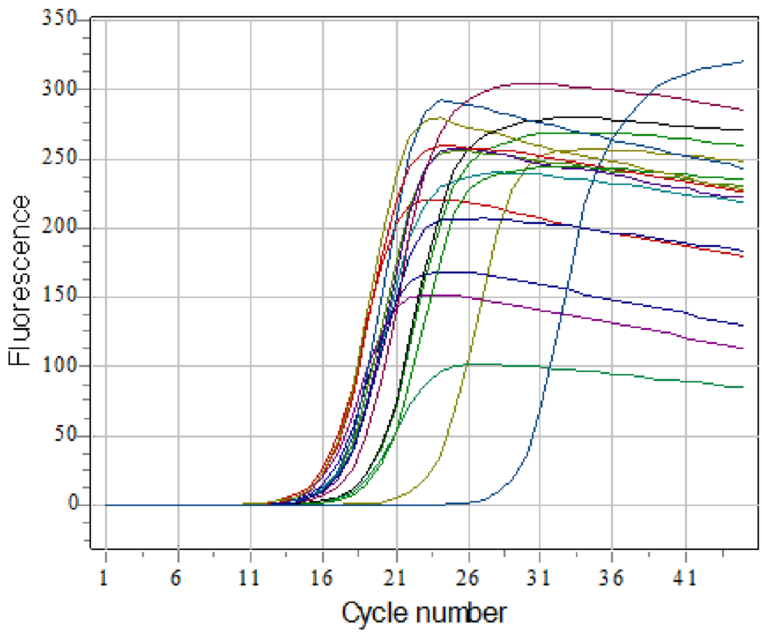


Figure (3-8c): *16SrRNA* urine dissociation curves by qPCR. The photograph was taken directly from qPCR machine

Bacteria with multiple drug resistance (MDR) have become a global issue worldwide, and hundreds of thousands of people's lives are threatened every year. The emergence of novel MDR strains and insufficient development of new antimicrobial agents are the major reasons that limit the choice of antibiotics for the treatment of bacterial infection. Thus, preserving the clinical value of current antibiotics could be one of the effective approaches to resolve this problem Law *et al*., 2019).

As1974 is the master regulator to moderate the expression of several drug resistance pathways, including membrane transporters and biofilm-associated antibiotic-resistant genes, and its expression is regulated by the methylation sites located at the 5' UTR of the gene. The sRNA could regulates the MDR pathways in clinical isolates of *P. aeruginosa*. Moreover, transforming bacterial drug resistance to hyper susceptibility using sRNA could be the potential approach for tackling MDR bacteria in the future (Coleman *et al*., 2021).

A study demonstrated that, numerous novel small RNAs that were down regulated in the MDR clinical isolates of *P. aeruginosa,* and many were overexpressed of one of these small RNAs (sRNAs), As1974, was able to transform the MDR clinical strain to drug hyper susceptibility (Law *et al*., 2019).

A study confirms our findings and showed that the expressions of these sRNAs were significantly down regulated in the multidrug-resistant strains compared with those in the drug-susceptible strains. Moreover, overexpression of As1974 sRNA could transformed the bacteria from resistant strains of aminoglycoside, meropenem, and some of the board-spectrum cephalosporin to drug-sensitive strains (Ferrara *et al*., 2020).

This is of significant importance because sRNAs have been considered as the potential antimicrobial molecules for multidrug resistance pathogens with the shortage of efficient antibiotics in clinical sectors, and proved the concept that the sRNA is capable of eliminating the drug resistance of clinical isolates of *P. aeruginosa*. In other words, sRNA treatment in combination with old non-functional antibiotics (those that have been shown to be resistant and cannot be used for treatment) may potentially be the strategic approach to target the widely spreading multidrug resistance of bacterial infection in the world. Although the delivery of the sRNA to the pathogen in the human body is still an unsolved problem, this approach indeed opens up a new direction for the potential strategy of treatment as well as future development of antimicrobial agents (Bojanovič *et al.*, 2017).

One of the functional features of sRNA is to act as a master regulator to control multiple cellular pathways simultaneously in order to provide an immediate response to the environmental changes such as stress. The regulation of As1974 was capable of modulating the gene expression of various pathways, including efflux pumps, cell wall turnover, biofilm formation, motility, and iron acquisition. Moreover, most of them are related to the multidrug resistance (Law *et al.*, 2019).

3.6 *miRNA30* gene in urine, and blood samples

The results showed in Table (3-6), (3-7) and Figure (3-9a, b), (3-10 a, b) demonstrated down-regulation of *MiRNA30* in urine and blood samples of patients (1.36±0.34, 0.47821±0.03678), compared to 30 samples appear healthy subjects (5.2463±1.213, 5.0432±1.7441) respectively.

In the current study, evaluated the expression of microRNAs (miRNAs) in the peripheral blood of UTI patients, determining the diagnostic efficacy of miRNA in predication of UTI response. Figure (3-9a, b) revealing there were down regulation when it compared to the control as the expression of microRNAs and it good to mention that the same scenario were seen with blood samples as it is affected equally.

Table (3-6): The expression of *miRNA30* gene in urine samples

MiR-30	ΔCT (mean ±SE)	ΔΔCT (mean ±SE)	Fold Change (mean ±SE)
Control	12.54± 1.29	-0.0267±0.0001	5.2463± 1.213
Patients	14.386± 2.31	2.162±0.241*	1.36±0.34**
P-value	**0.061**	**0.012**	**0.01**

**** Down regulation, * significant differences p<0.05.**

Table (3-7): The expression of *miRNA30* gene in blood samples

MiR-30	ΔCT (mean ±SE)	ΔΔCT (mean ±SE)	Fold Change (mean ±SE)
Controls	11.44± 1.441	-0.0234±0.00	5.0432± 1.7441
Patients	16.42± 1.9821*	3.21±0.634*	0.47821±0.03678**
p-value	**0.031**	**0.001**	**0.001**

****Down regulation, * significant differences p<0.05.**

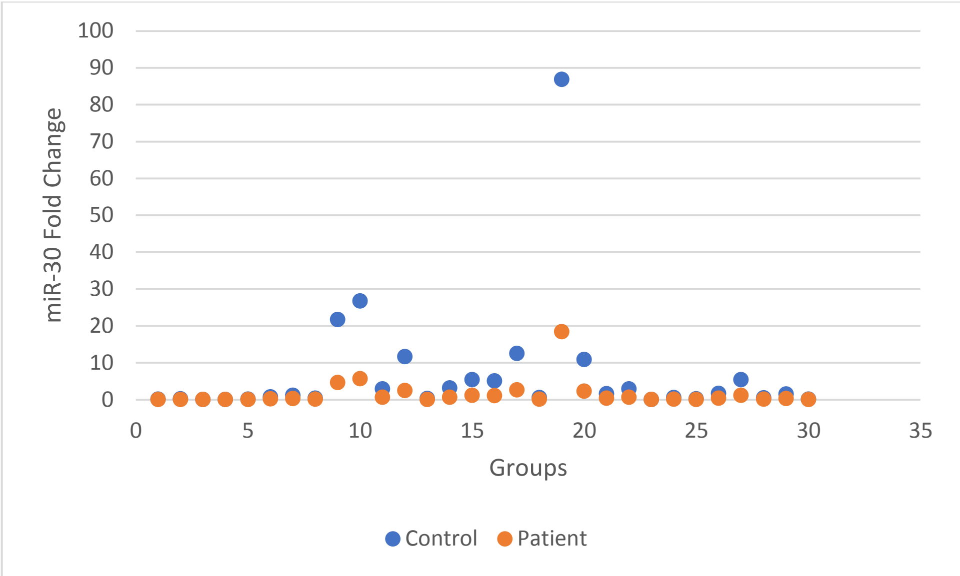


Figure (3-9a): The expression of *miRNA30* in UTI patient and controls in urine samples

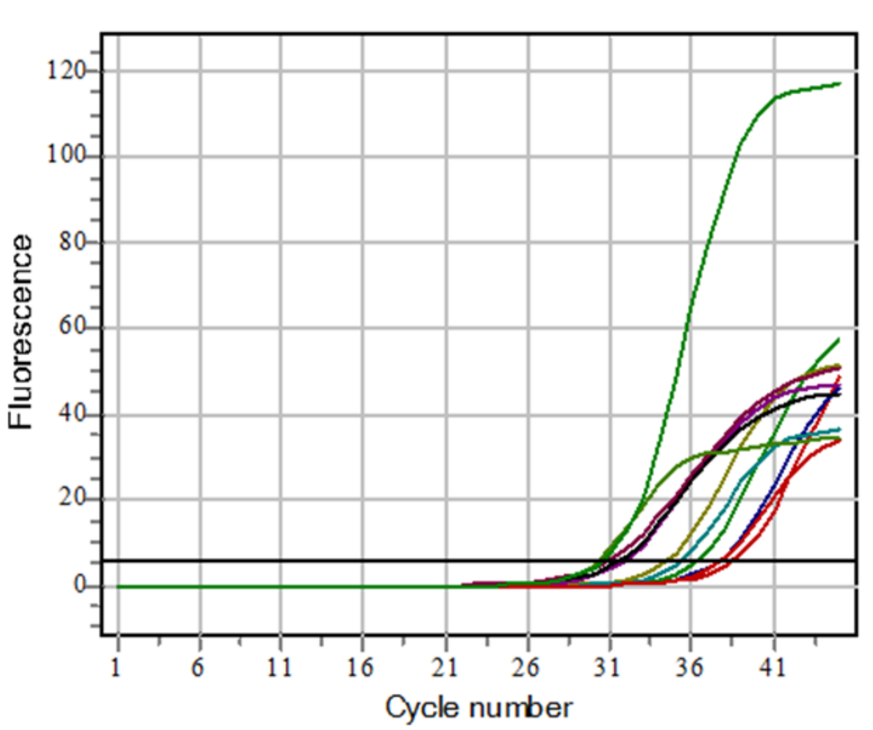


Figure (3-9b): *MiRNA30* urine dissociation curves by qPCR. The photograph was taken directly from qPCR machine

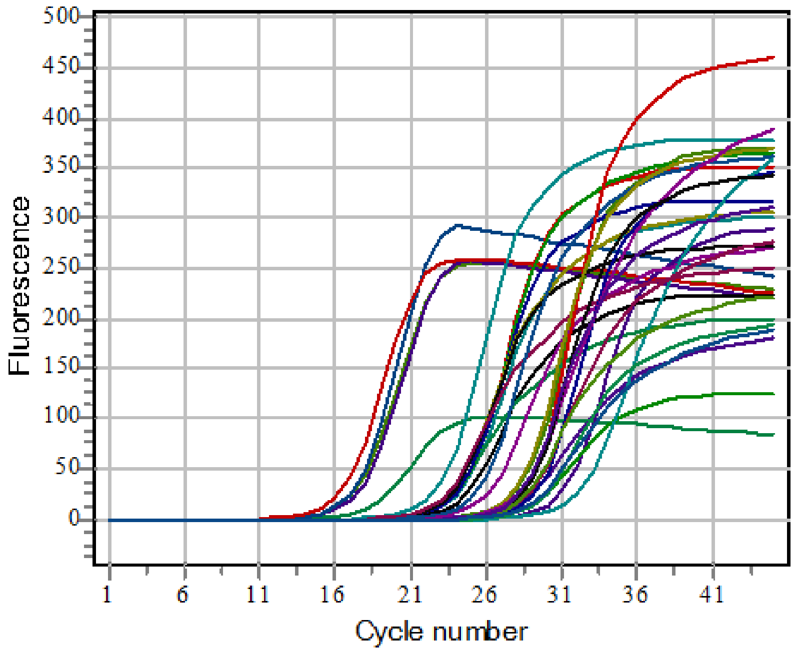


Figure (3-9c): *GADPH* blood and Urine dissociation curves by qPCR. The photograph was taken directly from qPCR machine

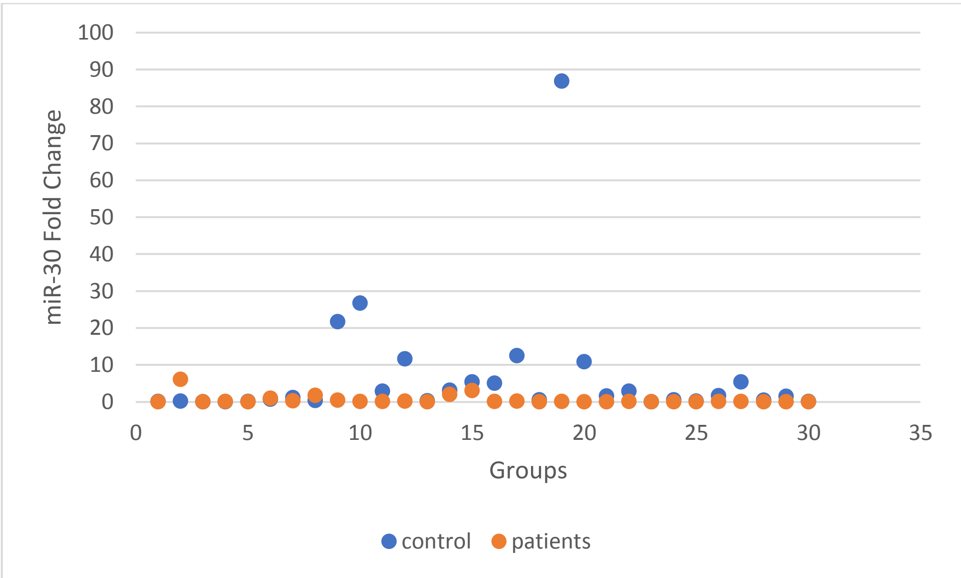


Figure (3-10a): The expression of *miRNA30* in blood samples in patients and controls

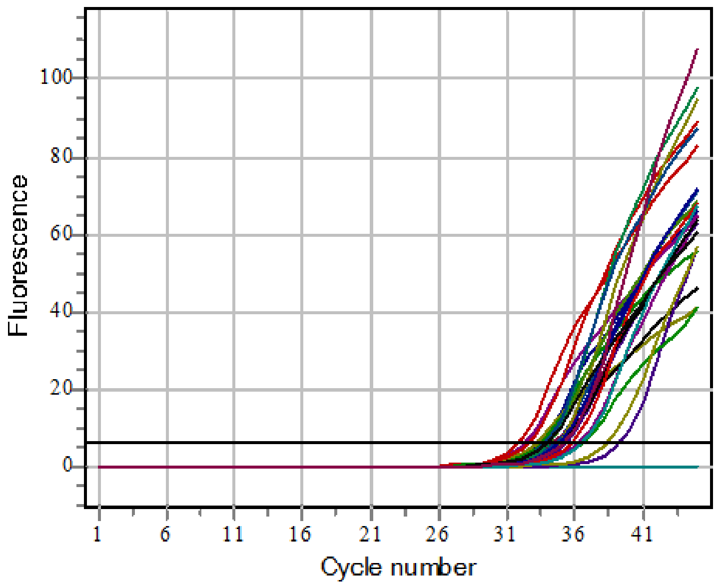


Figure (3-10b): *MiRNA30* in Blood dissociation curves by qPCR. The photograph was taken directly from qPCR machine

Although miRNAs have been described as important modulators of the host immune response against uropathogenic *Pseudomonas*, but their role in the macrophage interplay remains largely unclear. previous studies shown that miR-4458, miR-4667-5p, and miR-4668-5p and miR-30a-5P regulate autophagy-mediated elimination of *B. pseudomallei* by targeting ATG (Mishra *et al.*, 2020). miRNAs can also regulate the expression of Rab GTPases, such as Rab5a, Rab5c, and Rab11a. Therefore, involvement of miRNAs in regulating Rab GTPase undoubtedly deserves further investigation (Wang *et al.*, 2019).

There were limit investigation on the expression in miR-30-5p in *P. aeruginosa* infection, studies reported the role of miR-30 including the immune system, pro-inflammatory processes, apoptosis, cell cycle, and DNA replication and repair. Importantly, miRNA profiling revealed that four members of the miR-30 family are significantly down regulated in *B. pseudomallei*-infected macrophages (Hu *et al.*, 2019), which confirmed in present finding on the expression profile of miR-30-5P in uropathogenic *P. aeruginosa*. Further verified microarray data by qRT-PCR found that only the expression of miR-30b and miR-30c were down regulated whereas miR-30d and miR-30e were not significantly altered (Wang *et al.*, 2013). The miR-30 family members are expressed by genes localized in different genomic positions (Song *et al.*, 2014). In addition, they share a common seed sequence near the 5′ end but possess different compensatory sequences near the 3′ end (Wang *et al.*, 2019).

These different compensatory sequences allow miR-30 family members to target different genes and pathways, thus are often differentially expressed and regulated during biological processes. The expression levels of miR-30c, miR-30d and miR-30e were significantly decreased whereas the levels of miR-30a and miR-30b were not altered during osteoblast differentiation (Zhuang *et al.*, 2020).

Therefore, the differentially regulated expression pattern with the miR-30 family members during *P. aeruginosa* infection could be due to differences in sequence outside the seed region. However, the exact reason for these differences still needs to be investigated further Wang *et al.*, 2019). A study identified miR-30e-5p to be upregulated in different bacterial infections which enhances innate immunity to combat bacterial replication by targeting key negative regulators such as SOCS1 and SOCS3 of innate immune signaling pathways (Wang *et al.*, 2013). The finding suggested the down regulation of miR-30 in resistant bacteria could impair the Mucosal immunity and thus lead to bacterial colonization and pathogenicity, further studies required to investigated the miR-30 expression and its potential activity on cellular signaling pathway and thus could be key regulatory biomarker for uropathogenic bacterial infection.

Conclusions and Recommendations

Conclusions:

1. Resent study confirmed that all ages were susceptible to the UTIs, also females were suffered of UTIs more than males.
2. Antimicrobial effects very important in treatment of *P. aeruginosa*, high resistance present especially with Ticarcillin, Piperacillin, Imipenem, Meropenem, Gentamycin, Amikacin and Tobramycin.
3. The results demonstrated a significant over expression of HFQ binding protein in *P. aeruginosa* compared to sensitive clinical samples.
4. Resent study demonstrated down-regulation of As1974 in resistance, suggested that As1974 could be potential marker to transform the bacteria from resistance to sensitive.
5. The results showed down-regulation of Micro-30 in urine and blood samples compared to healthy subjects.
6. miRNA have been as important modulator of the host immune response against uropathogenic *P. aeruginosa*.
7. The sRNA could be potential approach for tackling MDR bacteria in the future.

Recommendations:

1. Further studies of the exact mechanism of distinct MiR-30 family member on regulating the development of different tissues and organs.
2. Study the role of MiR-30 family members of different types of tumors.
3. Study the role of MiR-30 in immunorgans development and disease-related immune response.
4. Further studies of the role of *HFQ* gene on the bacterial growth rate, observation of cellular morphology, changes in virulence.

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Appendix

Appendix

9/3/2022

Settings of analysis:

Date:
Protocol number:
Operator:
Run file:
Comment:

Test: **promega qotaq qpcr**

Amplification program:

1. 95.0 °C - :02:00
2. 95.0 °C - :00:15
   60.0 °C - :01:30

Qualitative

Number of the well	16SRNA urin CT Resistance	16SRNA urin CT Sensitive	CT, Fam urin HFQ Resis	CT, Fam urin HFQ Sensitive	CT, Fam urin AS1974 Sensitive	CT, Fam urin AS1974 Resis	Cp, Fam Mir-30 urin	GAPDH URIN CP, FAM	Mir-30 blood ct	Cp, Fam GAPDH blood
A1	16.8	13.2	31.3	33.84	34.8	37.5	37.6	17.3	38.9	20.28
A2	14.1	15.4	31.9	34.44	34.0	36.7	36.7	18.3	38	19.12
A3	12.2	13.2	32.6	35.14	32.2	34.9	38.8	17.6	40.1	18.58
A4	12.6	11.4	31.7	34.24	32.6	35.3	39.8	17.4	41.1	18.38
A5	14.6	14.9	32.5	35.04	34.5	37.2	35.9	16.5	37.2	17.48
A6	13.1	13.44	31.4	33.94	34.9	37.6	36.6	19.9	37.9	20.88
A7	12.2	13.38	33.1	35.64	33.6	36.3	33.4	17.3	34.7	18.28
A8	15.2	13.32	33.7	36.24	35.8	38.5	34.3	16.5	35.6	17.48
B1	11.4	13.26	31.6	34.14	34.3	37	38.0	26.2	39.3	27.18
B2	11.2	13.2	30.8	33.34	33.0	35.7	37.1	25.6	38.4	26.58
B3	11.3	13.14	31.2	33.74	34.5	37.2	35.0	20.3	36.3	21.28
B4	13.4	13.08	33.4	35.94	34.9	37.6	34.4	21.7	35.7	22.68
B5	11.2	13.02	31.1	33.64	33.6	36.3	39.7	21.4	41	22.38
B6	12.7	12.96	31	33.54	35.8	38.5	36.9	22.3	38.2	23.28
B7	12.2	12.9	32.3	34.84	36.2	38.9	34.8	21	36.1	21.98
B8	13.3	12.84	33.4	35.94	34.4	37.1	35.1	21.2	36.4	22.18
C1	11.1	12.78	32.4	34.94	33.1	35.8	35.6	23	36.9	23.98
C2	15.4	12.72	31.7	34.24	36.9	39.6	36.2	19	37.5	19.98
C3	13.6	12.66	32.5	35.04	33.1	35.8	34.8	25	36.1	25.98
C4	15.1	12.6	31.4	33.94	33.5	36.2	33.3	20.5	34.6	21.48
C5	13.3	12.54	33.1	35.64	32.2	34.9	33.8	18.2	35.1	19.18
C6	11.8	12.48	33.7	36.24	33.1	35.8	34.3	19.6	35.6	20.58
C7	12.4	12.42	31.6	34.14	34	36.7	39.5	17.3	40.8	18.28
C8	12.3	12.36	33.8	36.34	32.9	35.6	35.1	18	36.4	18.98
D1	27.1	12.3	35.5	38.04	35.6	38.3	35.8	17	37.1	17.98
D2	11.2	12.24	35.1	37.64	32.1	34.8	35.8	20.3	37.1	21.28
D3	12.7	12.18	34.4	36.94	31.9	34.6	35.8	22	37.1	22.98
D4	13.3	13.2	38.1	39	33.4	36.1	38.4	21	39.7	21.98
D5	11.4	15.4	30.3	32.84	34.2	36.9	36.6	20.9	37.9	21.88
D6	12.1	13.2	29.0	31.54	34.6	37.3	39.9	20.3	41.2	21.28

The expression of 16sRNA in sensitive *pseudomonas*

	16SRNA urin CT	CT, Fam urin	DCT	DDCT	F	16SRNA	CT, Fam urin	DCT	DDCT	F
	Sen	HFQ Sen				Resis	HFQ Resis			
1	13.2	33.84	20.64	-1.36	2.566852	16.8	31.3	14.5	-7.5	181.0193
2	15.4	34.44	19.04	-2.96	7.78124	14.1	31.9	17.8	-4.2	18.37917
3	13.2	35.14	21.94	-0.06	1.042466	12.2	32.6	20.4	-1.6	3.031433
4	11.4	34.24	22.84	0.84	0.558644	12.6	31.7	19.1	-2.9	7.464264
5	14.9	35.04	20.14	-1.86	3.630077	14.6	32.5	17.9	-4.1	17.14838
6	13.44	33.94	20.5	-1.5	2.828427	13.1	31.4	18.3	-3.7	12.99604
7	13.38	35.64	22.26	0.26	0.835088	12.2	33.1	20.9	-1.1	2.143547
8	13.32	36.24	22.92	0.92	0.528509	15.2	33.7	18.5	-3.5	11.31371
9	13.26	34.14	20.88	-1.12	2.17347	11.4	31.6	20.2	-1.8	3.482202
10	13.2	33.34	20.14	-1.86	3.630077	11.2	30.8	19.6	-2.4	5.278032
11	13.14	33.74	20.6	-1.4	2.639016	11.3	31.2	19.9	-2.1	4.287094
12	13.08	35.94	22.86	0.86	0.550953	13.4	33.4	20	-2	4
13	13.02	33.64	20.62	-1.38	2.602684	11.2	31.1	19.9	-2.1	4.287094
14	12.96	33.54	20.58	-1.42	2.675855	12.7	31	18.3	-3.7	12.99604
15	12.9	34.84	21.94	-0.06	1.042466	12.2	32.3	20.1	-1.9	3.732132
16	12.84	35.94	23.1	1.1	0.466516	13.3	33.4	20.1	-1.9	3.732132
17	12.78	34.94	22.16	0.16	0.895025	11.1	32.4	21.3	-0.7	1.624505
18	12.72	34.24	21.52	-0.48	1.394744	15.4	31.7	16.3	-5.7	51.98415
19	12.66	35.04	22.38	0.38	0.768438	13.6	32.5	18.9	-3.1	8.574188
20	12.6	33.94	21.34	-0.66	1.580083	15.1	31.4	16.3	-5.7	51.98415
21	12.54	35.64	23.1	1.1	0.466516	13.3	33.1	19.8	-2.2	4.594793
22	12.48	36.24	23.76	1.76	0.295248	11.8	33.7	21.9	-0.1	1.071773
23	12.42	34.14	21.72	-0.28	1.214195	12.4	31.6	19.2	-2.8	6.964405
24	12.36	36.34	23.98	1.98	0.25349	12.3	33.8	21.5	-0.5	1.414214
25	12.3	38.04	25.74	3.74	0.074842	21.1	35.5	14.4	-7.6	194.0117
26	12.24	37.64	25.4	3.4	0.094732	11.2	35.1	23.9	1.9	0.267943
27	12.18	36.94	24.76	2.76	0.147624	12.7	34.4	21.7	-0.3	1.231144
28	13.2	39	25.8	3.8	0.071794	13.3	38.1	24.8	2.8	0.143587
29	15.4	32.84	17.44	-4.56	23.58831	11.4	30.3	18.9	-3.1	8.574188
30	13.2	33.6	20.4	-1.6	3.031433	12.1	29	16.9	-5.1	34.29675

	CT, Fam urin	CT, Fam urin	DCT	DDCT	F	CT, Fam urin	CT, Fam urin	DCT	DDCT	F
	16S RNA urine Sen	AS1974 Sen				16S RNA urine	AS1974			
	Sen					Resis	Resis			
1	13.2	34.8	21.6	0.6	0.659754	16.8	37.5	20.7	-0.3	1.231144
2	15.4	34	18.6	-2.4	5.27803	14.1	36.7	22.6	1.6	0.32987
3	13.2	32.2	19	-2	4	12.2	34.9	22.7	1.7	0.30778
4	11.4	32.6	21.2	0.2	0.87055	12.6	35.3	22.7	1.7	0.30778
5	14.9	34.5	19.6	-1.4	2.63901	14.6	37.2	22.6	1.6	0.32987
6	13.44	34.9	21.4	0.46	0.72698	13.1	37.6	24.5	3.5	0.08838
7	13.38	33.6	20.2	-0.78	1.71713	12.2	36.3	24.1	3.1	0.11662
8	13.32	35.8	22.4	1.48	0.35848	15.2	38.5	23.3	2.3	0.20306
9	13.26	34.3	21.0	0.04	0.97265	11.4	37	25.6	4.6	0.04123
1	13.2	33	19.8	-1.2	2.29739	11.2	35.7	24.5	3.5	0.08838
1	13.14	34.5	21.3	0.36	0.77916	11.3	37.2	25.9	4.9	0.03349
1	13.08	34.9	21.8	0.82	0.56644	13.4	37.6	24.2	3.2	0.10881
1	13.02	33.6	20.5	-0.42	1.33792	11.2	36.3	25.1	4.1	0.05831
1	12.96	35.8	22.8	1.84	0.27932	12.7	38.5	25.8	4.8	0.03589
1	12.9	36.2	23.3	2.3	0.20306	12.2	38.9	26.7	5.7	0.01923
1	12.84	34.4	21.5	0.56	0.67830	13.3	37.1	23.8	2.8	0.14358
1	12.78	33.1	20.3	-0.68	1.60214	11.1	35.8	24.7	3.7	0.07694
1	12.72	36.9	24.1	3.18	0.11033	15.4	39.6	24.2	3.2	0.10881
1	12.66	33.1	20.4	-0.56	1.47426	13.6	35.8	22.2	1.2	0.43527
2	12.6	33.5	20.9	-0.1	1.07177	15.1	36.2	21.1	0.1	0.93303
2	12.54	32.2	19.6	-1.34	2.53151	13.3	34.9	21.6	0.6	0.65975
2	12.48	33.1	20.6	-0.38	1.30134	11.8	35.8	24	3	0.125
2	12.42	34	21.5	0.58	0.66896	12.4	36.7	24.3	3.3	0.10153
2	12.36	32.9	20.5	-0.46	1.37554	12.3	35.6	23.3	2.3	0.20306
2	12.3	35.6	23.3	2.3	0.20306	21.1	38.3	17.2	-3.8	13.9288
2	12.24	32.1	19.8	-1.14	2.20381	11.2	34.8	23.6	2.6	0.16493
2	12.18	31.9	19.7	-1.28	2.42839	12.7	34.6	21.9	0.9	0.53588
2	13.2	33.4	20.2	-0.8	1.74110	13.3	36.1	22.8	1.8	0.28717
2	15.4	34.2	18.8	-2.2	4.59479	11.4	36.9	25.5	4.5	0.04419
3	13.2	34.6	21.4	0.4	0.75785	12.1	37.3	25.2	4.2	0.05440

GAPDH	Cp,	DCT	DDCT	F	CT,	DCT	DDCT	F
URIN CP, FAM	URIN Control				MIR-30			
17.3	35.36	18.06	4.06	0.059954	37.6	20.3	6.3	0.012691
18.3	34.46	16.16	2.16	0.223756	36.7	18.4	4.4	0.047366
17.6	36.56	18.96	4.96	0.032129	38.8	21.2	7.2	0.006801
17.4	37.56	20.16	6.16	0.013985	39.8	22.4	8.4	0.00296
16.5	33.66	17.16	3.16	0.111878	35.9	19.4	5.4	0.023683
19.9	34.36	14.46	0.46	0.726986	36.6	16.7	2.7	0.153893
17.3	31.16	13.86	-0.14	1.101905	33.4	16.1	2.1	0.233258
16.5	32.06	15.56	1.56	0.339151	34.3	17.8	3.8	0.071794
26.2	35.76	9.56	-4.44	21.70567	38	11.8	-2.2	4.594793
25.6	34.86	9.26	-4.74	26.72281	37.1	11.5	-2.5	5.656854
20.3	32.76	12.46	-1.54	2.907945	35	14.7	0.7	0.615572
21.7	32.16	10.46	-3.54	11.63178	34.4	12.7	-1.3	2.462289
21.4	37.46	16.06	2.06	0.239816	39.7	18.3	4.3	0.050766
22.3	34.66	12.36	-1.64	3.116658	36.9	14.6	0.6	0.659754
21	32.56	11.56	-2.44	5.426417	34.8	13.8	-0.2	1.148698
21.2	32.86	11.66	-2.34	5.063026	35.1	13.9	-0.1	1.071773
23	33.36	10.36	-3.64	12.46663	35.6	12.6	-1.4	2.639016
19	33.96	14.96	0.96	0.514057	36.2	17.2	3.2	0.108819
25	32.56	7.56	-6.44	86.82268	34.8	9.8	-4.2	18.37917
20.5	31.06	10.56	-3.44	10.85283	33.3	12.8	-1.2	2.297397
18.2	31.56	13.36	-0.64	1.558329	33.8	15.6	1.6	0.329877
19.6	32.06	12.46	-1.54	2.907945	34.3	14.7	0.7	0.615572
17.3	37.26	19.96	5.96	0.016064	39.5	22.2	8.2	0.003401
18	32.86	14.86	0.86	0.550953	35.1	17.1	3.1	0.116629
17	33.56	16.56	2.56	0.169576	35.8	18.8	4.8	0.035897
20.3	33.56	13.26	-0.74	1.670176	35.8	15.5	1.5	0.353553
22	33.56	11.56	-2.44	5.426417	35.8	13.8	-0.2	1.148698
21	36.16	15.16	1.16	0.447513	38.4	17.4	3.4	0.094732
20.9	34.36	13.46	-0.54	1.453973	36.6	15.7	1.7	0.307786
20.3	37.66	17.36	3.36	0.097396	39.9	19.6	5.6	0.020617

GAPDH BLOOD CP, FAM	Cp, Fam Mir-BLOOD	DCT	DDCT	F
20.28	38.9	19.1	5.1	0.029157
19.12	38	11.4	-2.6	6.062866
18.58	40.1	19.5	5.5	0.022097
18.38	41.1	17.8	3.8	0.071794
17.48	37.2	20.4	6.4	0.011842
20.88	37.9	14.1	0.1	0.933033
18.28	34.7	16.1	2.1	0.233258
17.48	35.6	13.2	-0.8	1.741101
27.18	39.3	15.1	1.1	0.466516
26.58	38.4	17.8	3.8	0.071794
21.28	36.3	17.1	3.1	0.116629
22.68	35.7	16.3	2.3	0.203063
22.38	41	20.7	6.7	0.009618
23.28	38.2	13	-1	2.215252
21.98	36.1	12.4	-1.6	3.031433
22.18	36.4	17.1	3.1	0.116629
23.98	36.9	16.6	2.6	0.164938
19.98	37.5	21.5	7.5	0.005524
25.98	36.1	17.1	3.1	0.116629
21.48	34.6	19.4	5.4	0.023683
19.18	35.1	18.8	4.8	0.035897
20.58	35.6	17.7	3.7	0.076947
18.28	40.8	24.8	10.8	0.000561
18.98	36.4	18.5	4.5	0.044194
17.98	37.1	19.6	5.6	0.020617
21.28	37.1	17.7	3.7	0.076947
22.98	37.1	17.5	3.5	0.088388
21.98	39.7	21.8	7.8	0.004487
21.88	37.9	19.9	5.9	0.016746
21.28	41.2	19.5	5.5	0.022097

الخـــــــلاصة

تم جمع مائتي عينة سريرية من مرضى يعانون من التهابات المسالك البولية من كلا الجنسين، ١٢٩عينة من النساء و ٧١ عينة من الذكور الذين تتراوح أعمارهم بين ١٧ و٥٣ سنة، وتم تشخيصهم بأنهم مصابون بعدوى المسالك البولية في مستشفى بغداد التعليمي ومستشفى غازي الحريري والمختبرات المركزية في المدينة الطبية ومستشفى اليرموك في بغداد / العراق للفترة من تشرين الأول (٢٠٢٢) إلى كانون الاول (٢٠٢٣). ١٢٩ (٦٤.٥٪) من الإناث، بينما ٧١ (٣٥.٥٪) من الذكور. زرعت عينات البول لعزل وتم التعرف على بكتريا الزائفة الزنجارية، حيث ان ١٤٨ (٧٤٪) كانت موجبة ، بينما ٥٢ (٢٦٪)عينة لايوجد فيها نمو بكتيري. و١٤٨/٥٦ (٣٧.٨٤٪) من العزلات الموجبة كانت الزائفة الزنجارية، بينما ١٤٨/٩٢ (٦٢.١٦٪) تنتمي إلى أجناس أخرى من البكتيريا. في هذه الدراسة ان جميع العزلات البكتيرية من *P. aeruginosa* تميزت بالاختبارات المورفولوجية والميكروسكوبية والكيميائية الحيوية ونظام Vitek II، تم استخدام نظام (VITEK-2 GN Kit). تم إجراء اختبار الحساسية للعزلات البكتيرية ضد ١٢ مضادًا حيويًا محددًا بواسطة VITIK 2. ان نتائج المقاومة لعزلات *P. aeruginosa* ضد المضادات الحيوية اشارت إلى أن عزلات *P. aeruginosa* مقاومة لـ ٥٦ (١٠٠٪) لسبعة من المضادات الحيوية، وهي Ticarcillin و Piperacillin و Imipenem و Meropenem و Gentamycin و Amikacin و Tobramycin. كان الكوليستين أكثر العوامل فعالية ضد *P. aeruginosa* حيث أظهر نسبة حساسية حوالي ٢٢(٣٩.٢٩٪) يليه ١٧(٣٠،٣٦%) Ciprofloxacin١٠(١٧،٨٦%) /Ticarcillin clavulanic acid والسيفتازيديم ٧(١٢.٥٪) والسيفيبيم ٤(٧.١٤٪) على التوالي. في هذه الدراسة ، تم فحص التعبير عن جين HFQ في عينات البول، وأظهرت النتائج زيادة كبيرة في التعبير عن HFQ في مقاومة الزائفة الزنجارية مقارنة بالعينات السريرية الحساسة، والتي تُعطى عن طريق تغيير الطية بعد تطبيعها مع جين *16sRNA* عن طريق الطي (21.497±1.241) مقابل (0.04598± 1.92142) على التوالي. بالإضافة إلى ذلك ، فإن التعبير عن جين *AS1974* في عينات البول وجد تنظيمًا تنازليًا واضحًا لـ AS1974 في المقاومة بعد التطبيع مع 16sRNA مع تغيير أضعاف (0.6622±0.0465) مقابل (2.012±1.0243) على التوالي. ومع ذلك، أظهر جين *MiR-30* في البول والدم تنظيمًا منخفضًا لـ *MiR-30* في عينات البول والدم ()، مقارنة بالأشخاص الأصحاء (,1.36±0.34 (0.47821±0.03678) (5.2463±1.213, 5.0432±1.7441) على التوالي.

وزارة التعليم العالي والبحث العلمي
جـــــــــــامعة بــــــــــابل
كـــــــــــــــلية الطب

تأثير sRNA As1974 والبروتينات المرتبطة HFq على التعبير عن جين المقاومة والحمض النووي الريبي الصغير للمضيف miR-30 في مرضى المسالك البولية والمصابين بعدوى الزائفة الزنجارية

رسالة مقدمة إلى

مجلس كلية الطب / جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في العلوم /أحياء مجهرية طبية

من قبل

بشـــــــــــائر سعد جبار آلـ - فتله

بكالوريوس تحليلات مرضية /كلية العلوم /جامعة الكوفه/٢٠١٧

اشـــــــــــراف

الأستاذ الدكتـــــــــورة	الاستاذ المساعد الدكتور
الهام عباس بنيان الساعدي	رافد فـــــــاخرحسين

١٤٤٤هـ ٢٠٢٣ م