Original Article

# Effect of Small-Interfering RNA (As1974) and HFq-Binding Proteins on Resistance Gene and Host MicroRNA (miR-30C) Expression in *Pseudomonas aeruginosa*-Infected Patients from Iraq

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## Abstract

**Background:** MicroRNAs (miRNAs) are a class of small RNAs encoded by the genome that regulate the production of cellular mRNAs that include either incomplete or complete miRNA-binding sites. **Objectives:** To evaluate the impact of sRNA (As1974) and HFq-binding proteins on the expression of resistance gene and host miRNA (*miR-30C*) in Iraqi urinary tract infections (UTIs) patients infected with *Pseudomonas aeruginosa*. **Materials and Methods:** Patients with UTIs from Baghdad, Iraq's Baghdad Teaching Hospital, Ghazi Hariri Hospital, Central Laboratories in Medical City, and Al-Yarmouk Hospital were recruited during June 2022 and October 2022 to provide 200 clinical samples. **Results:** Out of 200 patients with UTIs, only 56 (38.14%) were diagnosed as *P. aeruginosa* from positive urine samples. Urine samples were analyzed for *HFQ* gene expression, and the results showed that HFQ is overexpressed in *P. aeruginosa*-resistant samples compared to sensitive clinical samples, as measured by fold change after normalization with housekeeping gene *16sRNA* by folding (21.4971.241 vs. 1.92142 0.04598). Furthermore, normalization of *As1974* gene expression in urine samples using 16sRNA revealed a downregulation of As1974 in resistance, with a fold change of 0.66220.0465 versus 2.0121.0243. The miR-30 gene was shown to be downregulated in urine and blood samples (1.360.34, 0.478210.03678) as compared to those of healthy subjects. **Conclusions:** All ages were susceptible to the UTIs, also females suffered from UTIs more than males. A significant over expression of HFQ-binding protein in *P. aeruginosa* compared to sensitive clinical samples. Downregulation of As1974 in resistance.

**Keywords:** HFq binding proteins, *miR-30C*, *Pseudomonas aeruginosa*, resistance gene, *sRNA (As1974)*, UTI

## Introduction

*Pseudomonas aeruginosa* is a type of Gram-negative bacteria that is ubiquitous in natural environments like water, soil, and even hot tubs. This bacterium can infect mammals and has been linked to a wide range of animal and human diseases.[1] This organism has the amazing ability to colonize ecological niches where resources are few, from water and soil to plant and animal tissues, because to its propensity for development in moist environments and its ability to use a wide variety of organic compounds.[2] The soluble pigments pyoverdin and pyocyanin are both produced by different strains of *P. aeruginosa*. The presence of pyocyanin indicates that *P. aeruginosa* is the cause of the suppurative infection.[3]

MicroRNAs (miRNAs) are a class of small RNAs encoded by the genome that regulate the production of cellular mRNAs that include either incomplete or complete

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miRNA-binding sites. The first step in the typical process of miRNA expression is the transcription of a capped, polyadenylated primary miRNA (pri-miRNA) transcript by RNA polymerase II.[4]

For the treatment of bacterial infections, RNA-based technologies are gaining popularity. Preclinical studies using tfgb1-targeting siRNAs have showed promising results in the fight against pulmonary tuberculosis.[5] However, numerous miRNAs, including several of those already described, offer fresh opportunities for the creation of effective antibacterial strategies. The creation of anti-miRNAs that target-specific miRNAs that facilitate bacterial infection may also reduce or halt the pathogen's host colonization. Antisense miRNAs are a class of synthetic single-stranded RNAs that target miRNAs for degradation.[6]

Recent research has identified a role for at least six small RNAs (sRNAs) in the regulation of drug resistance in *P. aeruginosa*, with various sRNAs having distinct regulatory mechanisms.[7] This is achieved through the following mechanisms: the sRNA As1974 is a significant regulator of the expression of numerous resistance pathways, including membrane transporters and biofilm-associated antibiotic resistance genes. Antibiotic-resistant bacteria can be converted to ones that are susceptible by the small RNA As1974.[8]

The expression of the master regulator As1974, which controls the expression of many drug-resistance pathways including membrane transporters and biofilm-associated antibiotic-resistant genes, is controlled. Methylation controls As1974 expression in all tested multidrug resistant (MDR) strains. All drug-resistant strains have lower levels of this gene relative to drug-susceptible strains. The drug resistance of a clinical strain could be overcome by causing hypersusceptibility through the overexpression of As1974. It forms strong bonds with Hfq.[9]

The Hfq protein is involved in post-transcriptional control of gene expression by interacting with regulatory bacterial small RNAs (sRNA). Several infectious bacteria's virulence and pathogenicity have been linked to Hfq.[10] It has been discovered that the Hfq RNA chaperone is present in nearly all of the sequenced bacterial species. Initially, it was shown to be a bacteriophage Q RNA replication factor in *Escherichia coli*.[11] Hfq serves multiple purposes, including binding bacterial sRNA and mRNA and promoting RNA–RNA interactions. In addition, Hfq has been shown to independently regulate gene expression by affecting mRNA polyadenylation.[12] Hfq governs the ribo-regulation of the expression of many genes in bacteria, in addition to binding RNAs, and affecting a number of proteins directly or indirectly.[13] Although certain bacteria, like *Staphylococcus aureus* and *Bacillus subtilis*, have two *hfq* genes, the majority of bacteria only have one.

Three different hfq gene sequences were discovered in *Bacillus anthracis*, and their roles in the bacterium were studied.[14] An *E. coli* mutant was created using hfq insertion mutations, proving the function and significance of Hfq. Reduced growth rates, altered cellular shape, and enhanced sensitivity to stress were just some of the pleiotropic phenotypic alterations displayed by the mutant.[15]

The aim of this study was to evaluate the impact of sRNA (As1974) and HFq-binding proteins on the expression of resistance gene and host miRNA (*miR-30C*) in Iraqi urinary tract infection (UTI) patients infected with *P. aeruginosa*.

## Materials and Methods

Two hundreds patients (129 females and 71 males) at age 17–53 years with UTIs were surveyed at four hospitals in Baghdad: Baghdad Teaching Hospital, Ghazi Hariri Hospital, Central Laboratories in Medical City, and Al-Yarmouk hospital. A total of two hundred clinical samples (mid-stream urine samples) were collected in sterile screw-cap containers during the period from June 2022 to October 2022. Each specimen was sent to the lab for further study as soon as possible and was cooled on the way there.

Thirty patients with severe UTI had 5 mm vein blood samples taken for miRNA research.

### Isolation and identification of *P. aeruginosa*

After centrifuging all urine samples at 1500 rpm for 5 min, the supernatant was discarded and the pellet was cultured in brain heart infusion broth medium (HIMEDIA/India) at 37°C for 24 h to encourage bacterial growth. Then, after 24 h in 37°C, they were streaked onto general and differential culture medium. Colonies that could not ferment lactose when streaked on MacConkey agar were chosen and re-cultured on new MacConkey agar plates to get truly pure, well-isolated colonies. After 24 further hour's incubation at 37°C, the acquired colonies were streaked onto plates of cetrimide agar to test for fluorescein and pyocyanin dye production. The Vitek2 system (Biomerieux, France, Card type: GN, AST-N222) was used to identify the bacteria, and morphological, microscopical, and biochemical testing confirmed the presence of the positive growth.

### Molecular analysis

The sequence of primers that used this study are illustrated in Table 1.

### EasyScript® first-strand cDNA synthesis super mix cat. no. AE301

All of the components for making cDNA from whole RNA or mRNA are included in the EasyScript® First-Strand

**Table 1: The sequence of primers used in the study**

| Primer | Sequence | Primer sequence 5′- - 3′ | $T_m$ (°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 | TAGGCGAGACATTTTTTTTTTTTTTTTTT | | |
| *16s RNA* | F | CAGCTCGTGTCGTGAGATGT | 62 | 55.0 |
| | R | G CGTAAGGGCCATGATGACTT | 60 | 50 |
| *GAPDH* | F | CACTAGGCGCTCACTGTTCTC | 60 | 60 |
| | R | AATCCGTTGACTCCGACCTT | 59 | 59 |

**Table 2: Polymerase chain reaction thermocycler conditions**

| qPCR steps | Temp. | Time | Cycle (s) |
|---|---|---|---|
| Initial activation | 95°C | 2 min | 1 |
| Denaturation | 95°C | 15 s | 40 |
| Annealing | 60°C | 60 s | |
| Extension | 90°C | 15 s | |

cDNA Synthesis SuperMix. The cDNA is generated in a time- and labor-saving manner using EasyScript® RT/RI Enzyme Mix and 2ES Reaction Mix. Allow to sit at 25° for 10 min. The next step is a 15-min incubation at 42°C (for qPCR) or at 42°C. We used Luna Script RT Master Mix Kit (5×) (BioLabs, England) to conduct qPCR, with RT mixtures containing 10 L of the master mix, 0.5 L of each forward and reverse primer, and 0.5 L of each cDNA template. After adding the forward and reverse primers, 5 µL of cDNA from each sample, and 4 µL of nuclease free water, the final amount was 20 µL. In this case, the GAPDH gene served as an *in vivo* reference standard. After activating the polymerase at 95°C for a full minute, the cDNA was subjected to 45 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 20 s, all while scanning the channels. Melting curve analysis was then performed based on the characteristics of the cDNA's separation as the number of denaturation cycles increased. The relative quantitative technique was used to calculate miRNA expression by normalizing target miRNA Ct values to the GAPDH reference gene. Folding = 2^-ΔΔCT

The reaction has been mixed thoroughly and centrifuged briefly to collect solutions at the bottom of polymerase chain reaction (PCR) tubes or plates and then have been stored on ice protected from light. Then after the preparation has finished the tubes placed into the thermal cycler which have been programmed as shown in Table 2.

In order to do qPCR, we used a GoTaq® qPCR Master Mix, Promega, USA Kit. Ten microliters of the master mix, five microliters of each forward and reverse primer, and an after adding the forward and reverse primers, 5 µL of cDNA from each sample, and 4 µL of nuclease free water, the final amount was 20 µL. Endogenous control materials, including urine, blood, and 16S RNA, were compared to the GAPDH gene. The melting curve analysis was based on the separation characteristics of double-stranded cDNA during cycles with increasing denaturing TM, and the qPCR was run at 95°C for 1 min to activate the polymerase, followed by 45 cycles of 95°C for 15 s to denaturate the double-stranded cDNA and 60°C for min of annealing steps with channel scanning. The expression levels of the target genes (HFQ, As1974, and MICRORNA-30) were calculated by the comparative Ct algorithm and folding = 2-CT analysis and normalized to the 16sRNA and GAPDH reference gene.

### Statistical analysis

The 25th revision of SPSS (SPSS, IBM Company, Chicago, IL 60606, USA) was used for the statistical analysis. Frequencies and percentages were used to illustrate categorical variables. The results were shown as (means ± SE) for continuous variables. The means of the two groups were compared using the student *t* test. If the probability value was not <0.05, it was not regarded to be statistically significant.[16]

### Ethical approval

Prior to the collection of samples, informed consent was acquired from all subjects engaged in this work. A local ethics committee examined and approved the study protocol, subject information, and permission form at College of Medicine, University of Babylon, Iraq, under the reference No. BMS/ 0296/016 on February 13, 2022.

## Results

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

The urine samples were cultured for *P. aeruginosa* isolation and identification, 148/200 (74%) revealed

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

To confirm the isolation of *P. aeruginosa*, the VITEK2 system was used (VITEK-2 GN Kit). With 56 samples, it was found 56 (100%) *P. aeruginosa*. 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), given by fold change after normalized with housekeeping gene *16sRNA* by folding (21.497 ± 1.241 vs 1.92142 ± 0.04598), respectively, as seen in Table 3 and Figure 1A and B.

This study has shown that As1974 is downregulated in resistance after normalization with 16sRNA, with fold change of 0.66220.0465 versus 2.0121.0243, respectively, suggesting that it could be a suitable marker to shift the bacteria from resistant to sensitive [Table 4 and Figures 2A and B, 3].

**Table 3: Expression of *HFQ* gene in pathogenic resistant and sensitive *Pseudomonas aeruginosa isolates* from 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 $P < 0.05$.

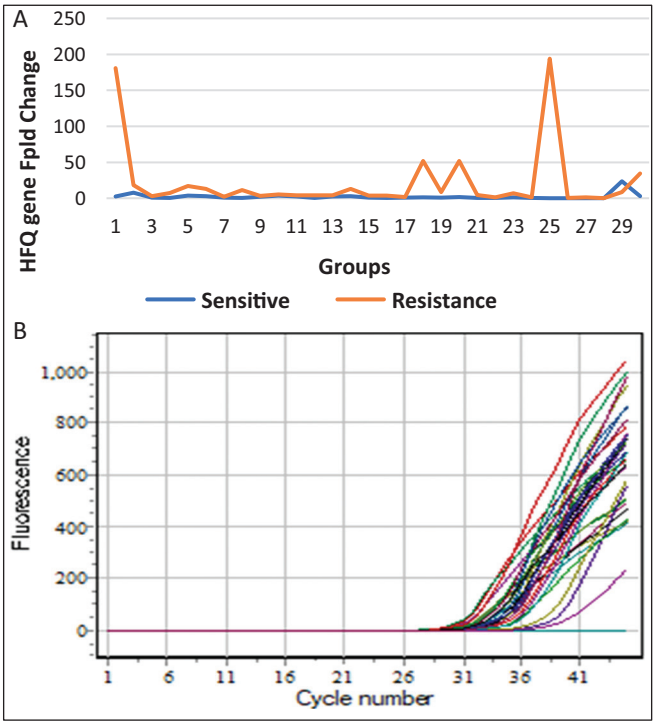


**Figure 1:** (A) The Expression of *HFQ* gene in sensitive and resistance *P. aeruginosa* in urine samples. (B) *HFQ* gene urine dissociation curves by qPCR

**Table 4: 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 *P. aeruginosa* | 19.93 ± 1.22 | −0.06 ± 0.001 | 2.012 ± 1.0243 |
| Resistance *P. aeruginosa* | 22.216 ± 3.15 | 2.014 ± 0.673 | 0.6622 ± 0.0465** |
| *P* value | 0.001 | 0.013 | 0.0001 |

** Downregulated

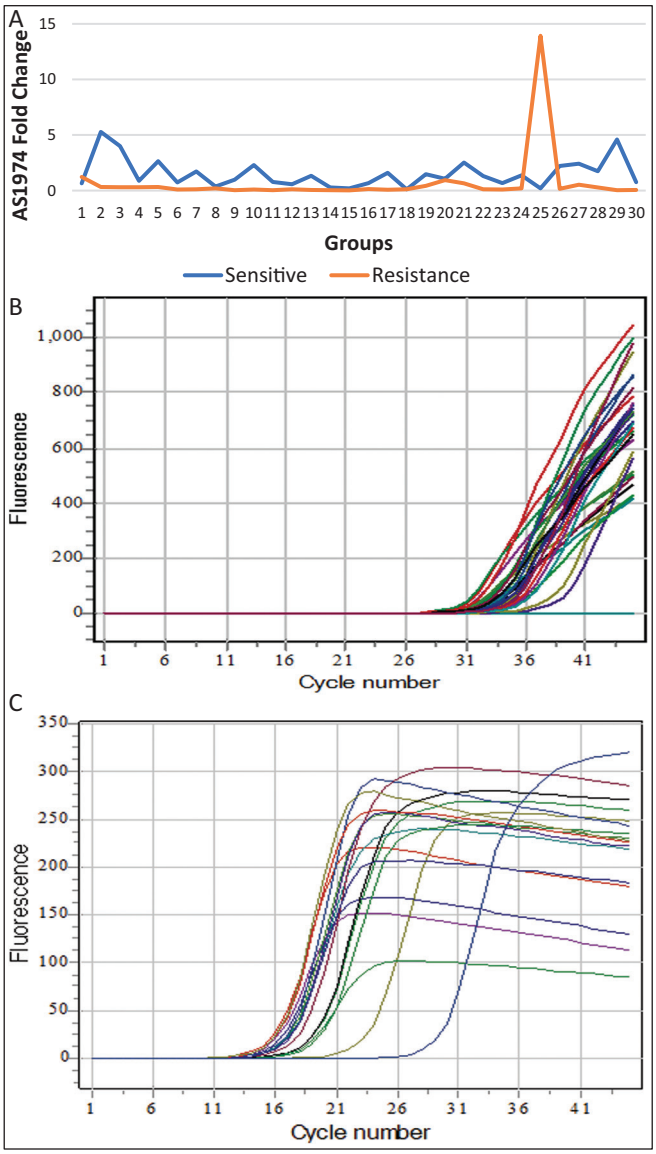


**Figure 2:** (A) The expression of *As1974* gene in sensitive and resistance *P. aeruginosa* in urine samples. (B) As1974 in urine dissociation curves by qPCR. (c) B.16sRNA urine dissociation curves by qPCR

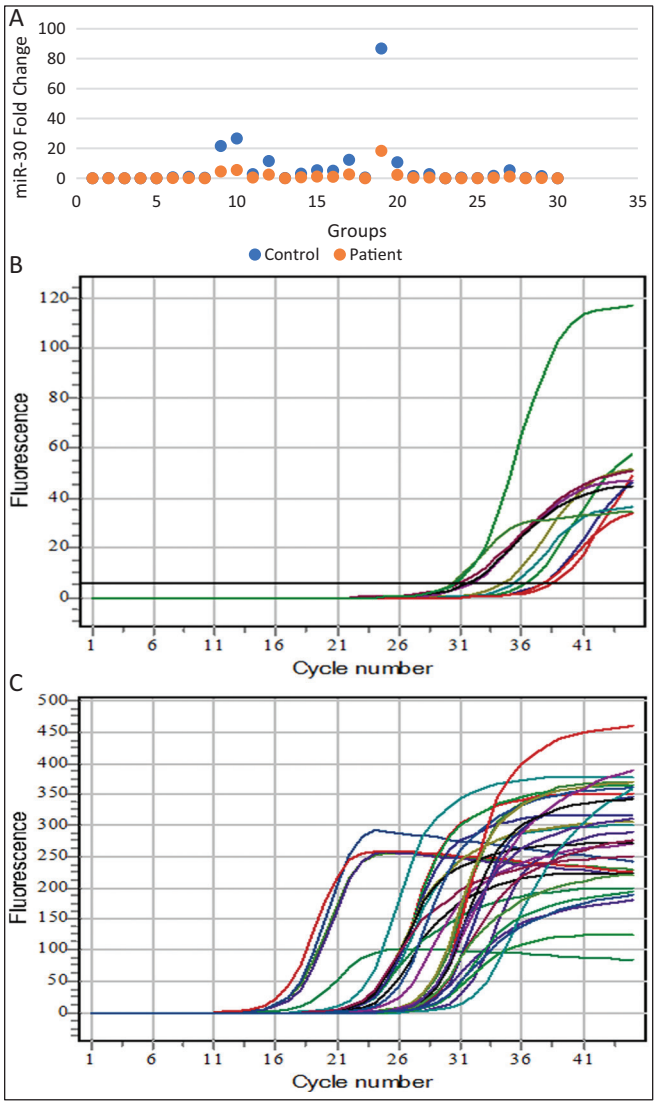


**Figure 3:** (A) The expression of *miRNA30* in UTI patient and controls in urine samples. (B) *MiRNA30* urine dissociation curves by qPCR. (C) GADPH blood and urine dissociation curves by qPCR

The results showed in Tables 5 and 6 and Figure 3A and B, 4A, B demonstrated downregulation 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. This study evaluated the expression of miRNAs in the peripheral blood of UTI patients, determining the diagnostic efficacy of miRNA in predication of UTI response. Figure 4A and B revealing there were downregulation when it compared to the control as the expression of miRNAs, and it is good to mention that the same scenario was seen with blood samples as it is affected equally.

## Discussion

This study's findings were consistent with those of AL-Khikani and Ayit,[17] 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 years) had the highest prevalence of UTIs. Researchers Almalki and Varghese[18] observed that *P. aeruginosa* produced an infection rate of 53 (36.05%) among those aged 11–20 years, 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.*[19] who found that females were more likely to be infected than males were (64.91% vs. 35.09%). This

**Table 5: 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 |

** Downregulation
* Significant differences $P < 0.05$

**Table 6: 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 |

**Downregulation
*Significant differences $P < 0.05$

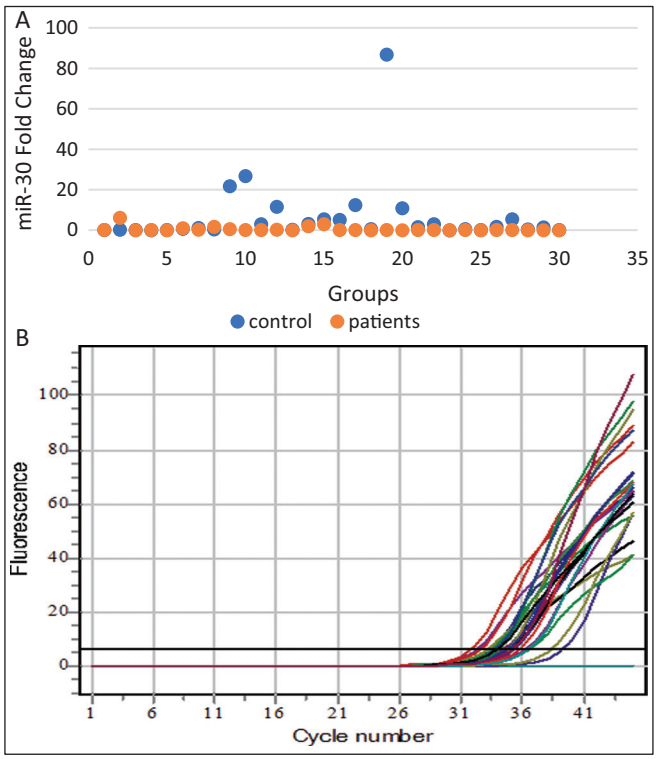


**Figure 4:** (A) The expression of *miRNA30* in blood samples in patients and controls. (B) *MiRNA30* in blood dissociation curves by qPCR

study's findings are 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. The findings corroborate the research by Lob *et al*.,[20] 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 García-Fernández *et al*.[21] Abdel Khalek *et al*.[22] 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*.[23] 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.[24] 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.[2] 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.[25] 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*.[26] 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. Strains of *P. aeruginosa* lacking the *hfq* gene are more resistant to multiple antibiotic classes. Data from transcriptome analysis showed that *Hfq* affects several pathways known to be implicated in antibiotic resistance. These pathways include import and efflux, energy metabolism, cell wall and LPS composition, and c-di-GMP levels. The capture of Hfq also increases the sensitivity to antibiotics.[27] Hfq has been demonstrated to inhibit CCR in *P. aeruginosa* by binding directly to catabolic mRNAs. For this reason, Crc, a protein involved in catabolite repression, stabilizes the RNA–Hfq complex and functions as a co-repressor. The effect of Hfq on bacteria's sensitivity to antibiotics is poorly understood.[26]

Hfq-mediated riboregulation via sRNA has been shown to modulate certain activities implicated in antibiotic sensitivity.[28] Deficiencies in ArcB, a component of the Arc AB-ToIc efflux pump, correlated with an *E. coli* hfq mutant strain's enhanced sensitivity to many hazardous chemicals.[29]

The resistance of *P. aeruginosa* PAO1 and PA14 hfq deletion strains to numerous types of antibiotics of clinical utility was mentioned by Chevalier *et al*.[30] An increase in antibiotic sensitivity was observed in most cases when the *hfq* gene was deleted. The levels of c-di-GMP and cellular energy metabolism are both affected by *Hfq*. Growth of the *hfq* deletion strains is slightly stunted, suggesting that the loss of Hfq has a deleterious effect on fitness overall. *P. aeruginosa* was found to have poor aminoglycoside uptake while it was in a dormant state.

The persistence of germs despite antibiotic therapy is linked to a condition known as phenotypic tolerance.[26] Based on the results of the transcriptome study, it is clear that Hfq regulates the expression of a wide variety of functions that influence resistance to antibiotics. Consequently, the observed enhanced susceptibility to antibiotics appears to result from a combination of physiological changes on the one hand and the modulation of specific antibiotic resistance determinants on the other.[31] It is still unclear whether Hfq exerts its regulatory influence on resistance genes/determinants through riboregulation or direct translational repression. *P. aeruginosa* hfq mutant strains, on the other hand, showed enhanced sensitivity to aminoglycosides, betalactams, fosfomycin, and polymyxin B.[26]

As a result, it is possible that Hfq regulates, at the posttranscriptional level, a number of functions giving susceptibility. Hfq is the primary RNA-binding protein in bacteria, and its interactions with mRNAs, either alone or in combination with regulatory small noncoding RNAs (sRNAs), influence mRNA translation and destruction. Despite the significant intraspecies genetic variation that is well known to exist, researchers typically only look at a single reference strain and believe that their results may apply to the entire species.[31]

As1974 is the master regulator that controls the expression of many drug-resistance pathways, including genes involved in membrane transport and antibiotic resistance in biofilm, through methylation sites in the genes' 5′ untranslated regions (UTR). Using sRNA to convert bacterial drug resistance to hypersusceptibility may be a future technique for treating MDR bacteria, as revealed by our study of the sRNA that govern the MDR pathways in *P. aeruginosa* clinical isolates.[8]

Hundreds of thousands of individuals are put in danger every year by bacteria that are resistant to numerous antibiotics. Antibiotic options for treating bacterial infections have been severely constrained in recent years due to the spread of antibiotic-resistant bacteria and a lack of new antimicrobial drug development. One possible solution to this issue could be to maintain the effectiveness of existing antibiotics in clinical settings. Expression of As1974 is controlled by methylation sites in its 5′ UTR, making it a master regulator of gene

expression for many drug-resistance pathways. These pathways include membrane transporters and antibiotic-resistant genes involved with biofilms. sRNA may control MDR pathways in *P. aeruginosa* clinical isolates.

Furthermore, sRNA may represent a future strategy for combating MDR bacteria by converting bacterial drug resistance to high sensitivity. One of the small RNAs (sRNAs) shown to be able to convert an MDR clinical strain to drug hyper sensitivity was identified as As1974. This sRNA was shown to be downregulated in the MDR clinical isolates of *P. aeruginosa*, while many others were overexpressed.[8] It is unknown what role miRNAs play in the interplay between macrophages and uropathogenic *Pseudomonas*, despite the fact that miRNAs have been recognized as significant modulators of the host immune response against this pathogen. According to earlier research, autophagy-mediated clearance of *B. pseudomallei* is regulated by miR-4458, miR-4667-5p, miR-4668-5p, and miR-30a-5P through targeting ATG10.[32]

Additionally, miRNAs can control the expression of Rab GTPases including Rab5a, Rab5c, and Rab11a. Therefore, miRNAs' potential roles in controlling Rab GTPase are intriguing and warrant more study.[33] Although research on miR-30-5p expression in *P. aeruginosa* infection has been limited, miR-30 has been shown to play a role in a variety of cellular processes, including immunity, inflammation, apoptosis, cell cycle regulation, and DNA replication and repair. Importantly, this finding on the expression profile of miR-30-5P in uropathogenic *P. aeruginosa* corroborated a previous miRNA profiling finding showing four members of the miR-30 family are considerably downregulated in *Burkholderia pseudomallei*-infected macrophages.[34]

The expression levels of miR-30b and miR-30c were found to be reduced, while those of miR-30d and miR-30e were not. This was confirmed by qRT-PCR from the original microarray data.[35] Genes in various subgenomic locations express members of the miR-30 family.[36] They both have similar seed sequences close to the 5′ end but distinct compensation sequences close to the 3′ end.[33] Members of the miR-30 family are commonly differentially expressed and regulated during biological processes because their unique compensatory sequences allow them to target a wide variety of genes and pathways. During the osteoblast development process, miR-30c, miR-30d, and miR-30e expression levels were considerably reduced, while miR-30a and miR-30b expression levels were not changed.[37-41]

## Conclusion

Recent study confirmed that all ages were susceptible to the UTIs, also females were suffered of UTIs more than males. A significant overexpression of HFQ-binding protein in *P. aeruginosa* compared to sensitive clinical samples. Downregulation of As1974 in resistance suggested that As1974 could be potential marker to transform the bacteria from resistance to sensitive and downregulation of Micro-30 in urine and blood samples compared to healthy subjects. The host immune response to uropathogenic *P. aeruginosa* has been shown to be significantly influenced by miRNA. The sRNA has promise as a future strategy for combating MDR bacteria.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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