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# Identification of rsbA Gene in Proteus Mirabilis Isolated from Patients with Respiratory Tract Infections and Determining their Antibiotic Resistance

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**Annotation: Background & aim**: P. mirabilis rarely causes lung infections or pneumonia, and it usually affects people who have chronic lung illness or chronic debilitation. The current study was conducted to determine the antibiotic resistance of P. mirabilis isolated from individuals with respiratory tract infections by identifying the rsbA gene.

**Materials & methods**: From January to July 2025, 270 specimens were taken from outpatients with respiratory tract infections who were exhibiting symptoms and visiting hospitals and private clinics in Kirkuk City. PCR was utilized to diagnose the rsbA gene, and each specimen was identified by phenotypic traits after being cultivated on various labeled culture media.

**Results**: The results indicated that when P. mirabilis was grown on MacConkey agar, 28 (10.4%) of the total specimens had positive growth results. On the other hand, the results showed that 243 (89.3%) of sputum samples were negative for bacterial growth of P. mirabilis out of a total of 270 samples. Biochemical tests for *P. mirabilis* bacteria appear to be negative for Indole, oxidase, while, *P. mirabilis* were positive for urease, citrate, motility, catalase and Kligler iron k/A H2S. *P. mirabilis* showed a high

resistance toward 78.6% Ceftriaxone, 85.7% Cefepime and 96.4% Ampicillin. Otherwise, *P. mirabilis* showed high sensitive toward 71.4% Gentamicin, 78.6% Imipenem, and 78.6% Amikacin respectively. After isolating DNA from P. mirabilis using an extraction and electrophoresis kit, it was found that 100% of P. mirabilis isolates contained the rsbA gene.

**Conclusions**: It is concluded from the results of the current work that Proteus mirabilis is one of the causes of respiratory tract infections and has shown high resistance to antibiotics. One of the most important reasons for its virulence is that it possesses virulence genes in high proportions. The study also showed that the bacteria possessed 100% of the rsbA gene, which is responsible for regulating swarming motility.

**Keywords:** P. mirabilis, rsbA, RTIs, virulence gene, antibiotic sensitivity

## Introduction

Proteus mirabilis is a rod-shaped, facultatively anaerobic, Gram-negative bacteria. Long recognized as a member of Enterobacteriaceae family, it is classified in the Gammaproteobacteria class (1). This motile bacterium was initially discovered by Gustav Hauser in 1885 (2). Its remarkable urease production, unique "swarming" behavior on agar plates, and quick and coordinated multicellular activity were first noted by Gustav Hauser. Its peritrichous flagella give it a characteristic "bull's-eye" pattern and aid in its movement across surfaces (3). Early in the twenty-first century, the bacterium's genome was sequenced, providing information about its metabolic adaptability to different conditions. Interestingly, P. mirabilis is present in a variety of environments, such as soil, water, and sewage, and it is essential to the breakdown of organic materials in these environments (4). However, it is mostly found in the digestive systems of both humans and animals since it is regarded as an essential component of the fecal flora (5). P. mirabilis has the ability to generate biofilms in addition to its virulence components. These features provide the pathogenic bacteria more pathogenicity and improve their ability to withstand drugs (6,7). Antibiotic resistance may result in poor treatment, a higher risk of patient death, and higher health expenses because of the additional hospitalizations, in addition to other reasons such the inappropriate use of antibiotics (7,8). Proteus mirabilis has evolved a number of virulence factors that aid in its growth and survival within the host. These virulence factors are linked to the capacity to cause disease (9). The quorum sensing rsbA gene encodes a sensory and functions as a protein sensor in the environment by promoting biofilm formation and controlling swarming movement (10). Without identifying the virulence genes, Kamil and Jarjes (11) examined the

distribution of ureR, the only gene that is in charge of P. mirabilis identification. In order to identify the rsbA gene in P. mirabilis isolated from individuals suffering from infections of the respiratory tract and ascertain their antibiotic resistance, the current investigation was implemented.

## Materials & Methods

### Sampling

From January to July 2025, 270 specimens were taken from outpatients with respiratory tract infections who were exhibiting symptoms and visiting Kirkuk City's hospitals and private clinics. Each specimen was cultivated on several labeled culture media for identification reasons. Phenotypic traits such as urease production in urea agar medium, non-lactose fermentation on MacConkey agar, and swarming movement on blood agar were used to identify the specimens. Tests utilizing catalase, motility, oxidase, and triple sugar iron agar media were carried out in order to further differentiate P. mirabilis from other possible microorganisms on the same media.

### Identification

The bacteria were identified based on a range of distinctive aspects, including morphological characteristics such as shape, size, and color on different culture media, and reactions with stains such as the Gram stain test to determine the type of bacteria (Gram-positive or Gram-negative). In addition, biochemical tests were performed like catalase, oxidase, indole, methyl red, and fermentation tests. The VITEK 2 apparatus (bioMerieux) was also used. All these assays helped to accurately identify the bacteria.

### Antibiotic susceptibility test

The Kirby-Bauer disk diffusion method was used to conduct AST. In this method, a Mueller-Hinton agar plate is prepared and filled with a sample of the bacteria to be tested. Disks containing different antibiotics are then placed on the surface of the agar. The plates are incubated at 37°C for 16–18 hours, allowing the bacteria to grow and form a halo of inhibition around the disks. The diameter of the inhibition halo is measured after the incubation period to determine the antibiotic's effectiveness against the bacteria.

### DNA extraction

Bacterial genomic DNA was extracted from isolates using a commercial genomic DNA extraction kit (Geneand/China), according to its resistance rating as specified by the manufacturer. Following extraction, concentration and purity were assessed using a Thermo Fisher Scientific NanoDrop spectrophotometer, where absorbance was measured at 260/280 nm to determine purity. After confirming sample quality, DNA amplification was performed using polymerase chain reaction (PCR) to amplify the target gene.

### Detection of rsbA gene using PCR

In this study, the virulence gene rsbA was assessed in P. mirabilis. Table 1 lists the oligonucleotide primers that were utilized to identify the species-specific region. For the gene, PCR amplification was carried out independently in a 25 µL reaction mixture.

Table (1): rsbA gene PCR assayprimers

| Primer | Primer sequence | Length (bp) | Ref. |
|---|---|---|---|
| rsbA-F | 5-TTGAAGGACGCGATCAGACC-3 | 467 | [12] |
| rsbA-R | 5- ACTCTGCTGTCCTGTGGGTA-3 | | |

The reaction for each gene was prepared using 12.5 µL of Taq PCR PreMix (2X), 1 µL of forward and reverse primers, and 3 µL of sampled DNA. The final volume was 25 µL with 7.5 µL of nuclease-free water. After electrophoresis on 2% agarose gel and Redsafe staining, primer sequences were obtained from Macrogen (Korea).

## Results and discussion

270 urine samples from patients with RTIs were used in the current investigation (table 2). According to the results, 29(10.7%) of the total samples showed positive results for the growth of *P. mirabilis* when it was cultured on MacConkey agar. On the other hand, the results showed that 243 (89.3%) of sputum samples were negative for bacterial growth of P. mirabilis out of a total of 270 samples.

**Table (2): Distributed of study samples according to UTI**

| | No. (%) **+ve culture** | No. (%) **-ve culture** | Total No.(%) |
|---|---|---|---|
| *P. mirabilis* | 29(10.7%) | 243(89.3%) | 270(100.0%) |

### *Proteus mirabilis* identification

*P. mirabilis* are gram-negative, motile bacilli. After an overnight incubation at 35–37°C, Proteus develops individual fermenting colonies on MacConkey agar (fig. 1) that do not contain lactose. The presence of bile salt in the medium prevents swimming or rippling movement, as shown in Figure (1). Biochemical tests for *P. mirabilis* bacteria appear to be negative for Indole, oxidase, while, *P. mirabilis* were positive for urease, citrate, motility, catalase and Kligler iron k/A H2S.

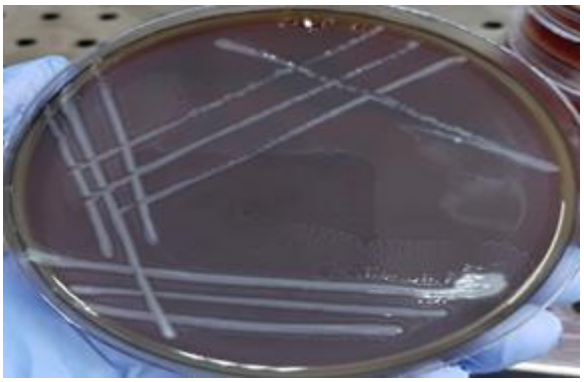

Figure (1): P. mirabilis colonies on MacConkey agar

### Antibiotic susceptibility

*P. mirabilis* showed a high resistance toward 78.6% Ceftriaxone, 85.7% Cefepime and 96.4% Ampicillin. Otherwise, *P. mirabilis* showed high sensitive toward 71.4% Gentamicin, 78.6% Imipenem, and 78.6% Amikacin respectively, as shown in table (3).

Table (3): Resistance and sensitivity of P. mirabilis to different antibiotics

Figure (1): P. mirabilis colonies on MacConkey agar

| **Antibiotics** | **Sensitive %** | **Intermediate %** | **Resistant %** | **P value** |
|---|---|---|---|---|
| Ampicillin | 0.0 | 3.6 | 96.4 | |
| Ceftriaxone | 21.4 | 0.0 | 78.6 | 0.001 |
| Gentamicin | 71.4 | 0.0 | 28.6 | |
| Ceftriaxone | 64.3 | 3.6 | 32.1 | |
| Cefepime | 14.3 | 0.0 | 85.7 | |
| Imipenem | 78.6 | 0.0 | 21.4 | |
| Ciprofloxacin | 67.9 | 3.6 | 28.5 | |
| Levofloxacin | 64.3 | 3.6 | 32.1 | |
| Azithromycin | 60.7 | 7.1 | 32.2 | |
| Trimethoprim | 28.6 | 3.6 | 67.8 | |
| Nalidixic acid | 35.7 | 0.0 | 64.3 | |
| Amikacin | 78.6 | 0.0 | 21.4 | |

A group of bactericidal medications known as β-lactam antibiotics have the β-lactam ring in their molecular structure. They fall into the following categories: monobactams, carbapenems, cephalosporins, penems (also called thiopenems), and penicillins. The chemical makeup of the ring fused to the βlactam pharmacophore unit, which creates a noncoplanar bicyclic scaffold, determines this classification (13). The current study's findings demonstrated a high level of resistance to the cefalosporins, specifically cefepime (85.7%) and ceftriaxone (78.6%). Study (14) from Diyala province, reported that 90% of isolates were resistant, concurred with these Ceftriaxone results. Additionally, 90.3% of the isolates had ceftriaxone resistance and 67.7% had cefepime resistance, according to Rout et al. (15). Cefepime resistance was determined to be approximately 90% by (16). However, study (17) from Iran observed that 10% of patients had ceftriaxone resistance, which was in contrast to this study. A high level of ampicillin resistance was also found in the study. There was 96.4% ampicillin resistance. Study of (20) from Saudi Arabia, study of (19), and (20) from Iraq, found that ampicillin resistance was 80%, which is consistent with this result. The fluoroquinolone antibiotic ciprofloxacin susceptibility rate was 67.9%, which is consistent with earlier research. Study (22) and (23) finding of 49% and 60.23%, respectively. However, this is somewhat different from Hussein et al. (19) from Iraq, who reported a higher level of sensitivity (69.8%), study of (24) and (25) from Turkey found lower levels (35% and 26%). since he discovered that 28.6% of people had gentamicin resistance. While study of (15) reported that amikacin and gentamicin resistance were different at 67.7% and 58%, respectively, his findings for netilmicin resistance were 45.1% in agreement with ours.

### *rsbA* gene detection

After isolating DNA from P. mirabilis using an extraction and electrophoresis kit, it was found that 100% of P. mirabilis isolates contained the rsbA gene, as shown in Figure 2.

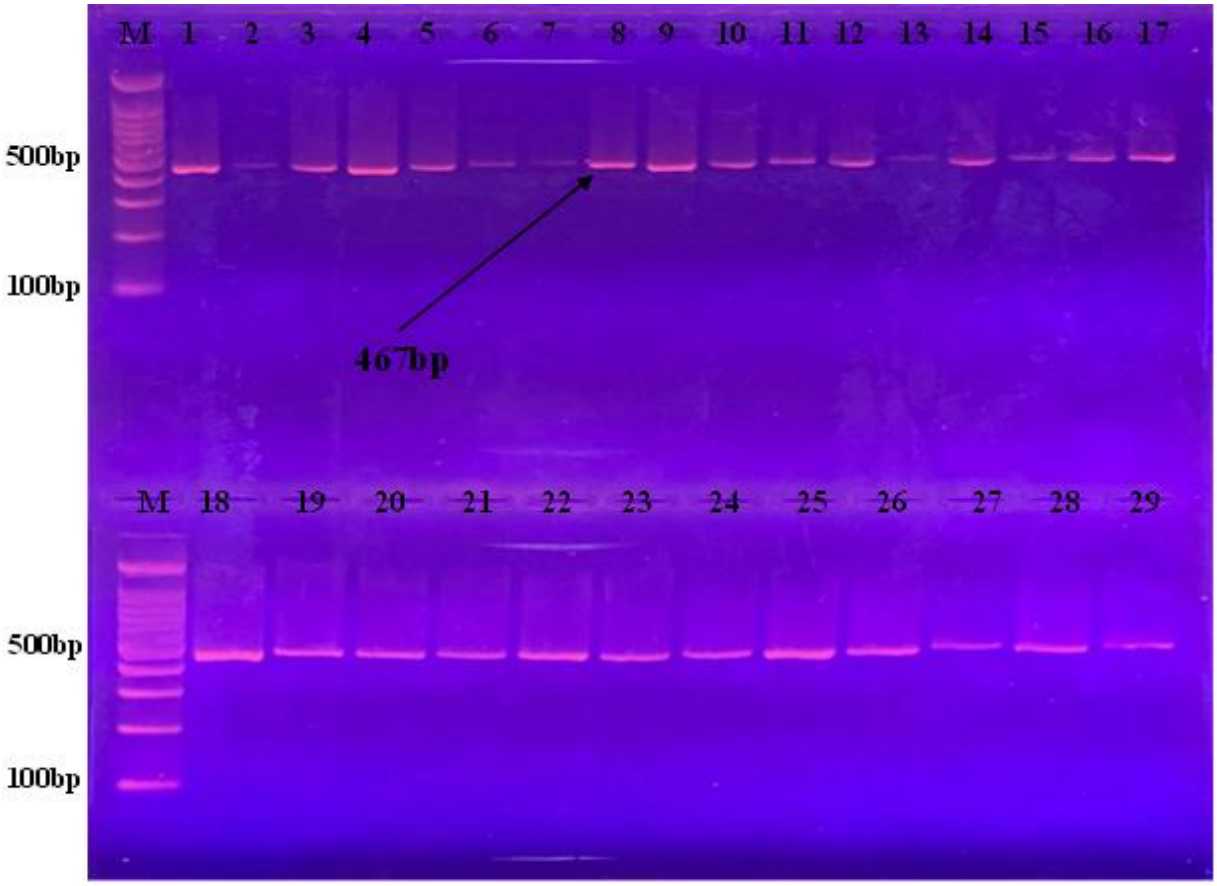


Figure (2): PCR amplification of 467bp rsbA gene by 2% agarose gel electrophoresis.

Ladder: M, Lane (1-29): PCR product of P. mirabilis isolates from sputum samples.

The rsbA gene plays a partial role in regulating bacterial crawling behavior, a collective behavior in which bacteria move in a coordinated manner across hard surfaces. The rsbA gene is thought to act as a protein-sensing agent in response to environmental conditions such as humidity and temperature, thus enhancing its adaptability to diverse environments (25). According to the results of the current study, the rsbA gene was found in all 29 (100%) of Proteus mirabilis isolates, exceeding the findings of a previous study (26) which showed that rsbA was present in 70% of Proteus mirabilis isolates. In another study (27), 53% of isolates were found to contain this gene. PCR analysis revealed that the rsbA gene, which contributes to biofilm formation, was associated with isolates from catheter-associated urinary tract infections, suggesting its role in increasing bacterial pathogenicity in medical settings. In a Brazilian study (28), the presence of several Pathogenic genes such as atfA, ptA, pmphA, ireA, zapA, mrpA, rsbA, and hpmA were present in all tested isolates. On the other hand, another study (29) showed that the ureA and flaA genes were present in 96.66% and 86.66% of the isolates, respectively, while the zapA, hpmA, and ureC genes were found in 100% of the isolates, reflecting the widespread presence of these pathogenic genes in Proteus mirabilis. These findings underscore the importance of pathogenic genes in enhancing the bacteria's ability to cause chronic infections and contribute to antibiotic resistance, making them a significant challenge in the treatment of urinary tract infections.

## Conclusions

It is concluded from the results of the current work that *P. mirabilis* is one of the causes of respiratory tract infections and has shown high resistance to antibiotics. One of the most important reasons for its virulence is that it possesses virulence genes in high proportions. The study also showed that the bacteria possessed 100% of the rsbA gene, which is responsible for regulating swarming motility.

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