

## Prevalence of Virulence Genes and Antibiotic Resistance in Clinical Isolates of *Proteus mirabilis*

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### Abstract

**Background:** *Proteus mirabilis* can be distributed in water, soil, and the human digestive tract. It is considered among the leading causes of urinary tract infections and can also cause bacteremia, as well as wound and respiratory infections. This study aimed to determine the antibiotic resistance profile and the number of virulence genes in local *P. mirabilis* isolates from various clinical samples.

**Methods and Results:** Between May 2024 and August 2024, a total of 250 samples were collected from various clinical sources, including wound swabs, sputum, urine, and pus, at different hospitals in Baghdad. To identify *P. mirabilis* isolates, biochemical tests and the VITEK 2 system were used. An antibiotic susceptibility test was performed using the disc diffusion technique. The *zapA*, *ureC*, *mrpA*, and *rsbA* genes were detected by PCR.

Seventy-three (29.2%) isolates out of 250 samples from different sources were identified as *P. mirabilis*. The results indicated that 73.6%, 29.3%, 6.2%, and 17.6% of pus, wound, sputum, and urine samples, respectively, were *P. mirabilis*. Of the isolates, 95.9% were resistant to nitrofurantoin, 75.3% to aztreonam, 91.8% to ampicillin, 67.1% to levofloxacin, 58.9% to ceftazidime, 52.1% to piperacillin, 27.4% to ciprofloxacin, 11.0% to imipenem, and 9.6% to amikacin. The rate of *zapA*, *ureC*, *mrpA*, and *rsbA* genes was 75.3%, 91.7%, 52.1%, and 79.4%, respectively.

**Conclusion:** This study showed that *P. mirabilis* is commonly found in sputum, urine, wounds, and pus samples, and that infections are more prevalent in men. It exhibits significant levels of antibiotic resistance, especially to nitrofurantoin (95.9%). The need for efficient infection control and antimicrobial stewardship to address multidrug-resistant *P. mirabilis* infections is highlighted by the rising antibiotic resistance, which has been linked to changes in bacterial enzymes and antibiotic misuse. (International Journal of Biomedicine. 2025;15(2):400-403.)

**Keywords:** *Proteus mirabilis* • antibiotic resistance • virulence genes

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### Introduction

*Proteus species* (spp.) are found in different environments around the world. They are found in soil, wastewater, and dung, where they help break down animal-derived organic matter.<sup>1</sup> They are also an essential element of the fecal flora.

Therefore, they may be found mainly in the digestive tracts of humans and animals.<sup>2</sup> It is one of the most famous Gram-positive bacteria, facultative anaerobes, and is rod-shaped. The first characteristics highlighted by Gustav Hauser in *P. mirabilis* are its prominent urease production, characteristic swarming activity on agar plates, and rapid,

multicellular coordinated activity. When moving across surfaces, it produces a characteristic “bull’s-eye” pattern due to its peritrichous flagellum that facilitates its movement.<sup>3</sup> It also causes urinary tract infections, bacteriuria, kidney stones, catheter obstruction, and acute pyelonephritis. The majority of complicated urinary tract infections are caused by *P. mirabilis* strains and uropathogenic *Escherichia coli*.<sup>4,5</sup> It also causes wound infections, burns, prostatitis, meningitis, and otitis media.<sup>6</sup> When infected with *P. mirabilis*, there are clinical symptoms such as abdominal discomfort, dysuria, which is characterized by irritation or a sense of burning during urination, a rise in the frequency of urination, turbid urine, fever, and chills, which are systemic indicators of infection.<sup>7</sup> The development and spreading of different mechanisms of resistance to antibiotics are constantly increasing in clinical bacterial strains, leading to a clinical problem, so the available treatment options are narrow.<sup>8,9</sup> In addition, many studies showed that *P. mirabilis* is resistant to multiple drugs worldwide.<sup>10</sup> *P. mirabilis* has several virulence factors such as swarming motion, fimbriae, urease, hemolysin, protease and as a result of these virulence factors, it can colonize and infect the host.<sup>11</sup>

This study aimed to determine the antibiotic resistance profile and the number of virulence genes in local *P. mirabilis* isolates from various clinical samples.

## Materials and Methods

During the period from May 2024 to August 2024, a total of 250 samples were obtained from different clinical sources (wound swabs, sputum, urine, and pus) of different hospitals in Baghdad. Following a direct inoculation in tryptone soy broth, the samples were streaked on McConkey agar and blood agar and plated for 24 hours at 37°C. The VITEK-2 system and biochemical assays were carried out to identify *P. mirabilis* isolates. The disc diffusion technique was used in the antibiotic susceptibility test, and the results were identified according to CLSI guidelines.<sup>12</sup> In this study, nine antibiotics were used: amikacin 30 µg, ampicillin 10 µg, aztreonam 10 µg, ceftazidime 30 µg, ciprofloxacin 5 µg, imipenem 10 µg, levofloxacin 5µg, nitrofurantoin 300 µg and piperacillin 100 µg. The virulence genes *zapA*, *ureC*, *mrpA*, and *rsbA* were detected by PCR using primers listed in Table 1.

**Table 1.**  
**Primer sequence.**

Primer	Sequence: 5' → 3'	Amplicon size (bp)	Ref.
<i>zapA</i>	F: ACCGCAGGAAAACATATAGCCC R: GCGACTATCTTCCGCATAATCA	540	<u>13</u>
<i>ureC</i>	F: GGGGGCAATTTCGGTGATGT R: TGGCGCATAAGCGACCATAC	319	<u>14</u>
<i>mrpA</i>	F: TTCTTACTGATAAGACATTG R: ATTTTCAGGAAACAAAAGATG	512	<u>15</u>
<i>rsbA</i>	F: TTGAAGGACGCGATCAGACC R: ACTCTGCTGTCTGTGGGTA	467	<u>16</u>

## Results

Seventy-three (29.2%) isolates out of 250 samples from different sources were identified as *P. mirabilis*. The results indicated that 73.6%, 29.3%, 6.2%, and 17.6% of pus, wound, sputum, and urine samples, respectively, were *P. mirabilis* (Table 2). Of the isolates, 95.9% were resistant to nitrofurantoin, 75.3% to aztreonam, 91.8% to ampicillin, 67.1% to levofloxacin, 58.9% to ceftazidime, 52.1% to piperacillin, 27.4% to ciprofloxacin, 11.0% to imipenem, and 9.6% to amikacin (Table 3). The rate of *zapA*, *ureC*, *mrpA*, and *rsbA* genes was 75.3%, 91.7%, 52%, and 79.4%, respectively (Table 4). The distribution of the virulence genes according to the source of samples was demonstrated in Table 5.

**Table 2.**  
**Distribution of *P. mirabilis* isolates.**

Source	Samples	Positive growth	<i>P.mirabilis</i>	Negative growth	Male	Female
Urine	74 (29.6%)	61 (82.4%)	13 (17.6%)	13 (17.6%)	43 (58.1%)	31 (41.8%)
Wound	58 (23.2%)	43 (74.1%)	17 (29.3%)	15 (25.9%)	35 (60.3%)	23 (39.6%)
Sputum	65 (26.0%)	14 (21.5%)	4 (6.2%)	51 (78.5%)	50 (76.9%)	15 (23.0%)
Pus	53 (21.2%)	45 (84.9%)	39 (73.6%)	14 (26.4%)	31 (58.4%)	22 (41.5%)
Total	250 (100%)	163 (65.2%)	73 (29.2%)	87 (34.8%)	159 (63.6%)	91 (36.4%)

**Table 3.**  
**Susceptibility of *P. mirabilis* isolates to selected antibiotics.**

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Amikacin	66 (90.4)	0 (0.0)	7 (9.6)
Ampicillin	4 ( 5.5)	2 (2.7)	67 (91.8)
Aztreonam	13 (17.8)	5 (6.8)	55 (75.3)
Ceftazidime	23 (31.5)	7 (9.6)	43 (58.9)
Ciprofloxacin	39 (53.4)	14 (19.2)	20 (27.4)
Imipenem	60 (82.2)	5 (6.8)	8 (11.0)
Levofloxacin	24 (32.9)	0 (0.0%)	49 (67.1)
Nitrofurantoin	2 (2.7)	1 (1.4)	70 (95.9)
Piperacillin	26 (35.6)	9 (12.3)	38 (52.1)

**Table 4.**  
**Detection of virulence genes.**

Gene	No. of isolates	%
<i>zapA</i>	55	75.3
<i>ureC</i>	67	91.7
<i>mrpA</i>	38	52.1
<i>rsbA</i>	58	79.4
<i>zapA</i> , <i>ureC</i> , <i>mrpA</i> , <i>rsbA</i>	19	26.0

**Table 5.**  
**Distribution of virulence genes according to the source.**

Source	<i>zapA</i> n (%)	<i>ureC</i> n (%)	<i>mrpA</i> n (%)	<i>rsbA</i> n (%)	All genes n (%)
Urine	11 (20)	13 (19.4)	8 (21.1)	10 (17.2)	3 (15.8)
Wound	13 (23.6)	15 (22.4)	9 (23.7)	17 (29.3)	5 (26.3)
Sputum	4 (7.3)	3 (4.5)	3 (7.9)	4 (6.9)	2 (10.5)
Pus	27 (49.1)	36 (53.7)	18 (47.4)	27 (46.6)	9 (47.4)
Total	55	67	38	58	19

Discussion

*Proteus* is the third most common cause of hospital-acquired infections. *Proteus* species are found in different environments and are a natural part of the human gastrointestinal flora.<sup>17</sup> This organism is an opportunistic pathogen that causes a wide range of infections. One of the global problems is the growing bacterial resistance to antibiotics. *Proteus mirabilis* shows high resistance to many antibiotics that can lead to multidrug resistance and failure of antimicrobial therapy.<sup>18</sup> In the current study, 39 (73.6%) isolates from pus samples were *P. mirabilis*. Our results are in line with a study by P. Snega Priya et al.,<sup>19</sup> which found that the pathogen with the highest pus isolation rate (82.86%) was *P. mirabilis*. Seventeen (29.3%) isolates from wound samples were *P. mirabilis*. This result is similar to a study by Elhoshi et al.,<sup>20</sup> which showed that 31% of wound swabs were *P. mirabilis* isolates. Our results revealed that 13 (17.6%) isolates from urine samples were positive for *P. mirabilis*, and this result corresponds to the result of Serry et al.,<sup>18</sup> who showed that *P. mirabilis* isolates from urine samples were 15.2%.- Only 4 (6.2%) were positive for *P. mirabilis* from sputum isolates. In contrast, Kamil et al.<sup>21</sup> stated that 20% of sputum isolates were *P. mirabilis*.- The distribution of *P. mirabilis* isolates was 159 (63.60%) in males and 91 (36.40%) in females. Because males were exposed to more adverse environmental factors than females, they were more vulnerable to bacterial infection.<sup>22</sup> The resistance to amikacin in 9.6% of isolates was close to a study by Salama et al.,<sup>1</sup> which stated that 8.3% of *P. mirabilis* isolates were amikacin resistant. Resistance to nitrofurantoin was the highest among the antibiotics under study, 95.9%. This agreed with Hussein et al.,<sup>4</sup> who reported that resistance to nitrofurantoin appeared in 88.9% of *P. mirabilis* isolates. Enzymatic modifications in bacteria and the excessive and inappropriate use of antibiotics may be connected to antibiotic resistance.<sup>23,24</sup> The *ureC* gene was found in 91.7% of *P. mirabilis* isolates; this is in line with Algammal et al.,<sup>2</sup> who reported that the *ureC* gene was detected in all isolates of *P. mirabilis*. The *rsbA* gene was detected in 79.4%, which was in line with a study by Phiri et al.,<sup>25</sup> who showed that the *rsbA* gene was detected in 89.5%. The *zapA* was detected in 75.3% and *mrpA* in 52% of the isolates, while a study by Tabatabaei et al.<sup>15</sup> found that *zapA* and *mrpA* were detected in 95% and 90% of the isolates, respectively.

However, there are significant differences in the frequency of virulence genes between studies, which might result in different recommendations for infection management.

**In conclusion**, our study showed that *P. mirabilis* is commonly found in sputum, urine, wounds, and pus samples, and that infections are more prevalent in men. It exhibits significant levels of antibiotic resistance, especially to nitrofurantoin (95.9%). The need for efficient infection control and antimicrobial stewardship to address multidrug-resistant *P. mirabilis* infections is highlighted by the rising antibiotic resistance, which has been linked to changes in bacterial enzymes and antibiotic misuse.

Competing Interests

The authors declare that they have no competing interests.

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