

Evaluation of biofilm activity, virulence genes, and antibiotic susceptibility profiles in clinical *Proteus mirabilis* isolates

İrem Unat¹, Tuğrul Hoşbul²

¹ Medical Microbiology Laboratory, Kelkit State Hospital, Gümüşhane, Türkiye

² Department of Medical Microbiology, Gülhane Faculty of Medicine, University of Health Sciences, Ankara, Türkiye

Abstract

Introduction: *Proteus mirabilis* is a common opportunistic pathogen in wound and urinary tract infections (UTIs), whose clinical significance is driven by biofilm formation. This virulence-mediated process provides a shield against antimicrobials and host immunity, promoting difficult-to-treat infections. This study aimed to elucidate the relationship between biofilm-forming capacity, presence of virulence genes, and antibiotic resistance profiles in *P. mirabilis* isolates.

Methodology: A total of 76 *P. mirabilis* isolates from urine and wound samples were analyzed. Isolates were identified using MALDI-TOF MS, antimicrobial susceptibility was tested via VITEK®2, biofilm formation was measured using the crystal violet method, and the presence of virulence genes (*ureC*, *ireA*, *zapA*, *rsbA*, *flaA*, *atfA*, *ucaA*, and *hpmA*) was analyzed by PCR.

Results: Wound isolates showed significantly enhanced biofilm production and higher resistance rates to multiple antibiotics. Moderate-to-strong biofilm production was significantly associated with catheterization, the presence of the *zapA*, *atfA*, and *ucaA* genes, and increased resistance to ciprofloxacin and trimethoprim-sulfamethoxazole (TMP-SXT). The presence of the *hpmA* gene showed a borderline association with TMP-SXT resistance. The presence of the *rsbA* gene was significantly associated with a reduced likelihood of ertapenem resistance, suggesting a role in modulating porin-mediated antibiotic uptake.

Conclusions: Biofilm-mediated resistance is a significant barrier to managing *P. mirabilis* infections, particularly in wounds and catheter-associated UTIs. The presence of specific genes in moderate-to-strong biofilm producers indicates their products are integral to the structural integrity and the functional capacity of the biofilm. The inverse relationship between *rsbA* and carbapenem resistance warrants further investigation as a biomarker for susceptibility.

Key words: *Proteus mirabilis*; virulence factors; biofilm; antibiotic resistance.

J Infect Dev Ctries 2026; 20(9):1351-1360. doi:10.3855/jidc.22641

(Received 14 November 2025 – Accepted 11 February 2026)

Copyright © 2026 Unat *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Discovered in 1885 by German microbiologist Gustav Hauser, the microorganism was named after Proteus, the son of the sea god Poseidon, who lived on the island of Pharos and was believed to be capable of changing his shape and form to escape his enemies [1]. The name derives from the mythological figure Proteus, reflecting the fact that *Proteus* spp. can adapt to various environments, such as liquid and solid surfaces, exhibiting characteristic metabolic features under changing conditions [2]. Within the *Proteus* species, *P. mirabilis* is of particular interest as it constitutes approximately 80–90% of all *Proteus*-related infections [3]. *P. mirabilis* is a facultative anaerobic, Gram-negative bacterium that naturally resides in the gastrointestinal flora of humans and animals, and is also found in the environment, including soil, water, and sewage [2]. An opportunistic pathogen by nature, *P. mirabilis* has been reported to induce infections across a wide array of bodily locations, posing a particular threat to complicated patients. Its broad spectrum of

involvement includes the urinary tract, wounds, gastrointestinal and respiratory tracts, and eyes. Furthermore, it contributes 3-5% of all nosocomial infections [3].

Proteus mirabilis can cause life-threatening infections due to various virulence factors and strategies for evading host defense mechanisms, such as enzymes like metalloprotease, urease, hemolysin, fimbrial and flagellar structures, and biofilm formation [4]. Biofilm is a structured community of bacterial cells encapsulated with a self-produced matrix of extracellular polymeric substance (EPS), providing protection against antibiotics and host immune responses [5]. This structure is highly significant, as biofilms are implicated in up to 80% of all chronic infections [6]. While *P. mirabilis* biofilms are well-known for causing stone formation in urinary tract infections (UTIs), their role in chronic wound infections is less understood. Although frequently found in venous ulcers and diabetic foot infections, knowledge about *P. mirabilis*'s biofilm-forming ability and host-pathogen

interactions within the chronic wound environment is highly limited [7,8].

This study aims to analyze the phenotypic expression of biofilm formation by *P. mirabilis* isolates obtained from urine and wound samples, alongside the presence of associated virulence genes (*ucaA*, *zapA*, *rsbA*, *flaA*, *hpmA*, *ureC*, *atfA*, *ireA*) and their relationship with antibiotic resistance. By elucidating the relationship between them, the objective is to contribute to the development of long-term strategies for the rational and empirical management of these infections.

Methodology

Bacterial strains

This study included 76 *P. mirabilis* isolates obtained from urine and wound samples collected at Gülhane Training and Research Hospital between January 2021 and March 2023. Only one isolate per patient was included, excluding duplicate isolates. Samples were cultured on 5% sheep blood agar and EMB agar, and incubated aerobically at 37 °C for 24 hours. Identification was confirmed using MALDI-TOF MS (Bruker Daltonics, Germany). Antibiotic susceptibility testing was performed using the VITEK®2 Compact system (bioMérieux, France) according to EUCAST guidelines [9]. Isolates were stored at -80 °C in 10% glycerol-BHI broth and subcultured on TSA for revival.

Biofilm-forming capacity

The biofilm-forming abilities of *P. mirabilis* isolates were evaluated using the crystal violet microtiter plate assay described by Merritt *et al* [10]. All isolates were cultured in BHI broth at 37 °C for 18 hours, then diluted 1:100 in TSB supplemented with 1% glucose. A volume of 200 µL of each diluted culture

Table 1. Classification of Biofilm Forming Capacities.

Average OD Values	Biofilm Forming Capacity
$OD_{\text{isolate}} \leq OD_{\text{cut-off}}^a$	Non biofilm production
$OD_{\text{cut-off}} < OD_{\text{isolate}} \leq 2OD_{\text{cut-off}}$	Weak
$2 OD_{\text{cut-off}} < OD_{\text{isolate}} \leq 4OD_{\text{cut-off}}$	Moderate
$4OD_{\text{cut-off}} < OD_{\text{isolate}}$	Strong

^a $OD_{\text{cut-off}} = OD_{\text{nc}} + 3SD$ (Optical density cut-off value, mean OD value of negative control + (3x standard deviation of negative control)).

was inoculated into four wells of 96-well flat-bottom microtiter plates. *Staphylococcus epidermidis* ATCC 35984 was used as a positive control, and sterile TSB with 1% glucose as a negative control. Plates were sealed with parafilm and incubated at 37 °C for 24 hours. After incubation, wells were washed three times with PBS to remove non-adherent cells, then air-dried at room temperature. Wells were stained with 0.1% crystal violet for 45 minutes and washed again. Bound dye was solubilized with 95% ethanol, and absorbance was measured at 630 nm using a microplate reader. For each isolate, the mean optical density (OD) was calculated. A cut-off OD ($OD_{\text{cut-off}}$) was defined as the mean OD of the negative control plus three standard deviations, and isolates were classified based on Table 1.

DNA Isolation

Template DNA for PCR was extracted using the DNA4PCR kit (RTechMed, Turkey) following the manufacturer's instructions. Isolates were first cultured on TSA at 37 °C for 18–24 hours. A bacterial suspension was prepared in 1 mL sterile distilled water from pure colonies, then centrifuged at $12,000 \times g$ for 3 minutes. The pellet was resuspended in 100 µL of kit buffer, vortexed, and incubated at 56 °C for 30 minutes, followed by heating at 100 °C for 8 minutes. After a final centrifugation at $12,000 \times g$ for 3 minutes, the supernatant containing DNA was collected and stored at -20 °C until use.

Table 2. Primers used in the study.

Virulence genes	Primers (5'→3')	Base pair (bp)	Hybridization temperature (°C)	References
<i>ureC</i>	F: GTTATTCGTGATGGTATGGG R: GTAAAGGTGGTTACGCCAGA	317	56.2	[11]
<i>ireA</i>	F: ACTACGATAACGAGCGCCAG R: GCCCTAACTGCGGGAATACG	681	60	[12]
<i>zapA</i>	F: ACCGCAGGAAAACATATAGCCC R: GCGACTATCTTCCGCATAATCA	540	59	[11]
<i>rsbA</i>	F: TTGAAGGACGCGATCAGACC R: ACTCTGCTGCTGTGGGTA	467	58	[11]
<i>flaA</i>	F: AGGATAAATGGCCACATTG R: CGGCATTGTTAATCGCTTT	417	54.2	[13]
<i>atfA</i>	F: CATAATTTCTAGACCTGCCCTAGCA R: CTGCTTGGATCCGTAATTTTAAACG	382	50	[12]
<i>ucaA</i>	F: GCTTTTACATCCCAGCGGT R: GCTGCATTGCTGGCTCATC	476	60	[12]
<i>hpmA</i>	F: TGGTATCGATGTTGGCGTTA R: GTGGTGCCCACTTTCAGATT	717	55	[14]

Polymerase Chain Reaction

The presence of *ureC*, *ireA*, *zapA*, *rsbA*, *flaA*, *atfA*, *ucaA*, and *hpmA* virulence genes in *P. mirabilis* isolates was investigated by PCR. Primer sequences, expected product sizes, and annealing temperatures are listed in Table 2. PCR reactions were prepared in 50 µL volumes, containing PCR buffer (1X) (Genaxxon, Germany), MgCl₂ (2.5 mM) (Genaxxon, Germany), dNTPs (0.2 mM) (Genaxxon, Germany), forward and reverse primers (0.5 mM each) (Genaxxon, Germany), Taq polymerase (1.5 U) (Genaxxon, Germany), and 2 µL template DNA. Amplification conditions consisted of an initial denaturation at 95 °C for 5 minutes, followed by 36 cycles of 95 °C for 30 seconds, gene-specific annealing for 30 seconds, and 72 °C for 60 seconds. A final extension step was performed at 72 °C for 8 minutes. Annealing temperatures for each gene are detailed in Table 2. Amplicons were analyzed by agarose gel electrophoresis using 1% agarose gel. Electrophoresis was performed at 100 V for 60 minutes. Bands were visualized under UV light using a Gel Doc EZ Imager (Bio-Rad, USA), and isolates showing bands of the expected size were considered positive (Table 2). Selected amplicons were sequenced commercially (Sentebiolab, Turkey) using the ABI Prism 3130 Genetic Analyzer. Sequencing results were analyzed using FinchTV (v1.4.0) and compared against the NCBI database using BlastN. The nucleotide sequences reported in this study have been deposited in the GenBank database under the accession numbers PX461479-PX461486.

Statistical analysis

Data analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated as mean, standard deviation,

median, minimum, maximum values, and percentages. Categorical variables were compared using the Pearson Chi-square test. Fisher's exact test was applied when the expected number of observations in any cell of the contingency table was below 5. To quantify the strength of associations, Odds Ratios (OR) and 95% Confidence Intervals (CI) were calculated. For 2 × 2 contingency tables containing zero cell counts, the Haldane-Anscombe correction was applied by adding a constant of 0.5 to each cell to enable calculation and prevent bias. A $p < 0.05$ was considered statistically significant.

Results

Bacterial Isolates and Patient Demographics

A total of 76 *P. mirabilis* isolates were included in this study, of which 41 (53.9%) were obtained from urine cultures, and 35 (46.1%) from wound cultures. Among the patients, 37 (48%) were male, and 39 (52%) were female, with ages ranging from 1 to 92 years; 58 (76.3%) patients were aged 16 years or older. In the UTI group, 27 (65.8%) were female, and 14 (34.1%) were male, while in the wound infection group, 23 (65.7%) were male and 12 (34.2%) were female.

Antibiotic Susceptibility Test Results

In general, isolates from urine samples exhibited higher susceptibility rates compared to those from wound samples. Notably, susceptibility to piperacillin-tazobactam, ertapenem, meropenem, and amikacin was high (> 90%) in both groups. However, resistance to ampicillin ($p = 0.012$; OR: 3.26; 95% CI: 1.27-8.37), ceftriaxone ($p = 0.010$; OR: 6.75; 95% CI: 1.35-33.79), and ciprofloxacin ($p = 0.002$; OR: 4.74; 95% CI: 1.75-12.85) was significantly higher among wound isolates compared to urine isolates. Additionally, resistance to amoxicillin-clavulanate ($p = 0.045$; OR: 3.04; 95% CI:

Table 3. Antibiotic susceptibility test results of isolates.

Antibiotics	Urine isolates (n = 41), n (%)			Wound isolates (n = 35), n (%)			p	OR (95% CI) ^e
	S ^a	I ^a	R ^a	S ^a	I ^a	R ^a		
Ampicillin	27 (65.9%)	0	14 (34.1%)	13 (37.1%)	0	22 (62.9%)	0.012^c	3.26 (1.27-8.37)
Amoxicillin-clavulanate	35 (85.4%)	0	6 (14.6%)	23 (65.7%)	0	12 (34.3%)	0.045^c	3.04 (1.00-9.26)
Piperacillin-tazobactam	41 (100%)	0	0	34 (97.1%)	0	1 (2.9%)	0.461 ^d	3.61 (0.14-91.44)
Cefuroxime	1 (2.4%)	36 (87.8%)	4 (9.8%)	0	26 (74.3%)	9 (25.7%)	0.066 ^c	3.20 (0.89-11.52)
Ceftazidime	39 (95.1%)	1 (2.4%)	1 (2.4%)	28 (80%)	2 (5.7%)	5 (14.3%)	0.089 ^d	6.67 (0.74-60.08)
Ceftriaxone	39 (95.1%)	0	2 (4.9%)	26 (74.3%)	0	9 (25.7%)	0.010^c	6.75 (1.35-33.79)
Cefepime	38 (92.7%)	2 (4.9%)	1 (2.4%)	27 (77.1%)	2 (5.7%)	6 (17.1%)	0.044^d	8.28 (0.94-72.51)
Ertapenem	41 (100%)	0	0	32 (91.4%)	0	3 (8.6%)	0.093 ^d	8.94 (0.45-179.28)
Meropenem	41 (100%)	0	0	34 (97.1%)	1 (2.9%)	0	0.093 ^d	ND ^f
Amikacin	40 (97.6%)	1 (2.4%)	0	32 (91.4%)	0	3 (8.6%)	0.093 ^d	8.94 (0.45-179.28)
Ciprofloxacin	32 (78%)	0	9 (22%)	15 (42.9%)	0	20 (57.1%)	0.002^c	4.74 (1.75-12.85)
TMP-SXT ^b	23 (56.1%)	0	18 (43.9%)	12 (34.3%)	0	23 (65.7%)	0.057 ^c	2.45 (0.96-6.22)

^a S: Susceptible at standard dose; I: Susceptible at increased exposure; R: Resistant; ^b TMP-SXT: Trimethoprim-sulfamethoxazole; ^c Pearson chi-square method was used; ^d Fisher's exact chi-square method was used. ^e OR: Odds Ratio; CI: 95% Confidence Interval ^f Not determined. Odds ratios for variables with zero cell counts were calculated using the Haldane-Anscombe correction.

1.00-9.26) and cefepime ($p = 0.044$; OR: 8.28; 95% CI: 0.94-72.51) exhibited a borderline statistical significance, suggesting a notable trend toward higher resistance in wound infections. Trimethoprim-sulfamethoxazole showed the lowest susceptibility rates in both groups. For statistical comparison, isolates categorized as “susceptible” and “susceptible, increased exposure” were grouped. Detailed resistance profiles are presented in Table 3, with significant differences highlighted in bold.

Biofilm Formation Capacity of Isolates

Assessment of the biofilm-forming capacity of the isolates showed that 40 isolates (52.6%) were weak, 22 isolates (28.9%) were moderate, and 14 isolates (18.4%) were strong biofilm producers. Among the urinary isolates, 24 (58.5%) were weak, 15 (36.6%) were moderate, and 2 (4.9%) were strong biofilm producers. For wound isolates, 16 (45.7%) were weak, 7 (20%) were moderate, and 12 (34.3%) were strong biofilm producers. A statistical risk assessment comparing strong biofilm producers against the combined group of non-producing, weak, and moderate producers revealed that wound isolates were significantly more likely to exhibit strong biofilm formation compared to urine isolates ($p = 0.001$; OR: 10.17; 95% CI: 2.09-49.55) (Table 4).

Among patients with urine cultures, 24.4% were catheterized. Isolates from these patients showed a distinct shift toward higher biofilm capacity (20% weak, 70% moderate, 10% strong) compared to non-catheterized patients (71% weak, 25.8% moderate, 3.2% strong). Statistical analysis grouping moderate and strong producers revealed that catheterization was significantly associated with enhanced biofilm production capacity ($p = 0.006$; OR: 9.77; 95% CI: 1.72-55.3).

In an investigation of the relationship between antibiotic susceptibility and biofilm formation, weak biofilm producers exhibited resistance to ampicillin (37.5%), amoxicillin-clavulanate (17.5%), cefuroxime (12.5%), ceftazidime (5%), cefepime (5%), ceftriaxone (7.5%), ertapenem (2.5%), ciprofloxacin (25%), and TMP-SXT (32.5%). Moderate and strong biofilm producers, however, showed higher resistance rates, notably to ampicillin (58.3%), amoxicillin-clavulanate

(30.6%), cefuroxime (27.8%), ceftriaxone (22.2%), cefepime (13.9%), ciprofloxacin (52.8%), and TMP-SXT (77.8%). Piperacillin-tazobactam (2.8%) and amikacin (8.3%) resistance were also observed in this group. No meropenem resistance was detected across any isolate. A statistically significant difference in ciprofloxacin ($p = 0.013$; OR: 3.35; 95% CI: 1.27-8.84) and TMP-SXT ($p < 0.001$; OR: 7.27; 95% CI: 2.60-20.31) resistance was identified between weak and moderate/strong biofilm producers.

Investigation of Virulence-Related Genes

The presence of virulence-associated genes in 76 *P. mirabilis* isolates was investigated. Among urine isolates, all (100%) were positive for *ureC*, *flaA*, and *rsbA*. Additionally, *hpmA* was detected in 39 (95.1%) isolates, *zapA* in 38 (92.7%), *atfA* in 36 (87.8%), *ireA* in 35 (85.4%), and *ucaA* in 31 (75.6%).

Among wound isolates, *ureC* was detected in all (100%), followed by *flaA* and *rsbA* in 34 (97.1%), *hpmA* in 33 (94.3%), *zapA* in 32 (91.4%), *atfA* and *ucaA* in 31 (88.6%), and *ireA* in 30 (85.7%) isolates. No statistically significant differences were observed between urine and wound isolates regarding the distribution of virulence genes ($p > 0.05$). Detailed data are presented in Figure 1.

Figure 1. Presence of Virulence Genes in Isolates.

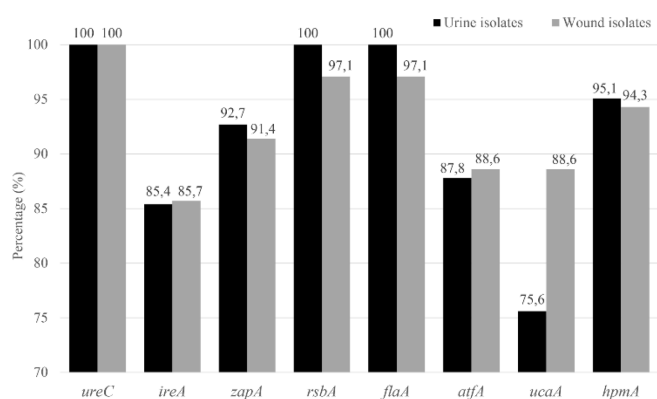


Table 4. Biofilm Forming Capacities of Isolates.

Biofilm Production	Urine isolates (n = 41), n (%)	Wound isolates (n = 35), n (%)	<i>p</i> ^a	OR (95% CI) ^b
Non biofilm production	-	-		
Weak	24 (58.5%)	16 (45.7%)	0.001	10.17 (2.09-49.55)
Moderate	15 (36.6%)	7 (20%)		
Strong	2 (4.9%)	12 (34.3%)		

^a Pearson chi-square method was used. ^b OR: Odds Ratio; CI: 95% Confidence Interval.

The comparison between the antibiotic resistance profiles and the presence of virulence genes revealed no statistically significant association between resistance to ampicillin, β -lactam/ β -lactamase inhibitor combinations, cephalosporins, carbapenems, amikacin, or ciprofloxacin and the presence of virulence genes ($p > 0.05$). However, the presence of the *rsbA* gene was associated with a significantly reduced likelihood of ertapenem resistance ($p = 0.039$; OR: 0.01; 95% CI: 0.00-0.35). Additionally, *hpmA* ($p = 0.041$; OR: 11.86; 95% CI: 0.62-228.43) was more prevalent in TMP-SXT-resistant isolates, demonstrating a borderline significant association.

The relationship between the presence of virulence genes and the isolates' biofilm-forming capacity was evaluated. Among the weak biofilm producers, *ureC* was detected in 40 (100%), *ireA* in 32 (80%), *zapA* in 34 (85%), *rsbA* and *flaA* in 39 (97.5%), *atfA* in 31 (77.5%), *ucaA* in 28 (70%), and *hpmA* in 36 (90%) isolates.

In moderate and strong biofilm producers, *ureC*, *zapA*, *rsbA*, *flaA*, *atfA*, and *hpmA* were detected in all 36 isolates (100%), *ireA* in 33 (91.7%), and *ucaA* in 34 (94.4%).

A statistically significant difference was observed between weak and moderate/strong biofilm producers in terms of the presence of *zapA* ($p = 0.026$, OR: 13.75; 95% CI: 0.75-253.46), *atfA* ($p = 0.003$; OR: 22.02; 95% CI: 1.23-393.59), and *ucaA* ($p = 0.006$; OR: 7.29; 95% CI: 1.50-35.31) genes. This relationship is summarized in Table 5, with statistically significant values shown in bold.

Discussion

Proteus mirabilis, naturally found in soil, water, and mammalian gut flora, is an opportunistic pathogen causing skin, wound, respiratory, and urinary tract infections [11]. A consortium of virulence factors, encompassing adhesins, swarming motility, enzymes like metalloproteinase and urease, mediates the development of biofilms. This process is central to the

emergence of antibiotic resistance and the chronicity of infection [12]. Therefore, understanding the relationship between the biofilm-forming ability of *P. mirabilis*, virulence mechanisms, and their impact on antibiotic resistance is crucial for defining effective treatment strategies.

The demographic distribution of the isolates revealed a distinct gender-based predilection depending on the infection site. A significant majority of urinary isolates were sourced from females (65.8%), while wound isolates showed a strong male predominance (65.71%). This observation corroborates existing literature that identifies females as the primary cohort for *P. mirabilis* UTIs and males for wound infections. The higher incidence of wound infections among males could reflect epidemiological trends, potentially linked to a greater engagement in professions with a higher risk of physical trauma [13,14].

The management of *P. mirabilis* infections is significantly hampered by antibiotic resistance. The findings revealed high resistance rates in urinary isolates to TMP-SXT (43.9%), ampicillin (37.1%), and ciprofloxacin (22%), corroborating some local surveillance data. However, the global resistance landscape for *P. mirabilis* is markedly variable. In contrast to findings of this study, many international studies report higher overall resistance and an increasing prevalence of extended-spectrum β -lactamase (ESBL) producers, underscoring the critical need for continuous, region-specific antimicrobial stewardship [15,16]. Wound isolates represented a more clinically challenging cohort, with substantially higher resistance to TMP-SXT (65.7%), ampicillin (62.9%), and ciprofloxacin (57.1%). This finding is consistent with global surveillance data, where, despite the continued efficacy of carbapenems, resistance to fluoroquinolones, TMP-SXT, aminoglycosides, and third-generation cephalosporins is escalating. This trend, largely driven by the dissemination of ESBL and AmpC β -lactamases, critically limits available therapeutic options [17].

Table 5. The Relationship Between the Presence of Virulence Genes and Biofilm Formation Capacities of Isolates.

Virulence genes	Weak biofilm producers (n = 40), n (%)	Moderate and strong biofilm producers (n = 36), n (%)	p^a	OR (95% CI) ^b
<i>ureC</i>	40 (100%)	36 (100%)	-	ND ^c
<i>ireA</i>	32 (80%)	33 (91.7%)	0.149	2.75 (0.67-11.29)
<i>zapA</i>	34 (85%)	36 (100%)	0.026	13.75 (0.75-253.46)
<i>rsbA</i>	39 (97.5%)	36 (100%)	1.0	2.77 (0.11-70.23)
<i>flaA</i>	39 (97.5%)	36 (100%)	1.0	2.77 (0.11-70.23)
<i>atfA</i>	31 (77.5%)	36 (100%)	0.003	22.02 (1.23-393.59)
<i>ucaA</i>	28 (70%)	34 (94.4%)	0.006	7.29 (1.50-35.31)
<i>hpmA</i>	36 (90%)	36 (100%)	0.117	9.00 (0.47-173.27)

^a Fisher's exact chi-square method was used. ^b OR: Odds Ratio; CI: 95% Confidence Interval; ^c Not determined. Odds ratios for variables with zero cell counts were calculated using the Haldane-Anscombe correction.

In this study, *P. mirabilis* isolates from wound infections were found to produce significantly stronger biofilms compared to urine isolates. Consistent with these findings, other studies have also reported that wound isolates form more robust biofilms and exhibit increased resistance to treatment [7,18,19]. This discrepancy has been hypothesized to be associated with variations in virulence factors depending on the site of isolation [19]. Another finding in this study is that the presence of an indwelling urinary catheter was significantly correlated with enhanced biofilm formation, suggesting that these abiotic surfaces provide a critical scaffold for *P. mirabilis* colonization and biofilm maturation in vivo. This finding is further supported by Tabatabaei *et al.*, who reported a significantly more pronounced biofilm production in isolates from catheterized versus non-catheterized patients [20]. Catheter surfaces provide a favorable environment for bacterial colonization and biofilm formation, which can lead to complications such as urinary tract infections, catheter blockage, sepsis, and antibiotic resistance [21].

The heterogeneous polymeric matrix, comprising 90% of the biofilm, confers a significant protective advantage, rendering bacteria up to 1000-fold more resistant and complicating 65% of all nosocomial infections [22]. This study confirmed this, finding that moderate-strong biofilm producers exhibited significantly higher resistance, particularly to ciprofloxacin ($p = 0.013$; OR: 3.35; 95% CI: 1.27-8.84) and TMP-SXT ($p < 0.001$; OR: 7.27; 95% CI: 2.60-20.31). This multifactorial resistance stems from the matrix limiting antibiotic diffusion, the presence of metabolically dormant cells, and quorum sensing-mediated gene expression [23–25]. Therefore, developing novel therapeutic strategies that bypass this complex protective barrier is of critical clinical importance.

The virulence and antimicrobial resistance of *P. mirabilis* are often genetically coupled, as operons encoding virulence factors are frequently co-localized with resistance determinants on mobile genetic elements, facilitating their co-selection [26]. In this study, despite a homogenous distribution of virulence genes across both cohorts, urinary isolates exhibited a significantly attenuated biofilm-forming capacity. This phenotypic divergence, despite similar genotypic potential, is likely attributable to the low catheterization rate in the patient group providing urine samples. This hypothesis is consistent with the principle that biofilm formation is critically influenced by environmental stimuli. As established by Donlan *et al.*, indwelling

catheters provide the rough, hydrophobic abiotic surfaces known to promote rapid microbial colonization [27]. This surface interaction is a key trigger for quorum sensing, a regulatory system that governs the expression of many biofilm-associated virulence factors [27].

The nickel-dependent metalloenzyme urease is a cornerstone of *P. mirabilis* pathogenicity, critically influencing both biofilm development and infection progression. This enzyme catalyzes the hydrolysis of urea into ammonia and carbon dioxide, a process that elevates urinary pH and promotes the precipitation of struvite and apatite crystals. These crystals become integrated into the biofilm matrix, leading to the catheter encrustation and blockage characteristic of *P. mirabilis* infections [28,29]. Consistent with its fundamental role, *ureC* gene was universally present (100%) in the isolate cohort. This ubiquity underscores the enzyme's indispensable contribution to pathogenesis, a conclusion strongly supported by functional studies where deletion of *ureC* results in a substantial attenuation of bacterial colonization [30].

Siderophore-mediated iron acquisition utilizes outer membrane proteins (OMPs) like IreA, which functions as the specific ferri-siderophore receptor. This uptake process is energy-dependent, utilizing the TonB complex for outer membrane transport and an ABC transporter for cytoplasmic entry. The *ireA* gene is notably upregulated under low-iron conditions [31–33]. The high prevalence of *ireA* in the isolates aligns with its known critical role in iron acquisition [34,35]. While no significant association was found between *ireA* prevalence and either antimicrobial resistance or biofilm strength ($p > 0.05$), this finding must be interpreted with caution, as this study assessed gene prevalence rather than differential expression levels. A functional study confirms that this iron uptake pathway is a prerequisite for biofilm maturation, as blocking it prevents stable adhesion [36]. Therefore, this pathway remains a crucial therapeutic vulnerability, and strategies like chelator-coated catheters are a promising anti-biofilm approach [36].

The secreted metalloprotease ZapA is an essential pathogenic determinant that subverts host immunity by degrading defense molecules (e.g., immunoglobulins, complement) and compromises tissue integrity [37]. While *zapA* was highly prevalent in the cohort, its mere presence did not correlate with antimicrobial resistance, a finding plausibly explained by the known redundancy of ZapA-independent evasion pathways [37–39]. The contribution of *zapA* to biofilm formation appears similarly complex. Although significantly associated with moderate-strong biofilm producers in this study,

another in vivo model (e.g., prostatitis) reports that *zapA* mutants retain wild-type biofilm capacity [40]. This discrepancy strongly suggests that the role of ZapA in biofilm architecture, unlike its direct proteolytic function, is highly contingent on specific environmental and tissue-specific stimuli.

Motility in *P. mirabilis* is a pivotal virulence attribute primarily governed by two genes: *flaA*, which encodes the primary flagellin subunit, and *rsbA*, which encodes a sensor kinase that regulates swarming and the expression of other virulence factors like urease, protease and hemolysin [41,42]. Their precise role in biofilm formation is complex; the necessity of flagella is debated and the mechanism by which *rsbA* modulates biofilm is unclear [42]. Some studies report flagella are crucial for initial surface attachment, while others demonstrate biofilm formation can still occur in *flaA* deletion mutants [43,44]. While this study found only a non-significant association of these genes in moderate-strong biofilm producers ($p > 0.05$), a notable correlation was identified where *rsbA* presence was significantly associated with a reduced risk of carbapenem resistance ($p = 0.039$; OR: 0.01; 95% CI: 0.00-0.35). This apparent paradox has a clear mechanistic basis, as RsbA-mediated systems have been reported to upregulate porin expression, thus facilitating antibiotic influx [45]. These findings position RsbA as a pleiotropic regulator, influencing virulence, biofilm modulation, and antimicrobial permeability.

The diverse fimbrial repertoire of *P. mirabilis* is essential for mediating adhesion to both host tissues and abiotic surfaces, a function that is fundamental to colonization and biofilm initiation [46]. The finding of a high prevalence of the *atfA* and *ucaA* adhesin genes aligns with this critical function. The significance of these fimbrial structures is multifaceted. A functional study using isogenic mutants has demonstrated that fimbriae, including the ATF and UCA types, are indispensable not only for initial attachment but also for the subsequent stabilization and structural integrity of the biofilm matrix [46]. Consistent with this structural role, the statistical analysis identified the *atfA* gene as the strongest predictor of moderate-to-strong biofilm formation (OR: 22.02; $p = 0.003$), highlighting its pivotal contribution to the maturation and stability of the biofilm structure.

P. mirabilis produces HpmA, a calcium-independent, pore-forming hemolysin that causes erythrocyte lysis, tissue damage, and contributes to invasion [47]. In the cohort, *hpmA* was nearly ubiquitous (> 94%) and showed a notable trend toward

association with TMP-SXT resistance ($p = 0.041$; OR: 11.86; 95% CI: 0.62-228.43). This association is likely indirect, stemming from HpmA-mediated immune evasion, which enhances bacterial persistence and compromises therapeutic efficacy [48]. In contrast to this association, the contribution of *hpmA* to biofilm formation appears ambiguous. This study found only a non-significant association between *hpmA* presence and strong biofilm production, a finding that aligns with conflicting reports in the literature, where its role seems highly contingent on environmental conditions or expression levels [49, 50].

Limitations

The findings of this study should be interpreted within the context of certain limitations. First, the investigation of virulence determinants was restricted to genomic detection via PCR; therefore, the lack of transcriptomic analysis limits the ability to correlate gene presence directly with functional expression levels within the biofilm matrix. Consequently, the observed discrepancies between genotypic profiles and phenotypic biofilm capacity may be attributed to regulatory mechanisms not captured in this study. Second, the study was conducted at a single center with a sample size ($n=76$) that, while sufficient for statistical validity in local surveillance, may limit the broader generalizability of the epidemiological data. Finally, molecular typing methods (e.g., MLST or PFGE) were not employed to assess clonal relatedness, which precludes definitive differentiation between the dissemination of specific high-risk clones and the horizontal transfer of resistance elements.

Conclusions

This study confirms that biofilm formation is a central driver of therapeutic failure in *P. mirabilis*, strongly correlating with increased resistance in wound and catheter-associated UTIs. The data reveal a complex genetic underpinning for this phenotype. While a borderline significant trend was observed between the *hpmA* gene and TMP-SXT resistance, a distinct and clinically relevant finding was the significant association between the presence of the *rsbA* gene and a reduced likelihood of ertapenem resistance.

This suggests a sophisticated regulatory network where *rsbA* may modulate porin expression, potentially serving as a genomic marker for carbapenem susceptibility. These findings underscore the critical need to move beyond standard susceptibility testing and to develop novel therapeutics that target the specific

virulence and biofilm regulatory pathways that enable *P. mirabilis* to persist.

Funding

This study was supported by the Scientific Research Projects Coordination Office of the University of Health Sciences (Project No: 2024/057).

Ethics Approval

Ethical approval for this study was obtained from the Gülhane Scientific Research Ethics Committee on September 26, 2023 (Approval No: 2023/329).

Authors' contributions

İrem Unat and Tuğrul Hoşbul both contributed to the conceptualization and methodology of the study. İrem Unat conducted the formal analysis, investigation, data curation, visualization and prepared the original manuscript draft. Tuğrul Hoşbul provided supervision and performed the final review and editing of the manuscript. All authors have read and approved the final manuscript.

Corresponding author

İrem Unat, MD
Medical Microbiology Specialist
Kelkit State Hospital
Gümüşhane, Türkiye
Email: irem.unat@icloud.com

Conflict of interest

No conflict of interest is declared.

References

- Sellaturay S, Nair R, Dickinson I, Sriprasas S (2012) *Proteus*: mythology to modern times. Indian J Urol 28: 388-391. doi: 10.4103/0970-1591.105748.
- Drzewiecka D (2016) Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol 72: 741–758. doi: 10.1007/s00248-015-0720-6.
- Palusiak A (2022) *Proteus mirabilis* and *Klebsiella pneumoniae* as pathogens capable of causing co-infections and exhibiting similarities in their virulence factors. Front Cell Infect Microbiol 12: 991657. doi: 10.3389/fcimb.2022.991657.
- Chakkour M, Hammoud Z, Farhat S, El Roz A, Ezzeddine Z, Ghseini G (2024) Overview of *Proteus mirabilis* pathogenicity and virulence. Insights into the role of metals. Front Microbiol 15: 1383618. doi: 10.3389/fmicb.2024.1383618.
- Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8: 623–633. doi: 10.1038/nrmicro2415.
- Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA (2018) Bacterial biofilm and associated infections. J Chin Med Assoc 81: 7–11. doi: 10.1016/j.jcma.2017.07.012.
- Majtan J, Bohova J, Horniackova M, Klaudiny J, Majtan V (2014) Anti-biofilm effects of honey against wound pathogens *Proteus mirabilis* and *Enterobacter cloacae*. Phytother Res 28: 69–75. doi: 10.1002/ptr.4957.
- Kwiecińska-Piróg J, Przekwas J, Majkut M, Skowron K, Gospodarek-Komkowska E (2020) Biofilm formation reducing properties of manuka honey and propolis in *Proteus mirabilis* rods isolated from chronic wounds. Microorganisms 8: 1823. doi: 10.3390/microorganisms8111823.
- The European Committee on Antimicrobial Susceptibility Testing (2025) Breakpoint tables for interpretation of MICs and zone diameters. Version 15.0. Available: <https://www.eucast.org>. Accessed: 20 October 2025.
- Merritt JH, Kadouri DE, O'Toole GA (2006) Growing and analyzing static biofilms. Curr Protoc Microbiol 00: 1B.1.1-1B.1.17. doi: 10.1002/9780471729259.mc01b01s00.
- Jacobsen SM, Shirliff ME (2011) *Proteus mirabilis* biofilms and catheter-associated urinary tract infections. Virulence 2: 460–465. doi: 10.4161/viru.2.5.17783.
- Armbruster CE, Mobley HLT, Pearson MM (2018) Pathogenesis of *Proteus mirabilis* infection. EcoSal Plus 8: esp-009-2017. doi: 10.1128/ecosalplus.esp-0009-2017.
- Tom IM, Agbo EB, Faruk UA, Umoru AM, Ibrahim MM, Umar JB, Haruna AB, Aliyu A (2018) Implication of *Proteus* spp in the pathology of nosocomial wound infection in Northeastern Nigeria. Int J of Pathog Res 1: 1–8. doi: 10.9734/ijpr/2018/v1i21258.
- Chen CY, Chen YH, Lu PL, Lin WR, Chen TC, Lin CY (2012) *Proteus mirabilis* urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. J Microbiol Immunol Infect 45: 228–236. doi: 10.1016/j.jmii.2011.11.007.
- Mo L, Wang J, Qian J, Peng M (2022) Antibiotic sensitivity of *Proteus mirabilis* urinary tract infection in patients with urinary calculi. Int J Clin Pract 2022: 1–6. doi: 10.1155/2022/7273627.
- Morrissey I, Hackel M, Badal R, Bouchillon S, Hawser S, Biedenbach D (2013) A review of ten years of the study for monitoring antimicrobial resistance trends (SMART) from 2002 to 2011. Pharmaceuticals 6: 1335–1346. doi: 10.3390/ph6111335.
- ElTaweel M, Said HS, Barwa R (2024) Emergence of extensive drug resistance and high prevalence of multidrug

- resistance among clinical *Proteus mirabilis* isolates in Egypt. Ann Clin Microbiol Antimicrob 23: 46. doi: 10.1186/s12941-024-00705-3.
18. Smith K, Perez A, Ramage G, Lappin D, Gemmell CG, Lang S (2008) Biofilm formation by Scottish clinical isolates of *Staphylococcus aureus*. J Med Microbiol 57: 1018–1023. doi: 10.1099/jmm.0.2008/000968-0.
 19. Wasfi R, El-Rahman OA, Mansour L, Hanora A, Hashem A, Ashour M (2012) Antimicrobial activities against biofilm formed by *Proteus mirabilis* isolates from wound and urinary tract infections. Indian J Med Microbiol 30: 76–80. doi: 10.4103/0255-0857.93044.
 20. Tabatabaei A, Ahmadi K, Namazi SA, Khosravi N, Badamchi A (2021) Virulence genes and antimicrobial resistance pattern in *Proteus mirabilis* strains isolated from patients attended with urinary infections to tertiary hospitals, in Iran. Afr Health Sci 21: 1677–1684. doi: 10.4314/ahs.v21i4.22.
 21. Zhang Q, Zong Q, Feng X, Luo M, Sun W, Zhai Y (2025) Antibacterial and antifouling materials for urinary catheter coatings. Acta Biomater 192: 28–47. doi: 10.1016/j.actbio.2024.12.040.
 22. Kwiecinska-Piróg J, Bogiel T, Skowron K, Wiecekowska E, Gospodarek E (2014) *Proteus mirabilis* biofilm - qualitative and quantitative colorimetric methods-based evaluation. Braz J Microbiol 45: 1423–1431. doi: 10.1590/S1517-83822014000400037.
 23. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkel R (2023) Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. Microorganisms 11: 1614. doi: 10.3390/microorganisms11061614.
 24. Singh S, Singh SK, Chowdhury I, Singh R (2017) Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. Open Microbiol J 11: 53–62. doi: 10.2174/1874285801711010053.
 25. Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55: 165–199. doi: 10.1146/annurev.micro.55.1.165.
 26. Liu L, Dong Z, Ai S, Chen S, Dong M, Li Q, Zhou Z, Liu H, Zhong Z, Ma X, Hu Y, Ren Z, Fu H, Shu G, Qiu X, Peng G (2023) Virulence-related factors and antimicrobial resistance in *Proteus mirabilis* isolated from domestic and stray dogs. Front Microbiol 14: 1141418. doi: 10.3389/fmicb.2023.1141418.
 27. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8: 881–890. doi: 10.3201/eid0809.020063.
 28. Li X, Zhao H, Lockett CV, Drachenberg CB, Johnson DE, Mobley HLT (2002) Visualization of *Proteus mirabilis* within the matrix of urease-induced bladder stones during experimental urinary tract infection. Infect Immun 70: 389–394. doi: 10.1128/IAI.70.1.389-394.2002.
 29. Wasfi R, Hamed SM, Amer MA, Fahmy LI (2020) *Proteus mirabilis* biofilm: development and therapeutic strategies. Front Cell Infect Microbiol 10: 414. doi: 10.3389/fcimb.2020.00414.
 30. Jones BD, Lockett C V, Johnson DE, Warren JW, Mobley HL (1990) Construction of a urease-negative mutant of *Proteus mirabilis*: analysis of virulence in a mouse model of ascending urinary tract infection. Infect Immun 58: 1120–1123. doi: 10.1128/iai.58.4.1120-1123.1990.
 31. D'Alessandro B, Lery LMS, Krüger WMA, Lima A, Piccini C, Zunino P (2011) Proteomic analysis of *Proteus mirabilis* outer membrane proteins reveals differential expression in vivo vs. in vitro conditions. FEMS Immunol Med Microbiol 63: 174–182. doi: 10.1111/j.1574-695X.2011.00839.x.
 32. Pearson MM, Sebaihia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, Abdellah Z, Arrowsmith C, Atkin B, Chillingworth T, Hauser H, Jagels K, Moule S, Mungall K, Norbertczak H, Rabinowitsch E, Walker D, Whithead S, Thomson NR, Rather PN, Parkhill J, Mobley HLT (2008) Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. J Bacteriol 190: 4027–4037. doi: 10.1128/JB.01981-07.
 33. Himpel SD, Pearson MM, Arewång CJ, Nusca TD, Sherman DH, Mobley HLT (2010) Proteobactin and a yersiniabactin-related siderophore mediate iron acquisition in *Proteus mirabilis*. Mol Microbiol 78: 138–157. doi: 10.1111/j.1365-2958.2010.07317.x.
 34. Phiri RM, Ertugrul MB, Bozdoğan B, Hoşbul T (2024) Evaluation of virulence genes in *Proteus* strains isolated from diabetic foot infections and urinary tract infections. J Infect Dev Ctries 18: 1559–1565. doi: 10.3855/jidc.18928.
 35. de Oliveira DW, Barboza MGL, Faustino G, Inagaki WTY, Sanches MS, Kobayashi RKT, Vespero EC, Rocha SPD (2021) Virulence, resistance and clonality of *Proteus mirabilis* isolated from patients with community-acquired urinary tract infection (CA-UTI) in Brazil. Microb Pathog 152: 104642. doi: 10.1016/j.micpath.2020.104642.
 36. Iribarnegaray V, González M, Caetano A, Platero R, Zunino P, Scavone P (2021) Relevance of iron metabolic genes in biofilm and infection in uropathogenic *Proteus mirabilis*. Curr Res Microb Sci 2: 100060. doi: 10.1016/j.crmicr.2021.100060.
 37. Belas R, Manos J, Suvanasthi R (2004) *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. Infect Immun 72: 5159–5167. doi: 10.1128/IAI.72.9.5159-5167.2004.
 38. Ewaoche IS, Tolulope A, Ibemologi A, Edmund E (2021) UreC and ZapA virulence genes amplification in clinical specimen of *Proteus mirabilis* in Bayelsa state, Nigeria. J Microbiol Exp 9: 15–20. doi: 10.15406/jmen.2021.09.00317.
 39. Alsaadi LAS, Ali IA, Abbas SA (2022) Molecular analysis of *Proteus mirabilis* virulence genes isolated from urinary tract infections from Baquba teaching hospital. AIP Conf Proc 2394: 020031. doi: 10.1063/5.0121411.
 40. Phan V, Belas R, Gilmore BF, Ceri H (2008) ZapA, a virulence factor in a rat model of *Proteus mirabilis*-induced acute and chronic prostatitis. Infect Immun 76: 4859–4864. doi: 10.1128/IAI.00122-08.
 41. Belas R (1994) Expression of multiple flagellin-encoding genes of *Proteus mirabilis*. J Bacteriol 176: 7169–7181. doi: 10.1128/jb.176.23.7169-7181.1994.
 42. Liaw SJ, Lai HC, Wang WB (2004) Modulation of swarming and virulence by fatty acids through the RsbA protein in *Proteus mirabilis*. Infect Immun 72: 6836–6845. doi: 10.1128/IAI.72.12.6836-6845.2004.
 43. Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME (2008) Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clin Microbiol Rev 21: 26–59. doi: 10.1128/CMR.00019-07.
 44. Scavone P, Iribarnegaray V, González MJ, Navarro N, Huerta NC, Wilde JJ, Härtel S, Zunino P (2023) Role of *Proteus mirabilis* flagella in biofilm formation. Rev Argent Microbiol 55: 226–234. doi: 10.1016/j.ram.2023.01.005.
 45. Zhang K, Liu L, Yang M, Chen C, Li X, Tian J, Luo C, Wang X, Wang M (2022) Reduced porin expression with EnvZ-OmpR, PhoPQ, BaeSR two-component system down-

- regulation in carbapenem resistance of *Klebsiella pneumoniae* based on proteomic analysis. *Microb Pathog* 170: 105686. doi: 10.1016/j.micpath.2022.105686.
46. Scavone P, Iribarnegaray V, Caetano AL, Schlapp G, Härtel S, Zunino P (2016) Fimbriae have distinguishable roles in *Proteus mirabilis* biofilm formation. *Pathog Dis* 74: ftw033. doi: 10.1093/femspd/ftw033.
 47. Cestari SE, Ludovico MS, Martins FH, da Rocha SPD, Elias WP, Pelayo JS (2013) Molecular detection of HpmA and HlyA hemolysin of uropathogenic *Proteus mirabilis*. *Curr Microbiol* 67: 703–707. doi: 10.1007/s00284-013-0423-5.
 48. Motamedi H, Asghari B, Tahmasebi H, Arabestani M (2018) Identification of hemolysine genes and their association with antimicrobial resistance pattern among clinical isolates of *Staphylococcus aureus* in West of Iran. *Adv Biomed Res* 7: 153. doi: 10.4103/abr.abr_143_18.
 49. Mishu NJ, Shamsuzzaman SM, Khaleduzzaman HM, Nabonee MA, Dola NZ, Haque A (2022) Association between biofilm formation and virulence genes expression and antibiotic resistance pattern in *Proteus mirabilis*, isolated from patients of Dhaka Medical College Hospital. *Arch Clin Biomed Res* 6: 50170257. doi: 10.26502/acbr.50170257.
 50. Sun Y, Wen S, Zhao L, Xia Q, Pan Y, Liu H, Wei C, Chen H, Ge J, Wang H (2020) Association among biofilm formation, virulence gene expression, and antibiotic resistance in *Proteus mirabilis* isolates from diarrhetic animals in Northeast China. *BMC Vet Res* 16: 176. doi: 10.1186/s12917-020-02372-w.