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Antibiogram Profiling with Prevalence Assessment of *Proteus mirabilis* Recovered from Hospital Settings

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Abstract:

Background: *Proteus mirabilis* is an opportunistic Gram-negative bacterium that may be present in hospital environments and is associated with several healthcare-associated infections, including urinary tract infections, wound infections, and burn infections. This study aimed to investigate the presence of *P. mirabilis* in clinical or hospital-related samples and to evaluate the antimicrobial susceptibility patterns of bacterial isolates

Materials and Methods: A total of 150 clinical samples and 100 environmental samples were collected from three hospitals in Babylon Province. The samples were cultured for bacterial growth. Bacterial isolates were identified using biochemical tests and the VITEK 2 automated system. antimicrobial susceptibility testing was performed.

Results: The results showed that 20 *Proteus mirabilis* isolates were obtained from a total of 150 clinical samples, while no *P. mirabilis* isolates were recovered from environmental samples. However, other bacterial species were isolated from environmental samples and identified using the VITEK 2 automated system. The results of antibiotic susceptibility test showed a resistance rate of 36.8% for cefotaxime, ceftazidime, and cefepime. Furthermore, a high resistance rate (100%) was observed against tigecycline. The resistance rate to avibactam, imipenem, and trimethoprim was 26.3%, and the resistance rates to ciprofloxacin, ampicillin, and ceftolozane were 21.1%, 15.8%, and 10.5%, respectively. Colistin showed no detectable activity against the tested *P. mirabilis* isolates.

Conclusion: *Proteus mirabilis* is an important opportunistic pathogen associated with healthcare-related infections. The isolates demonstrated variable antimicrobial resistance patterns, with notably high resistance to tigecycline and moderate resistance to cephalosporins.

Keywords: *proteus mirabilis*, isolation, identification, Antibiotic Resistance, hospital environments

1. INTRODUCTION

Proteus species are recognized as important opportunistic pathogens responsible for a significant proportion of urinary tract infections (UTIs) worldwide [1]. Among them, *Proteus mirabilis* is the most clinically relevant species due to its strong association with both community-acquired and hospital-acquired infections [2]. It is a Gram-negative bacterium characterized by urease production, motility, and the ability to form biofilms, which contribute to its persistence in the urinary tract and medical environments [3].

The ability of *Proteus spp.* to survive under different environmental and host conditions makes it a successful pathogen in both simple and complicated infections. These bacteria are commonly isolated from urine samples and are frequently associated with catheter-associated urinary tract infections (CAUTIs), wound infections, and other nosocomial infections[4]. Accurate isolation and identification using culture characteristics, microscopic examination, and biochemical tests remain essential for proper diagnosis [5].

In recent years, the emergence of antibiotic-resistant *Proteus* isolates has become a major clinical concern worldwide. Misuse and overuse of antibiotics have contributed to the development of multidrug-resistant strains [6], reducing the effectiveness of commonly used antimicrobial agents. Therefore, determining antimicrobial susceptibility patterns and evaluating resistance levels using standardized methods such as minimum inhibitory concentration (MIC) are essential for appropriate treatment strategies [7]. *Proteus spp.* infect humans due to several virulence factors, including urease, flagella, fimbriae, biofilm formation, and the production of toxins such as hemolysin and mirabilysin [8]. Consequently, the incidence of these bacteria is expected to be higher in hospitalized patients compared to outpatients. Antibiotic resistance in *Proteus mirabilis* is attributed to several mechanisms, most notably the production of β -lactamase enzymes, in addition to efflux pumps that expel antibiotics outside the bacterial cell, thereby reducing their intracellular concentration and leading to decreased therapeutic effectiveness[9]. The present study aims to isolate and identify *Proteus spp.* from clinical samples and environmental samples, and evaluate antibiotic susceptibility patterns of commonly used antibiotics.

2. MATERIALS AND METHODS

2.1 Samples Collection

A total of one hundred and fifty clinical samples were obtained from three hospitals in Babylon Province (Al-Imam Al-Sadiq Hospital, Marjan Teaching Hospital, and Al-Hilla Teaching Hospital) during the period from September 2025 to January 2026 from patients suffering from urinary tract infections (UTI), burns, and wound infections. Burn and wound samples were collected from patients prior to skin disinfection.

In addition, 100 environmental samples were collected from the same hospitals. The environmental samples consisted of 55 hospital wastewater samples and 45 environmental swab samples obtained from various hospital sources, including patient beds, medical equipment, surgical instruments, floors, bathrooms, tables, operating rooms, waste containers, sinks, walls, and healthcare workers' hands. All samples were collected following standard aseptic techniques to avoid contamination during collection, transport, and handling.

2.2 Isolation of *proteus mirabilis*

Urine, burn, and wound samples were collected. Urine samples were collected in sterile, leak-proof containers, while burn and wound samples were collected using sterile cotton swabs. All specimens were transferred into tubes containing 2 mL of tryptic soy broth. The samples were incubated at 37°C for 24 hours. After incubation, cultures were inoculated using a sterile loop by the streaking method onto MacConkey agar,

nutrient agar, and blood agar plates, and then incubated at 37°C for an additional 24 hours [10]. Sterile swabs moistened with Tryptic Soy Broth (TSB, Oxoid) were used to collect environmental samples from an area of approximately 10 × 10 cm of each surface. After collection, the swabs were placed into sterile tubes containing 2 mL of TSB for transport to the laboratory. The samples were then incubated in TSB before being cultured on selective and differential media for the isolation of *Proteus mirabilis* [11].

2.3 Identification of *proteus mirabilis*

The isolates were identified based on morphological characteristics, including colony size, shape, color, swarming behavior, odor, transparency, margin, and elevation. Microscopic examination was also performed based on Gram staining, colony patterns on different culture media, and pigment production. Identification of *Proteus mirabilis* isolates was further confirmed using biochemical tests, including lactose fermentation, indole test, oxidase test, sucrose fermentation, and catalase test, in addition to urease test, motility test, hydrogen sulfide (H₂S) production, methyl red (MR) test, Voges–Proskauer (VP) test, citrate utilization test, and triple sugar iron (TSI) agar test, alongside the VITEK 2 automated identification system. [12].

2.4 Antibiotic Susceptibility Testing for *proteus mirabilis*

This test was performed using the VITEK 2 automated system according to the manufacturer's instructions. The isolates were obtained from fresh overnight cultures (18–24 hours) grown on appropriate culture media, and a single pure colony was selected from each isolate for analysis. A bacterial suspension was then prepared by emulsifying the colony in sterile normal saline (0.45–0.5% sodium chloride solution). The turbidity of the suspension was adjusted to match the 0.5 McFarland standard using a turbidity meter. This step ensures standardization of the bacterial inoculum to obtain accurate and comparable results. Appropriate VITEK 2 AST cards designed for Gram-negative bacteria were selected. These cards contain a panel of antimicrobial agents at different concentrations used to evaluate the resistance pattern of *Proteus mirabilis*. The bacterial suspension was then loaded into the system's tubes, and the VITEK 2 cards were inserted into the instrument for automated processing. The system performs incubation and reading automatically by monitoring bacterial growth changes within the micro-wells of the card, allowing determination of the bacterial response to different antibiotics. The analysis typically takes 6 to 18 hours depending on the sample type and the card used. After completion of the analysis, the system automatically generated the results, classifying *Proteus mirabilis* isolates into three categories according to susceptibility: Sensitive (S), Intermediate (I), and Resistant (R) [13].

3. Results

3.1. Isolation of *Proteus mirabilis*

Out of 150 clinical samples, 90 samples (60%) showed bacterial growth, while 60 samples (40%) showed no growth after the incubation period. Among the samples that exhibited bacterial growth, 20 isolates (13.3%) were identified as *Proteus mirabilis* (Table 1). The highest proportion of isolates was obtained from urinary tract infection samples 13(65%), followed by wound samples 4(20%), and burn samples 3(15%)(Table

2). Other bacterial species isolated and identified from the clinical samples in the current study were: *Escherichia coli* (20 isolates)(28.57%), *Staphylococcus aureus*(15 isolates) (21.34%), *Pseudomonas aeruginosa* (12 isolates) (17.14%), *Klebsiella oxytoca*(8 isolates)(11.43), *Enterobacter hormaechei* (7 isolates) (10.00%), *Clostridium perfringens* (5 isolates) (7.14 %), *Salmonella typhimurium* (3 isolates) (4.29 %).

Table.1: Distribution of bacterial growth among 150 clinical samples.

Culture result	Number of samples (n)		Percentage (%)		P value
<i>Proteus mirabilis</i>	20	90	13.3	60	0.0001*
Other bacteria	70		46.7		
No growth (negative)	60		40.0		
Total	150		100		

* represent a significant difference at $p < 0.05$

Table.2: Number and percentages of *Proteus spp* in different clinical samples.

Samples	Number of isolates	%	P value
Urine	13	65%	0.011*
Wound	4	20%	
Burns	3	15%	
Total	20	100%	

* represent a significant difference at $p < 0.05$

For the environmental samples, 100 samples were collected, including 55 wastewater samples and 45 environmental swabs from different sites. No *Proteus spp.* isolates were detected in any of the samples. Although *Proteus spp.* was absent, other bacterial species were identified including: *Pseudomonas aeruginosa* (25 isolates)(25.0 %), *Staphylococcus spp* (15 isolates)(15.0 %), *klebsiella spp* (12 isolates)(12.0 %), *Salmonella spp* (10 isolates)(10.0 %), *Citrobacter amalonaticus* (8 isolates)(8.0 %), *Shigella spp*(7 isolates)(7.0 %), *Aeromonas sobria* (6 isolates)(6.0 %), *Moraxella spp* (5 isolates)(5.0 %), *Myroides spp* (4 isolates)(4.0 %), *Brevundimonas diminuta* (3 isolates)(3.0 %), *Campylobacter spp* (2 isolates)(2.0 %), *Oligella ureolytica* (3 isolates)(3.0 %).

3.2. Identification of *Proteus mirabilis*

After isolating the bacteria, they were cultured on several media, including MacConkey agar and blood agar, in order to examine the morphological characteristics of the colonies. On MacConkey agar, the colonies appeared pale and colorless due to their inability to ferment lactose, with smooth and non-mucoid surfaces. On blood agar, the colonies exhibited characteristic swarming motility, forming concentric waves across the surface of the medium. Some isolates also showed β -hemolysis, surrounded by a clear zone, indicating the lysis of red blood cells due to the production of hemolysins. (**Figure 1**).

For confirmation of identification, a series of biochemical tests was performed, and the results are presented in Table (3). All isolates showed positive reactions for the catalase test and negative reactions for the oxidase test. In addition, all isolates were unable to ferment sucrose and lactose. The Methyl Red (MR) test showed positive results, whereas the Voges–Proskauer (VP) test was negative. The isolates also gave negative results for the Simmons citrate test. Furthermore, all isolates demonstrated positive gelatin liquefaction, indicating their ability to hydrolyze gelatin. The Kligler Iron Agar (KIA) test revealed positive hydrogen sulfide (H_2S) production, and the urease test was also positive. These morphological and biochemical characteristics are consistent with the standard profile of *Proteus mirabilis*, thereby confirming the identification of the studied isolates [14].

Table (3) The of biochemical test used to identify *proteus mirabilis*

No	The Test	The result
1.	Catalase	+
2.	Oxidase	-
3.	Lactose fermentation	-
4.	Sucrose fermentation	-
5.	Methyl red	+
6.	Voges-proskauer	-
7.	citrate utilization	-
8.	Gelatin Liquefaction	+
9.	Kligler Iron Agar	+
10.	Urease agar	+

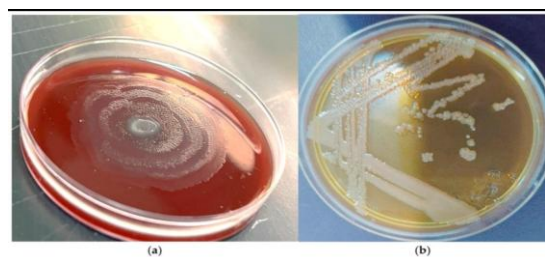


Figure 1: Morphological Characteristics of *proteus mirabilis* isolates on A: Blood agar B: MacConkey agar

3.3. Antibiotic Susceptibility Results of *Proteus mirabilis* Isolates

All the confirmed isolates of *Proteus mirabilis* were evaluated for susceptibility to 15 selected antibiotics . Susceptibility screening of the isolates was performed using the VITEK2 compact system. The results of drug susceptibility testing are shown in Table (4). The results showed a resistance rate of 36.8% for cefotaxime, Cefepime, and ceftazidime. Furthermore, the current findings demonstrated a markedly high resistance rate (100%) of *Proteus mirabilis* isolates to Tigecycline. However, the frequency of resistance to Ceftazidime/Avibactam, Imipenem, and Trimethoprim in *Proteus mirabilis* was (26.3%). The resistance rates of *Proteus mirabilis* isolates to Ciprofloxacin, Ampicillin, and ceftolozane were evaluated (21.1%, 15.8 %,10.5 % respectively) . Colistin showed no detectable activity against the tested *Proteus mirabilis* isolates.

Table (4) : Antimicrobial susceptibility rates among *Proteus mirabilis* isolated from clinical samples

Antibiotic	Clinical samples (n=19)					
	R	%	I	%	S	%
Ampicillin/Sulbactam (AMP)	3	15.8	-	-	16	84.2
Piperacillin/Tazobactam (TZP)	3	15.8	-	-	16	84.2
Cefotaxime (CTX)	7	36.8	-	-	12	63.2
Ceftazidime (CAZ)	7	36.8	-	-	12	63.2
Ceftazidime/Avibactam (CAZ/AVI)	5	26.3	-	-	14	73.7
Ceftolozane/Tazobactam (CTZ)	2	10.5	1	5.3	16	84.2
Cefepime (FEP)	7	36.8	-	-	11	57.9
Imipenem (IPM)	5	26.3	2	10.5	12	63.2
Meropenem (MEM)	1	5.3	-	-	18	94.7
Amikacin (AK)	0	0	4	21.1	15	78.9
Gentamicin (GEN)	1	5.3	4	21.1	14	73.7
Ciprofloxacin (CIP)	4	21.1	-	-	15	78.9
Tigecycline (TGC)	100	100	-	-	0	0
Colistin (CT)	0	0	0	0	0	0

Trimethoprim/Sulfamethoxazole (SXT)	5	26.3	-	-	14	73.7
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R : resistance; I: intermediate; S: sensitive

4. DISCUSSION

Different clinical samples were collected from wounds, burns, and urinary tract. 20 clinical isolates of the *proteus mirabilis* were obtained out of 150 samples, which constituted a percentage of (13.3% of positive samples). Similar studies have been done by other researchers, such as AL-joda et al. [15] who found that the percentage of clinical isolates was 19%, while Rafiq et al [16] found that the percentage of clinical isolates was 9.6%,;as for Al-Mudallal et al [17] who found that the percentage for clinical isolates reached 10%, Khan et al [18] obtained a percentage that reached 23%, Al-Kaim et al[19] obtained a percentage of 12.6%, and Abdullah et al [20] found the percentage of *proteus mirabilis* isolation of (17%).

For the environmental samples, no *Proteus spp.* isolates were detected in any of the samples. Although *Proteus spp.* was absent, other bacterial species were identified (*Aeromonas sobria* , *Myroides ssp* , *Citrobacter amalonaticus* , *Staphylococcus spp* , *Brevundimonas diminuta* | *vesicularis* , *Oligella ureolytica* , *Salmonella spp* , *klebsiella spp* , *Shigella spp* , *Pseudomonas aeruginosa* , *Campylobacter spp* , *Moraxella spp.*). This diversity reflects a significant microbial presence across hospital environmental samples. The highest bacterial loads were observed in wastewater and frequently touched surfaces, indicating potential hotspots for pathogen spread. The presence of these pathogenic bacteria in wastewater poses a direct threat to human health, as exposure can lead to gastrointestinal infections, skin infections, and respiratory diseases. Contaminated water serves as a major vehicle for disease transmission, impacting not only patients but also healthcare personnel and visitors. and studies indicate that around 1.1 billion individuals lack access to safe drinking water due to inadequate sanitation, resulting in a rise in waterborne infections, with 2.2 million fatalities annually attributed to these illnesses [21]. Waterborne illnesses induce significant gastrointestinal infections and diarrhea. In underdeveloped countries, circumstances are more severe than in wealthy nations due to inadequate management, unsanitary environments, and limited access to safe drinking water resources. Sewage dumping and chemical pollutants in water result in deterioration of water quality [22].

P. mirabilis is acknowledged as a rising healthcare issue due to its role as an opportunistic human pathogen, frequently located in the gastrointestinal tract, skin, and oral mucosa. Immunocompromised individuals or those receiving antibiotic treatment are especially vulnerable to its proliferation [23]. Antibiotics, extensively used in hospital settings, exert significant selection pressure on bacteria, leading to the development and dissemination of antibiotic resistance. Nevertheless, the role of antibiotics as the principal catalyst of antibiotic resistance in species occupying various ecological niches remains ambiguous, due to the chemical similarity between antibiotics and naturally occurring chemicals. Moreover, multiple bacterial resistance mechanisms are recognized to confer resistance to both metals and antibiotics [24].

Resistance of *Proteus mirabilis* to most antibiotics has developed rapidly and arises from a combination of reduced outer-membrane permeability and secondary resistance mechanisms, such as chromosomally or plasmid-encoded periplasmic β -lactamases and energy-dependent multidrug efflux pumps [25]. However,

significant geographic disparities exist in resistance rates across various antibiotic classes and specific drugs within each class [26]. Antibiotic susceptibility patterns provide a valuable framework for choosing the correct antibiotic. This information is crucial for devising effective ways to prevent antibiotic resistance [27]. The present study demonstrated variable resistance patterns among *Proteus mirabilis* isolates against the tested antibiotics. A relatively moderate resistance rate (36.8%), including cefotaxime, cefepime, and ceftazidime. This may be attributed to the production of extended-spectrum β -lactamases (ESBLs), which are commonly reported in *Proteus* species. These findings are consistent with previous studies that reported increasing resistance to cephalosporins among uropathogens. These findings partially agree with the study conducted by Nissanka et al [28] in Anbar Governorate, Iraq, which reported higher resistance rates exceeding 80% for these cephalosporins, while Ciprofloxacin resistance was approximately 40%, Imipenem, and meropenem resistance remained 26.3%, 5.3%. A global study published by Kristiansen et al [29], including 190 isolates also demonstrated high resistance to β -lactams and Ciprofloxacin, while carbapenem and certain cephalosporin resistance remained low (<10%) in some regions, reflecting the worldwide trend of increasing multidrug resistance in *P. mirabilis*. Additionally, a study published by Alp et al [30] documented moderate to high resistance to Ciprofloxacin (45–60%) and Trimethoprim–Sulfamethoxazole (40–55%), while cephalosporin resistance ranged from (50–70%), supporting the results of the current study and confirming the widespread occurrence of multidrug-resistant *P. mirabilis* isolates [31]. Resistance to cephalosporins may not be limited to the production of β -lactamases. Other mechanisms, such as alterations in outer membrane permeability, can hinder antibiotic penetration and access to target sites. This mechanism is particularly relevant in Gram-negative bacteria, as their outer membrane contains protein channels called porins, which restrict antibiotic entry [32]. Notably, a very high resistance rate (100%) was recorded for tigecycline, which may reflect intrinsic resistance or limited clinical effectiveness of this antibiotic against *Proteus mirabilis*, which is consistent with the findings of Zhang et al [33]. Although Tigecycline is generally considered one of the most effective broad-spectrum antibiotics and is often reserved as a last-ditch treatment for infections due to extensively drug-resistant gram-negative bacteria, the current findings indicate a marked reduction in its expected efficacy in the studied isolates. [34]. In contrast, lower resistance rates were observed for avibactam, imipenem, and trimethoprim (26.3%), indicating that these antibiotics may still retain some therapeutic efficacy, which is consistent with previous local reports by Armbruster et al [35], Bouhrour et al [36], and Al-Azzawi et al [37] who reported resistance rates of 15% and 16.2%, and 15.7% respectively. However, these results differ from Zhang et al. [38], who recorded 58% resistance, and Hafiz et al [39], who reported 25% resistance. Carbapenems are considered among the most effective antibiotics for treating Gram-negative bacterial infections due to their stability against β -lactamase hydrolysis and high permeability through the bacterial outer membrane [40]. Additionally, resistance to ciprofloxacin (21.1%), ampicillin (15.8%), and ceftolozane (10.5%) was relatively low compared to other antibiotics, the results are consistent with prior reports in Iraq by Omrana et al [41] showing 26.98% resistance. Resistance to Ciprofloxacin may arise from structural modifications in target enzymes, mutations in regulatory genes controlling efflux pumps, or a combination of both [42], [43] suggesting variable susceptibility profiles among the isolates. The lack of detectable activity of colistin against the tested isolates may be explained by the intrinsic resistance of *Proteus mirabilis* to polymyxins, which has been well documented in the literature. Overall, these findings highlight the emergence of multidrug resistance among *Proteus mirabilis*, emphasizing the need for continuous antimicrobial surveillance and rational antibiotic use.

CONCLUSION

P. mirabilis is a relevant pathogen in the selected hospitals in Babylon Governorate with considerable resistance to Tigecycline, Cefotaxime, Ceftazidime, and Cefepime. Other notable pathogens included *Pseudomonas aeruginosa*, and *Klebsiella oxytoca*, while *Enterobacter hormaechei*, *Clostridium perfringens*, and *Salmonella typhimurium*. These findings not only underscore the diverse etiological spectrum of bacterial infections within hospital settings but also reinforce the critical necessity for routine microbiological surveillance, locally guided antibiotic resistance monitoring, and the implementation of stewardship initiatives.

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