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RESEARCH ARTICLE OPEN ACCESS

Antibiotic susceptibility testing of *Proteus mirabilis* isolates from selected hospitals in Anbar governorate, Iraq

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Abstract
Objective: To investigate the antibiotic susceptibility profiles of *Proteus mirabilis* isolated from clinical samples in selected hospitals in Anbar Governorate, Iraq.
Methods: A cross-sectional study was conducted from September 2024 to January 2025. A total of 255 clinical samples were collected from patients attending Al-Ramadi Teaching Hospital, Hit General Hospital, and private clinics. Isolation and identification of *P. mirabilis* were performed using conventional biochemical methods and confirmed with the Vitek-2 Compact system. Antibiotic susceptibility testing was conducted via the Kirby-Bauer disk diffusion method according to CLSI 2024 guidelines. Data were analysed descriptively, and chi-square tests were used to assess demographic associations.
Results: Out of 255 samples, 15 (5.88%) yielded *P. mirabilis*. Isolates showed 100% sensitivity to carbapenems (meropenem, imipenem, and ertapenem). High resistance was noted to cefotaxime (100%), ceftriaxone (73.3%), and piperacillin (86.6%). Moderate susceptibility was observed for amikacin (53.3%) and ciprofloxacin (46.6%). Infections were more prevalent in males (73.3%), urban residents (66.7%), and individuals aged 11–20 years (33.3%). A significant association was found between gender and infection status ($p < 0.05$).
Conclusion: *Proteus mirabilis* isolates in Anbar Governorate exhibit high resistance to third-generation cephalosporins but retain full susceptibility to carbapenems. These findings underscore the urgent need for local antimicrobial resistance surveillance and implementation of stewardship programs. Clinicians should exercise caution in empirical antibiotic selection and prioritise culture-based therapy to minimise resistance spread.

Keywords: *Proteus mirabilis*, Antibiotic resistance, Carbapenems, Antimicrobial stewardship, Iraq

Plain English Summary

This study looked at how well antibiotics work against a type of bacteria called *Proteus mirabilis*, which can cause infections like urinary tract and wound infections. We collected samples from patients in several hospitals and clinics in Anbar Governorate, Iraq. We found that while this bacterium was not very common, it was completely resistant to some commonly used antibiotics, especially certain cephalosporins. However, it was still fully sensitive to a powerful group of antibiotics called carbapenems. Most infections were seen in males, teenagers, and people living in urban areas. These results are important because they show that doctors in the region need to be cautious about which antibiotics they prescribe. Using carbapenems carefully and only when truly necessary is essential to prevent the bacteria from becoming resistant to them as well. The study highlights the need for better monitoring of antibiotic resistance and more responsible use of antibiotics to protect public health.

Introduction

The genus Proteus, belonging to the family Morganellaceae and order Enterobacterales, comprises Gram-negative, facultative anaerobic bacilli that are commonly found in soil, water, and the intestinal flora of humans and animals.

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Among its five species (*P. mirabilis, P. vulgaris, P. penneri, P. hauseri*, and *P. myxofaciens*), *Proteus mirabilis* is the most frequently isolated in clinical settings, particularly in urinary tract infections (UTIs) and, to a lesser extent, in wound infections, otitis media, burns, and respiratory tract infections (1).

Globally, *P. mirabilis* has emerged as a significant opportunistic pathogen in both community and healthcare-associated infections, often affecting individuals with immunosuppression, anatomical abnormalities, or indwelling medical devices (2). Its ability to swarm across solid surfaces, form biofilms, and produce urease facilitates its pathogenesis, especially in catheter-associated UTIs (3).

Of increasing concern is the escalating antimicrobial resistance (AMR) of *P. mirabilis*. Like other members of Enterobacterales, this organism has demonstrated rising resistance to third-generation cephalosporins, aminoglycosides, and fluoroquinolones, primarily through mechanisms such as the production of extended-spectrum beta-lactamases (ESBLs), AmpC enzymes, and alterations in penicillin-binding proteins and porins (4, 5). The World Health Organisation has recently emphasised AMR as one of the top global public health threats, with carbapenem-resistant Enterobacterales representing a critical priority group for research and surveillance (6).

In recent years, bacterial resistance to drugs has been observed, which is linked to the availability and use of increasing amounts of broad-spectrum antibiotics, as well as their excessive and unregulated use. This will lead to a loss of therapeutic options and ultimately to an inability to control and treat the bacteria (7).

Despite this alarming trend, limited local data exist regarding the resistance patterns of *P. mirabilis* in many parts of Iraq, including Anbar Governorate. Most available studies have focused broadly on Enterobacterales or *Escherichia coli* and *Klebsiella pneumoniae*, leaving gaps in our understanding of *P. mirabilis* epidemiology and resistance dynamics in this region (8, 9).

This study aims to address this gap by investigating the antibiotic susceptibility patterns of *P. mirabilis* isolates obtained from clinical cases in selected hospitals and clinics in Anbar Governorate. By providing local surveillance data, the study seeks to inform empirical treatment decisions and contribute to ongoing national and global efforts to curb antimicrobial resistance.

Materials and Methods

Study Sites

This study was conducted between September 20, 2024, and January 29, 2025, in Anbar Governorate, Iraq. Clinical samples were collected from hospitalised patients and outpatients at Al-Ramadi Teaching Hospital, Hit General Hospital, and several private clinics.

Sample Collection

A total of 255 clinical samples were collected using a convenience sampling approach. The sample size was guided by existing literature and previous studies conducted in the region (10). The age of participants ranged from 1 to 65 years. Samples were obtained from urine (n=55), wounds (n=55), burns (n=48), middle ear (n=53), and upper respiratory tract (n=44). Swabs were immediately inoculated on Luria-Bertani (LB) agar and incubated at 37°C for 24 hours.

Isolation and Identification of P. mirabilis

Following initial incubation, isolates were sub cultured on MacConkey agar, blood agar, and HiCrome UTI agar. Morphological characteristics such as swarming on blood agar and non-lactose fermenting colonies on MacConkey agar were used for preliminary identification (11).

Gram staining and standard biochemical tests were performed, including catalase, oxidase, urease, IMViC, motility, and lactose fermentation tests (12). Identification was confirmed using the Vitek-2 Compact System (bioMérieux, France; version 08.01). Quality control strains, including *Escherichia coli* ATCC 25922, were used throughout to validate biochemical tests.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (13, 14). Ten antibiotics were tested: Cefotaxime (30 µg), Ceftriaxone (30 µg), Cefixime (5 µg), Meropenem (10 µg), Imipenem (10 µg), Ertapenem (10 µg), Piperacillin (100 µg), Aztreonam (30 µg), Amikacin (30 µg), and Ciprofloxacin (5 µg). Antibiotic discs were sourced from Himedia Company, India. Inhibition zone diameters were measured and interpreted following CLSI 2024 breakpoints. *E. coli* ATCC 25922 was used as a control strain.

Results

Isolation Frequency and Demographic Distribution

Out of 255 clinical samples, 15 (5.88%) were identified as *P. mirabilis*. The distribution of isolates by specimen type was: middle ear 5 (9.43%), urine 4 (7.27%), burns 3 (6.25%), wounds 2 (3.63%), and upper respiratory tract 1 (2.27%) (Table 1).

Table 1: Distribution of clinical specimens

Specimen Type	No. of Samples	% of Total
Urine	55	21.60%
Wound	55	21.60%
Burn	48	18.80%
Middle Ear	53	20.80%
Upper Respiratory Tract	44	17.20%
Total	255	100%

Demographically, the infection rate was higher among males (11/15, 73.3%) compared to females (4/15, 26.7%), and higher in urban residents (10/15, 66.7%) than in rural residents (5/15, 33.3%). The most affected age group was 11–20 years (5/15, 33.3%) (Table 2). A chi-square analysis found a statistically significant association between gender and *P. mirabilis* infection ($p < 0.05$).

Table 2: Distribution of samples by age group

Age Group (years)	No. of Samples	% of Total
1–10	46	18.00%
11–20	51	20.00%
21–30	73	28.60%
31–40	37	14.50%
41–50	29	11.30%
51–65	18	7.50%
Total	255	100%

Antibiotic Susceptibility Test

All isolates were tested against 10 antibiotics. The highest sensitivity was observed for carbapenems: Meropenem, Imipenem, and Ertapenem (15/15, 100%). Moderate sensitivity was found for Aztreonam (9/15, 60%), Amikacin (8/15, 53.3%), and Ciprofloxacin (7/15, 46.6%). High resistance was noted to Cefotaxime (15/15, 100%), Ceftriaxone (11/15, 73.3%), Cefixime (5/15, 33.3%), and Piperacillin (1/15, 6.6%) (Table 3).

Table 3: Antibiotic susceptibility of *P. mirabilis* isolates

Antibiotic	Sensitive n (%)	Intermediate Susceptibility n (%)	Resistant n (%)
Meropenem	15 (100%)	0	0
Imipenem	15 (100%)	0	0
Ertapenem	15 (100%)	0	0
Aztreonam	9 (60%)	1 (6.6%)	5 (33.3%)
Amikacin	8 (53.3%)	5 (33.3%)	2 (13.3%)
Ciprofloxacin	7 (46.6%)	0	8 (53.3%)
Cefixime	5 (33.3%)	1 (6.6%)	9 (60%)
Piperacillin	1 (6.6%)	1 (6.6%)	13 (86.6%)
Ceftriaxone	0	4 (26.6%)	11 (73.3%)
Cefotaxime	0	0	15 (100%)

All susceptibility data were interpreted using CLSI 2024 breakpoints

Figure 1 shows *P. mirabilis* swarming on blood agar and lactose non-fermenting colonies on MacConkey agar (high-resolution images; scale bar: 5 mm).

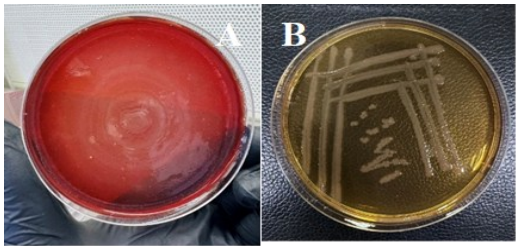

Figure 1: *P. mirabilis* swarming on blood agar (A) and lactose non-fermenting colonies on MacConkey agar (B) (high-resolution images; scale bar: 5 mm)

Figure 2 presents Gram-stained *P. mirabilis* rods at 1000x magnification (scale bar: 10 µm).

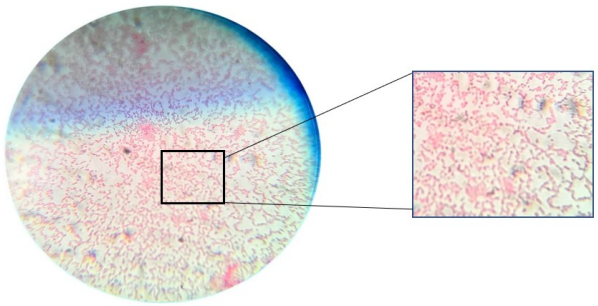

Figure 2: Gram-stained *P. mirabilis* rods at 1000x magnification (scale bar: 10 µm)

Key Findings Summary

P. mirabilis isolation rate was low (5.88%) but showed alarming resistance to multiple cephalosporins. Infections were more frequent among males and urban residents, particularly in adolescents. Carbapenems remain highly effective.

Discussion

The present study investigated the prevalence and antibiotic susceptibility of *Proteus mirabilis* isolated from clinical samples in Anbar Governorate. The overall isolation rate of 5.88% aligns with earlier regional findings by Owaied and Jabur. (15), but remains lower than those reported in Baghdad and Zakho (8, 10). This suggests a relatively modest distribution of *P. mirabilis* in the study area, though likely influenced by the limited sample size and regional variation.

High resistance was observed against third-generation cephalosporins, particularly cefotaxime (100%) and ceftriaxone (73.3%). This is consistent with studies conducted in Baghdad, where *P. mirabilis* isolates also showed complete resistance to cefotaxime (16), and aligns with reports from Thi-Qar and other parts of Iraq (15). The widespread resistance could be attributed to the extensive and often unregulated use of cephalosporins, leading to the selection of ESBL-producing strains (5, 9).

On the other hand, complete susceptibility to carbapenems (meropenem, imipenem, and ertapenem) reflects their retained efficacy and highlights their critical role as first-line therapy for serious infections caused by *P. mirabilis*. Similar findings were observed in studies from Duhok, Zakho, and Indonesia (8, 17, 18). However, this should not justify indiscriminate use of carbapenems, as overuse may hasten resistance development (4).

Moderate susceptibility rates to amikacin (53.3%) and ciprofloxacin (46.6%) further suggest these agents may still have utility in treating some infections, albeit cautiously. These values are similar to those reported by Al-Nabhani & Shami (16) and Allawi & Motaweq (19), indicating a concerning trend toward multidrug resistance.

Demographically, infection prevalence was higher among males (73.3%), adolescents aged 11–20 years (33.3%), and urban residents (66.6%). This demographic pattern may reflect higher rates of catheterization, behavioural risk factors, and healthcare contact among these subpopulations (Al-Ezzy et al., 2023) (20). While similar distributions have been reported (Pal et al., 2014) (21), further epidemiological investigations are needed to confirm causal factors.

Clinical Implications

The findings support the continued use of carbapenems for managing severe *P. mirabilis* infections. However, reliance on carbapenems must be balanced with antimicrobial stewardship strategies to prevent emerging resistance. Given the high resistance to third-generation cephalosporins, clinicians in this setting should avoid their empirical use unless supported by susceptibility testing. Implementation of local stewardship programs and regulation of antibiotic sales are urgently needed to curb resistance proliferation.

Study Limitations

This study has several limitations. First, the sample size of *P. mirabilis* isolates (n=15) is relatively small, limiting generalizability. Second, all samples were collected from a single governorate, potentially introducing geographic bias. Third, the study did not assess prior antibiotic exposure or comorbidities, which could have confounded susceptibility outcomes. Additionally, molecular typing and resistance gene profiling were not performed, limiting the understanding of underlying mechanisms.

Future research should involve larger multicentre studies incorporating molecular diagnostics and

surveillance of resistance gene transmission pathways.

Conclusion

This study demonstrates a concerning level of antibiotic resistance among *Proteus mirabilis* isolates in Anbar Governorate. All isolates showed complete sensitivity to carbapenems, meropenem, imipenem, and ertapenem, reaffirming their role as highly effective first-line treatments for serious infections caused by this organism. However, the study also revealed high levels of resistance to third-generation cephalosporins, notably cefotaxime and ceftriaxone, which underscores the urgency of implementing comprehensive local antimicrobial resistance (AMR) surveillance programs.

The findings also reflect significant demographic trends, with higher prevalence observed among males, adolescents, and individuals residing in urban areas. These insights provide an opportunity to develop targeted interventions for these high-risk groups.

Moving forward, healthcare providers in the region should incorporate these findings into clinical decision-making and prioritise antimicrobial stewardship efforts to avoid the overuse of broad-spectrum antibiotics. Policymakers should support the development of robust local AMR monitoring systems and promote evidence-based antibiotic prescribing practices to help curb the growing threat of drug-resistant *P. mirabilis*. a concerning level of antibiotic resistance among *P. mirabilis* isolates in Anbar Governorate. Key findings include:

High sensitivity to carbapenems: All isolates (100%) were susceptible to meropenem, imipenem, and ertapenem, underscoring their importance as first-line therapy.

Urgent need for AMR surveillance: The high resistance to cephalosporins, especially cefotaxime (100%) and ceftriaxone (73.3%), calls for expanded local surveillance and resistance monitoring programs.

Demographic trends: Infections were more common among males, adolescents, and urban residents, highlighting the need for targeted prevention strategies.

Given these findings, healthcare facilities in the region should adopt antimicrobial stewardship policies and discourage the overuse of broad-spectrum antibiotics, particularly cephalosporins. Policymakers and clinicians must prioritise sustained AMR surveillance, patient education, and adherence to evidence-based prescribing practices to mitigate the spread of resistant *P. mirabilis* strains.

List of Abbreviations

AMR: Antimicrobial Resistance
ATCC: American Type Culture Collection
CLSI: Clinical and Laboratory Standards Institute
ESBL: Extended-Spectrum Beta-Lactamase
LB: Luria-Bertani
UTI: Urinary Tract Infection
WHO: World Health Organisation

Declarations

Ethics Approval and Consent to Participate

The study was approved by the local ethics committee on December 26, 2024, under project number 267. Written and verbal informed consent was obtained from all participants or their legal guardians before sample collection. The study adhered to the principles of the Declaration of Helsinki.

Consent for Publication is not applicable.

Availability of Data and Materials

All data generated or analysed during this study are included in this published article. Further details are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no competing interests.

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Authors' Contributions

HTR: Conceptualisation, Methodology, Data Curation, Formal Analysis, Writing – Original Draft.

ADF: Investigation, Resources, Supervision, Writing – Review & Editing.

ALH: Validation, Visualisation, Project Administration, Writing – Review & Editing.

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