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Original Paper

Antimicrobial resistance and the multiple antibiotic resistance index of *Proteus mirabilis* from diabetic foot infections and a hospital environment: insight from an Algerian diabetology ward

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Fatima Zohra Henni*, Fella Hamaidi-Chergui, Yacine Boutekfa, Faiza Henni

Laboratory of Biotechnology, Environment and Health, Biology Department, University of Blida 1, Algeria

*Corresponding author, E-mail: henni_fatimazohra@univ-blida.dz

Abstract

Proteus mirabilis is an opportunistic pathogen commonly linked to diabetic foot infections and recognized for its persistence in healthcare environments. Its resistance to antimicrobials and ability to form biofilms contribute to treatment challenges and the spread of infections. This study compared clinical and environmental *P. mirabilis* isolates from an Algerian diabetology ward, analyzing their antimicrobial resistance profiles, multidrug resistance index, and biofilm-forming capacity. Over one year, 200 samples were collected 100 from diabetic foot infections and 100 from hospital surfaces. Swab samples underwent enrichment and selective culturing, with identification and confirmation via biochemical tests and an automated microbial system. Antimicrobial susceptibility was tested using disk diffusion, the multiple antibiotic resistance index was computed, and biofilm production was screened via two qualitative methods. Among 64 non-duplicate *P. mirabilis* isolates (39 clinical, 25 environmental), high multidrug resistance was observed, including > 80% resistance to ampicillin/amoxicillin, 68.75% to nalidixic acid, and 82.81% to cefazolin. Environmental isolates exhibited significantly higher multiple antibiotic resistance indices than clinical ones, indicating elevated resistance in hospital surfaces. All isolates formed biofilms. The prevalence of resistant, biofilm-producing *P. mirabilis* in both patients and the environment underscores hospitals as reservoirs for opportunistic pathogens. These results stress the importance of enhanced infection control and environmental surveillance to curb the dissemination of resistant strains in healthcare facilities.

Key words: biofilm, diabetic foot infections, hospital environment, multidrug-resistance, *Proteus mirabilis*.
Abbreviations: AK, amikacin; AMC, amoxicillin–clavulanic acid; AMP, ampicillin; AMX, amoxicillin; ATM, aztreonam; BHIB, Brain Heart Infusion Broth; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; CZ, cefazolin; ESBL, extended-spectrum beta-lactamase; ETP, ertapenem; FEP, cefepime; FOX, cefoxitin; IPM, imipenem; MAR, multiple antibiotic resistance; NA, nalidixic acid; SXT, trimethoprim-sulfamethoxazol.

Introduction

Proteus mirabilis is a Gram-negative, rod-shaped bacterium belonging to the Morganellaceae family. It is well known for its distinctive biological traits and its role as an important opportunistic pathogen in humans. This bacterium is especially recognized for its swarming motility, urease production, and its ability to cause various infections, including urinary tract infections, wound infections, and gastroenteritis (Armbruster et al. 2018; Mobley 2019; Janda, Abbott 2021). Beyond its clinical significance, *P. mirabilis* shows impressive survival skills in the hospital environment, where it can persist on surfaces and medical devices, acting as a reservoir for hospital-acquired infections (Wasfi et al. 2020; Scavone et al. 2023). Its unique swarming behaviour movement across solid surfaces in a coordinated way that forms concentric rings is closely tied to its virulence and its ability to colonize surfaces such as catheters (Armbruster et al. 2018; Mobley 2019; Gmiter, Kaca 2022).

Moreover, *P. mirabilis* is notorious for its high level of antibiotic resistance, which poses a serious challenge in clinical treatment. It carries resistance genes against many antibiotics, including beta-lactams, quinolones, and carbapenems. The rise of extended-spectrum beta-lactamase (ESBL)-producing strains has further complicated therapy for infections caused by this pathogen (Alqurashi et al. 2022; Chakkour et al. 2024; Lian et al. 2024). Its resistance mechanisms include producing

enzymes that modify antibiotics, activating efflux pumps, and mutating porins to reduce antibiotic entry. These resistance traits are often carried on mobile genetic elements like plasmids and integrons, which help spread resistance genes among bacteria (Alqurashi et al. 2022; Liu et al. 2023; Lian et al. 2024). Hospitals, especially high-risk units such as diabetology wards, are key reservoirs for multidrug-resistant pathogens, exposing vulnerable patients to infections from both clinical and environmental sources (Drees et al. 2008; Otter et al. 2011). In this setting, *P. mirabilis* is an increasing concern due to its combination of resistance, biofilm formation, and environmental persistence, all of which aid its spread.

Despite extensive investigation into antimicrobial resistance in clinical isolates of *P. mirabilis*, there remains a significant knowledge gap regarding its environmental prevalence and resistance patterns in North African healthcare settings, particularly in Algeria. This gap is concerning given *P. mirabilis* demonstrated persistence and dissemination in diverse environments, especially poultry-related ecosystems where resistance rates are critically high. Notably, recent research in eastern Algeria reported that *P. mirabilis* constituted 8.71% of enterobacterial isolates from poultry, with all isolates exhibiting multidrug resistance (Kamel et al. 2024). In addition, community-acquired urinary tract infections in Algiers revealed *P. mirabilis* in 13 of 133 positive cases, with 93.98% showing resistance to at least one tested antibiotic (Benmoumou et al. 2023). Furthermore, hospital wastewater studies indicate that 82.76% of *P. mirabilis* strains display multidrug resistance (Nojaya et al. 2022), underscoring the critical role of environmental reservoirs in the persistence and transmission of resistant strains within healthcare environments. Considering the escalating global threat of multidrug-resistant pathogens and the heavy clinical burden presented by infections such as diabetic foot ulcers, elucidating the links between environmental and clinical *P. mirabilis* isolates is essential to inform effective surveillance, control, and intervention strategies in Algeria and the wider North African region.

Therefore, this study aimed to compare the antimicrobial resistance profiles, multiple antibiotic resistance (MAR) index values, and biofilm-forming abilities of *P. mirabilis* strains isolated from diabetic foot infections and the hospital environment in an Algerian diabetology ward. The findings shed light on the hospital environment's potential role as a reservoir for resistant *P. mirabilis* strains and highlight the importance of integrated surveillance to control their spread.

Materials and methods

Samples collection

One hundred non-duplicate samples were collected from various sites and surfaces within the diabetology ward of a public tertiary care hospital located in Blida Province, northern Algeria, over a one-year period. Additionally, one hundred clinical samples were obtained from hospitalized patients, specifically from diabetic wound infections. All samples were collected using sterile swabs soaked in Brain Heart Infusion Broth (BHIB), and transported immediately to the microbiology laboratory under appropriate conditions for further analysis.

Enrichment and preliminary isolation of bacterial strains

The collected samples were incubated at 37 °C for 24 h in BHIB to enrich the bacterial population. Following overnight incubation, tubes exhibiting visible turbidity were identified as positive and subsequently examined using a light microscope. Portions of these turbid cultures were then inoculated onto selective and differential media, such as MacConkey agar, Hektoen enteric agar, and Chromagar, to isolate Gram-negative bacilli. The inoculated plates were incubated aerobically at 37 °C for a period of 24 to 48 h (Wu et al. 2024). Each sample was plated in duplicate (two plates per sample) to confirm growth. All samples were processed within two hours of collection to preserve microbial viability and minimize contamination.

Bacterial identification

Following incubation, bacterial colonies were initially inspected macroscopically to evaluate morphological characteristics such as colony size, shape, pigmentation, and swarming motility. Presumptive identification of *Proteus* species was based on distinctive traits including rapid swarming behavior, a characteristic ammonia-like odour, inability to ferment lactose on MacConkey agar, and hydrogen sulfide (H_2S) production on selective media. Gram staining was then performed for microscopic examination to verify the presence of Gram-negative bacilli exhibiting the typical rod-shaped morphology (Forbes et al. 2016).

Further biochemical characterization was performed using the API 20E system (bioMérieux, France), which facilitated metabolic profiling of the isolates through analysis of carbohydrate fermentation and enzymatic activities. Isolates identified as *Proteus mirabilis* by the API 20E system were subsequently confirmed using the VITEK® 2 Compact automated identification system (bioMérieux), following the manufacturer's protocol. This two-step verification process ensured accurate species-level identification prior to conducting antimicrobial susceptibility testing (Pincus 2010).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was carried out using the standard disc diffusion technique on Muller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI 2023) guidelines. The bacterial suspension was standardized to a 0.5 McFarland turbidity and evenly spread across the agar surface with a sterile swab. Antibiotic discs were applied in the following concentrations:

amoxicillin–clavulanic acid (AMC, 20/10 μg), cefotaxime (CTX, 30 μg), cefepime (FEP, 30 μg), imipenem (IPM, 10 μg), gentamicin (CN, 10 μg), amikacin (AK, 30 μg), ciprofloxacin (CIP, 5 μg), trimethoprim–sulfamethoxazole (SXT, 1.25/23.75 μg), ampicillin (AMP, 10 μg), amoxicillin (AMX, 25 μg), cefoxitin (FOX, 30 μg), cefazolin (CZ, 30 μg), aztreonam (ATM, 30 μg), ertapenem (ETP, 10 μg), nalidixic acid (NA, 30 μg), and chloramphenicol (C, 30 μg). After placing the discs on the inoculated plates, the samples were incubated at 37 °C for 18 to 24 h. The zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints. Each isolate was subjected to three independent antimicrobial susceptibility tests, and the average result was used for data analysis.

Multiple antibiotic resistance index calculation

The multiple antibiotic resistance (MAR) index was calculated based on the averaged results of the three independent tests for each *P. mirabilis* isolate to assess the extent of resistance to the antibiotics tested. The MAR index was computed using the formula:

$$\text{MAR index} = a / b,$$

where a represents the number of antibiotics to which the isolate exhibited resistance, and b is the total number of antibiotics tested against that isolate. A MAR index exceeding 0.2 was interpreted as indicative of contamination from high-risk sources characterized by frequent or inappropriate antibiotic use (Woh et al. 2023). This index was determined separately for isolates obtained from patients and those from the hospital environment, allowing for evaluation and comparison of antibiotic pressure in both contexts.

Biofilm formation assay

Isolates exhibiting a MAR index greater than 0.2 were selected to assess their biofilm-forming capacity using two complementary methods to improve the reliability of the results: the qualitative crystal violet microtiter plate assay and the Congo red agar method.

Biofilm production was assessed qualitatively using a microtiter plate assay based on crystal violet staining. Overnight bacterial cultures were diluted in tryptic soy broth, and 200 μL of the suspension was inoculated into sterile flat-bottom 96-well microplates. The plates were incubated at 37 °C for 24 h without shaking. After incubation, wells were gently washed with phosphate-buffered saline to remove planktonic cells. Subsequently, wells were stained with 0.1% crystal violet solution for 15 min, rinsed with distilled water, and air-dried. Biofilm formation was visually evaluated by the intensity of the violet staining adhering to the bottom and sides of the wells. Based on the depth and uniformity of staining, isolates were categorized as non-biofilm producers, or weak, moderate, or strong biofilm producers (Pui et al. 2017). This test was carried out in triplicate wells per isolate.

Simultaneously, isolates were streaked onto Congo red agar plates (in duplicate) prepared with brain heart infusion agar supplemented with 5% sucrose and 0.08% Congo red dye. After aerobic incubation at 37 °C for 24 to 48 h, colony morphology was examined. Biofilm-producing isolates developed black, dry, and crystalline colonies, whereas non-producers formed smooth red or pink colonies (Valente 2023; Vekatalaxmi 2023).

Ethical approval

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Ethical approval was obtained from the Bioethical Committee of Blida 1 university -Algeria and the Health Authorities of the Public Tertiary Care Hospital, Blida, Algeria. No formal registration number was issued, as the local ethics committees generally provide approval letters without numbering.

Statistical analysis

Statistical analysis was performed using SPSS version26. Categorical variables were compared using the *Chi*-square test or Fisher's exact test when appropriate. A p-value less than 0.05 was considered statistically significant.

Results

Isolation and identification of Proteus mirabilis

A total of 200 samples were collected throughout the study, comprising 100 samples from various environmental surfaces within the diabetology ward and 100 samples from hospitalized patients with diabetic wound infections. From these, 64 *P. mirabilis* isolates were obtained, yielding an overall isolation rate of 32%. Specifically, *P. mirabilis* was recovered from 25% of environmental samples and 39% of clinical samples (Table 1).

Initial identification of the isolates was performed based on colony morphology, swarming motility, and biochemical traits, followed by confirmation with the API 20E system. Definitive species-level identification was then confirmed using the VITEK® 2 Compact automated system. All 64 isolates were preserved for subsequent phenotypic analyses, which included antibiotic susceptibility testing, calculation of the MAR index, and evaluation of biofilm formation.

Antibiotic susceptibility test result

To evaluate the antibiotic resistance patterns of the isolated *P. mirabilis* strains, all 64 isolates were tested against a panel

Table 1. Distribution of positive *P. mirabilis* samples

Origin	Number of samples	Isolated *P. mirabilis*	
		Number	%
Hospital environment	100	25	25
Clinical samples	100	39	39

Table 2. Antibiotic resistance rates of *P. mirabilis* isolates

Antibiotic	Code	Clinical resistance (%)	Environmental resistance (%)	Overall resistance %
Amoxicillin clavulanic acid	AMC	85.00	80	82.81
Ampicillin	AMP	69.23	80	73.43
Amoxicillin	AMX	85.00	80	82.81
Aztreonam	ATM	36.00	80	53.13
Trimethoprim-sulfamethoxazole	SXT	61.50	60	61.00
Cefotaxime	CTX	28.20	80	48.43
Amikacin	AK	10.26	38	17.19
Gentamicin	CN	25.64	64	40.63
Ciprofloxacin	CIP	51.28	16	37.50
Cefoxitin	FOX	48.71	84	33.10
Nalidixic acid	NA	59.00	84	68.75
Cefazolin	CZ	79.48	88	82.81
Chloramphenicol	C	46.15	44	45.31
Imipenem	IMP	5.12	16	9.38
Ertapenem	ETP	0	0	0
Cefepime	FEP	0	0	0

of commonly used antimicrobial agents. This assessment aimed to determine the degree of resistance present in both clinical and environmental isolates and to identify potential multidrug-resistant strains. The antibiotic susceptibility profiles of the 64 *P. mirabilis* isolates, comprising 39 clinical and 25 environmental strains, are summarized in Table 2.

Overall, a high prevalence of resistance was observed for several antibiotics, particularly among β-lactams. Resistance rates were notably elevated for amoxicillin-clavulanic acid and amoxicillin, each at 82.81%, and ampicillin at 73.43%. Additionally, resistance to nalidixic acid and cefazolin was significant, with 68.75 and 82.81% of isolates resistant, respectively. Conversely, all isolates remained fully susceptible to ertapenem and cefepime, while resistance to imipenem was relatively low at 9.38%. Comparative analysis revealed that environmental isolates exhibited slightly higher resistance rates than clinical isolates for certain antibiotics, including aztreonam (80 vs. 36%), nalidixic acid (84 vs. 59%), and gentamicin (64 vs. 26%).

When analyzing the resistance profiles based on antibiotic classes, β-lactam antibiotics including penicillins (ampicillin, amoxicillin), β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanic acid), and cephalosporins (cefoxitin, cefazolin, cefotaxime) exhibited notably high resistance rates among both clinical and environmental isolates.

In contrast, carbapenems such as ertapenem and cefepime showed no detected resistance, suggesting that these antibiotics remain effective against *P. mirabilis* isolates in this setting. Aminoglycosides, fluoroquinolones, and other antibiotic classes displayed variable resistance rates, with environmental isolates often exhibiting higher resistance levels compared to clinical isolates.

Resistotype distribution

Analysis of antimicrobial resistance profiles identified multiple distinct resistotypes among both clinical and environmental *P. mirabilis* isolates. Resistotypes were classified based on the specific combinations of antibiotics to which each isolate demonstrated resistance. The distribution of the predominant resistotypes in clinical and environmental isolates is summarized in the following tables.

Among the environmental *P. mirabilis* isolates, eight unique resistotypes were detected. Several complex multidrug-resistant patterns were recurrent, especially those involving resistance to β-lactams, aminoglycosides, and fluoroquinolones. Overall, the environmental isolates frequently exhibited intricate multidrug-resistant profiles, indicating possible clonal spread within the hospital setting (Table 3).

A total of nine distinct resistotypes were identified among the clinical *P. mirabilis* isolates. The most common resistotypes included resistance to β-lactams (AMC, AMP, AMX), nalidixic acid, and cefazolin, with some patterns detected in multiple isolates. Overall, the majority of clinical isolates exhibited unique or less frequent resistance patterns, highlighting the diversity of antimicrobial resistance within patient-derived strains (Table 4).

Multiple antibiotic resistance (MAR) index

The MAR index was used to assess the level of multidrug resistance among both clinical and environmental *P. mirabilis* isolates. Clinical strains exhibited MAR index values ranging from 0.00 to 0.75, with an average value of 0.43. In comparison, environmental strains showed MAR index values between 0.19 and 0.75, with a higher mean MAR index of 0.53. Statistical analysis revealed a significant

Table 3. Resistotype distribution of environmental strains

Resistotype	Antimicrobial resistance profile	Number of isolates (%)
I	AMX/AMC/AMP/CZ/NA	5 (20)
II	AMX/AMC/AMP/CZ/NA/AK	4 (16)
III	AMX/AMC/AMP/CZ/NA/AK/CIP	3 (12)
IV	AMX/AMC/AMP/CZ/NA/AK/CIP/GN	2 (8)
V	AMX/AMC/AMP/CZ/NA/AK/CIP/GN/CTX	2 (8)
VI	AMX/AMC/AMP/CZ/NA/AK/CIP/GN/CTX/IPM	1 (4)
VII	AMX/AMC/AMP/CZ/NA/AK/CIP/GN/CTX/IPM/C	1 (4)
VIII	AMX/AMC/AMP/CZ/NA/AK/CIP/GN/CTX/IPM/C	1 (4)
IX	Other unique patterns	5 (20)

Table 4. Resistotype distribution of clinical strains

Resistotype	Antimicrobial resistance profile	Number of isolates (%)
I	AMC/AMP/AMX/NA/CZ	6 (15.4)
II	AMC/AMP/AMX/CTX/NA/CZ	4 (10.3)
III	AMC/AMP/AMX/NA/CZ/COT	4 (10.3)
IV	AMC/AMP/AMX/NA/CZ/AK/CIP	3 (7.7)
V	AMC/AMP/AMX/CTX/NA/CZ/CN	2 (5.1)
VI	AMC/AMP/AMX/NA/CZ/COT/AK/CIP/CN	2 (5.1)
VII	AMC/AMP/AMX/NA/CZ/CTX/CIP/CN/COT/AK	2 (5.1)
VIII	AMC/AMP/AMX/NA/CZ/CTX/CIP/CN/COT/AK/IPM	1 (2.6)
IX	AMC/AMP/AMX/NA/CZ/CTX/CIP/CN/COT/AK/IPM /C	1 (2.6)
X	Other unique patterns	14 (35.9)

difference in MAR index values between clinical and environmental *P. mirabilis* isolates ($p < 0.05$). This result confirms that environmental strains exhibited significantly higher levels of multidrug resistance compared to clinical strains (Table 5).

Biofilm formation assay

All *P. mirabilis* isolates included in this study were qualitatively evaluated for their biofilm-forming ability using two established methods: the Congo red agar assay and the microplate-based tube assay. The findings demonstrated that all isolates (100%) showed biofilm formation by at least one of these techniques.

Discussion

In this study, *P. mirabilis* was isolated from both clinical and environmental samples collected within the diabetology ward of an Algerian hospital. Notably, 39% of the clinical samples tested positive for *P. mirabilis*, underscoring its significant role in diabetic foot infections. Diabetic patients are particularly vulnerable to such infections due to impaired immune responses and delayed wound healing. Our findings are consistent with those of Jaber and Almiyah (2023) in Iraq, who reported a 32% prevalence of *P. mirabilis* in diabetic foot infections. Both studies emphasize the clinical prominence of this pathogen in diabetic wounds.

P. mirabilis expresses several virulence factors like urease, hemolysins, and proteases, which promote tissue destruction and biofilm formation, complicating infection management (Armbruster, Mobley 2012). The high prevalence in our clinical isolates suggests that *P. mirabilis* significantly contributes to the worsening of diabetic foot infections, emphasizing the need for targeted antimicrobial therapies and specialized wound care.

Additionally, *P. mirabilis* was recovered from 25% of environmental samples (hospital surfaces and equipment), confirming its ability to persist in healthcare environments and act as a potential reservoir for nosocomial infections. This aligns with Drzewiecka (2016), who showed that *P. mirabilis* can form biofilms on abiotic surfaces, enhancing

Table 5. MAR index results

Isolates	Mean	Minimum	Maximum
Hospital environment	0.53	0.19	0.75
Clinical	0.43	00	0.75

resistance to disinfectants. Environmental contamination raises the risk of cross-transmission, especially in settings with suboptimal hygiene practices, and poses a heightened threat to diabetic patients with prolonged hospital stays or frequent exposure to invasive devices.

Regionally, *P. mirabilis* is also prevalent in North African healthcare settings. In Egypt, for instance, this species represented up to 10% of clinical isolates among the *Proteus-Morganella-Providencia* group, with 5.5% isolated from urine samples and a 20.7% prevalence at Mansoura University Hospitals (El Tweel et al. 2024). Similar trends were observed in Morocco, particularly in ICUs, where up to 14% of hospital equipment was contaminated with carbapenem-resistant strains (Lahlou et al. 2023)

Our findings reveal alarmingly high resistance rates to β-lactams, especially amoxicillin-clavulanic acid (82.81%), amoxicillin (82.81%), and ampicillin (73.43%). These rates exceed WHO's 2020 global averages, where approximately 20% of *E. coli* strains from UTIs showed resistance to commonly used antibiotics (WHO 2023). The failure of amoxicillin-clavulanic acid designed to counteract β-lactamase activity suggests the involvement of more advanced resistance mechanisms such as extended-spectrum β-lactamases and AmpC β-lactamases (Paterson, Bonomo 2005; Angelis et al. 2020). The high resistance to cefazolin (82.81%) further supports this hypothesis, as first-generation cephalosporins are highly susceptible to hydrolysis by these enzymes.

These resistance trends are likely driven by the widespread and often unregulated use of β-lactam antibiotics in healthcare settings, which exerts selection pressure favouring resistant strains, particularly on contaminated surfaces.

In contrast, all isolates in our study remained fully susceptible to ertapenem, and only 9.38% showed resistance to imipenem. This contrasts with the rising trend of carbapenem-resistant Enterobacterales in other regions, as highlighted in the 2024 treatment guidelines for multidrug-resistant Gram-negative infections (Meletis 2016; Wise et al. 2023). The preserved susceptibility in our isolates indicates that mechanisms such as carbapenemase production or porin loss combined with β-lactamase activity are not yet widespread in our study population.

A key finding was the differential resistance pattern between environmental and clinical isolates. Environmental strains showed higher resistance to aztreonam (80 vs. 36%), nalidixic acid (84 vs. 59%), and gentamicin (64 vs. 26%). This aligns with the findings of Hua et al. (2020), who highlighted that environmental source are important reservoirs of resistance genes. These environments often expose bacterial populations to sub-inhibitory concentrations of antimicrobials, promoting the persistence and spread of resistance determinants (Larsson, Flach 2022).

Antimicrobial resistance in *P. mirabilis* isolates from North Africa is alarmingly high. Egyptian studies reported 73.33% multidrug resistance and 9.1% extensive drug resistance, with high resistance rates to ceftazidime (83.7%), nitrofurantoin (81%), and cefazolin (78%). Carbapenem susceptibility remained moderate (60 to 71%). Resistance was mainly associated with extended-spectrum β-lactamases (detected in 57.6% of isolates), carbapenemase genes such as *blaOXA-48* and *blaNDM-1*, and plasmid-mediated quinolone resistance. Integrons present in over 86% of isolates play a major role in horizontal gene transfer and resistance gene dissemination (El Tweel et al. 2024).

The MAR index values observed in this study provide crucial insights into the multidrug resistance patterns of *P. mirabilis* isolates. MAR indices > 0.2 indicate that isolates likely originate from areas of high antibiotic use or a high-risk source, making both our clinical (mean = 0.43) and environmental (mean = 0.53) isolates of significant concern from a public health perspective. These findings suggest that the hospital environment may act as a significant reservoir for highly resistant *P. mirabilis* strains. The elevated MAR index values among environmental isolates reflect a selective pressure likely due to the frequent use or misuse of antibiotics within healthcare settings. The MAR index range of 0.00 to 0.75 observed in clinical strains, with an average of 0.43, indicates substantial multidrug resistance within the clinical population. This finding aligns with recent studies on *P. mirabilis* clinical isolates where 73.33% of isolates were classified as multidrug resistant, consistent with our observations that the majority of clinical isolates exceeded the critical 0.2 threshold (Salama et al. 2021; Salama et al. 2025).

The detection of strains with MAR index values exceeding 0.2 in both groups emphasizes the urgent need for strict infection control policies and continuous antimicrobial resistance monitoring, not only among patients but also in the hospital environment.

All *P. mirabilis* isolates recovered in this study exhibited the ability to form biofilms, which is consistent with the well-documented biofilm-forming capacity of this species. Biofilm formation in *P. mirabilis* is a multifactorial process driven by several virulence mechanisms. First, the bacterium expresses a range of adhesion factors, including fimbriae, outer membrane proteins, and lipopolysaccharides, which promote initial attachment to both biotic and abiotic surfaces (Czerwonka et al. 2016; Wasfi et al. 2020). Moreover, the presence of flagella enhances swarming motility, facilitating surface colonization and promoting biofilm maturation, although biofilm formation can still occur even in the absence of functional flagella (Khanduri 2023; Scavone et al. 2023). Another critical factor is urease production, which contributes to biomineralization and the development of crystalline biofilms, particularly relevant in clinical settings such as catheter-associated infections (Wasfi et al. 2020). The ability of all isolates to form biofilms may also explain the high rates of antimicrobial resistance observed, as biofilm-embedded bacteria are known to exhibit significantly increased tolerance to both antibiotics

and the host immune response (Wasfi et al. 2020). These findings highlight the clinical importance of biofilm formation in *P. mirabilis* and emphasize the need for innovative therapeutic strategies, including anti-virulence approaches and bacteriophage therapy, to effectively combat biofilm-associated infections (Wasfi et al. 2020; Khanduri 2023).

Conclusions

This study underscores the critical role of the hospital environment in the dissemination of antimicrobial-resistant bacteria. The detection of *Proteus mirabilis* in both clinical (39%) and environmental (25%) samples from the diabetology ward highlights the ability of this opportunistic pathogen to survive on hospital surfaces and potentially contribute to infections. The high prevalence of multidrug-resistant isolates is particularly concerning, with over 80% of strains displaying resistance to key β-lactam antibiotics such as ampicillin and amoxicillin. Elevated resistance rates were also observed for nalidixic acid (68.75%) and cefazolin (82.81%), while MAR index analysis confirmed significantly higher levels of multidrug resistance among environmental isolates. The universal detection of biofilm-forming *P. mirabilis* strains within the hospital environment raises additional concerns regarding the persistence of these bacteria on inadequately disinfected surfaces and their potential role in infecting vulnerable patients. These findings reinforce the importance of incorporating environmental surveillance and control strategies as essential components of antimicrobial resistance management. Reducing the environmental spread of resistant bacteria is vital to limiting their clinical impact and preventing their dissemination beyond the hospital setting.

In addition, by incorporating available data from North African countries, this study fulfills its initial aim of situating Algerian findings within a broader regional context. Although information from neighboring countries remains scarce, the results presented here provide a valuable baseline for future regional surveillance and comparative analyses, thereby contributing to a better understanding of antimicrobial resistance trends across the region.

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