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Co-Resistance of Antibiotics and Heavy Metals in *Proteus mirabilis* Isolated from Hospital Environments

A thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَأَنْ لَّيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَى ﴿٣٩﴾
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Supervisors Certification

I certify that this thesis entitled “**Co-Resistance of Antibiotics and Heavy Metals in *Proteus mirabilis* Isolated from Hospital Environments**” had been carried out under my supervision at the University of Al-Qadisiya / College of Science / Department of Environmental Sciences in partial fulfillment of the requirements for obtaining a Master degree in Ecology

Supervisor Signature

Prof. Dr Aamal Ghazi Mahdi Al-Saadi

Date / /2026

Dedication

To Allah, the Almighty, the Eternal Creator, Originator of the heavens and the earth, and the One who governs this universe with perfect wisdom and absolute perfection.

To the Prophet of Mercy, Muhammad peace be upon him and his family

To the symbol of sacrifice and generosity, (My beloved father)

To my first teacher, who has always given me encouragement, happiness, and hope, and whose prayers have never ceased,(My dear mother)

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To all who were there for me, thank you for your help and encouragement , I dedicate this simple work.

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Summary

Proteus spp were Gram-negative opportunistic pathogens and were considered one of the important causes of nosocomial infections, particularly in hospitalized patients. This study aimed to investigate the prevalence of *Proteus spp.* in hospital environments, assessing their resistance to selected antibiotics and tolerance to heavy metals, and examining the potential association between antibiotic resistance and heavy metal tolerance. A total of 250 environmental and clinical samples were collected from three hospitals in Babylon Province during the period from September 2025 to January 2026. These hospitals included (Al-Hilla Teaching Hospital, Marjan Teaching Hospital, and Imam Al Sadiq Hospital). The collected samples were divided into 150 clinical specimens and 100 environmental samples. The clinical specimens included burns, wounds, and urinary tract infection samples. Burn and wound swabs were collected from the depth of the infected sites using standard aseptic procedures. Meanwhile, the environmental samples included 55 hospital wastewater samples and 45 swabs from different hospital surfaces and objects, such as patient beds, medical equipment, waste containers, tabletops, healthcare workers' hands, sinks, walls, taps, door handles, floors, countertops, nurses' workbenches, showers, handrails, bedside tables, oxygen cylinders, stethoscopes, medical trays, soap or hand-gel containers, and meal tables. All samples were collected carefully to prevent contamination during transportation and laboratory processing. The samples were cultured on MacConkey agar, blood agar, and nutrient agar, and bacterial isolates were identified using conventional biochemical tests and the VITEK 2 compact automated system. Antimicrobial susceptibility was determined using the minimum inhibitory concentration (MIC) method, while heavy metal resistance was evaluated using a microtiter plate assay based on MIC and IC₅₀ values. Molecular detection of antibiotic resistance genes was performed using Polymerase chain reaction

(PCR). The investigated genes included *acrA*, *acrB*, *blaTEM*, and *blaCTX-M*. Out of the 150 clinical samples, 90 samples showed bacterial growth, representing 60% of the total clinical specimens, while 60 samples showed no bacterial growth, representing 40%. Among the positive bacterial cultures, 20 isolates were identified as *Proteus mirabilis*, accounting for 13.3% of the total clinical samples. The highest proportion of *P. mirabilis* isolates was detected in urinary tract infection samples, with 13 isolates representing 65%, followed by wound samples with 4 isolates representing 20%, and burn samples with 3 isolates representing 15%.

In contrast, no *Proteus spp.* isolates were detected among the 100 environmental samples. However, several other bacterial species were recovered from these environmental specimens. The most frequently isolated species was *Pseudomonas aeruginosa* (25 isolates), *Staphylococcus spp* (15 isolates), *klebsiella spp* (12 isolates), *Salmonella spp* (10 isolates), *Citrobacter amalonaticus* (8 isolates), *Shigella spp* (7 isolates), *Aeromonas sobria* (6 isolates), *Moraxella spp* (5 isolates), *Myroides spp* (4 isolates), *Brevundimonas diminuta* (3 isolates), *Campylobacter spp* (2 isolates), *Oligella ureolytica* (3 isolates).

Antibiotic susceptibility testing revealed variable resistance patterns among the tested *P. mirabilis* isolates. The isolates showed a resistance rate of (36.8%) against cefotaxime, ceftazidime, and cefepime. A very high resistance rate was observed against tigecycline, reaching (100%). Resistance to avibactam, imipenem, and trimethoprim was recorded at (26.3%), while resistance rates to ciprofloxacin, ampicillin, and ceftolozane were (21.1%, 15.8%, and 10.5%), respectively. In addition, colistin showed no detectable activity against the tested *P. mirabilis* isolates. The MIC results confirmed high levels of resistance to several antibiotics, particularly tigecycline, cefotaxime, ceftazidime, and cefepime. Tigecycline MIC values ranged from (2 to 4 µg/mL), while

cefotaxime and ceftazidime MIC values ranged from (0.25 to 64 µg/mL and 0.12 to 64 µg/mL), respectively. Cefepime MIC values ranged from (0.12 to 16 µg/mL). The results demonstrated that the efflux pump-associated genes *acrA* and *acrB* were detected in all tested isolates, representing (100%), while β lactamase genes, *blaTEM* and *blaCTX-M* were detected in 7 isolates, representing (71.4%).

Regarding heavy metal tolerance test, the results showed that zinc had the highest tolerance level among the tested heavy metals, with MIC values ranging from (2.5 to 10 mg/mL) and IC₅₀ values ranging from (0.90 to 3.90 mg/mL). Chromium showed moderate tolerance, with a constant MIC value of (2.5 mg/mL) and IC₅₀ values ranging from (1.45 to 2.12 mg/mL). Copper showed lower resistance, with MIC values ranging from (0.62 to 2.5 mg/mL) and IC₅₀ values ranging from (0.31 to 0.86 mg/mL). Cadmium exhibited an MIC value of (1.25 mg/mL), with IC₅₀ values ranging from (0.347 to 0.726 mg/mL).

These findings indicate the coexistence of antibiotic resistance and heavy metal tolerance among clinically isolated *P. mirabilis*. This coexistence may contribute to the persistence and adaptation of resistant bacteria in hospital environments. Therefore, the results emphasize the need for stricter control of heavy metal discharge and continuous monitoring of both antibiotic and heavy metal resistance determinants in clinically important bacterial species.

List of Content

Section	Subject	Page no.
	Abstract	I
	List of content	IV
	List of Tables	VIX
	List of Figures	XII
	List of Abbreviation	XVI
Chapter One :Introduction		
1.1.	Introduction	1
1.2.	The aim of the study	5
Chapter Two : Literatures Review		
2.1.	History of <i>Proteus ssp</i>	6
2.2.	Environmental Roles and Application of the <i>Proteus ssp</i> .	7
2.3.	Clinical significance of <i>Proteus</i>	8
2.4.	Laboratory identification of <i>Proteus mirabilis</i>	9
2.5.	Characteristic of <i>Proteus ssp</i>	10
2.6.	Classification of <i>Proteus ssp</i>	12
2.7.	Pathogenesis	13
2.8.	Epidemiology of <i>P. mirabilis</i>	14

2.9.	Virulence Factors of <i>Proteus ssp</i>	14
2.9.1	Adhesion and biofilm formation	14
2.9.2	Motility	17
2.9.3	Urease Production	19
2.9.4	Lipopolysaccharide (LPS)	20
A-	O-antigens	20
B-	Core oligosaccharide	21
C-	Lipid A	21
2.9.5	Resistance to antibiotics	22
2.9.6	Fimbriae	25
2.9.7	Proteases	27
2.9.8	Hemolysin	27
2.10.	Genomics of <i>Proteus</i>	30
2.11.	Heavy metals resistance of <i>Proteus ssp</i>	32
2.12.	Metal acquisition system of <i>P. mirabilis</i>	33
2.13.	Unraveling <i>P. mirabilis</i> metallophores and their role in pathogenesis	34
2.14.	Mechanisms of heavy metal resistance by <i>proteus ssp</i>	35
2.14.1.	Extracellular permeability barrier	35
2.14.2.	Active transport by Efflux pump	36
2.14.3	Extracellular sequestration or precipitation	37

2.14.4.	Interacellular sequestration or accumulation	37
2.15.	Toxic Impacts of the heavy metal on the bacteria	38
2.16.	Heavy metal and Antibiotic resistance	38
Chapter Three :Materials & Methods		
3.1.	Materials	42
3.1. 1.	Apparatuses and equipment	42
3.1.2.	Chemical Materials	45
3.1.3.	culture Media	47
3.1.4.	Antibiotic disk	48
3.1.5	DNA amplification materials	49
3.1.5.1	Molecular kits	49
3.1.6	Primers	50
3.2	Methods	51
3.2.1	Study plan	51
3.3.	Preperation of the culture Media	52
3.3.1	MacConkey agar	52
3.3.2.	Nutrient broth	52
3.3.3.	Brain heart infusion agar	52
3.3.4	Brain Heart infusion broth	53
3.3.5.	Blood agar medium	53
3.3.6	Tryptic Soy broth	53
3.3.7	Peptone water medium	53
3.3.8.	MR-VP medium	54
3.3.9	Simmons' citrate medium	54

3.3.10	Kligler Iron agar medium	55
3.3.11	Urea agar medium	55
3.3.12	Gelatin liquefaction	56
3.3.13	Luria-Bertani broth (LB)	56
3.3.14	Luria-Bertani agar (LB)	56
3. 4	Samples collection	57
3.5	Identification of bacterial isolates	57
3.5.1	Morphological Characteristics	57
3.5.2.	Microscopic Characteristics	58
3.5.3	Biochemical identification	58
3.5.3.1	Catalase test	58
3.5.3.2	Oxidase test	58
3.5.3.3	Methyl-red test	59
3.5.3.4	Voges-proskauer test	59
3.5.3.5	Citrate utilization test	59
3.5.3.6	Gelatinase test	60
3.5.3.7	Urease test	60
3.5.3.8	Kligler Iron Agar test	60
3.5.4	Identification by vitek® 2 compact	61
3.6	Virulence factors assay	62

3.6.1	Haemolysin production	62
3.6.2	Urease production	62
3.7	Sterilization methods	62
3.8	Maintenance of Bacterial Isolates	63
3.8.1	Short Term Maintenance	63
3.8.2	Long Term Storage	63
3.9	Antibiotic sensitivity test	63
3.10	Colony count	64
3.11	Preparation of Standard Heavy Metal Solutions	65
3.12	Metal resistance profiles	65
3.13	Determination of IC ₅₀ Using Bacterial Viability Dose–Response Curves	66
3.14	Molecular detection of	67
3.14.1.	DNA Extraction	67
3.14.2.	Preparation of PCR primers	68
3.15	Amplification of antibiotic resistance genes in <i>p. mirabilis</i>	68
3.15.1.	Detection of PCR products by agarose gel electrophoresis	71
3.16	Statistical analysis	72
Chapter Four :Results & Discussion		
4.1	Isolation and identification of <i>Proteus ssp</i>	72
4.1.1	Clinical samples	72
4.1.2	Environmental samplers	78

4.2.	Identification of <i>Proteus ssp</i>	82
4.2.1	Cultural Features of <i>Proteus ssp</i>	82
4.2.2	Microscopic Examination of bacterial isolates	84
4.2.3	The biochemical Tests	84
4.2.3.1	Traditional Biochemical tests	84
4.2.3.2	Results of Vitek2 system	85
4.3	Antibiotic susceptibility test of <i>Proteus mirabilis</i> .	87
4.4	Minimum Inhibitory Concentrations (MIC) of Antibiotics Against Clinical Isolates	91
4.5	Molecular Detection of Antibiotic Resistance Genes	94
4.5.1	Detection of <i>acrA</i> Gene	94
4.5.2	Detection of <i>acrB</i> Gene	95
4.5.3	Detection of <i>TEM</i> Gene	96
4.5.4	Detection of <i>CTX-M</i> Gene	97
4.6	Testing of heavy metals tolerance of <i>proteus mirabilis</i>	100
4.6.1	chromium resistant bacteria	100
4.6.2	cadmium resistant bacteria	103
4.6.3	copper resistant bacteria	106

4.6.4	zinc resistant bacteria	108
References		117
Appendix (1): VITEK 2 Identification Reports for Environmental Samples.		A-1
Appendix (2) : Colony-Forming Unit (CFU/mL) Results of Hospital Wastewater Samples.		A-13
Appendix (3): VITEK 2 Compact Identification Reports of <i>Proteus mirabilis</i> Clinical Isolates.		A-14
الخلاصة		١

List of Tables

No. of table	Subject	Page no.
(2-6)	showing Scientific classification of <i>Proteus mirabilis</i>	12
(3- 1)	The apparatuses and equipment employed in the current investigation.	42
(3-2)	chemical materials used in this study	45
(3-3)	The culture media used in this study	47
(3- 4)	The Antibiotic disks employed in the current investigation	48
(3- 5)	Kits for molecular detection	49
(3- 7)	Primers, sequences and size.	50
(3- 8)	Preparation of PCR reaction mixture.	68

(3-9)	Program of PCR for blaTEM gene	69
(3-10)	Program of PCR for blaCTX-M gene	69
(3-11)	Program of PCR for (acrA,aceB) gene	70
(4-1)	Number and percentage of bacteria that isolated from clinical cases	72
(4-2)	Other bacterial species recovered from clinical samples	72
(4-3)	Number and percentages of <i>Proteus spp</i> in different clinical samples.	73
(4-4)	Distribution of <i>Proteus</i> -positive cases according to age groups	74
(4-5)	Distribution of <i>Proteus</i> -positive cases according to Sex	75
(4-6)	Bacterial species isolated from environmental samples	79
(4-7)	The of biochemical test used to identify <i>proteus mirabilis</i>	85
(4- 8)	Antimicrobial susceptibility rates among <i>Proteus mirabilis</i> isolated from clinical samples (n= 19)	87
(4-9)	MIC Values of Selected Antibiotics Against Clinical Isolates	92
(4-10)	MIC and IC50 value for Cr of different isolate <i>p. mirabilis</i> isolates	101
(4-11)	MIC and IC50 value for Cd of <i>p.mirabilis</i> isolates	104
(4-12)	MIC and IC50 value for Cu of different isolate	106
(4-13)	MIC and IC50 value for Zn <i>p.mirabilis</i> isolates	109

List of Figures

No. of figure	Subject	Page no.
(2- 9)	virulence factors of <i>Proteus mirabilis</i> .	30
(3- 1)	Chart flow of study plan	51
(4- 1)	Characteristics of <i>proteus</i> colonies on A: MacConkey agar B: Blood agar	83
(4-2)	Gram stain for bacterial cells for gram-negative bacteria.	84
(4-3)	Agarose gel electrophoresis of PCR products (256bp) obtained by using <i>acrA</i> specific primers. lanes 1-10 represent the clinical <i>proteus</i> isolates, Lane M represent DNA ladder.	95
(4-4)	Agarose gel electrophoresis of PCR products (432bp) obtained by using <i>acrB</i> specific primers. lanes 1-10 represent the clinical <i>proteus</i> isolates, Lane M represent DNA ladder	96
(4-5)	Agarose gel electrophoresis of PCR products (500 bp) obtained using TEM specific primers. Lanes 1, 2, 3, 6, and 7 represent positive bacterial isolates, whereas lanes 4 and 5 represent negative isolates. Lane M represents the DNA ladder.	97
(4-6)	Agarose gel electrophoresis of PCR products (600 bp) obtained using CTX-M specific primers. Lanes 1, 2, 3, 6, and 7 represent positive bacterial isolates, whereas lanes 4 and 5 represent	98
(4-7)	MIC and IC50 value for Cr of <i>p. mirabilis</i> is isolates	102
(4-8)	Dose–response curves showing cell viability of isolates across a concentration gradient of Cr (0–20 mg/mL)	103

(4-9)	MIC and IC50 value for Cd of <i>p.mirabilis</i> isolates	105
(4-10)	Dose–response curves showing cell viability of isolates across a concentration gradient of Cd (0–20 mg/mL)	105
(4-11)	MIC and IC50 value for Cu of <i>p.mirabilis</i> isolates	107
(4-12)	Dose–response curves showing cell viability of isolates across a concentration gradient of Cu (0–20 mg/mL)	108
(4-13)	MIC and IC50 value for Zn <i>p.mirabilis</i> isolates	110
(4-14)	Dose–response curves showing cell viability of isolates across a concentration gradient of Zn (0–20 mg/mL)	110

List of Abbreviation

Symbol	Description
CLSI	Clinical and Laboratory Standards Institute
<i>P.mirabilis</i>	<i>Proteus mirabilis</i>
MDR	Multidrug Resistance
VITEK 2 system	vital index of traditional environmental knowledge
UTIs	Urinary tract infection
TSA	Tryptic soy agar
µm	Micrometre
µL	Microlitre
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
WHO	World Health Organization
UV	Ultraviolet
bp	Base pair
CFU	High colony forming unit
MR	Methyl Red
ROS	Reactive Oxygen Species
NCBI	National Center for Biotechnology Information
ATB	Antibiotic
ARGs	Antibiotic-Resistant Genes
HEs	Hospital Effluents
HWW	Hospital Wastewater

Chapter One :Introduction

1.1.Introduction

Species of the genus *Proteus* were classified within the family Morganellaceae (Muhsal *et al.*, 2025). All species were motile, lactose-negative, urease-producing, Gram-negative, rod-shaped bacteria that differentiated from ordinary enterobacterial bacilli into very elongated rods adorned with many flagella, resulting in swarming colonies (Bennett *et al.*, 2019; Armbruster *et al.*, 2012; Schaffer *et al.*, 2017). They were prevalent in nature and were found in surface water, wastewater, soil, on crops, and among the decomposing flora of animal organic matter (Ahmad *et al.*, 2016).

They inhabit the skin and mucous membranes as saprotrophic organisms. also reside in the human and animal digestive tract as part of the normal microbiota (Hurst, 2019) .

The genus *Proteus* comprises many species, specifically: *Proteus alimentorum*, *Proteus cibarius*, *Proteus columbae*, *Proteus faecis*, *Proteus hauseri*, *Proteus mirabilis*, *Proteus penneri*, *Proteus terrae*, and *Proteus vulgaris* (Dai *et al.*, 2019). Despite the several species under the genus *Proteus*, the most clinical strains identified are *Proteus mirabilis* and *Proteus vulgaris*. The quantity and variety of organisms within the genus *Proteus* fluctuate according on their habitat (Drzewiecka, 2016).

Limited research has concentrated on *Proteus* species extracted from aquatic ecosystems. These settings facilitate the development of antibiotic resistance in microorganisms. Souna *et al.*,(2011) asserts that consistent evaluation of the sensitivity of major bacterial species to commonly used antibiotics is crucial.

Hospital wastewater (HWW) significantly differs from wastewater emitted by other sources and is both harmful and contagious. It encompasses a broad spectrum of various micro- and macropollutants released by surgical rooms,

wards, labs, laundry facilities, polyclinics, research units, radiology departments, and the medical and nutritive solutions used in microbiology laboratories (Al-Enazi, 2017).

The Hospital wastewater presents a significant hazard to people regarding the transmissibility and rapid proliferation of infectious illnesses inside healthcare facilities, society, hospital staff, and the environment (Akin, 2017). Various solvents, medicines, and radionuclides are used in hospitals for diagnosis, disinfection, and research purposes. Following medication use, several substances remain nonmetabolized or are partly assimilated in the human body, subsequently being excreted unchanged and into wastewater (Ram *et al.*, 2020).

The World Health Organization has declared that antibiotic resistance is on the growing, and we are rapidly depleting our therapeutic alternatives (Barancheshme *et al.*, 2018). In Europe, 20–30% of hospitalized patients get antibiotic (ATB) therapy throughout their stay. This establishes favorable circumstances for the emergence of antimicrobial resistance owing to significant selection pressure. Furthermore, harmful microorganisms might spread throughout the hospital via healthcare professionals. Antibiotic-resistant bacteria (ARB) may spread from these institutions via sick patients and the sewage system. The problem may be exacerbated when effluents from healthcare institutions are dumped straight into the sewer network without previous treatment. Sewerage networks gather wastewater from houses as well as hospitals, mental clinics, retirement homes, and other healthcare institutions, which are significant sources of pharmaceuticals. Hospital effluents (HEs) are classified as a distinct category due to their very dangerous and poisonous nature. These categories of wastewater include a mixture of antibiotics, disinfectants, metabolized pharmaceuticals (Mackul'ak *et al.*, 2015) and both sensitive and resistant microorganisms from hospitalized patients (Le *et al.*, 2016). Antibiotics at subinhibitory concentrations found in wastewater function

as signaling molecules and regulatory agents, promoting horizontal gene transfer, mutagenesis, and the DNA repair response in bacteria, as well as facilitating the selection of resistant bacterial strains (Jutkina *et al.*, 2016). This renders HEs crucial and influential contributors to the proliferation and advancement of ATB resistance in wastewater and the environment.

Hospital wastewaters were intricate effluents comprising antibiotic compounds, metabolized pharmaceuticals, disinfectants (Emmanuel *et al.*, 2005), human excreta, and microorganisms, which might have harbored multidrug-resistant (MDR) genes (Chang *et al.*, 2010; Galvin *et al.*, 2010; Chagas *et al.*, 2011). Consequently, hospital wastewaters were regarded as hotspots for antibiotic resistance, creating an environment conducive to the exchange of antibiotic resistance genes (Kunhikannan *et al.*, 2021).

Hospital wastewater was a considerable contributor to the environmental burden of antibiotics and, subsequently, antibiotic resistance (Kümmerer, 2004; Zhang *et al.*, 2009; Davies & Davies, 2010; Harris *et al.*, 2012). Antibiotic resistance genes (ARGs) were ubiquitous in many settings and were regarded as a growing contaminant and an ecological concern (Kümmerer, 2004; Engemann *et al.*, 2008). Environmental ARGs might have operated as a reservoir and might have been horizontally transmitted to human-associated bacteria, hence contributing to the growth of antibiotic resistance (Khan *et al.*, 2013).

Proteus bacteria were widely distributed across diverse environmental niches, including soil, water, decomposing organic matter, as well as the gastrointestinal tract of humans and animals. They played a significant role in the biological decomposition of organic materials, contributing to the recycling of nutrients within ecosystems. Moreover, their presence in water could serve as a potential indicator of fecal contamination, highlighting their relevance in

environmental health and water quality monitoring (Jamil *et al.*, 2022).

These bacteria also exhibited a remarkable capacity to adapt to varying environmental conditions, which was associated with their genetic plasticity and the production of diverse enzymes, such as urease and proteolytic enzymes, thereby facilitating their persistence and dissemination across multiple habitats. *Proteus* species represented an increasing concern in public health due to their ability to develop resistance to a broad spectrum of antibiotics. Numerous recent studies had reported strains resistant to β -lactams, fluoroquinolones, and certain carbapenems. This posed a significant therapeutic challenge, particularly in healthcare settings (Wasfi *et al.*, 2022).

Furthermore, their capacity to form biofilms enhances their resistance to treatment, as these structures protect bacterial cells from the effects of antibiotics and the host immune system (Qiao *et al.*, 2020).

Co-resistance to heavy metals and antibiotics in *Proteus* represents a clear example of the complex interplay between environmental pollution and clinically significant antibiotic resistance. Through the genetic linkage between heavy metal resistance determinants and antibiotic resistance determinants, in addition to the role of efflux pump systems and biofilm formation, this bacterium has developed a remarkable ability to adapt to dual environmental and therapeutic pressures (Niu *et al.*, 2024).

Accordingly, addressing this phenomenon requires an integrated strategy that combines both environmental and clinical perspectives, based on the understanding that heavy metal pollution is not merely an environmental issue, but a major driving factor in the development and dissemination of multidrug resistance in medically important bacteria (Fritzenwanker *et al.*, 2025).

1.2.The aim of the study

Investigate the co-occurrence (co-resistance) of antibiotics and heavy metals in *Proteus spp* through:

1. Isolation of *Proteus spp* from different hospital settings.
2. Identification of *Proteus spp* using microscopic, culture and Biochemical tests.
3. Determination of *Proteus* susceptibility to various antibiotics belonging to different classes using Vitek system.
4. Determination of *Proteus* tolerance to some heavy metals by MIC and LC50.
5. Detection of some antibiotic resistance genes using uniplex PCR.

Chapter Two : Literatures Review

2. Review of Literatures

2.1. History of *proteus* spp

The genus *Proteus* was initially characterized in 1885 by German microbiologist Gustav Hauser, who demonstrated their capacity to swarm over solid substrates (Drzewiecka, 2016). The name *Proteus* originates from Homer's "Odyssey," referring to the character *Proteus*, who possessed the power of shape-shifting and infinite metamorphosis. Hauser identified two species within the genus: *Proteus vulgaris* and *Proteus mirabilis* (Manos *et al.*, 2007a).

The swarming capability, associated with the transformation of short swimmer cells into elongated, multinucleated, and hyper-flagellated swarmer cells, is particularly evident in the second species. This may be a potential origin of the name *P. mirabilis*, which translates as astonishing, remarkable, or splendid in Latin. Hauser may have seen *P. vulgaris* as more prevalent and commonplace; hence, he assigned it a Latin name signifying broad and customary. The capacity for swarming development is employed in a straightforward and efficient Dienes test to distinguish between *Proteus* spp. strains, exhibiting discriminatory power akin to ribotyping (Pfaller *et al.*, 2000).

The genus *Proteus* comprises Gram-negative, facultative anaerobic, heterotrophic, and proteolytic rods that serve as opportunistic pathogens in humans. The taxonomic categorization of these microorganisms has undergone multiple revisions. Recently, the sole *Proteus* species lacking clinical relevance, *Proteus myxofaciens*, has been proposed for reclassification from the genus *Proteus* to a new genus, *Cosenzaea* (Rainey *et al.*, 2015). Notably, the exclusion of numerous species from the genus *Proteus*, resulting in the establishment of the new genera *Providencia* and *Morganella*, is significant. The three closely related genera constitute the tribe Proteeae within the family Enterobacteriaceae (Manos *et al.*, 2007a).

Bacteria of the genus *Proteus* can be distinguished by their O-antigen variability, however serotyping is not part of the standard diagnostic procedures for these rods. To far, 80 O-antigenic serogroups have been identified within the genus, with some subdivided into subgroups (Arbatsky *et al.*, 2013a), (Knirel *et al.*, 2011), (Siwińska *et al.*, 2018), and numerous additional O serotypes continue to be uncovered. The chemical structure of the sugar component of lipopolysaccharides may significantly influence the adaptation of *Proteus spp.* to environmental conditions and augment their pathogenicity, as certain O serotypes are more prevalent and frequently isolated from clinical sources than others (Arbatsky *et al.*, 2013b), (Drzewiecka *et al.*, 2010), (Drzewiecka *et al.*, 2004).

2.2 Environmental Roles and Application of the *protues spp*

Proteus spp. as an element of intestinal microflora of humans and animals, are often treated as zymogens in soil or water. In fact, these proteolytic microorganisms come to these environments with feces or waste, and after digestion of approachable organic matter, they decay due to the lack of nutrients. (Hawkey, 2008).

Nonetheless, these bacteria are also present in habitats such as autochthones, where they are well adapted to environmental circumstances and display rare and outstanding metabolic characteristics, although this element of the genus *Proteus*'s lifecycle is less recognized.

Although *Proteus spp* were recognized as opportunistic pathogens, they exhibit more beneficial characteristics in natural habitats. They may function as effective and specialized plant-growth-promoting rhizobacteria (PGRP) or as bioremediators of hydrocarbons, pesticides, herbicides, aromatic chemicals, azo dyes, and heavy metals in polluted settings. It may be isolated from multiple

sites, including: multiple nosocomial and ,burn, wounds infections, urinary tract infections, and pulmonary infections; from the medical equipment, such as inhalers, dialysis equipment, respirators, anesthesiology equipment, vaporizers and from toilets and sinks (Azam,and Khan , 2019).

2.3. Clinical significance of *proteus*

Proteus spp. infect humans owing to its several virulence factors, including the enzyme urease, flagella, fimbriae, biofilm development, and the production of toxins such as hemolysin and mirabilysin (Armbruster *et al.*, 2018a).

Consequently, the incidence of this bacteria was anticipated to be greater in hospitalized patients compared to outpatients (Hassuna *et al.*, 2025).

Therefore, treatment modalities for these individuals are essential for managing infections caused by *proteus spp.*

Proteus species have intrinsic resistance to nitrofurantoin and tetracycline, (Mirzaei *et al.*, 2021) resulting in a significant resistance rate of 91% and 98% to nitrofurantoin and tetracycline, respectively.

The urease-producing *P. mirabilis* is recognized for its capacity to transform into elongated swarm cells and exhibit a characteristic bull's-eye movement pattern on agar plates (Drzewiecka, 2016),(Pearson *et al.*, 2010). This bacteria causes infections in wounds, the gastrointestinal system, and the urinary tract, although it is most recognized for causing catheter-associated urinary tract infections (CAUTI).

Proteus mirabilis may cause respiratory tract infections, bacteremia, meningitis, cystitis, acute pyelonephritis, and renal calculi (Fox-Moon *et al.*, 2024),(Armbruster *et al.*, 2018b). Moreover, *proteus* infection has been associated with autoimmune diseases. comprising rheumatoid arthritis (Fox-Moon *et al.*, 2024). The pathogenicity of *Proteus* species necessitates many

virulence factors, including mannose-resistant *Proteus*-like (MR/P) fimbriae, mannose-resistant Klebsiella-like hemagglutinin (MR/K), *P. mirabilis* fimbriae (PMF), and the urease enzyme. (Grahl *et al.*, 2021).

2.4. Laboratory identification of *proteus mirabilis*

P. mirabilis is a highly motile and swarming bacterial species. *P. mirabilis* has a characteristic swarming phenotype on culture media, such as blood agar, that encompasses the entire agar plate. The swarming phenotype of *P. mirabilis* is visually manifested as growing waves or rings on the agar medium's surface (Ifeanyichukwu *et al.*, 2015). Urine, sputum, and pus are specimens obtained when *P. mirabilis* infection is suspected. *P. mirabilis* is culturable and can be isolated on blood agar, MacConkey agar, or eosin-methylene blue (EMB) agar, where it generates non-lactose fermenting pale or colorless swarming colonies (Schaffer and Pearson, 2015).

P. mirabilis exhibits swarming on blood agar plates and emits a fishy odor following aerobic incubation. While the addition of specific chemicals to culture media can prevent or limit swarming in *Proteus* species, this method may also impede the growth of other clinically significant bacterial pathogens present in the specimen under investigation. *P. mirabilis* is biochemically characterized as urease positive, indole negative, and capable of producing H₂S on triple sugar iron agar (TSIA) slants. Other *Proteus* species, however, yield good results in the indole test. *P. mirabilis* does not swarm on Cystine–Lactose–Electrolyte-Deficient agar due to the medium's electrolyte deficiency. Swarming in *P. mirabilis* is inhibited on MacConkey agar plates due to the presence of bile salts in the medium, which deter swarming incubated at 37°C for 18-24 h. (Madigan *et al.*, 2008).

The conventional laboratory identification of *Proteus spp.* relies on a combination of morphological characteristics, biochemical tests, and distinctive phenotypic features. These bacteria are Gram-negative bacilli belonging to the

family Enterobacterales. They are characterized by their ability to exhibit swarming motility on solid culture media, which represents one of their most distinctive phenotypic traits. They also form characteristic colonies with concentric ring-like patterns spreading across the surface of agar (Janda & Abbott, 2021).

Biochemically, *Proteus spp.* are strongly urease positive, leading to an increase in environmental pH due to the hydrolysis of urea. They are also typically phenylalanine deaminase positive, an important test for differentiating members of Enterobacterales. In addition, they produce hydrogen sulfide (H₂S positive) on TSI medium, while indole reactions vary depending on the species, being negative in *P. mirabilis* and positive in *P. vulgaris*. They are generally non-lactose fermenters on MacConkey agar, which aids in distinguishing them from many other enteric bacteria (Chakkour *et al.*, 2024).

Clinically, *Proteus spp.* are commonly associated with urinary tract infections and are known for their ability to contribute to the formation of urinary stones (struvite calculi) due to urease activity and the resulting alkalinization of urine. (Drzewiecka, 2016).

Clinical laboratories employ many automated identification techniques, such as API 20E and VITEK 2, for the prompt and reliable identification of *Proteus* species in clinical isolates (Mahmud *et al.*, 2018). The MALDI-TOF MS technology has been implemented in microbiological diagnostic laboratories for accurate species identification by protein fingerprinting, facilitated by technical developments. Research has shown its efficacy in distinguishing closely similar species within the *Proteus* genus (Singhal *et al.*, 2015).

2.5. Characteristic of *proteus spp*:

P. mirabilis, a Gram-negative, dimorphic bacterium, is a genus of motile, aerobic, facultative anaerobic rods within the Enterobacteriaceae family,

associated with uncomplicated urinary tract infections caused by strains of *P. mirabilis* (Chakkour *et al.*, 2024). *Proteus* strains were examined for a diverse array of biochemical responses; all strains exhibited swarming behavior and shown the ability to digest casein and liquefy gelatin. The *Proteus* genus is categorized as an opportunistic pathogen. It is present in the digestive tracts of people and animals, as well as in the environment (Jacobsen *et al.*, 2008). It has captivated scientists for decades because of its capacity to convert from microscopic rods into elongated, multinucleated swarmer cells possessing thousands of flagella (Armbruster & Mobley, 2012).

At 36°C, *Proteus* species can navigate both solid and semisolid surfaces. Due to the characteristics of fermentation metabolism, they may thrive and multiply in both aerobic and anaerobic conditions (Christopher *et al.*, 2012). *Proteus* species play a crucial function in the natural environment by facilitating the decomposition of organic material derived from animal sources (Drzewiecka, 2016).

Nonetheless, their morphology is always evolving, facilitating the formation of the so-called long cells. Characteristic at temperatures ranging from 20 to 37 °C. Their size typically varies from 10 to 80 µm in diameter and 1.0 to 3.0 µm in length, featuring many cilia. They typically emit an ammonia odor (Buckle, Jane 2015; Veflen Olsen, N.; Motarjemi, Y, 2014). Surface alongside nucleoids that are not partitioned by a septum (Armbruster *et al.*, 2018).

When bacteria divide and individual cells are isolated from one another, the process concludes and results in the formation of short rods (Khaled *et al.*, 2021).

P. mirabilis is typically lactose-negative, indole-negative, H₂S-producing on triple sugar iron (TSI) agar, and maltose-negative (Pearson, 2019).

These bacteria also possess a variety of virulence factors and enzymes, such as urease, which hydrolyzes urea into CO₂ and NH₃, and protease, which degrades

proteins. In addition, they produce toxins, including hemolysin and (*Proteus* toxin agglutinin) (Pta) (Rozalski *et al.*, 2012 ; Cestari *et al.*, 2013).

Clinical strains of *P. mirabilis*, akin to other Enterobacterales, have undergone substantial evolution. Resistance to antimicrobial agents in recent years decades (Literacka *et al.*, 2019).

2.6. Classification of *proteus spp*

The scientific Classification of *P.mirabilis* is as follows, according to the second edition of Bergey's Manual of Systematic Bacteriology (Garrrity *et al.*, 2012):

Table(2.6) showing Scientific classification of *Proteus mirabilis*

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	<i>Proteus</i>
Species	<i>p.mirabilis</i> , <i>p.vulgaris</i> , <i>p.penneri</i> , etc.

2.7. Pathogenesis

Proteus are opportunistic bacterial pathogens that, under conducive circumstances, induce urinary tract infections (UTIs), frequently linked to severe urinary tract infections (Abbott, 2007). They typically impact the upper urinary system, a frequent location of infection, resulting in conditions like

urolithiasis, cystitis, and acute pyelonephritis. Infrequent instances of bacteraemia linked to urinary tract infections involving *Proteus spp.* have been documented. Additional illnesses encompass septicaemia, wound infections, neonatal or newborn meningitis, and rheumatoid arthritis (O'hara *et al.*, 2000; Ronald, 2002).

Janda *et al.* (2006) and Carroll *et al.* (2022) examined endocarditis caused by *Proteus* species, whereas Okimoto *et al.* (2010) documented pneumonia owing to *P. mirabilis*.

Bloch *et al.* (2010) documented brain abscesses associated with *P. vulgaris* bacteremia. It is important to emphasize that *Proteus* bacteria are responsible for urinary tract infections with greater frequency. This category of infections is categorized as uncomplicated or complex. Uncomplicated infections arise in patients who were otherwise healthy, while complicated infections typically occur in individuals with urinary catheters, structural or functional abnormalities in the urinary tract, concurrent illnesses, immunocompromised states, or following surgical procedures in the urogenital system. Complex urinary tract infections may be polymicrobial and are typically induced by Gram-negative bacteria such as *Proteus spp.*, *Providencia stuartii*, *Morganella morganii*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, in addition to certain Gram-positive bacteria (Nielubowicz and Mobley, 2010). *Proteus* species can induce hematogenous and ascending infections, with the latter being more prevalent among these microbes (Jacobsen *et al.*, 2008).

2.8. Epidemiology of *P.mirabilis*

In 2019, about 404.6 million individuals globally suffered from urinary tract infections (UTIs), resulting in almost 236,786 fatalities and contributing to 5.2 million (4.5 – 5.7) disability-adjusted life years (DALYs) (Zhilin Zeng *et al.*,

2021). More than 60% of women experience at least one symptomatic urinary tract infection (UTI) throughout their lifetime, but the frequency of UTIs in men is considerably lower, especially affecting those with urologic structural abnormalities and older adult men (Martha and Edgardo Castillo-Pino, 2019).

Urinary tract infections (UTIs) are prevalent in females, particularly during pregnancy and in premenopausal and postmenopausal women. UTIs are linked to considerable morbidity and death, adversely impacting the quality of life of affected individuals. Antibiotic therapy is an effective treatment modality. It diminishes the duration of symptoms, the emergence of resistance, bad effects of antibiotics, and other related issues, hence enabling a research foundation to explore alternate methods for managing UTIs. Natural methods have been extensively employed for the management of many ailments to alleviate symptoms (Fazly Bazzaz *et al.*, 2021).

2.9. Virulence Factors of *Proteus Spp*

2.9.1. Adhesion and biofilm formation

The primary event in the pathogenesis of bacterial infectious illnesses is the colonization of the host epithelium, often facilitated by fimbrial and nonfimbrial adhesins (Beachey, 1981; Soto & Hultgren, 1999) (Figure 2-1).

Urinary tract infections (UTIs) attributed to *P. mirabilis* often occur via an ascending route, when bacterial cells migrate from the gastrointestinal tract to various parts of the urinary system (Armbruster and Mobley, 2012). The primary factor in beginning infection is the adherence of cells to biotic and/or abiotic surfaces, typically including a catheter in the case of *P. mirabilis*. cells bind to proteins produced from bodily fluids that cover catheter surfaces (Wasfi *et al.*, 2020). Adhesion transpires via specialized fimbrial and non-fimbrial adhesins. Irrespective of the bonding mechanism to the surface, interactions typically stem from fundamental physicochemical forces, with cells adhering to

the substrate through nonspecific, reversible interactions, including gravitational, electrostatic, and hydrophobic van der Waals forces (Berne *et al.*, 2015).

Adherence is a crucial factor in the development of urinary tract infections, similar to several bacterial illnesses (Sareneva *et al.*, 1990). Evidence indicates that variations in the capacity of host epithelial cells to adhere to bacteria via Fimbriae certain adhesins affect susceptibility to infections (Daifuku & Stamm, 1986). have been shown to facilitate the adhesion of *P. mirabilis* to uroepithelial cells (Rozalski *et al.*, 1997; Coker *et al.*, 2000).

The preliminary attachment of *P. mirabilis* cells to biotic and abiotic surfaces results in the development of a bacterial biofilm (Drzewiecka *et al.*, 2022).

Biofilm production transpires by the aggregation of microbial cells and their adherence to host tissue surfaces in an aqueous medium. These biofilms are composed of extracellular polymers, namely polysaccharides, which are crucial for biofilm formation, resistance to antimicrobial treatments, and bacterial adhesion to surfaces. The production of biofilms is regarded as a fundamental and critical precursor to bacterial pathogenicity, since it augments the capacity of germs to inhabit host tissues (Kokare *et al.*, 2009; Lynch *et al.*, 2003).

The extracellular polymeric matrix safeguards bacterial cells against environmental stresses, including desiccation, food scarcity, pH fluctuations, osmotic pressure, and toxic substances (Flemming *et al.*, 2016).

Biofilm is a complex structure whereby bacterial cells are encased by biopolymers known as extracellular polymeric substances (EPS). Biofilm serves as an effective barrier against external physical, chemical, and biological influences, including antimicrobial agents. Consequently, biofilm is a considerable medical challenge. In the instance of *P. mirabilis*, biofilm mostly develops on catheter surfaces. Similar to other biofilm-forming

bacteria, the biofilm development by *P. mirabilis* correlates with increased antibiotic resistance (Aiassa *et al.*, 2012).

Extracellular polymeric substances (EPS) primarily include polysaccharides, proteins, extracellular DNA, glycoproteins, lipids, lipoteichoic acids, and lipopolysaccharides (LPS). Water, which serves a protective function, also comprises a significant percentage of the biofilm matrix (Flemming *et al.*, 2023).

Biofilms are highly hydrated structures, containing approximately 97% water, with an average thickness ranging between 0.2 and 1.0 mm (Boudarel *et al.*, 2018; Czyzewska-Dors *et al.*, 2025).

The composition of the EPS defines the biofilm properties (Boudarel *et al.*, 2018). However, the components of *P. mirabilis* EPS have not been determined in detail (Boudarel *et al.*, 2018).

Biofilms provide significant medical hazards since bacterial cells inside their matrix may withstand host immune responses and antibiotic therapies. *Proteus mirabilis* may build biofilms on urinary catheters (Vestby *et al.*, 2020).

This transpires due to the deposition of minerals, particularly calcium and magnesium, inside the biofilm matrix. The aggregation of these ions promotes the crystallization of deposits via the urease enzyme generated by bacteria, therefore obstructing urinary catheters and enabling bacterial ascent from the catheter surface into the bladder (Stickler, 2008).

These biofilms are linked to several clinically relevant illnesses in humans, including chronic otitis media, osteomyelitis, wound infections, and infections related to burns (Costerton *et al.*, 1999). Biofilms formed by *P. mirabilis* may lead to significant problems in catheterized patients, as shown by research indicating that 93.75% of *P. mirabilis* isolates have biofilm formation capability (Ali, 2012).

Furthermore, studies demonstrate a significant correlation between biofilm development and other virulence factors of *P. mirabilis*. Qaddoori *et al.* (2018) found that all examined isolates had the ability to produce many biofilm-associated components, such as extracellular enzymes and adhesion molecules, which facilitate bacterial persistence and survival under varied environmental circumstances (Alkallas *et al.*, 2020).

2.9.2 Motility

Conflicting findings exist about the significance of flagella in the infection process. Flagella facilitate bacterial movement, indicating their necessity for the pathogenicity of uropathogens, especially during the ascent infections (Shirtliff and Leid, 2009). Flagella on the surfaces of bacterial pathogens are generally believed to facilitate initial colonisation and spread within the host adhesion, and the perception of the extracellular environment. For *Proteus* species, these surface features are crucial in the action termed swarming, a defining trait of these animals (Gmiter and Kaca, 2022).

Swarming may contribute to the migration of *Proteus* strains on to catheter materials. Flagella serve as H antigens and exhibit significant immunogenicity (Jacobsen *et al.*, 2008).

Bacteria can evade the human immune response through the process of antigenic variation. This behavior is predicated on the rearrangement of flagellin genes. Flagellar antigenic site variants enable bacteria to circumvent the effects of secretory IgA antibodies targeting these structures (Belas and Suvanasuthi, 2005). Differentiation of swarm cells is crucial for virulence of *Proteus* strains in urinary tract infections, as various virulence factors, such as flagellin, urease, hemolysin HmpA, and IgA metalloprotease ZapA, are elevated in differentiated swarmer cells compared to swimmer cells (Fraser *et al.*, 2002).

Swarming is a phenomenon of multicellular differentiation enabling a bacterial population for travel coordinately across a solid surface. The mobility of *Proteus* species to new places is significant and likely facilitates their colonization of macroorganisms. It encompasses intercellular signaling and multicellular interactions, linked to the potential for morphological differentiation of bacteria based on growth conditions. In the generation of swarm cells, DNA replication transpires without septation, leading to the emergence of elongated, polyploid cells (Williams and Schwarzhoff, 1978). At certain intervals, these bacteria temporarily halt their movement, experiencing a process termed consolidation, which transforms them into a shorter morphotype (Gué *et al.*, 2001). Thereafter, they re-differentiate into swarm cells, characterized by their exceptional length (generally 20–80 µm) and hyper-flagellation (Gué *et al.*, 2001; Strating *et al.*, 2012).

The manifestation of levels during swarming underscores the dynamic character of this process (Allison *et al.*, 1992; Fraser *et al.*, 2002).

Proteus are dimorphic bacteria that, in liquid conditions, exhibit motility as peritrichously flagellated small rods of 1.0 to 2.0 µm in length, possessing 6 to 10 flagella. These bacteria are referred to as swimming cells. Upon transfer to solid medium, these short rods transform into extended (20-80 µm in length), multinucleate, hyper-flagellate, nonseptate swarmer cells. The latter migrate away from the site of inoculation when the swarmer cell population diminishes on solid surfaces. Subsequently, the process of consolidation occurs. During this phase of rapid proliferation, the elongated rods decompose into shorter bacteria. Differentiation and dedifferentiation processes in the bacteria of *Proteus* are cyclical. This leads to the development of distinct rings of bacterial growth in the agar plate (Verstraeten *et al.*, 2008; Morgenstein *et al.*, 2010; Rozalski and Staczek, 2010).

2.9.3. Urease production

“Urease” plays a crucial function in pathogenesis (Griffith *et al.*, 1976). Urease catalyzes the enzymatic decomposition of urea into ammonia and carbon dioxide, resulting in an increase in local pH owing to ammonia generation (Kappaun *et al.*, 2018).

The alkaline pH initiates a series of processes in the urinary system, culminating in the precipitation of calcium and magnesium ions and the production of urinary calculi, such as magnesium ammonium phosphate (struvite) and calcium phosphate (apatite) (Armbruster *et al.*, 2018).

The significant correlation between *P. mirabilis* virulence and urease activity highlights the serious risk presented by urinary stones (Jones *et al.*, 2005; Munns and Amawi, 2010; Chew *et al.*, 2012).

These stones may attain significant diameters and impede urine flow, leading to tissue injury (Li *et al.*, 2002). The ramifications include urinary catheters, whereby precipitated minerals may amalgamate with bacteria, resulting in a crystalline biofilm that progressively obstructs urine flow. This intricate interaction provides a conducive environment for bacteria to infiltrate stones, providing protection against drugs and immunological responses (Armbruster *et al.*, 2014).

In the setting of UTIs, urease activity is influenced by factors beyond *P. mirabilis* alone. Cases of polymicrobial infections, such as the experimental co-infection of mice with *P. mirabilis* and urease-positive *P. stuartii*, result in an increase in urolithiasis and bacteremia (Tasić *et al.*, 2021).

Although bacterial burdens are similar to those in mono-species infections, the combined actions synergistically augment the total pathogenicity potential (Morris *et al.*, 2019).

Recognizing the pivotal function of urease in the pathogenicity of *P. mirabilis*,

current research is concentrating on this enzyme for the development of clinically significant inhibitors. Furthermore, recognizing that the bacterium's ability to produce urinary stones and crystalline biofilms depends on an alkaline pH, an additional approach to avert catheter obstruction entails urine acidification (Jacobsen *et al.*, 2008).

2.9.4. Lipopolysaccharide (LPS)

Lipopolysaccharide consists of three genetically and structurally unique components: the O-specific chain (O-antigen, O-specific polysaccharide), the core oligosaccharide, and lipid A, which anchors the LPS molecule to the bacterial outer membrane (Mohammed, 2014).

LPS encompassing all three areas is synthesized by smooth strains of bacteria. Rough strains produce lipopolysaccharides (LPS) that include lipid A and either the complete core area or a portion thereof (Huang *et al.*, 2012).

All three areas of LPS from *Proteus* have been examined (Rozalski, 2008; and Rozalski *et al.*, 2002).

A- O-antigens

The structural variations in O-antigens provide a foundation for the serological classification of *Proteus* strains. The current serological classification system has 78 serogroups (Drazewiecka *et al.*, 2008 and 2010; Knirel *et al.*, 2011). The incidence of specific *Proteus* O serogroups among clinical isolates have fluctuated as time went on. Clinical isolates were previously identified as belonging to serogroups O3, O10, O11, O13, O23, O27, and O30. Wang *et al.* (2010) and Kaca *et al.* (2017) have identified five probable *Proteus* O-antigen gene clusters and *Proteus*-specific genes in strains categorized into five serogroups. The O-specific polysaccharide chain located on the outside of bacteria contributes to glycocalyx development. The glycocalyx facilitates bacterial growth in the form of a biofilm on solid surfaces. Bacterial biofilm is

characterized like a bacterial colony encased in a matrix that adheres to surfaces. Bacteria encased are in a capsule of glycocalyx shielded from the effects of antibodies and other immune mechanisms (Donlan and Costerton, 2002; Vestby *et al*, 2020).

B- Core oligosaccharide

The core area of *Proteus* lipopolysaccharides was examined using rough or smooth mutants variants categorized into several serogroups. Core structural diversity is distinctive to *Proteus spp.*, differentiating it from *E. coli* and *Salmonella* (Vinogradov *et al.*, 2002).

The *Proteus* core area consists of two components: an inner section shared among multiple strains and an outer section distinguished by structural variability across different strains. The common component is not uniform across all *Proteus* strains and exists in three variants known as glycoforms I-III. The outer section of the central zone (outer core) possesses an oligosaccharide specific to certain *Proteus* strains (Caroff, 2020).

C- Lipid A

LPS, secreted by harmful bacteria in the course of infection, elicits a range of biological actions. Consequently, LPS is referred to as an endotoxin. The biological action mechanism of endotoxin is prevalent among most bacteria. The biological domain is lipid A (Łukasiewicz and Ługowski, 2003). *Proteus* lipid A comprises a disaccharide of glucosamine modified with phosphate groups and fatty acids. It also includes 4-amino 4-deoxy-L-arabinopyranose (L-Arap4N), which quantitatively replaces the esterified phosphate moiety of the glucosamine section. The lipopolysaccharides (LPS) of certain bacterial forms enhance their resistance to the bactericidal effects of serum (Kwil, 2003; Mielnik *et al.*, 2004; Kaca *et al.*, 2009).

2.9.5. Fimbrea

The virulence of *P. mirabilis* is associated with various factors, including flagella-mediated swarming motility, Fimbriae are external structures present on the surface of the bacterial cell wall and play a crucial role in adherence to host cell surfaces. This adhesion represents a fundamental step in bacterial colonization and the establishment of infection, particularly under conditions of continuous urine flow. (Mobley, 2001).

The colonization of the bladder and kidney clearly increases an organism's capacity to induce infection in the urinary system. Multiple findings indicate the involvement of fimbriae in the colonization of *P. mirabilis* (Pellegrino *et al.*, 2003). The adherence of *P. mirabilis* to renal and uroepithelial cells is mediated by fimbriae (Svamborg *et al.*, 1980; Wray *et al.*, 1986).

Proteus mirabilis exemplifies a unique instance in which many forms of fimbriae may be concurrently produced by a single cell (Mobley & men). In 1991, urinary tract infections accounted for 9.6 million physician visits and 1.5 million hospital admissions in the United States (Gmiter and Kaca, 2022).

The genomic investigation of *P. mirabilis* has uncovered a range of sticky structures that surpass the well-examined chaperone-usheer fimbriae. The bacterium's genome contains genes that encode one or two possible type IV pili. This suggests that the bacteria possess a varied array of adhesins that are crucial to their pathogenicity (Silverblatt, 1974; Jacobsen *et al.*, 2018).

2.9.6. Proteases

P. mirabilis is a bacterium where proteases play a crucial role in its ability to cause infections and its survival, particularly in the urinary system. This environment presents a significant challenge for bacteria due to the robust defenses provided by the human body, such as antibodies and antimicrobial peptides (Ghaidaa *et al.*, 2024).

Among the proteases produced by *P. mirabilis*, ZapA (mirabilysin) is a potent metalloprotease that excels at degrading a wide variety of host proteins in vitro. This protease demonstrates an exceptional ability to break down numerous proteins required by the immune system to fight infection, contributing to the bacterium's ability to evade the immune response (Walker *et al.*, 1999; Belas *et al.*, 2004).

The significance of this discovery lies in the fact that finding effective chemical inhibitors for ZapA offers the potential to target it as a therapeutic goal. This discovery provides an important foundation for further research into novel therapeutic strategies that could help mitigate *P. mirabilis* infections (Belas *et al.*, 2004).

Additionally, the pathogenesis of *P. mirabilis* is mediated by proteases, with ZapA being particularly notable for its ability to degrade a wide range of host proteins in vitro. This versatility highlights its critical role in evading the host's immune response, reinforcing its importance as a major virulence factor. (Belas *et al.*, 2004).

2.9.7. Hemolysin

Proteus rods generate three varieties of cytolysins: HpmaA and HlyA hemolysins, together with Pta cytotoxic agglutinin. HpmaA is a 166 kD protein, a cell-bound haemolysin, calcium independent produced by bacteria and activated through a mechanism mediated by HpmaB. This hemolysin is a member of the pore-forming toxins family and exhibits cytotoxicity towards several cell types.

HlyA is synthesized by strains of *P. vulgaris*, *P. penneri*, and *P. hauseri*. It is a highly cytotoxic, calcium-dependent extracellular toxin of 110 kDa that is part of the RTX protein family (RTX - repeat in toxin) (Al-Duliami *et al.*, 2011).

The hly operon, essential for the extracellular and synthesis release of

hemolysin, has four structural genes organized sequentially as hlyC, hlyA, hlyB, and hlyD (Koronakis *et al.*, 2003). The gene hlyA codes for the 110-kDa haemolysin protein (pro-HlyA), an inactive precursor of the mature toxin. The transformation of pro-HlyA into haemolysin (HlyA) hemolytically active occurs in the bacterial cytoplasm and is facilitated by HlyC. The combination of HlyB, HlyD, and TolC facilitates the secretion of hemolysin across the bacterial cell wall (Holland *et al.*, 2005). The product of the hlyC gene is thought to perform twin roles: (i) activation and (ii) transport of hemolysin across the periplasm through the cytoplasmic membrane (Patrick *et al.*, 2010).

Haemolysin has been identified as a virulence component in *Proteus*, since strains exhibiting elevated production of haemolysin demonstrate more lethality compared to those with diminished production; the *Proteus* rod cytotoxicity towards human kidney epithelial cells is predominantly attributed to haemolysin (Armbruster *et al.*, 2014).

Haemolysin is believed to promote bacterial dissemination within the kidney and the onset of pyelonephritis during ascending urinary tract infections (Coker *et al.*, 2000).

Proteus toxic agglutinin (Pta), encoded by ICEPm1, is a bifunctional outer-membrane autotransporter that facilitates cell–cell aggregation and possesses a catalytically active α -domain (subtilisin-like alkaline protease domain) able to lyse renal and urinary bladder cells (Wiles *et al.*, 2008).

Hemolysin are extracellular enzymes produced by bacteria that target erythrocytes, leading to the lysis of red blood cells by disrupting the cell membrane. A multitude of harmful bacteria synthesize haemolysins as a component of their virulence factors. These enzymes may lyse many kinds of red blood cells, including human, sheep, and rabbit erythrocytes, contingent upon the bacterial species involved (Liaw *et al.*, 2000).

Research has shown a correlation between haemolysin production in *P. mirabilis* and its capacity to colonize and proliferate inside host tissues. During

infection, the bacteria generates cytotoxic and immunogenic haemolysins, which augment its virulence and facilitate renal tissue damage, including a documented association between haemolysin activity and kidney stone development (Gabidullin *et al.*, 1990).

p. mirabilis utilizes many techniques to circumvent host defenses, including the development of a Serratia-type calcium-independent hemolysin, encoded by the *hpmA* gene. This hemolysin has the ability to lyse both nucleated and enucleated red blood cells, underscoring its extensive cytolytic activity (Swihart and Welch, 1990; Uphoff and Welch, 1990; Cestari *et al.*, 2013).

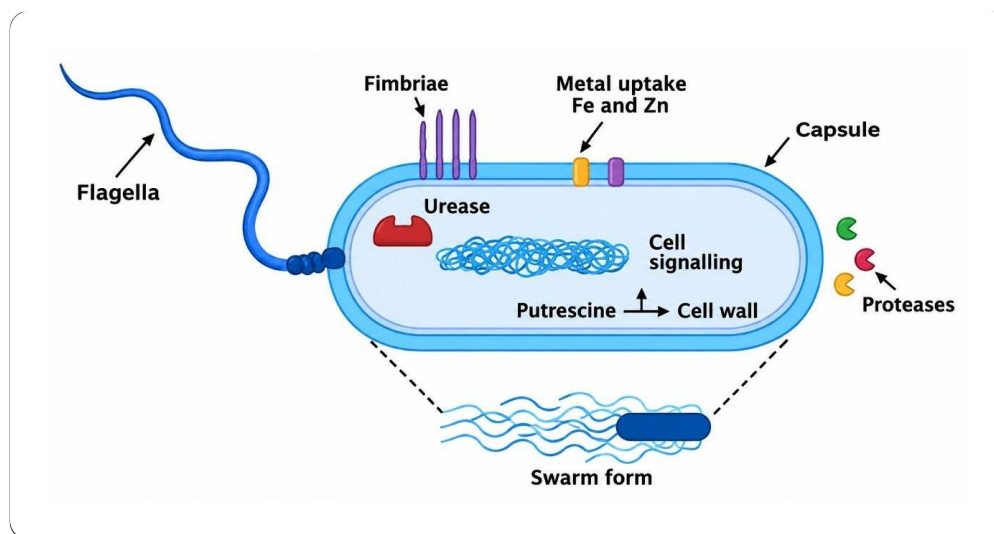
The membrane transporter that facilitates hemolysin secretion is encoded by *hpmB* and demonstrates significant conservation across *P. mirabilis* isolates, underscoring the critical importance of this hemolytic mechanism in the bacterium's pathogenicity (Fraser *et al.*, 2002).

This cytolytic toxin is crucial for invading host tissues and initiating infections.

The capacity to lyse red blood cells is significant, as it not only liberates vital resources from host cells but also establishes a conducive environment for the fast growth of the bacteria inside the host (Fraser *et al.*, 2002).

previous research has shown that hemolysin-deficient *P. mirabilis* mutants have diminished virulence, highlighting the importance of this toxin in the bacterium's overall pathogenicity (Alamuri *et al.*, 2010; Armbruster *et al.*, 2018).

In addition to its function in nutrition acquisition and tissue invasion, hemolysin is also crucial to immune evasion. By breaking host cell membranes, hemolysin promotes the dissemination of *P. mirabilis* inside the host, enabling the bacteria to circumvent immune cells. Consequently, exacerbating the intensity and duration of illnesses. The many roles of hemolysin underscore its significance as a principal virulence factor in the pathogenic approach of *P. mirabilis* (Norsworthy and Pearson, 2017).



Figure(2-1) virulence factors of *p. mirabilis* (Chakkour *et al.*, 2024).

2.9.8. Resistance to antibiotics

The pathogenesis of urinary tract infections and the antibiotic resistance of uropathogens have evolved in recent years, affecting both community and nosocomial infections (Manges *et al.*, 2006; Ghaly *et al.*, 2009). *Proteus spp.* are typically vulnerable to broad spectrum cephalosporins, aminoglycosides, and imipenem (Abbott, 2007).

P. mirabilis is susceptible to trimethoprim-sulfamethoxazole, ampicillin, amoxicillin, and piperacillin. Additionally, *P. vulgaris* and *P. penneri* are susceptible to cefoxitin, cefepime, and aztreonam. *P. mirabilis* has resistance to nitrofurantoin. *P. vulgaris* and *P. penneri* exhibit resistance to piperacillin, amoxicillin, ampicillin, cefoperazone, cefuroxime, and cefazolin. Resistance to β -lactam antibiotics in *Proteus* is on the rise (Karlowsky *et al.*, 2002).

β -Lactamases, classified under the Enzyme Commission number EC 3.5.2.6, are hydrolytic enzymes produced by Gram-negative bacteria, including *Proteus mirabilis*, that confer resistance to β -lactam antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems. These antibiotics

share a common structural feature, namely the β -lactam ring, which is essential for their antibacterial activity. β -Lactamases inactivate these agents by hydrolyzing the β -lactam ring, thereby abolishing their ability to inhibit penicillin-binding proteins (PBPs) and disrupt bacterial cell wall synthesis (Hassuna *et al.*, 2025).

Molecularly, β -lactamases are classified into four main classes (A, B, C, and D) according to the Ambler classification system. Classes A, C, and D possess a serine residue at their active site, whereas Class B enzymes are metallo- β -lactamases that require zinc ions for catalytic activity. In *Proteus mirabilis*, resistance to β -lactam antibiotics is most commonly mediated by Class A enzymes, particularly those encoded by the blaTEM and blaCTX-M genes. The blaTEM gene represents one of the earliest and most widespread β -lactamase genes identified and includes numerous allelic variants resulting from point mutations that expand substrate specificity. Similarly, blaCTX-M genes encode extended-spectrum β -lactamases (ESBLs), which are capable of hydrolyzing third-generation cephalosporins such as cefotaxime and ceftriaxone, significantly limiting therapeutic options in urinary tract infections. (Jaidane *et al.*, 2025).

These β -lactamase genes may be located on chromosomes or plasmids, and their plasmid-mediated transfer plays a crucial role in the rapid dissemination of resistance among Enterobacterales in clinical settings. The high mobility of bla genes represents a major challenge in the treatment of *Proteus mirabilis* infections, particularly in hospital-associated and catheter-associated urinary tract infections (Billard-Pomares *et al.*, 2020).

In addition to enzymatic degradation mechanisms, multidrug resistance in *Proteus mirabilis* may also involve active efflux systems. The AcrAB-TolC efflux pump, encoded by the acrA and acrB genes, is a well-characterized

resistance-nodulation-division (RND) transporter system that expels a wide range of structurally unrelated antibiotics from the bacterial cell. Overexpression of AcrAB contributes to decreased intracellular antibiotic accumulation and enhances resistance not only to β -lactams but also to fluoroquinolones, tetracyclines, and other antimicrobial classes. Therefore, the combined presence of β -lactamase genes (*bla*TEM, *bla*CTX-M) and efflux pump determinants (*acrA*, *acrB*) may synergistically contribute to the multidrug-resistant phenotype observed in clinical isolates of *Proteus mirabilis*. (Soliman *et al.*, 2024).

Cephalosporins were first discovered by the Italian scientist Giuseppe Brotzu in 1948. These antibiotics are structurally composed of a β -lactam ring fused with a dihydrothiazine ring, forming the core nucleus known as 7-aminocephalosporanic acid (7-ACA). This nucleus contains two variable side chains (R1 and R2), which differ depending on the specific derivative (Jaafar *et al.*, 2025).

Cephalosporins are chemically classified into two major groups. The first group is the oxyiminocephalosporins, which include Cefotaxime, Ceftriaxone, and Ceftazidime. These compounds are characterized by their enhanced resistance to β -lactamase enzymes. The second group comprises cephamycins, such as Cefoxitin and Cefotetan, which possess a methoxy group that contributes to their increased stability against bacterial enzymatic degradation (Hamed *et al.*, 2021).

In addition, cephalosporins are classified into generations based on their antibacterial spectrum and chemical structure. The first generation includes agents such as Cephalexin and Cephalothin, which exhibit strong activity against Gram-positive bacteria but limited efficacy against Gram-negative organisms (Jaafar *et al.*, 2025).

The second generation includes antibiotics such as Cefuroxime and Cefoxitin, which demonstrate improved stability against β -lactamase enzymes and enhanced activity against Gram-negative bacteria (Tamma *et al.*, 2022).

The third generation comprises more advanced cephalosporins, including Cefotaxime, Ceftriaxone, and Ceftazidime. These agents are characterized by their high resistance to β -lactamases and their ability to penetrate the outer membrane of Gram-negative bacteria (Shaikh *et al.*, 2015). Notably, Cefotaxime exhibits strong activity against *Proteus mirabilis*, although its efficacy may vary against other species such as *Proteus vulgaris* (Tamma *et al.*, 2022).

The fourth generation, represented by Cefepime, is distinguished by its high stability against both chromosomally and plasmid-mediated β -lactamases, in addition to its broad spectrum activity against both gram positive and gram negative bacteria (Brunton, 2022).

2.10. Genomics of *proteus*

P. mirabilis inhabits many habitats and has been recognized for its strain heterogeneity in swarming behavior and LPS O-antigen structure (Williams *et al.*, 1978)–(Manos *et al.*, 2007b). Based on these studies, it seems that this species may exhibit significant variety in its genomic composition, akin to the mosaic pangenome of *E. coli* (Welch *et al.*, 2002), (Touchon *et al.*, 2009). Nonetheless, many lines of evidence indicate a significant conservation of the *P. mirabilis* chromosome (Potter *et al.*, 2023).

Proteus mirabilis has a moderately sized genome compared to other Enterobacterales, typically ranging from about 3.8 to 4.3 Mbp in length depending on the strain sequenced. Several complete genome assemblies of clinical isolates show genome sizes such as 3.85 Mbp, 3.99 Mbp, and up to

4.31 Mbp, reflecting natural genomic variation among isolates. (Lian *et al.*, 2025).

The *P. mirabilis* genome usually consists of a single circular chromosome with a guanine–cytosine (G+C) content of approximately 38.6–39.2%, which is characteristic of this species. (Lian *et al.*, 2025). The chromosome encodes thousands of protein-coding sequences (e.g., approximately 3,400–4,000 CDSs), including genes involved in basic metabolism, motility, adhesion, and virulence, enabling the bacterium to adapt to diverse environments and cause urinary tract infections. (Mohammed *et al.*, 2024).

In addition to the chromosomal genes, some *P. mirabilis* genomes contain plasmids of variable sizes that may carry additional genes, including those associated with antimicrobial resistance. The flexibility of the *P. mirabilis* genome is further enhanced by the presence of mobile genetic elements such as insertion sequences, transposons, and genomic islands, which facilitate horizontal gene transfer. These features contribute to the genetic diversity of strains and play important roles in the acquisition and spread of resistance and virulence determinants. (Mohammed *et al.*, 2024).

previous studies have also revealed that many of the genomic islands and mobile elements in *P. mirabilis* harbor antimicrobial resistance genes and virulence-associated loci. These elements facilitate the rapid dissemination of resistance determinants across isolates, contributing to the emergence of multidrug-resistant *P. mirabilis* strains in clinical settings. The combined effect of genomic plasticity and the presence of virulence and resistance genes underscores the adaptability and pathogenic success of *P. mirabilis* as an opportunistic pathogen. (Hao *et al.*, 2023).

2.11. Heavy metals resistance of *proteus spp*

Heavy metals are considered significant environmental contaminants owing to their detrimental impact on flora, fauna, humans, and microbes. Bharti (2012) asserts that heavy metals, including arsenic, lead, cadmium, nickel, mercury, selenium, cobalt, antimony, vanadium, zinc, platinum, palladium, and rhodium, are very dangerous even in minimal quantities. Heavy metal contamination of the environment and exposure to metals such as mercury, cadmium, and lead is an increasing concern global issue. Human exposure to heavy metals has significantly increased in recent decades due to a substantial growth in their use in industrial processes and products (Alobaidi *et al.*, 2025).

Microorganisms capable of thriving in elevated concentrations of heavy metals are of significant interest as bioremediation agents due to their ability to facilitate various transformation processes (Adamis *et al.*, 2004).

Bacterial resistance and metal tolerance capabilities are common phenomena that may be used for environmental bioremediation. Consequently, these resistant bacteria may represent one of the most viable biotechnological alternatives. Nonetheless, the dangers presented by antibiotic-resistant and heavy metal-tolerant bacteria may be used in detoxification procedures to turn a dangerous substance into a safe form by establishing biotransformation mechanisms. (Liu, 2011).

2.12. Mechanisms of heavy metals resistance by *proteus spp*

The historical presence of heavy metals in the environment, coupled with their pronounced toxicity and resistance to biodegradation unlike organic pollutants, compelled microorganisms in the early stages of evolution to adapt and devise mechanisms for transforming heavy metals into less hazardous compounds to preserve ionic equilibrium. This adaptation facilitated the emergence of resistant microorganisms. Furthermore, these organisms have evolved novel

resistance mechanisms in response to heightened pollution due to increased human activities in recent years (Nies, 2019). Bacteria possess many methods to withstand heavy metals (Prabhakaran *et al.*, 2016).

2.12.1.Extracellular permeability barrier

The cell wall or extracellular matrix serves as a barrier that inhibits the ingress of metal ions into the cell, while various bacterial groups can sequester metal ions via ionizable functional groups present in the cell wall or extracellular structures, such as carboxyl, amino, phosphate, and hydroxyl groups, which confer electronegative characteristics to the bacterial cell surface (Nies, 2019). The attachment of heavy metals to the bacterial cell surface facilitates metal immobilization, as numerous positively charged heavy metals, including cadmium and zinc, interact with negatively charged functional groups on the bacterial cell surface, such as carboxyl groups, amines, amides, phosphates, and hydroxyls. The adsorption of minerals onto the bacterial cell surface significantly influences the distribution of minerals, particularly in aquatic ecosystems. The capacity of bacterial cells to adsorb metals has been refined into a method for extracting minerals from contaminated wastewater (Ianeva, 2009).

Bacterial cell walls include negatively charged functional groups that serve as efficient binding sites for heavy metals. In Gram-negative bacterial cell walls, the carboxyl groups and phosphate groups in the outer membrane are crucial for the binding process (Jarosławiecka and Piotrowska-Seget, 2014; Prabhakaran *et al.*, 2016).

Bacteria synthesize extracellular polysaccharides, slime layers, exopolymers, or capsules that generate various extracellular structures. These structures include negatively charged functional groups, such as the carboxylic group, which efficiently bind positively charged heavy metals like Cd, therefore obstructing

their ingress into the bacterial cell. These aggregates continue to function post-mortem of the bacteria, serving a crucial role in the biosorption process (Gadd, 2010).

2.12.2.Active transport by Efflux pump

Bacteria use an efflux pump to avert the accumulation of harmful heavy metals inside cells. Efficiently expel metal ions from the cell using energy derived from the hydrolysis of adenosine triphosphate (ATP) or by employing a chemiosmotic gradient (Silver & Phung, 2005).

Specialized transport systems are triggered and formed by cells only during periods of starvation and certain metabolic states; however, a rise in heavy metal concentration may negate this specialization, allowing these ions to traverse via unspecialized fast transport systems (Al-Khafaji, 2008).

2.12.3.Extracellular sequestration or precipitation

It involves the entrapment of metal ions by cellular substances in the plasma space or the outer membrane, or the deposition of metal ions as insoluble complexes. Bacterial species move metal ions from the cytoplasm to sequester them inside the plasma space. *Proteus spp.* may sequester heavy metals by synthesizing biosurfactants, which form complexes with heavy metals such as Cd and zinc. hence diminishing their prevalence in the liquid phase. The complex is non-toxic to bacterial cells. Bacterial cells synthesize biosurfactants that are either affixed to their surface or secreted into the surrounding media (Mathuriya and Yakhmi, 2014).

2.12.4.Interacellular sequestration or accumulation

The sequestration of metal ions by cellular molecules results in their accumulation inside the cytoplasm, manifesting as dense compounds detectable by electron spectroscopy. Metallothioneins safeguard bacterial metabolic

processes while simultaneously sequestering harmful heavy metals (Naik & Dubey, 2017).

Proteus spp. also exhibits resistance to heavy metals via bioaccumulation, an active mechanism of metal accumulation. Bioaccumulation is influenced by cellular metabolic activity, metal toxicity and availability, metal ion specification, genetic adaptations, and the physiological and structural characteristics of cells. Upon accumulating inside the cell, the speciation of complex heavy metal ions is reduced to a less hazardous form by specific oxidation processes and specialized enzymes (Abdelbary *et al.*, 2019; Gupta *et al.*, 2016).

2.13.Metal acquisition systems of *P. Mirabilis*

Proteus mirabilis has exceptional metal acquisition mechanisms, exemplifying microbial adaptability in difficult conditions, particularly in host-pathogen interactions. *P. mirabilis*, has developed intricate processes to get vital metals such as iron, zinc, and manganese, which are needed for its survival and pathogenicity. *P. mirabilis* utilizes advanced metal acquisition mechanisms, especially for iron, a micronutrient often sequestered by host defenses that limit metal availability (Cassat and Skaar, 2013).

The bacterium uses specialized transport systems to maintain appropriate intracellular concentrations of essential metal ions. These systems help regulate metal uptake, distribution, storage, and export, thereby preventing both metal deficiency and toxic accumulation. In addition to essential metals, *P. mirabilis* may also be exposed to toxic or excessive concentrations of heavy metals in contaminated environments, such as cadmium, chromium, zinc, and copper. (Moble, 2015).

Bacteria typically need about 10^{-7} to 10^{-5} M iron and 10^{-15} to 10^{-16} M zinc for optimum development. Mn^{2+} (total concentration of about 2 μM) is assimilated

by microorganisms for use in Mn-specific enzymes and may also be exchanged with iron at the metal-binding sites of some Fe proteins. Nickel (Ni), often used by bacteria for anaerobic development, is present at concentrations below 5 μM under aerobic settings. The total Ni^{2+} concentration in the cell may rise during anaerobic development when Ni-containing hydrogenase is produced (Loutet *et al.*, 2015).

2.14.Unraveling *P. mirabilis* metallophores and their role in pathogenesis

Metallophores are little molecules produced by bacteria that function as crucial agents in the procurement of essential metal ions from their environment. These chemicals are essential in several biological processes, primarily in bacterial pathogenesis (Ghssein and Ezzeddine, 2022).

P. mirabilis exemplifies the complex use of metallophores by pathogens to enhance their pathogenicity. *P. mirabilis* utilizes metallophores to extract metal ions, especially iron, from the host environment during pathogenesis. Iron is a crucial nutrient essential for bacterial growth and survival, but its availability inside the host is perpetually limited by the immune system's strategy of iron sequestration, a defensive tactic against bacterial invasion (Miethke and Marahiel, 2007).

In addition to iron uptake, metallophore-associated mechanisms in *P. mirabilis* are involved in the acquisition and homeostasis of other transition metals essential for bacterial growth and virulence. Zinc, for example, is required for the activity of numerous enzymes and regulatory proteins; *P. mirabilis* employs high-affinity zinc uptake systems, such as the ZnuABC transporter, to ensure adequate intracellular zinc under conditions where host sequestration limits its availability. Disruption of zinc acquisition pathways has been shown to impair bacterial growth and reduce virulence in experimental models, underscoring the

importance of zinc homeostasis in pathogenesis. Unlike iron and zinc, copper is both an essential cofactor for certain bacterial enzymes and a potent antimicrobial metal at elevated concentrations. To manage this duality, *P. mirabilis* deploys copper efflux pumps and copper-binding proteins that work in concert with metallothionein systems to maintain copper at sub-toxic levels while preserving its utility as an enzymatic cofactor (González-Álvarez *et al.*, 2023).

Under conditions of metal scarcity, metallothionein production is upregulated to facilitate the scavenging of essential metal ions; but environmental factors such as pH, temperature, oxygen availability, and the presence of competing ligands can influence metallothionein synthesis. These factors regulate transcription factors, which bind to specific DNA sequences to activate metallothionein biosynthesis genes. Complex regulatory networks involving transcription factors, small RNAs, and signaling molecules further fine-tune metallothionein production in response to cellular needs (Berg *et al.*, 2020).

2.15.Toxic Impacts of the heavy metal on the bacteria

Heavy metals impact microorganisms, including bacteria. Their primary impacts include the destruction of cell membranes when their concentration exceeds the minimum inhibitory concentration of the organism, and the influence of metals, which typically interacts with other parameters such as temperature and pH (Al-Khafaji, 2008).

Heavy metals, particularly positively charged ions with high atomic weights like cadmium and mercury, attach to thiol groups in enzymes and proteins when present within cells. They can also interact with hydroxyl or phosphate groups in DNA, resulting in a conformational alteration of proteins. Nucleic acids and the impairment of their function, exemplified by the non-specific binding of

cadmium to DNA, result in a single-strand break (Fashola *et al.*, 2016). Ionic interactions may cause heavy metal ions to interfere with similarly charged base metal ions, disturbing essential metals that serve critical roles in bacterial organisms, exemplified by the interaction of cadmium with zinc, which binds to and replaces the enzyme. Cadmium can substitute for calcium and magnesium, hence impeding enzyme activity (Johansen *et al.*, 2018). Another impact is the binding of heavy metals to the glutathione molecule, inhibiting its functionality. Heavy metals also influence oxidative phosphorylation and membrane permeability (Mendez *et al.*, 2019).

2.16. Heavy metals and Antibiotic resistance

The discovery of antibiotics and their utilization in treating infectious diseases has fundamentally transformed public health. Nonetheless, the incessant application of antibiotics, along with the perpetual adaptive potential of microorganisms, established the foundation for the selective proliferation and development of antibiotic-resistant bacteria. This has finally precipitated the emergence of multidrug-resistant microorganisms (MDR)' (Alam *et al.*, 2022).

The medical community faces a challenge in treating microbial illnesses with the existing antibiotics due to the unprecedented rate of resistance spreading among pathogenic bacteria (Aslam *et al.*, 2018). Failure of antibiotic treatment results in hundreds of thousands of fatalities annually. The WHO has designated antibiotic resistance as a global health concern that, if not immediately addressed, could precipitate a post-antibiotic age, highlighting the selection and evolution of antibiotic resistance. A range of other chemicals, including heavy metals present in the natural environment, may initiate this process (Murray *et al.*, 2022).

Bacteria are continuously exposed to heavy metals in various environments, which can promote the survival of metal-tolerant cells through genetic

adaptations and mutations (Sugitha *et al.*, 2025). Resistance to heavy metals in pathogenic bacteria has been linked to the acquisition of extrinsic resistance determinants, often carried on mobile genetic elements. Importantly, antibiotic resistance genes (ARGs), including those associated with Class 1 integrons and gene cassettes, are frequently co-located with heavy metal resistance genes, facilitating their co-selection and dissemination. This genetic linkage suggests that exposure to heavy metals may contribute to the persistence and spread of antibiotic resistance even in clinical settings (Sugitha *et al.*, 2025).

Such co-selection raises significant concerns regarding the persistence and dissemination of antibiotic resistance determinants in pathogenic bacteria. The genetic linkage between heavy metal resistance and antibiotic resistance genes facilitates their maintenance and transfer within bacterial populations. Early studies demonstrating the association between resistance to multiple antibiotics and heavy metals date back to the 1960s (Moore, 1960; Richmond and John, 1964).

Thereafter, several studies documented the co-selection and proliferation of antibiotic-resistant bacteria following exposure to heavy metals. Metals such as zinc, copper, lead, and cadmium were associated with the maintenance and dissemination of antibiotic resistance genes (ARGs) in bacterial populations (Bhattacharya *et al.*, 2000). Exposure to subinhibitory concentrations of heavy metals may promote cross-resistance to antibiotics through mechanisms such as horizontal gene transfer (HGT) of mobile genetic elements (MGEs) and bacterial mutagenesis (Pathak & Gopal, 2005; Li *et al.*, 2021). Relatively low concentrations of heavy metals may therefore exert sufficient selective pressure to contribute to antibiotic resistance (Chen *et al.*, 2015; Stepanauskas *et al.*, 2005).

Heavy metals are widely distributed in the environment as a result of human activities, including mining, industrial operations, and agricultural practices,

and may accumulate in soil and aquatic environments (Buelow *et al.*, 2021). Their concentrations and bioavailability may also increase under changing environmental conditions, potentially contributing to antimicrobial resistance through co-resistance mechanisms (Magnano San Lio *et al.*, 2023). Associations between metal contamination and antibiotic resistance have been reported in agricultural waste sites, industrially contaminated areas, and environments receiving poorly treated wastewater and biosolids (Knapp *et al.*, 2021). At high concentrations, metals can induce cellular stress and defense mechanisms, while their presence may exert selective pressure that favors the persistence and dissemination of resistant bacteria.

Chapter Three :Materials & Methods

3. Material and Methods

3.1. Materials

3.1. 1. Apparatuses and equipment

Table (3-1) lists the apparatuses and instruments used in the current investigation, along with their manufacturers and countries of origin.

Table (3- 1) The apparatuses and equipment employed in the current investigation.

No.	Instruments& Materials	Company	Country
1.	Incubator	Memmert VWR	Germany USA
2.	<i>oven</i>	Memmert	Germany
3.	<i>Autoclave</i>	Memmert	Germany
4.	<i>Benson burner</i>	Memmert	Germany
5.	<i>Water bath</i>	Memmert	Germany
6.	Vitek system	BioMerieux	USA
7.	Eppendorf 5415 D centrifuge micro	eBay Inc	USA
8.	Eppendorf 5417 R micro centrifuge Refrigerated	eBay Inc	USA
9.	Micropipette tips	Sterellin Ltd.	UK
10.	High- speed centrifuge	Eppendorf	Germany
11.	PCR tubes	Eppendorf	Germany

12.	Light microscope	Olympus Carl Zeiss	Japan Germany
13.	Micro imaging microscope	Carl Zeiss	Germany
14.	Micropipette 0.5-10,1-20µl, 5-50 µl,10-200 µl,100-1000µl	CYAN VWR	Belgium USA
15.	Platinum wire loop	Himedia Thomas Scientific	India USA
16.	Wooden sticks	Supreme	China
17.	Refrigerator	Concord	Italy
18.	Stirrer /Hot Plate	Labtech Corning	Korea USA
19.	Plastic test tubes	VWR	USA
20.	Milipore filter(0.45µm)	VWR	USA
21.	PCR System/Conventional Thermo cycler	MJ Reasearch Bioneer	USA Korea
22.	Gel Electrophoresis	Shando, scientific co. BIO-RAD	UK USA
23.	Hood	Labogene	Denmark
24.	Ultraviolet transilluminator	Dahan San. Gabriel	Korea CA
25.	Mini vortex	CYAN Fisher Scientific	Belgium USA
26.	Microwave	Kenmore	USA
27.	Digital dry bath(42 °C,50°C,100 °C)	Corning LSE	USA
28.	Rocker incubator	Boucker Scientific	USA

29.	Shaker	Boucker Scientific	USA
30.	Sensitive electric balance(mg, g)	Sartorius Fisher Scientific	Germany USA
31.	Gel documentation system, Microputser	Bio Rad	USA
32.	Camera	Canon	Japan
33.	Disposable petri dishes	Al-Hani company	Lebenon
34.	Sterilized cotton swabs	Sterile EO.	China
35.	Disposable syringe 3ml ,5ml, 10ml	Sterile EO.	China
36.	Milipoer filter paper	Millipore	USA
37.	Automatic microtiter plate washer	HUMAN	Germany
38.	Centrifuge	Hittch	Germany
39.	Electric oven	SAKURA	Japan
40.	Ependorff tubes (1.5ml)	Ependorff	Germany
41.	Fixed and adjustable mi-cropipettes Assistant (As-sipette)	Ependorff	Germany
42.	Flasks of different Sizes	Pyrex	Germany
43.	Incubator	YAMATO	Japan
44.	Laminar flow UV-cabinet	Labtech	Korea
45.	Transport cotton swap	CITO SWAB	China

46.	UV-Vis spectrophotometer	Shimadzu	Japan
47.	Vortex Mixer	Heidolph	Germany

3.1.2. Chemical Materials

Chemical materials that are used in this study are showed in Table (3-2).

Table (3-2): chemical materials used in this study

N0.	Chemicals	Company/country
1.	NaCl, KOH, Na ₂ HPO ₄ , KH ₂ PO ₄ , K ₂ HPO ₄ , NH ₄ Cl, MgSO ₄ , CaCl ₂ , NaH ₂ PO ₄ , H ₂ O ₂ , KCN, Trypton, Yeast extract, HCl	Merk Darmstadt-Germany
2.	Alpha-nephthol	BDH – England
3.	P-Dimethyl aminobenzaldehyde	BDH – England
4.	Peptone	BDH – England
5.	Methyl red indicator solution	BDH – England
6.	Glycerol	BDH – England
7.	Isoprobanol	BDH – England
8.	99% , 95% and 70% alcohol (Ethanol)	BDH –England & Flukachemika- Switzerland
9.	Urea solution (40%)	Flukachemika- Switzerland
10.	Aspartic acid	Vegetadrog –France
11.	Tannic acid	Fisher Scientific-USA
12.	Lauric acid	Fisher Scientific-USA

13.	Myristic acid	Fisher Scientific-USA
14.	palmitic acid	Fisher Scientific-USA
15.	Stearic acid	Fisher Scientific-USA
16.	Oleic acid	Fisher Scientific-USA
17.	P- nitrophenylglycerol	Fisher Scientific-USA
18.	Sodium azide	Fisher Scientific-USA
19.	Urea solution 40%	Fisher Scientific-USA
20.	Gram stain set	Ward's Natural Science -USA
21.	Resveratrol	Thomas Scientific-USA
22.	D-Mannose reagent , Sodium nitroprusside, Potassium cyanide	VWR-USA
23.	Gelatin, Trichloroacetic acid (5%)	Sigma-Germany
24.	Ethidium Bromide	Bio Basic –USA& Alfa Aesar-USA
25.	DNA purification kit for sequencing	Thermo Fisher – USA

3.1.3. Culture Media

The culture media that are used in thos study showed in Table (3-3).

Table (3-3): The culture media used in this study

No.	Media	Company/country
1.	MacConkey agar	Himedia - Accumax India
2.	Blood agar base	Himedia - Accumax India
3.	MR-VP broth	Himedia - Accumax India
4.	Peptone broth	Himedia - Accumax India
5.	Urea agar base	Diffco – Michigan
6.	Simmons citrate agar	Diffco – Michigan
7.	Nutreint broth	Ward's Natural Science-USA VWR- USA
8.	Nutreint agar	Ward's Natural Science-USA VWR- USA
9.	Tryptic soy broth	Ward's Natural Science-USA VWR- USA
10.	Luria bertani broth &agar	Ward's Natural Science-USA VWR- USA

3.1.4 DNA amplification materials

3.1.4.1 Molecular kits

Molecular kits:

Table (3-4) showed kits that were used in molecular study with their companies and countries of origin.

Table (3- 4) Kits for molecular detection.

No.	Types of kit	Company	Origin
1.	DNA Extraction Kit	Favorgen	Taiwan
2.	GoTaq® Green Master Mix	Promega	U.S.A

3.1.5 Primers

The following primers were used in this study for the molecular detection of (*acrA*, *acrB*, *TEM*, and *CTX- M*) genes in *Proteus mirabilis* isolates, as listed in Table (3-5).

Table (3-5) Primers, sequences and size.

Gene	Sequence (5' - 3')	Product size (bp)	References
<i>acrA</i>	F: ATCACCTTTCGCACTGTCGT R: CGACAAACAGGCCCAACAAG	256	Ali and Al-Dahmoshi (2022)
<i>acrB</i>	F: CATAAACACGCCCTGGTCCT R: GCTACCCGTAAGTCGATGGG	432	Ali and Al-Dahmoshi (2022)
<i>CTX-M</i>	F:ACAGCAGATAATTGCAA R: AACCAGATCACCGCGATA	599	Bajaj <i>et al.</i> (2015)
<i>TEM</i>	F:TCAACAGCGGTAAGATCCTTGA R: TGCAACTTTATCCGCCTCCA	500	Bajaj <i>et al.</i> (2015)

3.2 Methods

3.2.1 Study plan:

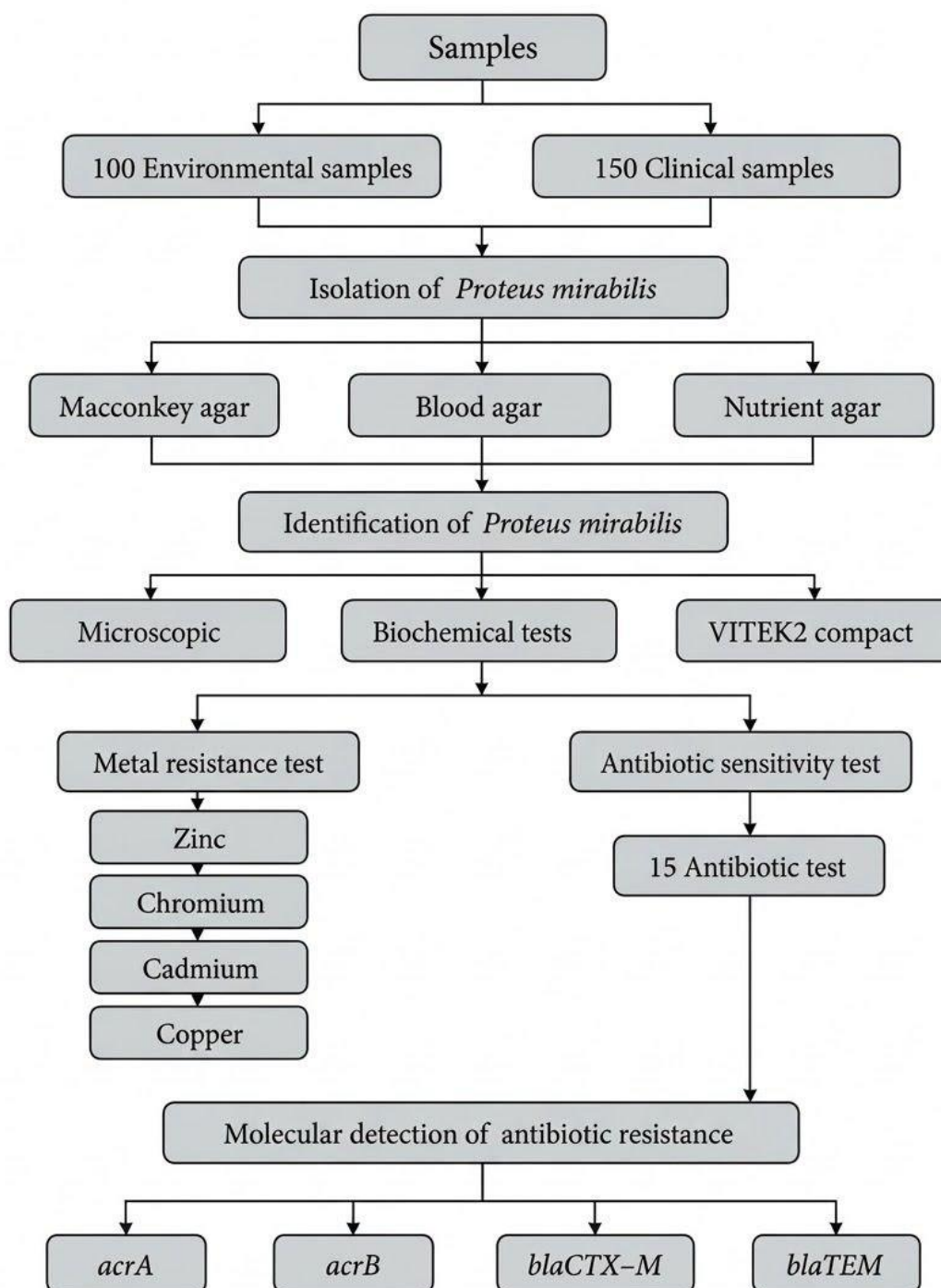


Figure (3- 1) Chart flow of study plan

3.3 Preperation of the culture Media

3.3.1. MacConkey agar

MacConkey agar medium was prepared by dissolving 51.5 gm in 1000 ml D.W. Then the medium was sterilized by autoclaving at a temperature of 121°C and a pressure of 15 p.s.i. for 15 min, and the medium was then cool down to 45°C. It is used to differentiate lactose fermenting from lactose non-fermenting gram-negative bacteria (Jung and Hoilat, 2020).

3.3.2. Nutrient broth

Nutrient broth was prepared by dissolving 8 g of nutrient broth powder in 1 liter of distilled water. The solution was stirred thoroughly until complete dissolution of the powder. It was then dispensed into test tubes or flasks as required and sealed with cotton plugs or screw caps. The media were sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to room temperature before use. This medium was used for the cultivation and preservation of bacterial isolates (Tortora *et al*, 2022).

3.3.3. Brain heart infusion agar

This medium was prepared by dissolving 38 g in 1000 ml of distilled water. The medium was heated with continuous stirring until complete dissolution, then sterilized by autoclaving at 121°C and a pressure of 15 p.s.i. for 15 minutes. After sterilization, the medium was allowed to cool to 45–50°C, then poured into sterile Petri dishes and left to solidify. This medium was used for the cultivation and isolation of the bacterial isolates under study (Atlas *et al*, 2010).

3.3.4. Brain Heart infusion broth

This medium was prepared by dissolving 37 gm in 1000 ml D.W. Then the medium was sterilized by autoclaving at a temperature of 121°C and a

pressure of 15 p.s.i. for 15 min, and then cool down to 45C°. This medium was used for maintaining bacterial isolated as standard for a long time (Forbes *et al.*, 2007).

3.3.5. Blood agar medium

Blood agar media were prepared as per the manufacturing company instructions sterilized by autoclaving and cooled to 45C° then 5% of fresh human blood was added. It is an enrichment , bacterial growth medium, and it is used for bacterial isolation, and identification as well as determination the type of hemolysis (Niederstebruch *et al.*, 2017).

3.3.6. Tryptic Soy broth

This medium was formulated dissolve 30 g dehydrated medium in 1 L deionised water, followed by sterilization via autoclave at 121°C for 15 minutes. This medium served as an growth medium for the growth of bacterial isolates. (Kusumawati *et al.*, 2025).

3.3.7. Peptone water medium

The medium was made by dissolving 8g of peptone in 1000 ml of distilled water, thereafter distributed in test tubes, and autoclaved. It was utilized to demonstrate the bacterial capacity to breakdown tryptophan amino acid into indole (Patel, 2024).

3.3.8. MR-VP medium

The Methyl Red–Voges Proskauer (MR-VP) broth was prepared according to standard microbiological procedures. The medium was formulated per liter of distilled water using 7.0 g peptone, 5.0 g dextrose (glucose), and 5.0 g dipotassium phosphate (K₂HPO₄). The components were dissolved in distilled water with continuous stirring and gentle heating until complete dissolution, and the final volume was adjusted to 1000 mL. The pH of the medium was adjusted

to 6.9 ± 0.2 at room temperature. The medium was dispensed into sterile culture tubes and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After sterilization, the tubes were allowed to cool to room temperature and stored at $2\text{--}8^{\circ}\text{C}$ until use. This medium was used for the differentiation of enteric bacteria based on glucose fermentation in the MR test and acetoin production in the VP test (Cappuccino and Welsh, 2020).

3.3.9. Simmons' citrate medium

Simmons citrate agar was prepared according to standard microbiological methods by dissolving the following components per liter of distilled water: 2.0 g sodium citrate, 1.0 g ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), 1.0 g dipotassium phosphate (K_2HPO_4), 0.2 g magnesium sulfate (MgSO_4), 5.0 g sodium chloride (NaCl), 15.0 g agar, and 0.08 g bromothymol blue as a pH indicator (Forbes *et al.* 2022). All components were dissolved in approximately 900 mL of distilled water with continuous stirring and gentle heating until complete dissolution, and the final volume was adjusted to 1000 mL. The pH was adjusted to 6.8 ± 0.2 at room temperature. The medium was sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After sterilization, it was cooled to $45\text{--}50^{\circ}\text{C}$ and poured into sterile test tubes in a slanted position to form agar slants. The medium was stored at $2\text{--}8^{\circ}\text{C}$ until use.

This medium was used to determine the ability of bacterial isolates using citrate as the only source of carbon, indicated by a change in colour from green to blue due to alkalinization of the medium (MacFaddin, 2003).

3.3.10. Kligler Iron agar medium

Kligler Iron Agar (KIA) was prepared according to standard microbiological procedures by dissolving the following components per liter of distilled water: 10.0 g peptone, 3.0 g yeast extract, 5.0 g sodium chloride

(NaCl), 1.0 g lactose, 0.1 g glucose, 0.5 g ferric ammonium citrate, 0.2 g sodium thiosulfate, 0.024 g phenol red as a pH indicator, and 12.0 g agar (Tortora *et al*, 2022). All components were dissolved in approximately 900 mL of distilled water with continuous stirring and gentle heating until complete dissolution. The final volume was adjusted to 1000 mL, and the pH was adjusted to 7.4 ± 0.2 at room temperature. The medium was sterilized and stored at 2–8°C until use.

This medium was used for the differentiation of Gram-negative enteric bacteria based on fermentation of glucose and lactose and hydrogen sulphide (H₂S) production (Atlas, 2010).

3.3.11. Urea agar medium

medium was formulated by incorporating 15 ml of urea solution, sterilized using filtering (0.22 µm), into 100 ml of urea agar base, sterilized through autoclaving at 121°C for 15 minutes and subsequently cooled to 50°C. The pH was modified to 7.1, after which the medium was allocated into sterile test tubes and permitted to solidify in a slanted configuration. This medium was utilized to evaluate the capacity of bacteria to synthesize the urease enzyme (Mahon and Manuselis, 2007).

3.3.12. Gelatin liquefaction medium

One hundred twenty grams of gelatin were incorporated into 1000 ml of warm nutrient broth at 50°C, brought to a boil to ensure complete dissolution, and subsequently pipetted into test tubes. Autoclaved at 15 psi (121°C) for 15 minutes for sterilization. Permit the tubed medium of cooling while positioned up right. This medium was utilized to assess the capacity of microorganisms for liquefaction of gelatin (Isenberg and Garcia, 2004).

3.3.13. Luria-Bertani broth (LB)

This medium was created by dissolving 10g of tryptone, 5g of yeast extract, and 10g of NaCl in 1000ml of distilled water. The pH was adjusted to 7.2 and subsequently sterilized using autoclaving at 121°C for 15 minutes. This medium was utilized to culture bacterial isolates (Bertani, 2004).

3.3.14. Luria-Bertani agar (LB)

LB medium was prepared as above for LB broth, then 10 g/L agar were added before being autoclaved. It is used for the cultivation bacterial strain. (Bertani, 2004).

3.4. Samples collection

A total of 150 clinical samples were collected from Al-Hilla Teaching Hospital, Marjan Teaching Hospital, and Imam Al-Sadiq Hospital during the period from September 2025 to January 2026. The collected specimens comprised urine samples obtained from patients diagnosed with urinary tract infections, (including both catheterized and non-catheterized individuals), in addition to swab samples taken from burns and wounds at the swabs were taken from the burn or wound depth.

Midstream urine specimens were gathered in sterile, screw-capped containers. Subsequently, the urine samples were inoculated onto appropriate culture media and incubated aerobically at 37 °C for 24 hours.

one hundred environmental swabs samples were collected including hospital wastewater and environmental swabs including patient beds, medical equipment, waste containers, tabletops, hands of healthcare personnel, sinks, and walls. Swabs were used for collecting the organisms present in an area of 10X10 cm of each surface. The swabs were first humidified in Tryptic Soy

Broth (TSB - Oxoid), then used to sample, and transported in 2 ml of TSB tubes and then culture in the laboratory (Pedro Miguel de Abreu *et al.*2014) .

3.5. Identification of bacterial isolates

3.5.1. Morphological Characteristics

One colony was extracted from each main positive culture. The identification relied on morphological characteristics, including colony size, shape, color, swarming behavior, odor, translucency, edge, and texture elevation. (Sherwood *et al.*, 2017).

3.5.2. Microscopic Characteristics

Gram stain is used to evaluate the microscopic characteristics of the isolated bacteria (Du *et al.*, 2018). Gram stain is used to identify bacterial cells' shape, size, and arrangement and interaction with Gram stain.

3.5.3 Biochemical identification

For the purpose of diagnosing isolates of *P. mirabilis*, the following tests used:

3.5.3.1 Catalase test

This test was used to determine whether bacteria could produce the catalase enzyme, which breaks down H_2O_2 into oxygen and water. A loopful of colony growth was transferred to the surface of a clean, dry glass slide, and a drop of (3 percent) was hydrogen peroxide gently mixed . The presence of gaseous bubbles indicates a positive result. The creation of oxygen bubbles revealed the existence of the enzyme in the bacterial isolate, whereas the absence of bubble generation indicates the absence of catalase (Forbes *et al.*, 2007).

3.5.3.2 Oxidase test

The oxidase test was performed by transferring a single bacterial colony onto filter paper using a sterile wooden applicator stick, followed by the addition of

2–3 drops of 1% oxidase reagent. The development of a dark purple color within 10 seconds was considered a positive reaction, whereas the absence of color change indicated a negative result. *Proteus* species showed negative oxidase activity, consistent with their classification within the Enterobacteriaceae family (Forbes *et al.*, 2007).

3.5.3.3 Methyl-red test

The Methyl Red (MR) test was performed to evaluate the ability of *Proteus mirabilis* to produce stable acidic end products from glucose fermentation. A pure colony was inoculated into MR-VP broth and incubated at 37°C for 24–48 hours. After incubation, 3–5 drops of methyl red reagent were added. The appearance of a red color indicated a positive result ($\text{pH} \leq 4.4$), confirming mixed acid fermentation, whereas a yellow color indicated a negative result. *Proteus mirabilis* typically shows a positive MR reaction, supporting its biochemical identification.(Budiarto *et al.*, 2021) .

3.5.3.4 Voges-proskauer test

The Voges–Proskauer (VP) test was performed by inoculating MR-VP broth with the bacterial culture, followed by incubation at 37°C for 24–48 hours. After incubation, 0.5 mL of α -naphthol reagent and 0.2 mL of potassium hydroxide (KOH) solution were added to each tube with gentle shaking. The tubes were then left undisturbed for 10–15 minutes to allow color development. The appearance of a pink color indicates a positive reaction, which reflects the production of acetoin (acetyl methyl carbinol) (Sastry & Bhat, 2018).

3.5.3.5 Citrate utilization test

The Citrate utilization test was performed to assess the ability of *Proteus mirabilis* to use citrate as a sole carbon source. The isolate was inoculated onto Simmons citrate agar and incubated at 37°C for 24–48 hours. A positive result

was indicated by growth with a color change of the medium from green to blue, while no growth and no color change indicated a negative result. *Proteus mirabilis* typically gives a negative citrate reaction, supporting its biochemical identification. (Riddell & Sanyal, 2025).

3.5.3.6 Gelatinase test

The Gelatinase test was performed to evaluate the ability of *Proteus mirabilis* to hydrolyze gelatin by producing gelatinase enzyme. The isolate was inoculated into nutrient gelatin medium and incubated at 37°C for 24–48 hours. After refrigeration at 4°C for 15–30 minutes, liquefaction of the medium indicated a positive result, while solidification indicated a negative reaction. *Proteus mirabilis* typically shows a positive gelatinase activity. (Riddell & Sanyal, 2025).

3.5.3.7 Urease test

The urease test was performed by inoculating urea agar slants with the bacterial suspension by streaking the whole surface of the slant. The tubes were incubated with loosened caps at 37°C for 16–24 hours. After incubation, the medium was examined for color change. Urease production was indicated by a change in the color of the medium from yellow or orange-yellow to pink, indicating a positive urease reaction (Tille, 2022).

3.5.3.8 Kligler Iron Agar test

Kligler's Iron Agar (KIA) test was performed by inoculating the medium with a pure bacterial colony using a sterile inoculating needle. The butt of the medium was stabbed first, followed by streaking the slant surface. The inoculated tubes were incubated at 37°C for 18–24 hours. The test was used to assess glucose and lactose fermentation, hydrogen sulfide (H₂S) production, and gas

production by *Proteus mirabilis*. Results were interpreted based on changes in the slant and butt reactions. A red slant with a yellow butt (K/A) indicated glucose fermentation only, whereas a yellow slant with a yellow butt (A/A) indicated fermentation of both glucose and lactose. A red slant with a red butt (K/K) indicated no fermentation of either sugar. The formation of a black precipitate indicated H₂S production; in such cases, the butt was interpreted as acidic even if the yellow color was masked by blackening. Gas production was observed as cracks, bubbles, or lifting of the agar medium (Budiarto *et al.*, 2021).

3.5.4. Identification by vitek® 2 compact

Following biochemical and Gram staining tests to confirm the identification of *Proteus*, the samples were grown on MacConkey agar and incubated at 37 °C for 24 hours to achieve pure colonies for Vitek® 2 Compact Identification. The procedure was as follows:

A sterile disposal plastic loop was employed to extract pure colonies from the MacConkey agar medium and transfer them into a can tube containing approximately 3 ml of sterile saline solution (NaCl 0.45% to 0.50%, pH 4.5 to 7.0), which was subsequently mixed by vortexing.

The turbidity of the suspension was quantified using Densichek. The turbidity must range from 0.50 to 0.63 GN (Gram Negative) cards were infected with bacterial suspension. A tube carrying the suspension was positioned in a specialized rack (cassette), and the identification card was placed alongside it.

Each card possesses a unique identifier (code) obtained by the Vitek scanner, followed by the completion of a form detailing the sample information contained within each tube, facilitated by a specialized computer linked to the Vitek® 2 compact. A specialized rack was positioned within the filling door, awaiting a brief interval, while the tubes were filled with samples according to

the specific mechanism of the Vitek® 2 compacts; subsequent to filling, the tubes were severed. The Vitek ® 2 compact signals the conclusion of the procedure with a distinctive sound.

The rack with the samples was manually moved to the loading door for incubation at 37 °C, with findings provided after 6 to 12 hours. The test report was generated for each card within the incubator in accordance with the manufacturer's instructions for the Vitek ® 2 compact.

3.6. Virulence factors assay

3.6.1. Haemolysin production

Most haemolytic bacteria were evaluated by culturing on 5% human blood agar at 37°C for 24 hours. Blood Agar is regarded as a differential medium for hemolysis, categorizing microorganisms into three groups based on their capacity to degrade hemoglobin or red blood cells: alpha-hemolysis (α -hemolysis), characterized by a green to light-brown halo surrounding the colonies; beta-hemolysis (β -hemolysis), marked by a clear zone around the colonies; and gamma-hemolysis (γ -hemolysis), where no hemolysis is detected (Dulczak and Kirk, 2005).

3.6.2. Urease production

I urea slant was inoculated with a bacterial suspension over the entire surface and incubated the tubes with loose caps at 37°C, and subsequently observed the color change of the medium after 16 hours of incubation. Urease production was shown by a change in medium color to pink (Winn *et al.*, 2006).

3.7. Sterilization methods

Dry sterilization is employed to sterilize glassware at temperatures ranging from 160 to 180 °C for a duration of 2 to 3 hours, whereas moist heat sterilization is

utilized for sterilizing media and specific solutions that are heat-stable. This was conducted in an autoclave at a pressure of 15 psi at 121 °C for 15 minutes. Filtration with 0.22 µm diameter Millipore filters was employed for fluids susceptible to heat denaturation (Levinson, 2025).

3.8. Maintenance of Bacterial Isolates

3.8.1. Short Term Maintenance

A single pure colony of bacterial isolate was streaked onto nutrient agar slants and incubate at 37°C for 24 hours. Subsequently, stored in a refrigerator at 4°C. This technique was employed to preserve bacterial isolates for a duration of two to three weeks for slants (Prescott and Harley, 2002).

3.8.2 Long Term Storage

Bacteria preserved for six months in nutrient broth or brain heart infusion containing 15% glycerol at -20 °C without loss of viability. 0.15ml of sterilized glycerol was incorporated into bacterial cultures in tiny vials of nutrient broth or brain heart infusion, achieving a final volume of 1 ml, and subsequently stored at -20 °C (Vandepitte *et al.*, 2003).

3.9. Antibiotic Susceptibility test

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) (Paluch *et al*, 2023).

3.10. Colony count

The total viable bacterial count was determined using the spread plate technique. Serial ten-fold dilutions of the bacterial suspension were prepared using sterile normal saline under aseptic conditions. From appropriate dilutions, 0.1 mL of each dilution was aseptically transferred onto the surface of sterile nutrient agar plates. The inoculum was evenly distributed over the agar surface using a sterile L-shaped glass spreader, ensuring uniform distribution of bacterial cells.

The plates were allowed to absorb the inoculum for a few minutes and then incubated in an inverted position at 37°C for 24 hours. After incubation, plates containing 30–300 colonies were selected for accurate enumeration. Colonies were counted manually, and the results were expressed as colony-forming units per milliliter (CFU/mL).

Colonies of the spread plate cultures were directly counted (only plates having 30–300 colonies) and recorded as a function of colony forming units per milliliter (cfu/mL) (Jadhav *et al.*, 2024) using the following formula :

$$\text{CFU/ml} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (ml)}}$$

3.11. Preparation of Standard Heavy Metal Solutions

Standard solutions of four heavy metals (ZnCl_2 , CuCl_2 , CdCl_2 , CrCl_2) were prepared by dissolving 200 mg of each metal in 10 mL of deionized distilled water to obtain a stock concentration of 20 mg/mL. The solutions were thoroughly mixed until complete dissolution was visually confirmed, and the pH of each prepared solution was checked before use. Subsequently, the solutions were sterilized by filtration through 0.22 μm membrane filters and stored in tightly sealed sterile containers at 4°C until use (Lima *et al.*, 2012). Different concentrations were then prepared by dilution using the equation ($C_1V_1 = C_2V_2$), where the basic concentrations were diluted by adding deionized distilled water.

3.12. Metal resistance profiles

Proteus mirabilis isolates were assessed for their tolerance to selected heavy metal salts, including zinc chloride (ZnCl_2), copper(II) chloride (CuCl_2), cadmium chloride (CdCl_2), and chromium(II) chloride (CrCl_2), at final concentrations of (0.625, 1.25, 2.5, 5, 10, 20 mg/mL). Wells without metal treatment were used as controls. Overnight bacterial cultures grown for 24 h

were adjusted to a 0.5 McFarland turbidity standard and then diluted with sterile nutrient broth to obtain a final inoculum of 5×10^5 CFU/mL. The final inoculum was added to 96-well microtiter plates containing nutrient broth supplemented with the respective heavy metal concentrations. All experiments were performed in technical triplicates. The plates were incubated at 28°C for 24–48 h under aerobic conditions to evaluate heavy metal tolerance under environmental growth conditions. Bacterial growth was recorded at both 24 and 48 h. Bacterial growth was evaluated by determining percentage cell viability relative to the control. Bacterial growth inhibition was evaluated using the broth microdilution method by determining MIC and IC₅₀ values. After incubation, bacterial growth was quantified by measuring optical density at 600 nm (OD₆₀₀) using a microplate reader. Cell viability (%) was calculated relative to the untreated control wells, and IC₅₀ was defined as the heavy metal concentration that reduced bacterial viability by 50%. Higher cell viability indicated greater bacterial tolerance to heavy metals, whereas lower values indicated growth inhibition and increased sensitivity. The MIC endpoint was defined as the lowest heavy metal concentration that produced $\geq 90\%$ inhibition of bacterial growth compared with the untreated control, based on OD₆₀₀ readings.

3.13. Determination of IC₅₀ Using Bacterial Viability Dose–Response Curves

The IC₅₀ value was determined based on dose–response curves constructed by plotting the percentage of cell viability of the bacterial isolates against increasing concentrations of the tested compound (0–20 mg/mL). The bacterial isolates were exposed to different concentrations, and the cell viability was measured for each concentration. Subsequently, a relationship between concentration and percentage viability was established by constructing a

graphical dose–response curve. The IC₅₀ value was defined as the concentration of the tested compound that reduced cell viability to 50%, and it was estimated from the curve using statistical regression analysis.

3.14. Molecular detection

3.14.1. DNA Extraction

The method was prepared according to the genomic DNA purification Kit provided by the manufacturing company (Favorgen, Taiwan).

1. One milliliter of an overnight bacterial culture was transferred into a sterile 1.5 mL microcentrifuge tube and centrifuged at 14,000 rpm for 2 min. The supernatant was completely discarded, and the bacterial pellet was retained.
2. The bacterial pellet was resuspended in 200 µL FATG1 Buffer by vortexing until completely mixed.
3. Twenty µL of Proteinase K was added to the sample mixture and mixed thoroughly by vortexing.
4. The sample was incubated at 60°C until complete lysis was achieved (approximately 1–3 h), with occasional vortexing during incubation.
5. Two hundred µL of FATG2 Buffer was added to the lysed sample, mixed thoroughly by pulse-vortexing, and incubated at 70°C for 10 min.
6. Two hundred µL of 96–100% ethanol was added to the sample and mixed thoroughly by vortexing.
7. The mixture was briefly centrifuged to remove any droplets from the inner surface of the tube lid.
8. The mixture, including any precipitate, was carefully transferred to a FATG Mini Column placed in a collection tube and centrifuged at 14,000 rpm for 1

min. The flow-through was discarded, and the column was transferred to a new collection tube.

9. Four hundred μL of W1 Buffer was added to the FATG Mini Column, followed by centrifugation at 14,000 rpm for 1 min. The flow-through was discarded.
10. Seven hundred and fifty μL of Wash Buffer was added to the column and centrifuged at 14,000 rpm for 1 min. The flow-through was discarded.
11. The column was centrifuged at 14,000 rpm for an additional 3 min to remove residual wash buffer.
12. The dried FATG Mini Column was transferred into a clean sterile 1.5 mL microcentrifuge tube. One hundred μL of preheated Elution Buffer was added directly onto the center of the membrane. The column was left for 3 min to allow complete absorption of the buffer and then centrifuged at 14,000 rpm for 2 min to elute the genomic DNA.
13. The extracted DNA was stored at -20°C until further molecular analysis.

3.14.2 Preparation of PCR primers

The primers for antibiotic resistance genes (*acrB*, *acrA*, *blaCTX-M*, *blaTEM*) were selected as shown in Table (3-7) and their sequences were verified using NCBI.

Lyophilized primers were obtained from Alpha DNA (Koren). The primers were dissolved in TE (Tris-EDTA) buffer to prepare a stock solution at a concentration of 100 μM , which was stored at -20°C .

Working solutions for each primer were prepared separately at a concentration of 10 μM by diluting 10 μL of the stock solution with 90 μL of nuclease free water, mixed using a vortex, and stored at 20°C until use.

3.15. Amplification of antibiotic resistance genes in *p. mirabilis*

PCR amplification was performed to detect the presence of antibiotic resistance genes (*acrA*, *acrB*, *blaCTX-M* and *blaTEM*) among *Proteus* isolates. Specific primers targeting each gene were used in individual PCR reactions. Amplification was carried out in a total reaction volume of 25 µL containing 12.5 µL of 2× PCR master mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1µL of genomic DNA template, and nuclease-free water to complete the final volume(8.5 µL) as shown in table (3-7).

amplification of the β-lactamase gene (*blaTEM*), PCR was performed under the following conditions: initial denaturation at 95°C for 2 minutes, Followed by denaturation at 95°C for 1 min, annealing at 58°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 minutes. (Bajaj *et al.*, 2015) as shown in table (3-8).

amplification of the β-lactamase gene (*blaCTX-M*), PCR was performed under the following conditions: initial denaturation at 95°C for 2 minutes, Followed by denaturation at 95°C for 1 min, annealing at 63°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 minutes. (Bajaj *et al.*, 2015) as shown in table (3-9).

For detection of the efflux pump genes (*acrA* and *acrB*), the thermocycling conditions consisted of an initial denaturation at 95°C for 2 minutes, Followed by denaturation at 95°C for 1 min, annealing at 58.3°C for *acrA* and 60.3°C for *acrB* for 30 sec, and extension at 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. (Ali and Al-Dahmoshi 2022) as shown in table (3-10).

Table (3- 7) Preparation of PCR reaction mixture.

PCR	Volume (μL)
Master mix	12.5 μl
Reverse primer	1 μl
Forward primer	1 μl
DNA template	2 μl
Nuclease -free water	8.5 μl
Total	25 μl

Table (3-8) Program of PCR for *blaTEM* gene

Step	Temperature (°C)	Time	No. of cycle
Initial denaturation	95°C	2min	1Cycle
Denaturation	95°C	1 min	30Cycle
Annealing	58°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	5min	1Cycle
Hold Temperature	4°C	–	

Table (3-9) Program of PCR for *blaCTX-M* gene

Step	Temperature (°C)	Time	No. of cycle
Initial denaturation	95°C	2min	1Cycle
Denaturation	95°C	1 min	
Annealing	63°C	30 sec	

Extension	72°C	1 min	30Cycle
Final extension	72°C	5min	1Cycle
Hold Temperature	4°C	–	

Table (3-10) Program of PCR for (*acrA,aceB*) gene.

Step		Temperature (°C)	Time	No. of cycle
Initial denaturation		95°C	2min	1Cycle
Denaturation		95°C	1 min	30Cycle
Annealing	<i>acrA</i>	58.3°C	30 sec	
	<i>acrB</i>	60.3°C		
Extension		72°C	1 min	
Final extension		72°C	5min	1Cycle
Hold Temperature		4°C	—	

3.15.1. Detection of PCR products by agarose gel electrophoresis

To confirm the existence of DNA after PCR, the following method was done according to (Sambrook and Russel, 2001) :-

1- Preparing the agarose gel

Agarose was prepared by dissolving 1 g of agarose in 100ml of TBE (1X).The mixture was heated until agarose dissolved by using a microwave, left until cool, then 2 µl of the ethidium bromide was added into the agarose gel before pouring.

2- The casting of agarose gel

A- The casting tray was prepared with the cathode up and anode down. The gel casting was placed inside a casting tray.

B- The comb was placed at one end of the tray.

C- Agarose solution was poured into the casting gel and let to cool until it hardens.

D- After hardening, combs are carefully removed.

E- TBE buffer was added to the casting tray until buffer over the gel.

3- Loading and Running DNA on Agarose Gel

A- 2 μ l loading dye (bromophenol blue) was mixed with 5 μ l of the DNA product, then loaded into the well.

B- Before the electricity was turned on the position of the cathode and anode were checked.

C- The power supply was turned on at 70V for 60 min until the blue dye approaches the end of the gel.

D- DNA bands were detected and examined under UV transilluminator, then photographed by a digital camera. DNA product was stored at -20 ° until it can be used later.

3.16. Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Chi-square test was used to study association between any two categorical variables. Odds ratio and 95% confidence interval was estimated to measure risk. The level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less (Daniel, 2009).

Chapter Four :Results & Discussion

4.1 Isolation and identification of *proteus ssp*

4.1.1. clinical samples

In this study, a total of 150 clinical samples were collected from patients of both sexes and different age groups. By performing laboratory and biochemical tests, 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*, whereas the remaining isolates belonged to other bacterial species (table 4-1) (table 4-2). The highest proportion of isolates was obtained from urinary tract infection samples 13(65%), followed by wound amples 4(20%), and burn samples 3(15%). (table 4-3).

(Table 4-1): Number and percentage of *Proteus mirabilis* that isolated from clinical cases

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 4-2): Other bacterial species recovered from clinical samples

Bacterial species	Number of isolates	Percentage (%)
<i>Escherichia coli</i>	20	28.57
<i>Staphylococcus aureus</i>	15	21.43
<i>Pseudomonas aeruginosa</i>	12	17.14
<i>Klebsiella oxytoca</i>	8	11.43

<i>Enterobacter hormaechei</i>	7	10.00
<i>Clostridium perfringens</i>	5	7.14
<i>Salmonella typhimurium</i>	3	4.29
Total	70	100.00

Table (4-3): 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 $p < 0.05$

Table (4-4) shows the distribution of *Proteus*-positive cases according to age groups and gender. The results indicate that the highest percentage of infection was recorded in the age group (40–50) years at (35%), followed by the (30–40) age group at (30%), then the (50–60) age group at (20%), while the lowest percentage was observed in the (20–30) age group at (15%).

The higher rate of infection in the middle age groups (30–50 years) may be attributed to increased exposure to risk factors such as recurrent urinary tract infections and medical interventions, in addition to physiological changes that may affect immune system efficiency compared to younger individuals. (Aggarwal & Leslie, 2025).

the results showed no significant differences among the age groups, as the P-value was (0.99), which is higher than (0.05). This indicates that the variations

in infection rates between age groups are likely random and not statistically significant.

Overall, these findings suggest that although there is an apparent variation in infection distribution across age groups, this variation is not statistically significant.

Table (4-4): Distribution of *Proteus*-positive cases according to age groups

Age Ranges (Years)	<i>Proteus</i> -positive (n)	Percent age (%)	P value
20-30	3	15%	0.99 NS
30-40	6	30%	
40-50	7	35%	
50-60	4	20%	
Total	20	100	

* represent a significant difference at $p < 0.05$. NS: not significant

Table (4-5) presents the distribution of patients according to sex and the distribution of *Proteus*-positive cases. Among the 150 patients included in the study, 90 (60%) were females and 60 (40%) were males. A total of 20 *Proteus*-positive cases were identified, including 14 (70%) females and 6 (30%) males. Although the proportion of *Proteus*-positive cases was higher among females compared with males, the difference was not statistically significant ($P = 0.33$). This difference may be attributed to physiological and anatomical factors, as the female urethra is shorter and closer to the anus than in males, facilitating the ascension of intestinal flora to the urinary tract and increasing the risk of urinary tract infections (UTIs). Additionally, the female urethra is less effective in preventing bacterial entry and colonization. (Baimakhanova *et al.*, 2025).

(Table. 4-5) Distribution of *Proteus*-positive cases according to Sex

Sex	No. of Patients (Total)	Prevalence (%)	Proteus-positive (n)	Distribution of Proteus-positive cases (%)	P value
Female	90	%60	14	%70	0.33 NS
Male	60	%40	6	%30	
Total	150	%100	20	%100	

n:numberofcases

* represent a significant difference at $p < 0.05$. NS: not significant

The findings of the present study are consistent with several recent investigations that have highlighted the clinical significance of *Proteus spp.* as one of the common causative agents of urinary tract infections (UTIs). In a large-scale study conducted in the Mardan region of Pakistan in 2024, Abdullah (2024). reported that *Proteus spp.* accounted for 17% of urinary isolates from patients with UTIs, confirming the prominent role of this bacterium among uropathogens in recent clinical studies.

Similarly, a recent study published in Minya Governorate, Egypt, in 2025 by al-Sayed (2025) demonstrated that *Proteus spp.* were isolated from 20% of UTI cases, with a higher prevalence observed in catheter-associated samples compared to non-catheterized specimens, which further supports its association with hospital-acquired infections.

AL-joda *et al*, (2021) reported that *Proteus mirabilis* represented 19% of bacterial isolates recovered from patients with UTIs. This finding aligns with the present study in confirming the frequent occurrence of this bacterium, although the isolation rates varied depending on population characteristics and geographical location.

The current results also agree with several previous Iraqi studies. Khan *et al* (2022) successfully isolated *Proteus mirabilis* from urine samples at a rate of 10%. Likewise, Saleh *et al*, (2022) and Jasim *et al*, (2024) reported isolation rates of 12.6%, and 9.6%, respectively, while Rafiq *et al*, (2026) recorded a higher rate of 19% .

On the other hand, lower isolation rates were reported in Baghdad by Al-Mudallal *et al*, (2021) and in Diyala by AL-Ezzy *et al*, (2023), at 8% and 6%, respectively. Internationally, Suhartono *et al*, (2022) in Indonesia reported an isolation rate of 7.4% for *Proteus mirabilis* from urine samples.

In contrast, several studies reported markedly higher isolation rates compared to the present study. Muhanna *et al*, (2020) in Maysan, Jaber and Almiyah (2022) in Al- Abdulrraziq, and Al-Nabhani and Shami (2023) documented isolation rates of 37%, 32%, and 35.3%, respectively.

Furthermore, several previous Iraqi studies have reported the presence and clinical importance of *Proteus mirabilis* in urinary tract infections (UTIs). Owaied and Jabur (2022) reported that *P. mirabilis* accounted for approximately 8.6% of clinical isolates obtained from patients in Thi-Qar province, with a significant proportion of isolates recovered from urinary tract infections. Similarly, Al-Khalidy and Aburesha (2023) identified *P. mirabilis* in 60 isolates (35.2%) out of 170 urine samples collected from UTI patients in Baghdad hospitals, indicating a relatively higher prevalence in clinical settings

Additionally, recent studies have reported variable isolation rates of *Proteus spp.* from patients with UTIs. For instance, a systematic review and meta-analysis from Iran by Shafaei Fallah *et al*, (2024) reported a pooled prevalence of 9.3% for *Proteus spp.* among urinary isolates from patients with UTIs. Similarly, a study in Egypt by Abdelwahab *et al* (2024) observed *Proteus spp.* as one of the common uropathogens in hospitalized patients, though the exact

percentage varied across different settings. This difference in prevalence rates among several studies can be attributed to differences in hygienic practices, population characteristics, and geographical location

The present study demonstrated that *Proteus* infection was more common in females (15.56%) than males (10.00%). This finding is consistent with the study conducted by Foxman (2014), who reported that urinary tract infections, including those caused by *Proteus spp.*, are significantly higher in females, with approximately 70–80% of cases occurring in women due to anatomical and physiological factors.

Similarly, Flores-Mireles *et al*, (2015) found that female patients accounted for nearly 65% of *Proteus mirabilis* urinary tract infections, attributing this to the shorter urethra and easier bacterial ascension in females.

In addition, Schaffer *et al*, (2013) reported that the proportion of female patients infected with *Proteus* ranged between 65–72% of total cases, supporting the findings of the current study.

In another study conducted by Armbruster and Mobley (2012), *Proteus mirabilis* represented nearly 60% of urinary isolates in females compared to a much lower rate in males, particularly in uncomplicated urinary tract infections.

In addition, Jacobsen *et al*, (2008) reported that *Proteus mirabilis* was isolated more frequently in adult females, representing about 60–75% of total positive urinary isolates.

Regarding age distribution, the current study showed that the highest infection rate was observed in the age group 30–50 years. This agrees with the findings of Klein and Hultgren (2020), who stated that UTIs caused by *Proteus spp.* are most prevalent in adults aged 30–60 years, with an incidence rate of

approximately 40–55%, mainly due to increased exposure to healthcare settings and risk factors such as catheterization.

Furthermore, O'Hara *et al*, (2000) reported that middle-aged and elderly patients showed higher susceptibility to *Proteus* infections, with rates reaching up to 50% in complicated urinary tract infections.

Moreover, This finding is in agreement with Armbruster & Mobley, (2012), who found that the highest incidence of *Proteus* infection occurred in adults aged 30–60 years, with a prevalence of approximately 45–55%.

In addition, Marrs *et al*, (2005) reported that infections were more frequent in middle-aged and elderly individuals, with positive isolate rates reaching about 50%, mainly due to increased exposure to urinary catheterization and weakened immune response.

4.1.2. Environmental samples

A total of 100 environmental samples were collected, including 55 hospital wastewater samples from three hospitals (Al-Hilla Teaching Hospital, Marjan Teaching Hospital, and Imam Al-Sadiq Hospital), as well as 45 environmental swab from various locations. These included patient beds, medical equipment, waste containers, tabletops, healthcare workers' hands, sinks, and walls. All samples were collected following standard aseptic procedures to prevent contamination during transportation and handling.

All samples were subjected to confirmatory laboratory tests, including culture on selective and non-selective media, incubation under appropriate conditions, and identification using the VITEK system.

No *Proteus spp.* isolates were detected in any of the samples, as reported in Appendix (1).

Although *Proteus spp.* was absent, other bacterial species were identified, including (*Pseudomonas aeruginosa* , *Staphylococcus spp*, *klebsiella spp*, *Salmonella spp* , *Citrobacter amalonaticus* , *Shigella spp* , *Aeromonas sobria* , *Moraxella spp*, *Myroides ssp* , *Brevundimonas diminuta* , *Campylobacter spp*, *Oligella ureolytica*). as show in Table (4-6).

Table (4-6) : Bacterial species isolated from environmental samples

Bacterial species	Number of isolates	Percentage (%)
<i>Pseudomonas aeruginosa</i>	25	25.0
<i>Staphylococcus spp</i>	15	15.0
<i>Klebsiella spp</i>	12	12.0
<i>Salmonella spp</i>	10	10.0
<i>Citrobacter amalonaticus</i>	8	8.0
<i>Shigella spp</i>	7	7.0
<i>Aeromonas sobria</i>	6	6.0
<i>Moraxella spp</i>	5	5.0
<i>Myroides spp</i>	4	4.0
<i>Brevundimonas diminuta</i>	3	3.0
<i>Campylobacter spp</i>	2	2.0
<i>Oligella ureolytica</i>	3	3.0
Total	100	100.0

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 (Al-Otaibi *et al.*, 2025). 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 (Prüss-Ustün *et al.*, 2019).

These microbial contaminants not only threaten human health but also impact aquatic organisms, disrupting ecosystems. Improving water management, enforcing stricter pollution regulations, and developing advanced water treatment technologies are critical steps to reduce the burden of waterborne diseases (Ali *et al.*, 2025).

High colony-forming unit (CFU/mL) values in wastewater indicate a significant level of viable microbial contamination, highlighting the importance of effective water safety and treatment measures. In this study, CFU/mL values indicated a high viable bacterial load in hospital wastewater samples. This finding is expected, as hospital wastewater typically contains organic matter, human excreta, pharmaceutical residues, disinfectant remnants, and microorganisms originating from patients, healthcare workers, laboratories, and clinical wards. These conditions create a nutrient-rich environment that promotes the survival and proliferation of various bacterial groups. (Lohbrunner *et al.*, 2026).

High colony-forming unit (CFU/mL) values were observed in the hospital wastewater samples. The highest recorded count reached 81×10^3 CFU/mL. Two additional samples showed high bacterial loads of 61×10^3 and 54×10^3 CFU/mL, whereas the remaining countable samples ranged from 1.62×10^3 to 40×10^3 CFU/mL as shown in appendix (2). These elevated counts may be attributed to the nutrient-rich nature of hospital wastewater, which contains organic matter, pharmaceutical residues, human excreta, and a wide variety of microorganisms. Such conditions provide a favorable environment for bacterial survival and proliferation (Al-Gheethi *et al.*, 2021).

Compared with previous studies, some of the recorded counts were higher than those reported by Okafor *et al* (2023), who found bacterial counts in hospital wastewater ranging from 2.4×10^5 to 6.5×10^5 CFU/mL. This increase may be attributed to the collection of samples directly from untreated hospital wastewater, which is characterized by high microbial and organic loads (Asfaw *et al.* 2017).

The differences observed between this study and previous investigations may be attributed to variations in hospital size, patient number, ward type, sampling season, sampling location, wastewater treatment status, dilution methods, culture media, incubation conditions, and bacterial groups analyzed. Therefore, direct numerical comparisons of CFU/mL values should be interpreted cautiously. Nevertheless, the general trend remains consistent with prior research, indicating that hospital wastewater may contain substantial viable bacterial loads and serve as a reservoir for pathogenic and opportunistic microorganisms (Verlicchi *et al.*, 2020).

Overall, the elevated CFU/mL values demonstrate that hospital wastewater represents a heavily contaminated microbial environment. The predominance of *Pseudomonas aeruginosa*, together with enteric and opportunistic bacteria, indicates that untreated hospital wastewater can act as an important reservoir for

pathogenic microorganisms. Consequently, these findings highlight the necessity for effective wastewater treatment, disinfection, and continuous microbiological monitoring prior to environmental discharge. These results are consistent with earlier studies showing that hospital water systems can harbor significant bacterial populations depending on sanitation practices and source type (Yin *et al.*, 2023). Elevated bacterial levels in hospital water may indicate potential contamination risks and emphasize the need for routine monitoring and effective disinfection procedures (Hounmanou *et al.*, 2026).

4.2. Identification of *proteus spp* .

4.2.1. Cultural Features of *proteus spp*.

The bacterial isolates were identified based on their morphological and cultural characteristics following growth on selective and differential media. On MacConkey agar the colonies appeared pale and colorless, confirming the inability of the isolates to ferment lactose. The colonies were circular in shape with entire margins, low to slightly raised elevation, and a smooth, translucent surface. The colony diameter ranged between 1–3 mm after 18–24 hours of incubation Figure (4-1A). The absence of swarming motility on this medium can be attributed to the presence of bile salts, which inhibit the rapid motility of *Proteus* species and facilitate differentiation from lactose-fermenting organisms such as *Escherichia coli* (Ali *et al.*, 2025).

In contrast, growth on Blood agar revealed characteristic cultural features of *Proteus* species. The isolates demonstrated the distinctive swarming phenomenon, whereby bacterial growth spread extensively over the surface of the medium in concentric waves, forming a thin, translucent film. Prior to the onset of swarming, individual colonies were relatively small, measuring approximately 1–2 mm in diameter, pale in color, circular in shape, with smooth margins, low to slightly raised elevation, and a smooth, translucent surface.

Following the initiation of swarming, the colony margins became irregular, while the growth extended to cover a wide area of the agar surface. Some isolates also showed β -hemolysis, surrounded by a clear zone, indicating the lysis of red blood cells due to the production of hemolysins as shown in Figure (4- 1B), (Mahmood *et al.*, 2025).

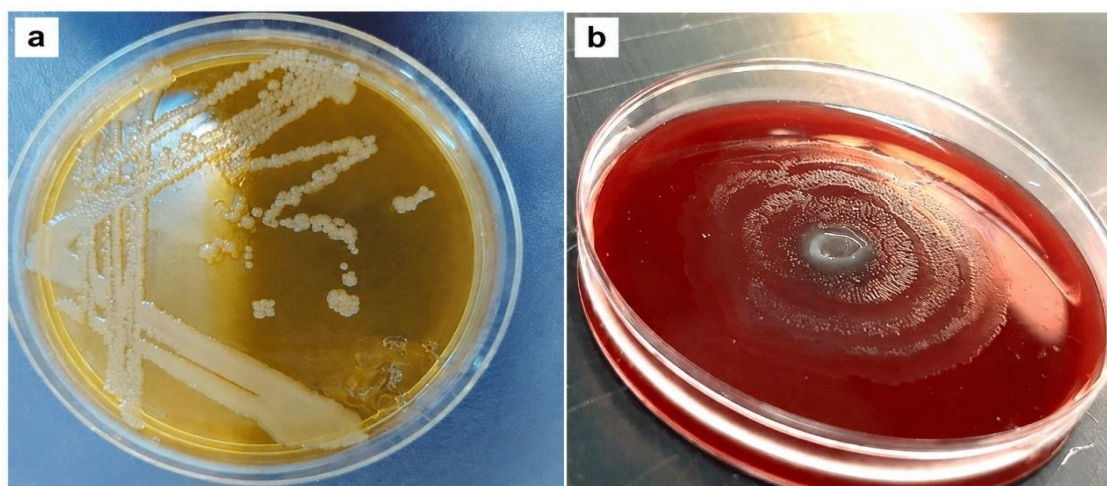


Figure (4-1) Characteristics of *proteus* colonies on A: MacConkey agar B: Blood agar

4.2.2. Microscopic Examination of bacterial isolates.

The Gram-stained bacterial cells appeared as short rod-shaped, Gram-negative bacteria, and they were non-spore-forming. (Jaber, 2022)

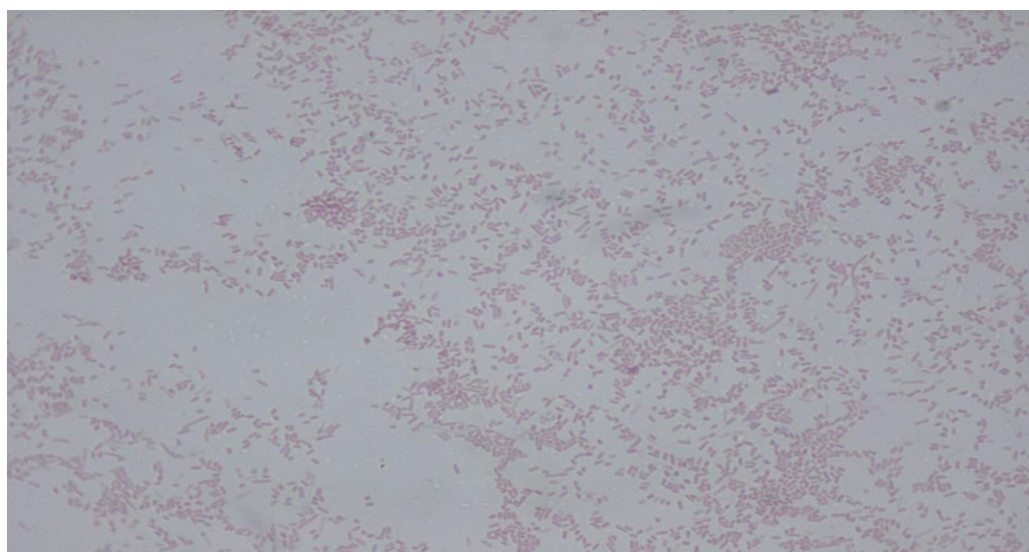


Figure (4-2) Gram stain for bacterial cells for gram-negative bacteria.

4.2.3. The biochemical Tests

4.2.3.1. Traditional Biochemical tests

For confirmation of identification, a series of biochemical tests was performed, and the results are presented in Table (4-7). 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 (H₂S) production, and the urease test was also positive. These biochemical characteristics are consistent with the standard profile of *Proteus mirabilis*, there by confirming the identification of the studied isolates (Hareza *et al.*, 2025).

Table (4-7) 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	+

4.2.3.2. Results of vitek2 system

Nineteen samples of *Proteus mirabilis* were diagnosed by the VITEK 2 Compact system based on biochemical tests after preliminary identification using culture media. Fourteen of 19 isolates were identified with a 98% probability, 3 of 19 isolates with a 96% probability, and 2 of 19 isolates with a 92% probability. Appendix (3) shows the full results of the VITEK 2 system.

Diagnosis using VITEK 2 Compact system gave a probability ratio for the genus and species of *Proteus mirabilis* bacteria between 92–98%. The system used a special diagnostic kit containing 47 wells inoculated with a 24-hour bacterial suspension, allowing accurate identification of most bacterial types. Minor discrepancies may occur due to contamination or the presence of more than one type of bacteria because the VITEK 2 system relies on multiple biochemical tests, which may reach up to 96 tests depending on the bacterial type, whether Gram-negative or Gram-positive.

The findings of the present study are consistent with previous reports that demonstrated the high accuracy of the VITEK 2 Compact system in the identification of Gram-negative bacteria, including *Proteus mirabilis*. Funke and Funke-Kissling (2005) reported that the VITEK 2 system provides reliable identification with accuracy exceeding 90% for most clinically relevant bacteria. Similarly, Ling *et al.* (2001) found that the VITEK 2 system showed a high level of agreement with conventional biochemical methods, with identification accuracy reaching up to 95% for Gram-negative isolates.

Furthermore, the Manual of Clinical Microbiology (2019) indicated that automated identification systems such as VITEK 2 are capable of achieving accuracy rates above 90%, particularly when pure bacterial cultures are used. However, these studies also noted that slight variations in accuracy may occur in cases of mixed infections or when dealing with atypical biochemical profiles, which may reduce the reliability of identification results. Hoshina *et al.* (2016) showed that the VITEK 2 system was able to identify *Proteus mirabilis* with an accuracy of between 95% and 98%. Some minor variations were observed in cases that contained mixed infections or bacteria with atypical biological characteristics. In a study by Singh *et al.* (2011), they noted that the accuracy of identifying *Proteus* using VITEK 2 was about 98%, but some differences were observed in isolates that had unusual biotypes or atypical growth. In a study by Alami *et al.* (2020), the VITEK 2 Compact system was evaluated in samples containing mixed infections. The study found that the system offered accuracy ranging from 96% to 98% in normal cases, but in cases of mixed infections, the results were more variable.

4.3. Antibiotic susceptibility test of *Proteus mirabilis*

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 (4-8). 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- 8) Antimicrobial susceptibility rates among *Proteus mirabilis* isolated from clinical samples (n= 19)

Antibiotic		Clinical samples (n= 19)		
No.		R	I	S
1.	Ampicillin/Sulbactam (AMP)	15.8	-	84.2
2.	Piperacillin/Tazobactam (TZP)	15.8	-	84.2
3.	Cefotaxime (CTX)	36.8	-	63.2
4.	Ceftazidime (CAZ)	36.8	-	63.2
5.	Ceftazidime/Avibactam(CAZ/AVI)	26.3	-	73.7
6.	Ceftolozane/Tazobactam (CTZ)	10.5	5.3	84.2
7.	Cefepime (FEP)	36.8	-	57.9
8.	Imipenem (IPM)	26.3	10.5	63.2
9.	Meropenem (MEM)	5.3	-	94.7
10.	Amikacin (AK)	0	21.1	78.9
11.	Gentamicin (GEN)	5.3	21.1	73.7
12.	Ciprofloxacin (CIP)	21.1	-	78.9
13.	Tigecycline (TGC)	100	-	0
14.	Colistin (CT)	100	-	0
15.	Trimethoprim/Sulfamethoxazole (SXT)	26.3	-	73.7

R : resistance; I: intermediate; S: sensitive

Antibiotic susceptibility patterns provide a valuable framework for choosing the correct antibiotic. This information is crucial for devising effective ways to prevent antibiotic resistance (Birgand *et al*, 2018). 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* (2025) 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% and 5.3%. A global study published by Kristiansen *et al* (2026), 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* (2004). 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.

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 (2002). 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* (2026).

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. (Hassuna *et al*, 2025). In contrast, lower resistance rates were observed for Ceftazidime / 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* (2012), Bouhrour *et al* (2024), and Al-Azzawi *et al* (2023) who reported resistance rates of 15% and 16.2%, and 15.7% respectively. However, these results different from Zhang *et al* (2026), who recorded 58% resistance, and Hafiz *et al* (2024), 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 (Munday *et al*, 2004). Additionally, resistance to ciprofloxacin (21.1%), ampicillin (15.8%), and ceftolozane (10.5%) was relatively low compared to other antibiotics, the result are consistent with prior reports in Iraq by Omrana *et al* (2024) 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. Similar findings were reported by Soliman *et al* (2024), Filipiak *et al* (2020), 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.

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 (Soliman *et al*, 2024). 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 a major factor driving the development of resistance in bacterial species occupying various ecological niches may vary depending on environmental conditions, as resistance can also be influenced by naturally occurring antimicrobial compounds, environmental chemical substances, and other selective pressures. Moreover, multiple bacterial resistance mechanisms are recognized to mediate resistance to antibiotics (Alam *et al*, 2022).

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 (Zhang *et al*, 2026) However, significant geographic disparities exist in resistance rates across various antibiotic classes and specific drugs within each class (Hassuna *et al*, 2025).

4.4. Minimum Inhibitory Concentrations (MIC) of Antibiotics Against Clinical Isolates

Nineteen clinical isolates of *P. mirabilis* were included in this study and subjected to MIC determination according to the Clinical and Laboratory

Standards Institute breakpoints (CLSI, 2025). The isolates demonstrated variable resistance profiles. Notably, all tested *Proteus mirabilis* isolates showed elevated tigecycline MIC values ranging from 2 to 4 µg/mL. Resistance to cefotaxime was observed, with MIC values ranging from ≤ 0.25 to 64 µg/mL, where isolates with MIC values ≥ 4 µg/mL were considered resistant. Similarly, resistance to ceftazidime was detected, with MIC values ranging from ≤ 0.12 to 64 µg/mL and resistance defined at ≥ 32 µg/mL. Cefepime resistance was also observed, with MIC values ranging from ≤ 0.12 to 16 µg/mL and resistance defined at ≥ 16 µg/mL.

For carbapenems, imipenem MICs ranged from 0.5 to 16 µg/mL, with resistance observed based on the applied interpretive criteria. This result should be interpreted cautiously, as *Proteus spp.*, including *P. mirabilis*, may exhibit naturally elevated imipenem MICs or reduced susceptibility compared with other Enterobacterales. Meropenem MICs ranged from 0.25 to 8 µg/mL, with resistance detected. Ciprofloxacin MICs ranged from 0.06 to 4 µg/mL, and resistance was observed based on the resistance breakpoint of ≥ 1 µg/mL. Amikacin showed MICs between 2-16 µg/mL, all within the susceptible range (≤ 16 µg/mL), while gentamicin MICs ranged from ≤ 1 to 16 µg/mL, with resistance defined at ≥ 8 µg/mL.

Trimethoprim/sulfamethoxazole MIC values ranged from ≤ 20 to 320 µg/mL according to the VITEK output format, and resistance was detected based on the corresponding resistance breakpoint of $\geq 4/76$ µg/mL. Colistin susceptibility was not interpreted because *P. mirabilis* is intrinsically resistant to colistin; therefore, no MIC-based susceptibility category was assigned. Piperacillin/tazobactam demonstrated good activity, with MIC values ≤ 4 µg/mL, well within the susceptible range (≤ 16 µg/mL). Ampicillin/sulbactam MICs ranged from ≤ 2 to 8 µg/mL, with resistance defined at ≥ 32 µg/mL, indicating susceptibility in most isolates.

Overall, the results revealed variable antimicrobial susceptibility patterns among the clinical *Proteus mirabilis* isolates, particularly highlighting resistance to tigecycline, cephalosporins, including cefotaxime, ceftazidime, and cefepime, and ciprofloxacin, while maintaining susceptibility to amikacin and piperacillin/tazobactam.

Table (4-9): MIC Values of Selected Antibiotics Against Clinical Isolates

N0.	Antibiotic	MIC (µg/mL)	CLSI Breakpoint	
			Susceptible breakpoint	Resistant breakpoint
1.	Ampicillin/Sulbactam	≤ 2-8	≤ 8	≥ 32
2.	Piperacillin/Tazobactam	≤ 4	≤ 16	≥ 64
3.	Cefotaxime	≤ 0.25-64	≤ 1	≥ 4
4.	Ceftazidime	≤ 0.12- 64	≤ 8	≥ 32
5.	Ceftazidime/Avibactam	≤ 0.12- 16	≤ 8	≥ 16
6.	Ceftolozane/Tazobactam	≤ 0.25 - 32	≤ 4	≥ 8
7.	Cefepime	≤ 0.12 -16	≤ 2	≥ 16
8.	Imipenem	≥ 0.5-16	≤ 4	≥ 8
9.	Meropenem	≥ 0.25 - 8	≤ 4	≥ 8
10.	Amikacin	2-16	≤ 16	≥ 64
11.	Gentamicin	≤ 1-16	≤ 4	≥ 8
12.	Ciprofloxacin	0.06 - 4	≤ 0.25	≥ 1
13.	Tigecycline	2-4	≤ 1	> 1
14.	Colistin	-	≤ 2	≥ 4
15.	Trimethoprim/Sulfamethoxazole	≤ 20- 320	≤ 2/38	≥ 4/76

The results of this study showed table (4-9) that all the tested isolates exhibited high-level resistance to Tigecycline, with MIC values ranging between 2–4 µg/mL, which is consistent with the findings of Lu *et al* (2008), which reported elevated MIC values of Tigecycline in *Proteus* isolates. Regarding cephalosporins, the *Proteus mirabilis* isolates in the current study exhibited elevated MIC values ranging from 0.25 to 64 µg/mL for Cefotaxime, ≤ 0.12 - 64 for Ceftazidime, and 0.12-16 µg/mL for Cefepime, indicating reduced susceptibility. These findings are consistent with those of Murugaiyan, J *et al* (2022), who reported high MICs for cephalosporins in resistant isolates from various clinical sources. Furthermore, the study by Al-Bassam & Al-Kazaz (2013) from Iraq supports these results, as similar elevated MIC ranges were recorded for cephalosporins in local *Proteus mirabilis* isolates. In addition, recent research, such as Owaied & Jabur (2022) demonstrated that high MICs for cephalosporins are common among resistant isolates. Regarding carbapenems, the MIC values ranged from ≥ 0.5 -16 µg/mL for Imipenem and ≥ 0.25 – 8 for Meropenem, which is partially consistent with the results of local studies, including Hamprecht *et al* (2023), and Kanzari *et al* (2018). The MIC values for Gentamicin ranged from 1 to 16 µg/mL, while those for Amikacin ranged from 2 to 16 µg/mL, indicating moderate susceptibility. These results are consistent with a local study of Stolarek, *et al* (2022). As for Trimethoprim–Sulfamethoxazole, the isolates exhibited a very wide range of MIC values, less than 20 µg/mL and greater than 320 µg/mL, indicating the presence of highly resistant strains. These results are consistent with a global study by Hassuna *et al* (2025) in France, as well as regional studies that reported elevated MIC values for this antibiotic in resistant *Proteus* isolates.

4.5.Molecular Detection of Antibiotic Resistance Genes

Polymerase chain reaction (PCR) was used for the molecular detection of antibiotic resistance genes (*acrA*, *acrB*, *TEM*, and *CTX-M*) among *Proteus*

mirabilis isolates. The amplified PCR products were analyzed by agarose gel electrophoresis to determine the presence or absence of the target genes. The results are illustrated in Figures (4-3),(4-4), (4-5), (4-6).

4.5.1. Detection of *acrA* Gene

PCR analysis revealed the presence of the *acrA* gene in all *P. mirabilis* isolates, with an amplicon size of (256bp). All tested isolates showed positive amplification, indicating a prevalence rate of (100%), as shown in Figure (4-3). This finding suggests a widespread distribution of efflux pump-related resistance mechanisms among the isolates.

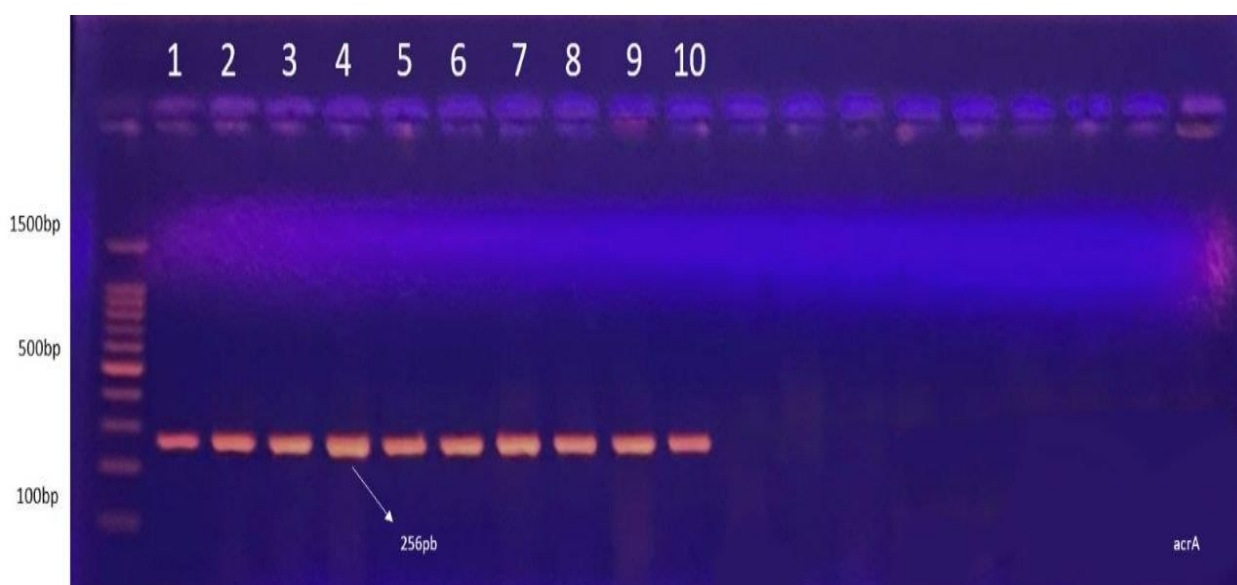


Figure (4- 3) Agarose gel electrophoresis of PCR products (256bp) obtained by using *acrA* specific primers. lanes 1-10 represent the clinical *proteus* isolates, Lane M represent DNA ladder.

4.5.2. Detection of *acrB* Gene

The *acrB* gene was successfully detected in all tested isolates, producing a PCR product of (432 bp), as illustrated in Figure (4-4). The detection rate was

(100%), indicating a strong presence of the AcrAB-TolC efflux system, which plays a major role in multidrug resistance among these isolates.

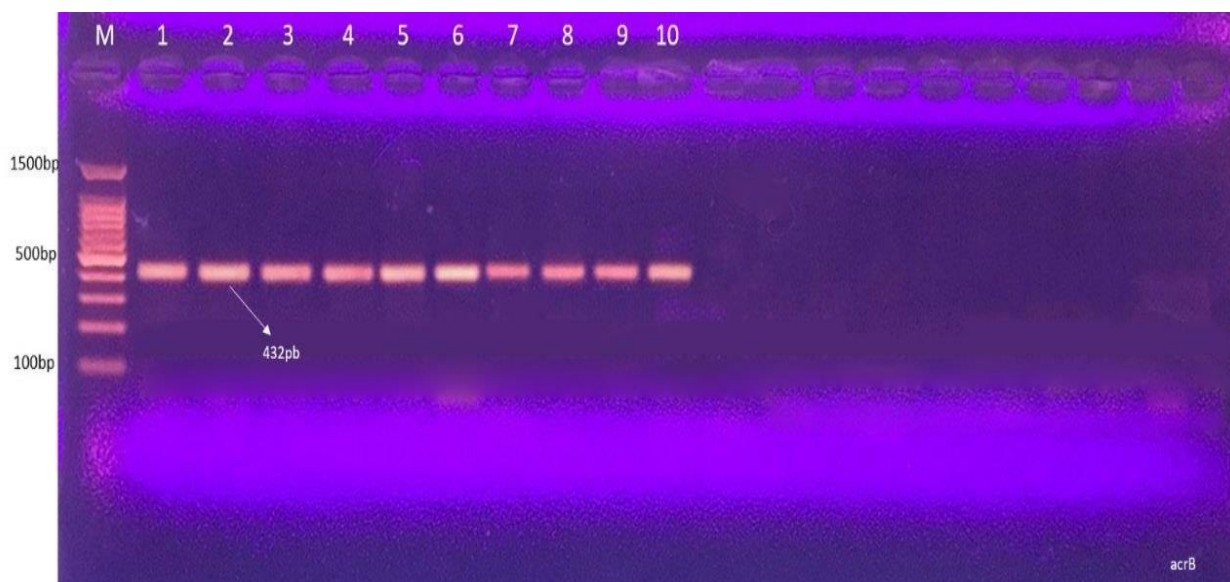


Figure (4- 4) Agarose gel electrophoresis of PCR products (432bp) obtained by using *acrB* specific primers. lanes 1-10 represent the clinical *proteus* isolates, Lane M represent DNA ladder.

4.5.3. Detection of *TEM* Gene

PCR analysis targeting the *TEM* β -lactamase gene revealed positive amplification in five out of seven bacterial isolates (isolates 1, 2, 3, 6, and 7), corresponding to (71.4%) positivity. Specific DNA bands at the expected amplicon size of (500 bp) were observed in these isolates, while no amplification bands were detected in isolates (4 and 5). These findings indicate the presence of the *TEM* gene in the majority of tested isolates, suggesting their potential ability to produce β -lactamase enzymes that hydrolyze β -lactam antibiotics and contribute to antimicrobial resistance. The results are illustrated in Figure (4-5).

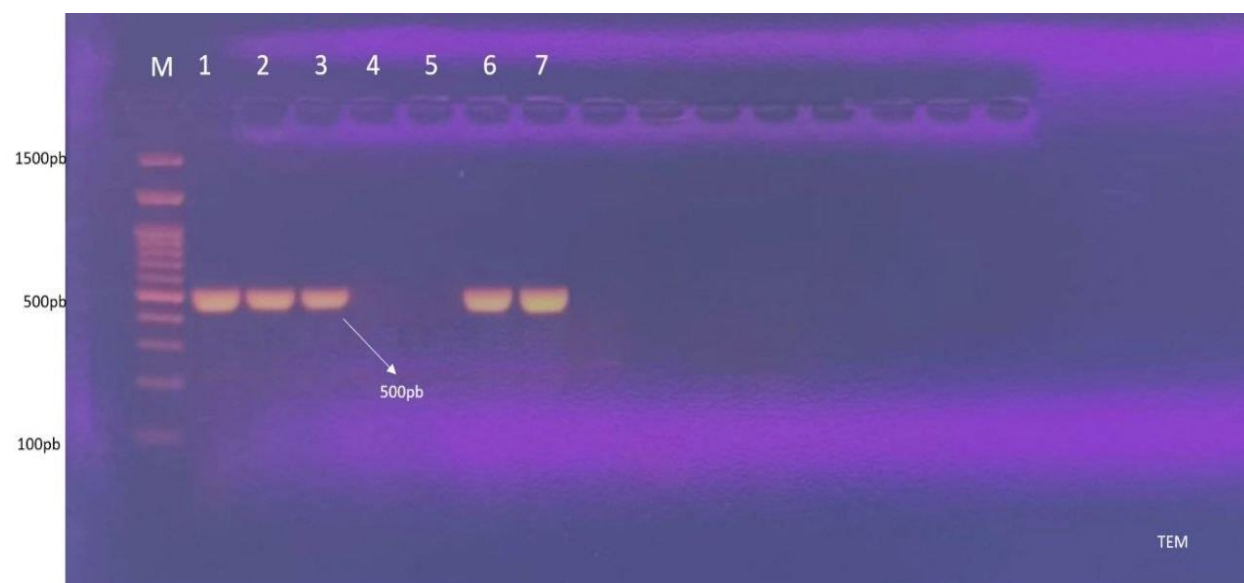


Figure (4-5): Agarose gel electrophoresis of PCR products (500 bp) obtained using *TEM* specific primers. Lanes 1, 2, 3, 6, and 7 represent positive bacterial isolates, whereas lanes 4 and 5 represent negative isolates. Lane M represents the DNA ladder.

4.5.4. Detection of *CTX-M* Gene

Molecular screening for the *CTX-M* gene revealed positive amplification products of the expected molecular size (600 bp) in five out of seven isolates (isolates 1, 2, 3, 6, and 7), corresponding to (71.4%) positivity. No amplification was detected in isolates (4 and 5). The detection of the *CTX-M* gene among the tested isolates indicates the potential presence of extended-spectrum β -lactamase (ESBL)-producing bacteria, which may confer resistance to extended-spectrum cephalosporins and other β -lactam antibiotics. These results are shown in Figure (4-6).

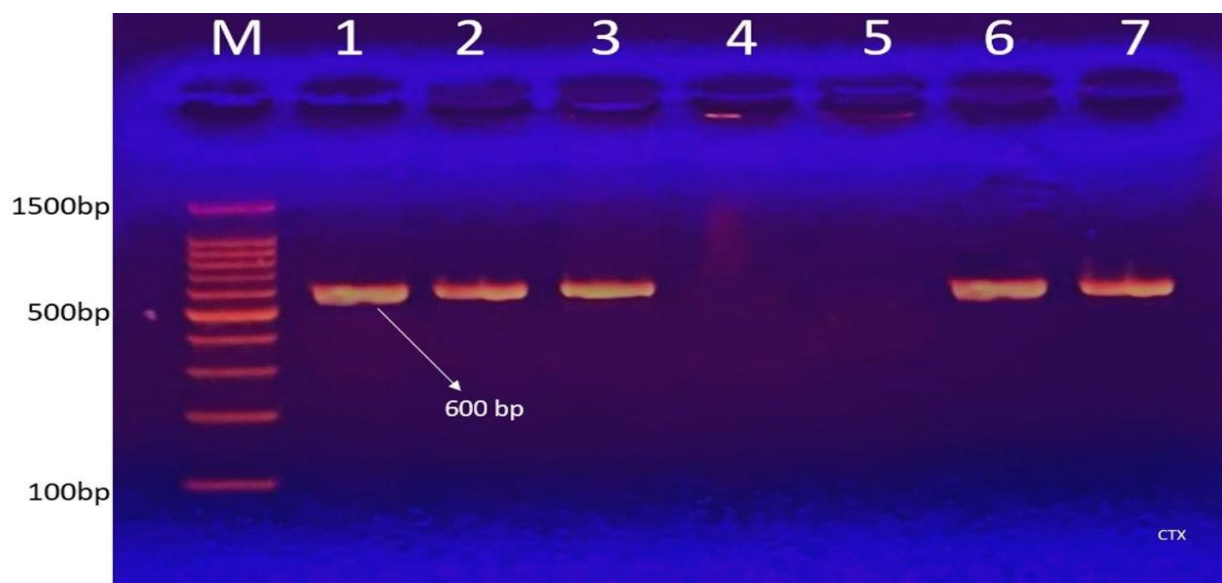


Figure (4-6): Agarose gel electrophoresis of PCR products (600 bp) obtained using *CTX-M* specific primers. Lanes 1, 2, 3, 6, and 7 represent positive bacterial isolates, whereas lanes 4 and 5 represent negative isolates. Lane M represents the DNA ladder.

Table (4-10): Detection of antibiotic resistance genes among *Proteus mirabilis* isolates by PCR

Gene	No.Positive	No.Negative	Total	%
<i>acrA</i>	10	0	10	100
<i>acrB</i>	10	0	10	100
<i>TEM</i>	5	2	7	71.4
<i>CTX-M</i>	5	2	7	71.4

The current study revealed a high prevalence of antibiotic resistance determinants among *p. mirabilis* isolates, with all samples harboring the *acrA* and *acrB* efflux pump genes (100%), and a majority demonstrating the presence of *TEM* and *CTX-M* β -lactamase genes (71.4% each). The universal detection of

acrA and *acrB* genes underscores the widespread distribution of the AcrAB-TolC efflux system among *P. mirabilis* isolates in this setting. This observation is consistent with recent studies, such as Zhang *et al.* (2021) and Ramos *et al.* (2020), which highlighted the global dissemination of efflux pump systems in Gram-negative bacteria and their central role in mediating resistance to various antibiotic classes, including fluoroquinolones, tetracyclines, and some β -lactams. Compared to previous reports, where *acrA* and *acrB* gene prevalence ranged from 70% to 95% (Albayati *et al.*, 2017), the 100% detection rate observed here may reflect selective pressure from extensive antibiotic use in the study environment or possibly the clonal spread of highly resistant strains. Similar findings were reported by Alzahrani *et al.* (2026), who observed a high prevalence (88%) of efflux pump genes in *P. mirabilis* from Egyptian hospitals, while Kumar *et al.* (2022) documented slightly lower rates (80–85%) in clinical isolates from India. This suggests that the prevalence in the current study is among the highest reported so far, potentially attributable to local antibiotic prescribing patterns or infection control practices.

The detection rates for *TEM* and *CTX-M* β -lactamase genes (71.4% each) are also notable. The prevalence of *TEM*-type enzymes aligns with findings by Al-Khoshaba *et al.*, (2025) in Iraq, who reported similar rates in clinical isolates, and slightly exceeds the global average of 50–65% reported by (Zhang *et al.*, 2024). In contrast, studies from Europe by Blanc *et al.*, (2020) reported *TEM* gene prevalence as low as 40%, while El-Kholy *et al.*, (2024) in North Africa found rates comparable to those in this study. Regarding the *CTX-M* gene, associated with extended-spectrum β -lactamases (ESBLs), the results are consistent with Ahmed *et al.* (2018), who documented a rising trend of *CTX-M*-type ESBLs in *Proteus* species, particularly in hospital-acquired infections. However, some studies, such as Rodriguez-Baño *et al.* (2010) in Europe, reported lower prevalence rates below 50%, while a study from South America

by Vivas *et al.*, (2020) reported higher rates in certain hospital settings, highlighting the influence of geographic and epidemiological factors on resistance gene distribution.

The joint detection of efflux pumps and β -lactamase genes in the same isolates supports the existence of multilayered resistance mechanisms. This phenomenon has been discussed by Abo-Amer *et al.* (2023), who emphasized that efflux pumps not only reduce antibiotic susceptibility directly but may also facilitate the acquisition and persistence of additional resistance genes, further limiting therapeutic options. In clinical practice, this could mean that infections caused by such *P. mirabilis* strains are more likely to be multidrug-resistant, complicating treatment and increasing the risk of therapeutic failure.

Moreover, the data from this study reflect the global trend of increasing multidrug-resistant (MDR) rates among Gram-negative pathogens, as highlighted in WHO reports and regional surveillance programs. The findings also align with Zhang *et al.* (2019), who observed that the spread of efflux and β -lactamase genes is often driven by the overuse of broad-spectrum antibiotics in both hospital and community settings. The 100% prevalence of efflux genes in this study is higher than some global averages, indicating a potential local outbreak or unique selective pressures. The *TEM* and *CTX-M* gene rates are among the higher end of reported values, suggesting a significant ESBL and β -lactamase burden compared to regions with stricter antibiotic regulation. Previous studies reported *Proteus mirabilis* as less likely than *Escherichia coli* or *Klebsiella* to harbor ESBL genes; however, our findings suggest this gap may be narrowing, which is a public health concern. In conclusion, the results of this study not only confirm the pervasive presence of efflux and β -lactamase genes in local *P. mirabilis* isolates but also highlight the risk of multidrug resistance in clinical settings. These findings are in line with, and in some cases exceed, prevalence rates reported in recent literature, emphasizing the urgent need for

robust antimicrobial stewardship and continuous surveillance to prevent further spread of such resistance determinants.

4.6. Testing of heavy metals tolerance of *proteus mirabilis*

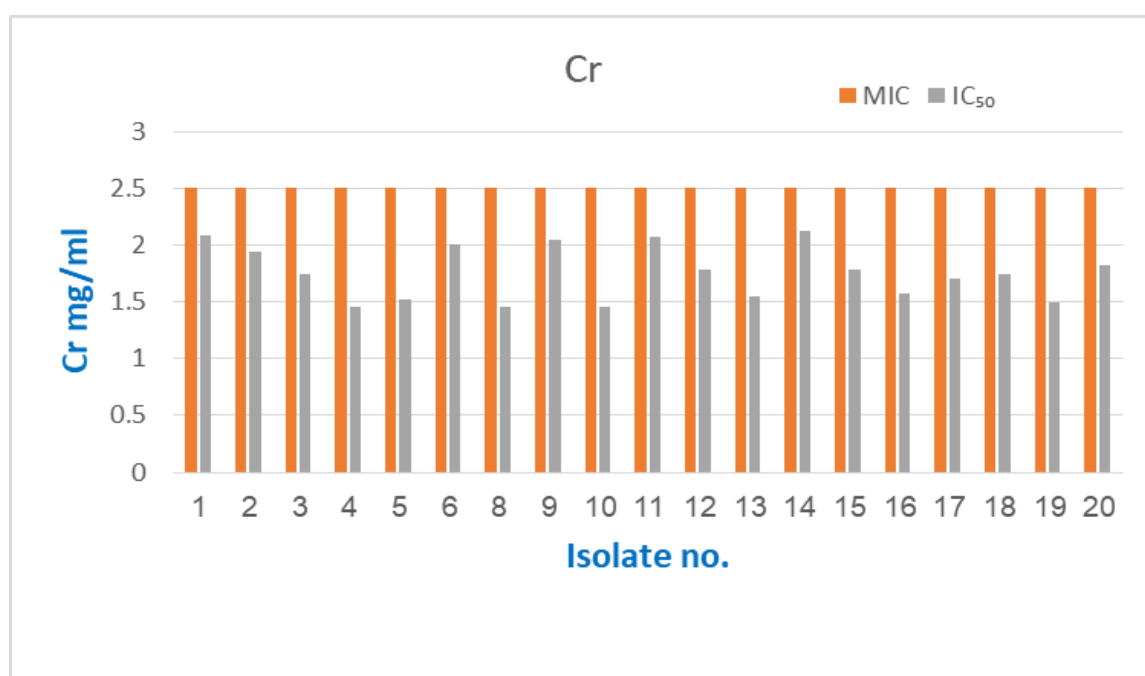
4.6.1 Chromium resistant bacteria

Minimum Inhibitory Concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) of chromium chloride ($CrCl_2$) were determined for different bacterial isolates, as shown in Table (4-11). The results revealed that all 19 isolates showed an MIC value of 2.5 mg/mL, indicating a uniform inhibitory endpoint among all tested isolates. In contrast, the IC_{50} values varied among the isolates, ranging from 1.45 to 2.12 mg/mL, reflecting differences in chromium tolerance among the isolates. Figure (4-7), Figure(4-8) demonstrates dose–response curves showing cell viability of isolates across a concentration gradient of Cr (0–20 mg/mL) .

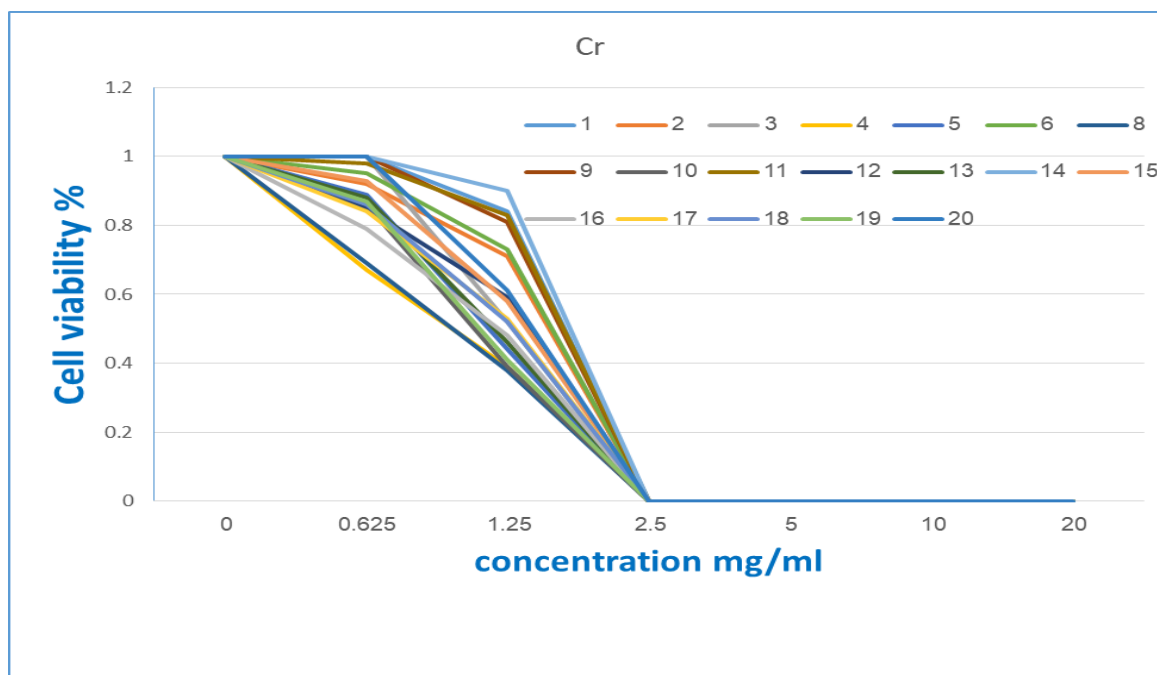
Table (4-11): MIC and IC_{50} value for Cr of different isolate *p. mirabilis* isolates

No. of isolates	MIC (mg/ml)	IC_{50} (mg/ml)	p-value
1	2.5	2.08	<0.001***
2	2.5	1.94	
3	2.5	1.74	
4	2.5	1.46	
5	2.5	1.52	
6	2.5	2.01	
7	2.5	1.45	
8	2.5	2.05	
9	2.5	1.46	
10	2.5	2.07	
11	2.5	1.79	

12	2.5	1.55	
13	2.5	2.12	
14	2.5	1.78	
15	2.5	1.58	
16	2.5	1.71	
17	2.5	1.74	
18	2.5	1.49	
19	2.5	1.83	



Figure(4-7):MIC and IC₅₀ value for Cr of *p. mirabilis* is isolates



Figure(4-8): Dose–response curves showing cell viability of isolates across a concentration gradient of Cr (0–20 mg/mL)

4.6.2 Cadmium resistant bacteria

Minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) of cadmium chloride ($CdCl_2$) were determined for the 19 clinical *P. mirabilis* isolates, as shown in Table (4-12). The results revealed that all 19 isolates showed an MIC of 1.25 mg/mL, indicating a uniform inhibitory endpoint among the isolates. In contrast, the IC_{50} values varied among the isolates, ranging from 0.347 to 0.726 mg/mL, reflecting differences in cadmium susceptibility among the tested isolates. Figure (4-9), Figure(4-10) demonstrates Dose–response curves showing cell viability of isolates across a concentration gradient of Cd (0–20 mg/mL).

Table (4-12): MIC and IC₅₀ value for Cd of *p.mirabilis* isolates

No. of isolates	MIC (mg/ml)	IC ₅₀ (mg/ml)	p-value
1	1.25	0.351	<0.001***
2	1.25	0.406	
3	1.25	0.363	
4	1.25	0.488	
5	1.25	0.53	
6	1.25	0.396	
7	1.25	0.406	
8	1.25	0.638	
9	1.25	0.68	
10	1.25	0.453	
11	1.25	0.428	
12	1.25	0.376	
13	1.25	0.391	
14	1.25	0.401	
15	1.25	0.381	
16	1.25	0.411	
17	1.25	0.347	
18	1.25	0.726	
19	1.25	0.613	

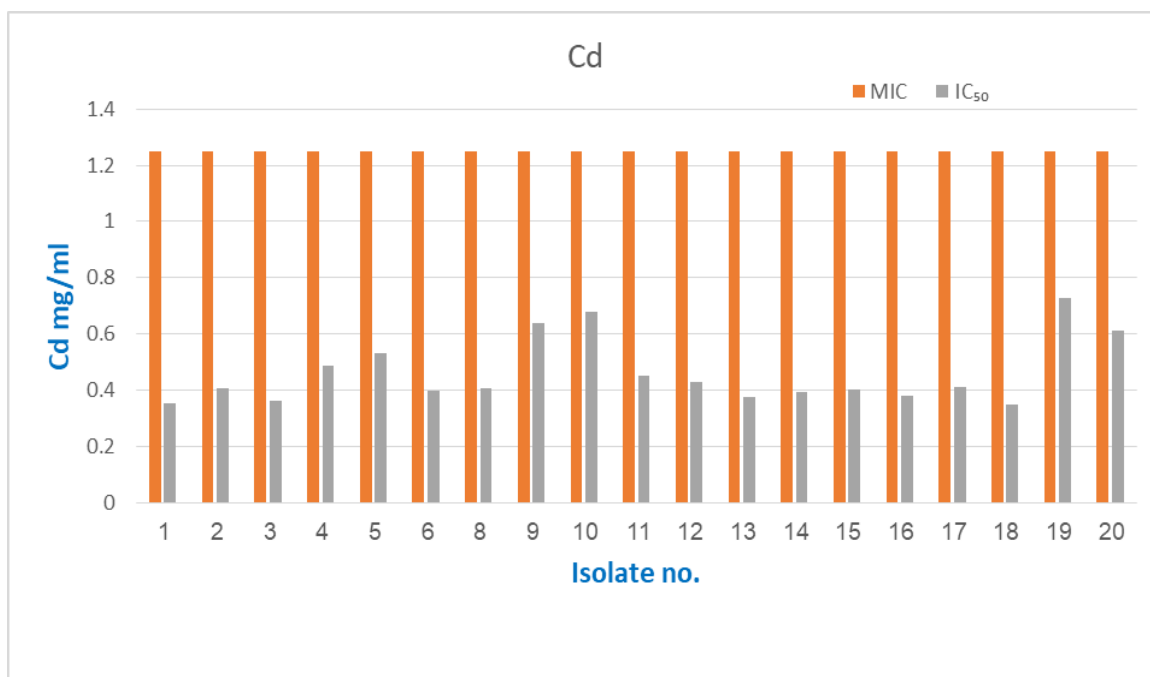
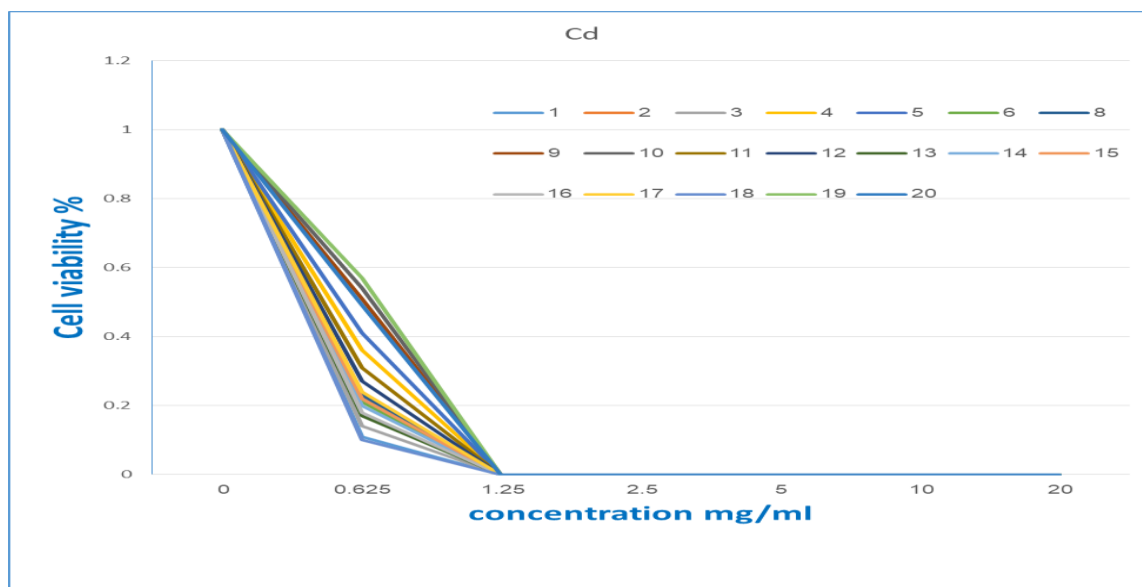


Figure (4-9): MIC and IC₅₀ value for Cd of *p.mirabilis* isolates



Figure(4-10): Dose–response curves showing cell viability of isolates across a concentration gradient of Cd (0–20 mg/mL)

4.6.3. Copper resistant bacteria

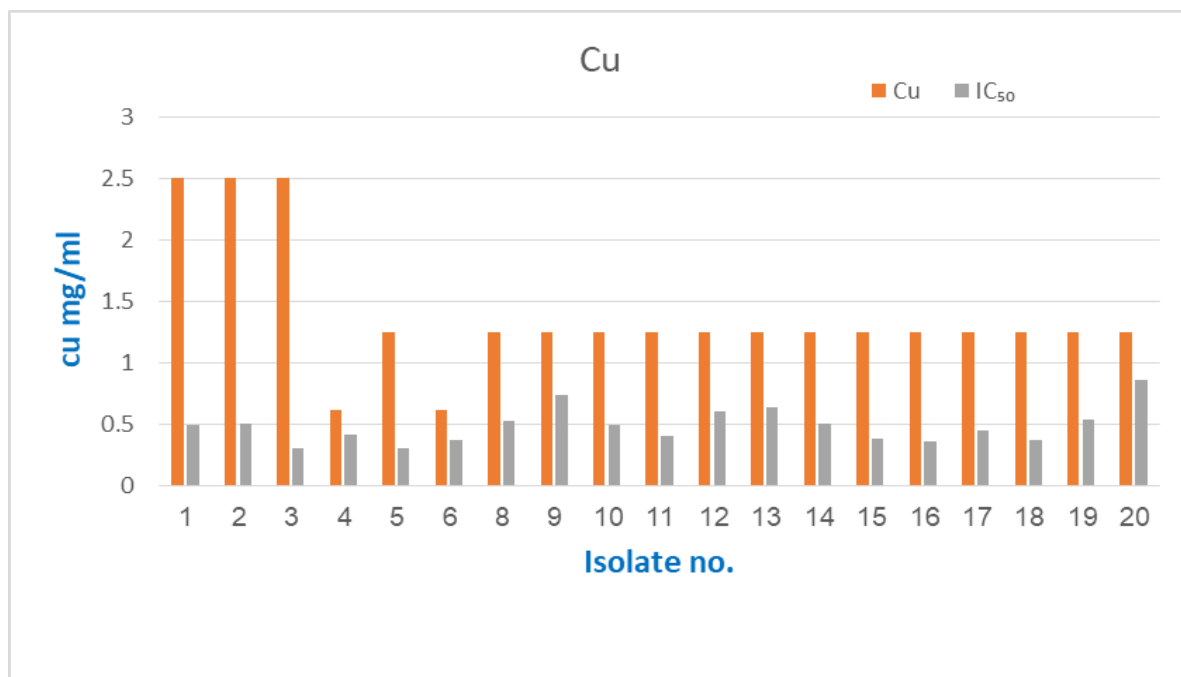
The minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC₅₀) of copper chloride (CuCl₂) were determined for the 19 clinical *P. mirabilis* isolates, as shown in Table (4-13). The results showed that the MIC values varied among the isolates, ranging from 0.62 to 2.5 mg/mL.

Most isolates showed an MIC of 1.25 mg/mL, while two isolates showed a lower MIC value of 0.62 mg/mL, indicating higher sensitivity to the tested copper compound. In contrast, three isolates showed a higher MIC value of 2.5 mg/mL. Meanwhile, the IC₅₀ values ranged from 0.31 to 0.86 mg/mL, reflecting differences in copper tolerance among the bacterial isolates. These findings suggest moderate copper tolerance in some isolates, with notable variation in response among the tested isolates. Figure(4-11), Figure(4-12) demonstrates Dose–response curves showing cell viability of isolates across a concentration gradient of Cu (0–20 mg/mL).

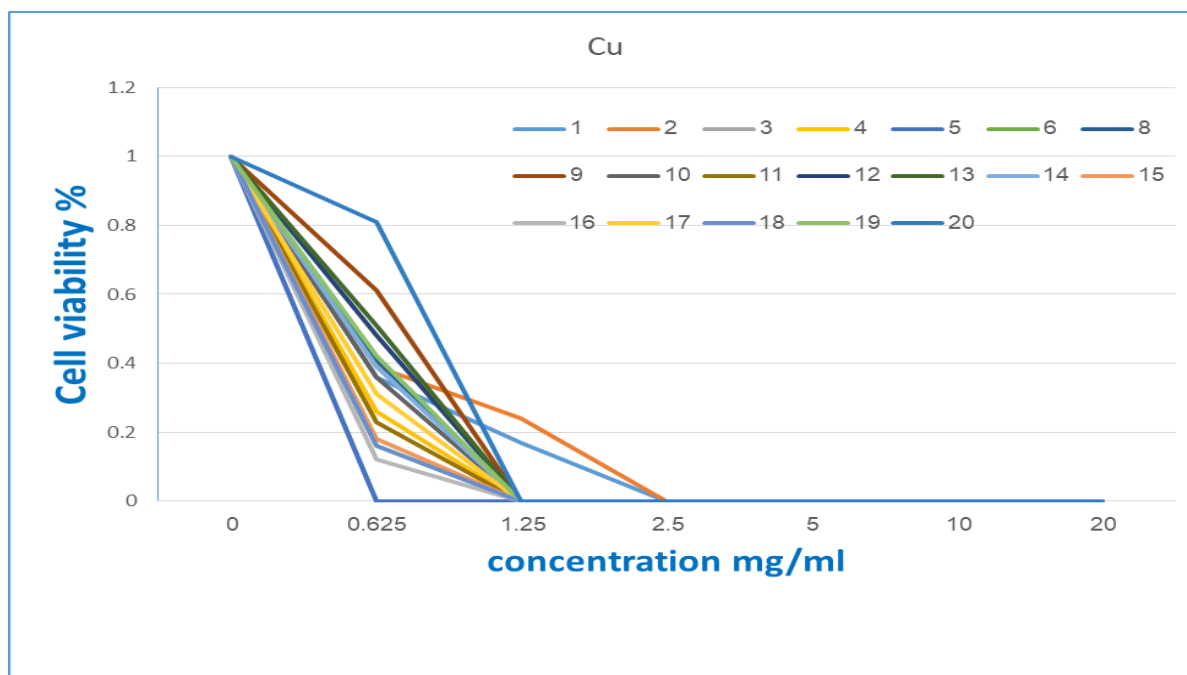
Table (4-13): MIC and IC₅₀ value for Cu of different isolate

No. of isolates	MIC (mg/ml)	IC ₅₀ (mg/ml)	p-value
1	2.5	0.49	<0.001***
2	2.5	0.51	
3	2.5	0.31	
4	0.62	0.42	
5	1.25	0.31	
6	0.62	0.37	
7	1.25	0.53	
8	1.25	0.74	
9	1.25	0.49	
10	1.25	0.41	
11	1.25	0.6	
12	1.25	0.64	
13	1.25	0.51	
14	1.25	0.38	
15	1.25	0.36	
16	1.25	0.45	

17	1.25	0.37	
18	1.25	0.54	
19	1.25	0.86	



Figure(4-11):MIC and IC₅₀ value for Cu of *p.mirabilis* isolates



Figure(4-12): Dose–response curves showing cell viability of isolates across a concentration gradient of Cu (0–20 mg/mL)

4.6.4. Zinc resistant bacteria

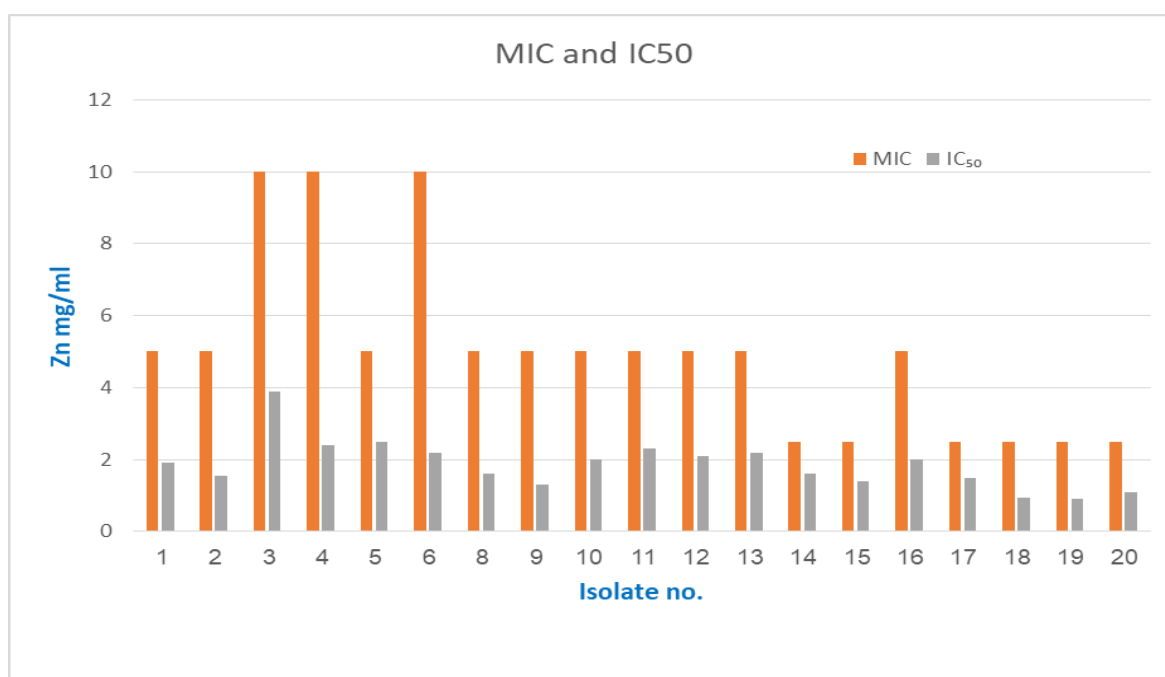
The minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) of zinc chloride ($ZnCl_2$) were determined for different bacterial isolates, as shown in Table (4-14).

The results showed that the MIC values varied among the isolates, ranging from 2.5 to 10 mg/mL, indicating that the bacterial isolates showed different levels of zinc tolerance. In contrast, the IC_{50} values differed between isolates, ranging from 0.90 to 3.90 mg/mL, reflecting differences in zinc tolerance among the bacterial isolates. Figure(4-13), Figure(4-14) demonstrates Dose–response curves showing cell viability of isolates across a concentration gradient of Zn (0–20 mg/mL).

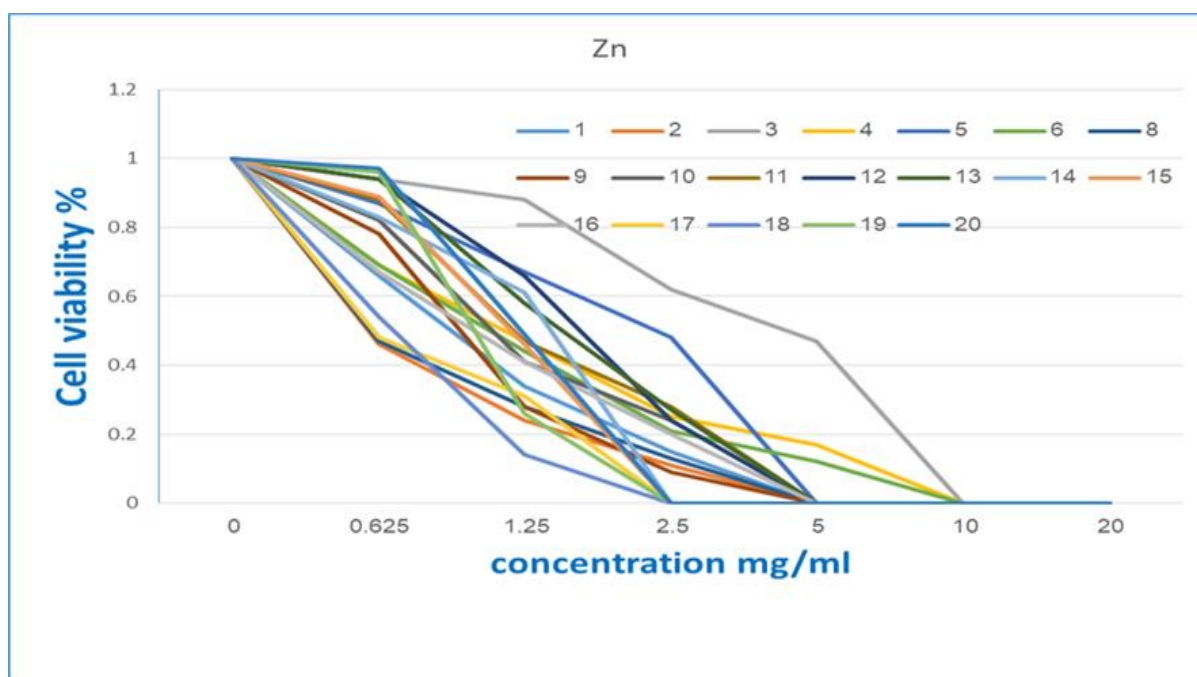
Table (4-14): MIC and IC_{50} value for *Zn p.mirabilis* isolates

No. of isolates	MIC (mg/ml)	IC_{50} (mg/ml)	p-value
1	5	1.9	<0.001***
2	5	1.55	
3	10	3.9	
4	10	2.4	
5	5	2.5	
6	10	2.2	
7	5	1.6	
8	5	1.3	
9	5	2	
10	5	2.3	
11	5	2.1	
12	5	2.2	
13	2.5	1.6	

14	2.5	1.4	
15	5	2	
16	2.5	1.5	
17	2.5	0.95	
18	2.5	0.9	
19	2.5	1.1	



Figure(4-13):MIC and IC₅₀ value for Zn *p.mirabilis* isolates



Figure(4-14): Dose–response curves showing cell viability of isolates across a concentration gradient of Zn (0–20 mg/mL)

the present results showed that *Proteus mirabilis* isolates exhibited variable resistance patterns toward different heavy metals, including zinc, chromium, copper, and cadmium, reflecting differences in adaptive capacity and detoxification mechanisms among the tested isolates. Zinc showed the highest level of tolerance compared to the other tested metals, with MIC values ranging from 2.5 to 10 mg/mL and IC₅₀ values ranging from 0.90 to 3.90 mg/mL. Chromium exhibited a lower tolerance level than zinc, with a constant MIC value of 2.5 mg/mL, while IC₅₀ values ranged from 1.45 to 2.12 mg/mL. Copper showed intermediate to low tolerance compared with the previously mentioned metals, with MIC values ranging from 0.62-2.5 mg/mL and IC₅₀ values ranging from 0.31 to 0.86 mg/mL. Cadmium exhibited the strong inhibitory effect and relatively low resistance among the tested metals, with a constant MIC value of 1.25 mg/mL and IC₅₀ values ranging from 0.347 to 0.726 mg/mL. For all four metals, the p-value of < 0.001 indicates that the difference between MIC and

IC₅₀ is highly statistically significant. This confirms that for each metal, the concentration required for complete growth inhibition is consistently and meaningfully higher than that required for 50% inhibition, validating the experimental dose-response model.

These results suggest that the isolates can tolerate varying concentrations of each metal, with partial differences in resistance likely attributable to intracellular defense mechanisms.

The findings of the present study are consistent with several previous studies addressing bacterial resistance to heavy metals, which enhances the reliability of the results and supports the understanding of variability in resistance patterns depending on metal type. For instance, Abbas *et al* (2025). and Nnaji *et al* (2024) reported, in a study on bacterial isolates from the Shatt Al-Arab River, significant variation in minimum inhibitory concentration (MIC) values for heavy metals. The MIC values ranged from 0.5 and 2.25 mg/L for metals such as zinc and lead, which is comparable to the wide variation observed in zinc MIC values (2.5–10 mg/mL) in the present study. Similarly Islam *et al* (2014), Sanuth *et al* (2016) demonstrated that *proteus mirabilis* isolates from agricultural soils were capable of tolerating high concentrations of zinc up to 35 mg/mL and chromium up to 12 mg/mL whereas copper tolerance was lower at approximately 5 mg/mL, These findings are in agreement with the current results, which showed relatively higher resistance to zinc compared to copper and chromium among several isolates. Furthermore, Sanuth & Adekanmbi (2016) and Zhang *et al* (2013) reported that certain *proteus mirabilis* isolates exhibited high MIC values for copper 3200 µg/mL and zinc 2600 µg/mL while cadmium showed comparatively lower MIC values 1000 µg/mL.

Across all four metals, IC₅₀ values consistently showed greater inter-isolate variability than MIC values. This pattern is biologically significant. MIC

represents an all-or-nothing growth endpoint requiring complete metabolic arrest, which often relies on constitutive, conserved mechanisms. In contrast, IC₅₀ reflects sub-lethal metal concentrations that bacteria can partially tolerate through inducible stress responses, including: up regulation of efflux pumps (e.g., CadA, CopA, ZntA, ChrA), Production of metal-chelating thiols (glutathione, metallothioneins), activation of ROS-scavenging enzymes (catalase, superoxide dismutase), and changes in membrane permeability. Strains with higher IC₅₀ values for a given metal likely have more efficient or more rapidly inducible versions of these systems (Harrison *et al*, 2007). This highlights the variations in metal resistance among bacterial isolates and corroborates the conclusion that bacterial tolerance is affected by physiological properties and environmental conditions . Together, these studies support the notion that heavy metal resistance is a variable trait based on the type of metal and bacterial isolate. It was found that metals such as zinc and chromium have higher MIC and IC₅₀ values than cadmium and copper and this finding is in agreement with the present study. The variation in MIC and IC₅₀ values for different metals and isolates supports differences in metal toxicity and the presence of several cellular defense systems including efflux pumps, metal binding proteins and antioxidant systems that enable bacterial survival in metal contaminated environments . Zinc (Zn²⁺) is a vital element but becomes toxic at elevated concentrations. In bacteria, homeostasis is regulated by Cation Diffusion Facilitator (CDF) transporters and certain RND efflux pump systems that expel surplus zinc from the cytoplasm to avoid metabolic disturbance. (Nies *et al*, 2003). Zinc is particularly concerning as it has been demonstrated to co-select for carbapenem resistance, given that metallo-β-lactamases (MBLs) like NDM-1 and IMP necessitate zinc as a cofactor for their enzymatic function. Consequently, environmental zinc contamination may directly promote the selection of carbapenem-resistant *P.mirabilis* already recognized for harboring metallo-beta-lactamases in clinical environments. (Walsh *et al*, 2005).

Chromium (Cr^{6+}) is highly toxic and carcinogenic and bacteria have two major resistance strategies, enzymatic reduction of hexavalent chromium (Cr^{6+}) to less toxic trivalent form (Cr^{3+}) and the activity of ChrA-like efflux transporters that export chromate ions outside the cell, thereby reducing intracellular accumulation (Ramírez-Díaz *et al.*, 2008). Chromium resistance determinants are also embedded in integron structures alongside gene cassettes conferring resistance to aminoglycosides, trimethoprim, and sulfonamides (Ramírez-Díaz *et al.*, 2008). Furthermore, the toxicity of copper (Cu^{2+}) is mainly due to oxidative stress induced by the production of reactive oxygen species (ROS). Therefore, *Proteus mirabilis* exports copper ions out of the cell using the CopA system (P-type ATPase) in addition to intracellular metal-binding proteins to reduce copper toxicity (Andrei *et al.*, 2020). Copper resistance genes (*cus*, *cop*) are frequently found on the same plasmids as antibiotic resistance genes in Enterobacteriaceae, and exposure to copper has been demonstrated to enrich multidrug-resistant strains in vitro and in animal models (Pal *et al.*, 2017). *Proteus mirabilis* also utilizes both efflux pumps and intracellular metal-binding proteins to mitigate the toxicity of cadmium (Cd^{2+}), a highly toxic heavy metal. Efflux systems such as CzcCBA are involved in the exclusion of cadmium ions from the cell, thereby minimizing intracellular accumulation, reducing oxidative stress and maintaining cellular homeostasis (Nies *et al.*, 2003). Genes of resistance to cadmium are often associated to Tn21-type transposons and class 1 integrons, which are important vehicles for the dissemination of antibiotic resistance in clinical and environmental bacteria. (Liebert *et al.*, 1999). The association between resistance to heavy metals and antibiotics is of the highest relevance in the context of environmental and clinical microbiology. The four metals examined in this study are known to co-select for antibiotic resistance through three main mechanisms: co-resistance (shared mobile genetic elements), cross-resistance (single mechanism that confers tolerance to both metals and antibiotics) and regulatory linkage (metal stress inducing antibiotic

resistance pathways) (Baker-Austin *et al.*, 2006) Most importantly, the well-characterized co-selection mechanisms that connect heavy metal resistance with antibiotic resistance raise serious concerns about the role of environmental heavy metal pollution as a driver of antimicrobial resistance. These findings emphasize the need for more stringent regulation of heavy metal discharge and regular surveillance of metal resistance in clinically relevant pathogens.

Conclusions & Recommendations

Conclusions :

1. *P. mirabilis* is a relative pathogen associated with health care related infections.
2. Other notable pathogens include *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Enterobacter hormaechei*, *clostridium perfringens*, *staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*.
3. *P. mirabilis* isolates demonstrated multidrug resistance patterns (MDR) with notably high resistance to tigecycline and moderate resistance to cephalosporins.
4. The presence of efflux pump genes (*acrA* and *acrB*) and β -lactamase genes (*TEM*) and (*TX-M*) in *P. mirabilis* isolates.
5. *P. mirabilis* exhibited varying levels of heavy metal resistance with the highest resistance observed to zinc, followed by chromium, copper, cadmium.

Recommendations:

1. The importance of evaluating and improving methods of preventing infection with *proteus mirabilis* and following sterilization methods accurately .
2. The study of the expression of antibiotic resistance genes and their induction mechanisms.
3. The study of heavy metal resistance and its role in the survival and spread of bacteria.
4. The study of the detection and expression of metal resistance genes and their induction methods.
5. The coexistence of antibiotic and heavy metal resistance calls for tighter regulation on heavy metal discharge and emphasize the importance of regularly monitoring metal resistance determinants in clinically relevant bacterial species.

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Appendix (1) : VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer: Microbiology Chart Report Printed June 11, 2025 1:08:22 PM AST

Patient Name: Patient ID:
 Location: Physician:
 Lab ID: 251272 Isolate Number: 1

Organism Quantity:
Selected Organism : Citrobacter amalonaticus
 BP Infection Site:

Source: Collected:

Comments:

Identification Information		Analysis Time: 4.83 hours	Status: Final
Selected Organism		88% Probability	Citrobacter amalonaticus
ID Analysis Messages		Bionumber: 4635711544502211	

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	+
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

A-(3) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:	Microbiology Chart Report	Printed June 20, 2025 2:18:59 PM AST
Patient Name:		Patient ID:
Location:		Physician:
Lab ID: 261172		Isolate Number: 1
Organism Quantity:		
Selected Organism : <i>Oligella ureolytica</i>		
BP Infection Site:		
Source:		Collected:

Comments:	

Identification Information	Analysis Time: 9.97 hours	Status: Final
Selected Organism	Oligella ureolytica	
	Bionumber:	0000001000000000
ID Analysis Messages		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

A-(4) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:		Microbiology Chart Report		Printed June 11, 2025 1:08:40 PM AST	
Patient Name:				Patient ID:	
Location:				Physician:	
Lab ID: 251273				Isolate Number: 1	
Organism Quantity:					
Selected Organism : Moraxella group					
BP Infection Site:					
Source:				Collected:	
Comments:					
Identification Information		Analysis Time: 7.77 hours		Status: Final	
Selected Organism		99% Probability		Moraxella group	
ID Analysis Messages		Bionumber:		0000000000100000	
Biochemical Details					
2	APPA	-	3	ADO	-
10	H2S	-	11	BNAG	-
17	BGLU	-	18	dMAL	-
23	ProA	-	26	LIP	-
33	SAC	-	34	dTAG	-
40	ILATk	+	41	AGLU	-
46	GlyA	-	47	ODC	-
58	O129R	-	59	GGAA	-
4	PyrA	-	12	AGLTp	-
19	dMAN	-	27	PLE	-
35	dTRE	-	36	CIT	-
42	SUCT	-	43	NAGA	-
48	LDC	-	53	IHISa	-
61	IMLTa	-	62	ELLM	-
5	IARL	-	7	dCEL	-
13	dGLU	-	14	GGT	-
20	dMNE	-	21	BXYL	-
29	TyrA	-	31	URE	-
37	MNT	-	39	5KG	-
44	AGAL	-	45	PHOS	-
56	CMT	-	57	BGUR	-
64	ILATa	-			

A-(5) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer: _____

Patient Name: _____

Location: _____

Lab ID: 251271

Organism Quantity: _____

Selected Organism : Citrobacter amalonaticus

BP Infection Site: _____

Source: _____

Collected: _____

Comments: _____

Identification Information	Analysis Time: 4.85 hours	Status: Final
Selected Organism	95% Probability	Citrobacter amalonaticus
ID Analysis Messages	Bionumber: 4605410544502211	

2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	+
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	(+)	64	ILATa	-			

A-(6) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:		Microbiology Chart Report		Printed June 11, 2025 1:09:17 PM AST													
Patient Name:				Patient ID:													
Location:				Physician:													
Lab ID: 2512261				Isolate Number: 1													
Organism Quantity:																	
Selected Organism : Aeromonas sobria																	
BP Infection Site:																	
Source:				Collected:													
Comments:																	
Identification Information		Analysis Time: 7.78 hours		Status: Final													
Selected Organism		87% Probability		Aeromonas sobria													
		Bionumber:		0627611050404220													
ID Analysis Messages																	
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	(-)	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	(+)	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

A-(7) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:		Microbiology Chart Report		Printed June 11, 2025 1:08:58 PM AST	
Patient Name:				Patient ID:	
Location:				Physician:	
Lab ID: 251274				Isolate Number: 1	
Organism Quantity:					
Selected Organism : Myroides spp					
BP Infection Site:					
Source:				Collected:	
Comments:					
Identification Information		Analysis Time: 8.73 hours		Status: Final	
Selected Organism		95% Probability		Myroides spp	
		Bionumber:		5042000100140220	
ID Analysis Messages					
Biochemical Details					
2	APPA	+	3	ADO	-
10	H2S	-	11	BNAG	-
17	BGLU	-	18	dMAL	-
23	ProA	-	26	LIP	-
33	SAC	-	34	dTAG	-
40	ILATk	+	41	AGLU	-
46	GlyA	-	47	ODC	-
58	O129R	-	59	GGAA	+
			4	PyrA	+
			12	AGLTp	+
			19	dMAN	-
			27	PLE	-
			35	dTRE	-
			42	SUCT	-
			50	IMLTa	-
			5	IARL	-
			13	dGLU	-
			20	dMNE	-
			29	TyrA	+
			36	CIT	-
			43	NAGA	-
			51	ELLM	-
			7	dCEL	-
			14	GGT	+
			21	BXYL	-
			28	URE	-
			37	MNT	-
			44	AGAL	-
			52	CMT	+
			60	ILATa	-
			9	BGAL	-
			15	OFF	-
			22	BAlap	-
			31	dSOR	-
			39	5KG	-
			45	PHOS	+
			53	BGUR	-

A-(8) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:		Microbiology Chart Report		Printed June 20, 2025 2:18:59 PM AST													
Patient Name:				Patient ID:													
Location:				Physician:													
Lab ID: 261172				Isolate Number: 1													
Organism Quantity:																	
Selected Organism : Pseudomonas aeruginosa																	
BP Infection Site:																	
Source:				Collected:													
Comments:																	
Identification Information		Analysis Time: 9.97 hours		Status: Final													
Selected Organism		Pseudomonas aeruginosa															
ID Analysis Messages		Bionumber: 0000001000000000															
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

A-(9) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:		Microbiology Chart Report		Printed June 20, 2025 2:18:59 PM AST													
Patient Name:				Patient ID:													
Location:				Physician:													
Lab ID: 261172				Isolate Number: 1													
Organism Quantity:																	
Selected Organism : Klebsiella spp																	
BP Infection Site:																	
Source:				Collected:													
Comments:																	
Identification Information		Analysis Time: 5.85 hours		Status: Final													
Selected Organism		Klebsiella spp															
ID Analysis Messages		Bionumber:		0000001000000000													
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	+	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

A-(10) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:		Microbiology Chart Report		Printed June 20, 2025 2:18:59 PM AST																																																																																																																																																	
Patient Name:				Patient ID:																																																																																																																																																	
Location:				Physician:																																																																																																																																																	
Lab ID: 261172				Isolate Number: 1																																																																																																																																																	
Organism Quantity:																																																																																																																																																					
Selected Organism : Staphylococcus spp																																																																																																																																																					
BP Infection Site:																																																																																																																																																					
Source:				Collected:																																																																																																																																																	
Comments:																																																																																																																																																					
Identification Information		Analysis Time:		10.39 hours																																																																																																																																																	
Selected Organism		Status:		Final																																																																																																																																																	
ID Analysis Messages		Bionumber:		0000001000000000																																																																																																																																																	
Biochemical Details <table border="1"> <tr> <td>2</td><td>APPA</td><td>+</td><td>3</td><td>ADO</td><td>-</td><td>4</td><td>PyrA</td><td>-</td><td>5</td><td>IARL</td><td>-</td><td>7</td><td>dCEL</td><td>-</td><td>9</td><td>BGAL</td><td>+</td> </tr> <tr> <td>10</td><td>H2S</td><td>-</td><td>11</td><td>BNAG</td><td>-</td><td>12</td><td>AGLTp</td><td>-</td><td>13</td><td>dGLU</td><td>+</td><td>14</td><td>GGT</td><td>-</td><td>15</td><td>OFF</td><td>-</td> </tr> <tr> <td>17</td><td>BGLU</td><td>+</td><td>18</td><td>dMAL</td><td>+</td><td>19</td><td>dMAN</td><td>+</td><td>20</td><td>dMNE</td><td>-</td><td>21</td><td>BXYL</td><td>-</td><td>22</td><td>BAlap</td><td>-</td> </tr> <tr> <td>23</td><td>ProA</td><td>+</td><td>26</td><td>LIP</td><td>+</td><td>27</td><td>PLE</td><td>-</td><td>29</td><td>TyrA</td><td>-</td><td>31</td><td>URE</td><td>+</td><td>32</td><td>dsOR</td><td>-</td> </tr> <tr> <td>33</td><td>SAC</td><td>+</td><td>34</td><td>dTAG</td><td>+</td><td>35</td><td>dTRE</td><td>+</td><td>36</td><td>CIT</td><td>-</td><td>37</td><td>MNT</td><td>-</td><td>39</td><td>5KG</td><td>-</td> </tr> <tr> <td>40</td><td>ILATk</td><td>-</td><td>41</td><td>AGLU</td><td>-</td><td>42</td><td>SUCT</td><td>+</td><td>43</td><td>NAGA</td><td>-</td><td>44</td><td>AGAL</td><td>-</td><td>45</td><td>PHOS</td><td>+</td> </tr> <tr> <td>46</td><td>GlyA</td><td>+</td><td>47</td><td>ODC</td><td>-</td><td>48</td><td>LDC</td><td>-</td><td>53</td><td>IHISa</td><td>-</td><td>56</td><td>CMT</td><td>-</td><td>57</td><td>BGUR</td><td>+</td> </tr> <tr> <td>58</td><td>O129R</td><td>+</td><td>59</td><td>GGAA</td><td>-</td><td>61</td><td>IMLTa</td><td>-</td><td>62</td><td>ELLM</td><td>-</td><td>64</td><td>ILATa</td><td>-</td><td></td><td></td><td></td> </tr> </table>						2	APPA	+	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+	10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-	17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	-	21	BXYL	-	22	BAlap	-	23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	-	31	URE	+	32	dsOR	-	33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-	40	ILATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+	46	GlyA	+	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	+	58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			
2	APPA	+	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+																																																																																																																																				
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-																																																																																																																																				
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	-	21	BXYL	-	22	BAlap	-																																																																																																																																				
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	-	31	URE	+	32	dsOR	-																																																																																																																																				
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-																																																																																																																																				
40	ILATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+																																																																																																																																				
46	GlyA	+	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	+																																																																																																																																				
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-																																																																																																																																							

A-(11) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:	Microbiology Chart Report	Printed June 20, 2025 2:18:59 PM AST															
Patient Name:		Patient ID:															
Location:		Physician:															
Lab ID: 261172		Isolate Number: 1															
Organism Quantity:																	
Selected Organism : Campylobacter spp																	
BP Infection Site:																	
Source:		Collected:															
Comments:																	
Identification Information	Analysis Time: 4.85 hours	Status: Final															
Selected Organism	Campylobacter spp																
ID Analysis Messages	Bionumber: 0000001000000000																
Biochemical Details																	
2	APPA	+	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

A-(12) VITEK 2 Identification Reports for Environmental Samples

bioMérieux Customer:	Microbiology Chart Report	Printed June 20, 2025 2:18:59 PM AST
Patient Name:		Patient ID:
Location:		Physician:
Lab ID: 261172		Isolate Number: 1
Organism Quantity:		
Selected Organism : Salmonella spp		
BP Infection Site:		
Source:		Collected:

Comments:	

Identification Information	Analysis Time:	7.77 hours	Status:	Final
Selected Organism	Salmonella spp			
	Bionumber:	0000001000000000		
ID Analysis Messages				

Biochemical Details																	
2	APPA	+	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

A:13-Appendix (2) : Colony-Forming Unit (CFU/mL) Results of Hospital Wastewater Samples

Table (1) (Colony Forming Units) for the Marjan teaching hospital samples

Sample No.	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	TNTC	TNTC	8.1×10^3	-	-	-
2.	TNTC	TNTC	39×10^3	-	-	-
3.	TNTC	TNTC	54×10^3	-	-	-
4.	TNTC	TNTC	40×10^3	-	-	-
5.	TNTC	TNTC	61×10^3	-	-	-
Mean $\pm$ SD = $(48.5 \pm 10.8) \times 10^3$ CFU/mL						

Table (2) (Colony Forming Units) for the Al- Hilla teaching hospital samples

Sample No.	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	TNTC	TNTC	1.62×10^3	-	-	-
2.	TNTC	TNTC	6×10^3	-	-	-
3.	TNTC	TNTC	15×10^3	-	-	-
4.	TNTC	TNTC	81×10^3	-	-	-
5.	TNTC	TNTC	25×10^3	-	-	-
Mean $\pm$ SD = $(31.8 \pm 33.7) \times 10^3$ CFU/mL						

Table (3) (Colony Forming Units) for the Imam Al-sadiq hospital samples

Sample No.	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
1.	TNTC	TNTC	12×10^3	-	-	-
2.	TNTC	TNTC	6.1×10^3	-	-	-
3.	TNTC	TNTC	9×10^3	-	-	-
4.	TNTC	TNTC	15×10^3	-	-	-
5.	TNTC	TNTC	3×10^3	-	-	-
Mean $\pm$ SD = $(9.8 \pm 5.1) \times 10^3$ CFU/mL						

A-14: Appendix (3) : VITEK 2 Compact Identification Reports of *Proteus mirabilis* Clinical Isolates

bioMérieux Customer: Microbiology Chart Report Printed Dec 7 2025 9:45 AM CDT

Patient Name: **hussna mohammed** Patient ID: 202665 1
 Location: Lab ID: 202665 1 Physician:
 Organism Quantity: Selected Organism : **Proteus mirabilis** Isolate Number: 1
 Source: Collected:

Comments:

Identification Information		Analysis Time: 9.27 hours	Status: Final
Selected Organism		99% Probability Proteus mirabilis	
ID Analysis Messages		Bionumber: 0033011341553271	

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Page 1 of 1

bioMérieux Customer: Microbiology Chart Report Printed Dec 7 2025 10:57:46 AM CDT

Patient Name: **hussna mohammed** Patient ID: 202665 1
 Location: Lab ID: 202665 1 Physician:
 Organism Quantity: Selected Organism : **Proteus mirabilis** Isolate Number: 1
 Source: Collected:

Comments:

Identification Information		Analysis Time: 9.27 hours	Status: Final
Selected Organism		99% Probability Proteus mirabilis	
ID Analysis Messages		Bionumber: 0033011341553271	

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Page 1 of 1

Comments:	
Identification Information	Analysis Time: 9.45 hoursStatus: Final
Selected Organism	99% ProbabilityProteus mirabilis
ID Analysis Messages	Bionumber: 0033011341553271
Biochemical Details	
2	APPA - 3
10	H2S + 11
17	BGLU - 18
23	ProA + 26
33	SAC - 34
40	ILATk + 41
46	GlyA + 47
58	O129R + 59
4	ADO - 4
12	BNAG + 12
19	dMAL - 19
27	LIP - 27
35	dTAG - 35
42	AGLU - 42
48	ODC + 48
61	GGAA + 61
5	PyrA - 5
13	AGLTp - 13
20	dMAN - 20
29	PLE - 29
36	dTRE + 36
43	SUCT + 43
53	LDC - 53
62	IMLTa + 62
7	IARL - 7
14	dGLU + 14
21	dMNE + 21
29	TyrA + 31
37	CIT + 37
44	NAGA + 44
56	IHIsa - 56
64	ELLM + 64
9	dCEL - 9
15	GGT + 15
22	BXYL - 22
32	URE + 32
39	MNT - 39
45	AGAL - 45
57	CMT + 57
	ILATa -
	BGAL -
	OFF -
	BAIap -
	dSOR -
	SKG -
	PHOS +
	BGUR -

Comments:	
Identification Information	Analysis Time: 9.27 hoursStatus: Final
Selected Organism	99% ProbabilityProteus mirabilis
ID Analysis Messages	Bionumber: 0033011341553271
Biochemical Details	
2	APPA - 3
10	H2S + 11
17	BGLU - 18
23	ProA + 26
33	SAC - 34
40	ILATk + 41
46	GlyA + 47
58	O129R + 59
4	ADO - 4
12	BNAG + 12
19	dMAL - 19
27	LIP - 27
35	dTAG - 35
42	AGLU - 42
48	ODC + 48
61	GGAA + 61
5	PyrA - 5
13	AGLTp - 13
20	dMAN - 20
29	PLE - 29
36	dTRE + 36
43	SUCT + 43
53	LDC - 53
62	IMLTa + 62
7	IARL - 7
14	dGLU + 14
21	dMNE + 21
29	TyrA + 31
37	CIT + 37
44	NAGA + 44
56	IHIsa - 56
64	ELLM + 64
9	dCEL - 9
15	GGT + 15
22	BXYL - 22
32	URE + 32
39	MNT - 39
45	AGAL - 45
57	CMT + 57
	ILATa -
	BGAL -
	OFF -
	BAIap -
	dSOR -
	SKG -
	PHOS +
	BGUR -

Comments:																			
Identification Information				Analysis Time:				7.77 hours				Status:				Final			
Selected Organism				99% Probability				Proteus mirabilis											
ID Analysis Messages				Bionumber:				0033011341553271											
Biochemical Details																			
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-		
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-		
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-		
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-		
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+		
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-		
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-					

Comments:																			
Identification Information				Analysis Time:				7.77 hours				Status:				Final			
Selected Organism				99% Probability				Proteus mirabilis											
ID Analysis Messages				Bionumber:				0033011341553271											
Biochemical Details																			
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-		
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-		
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-		
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-		
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+		
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-		
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-					

bioMérieux Customer:Microbiology Chart ReportPrinted Dec 7 2025 9:45 AM CDT

Patient Name: hussna mohammedPatient ID: 202665 1Location:Physician:Lab ID: 202665 1Isolate Number: 1

Organism Quantity:Selected Organism : Proteus mirabilis

Source:Collected:

Comments:																	
Identification Information						Analysis Time:				7.78 hours				Status:		Final	
Selected Organism						99% Probability				Proteus mirabilis							
ID Analysis Messages						Bionumber:				0033011341553271							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

bioMérieux Customer:Microbiology Chart ReportPrinted Dec 7 2025 9:45 AM CDT

Patient Name: hussna mohammedPatient ID: 202665 1Location:Physician:Lab ID: 202665 1Isolate Number: 1

Organism Quantity:Selected Organism : Proteus mirabilis

Source:Collected:

Comments:																	
Identification Information				Analysis Time:				7.78 hours				Status:		Final			
Selected Organism				99% Probability				Proteus mirabilis									
ID Analysis Messages				Bionumber:				0033011341553271									
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Comments:																	
Identification Information				Analysis Time:				8.73 hours				Status:		Final			
Selected Organism				99% Probability				Proteus mirabilis									
ID Analysis Messages				Bionumber:				0033011341553271									
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BALap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Comments:																	
Identification Information						Analysis Time:				8.73 hours				Status:		Final	
Selected Organism						99% Probability				Proteus mirabilis							
ID Analysis Messages						Bionumber:				0033011341553271							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BALap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

bioMérieux Customer:Microbiology Chart ReportPrinted Dec 7 2025 10:39:00 AM CDT

Patient Name: hussna mohammedPatient ID: 202665 1
Location:Physician:
Lab ID: 202665 1Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus mirabilis

Source:Collected:

Comments:																	
Identification Information				Analysis Time: 9.27 hours								Status: Final					
Selected Organism				99% Probability Bionumber: 0033011341553271													
ID Analysis Messages																	
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed Dec 7 2025 10:39 AM CDT

Patient Name: hussna mohammed

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : Proteus mirabilis

Source:

Collected:

Comments:

Identification Information

Selected Organism

ID Analysis Messages

Analysis Time:

99% Probability

Bionumber:

9.27 hours

Proteus mirabilis

0033011341553271

Status:

Final

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Patient Name: hussna mohammed

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : Proteus mirabilis

Source:

Collected:

Comments:	

Identification Information	Analysis Time: 12.37 hours	Status: Final
Selected Organism	99% Probability Bionumber: 0033011341553271	Proteus mirabilis
ID Analysis Messages		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Patient Name: hussna mohammed

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : Proteus mirabilis

Source:

Collected:

Comments:	

Identification Information	Analysis Time: 12.37 hours	Status: Final
Selected Organism	99% Probability Bionumber: 0033011341553271	Proteus mirabilis
ID Analysis Messages		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Patient Name: hussna mohammed

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : Proteus mirabilis

Source:

Collected:

Comments:	

Identification Information	Analysis Time: 12.37 hours	Status: Final
Selected Organism	99% Probability	Proteus mirabilis
ID Analysis Messages	Bionumber:	0033011341553271

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Patient Name: hussna mohammed

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : Proteus mirabilis

Source:

Collected:

Comments:	

Identification Information	Analysis Time: 9.27 hours	Status: Final
Selected Organism	99% Probability	Proteus mirabilis
ID Analysis Messages	Bionumber:	0033011341553271

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed January 15, 2026 10:57:46 AM CST

Patient Name: **hussna mohammed**

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : **Proteus mirabilis**

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	9.27 hours	Status:	Final
Selected Organism	99% Probability	Proteus mirabilis		
ID Analysis Messages	Bionumber:	0033011341553271		
	Study Period for this specimen: September 2025 to January 2026			

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Page 1 of 1

bioMérieux Customer:

Microbiology Chart Report

Printed June 5 2026 10:57:46 AM CDT

Patient Name: **hussna mohammed**

Patient ID: 202665 1

Location:

Physician:

Lab ID: 202665 1

Isolate Number: 1

Organism Quantity:

Selected Organism : **Proteus mirabilis**

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	9.27 hours	Status:	Final
Selected Organism	99% Probability	Proteus mirabilis		
ID Analysis Messages	Bionumber:	0033011341553271		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Page 1 of 1

bioMérieux Customer:Microbiology Chart ReportPrinted Dec 5 2025 10:57:46 AM CDT

Patient Name: hussna mohammedPatient ID: 202665 1Location:Physician:Lab ID: 202665 1Isolate Number: 1

Organism Quantity:Selected Organism : Proteus mirabilis

Source:Collected:

Comments:	

Identification Information	Analysis Time: 9.27 hours	Status: Final
Selected Organism	99% Probability	Proteus mirabilis
ID Analysis Messages	Bionumber:	0033011341553271

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHIsa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

الخلاصة

تُعد *Proteus spp* من مسببات الأمراض الانتهازية سالبة الغرام، وتُعتبر واحدة من الأسباب المهمة للعدوى المكتسبة من المستشفيات، ولا سيما لدى المرضى الراقدين في المستشفيات. هدفت هذه الدراسة إلى التحري عن انتشار *Proteus spp* في البيئات الاستشفائية، وتقييم مقاومتها لمجموعة مختارة من المضادات الحيوية وتحملها للمعادن الثقيلة، بالإضافة إلى دراسة العلاقة المحتملة بين مقاومة المضادات الحيوية وتحمل المعادن الثقيلة. تم جمع ما مجموعه 250 عينة بيئية وسريرية من ثلاثة مستشفيات في محافظة بابل خلال الفترة من أيلول 2025 إلى كانون الثاني 2026. وشملت هذه المستشفيات (مستشفى الحلة التعليمي، مستشفى مرجان التعليمي، ومستشفى الإمام الصادق). قُسمت العينات المجمعة إلى 150 عينة سريرية و100 عينة بيئية. تضمنت العينات السريرية عينات الحروق والجروح وعدوى المسالك البولية تم جمع مسحات الحروق والجروح من عمق مواقع الإصابة باستخدام إجراءات التعقيم القياسية. في حين تضمنت العينات البيئية 55 عينة من مياه الصرف الصحي للمستشفيات و45 مسحة من الأسطح والأدوات المختلفة داخل المستشفى، مثل أسرة المرضى، والمعدات الطبية، وحاويات النفايات، والطاولات، وأيدي العاملين الصحيين، والمغاسل، والجدران، والصنابير، ومقابض الأبواب، والأرضيات، والأسطح المنضدية، ومكاتب عمل الممرضين، والحمامات، والدرازين، والطاولات الجانبية للأسرة، واسطوانات الأوكسجين، والسماعات الطبية، والصواني الطبية، وحاويات الصابون أو جل اليدين، وطاولات تقديم الوجبات. جُمعت جميع العينات بعناية لمنع حدوث التلوث أثناء النقل والمعالجة المختبرية. تمت زراعة العينات على أوساط MacConkey agar و Blood agar و Nutrient agar، وتم التعرف على العزلات البكتيرية باستخدام الاختبارات الكيميائية الحيوية التقليدية ونظام VITEK 2 الآلي. تم تحديد حساسية المضادات الحيوية باستخدام طريقة الحد الأدنى للتركيز المثبط (MIC)، بينما تم تقييم مقاومة المعادن الثقيلة باستخدام اختبار صفحية الآبار الدقيقة المعتمد على قيم MIC و IC₅₀ كما تم إجراء الكشف الجزيئي عن جينات مقاومة المضادات الحيوية باستخدام تقنية تفاعل البوليميراز المتسلسل (PCR) وشملت الجينات التي تم فحصها *acrA* و *acrB* و *blaTEM* و *blaCTX-M*.

من أصل 150 عينة سريرية، أظهرت 90 عينة نمواً بكتيرياً، وهو ما يمثل 60% من إجمالي العينات السريرية، بينما لم تظهر 60 عينة أي نمو بكتيري، وتمثل 40% ومن بين المزارع البكتيرية الموجبة، تم تشخيص 20 عينة على أنها *Proteus mirabilis*، والتي شكلت 13.3% من إجمالي العينات السريرية. تم تسجيل أعلى نسبة من عزلات *P. mirabilis* في عينات عدوى المسالك

البولية، حيث بلغت 13 عزلة بنسبة 65%، تلتها عينات الجروح بـ 4 عزلات بنسبة 20%، ثم عينات الحروق بـ 3 عزلات بنسبة 15% .

على العكس من ذلك، لم يتم الكشف عن أي عزلات من *Proteus spp* ضمن العينات البينية البالغ عددها 100 عينة ومع ذلك، تم عزل العديد من الأنواع البكتيرية الأخرى من هذه العينات البينية. وكانت أكثر الأنواع عزلاً هي *Pseudomonas aeruginosa* بعدد 25 عزلة تلتها *Staphylococcus spp* بعدد 15 عزلة و *Klebsiella spp* بعدد 12 عزلة و *Salmonella spp* بعدد 10 عزلات و *Citrobacter amalonaticus* بعدد 8 عزلات و *Shigella spp* بعدد 7 عزلات و *Aeromonas sobria* بعدد 6 عزلات و *Moraxella spp* بعدد 5 عزلات و *Myroides spp* بعدد 4 عزلات و *Brevundimonas diminuta* بعدد 3 عزلات و *Campylobacter spp* بعدد 2 عزلة و *Oligella ureolytica* بعدد 3 عزلات .

أظهر اختبار حساسية المضادات الحيوية وجود أنماط مقاومة متباينة بين عزلات *P. mirabilis* المختبرة حيث أظهرت العزلات معدل مقاومة بلغ (36.8%) تجاه كل من cefotaxime و cefepime و tigecycline ولوحظ معدل مقاومة مرتفع جداً تجاه tigecycline، حيث بلغ (100%) كما سجلت المقاومة تجاه avibactam و imipenem و trimethoprim و نسبة (26.3%)، بينما بلغت معدلات المقاومة تجاه ciprofloxacin و ampicillin و ceftolozane و نسب (21.1% و 15.8% و 10.5%) على التوالي. بالإضافة إلى ذلك، لم يُظهر colistin أي فعالية قابلة للكشف ضد عزلات *P. mirabilis* المختبرة. وأكدت نتائج MIC وجود مستويات عالية من المقاومة تجاه العديد من المضادات الحيوية، وبشكل خاص tigecycline و cefotaxime و ceftazidime و cefepime تراوحت قيم MIC الخاصة بـ tigecycline بين (2–4 µg/mL)، بينما تراوحت قيم cefotaxime و ceftazidime بين (0.25–64 µg/mL) و (0.12–64 µg/mL) على التوالي. أما قيم MIC الخاصة بـ cefepime فقد تراوحت بين (0.12–16 µg/mL) أظهرت النتائج أن الجينات المرتبطة بمضخات الإخراج *acrA* و *acrB* كانت موجودة في جميع العزلات المختبرة، بنسبة (100%)، بينما تم الكشف عن جينات إنزيمات البيتا لاكتاميز *blaTEM* و *blaCTX-M* في 7 عزلات بنسبة (71.4%) .

فيما يتعلق باختبار تحمل المعادن الثقيلة، أظهرت النتائج أن الزنك يمتلك أعلى مستوى من التحمل بين المعادن الثقيلة المختبرة، حيث تراوحت قيم MIC بين (2.5–10 mg/mL)، بينما تراوحت قيم IC_{50} بين (0.90–3.90 mg/mL) أظهر الكروم مستوى متوسطاً من التحمل، مع قيمة MIC ثابتة بلغت 2.5 mg/mL ، وتراوحت قيم IC_{50} بين (1.45–2.12 mg/mL) أظهر النحاس مقاومة أقل،

حيث تراوحت قيم MIC بين (0.62–2.5 mg/mL)، بينما تراوحت قيم IC₅₀ بين (0.31–0.86 mg/mL) أما الكادميوم فقد أظهر قيمة MIC بلغت (1.25 mg/mL) ، مع تراوح قيم IC₅₀ بين (0.347–0.726) mg/mL .

تشير هذه النتائج إلى وجود تزامن بين مقاومة المضادات الحيوية وتحمل المعادن الثقيلة لدى عزلات *P. mirabilis* المعزولة سريرياً وقد يسهم هذا التزامن في تعزيز بقاء وتكيف البكتيريا المقاومة في البيئات الاستشفائية. لذلك، تؤكد النتائج على ضرورة فرض رقابة أكثر صرامة على تصريف المعادن الثقيلة، والاستمرار في مراقبة محددات مقاومة المضادات الحيوية والمعادن الثقيلة في الأنواع البكتيرية ذات الأهمية السريرية.



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المقاومة المشتركة للمضادات الحيوية والمعادن الثقيلة في بكتريا *Proteus mirabilis* المعزولة من بيئة المستشفيات

رسالة مقدمة الى

مجلس كلية العلوم - جامعة القادسية كجزء من متطلبات نيل شهادة الماجستير في علوم البيئة

من قبل

حسنى محمد ناجي البيرماني

جامعة القاسم الخضراء / كلية العلوم / بكالوريوس

علوم البيئة (2021-2022)

اشراف

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