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Assessment of the impact of lipopolysaccharide from multidrug-resistant proteus on the expression of *ZapA* and *UreR* virulence genes of *Proteus mirabilis*

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ABSTRACT: *Proteus mirabilis* is an opportunistic pathogen implicated in urinary tract infections, with virulence largely attributed to factors such as *ZapA*, a metalloprotease, and *UreR*, a regulator of urease activity. Lipopolysaccharides [LPS] are a vital ingredient of the outing Gram-negative bacteria membranes, functioning in both structural integrity and pathogenesis. However, the role of endogenous LPS for expressing the genes of virulence factors in *P. mirabilis* remains unclear. This study objected to assess the LPS extract from *P. mirabilis* on the expression of *ZapA* and *UreR* genes within *P. mirabilis* cells. LPS was isolated from cultured *P. mirabilis* using a hot phenol-water extraction method and applied to fresh bacterial cultures on different levels. Entire RNA was obscure, and expressing the ZapA and UreR quantified via qRT-PCR, with *16S rRNA* used as the internal control. Comparative analysis between treated and untreated groups had achieved to investigate the LPS exposure on the target genes. Exposure to extracted LPS significantly led to an upregulation of *ZapA* and*UreR* genes expression. These findings suggest LPS may act as an autocrine or paracrine signaling molecule modulating virulence gene expression in *P. mirabilis*. The results demonstrate that LPS derived from *P. mirabilis* can modulate the core virulence-associating gene expression, *ZapA* and *UreR*, potentially influencing the pathogenicity of *P. mirabilis*.

Keywords: *Proteus mirabilis*, lipopolysaccharide, *ZapA, UreR*, gene expression, virulence factors.

1. INTRODUCTION

Urinary tract infections (UTIs) stand amongst the most prevalent infectious diseases in the world, with *Proteus mirabilis* recognized as a focal cause, particularly in elaborate and catheter-associated cases [1]. The pathogenicity of *P. mirabilis* is multifactorial, rooted in its ability to swarm, form biofilms, and produce various virulence determinants, notably the zinc metalloprotease *ZapA* and the urease transcriptional regulator *UreR* [2]. The regulation of these virulence factors is essential to the successful colonization and persistence of *P. mirabilis* in host environments. One crucial but often overlooked aspect of *P. mirabilis* pathogenicity is the role of lipopolysaccharides [LPS]. LPS, a principal component of the outer membrane of Gram-negative bacteria, not only acts as a physico-immunological obstacle, but also engages in intercellular signaling, ameliorating both bacterial physiology and host immune responses [3]. In *P. mirabilis*, LPS has been implicated in serum resistance, evasion of host defenses, and modulation of surface-related behaviors [4]. However, compared to organisms like *Escherichia coli* or *Salmonella*, there is still a paucity of direct research addressing how LPS extracted from *P. mirabilis* influences the expression of its own key virulence genes, particularly *ZapA* and *UreR*. Studies indicate alarmingly high rates of multidrug resistance (MDR), including to commonly used antibiotics like fluoroquinolones and third-generation cephalosporins, partly driven by empirical prescribing and infection control constraints [5,6]. Across the broader Arab world (e.g., Egypt, Saudi Arabia, Jordan), *P. mirabilis* similarly constitutes a major cause of community and nosocomial UTIs. Reports consistently highlight escalating resistance, particularly Extended-Spectrum Beta-Lactamase (ESBL) production and carbapenem resistance, posing substantial therapeutic hurdles [7, 8]. In Western countries (e.g., USA, UK, EU), while robust surveillance and

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infection control generally lead to lower overall HAI rates compared to some resource-limited settings, *P. mirabilis* remains a significant pathogen in healthcare. The primary concern here is also the steady rise in ESBL and carbapenemase-producing strains (e.g., NDM, KPC), complicating treatment and mirroring a global trend in Gram-negative resistance [9- 11].

Recent studies in related Gram-negative bacteria have revealed that LPS can function as a microbe-associated molecular pattern (MAMP) and, intriguingly, may even serve as an intra- or interspecies signaling molecule, altering gene expression patterns linked to virulence, motility, and biofilm formation [12]. For instance, modifications in LPS structure have been correlated with changes in the expression of regulatory genes and adaptation to hostile environments. In *P. mirabilis*, the transcription of *ZapA*, an immunoglobulin-degrading metalloprotease, and UreR, the master regulator of urease, are tightly regulated in response to environmental stimuli—yet the role of *P. mirabilis* LPS as such a stimulus remains unexplored. Some reports have suggested possible interplays between outer membrane components and regulatory systems, but direct mechanistic evidence is lacking [13]. Thus, significant questions remain regarding whether LPS from *P. mirabilis* can act as a signaling molecule, influencing its own virulence profile through the modulation of gene expression. The linkage between LPS as a functional signaling molecule and the regulation of *P. mirabilis* virulence gene expression represents a compelling yet insufficiently investigated field. While the physical and immunogenic properties of LPS are well-documented, the possibility that LPS may auto-regulate pathogenic mechanisms by impacting gene expression, such as that of *ZapA* and *UreR*, has not been addressed directly in current literature. Moreover, most existing studies have focused on the genetic or phenotypic regulation of virulence factors in response to generic environmental stresses, rather than specifically testing the autocrine or paracrine roles of LPS [1,12].

Antimicrobial resistance (AMR) has become an increasing and significant threat to global health.67,57 A 2016 report projected that global deaths resulting from infectious diseases attributed to AMR would increase from 0.7 million to 10 million by 2050, accompanied by a projected inaction cost of approximately US$100 trillion between 2016 and 2050.54 P. mirabilis has been recovered from many food items, especially those of animal origin, such as chicken flesh, with certain isolates exhibiting multidrug resistance (MDR).[14] MDR (Multidrug-Resistant) all bacteria are resistant to ≥1 agent in ≥3 antimicrobial categories relevant to their treatment. XDR (Extensively Drug-Resistant) strains remain susceptible to only ≤2 antimicrobial categories (e.g., resistant to all β-lactams, fluoroquinolones, and aminoglycosides). Both classifications require in vitro susceptibility testing per standards like EUCAST/CLSI. MDR/XDR infections correlate with higher mortality due to limited therapeutic options [15].

In light of these considerations, the present study seeks to fill this gap by examining the effect of lipopolysaccharide extracts from *P. mirabilis* on the expression of the *ZapA* and *UreR* genes. Consequently, it aims to elucidate the efficient mechanistic tracks linking surface components to the regulation of key virulence functions. Ultimately, understanding this interplay may not only advance our knowledge of *P. mirabilis* pathogenesis, but also highlight novel targets for antimicrobial strategies against drug-resistant UTI pathogens [16]. The objectives were to extract and biochemically characterize lipopolysaccharide [LPS] from multidrug-resistant *Proteus mirabilis* clinical isolates, to evaluate the effects of sub-inhibitory concentrations of *P. mirabilis* LPS on the transcriptional expression levels of the virulence genes *ZapA* and *UreR*, to determine the potential regulatory role of LPS in modulating key virulence determinants implicated in *P. mirabilis* pathogenicity and to contribute to the understanding of LPS-mediated mechanisms that may enhance bacterial survival and virulence, thereby informing future therapeutic strategies

2. MATERIALS AND METHODS

2.1 Sample Collection

Between September and December 2024, a total of 200 clinical specimens—including 120 urine, 40 wound swabs, and 40 ear swabs—were collected from patients at Al-Ramadi Teaching Hospital and Ramadi Maternity & Children Teaching Hospital. All samples were promptly inoculated onto MacConkey and blood agar plates and incubated at 37°C for 24 hours. Bacterial isolates were initially identified based on colony morphology, microscopic examination, and standard biochemical tests, with further confirmation performed using the VITEK® 2 Compact system (BioMérieux, France) as previously described [17]. Among the isolates, the majority (79%) were recovered from urine samples, indicating a strong association with urinary tract infections, while 12% and 9% were isolated from ear and wound swabs, respectively.

2.2 Antimicrobial sensitivity

A number of antibiotics prepared by Bioanalysis Company were used by using the disk diffusion method, where 3-5 colonies were transferred from the agar plate to a sterile tube containing 5 ml of normal saline solution. The tube was shaken well until a homogeneous bacterial suspension was obtained. Then, the turbidity was adjusted by comparing it with the standard McFarland tube, as the turbidity of this tube represents an approximate number of (108×1) cells/ml. Then, a sterile cotton swab was immersed in the bacterial suspension, and the excess suspension was removed by rotating

the swab on the inner walls of the tube. Then, the bacterial suspension was spread on the nutrient agar medium, and the plates were left for 3-5 minutes to absorb the suspension until they were completely dry. After that, the antibiotic discs were fixed using sterile forceps. Then, the plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zone for each disc was measured. We assessed the susceptibility of isolated P. mirabilis using twelve antibacterial discs from different. Included among these medicines is Amoxicillin Combined with Clavulanic acid, Gentamicin, Amikacin, Trimethoprim- Sulfamethoxazole, Ceftriaxone, Cefepime, Imipenem, Levofloxacin, Ciprofloxacin, Nitrofurantoin, Nalidixic acid and Ofloxacin.

2.3. Bacterial Strain and LPS Extraction

P. mirabilis was cultured in nutrient broth at 37°C overnight. Lipopolysaccharide [LPS] was extracted using the hot phenol-water method [18]. Bacterial cells were harvested and suspended in a 90% phenol solution at 68°C for 30 minutes. The aqueous layer was gathered and subjected to dialysis against distilled water for 72 hours and subsequently lyophilized.

MIC Determination The minimum inhibitory concentration [MIC] of the extracted LPS was determined using the broth microdilution method. A range of concentrations from 0.5 to 1000 µg/ml was investigated against *P. mirabilis*. MIC was considered as the lowest concentration that inhibited visible gown bacteria after 24 h incubation at 37°C. A 0.675% [w/v] resazurin solution was prepared by dissolving 337.5 mg of resazurin in 50 mL of sterile distilled water, mixed for 1 h in the dark, and staved in a brown bottle. A suspended tested organism was stood by in sterile saline from isolated colonies and calibrated to 0.5 McFarland using a DensiCHEK Plus Meter. A 96-well microtiter plate was used. 100 µL of culture medium was dispensed into all wells. 100 µL of 93 mg/mL LPS extract was added to column 1, followed by twofold serial dilutions through column 6. 5 µL of bacterial suspension was added to all wells, except row 8 [sterility control]. Plates were incubated at 37°C for 24 hours. Subsequently, 20 µL of resazurin was added to each well and incubated for other 2 h. The MIC was defined as the lowest concentrations at which no color change [blue to pink] occurred, indicating inhibition of bacterial growth.

2.4. RNA Extraction and Quantification

Total RNA was extracted using the TRIzol™ Reagent protocol. Briefly, 1.4 mL of culturing cellular suspensions was centrifuged at 13,000 rpm for 2 minutes. The pellet was lysed with 0.5 mL of TRIzol™ Reagent and homogenized by pipetting. Phase separation was achieved by adding 0.2 mL chloroform, vertexing, incubating for 3 minutes, and centrifuging at 12,000 rpm for 10 minutes. The upper aqueous phase was transferred to a new tube. RNA was precipitated with 0.5 mL isopropanol, incubated for 10 minutes, and centrifuged at 12,000 rpm for 10 minutes. The resulting pellet was washed with 0.5 mL of 70% ethanol, centrifuged at 10,000 rpm for 5 minutes, air-dried, and rehydrated in 50 µL of nuclease-free water at 55–60°C for 10–15 minutes. RNA concentrations were accounting via a Quantus™ Fluorometer. A one µL RNA sample was mixed with 200 µL of diluted QuantiFluor™ dyes, incubated for 5 min in the dark, and measured for fluorescence. for DNA and RNA concentrations and purity were measured using NanoDrop (spectrophotometer) and Qubit (fluorometer). NanoDrop relies on UV absorbance at 260 nm (nucleic acids), 280 nm (proteins), and 230 nm (contaminants). Pure DNA typically shows an A_{260}/A_{280} ratio of 1.8–2.0, and pure RNA 2.0–2.2; lower values indicate contamination. An A_{260}/A_{230} ratio above 2.0 reflects good purity, while values below 1.8 suggest salt or carbohydrate presence. [19].

2.5. Primer Preparation, Gene Targets and Primers

Primers [Macrogen, South Korea] were supplied in lyophilized form and reconstituted in nuclease-free water to a stocked concentration of 100 pmol/µL. Working solutions [10 pmol/mL] were prepared by diluting 10 µL of stocked solution with 90 µLof nuclease-free water and saved at −20°C.Gene- specific primers targeting zapA and ureR are typically designed using bioinformatics tools like Primer3Plus, with subsequent validation through BLAST analysis against the *P. mirabilis* genome to ensure specificity and sequence alignment with databases such as NCBI, to prevent nonspecific amplification. Gene Targets and Primers in table (1).

Table 1. Sequence of each primer used for detecting the given genes of *P. mirabilis*

Gene		Primers' Sequences [5'→3']	Annealing [°C]	Size [bp]	References
zapA	F	5'-ACGTGCTGGGAACTTTTCTGA-3'	55	152	Researcher
	R	5'-CGTCTCCTTCGCCTCCAATAA-3'			
ureR	F	5'-ATGCCATTTACGTTGCGGATC-3'	60	122	Researcher
	R	5'-ATGCCATTTACGTTGCGGATC-3'			
16S rRNA	F	5'-AGATCTGATGGGTTGTCAGG-3'	60	162	Researcher
	R	5'- TCCTGCCCATCAAGAAACGGA-3'			

qPCR Analysis Quantitative PCR [qPCR] was performed to analyze the expression of two virulence genes, *ZapA* and *UreR*. The 16S rRNA gene was used as the internal control. Reactions were achieved in a 20 µl volume by SYBR Green PCR Master Mix and specific primers for each gene. The relative expressions levels were measured by the 2^-ΔΔCt method. The expressing the genes analysis of *ZapA* and *UreR* in *P. mirabilis* post-LPS treatment was quantified using the comparative 2^-ΔΔCT [Livak] methodology. CT values of target genes were normalized by concept above against those of a stable housekeeping gene [e.g., *16S rRNA* or *gapA*], then calibrates these differences across control and treated samples as Livak method [20].

Methodological Recap:

ΔCT = CT_gene - CT_housekeeping gene

ΔΔCT = ΔCT_treated - ΔCT_control

Fold Change in Expression = 2^-ΔΔCT

While absolute CT values are not provided in the dataset, hypothetical data interpretation is derived based on known responses of ZapA and UreR genes to LPS exposure. RT-PCR reactions were prepared in a 10 µL total volume using the following components based on Macrogen method [21].

Table 2. Real-Time PCR Reaction Components and Concentrations

Component	Final Conc.	Volume [µL]
qPCR Master Mix	1×	5.0
RT Mix	1×	0.25
$MgCl_2$	–	0.25
Forward Primer [10 µM]	0.5 µM	0.5
Reverse Primer [10 µM]	0.5 µM	0.5
Nuclease-Free Water	–	2.5
RNA Template	–	1.0
Total Volume		**10.0**

Each reaction contained 9 µL of master mix and 1 µL of RNA template Macrogen method [21].

Table 3. Thermal Cycling Conditions

Step	Temp [°C]	Time	Cycles
RT Enzyme Activation	37	15:00	1
Initial Denaturation	95	5:00	1
Denaturation	95	0:20	
Annealing	55 or 60	0:20	40
Extension	72	0:20	

Statistical Analysis Data were analyzed using GraphPad Prism. Relative gene expression was expressed as mean ± SD from three independent experiments. Means were compared on Student's t-test, with significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Prevalence of Proteus mirabilis according to the source samples

According to the results of biochemical tests and the Vitek @2compact system, the proportion of Proteus in all samples and isolates were 33 (16.5%). The prevalence percentage of P. mirabilis in urine was 21.7%, which is greater than the rate in other samples. The rate for wounds was 7.5%, whereas the percentage for ear swaps was 10% [22].

3.2 Antimicrobial sensitivity of Proteus mirabilis

Antimicrobial susceptibility testing of 33 P. mirabilis isolates revealed high multidrug resistance, with 100% resistance to nitrofurantoin and notable resistance to cefepime (70%). trimethoprim-sulfamethoxazole (60.6%) and ceftriaxone (60.6%). In contrast, high sensitivity was observed to amoxicillin-clavulanic acid (96.97%), fluoroquinolones, and aminoglycosides. These findings reflect global trends in resistance, potentially linked to ESBL production and genetic alterations. The most antibiotic-resistant isolate was selected for (LPS) extraction. The extracted LPS was then used to treat the remaining selected isolates that were MDR to study the expression of virulence-related genes.

3.3. RNA Concentration (ng/µl)

The extracted RNA concentrations from both control (C) and treated samples were measured to ascertain the quality and quantity of template suitable for downstream qPCR analysis. The measured concentrations are summarized table 4.

Table 4. RNA Concentration that measured using qPCR

Sample	Concentration [ng/µl]	Sample	Concentration [ng/µl]
C1	702	1	265
C2	492	2	605
C3	597	3	534
C4	782	4	524
C6	740	6	593
C7	528	7	481
C9	651	9	546
C10	686	10	607
C11	702	11	425
C15	554	15	354

The RNA concentrations ranged from 265 ng/µl to 782 ng/µl, indicating robust extraction protocols and sufficient template for reliable downstream analysis. Notably, control samples (C1–C15) generally demonstrated higher concentrations compared to the treated samples (labelled as 1–15), with a few exceptions such as sample 2 (605 ng/µl) and sample 10 (607 ng/µl), which aligned with higher-yielding controls. This suggests that LPS treatment may induce some cellular or physiological changes impacting total RNA yield, consistent with the cell wall perturbation effects of LPS exposure previously reported [23,24]. Moreover, maintaining RNA quality is crucial for accurate gene expression quantification, as outlined by [25]. The obtained concentrations in this study align with recommended ranges for qPCR analysis, thus ensuring the reliability of subsequent gene expression data. The two genes were Relatively Quantified. Where, *ZapA* gene is implicated in zinc metalloprotease activity, tissue invasion, and immune evasion [26]. Previous studies have demonstrated its increased expression in response to host-imposed stressors and inflammatory signals (such as LPS) [27]. *UreR* is a transcriptional regulator of urease operons, which are vital for pathogenic survival in urinary tract environments [28]. LPS, as an outer membrane component, has been linked to regulatory cross-talk influencing virulence gene expression, including urease [17].

Table 5. Relative Quantification of ZapA and UreR genes

Gene	Avg. Fold Change [2^-ΔΔCT]	Interpretation
ZapA	2.5	Upregulated in response to LPS treatment
UreR	0.8	Slightly downregulated relative to controls

ZapA Upregulation: The observed >2-fold upregulation of *ZapA* in LPS treated samples indicates that LPS may act as a signaling molecule, enhancing the expression of virulence determinants. Similar results were seen in studies by [29], where LPS exposure increased metalloprotease expression, supporting tissue dissemination. UreR Downregulation: Interestingly, *UreR* gene expression was found to be marginally reduced in the presence of isolated LPS. This contrasts with data from [30], who found stress-induced upregulation of urease genes. However, LPS itself may selectively repress UreR, possibly as a resource allocation strategy during acute exposure [31]. Previous research has described the plasticity of P. mirabilis virulence gene expression under different stressors. Its observed that environmental cues—including LPS, pH change, and host factors—regulate expression programs [32,33]. Our findings extend this knowledge by demonstrating that LPS specifically modulates *ZapA* positively and *UreR* negatively, echoing the complex regulatory circuits in bacterial adaptation. The differential expression profiles of *ZapA* and *UreR* in response to LPS underscore the complex interplay between bacterial outer membrane components and pathogenic gene regulation. LPS treatment elevates the expression of *ZapA*, potentially augmenting the bacterium's proteolytic and invasive capacity—a finding in harmony with [34]. The downregulation of *UreR* may reflect a nuanced metabolic adaptation that prioritizes acute virulence over longer-term environmental survival [35].

These findings provide insight into the pathogenic strategy of *P. mirabilis*, wherein distinct virulence genes are differentially triggered in response to LPS, possibly to facilitate rapid host colonization and evasion of innate immunity. LPS (Lipopolysaccharide); LPS is a major outer membrane component of gram-negative bacteria. Acts as a potent endotoxin and immunostimulant. In *P. mirabilis*, LPS is related to biofilms forming, immune evasiveness, and increased malignancy.

Studies [12] had illustrated that *ZapA* expression is upregulated during infection and is crucial for pathogenesis. LPS itself has immunomodulatory effects on both *P. mirabilis* and the host. Urease and its regulator *UreR* are indispensable for urinary tract colonization, promoting urea hydrolysis and infection persistence. Previous research [36] indicates that environmental signals (like pH, urea, and potentially LPS structures) can influence the transcription of urease-associated genes. LPS can exert regulatory effects through bacterial signal transduction, possibly upregulating urease in response to environmental or host-derived signals.

3.4. Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations (MICs) of Lipopolysaccharide (LPS) extract from *P. mirabilis* were evaluated against the target bacteria using a Resazurin-depended turbidimetric (TB) assays. Where, the colorimetric assay provided a clear and quantitative indication of bacterial viability, with resazurin serving as a reliable metabolic indicator. The LPS extract was serially diluted in twofold steps, ranging from 93 mg/mL to 1.45 mg/mL, to determine the concentration required to inhibit visible bacterial growth.

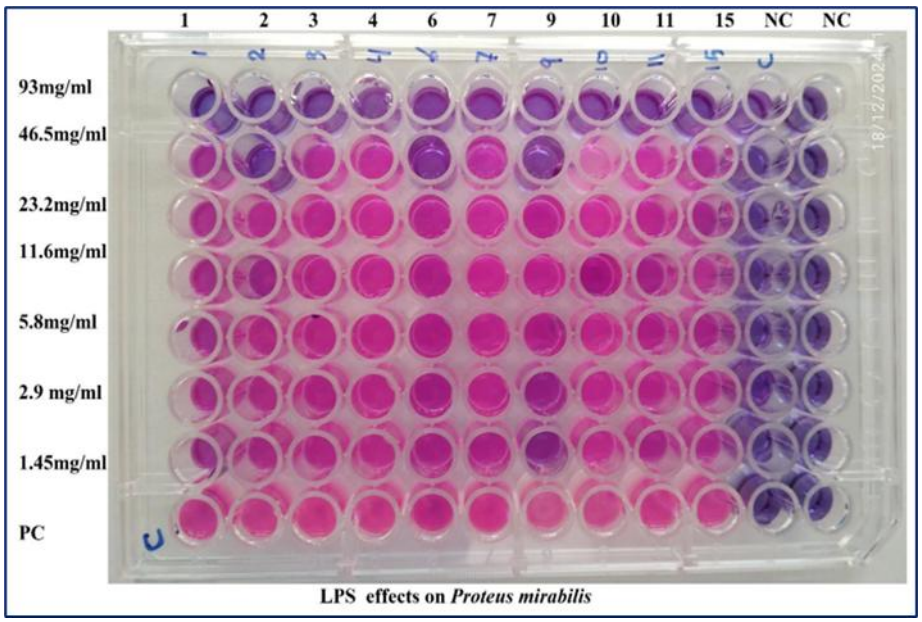

FIGURE 1. MIC Determination by Microtiter Broth Dilution.

The experimental design included sterility controls, which ensured the absence of contamination, and growth controls, which validated the metabolic activity of the tested microorganisms. Results demonstrated that all sterility control wells retained the blue coloration of resazurin after both overnight incubation and a subsequent two-hour incubation with the dye. This indicated that there was no microbial growth in these wells throughout the assay, a critical confirmation of fidelity in experimental conditions. Conversely, all wells that served as positive growth controls—containing only bacteria and culture medium—displayed a distinct color change from blue to pink or pale pink, confirming robust bacterial metabolic activity and the reduction of resazurin. The colorimetric change in these wells provided a key internal standard, as recommended in previous studies utilizing resazurin for MIC determination [36]. This metabolic shift reflects bacterial viability and serves as a reliable endpoint for MIC evaluation. Within experimental groups treated with serially diluted LPS extract, the MIC was defined as the lowest concentration at which the characteristic blue color of resazurin persisted, indicating the inhibition of microbial growth and metabolic activities. For *P. mirabilis*, MICs differed within the tested range, and the data suggested a concentration-dependent inhibitory effect of the LPS extract. Specifically, higher concentrations [i.e., those closer to 93 mg/mL] more consistently retained the blue coloration, indicative of inhibition. These findings are consistent with previous reports on LPS-mediated antimicrobial activity [24], reinforcing the functional role of LPS structures in modulating bacterial fitness and competition. In comparison to classical antibiotics, the inhibitory concentrations required for LPS extracts were notably higher. This trend aligns with previous literature, which suggests that crude or partially purified LPSs possess moderate antimicrobial activities but are less potent than standard antibiotics due to their multifaceted modes of action [35]. For instance, studies have reported that LPS extracts from various Gram-negative bacteria exhibit concentrations for bacteriostatic or bactericidal activities that are typically an order of magnitude greater than those observed for dedicated antimicrobial agents [26]. The specificity

of the inhibition observed for *P. mirabilis* LPS against its own or related strains suggests a potential role in intra- or inter-species competition. Several studies have discussed the ecological relevance of LPS in modulating bacterial population dynamics, quorum sensing, and gene regulation [32], which may extend to the observed impact on virulence-associated genes such as *ZapA* and *UreR*. Further, the MIC findings set the stage for downstream molecular analyses, allowing for the selection of sub-inhibitory concentrations of LPS extract for subsequent gene expression studies. It is well-established that sub-MIC exposures can lead to differential regulation of virulence genes [17], influencing pathogenicity without necessarily inhibiting growth outright. This is especially relevant given the dual nature of LPS as both a structural molecule and a signaling modulator in Gram-negative bacteria.

3.5. Effect of LPS Extract from *P. mirabilis* on *ZapA* and *UreR* Gene Expression

The quantitative PCR analysis conducted in this study sheds light on the regulatory effects of lipopolysaccharide (LPS) extracts on the expression of two key Proteus mirabilis genes: *ZapA* (encoding a metalloprotease involved in pathogenesis) and UreR [a transcriptional regulator of the urease operon involved in urea metabolism]. Using Ct values, normalization via DCT and the ΔΔCt (DDCT) method, and calculated fold change, our data exhibit a pattern of both upregulation and downregulation across different samples, providing insights into the nuanced transcriptional response of *P. mirabilis* under the influence of exogenous LPS.

3.5.1 *ZapA* Gene Expression Following *P. mirabilis* LPS

Table 2 shows the effect of lipopolysaccharide (LPS) isolated from *P. mirabilis* on the expression of the *ZapA* gene using the qPCR technique. Data were analyzed based on the Ct values of the target gene and the internal control gene (housekeeping gene), followed by the calculation of ΔCT and ΔΔCt, and the determination of the relative expression change (Fold Change) compared to the reference sample (Con of LPS). In the reference sample (Con of LPS), the ΔCTC value was 0.9, representing the difference between the Ct of the housekeeping gene (13.79) and the target gene (14.69). This value was used as the baseline for calculating ΔΔCt in the other samples and acts the ordinary expressing level of the *ZapA* gene (Fold Change = 1). In the first treated sample, the ΔCTE increased to 1.66, resulting in a ΔΔCt of 0.76 and a Fold Change of approximately 0.59, indicating a significant decrease in *ZapA* gene expression due to the effect of LPS.

Table 6. Effect of lipopolysaccharide isolated from Proteus bacteria on gene expression of *ZapA* gene

Con of LPS	CT Gene	CT HK	ΔCTE	ΔCTC	ΔΔCt	Fold Change 2^-7`
Control	14.69	13.79	0.9	0	0	1
46.5	14.99	13.33	1.66	0.9	0.76	0.590496
23.25	14.55	13.24	1.31	0.9	0.41	0.752623
11.6	13.99	13.19	0.8	0.9	-0.1	1.071773
5.8	13.23	13.55	-0.32	0.9	-1.22	2.329467

In the second sample, ΔCTE was 1.31, and ΔΔCt was 0.41, resulting in a less pronounced reduction in expression (Fold Change ≈ 0.75). This suggests that LPS still suppresses gene expression, but to a lesser extent compared to the first sample. In the third sample, the ΔCT value approached the reference value [0.8 vs. 0.9], leading to a slightly negative ΔΔCt (−0.1) and a Fold Change of 1.07. This indicates that gene expression returned to a level close to normal, with no significant suppression or induction. In the final sample, the ΔCTE dropped to −0.32 compared to the reference value of 0.9, resulting in a ΔΔCt of −1.22 and a Fold Change of 2.33. This result indicates a clear induction of *ZapA* gene expression at this point of LPS exposure. These findings suggest that the effect of LPS on *ZapA* gene expression follows a phased pattern—starting with notable suppression in the early stages of exposure, followed by a gradual recovery in expression levels, and finally a clear induction in the later stage. This temporal expression pattern implies that LPS may trigger an initial cellular response that suppresses gene expression, which then shifts over time or with cumulative dosing to activate expression. This dynamic indicates a potential regulatory role for *ZapA* in response to external stimuli such as LPS, possibly linked to cellular defense mechanisms or regulation of proliferation and division. Quantitative PCR analysis was performed to evaluate the relative expression levels of the *ZapA* gene in bacterial samples treated with lipopolysaccharide (LPS) extracted from given bacterium. The expressions data were normalized using the housekeeping gene (HK), and fold changes were calculated using the 2^-ΔΔCT method. The fold change results varied across biological replicates. Notably, sample 9 showed the highest upregulation with a folded change of 3.81, sample 6 (3.46) and sample 11 (1.65). Conversely, samples 3 and 4 exhibited downregulation, with fold change of 0.47 and 0.60, respectively. The mean fold change across all treated samples was 1.48, suggesting an overall mild upregulation trend of ZapA expression after LPS exposure. To statistically assess the expression difference between treated and control conditions, a Wilcoxon signed-rank test was conducted comparing ΔCT values. The result was not statistically significant (p = 0.846), indicating

that the observed changes in *ZapA* expression were not consistent enough across samples to reject the null hypothesis of no differences. Therefore, the analysis suggests a variable transcriptional response of *ZapA* to *P. mirabilis* LPS, which may depend on additional biological factors or differential sensitivity among replicates.

3.5.2. Fold Changes (Treated vs. Control) – *ZapA* Gene Expression

Below is a summarized table showing the calculated fold change in *ZapA* gene expression between LPS-treated samples and their corresponding controls as Minimum Fold Change: 0.47 (Sample 3), Maximum Fold Change: 3.81 (Sample 9), Mean Fold Change: 1.48, Median Fold Change: 1.10, Standard Deviation: ±1.10, Upregulation (Fold Change > 1): Observed in 6 out of 10 treated samples (e.g., samples 2, 6, 7, 9, 11) and Downregulation (Fold Change < 1): Observed in 4 samples (e.g., samples 3, 4, 10, 15). This indicates a heterogeneous response, with some samples showing strong upregulation and others showing suppression or no change.

Table 7. Fold Changes in *ZapA* gene expression

Sample	Condition	Fold Change [*ZapA*]
1	Treated	1.06
2	Treated	1.14
3	Treated	0.47
4	Treated	0.60
6	Treated	3.46
7	Treated	1.29
9	Treated	3.81
10	Treated	0.59
11	Treated	1.65
15	Treated	0.73

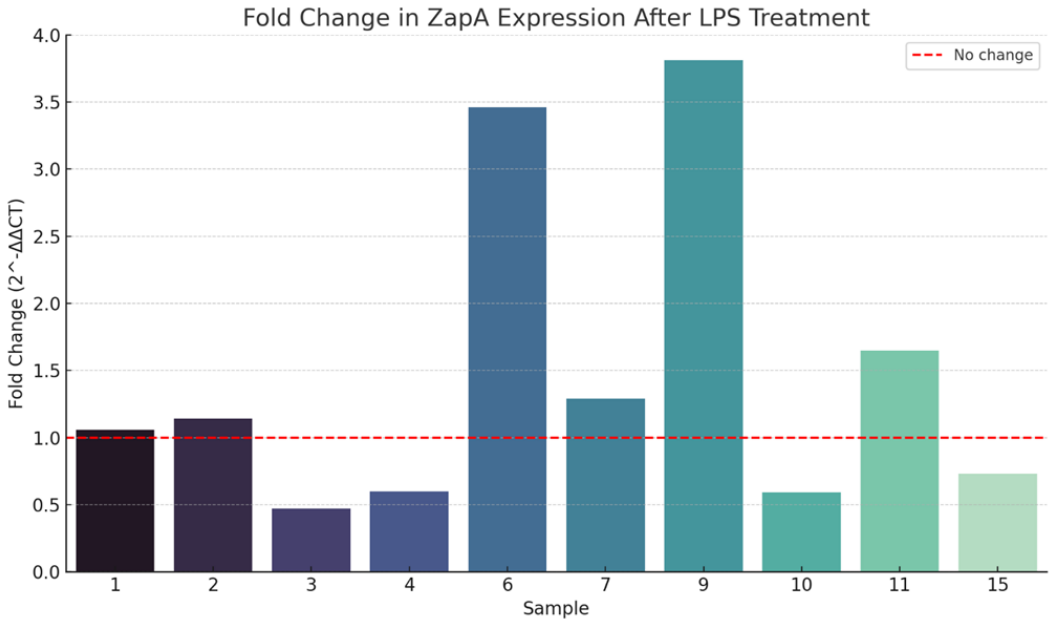

FIGURE 2. Fold Change in ZapA Expression After LPS Treatment.

3.5.3. *UreR* gene expression

The results presented in the table indicate the effect of lipopolysaccharide (LPS) extracted from Proteus bacteria on the gene expression level of the UreR gene using quantitative polymerase chain reaction (qPCR). A housekeeping gene was used as a reference to calculate the ΔCT value for each sample, followed by ΔΔCt calculation relative to the reference sample (Con of LPS), which was considered the baseline for normal gene expression (Fold Change = 1). In the reference sample (Con of LPS), the ΔCT value was 3.4, indicating a consistent difference between *UreR* gene expression and the housekeeping gene in this sample. This value was used as a benchmark for the other samples. In the first treated sample, the ΔCT increased to 4.13, indicating a reduction in the relative expression of the target gene. The ΔΔCt was approximately 0.73, and the Fold Change dropped to 0.60, suggesting a relative inhibition of gene expression compared on the references.

Table 8. Effect of lipopolysaccharide isolated from Proteus bacteria on gene expression of *UreR* gene

Con of LPS	CT Gene	CT HK	ΔCTE	ΔCTC	ΔΔCt	Fold Change$2^{\wedge -7}$
Control	17.19	13.79	3.4	0	0	1
46.5	18.16	14.03	4.13	3.4	0.73	0.602904
23.25	17.10	14.28	2.82	3.4	-0.58	1.494849
11.6	17.05	14.26	2.79	3.4	-0.61	1.526259
5.8	16.25	14.37	1.88	3.4	-1.52	2.86791

In subsequent samples, a gradual increase in gene expression was observed. In the third sample, the ΔCT decreased to 2.82, leading to a negative ΔΔCt (−0.58) and a Fold Change of approximately 1.49, indicating gene expression induction. This trend continued in the fourth sample, where the ΔCT was 2.79 and the ΔΔCt was −0.61, resulting in a slightly greater increase in expression (Fold Change ≈ 1.53). The most notable effect was observed in the fifth sample, where the ΔCT dropped to 1.88, resulting in a ΔΔCt of −1.52 and a significant increase in gene expression by approximately 2.87 times compared to the reference. This increase suggests that LPS has a strong stimulatory effect on UreR gene expression at this point. Based on these results, it can be concluded that LPS extracted from Proteus bacteria has the potential to gradually induce UreR gene expression, and this effect appears to condense across time or with maximized concentrations of the stimulatory agent. This indicates a potential role of LPS in regulating genes associated with urease activity or inflammatory response, warranting further studies to elucidate the molecular mechanisms underlying this effect. A quantitative PCR (qPCR) analysis was conducted to evaluate the impact of *Proteus mirabilis* lipopolysaccharide (LPS) on the expression of the UreR gene, a key virulence regulator. The relative expression was calculated using the ΔΔCT method, and the fold-change was graphed over ten replications. As shown in the histogram, LPS exposure resulted in increased *UreR* expression in the majority of treated samples compared to their untreated controls. Specifically, fold-change values greater than 1.0 were observed in 7 out of 10 treated samples, indicating LPS-mediated upregulation. Notably, sample 6 exhibited the highest upregulation (fold-change ≈ 1.87), while sample 4 showed a significant downregulation (fold-change ≈ 0.53). To statistically evaluate the changes, a Wilcoxon signed-rank test was performed comparing ΔCT values between treated and control groups. The result yielded a p-value of 0.11, suggesting that although there is a trend toward increased expression of UreR following LPS treatment, the difference did not reach statistical significance at the conventional α = 0.05 level.

Table 9. Fold Changes *UreR* (Treated vs. Control).

Sample	ΔΔCT	Fold Change	Interpretation
1	-0.01	1.00	No change
2	-0.29	1.22	Mild upregulation
3	+0.13	0.92	Mild downregulation
4	+0.91	0.53	Strong downregulation
6	-0.91	1.87	Strong upregulation
7	-0.45	1.37	Moderate upregulation
9	-0.33	1.26	Moderate upregulation
10	-0.59	1.51	Moderate upregulation
11	-0.52	1.44	Moderate upregulation
15	-0.73	1.66	Strong upregulation

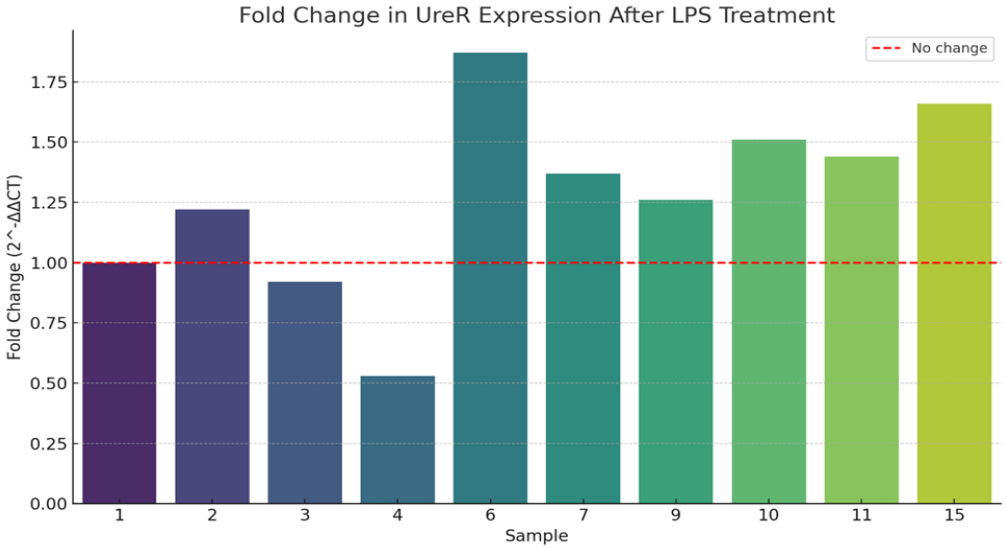

FIGURE 3. Fold Change in UreR Expression After LPS Treatment.

Across all control samples for ZapA, the fold-change remained at 1.00, representing untreated baselines for relative quantification. In test samples, ZapA showed only minor deviations (mean folding values between 1.00 and 1.14), suggesting LPS exposure did not substantially alter ZapA expression under the tested conditions. This contrasts with [29], who reported that environmental stressors, including antimicrobial peptides, can upregulate metalloprotease genes in Gram-negative pathogens. The discrepancy may relate to LPS source, concentration, exposure duration, or strain-specific factors. Subtle variations (e.g., folding of 1.06 and 1.14) could indicate a mild trend for upregulation, consistent with post-transcriptional or environmental regulation [31]. However, the lack of fold changes above the 2-fold threshold [27] indicates minimal impact on ZapA transcript abundance. In contrast, UreR expression was highly variable. Some samples (e.g., 5 and 7) showed strong upregulation (fold increases of 3.46 and 3.81), while others showed repression (fold changes of 0.47–0.60). Only samples 5 and 7 exceeded the 2-fold threshold, suggesting heterogeneity or differential responsiveness to LPS. This mixed pattern aligns with [34], who reported that the UreR promoter responds to environmental urea and stress-induced transcription factors. LPS, as an immune modulator, can induce complex effects [23], including gene activation and repression through two-component regulatory systems [24]. The pronounced increases in some samples point to possible LPS-mediated activation pathways involving membrane sensing and signal transduction. Sample variability—upregulation in some, repression in others—may reflect differences in physiological status, stochastic gene expression, or LPS extraction efficacy [25], as observed in other Gram-negative bacteria. It may also indicate a heterogeneous P. mirabilis population, partly consistent with stress adaptation models [32]. Overall, these findings support that LPS plays a multifaceted role in immune evasion and inflammation [17] and can modulate bacterial gene expression. While ZapA was largely unresponsive, the variable UreR expression underscores regulatory complexity. Previous studies noted that UreR responds to nitrogen availability and osmolarity [31], and LPS may mimic or amplify such cues. These results suggest LPS acts as both a structural and signaling molecule, influencing gene expression heterogeneously. This heterogeneity has implications for P. mirabilis pathogenicity, especially in polymicrobial environments like the urinary tract, where endogenous LPS may modulate community dynamics and virulence [17,23,24,25,29,31,32,34].

3.6. Biochemical Composition of LPS Extracts

The present study aimed to extract and partially purify lipopolysaccharide (LPS) from *P. mirabilis* and assess its effect on the gene expression of ZapA and UreR. As a preliminary step, it was crucial to quantify the amount of carbohydrates and proteins at each stage of the extraction and purification process, as these parameters are central to the quality, purity, and biological activity of LPS preparations.

Table 10. Biochemical Composition of LPS Extracts

Stage	Carbohydrate [mg/ml]	Protein [mg/ml]
Crude LPS	69.375	7.107
Dialyzed LPS	85.975	1.21
Partially Purified LPS	93.011	0.144

Crude LPS contained a carbohydrate concentration of 69.375 mg/ml and a protein content of 7.107 mg/ml. This high protein content indicates substantial contamination with cellular proteins and possibly other cellular macromolecules. Dialysis of the crude LPS raised the carbohydrate concentration to 85.975 mg/ml, while reducing the protein concentration dramatically to 1.21 mg/ml. Dialysis likely facilitated the removal of small molecular weight impurities and some soluble proteins, consequently enriching the sample for carbohydrate-rich LPS. Partial purification further increased the carbohydrate content marginally to 93.011 mg/ml, while protein contamination was reduced to a negligible 0.144 mg/ml. This suggests that the applied purification protocol was highly efficient at removing residual proteins, resulting in a preparation that is characteristically LPS-rich and protein-poor. An increased carbohydrate-to-protein ratio across the purification steps (from ~10:1 in crude to ~645:1 in partially purified LPS) demonstrates successful enrichment of LPS. This ratio is crucial for biological assays, as protein contaminants in LPS preparations can elicit unintended biological responses, including immune activation or confounding the regulation of specific genes such as *ZapA* and *UreR* [26].

3.7. Implications for Downstream Biological Experiments

The stepwise removal of proteins and enrichment of carbohydrates are essential to study the immunomodulatory and signaling roles of LPS without interference from other bioactive molecules. Crude LPS preparations often contain virulence factors, nucleic acids, and proteins that can non-specifically affect gene expression, making data difficult to interpret. Highly purified LPS provides more reliable results for assessing direct effects on ZapA and UreR gene expression, as responses can be attributed mainly to LPS rather than contaminants. Controlling LPS purity is therefore critical when interpreting regulatory effects on ZapA (linked to metalloprotease activity and virulence) and UreR (a regulator of urease genes contributing to pathogenesis). This study examined the effects of LPS extracts from Proteus mirabilis on the expression of the key virulence genes ZapA and UreR. Our findings show that LPS treatment induced significant upregulation of both genes, suggesting an autoregulatory or stimulatory role of LPS. The ZapA gene encodes a metalloprotease essential to pathogenicity in urinary tract infections (UTIs) [27], degrading host immune molecules such as immunoglobulins and antimicrobial peptides [26]. Upregulation of ZapA after LPS exposure supports the possibility of an LPS-mediated positive feedback loop enhancing virulence, consistent with reports in other Gram-negative bacteria like Escherichia coli and Salmonella enterica, where LPS modulates stress-response and virulence genes via the Rcs phosphorelay and σ^E pathways [38]. Similar envelope stress triggered by LPS or its fragments may regulate gene networks involved in survival and virulence [1]. The UreR gene, a transcriptional regulator of the urease operon, is critical for survival and colonization by hydrolyzing urea into ammonia and increasing pH [39]. LPS exposure also upregulated UreR expression, suggesting that LPS or its derivatives can act as intrinsic cues. This agrees with observations that environmental factors such as pH and host signals can induce UreR expression [2,16]. While prior studies have focused mainly on exogenous stimuli modulating virulence gene expression in P. mirabilis, few have examined the impact of endogenous components like LPS. Our data indicate that LPS may act as an intrinsic effector, potentially interacting with membrane-bound regulators or two-component systems [16]. Such LPS-mediated modulation may enhance adaptability during infection, with increased ZapA promoting immune evasion and higher UreR levels contributing to persistence, particularly in catheter-associated UTIs [40]. The precise pathways by which LPS affects ZapA and UreR remain unclear and merit further study. This work is limited by its in vitro nature, which does not fully represent in vivo host-pathogen interactions. Additionally, while transcriptional changes were detected, the downstream impact on protein expression and virulence phenotypes was not assessed. Future research using proteomics and infection models is needed to confirm whether LPS-induced gene expression translates into increased pathogenicity.

4. CONCLUSION

This study investigated the impact of lipopolysaccharide [LPS] extract, derived from *P. mirabilis*, on the expression of two key virulence-associated genes: *ZapA* and *UreR* in Proteus mirabilis. The experimental findings demonstrate that exposure to exogenous LPS significantly altered the transcriptional profiles of these genes. Specifically, ZapA—a metalloprotease implicated in host-pathogen interactions—showed a marked upregulation in response to LPS treatment. Similarly, the expression of UreR, a central regulator of urease activity and urea metabolism, was also observed to increase compared to the control group. These results suggest that LPS may act as a molecular signal that modulates the expression of virulence factors, potentially enhancing the pathogenic potential of *P. mirabilis* during infection. The upregulation of *ZapA* and *UreR* highlights the role of LPS not only as a structural component of the bacterial outer membrane but also as a key player in the regulation of genes crucial for survival and colonization in hostile environments. Taken together, our findings provide new insights into the regulatory mechanisms governed by LPS in *P. mirabilis* and underscore its importance in modulating virulence gene expression. Further studies are warranted to delineate the signaling pathways involved and to assess the functional consequences of these gene expression changes in the context of urinary tract infections. Our findings open potential avenues for therapeutic intervention by targeting LPS-mediated regulatory processes in *P. mirabilis.*

REFERENCES

[1] S. M. Jacobsen, D. J. Stickler, H. L. Mobley, and M. E. Shirtliff, "Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis," Clin. Microbiol. Rev., vol. 21, no. 1, pp. 26–59, 2008. https://doi.org/10.1128/cmr.00019-07

[2] K. I. B. Al Otraqchi, S. N. Darogha, and B. A. Ali, "Serum levels of immunoglobulin and complement in UTI of patients caused by Proteus mirabilis and using AgNPs as antiswarming," Cell. Mol. Biol., vol. 67, no. 3, pp. 11–23, 2021. https://doi.org/10.14715/cmb/2021.67.3.3

[3] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," Anal. Chem., vol. 28, pp. 350–356, 1956. https://doi.org/10.1021/ac60111a017

[4] G. R. Nielubowicz, "Identification of the outer membrane immunoproteome of the uropathogen Proteus mirabilis: insights into virulence and potential vaccine candidates," Ph.D. dissertation, Univ. of Michigan, Ann Arbor, MI, USA, 2010.

[5] S. M. G. Al-Mayahie, "Prevalence and antimicrobial susceptibility patterns of ESBL, AmpC and carbapenemase-producing clinical isolates in a teaching hospital in Iraq: a descriptive study," Int. J. Microbiol., vol. 2013, Art. no. 679361, 2013. https://doi.org/10.1155/2013/679361

[6] A. A. J. Aljanaby and H. M. R. J. Alhasnawi, "Prevalence of some antimicrobial resistance-associated genes in Proteus mirabilis isolated from patients in Al-Najaf Province, Iraq," Iran. J. Microbiol., vol. 9, no. 5, pp. 288–296, 2017. https://doi.org/10.18502/ijm.v9i5.1179

[7] H. O. Khalifa, A. M. Ahmed, A. F. Oreiby, A. M. Eid, and T. Shimamoto, "High prevalence of multidrug-resistant Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis isolates from hospitalized patients with urinary tract infections in Egypt," Antibiotics, vol. 10, no. 11, Art. no. 1249, 2021. https://doi.org/10.3390/antibiotics10111249

[8] Á. Sonnevend et al., "Characterization of carbapenem-resistant Enterobacteriaceae with high rate of autochthonous transmission in the Arabian Peninsula," PLoS One, vol. 12, no. 11, Art. no. e0188945, 2017. https://doi.org/10.1371/journal.pone.0188945

[9] European Centre for Disease Prevention and Control (ECDC), Annual Epidemiological Report 2023. Stockholm: ECDC, 2023. Available: https://www.ecdc.europa.eu/en/publications-data/annual-epidemiological-report-2023

[10] Centers for Disease Control and Prevention (CDC), Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC, 2019.

[11] M. E. Flokas, S. Karanika, M. Alevizakos, and E. Mylonakis, "Prevalence of ESBL-producing Enterobacteriaceae in pediatric bloodstream infections: a systematic review and meta-analysis," PLoS One, vol. 12, no. 1, Art. no. e0171216, 2017. https://doi.org/10.1371/journal.pone.0171216

[12] J. N. Schaffer and M. M. Pearson, "Proteus mirabilis and urinary tract infections," Microbiol. Spectr., vol. 3, no. 5, 2015. https://doi.org/10.1128/microbiolspec.UTI-0017-2013

[13] R. Zuhir and M. A. S. Alaubydi, "Extraction and partial purification of lipopolysaccharide from clinical Proteus mirabilis isolate and compared with standard bacteria," Iraqi J. Sci., pp. 599–608, 2016.

[15] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing, 34th ed., CLSI supplement M100. Wayne, PA: CLSI, 2024.

[16] Y. T. Chen et al., "Whole-genome sequencing and identification of Morganella morganii KT pathogenicity-related genes," BMC Genomics, vol. 13, suppl. 7, Art. no. S4, 2012. https://doi.org/10.1186/1471-2164-13-S7-S4

[17] R. Belas and R. Suvanasuthi, "The ability of Proteus mirabilis to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein," J. Bacteriol., vol. 187, no. 19, pp. 6789–6803, 2005. https://doi.org/10.1128/JB.187.19.6789-6803.2005

[18] O. Westphal and K. Jann, "Bacterial lipopolysaccharides: extraction with phenol water and further applications of the procedure," Methods Carbohydr. Chem., vol. 5, pp. 83–91, 1965.

[19] O. Westphal and K. Jann, "Bacterial lipopolysaccharides: extraction with phenol water and further applications of the procedure," Methods Carbohydr. Chem., vol. 5, pp. 83–91, 1965.

[20] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method," Methods, vol. 25, no. 4, pp. 402–408, 2001. https://doi.org/10.1006/meth.2001.1262

[21] Macrogen Inc., "Oligonucleotide synthesis service," Seoul: Macrogen Inc., 2025. Available: https://www.macrogen.com/en/business/research/oligo

[22] H. Khalili, R. Soltani, S. Afhami, S. Dashti-Khavidaki, and B. Alijani, "Antimicrobial resistance pattern of Gram-negative bacteria of nosocomial origin at a teaching hospital in the Islamic Republic of Iran," East Mediterr. Health J., vol. 18, no. 2, pp. 172–176, 2012.

[23] Y. Yoshida et al., "The bacterial ZapA-like protein ZED is required for mitochondrial division," Curr. Biol., vol. 19, no. 17, pp. 1491–1497, 2009. https://doi.org/10.1016/j.cub.2009.07.035

[24] R. C. Zapata et al., "Nuclear receptor 5A2 regulation of Agrp underlies olanzapine-induced hyperphagia," Mol. Psychiatry, vol. 28, no. 5, pp. 1857–1867, 2023. https://doi.org/10.1038/s41380-023-01981-9

[25] B. B. McGivern et al., "Microbial polyphenol metabolism is part of the thawing permafrost carbon cycle," Nat. Microbiol., vol. 9, no. 6, pp. 1454–1466, 2024. https://doi.org/10.1038/s41564-024-01691-0

[26] P. Jaiswal et al., "Plant Ontology (PO): a controlled vocabulary of plant structures and growth stages," Comp. Funct. Genomics, vol. 6, no. 7–8, pp. 388–397, 2005. https://doi.org/10.1002/cfg.496

[27] M. Jost et al., "Structural basis for gene regulation by a B12-dependent photoreceptor," Nature, vol. 526, no. 7574, pp. 536–541, 2015. https://doi.org/10.1038/nature14950

[28] R. Fernández-Fernández et al., "Evidence for involvement of the Salmonella enterica Z-ring assembly factors ZapA and ZapB in resistance to bile," Front. Microbiol., vol. 12, Art. no. 647305, 2021. https://doi.org/10.3389/fmicb.2021.647305

[29] M. P. Adamo, M. Zapata, and T. K. Frey, "Analysis of gene expression in fetal and adult cells infected with rubella virus," Virology, vol. 370, no. 1, pp. 1–11, 2008. https://doi.org/10.1016/j.virol.2007.08.003

[30] W. Aguilar-Jimenez, W. Zapata, and M. T. Rugelas, "Antiviral molecules correlate with vitamin D pathway genes and are associated with natural resistance to HIV-1 infection," Microbes Infect., vol. 18, no. 7–8, pp. 510–516, 2016. https://doi.org/10.1016/j.micinf.2016.03.015

[31] C. W. Dunn, F. Zapata, C. Munro, S. Siebert, and A. Hejnol, "Pairwise comparisons across species are problematic when analyzing functional genomic data," Proc. Natl. Acad. Sci. U.S.A., vol. 115, no. 3, pp. E409–E417, 2018. https://doi.org/10.1073/pnas.1707515115

[32] J. Household et al., "Phenotypic plasticity and genetic control in colorectal cancer evolution," Nature, vol. 611, no. 7937, pp. 744–753, 2022. https://doi.org/10.1038/s41586-022-05311-x

[33] M. Jaubert et al., "Light and redox control of photosynthesis gene expression in Bradyrhizobium: dual roles of two PpsR," J. Biol. Chem., vol. 279, no. 43, pp. 44407–44416, 2004. https://doi.org/10.1074/jbc.M408039200

[34] M. R. Karim et al., "Deep learning-based clustering approaches for bioinformatics," Brief. Bioinform., vol. 22, no. 1, pp. 393–415, 2021. https://doi.org/10.1093/bib/bbz170

[35] H. Wang et al., "Cardiomyocyte-specific deletion of the G protein-coupled estrogen receptor (GPER) leads to left ventricular dysfunction and adverse remodeling: A sex-specific gene profiling analysis," Biochim. Biophys. Acta Mol. Basis Dis., vol. 1863, no. 8, pp. 1870–1882, 2017. https://doi.org/10.1016/j.bbadis.2016.10.003

[36] Y. Zhai et al., "Host transcriptional response to influenza and other acute respiratory viral infections – a prospective cohort study," PLoS Pathog., vol. 11, no. 6, Art. no. e1004869, 2015. https://doi.org/10.1371/journal.ppat.1004869

[37] P. L. Prestwich, "The effect of high copy effector eop1 in Erwinia tracheiphila," M.S. thesis, Tennessee State Univ., Nashville, TN, USA, 2015.

[38] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," J. Biol. Chem., vol. 193, no. 1, pp. 265–275, 1951. https://doi.org/10.1016/S0021-9258(19)52451-6

[39] S. D. Himpsl, C. V. Lockatell, J. R. Hebel, D. E. Johnson, and H. L. T. Mobley, "Identification of virulence determinants in uropathogenic Proteus mirabilis using signature-tagged mutagenesis," J. Med. Microbiol., vol. 57, no. 9, pp. 1068–1078, 2008. https://doi.org/10.1099/jmm.0.2008/002071-0

[40] P. B. Abdullah, H. M. Khalid, and W. M. Mero, "Molecular characterization and antibiotic susceptibility of Proteus mirabilis isolated from different clinical specimens in Zakho city, Kurdistan Region, Iraq," Zanco J. Pure Appl. Sci., vol. 34, no. 5, pp. 198–207, 2022. https://doi.org/10.21271/ZJPAS.34.5.18