Original Article

Role of MAPK p38 Gene Expression among UTI Patients

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Abstract

Background: Urinary tract infections (UTIs) are an inflammation of the urinary tract, caused mainly by acquired infections. The main cause of UTIs is *Proteus mirabilis* and is linked to very serious and complicated conditions. Anti-microbial drug resistance has become a global concern recognized by the World Health Organization. Mitogen-activated protein kinases (MAPKs) operate upstream of many proinflammatory pathways, the regulation of inflammation and the inflammatory response associated with MAPK p38 signaling, and the expression of proinflammatory molecules. **Objective:** Detection of MAPK p38 gene expression by using real-time PCR (relative gene expression) among UTI patients with multidrug-resistant *Proteus mirabilis*. **Materials and Methods:** Mid-stream urine samples were collected from patients suspected to get UTI. A total of 280 urine specimens and 40 blood specimens (2–3 mL) of venous blood were attained by venipuncture from all subjects (patients and controls) involved in this study, admitted to Al-Hilla Teaching Hospital from both sex and different ages during the period from May 2023 to October 2023. The Vitek2 system was used for identification and antibiotic susceptibility of bacterial isolates. RNA extraction from fresh whole blood specimens was followed by the detection of gene expression of MAPK p38 by real PCR technique. **Results:**Results showed that according to microscopical, phenotypic, biochemical tests, and VITEK2 system, out of the whole urine samples, only 25/280 (8.93%) isolates were confirmed to be *Proteus mirabilis*. Antibiotic susceptibility for *Proteus mirabilis* isolates against 17 antibiotics showed maximum resistance to both tigecycline and nitrofurantoin as 100%, with 92% resistance to imipenem. Minimum resistance was for cefoxitin, ciprofloxacin, gentamicin, and ertapenem at 12% and amikacin, cefepime, piperacillin at 4%. The present study showed that MAPK p38 was upregulated and highly expressed among patients with UTIs caused by *Proteus mirabilis* in comparison with control. **Conclusion:** The expression of MAPK p38 gene was increased in UTI patient with MDR *Proteus mirabilis* when compared with the control group. *Proteus mirabilis* exhibited high resistance to both tigecycline and nitrofurantoin.

Keywords: Antibiotic susceptibility test, gene expression, MAPK p38 gene, real-time PCR

Introduction

Urinary tract infections (UTIs) are significantly associated with morbidity, especially in pregnant women and children that high-risk groups, and even people with weakened immune systems. Bacteria invade and colonize broad-spectrum parts, including the bladder and urethra ureter that part from the urinary system. Dysuria is a clinical manifestation of UTIs, which can lead to hypertension and renal failure.[1] *Proteus mirabilis*, an agent of catheter biofilm growth, quickly contaminates the surface of a recently implanted urinary catheter.[2]

Antibiotic-resistant bacteria are becoming a greater worldwide hazard to public health, according to the WHO. A possible source of MDR bacteria has been found to be antibiotic misuse, especially with regard to broad-spectrum antibiotics.[3,4]

Multidrug-resistant (MDR) bacteria are the spread of gram-negative, a public health issue.[5] In recent years, *Proteus mirabilis* is the MDR Enterobacteriaceae with the greatest extended-spectrum beta-lactamases (ESBL) and carbapenemase synthesis implicated in UTIs. Antibiotic resistance is caused by bacteria-building biofilms due to

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limited antibiotic penetration and expression of resistant genes, as a result of these factors, indwelling medical devices (IMDs) are the most sensitive to biofilm producing microbial colonizers.[6]

Mitogen-activated protein kinase (MAPK p38) is a vital component of many biological processes. To control the host's immunological response, the kinase is triggered in response to environmental stressors such as inflammation and bacterial infections. However, recent research has shown that pathogens may control MAPK p38 signaling to their advantage in order to either cause or avoid host cell death.[7] One of three distinct families of MAPKs (p42/44 or ERK kinase, JNK kinase, and p38 kinase) is MAPK p38, is currently targeted for anti-inflammatory therapy, and is a critical enzyme for cytokine TNF production.[8] The MAPKs p38 are activated by stress and play key roles in balancing cell survival and death in response to both extracellular and intracellular stresses.[9]

Aim of study

The aim of this study is to detect the expression of MAPK p38 gene by using real-time PCR (relative gene expression) among UTI patients with MDR *Proteus mirabilis.*

Materials and Methods

Patients

In a cross-sectional study, a total of 280 urine samples were collected from patients suspected by the urologists of having UTI and admitted to Al-Hilla Teaching Hospital from both sexes and different ages during the period from May 2023 to October 2023, followed by 20 blood specimens from UTI-infected patients by *Proteus*.

Control

Twenty individuals with the clinical diagnosis are suggested to be free from any signs and symptoms of UTI infection, with negative urine cultures, agreed to participate in this study as a control. Blood specimens were taken from all those participants for RNA extraction in order to be used for molecular study.

Inclusion criteria

Patients with signs and symptoms of UTI who met the diagnostic criteria were included, with only those diagnosed with *Proteus mirabilis* positive culture.

Exclusion criteria

This study excluded individuals with any chronic diseases, other evident infectious diseases, any bacterial growth other than *Proteus mirabilis* and fungal growth, and those who refused to participate.

Blood specimens collection

Aseptically, 2–3 mL of venous blood were attained by venipuncture from all subjects (patients and controls) involved in this study for RNA extraction for molecular study and gene expression.

RNA extraction from human blood

The RNA extraction from fresh whole blood specimens was carried out according to the manual of the manufacturer of Promega company.

PCR amplification

Primer pairs used in this study [listed in Table 1] were purchased from Macrogen, Korea in lyophilized form and rehydrated by nuclease-free water to be used for conventional PCR according to the conditions at Table 1.

Gene expression procedure

1. Prepared all reagents RNA, primers, and GoTaq 1-step RT-qPCR reaction mix.
2. Thawed the components of the GoTaq 1-step RT-qPCR system, the RNA templates, and the primer pair on ice, at room temperature or 37°C. Immediately mix each thawed component thoroughly.
3. Added 16.3 µL from the master mix component to the PCR tube.
4. 3.7 µL from RNA added to PCR tube containing master mix component.
5. Mixed gently after each addition by vortex.
6. Transferred plate into the preprogrammed instrument. Start the run immediately.
7. When the run is completed, collect the data and analyze the results.

Gene expression calculated

The data of the gene of interest relative to some calibrator or internal control gene is relative to gene expression. This method A widely used to present relative gene expression is the comparative CT method also referred to as the $2^{-\Delta\Delta cT}$ method.[10]

Table 1: Primers sequence and condition used in RT-qPCR P38 gene expression

Primer name	Sequence 5'-3'	Step	Temp/Time/c
P38-177b-F	(5'-TGTTCAGAGGAGCCAATCC-3)	Reverse transcription	37°C 15 min
P38-177b-R	(5'-GCTCCATGTCCTCAAAGCTC-3)	Reverse transcription inactivation and GOTaq DNA polymerase activation	95°C 10 min
		Denaturation	95°C 10 s
		Annealing	57°C 30 s
		Extension	72°C 30 s

Ethical approval

This study was approved by the committee of publication ethics at the College of Medicine, Babylon Province, Iraq, under reference No. BMS/0203/016. Verbal consent was obtained from patients. This is necessary ethical approval in all groups of study. The statistical analysis and descriptive statistics were proceeded and analyzed by using *t* test and Chi-square. *P* value ≤ 0.01 was considered to be all analyzes which were performed with statistical package for the social sciences (SPSS) for Windows version 26.

Result

Demographic characteristics of patients with UTI

The present study comprised 280 patients with UTIs as suggested by the urologists. The mean age of patients was 34.29 ± 13.27 years, ranging from 10–70 years.

Antibiotic susceptibility and identification of *Proteus mirabilis* isolates

Proteus mirabilis isolates identification and detection of antibiotic susceptibility using VITEK2 system, as results showed that out of the whole urine samples, only 25/280 (8.93%) isolates were confirmed to be *Proteus mirabilis*, as shown in Figure 1.

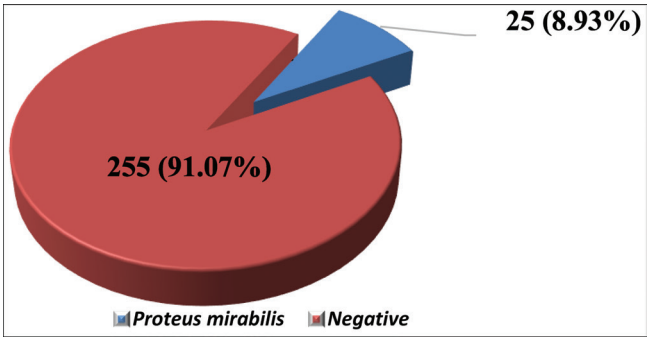

Figure 1: Percentage of *Proteus mirabilis* isolated from UTI patients

Later on, susceptibility was detected against 17 antibiotics used in this study as shown in Figure 2. Maximum resistance was observed for both tigecycline and nitrofurantoin as 100%, with 92% resistance to imipenem. Approximately 50% of isolates showed resistance to amoxicillin, ceftazidime, and cefazolin, while about 40% of *Proteus* were resistant to ampicillin and ceftriaxone. On the other hand, approximately 25% of *Proteus* were resistant to levofloxacin and trimethoprim, whereas the minimum resistance was for cefoxitin, ciprofloxacin, gentamicin, and ertapenem at 12% and amikacin, cefepime, piperacillin at 4%, this minimum resistance is highly sensitive for these antibiotics.

Gene expression of MAPK p38 by RT-PCR quantitation

For a total of 20 blood patient samples and 20 blood control samples, RNA was extracted to detect the gene expression of MAPK p38 by RT-qPCR (relative gene expression [2ΔCt] methods. In this method, the expression levels of MAPK p38 gene in the test sample as well as in control sample were normalized with house-keeping gene (SFRP-1) as shown in Figure 3.

Normalizing reaction cycle thresholds (CTs) are used to determine the relative expression levels of target genes to the housekeeper genes (SFRP-1). The relative mRNA expression levels of target genes were calculated using PCR efficiencies and mean crossing point deviations between samples and controls that were determined by ΔCT values. These values were used in the ΔΔCt equation for calculation.

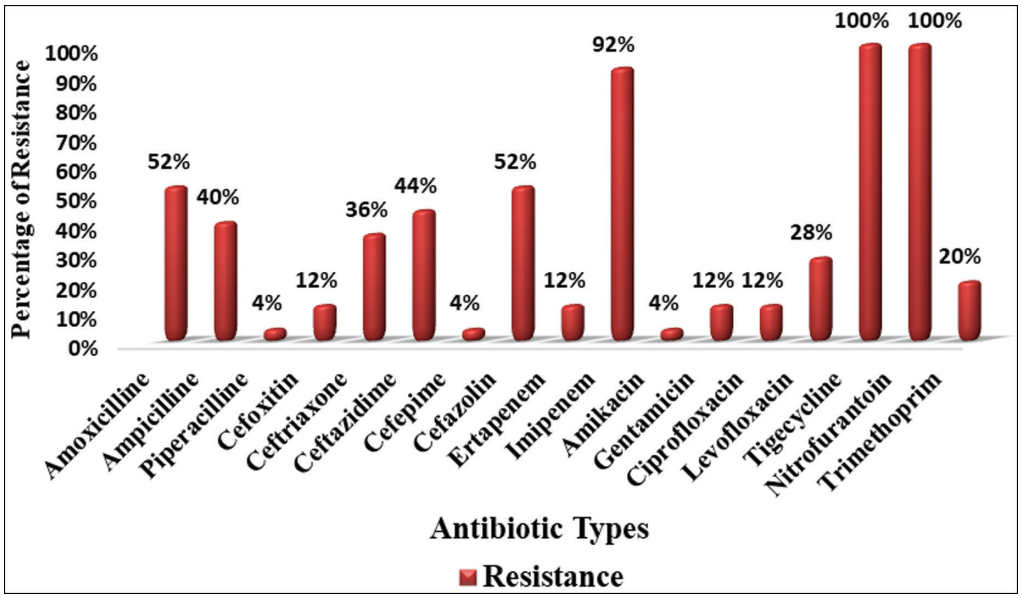

Figure 2: Frequency distribution of antibiotic resistance for *Proteus mirabilis* isolates isolated from UTI, against 17 antibiotics by VITEK2 system

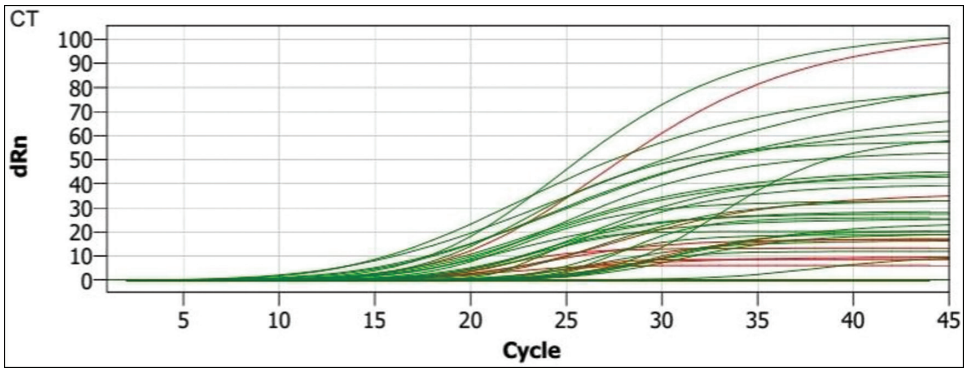

Figure 3: MAPK p38 gene expression level. This is the first 20 samples that represents amplification of reference gene (SFRP-1) and represents amplification of samples patients and control samples

Table 2: MAPK p38 fold gene expression in control and patients versus the reference gene (SFRP-1)

Group	No	Expression level of p38			*P* value
		Mean	SD	SE	
Patient	20	5.23	1.004	0.224	0.0001
Control	20	1.35	0.93	0.208	

SD: standard deviation, SE: standard error independent samples *t* test

Table 3: MAPK p38 (CT) expression in control and patients versus the reference gene (SFRP-1)

Group	No	Expression level of CT			*P* value
		Mean	SD	SE	
Patient	20	24.2	1.98	0.444	<0.05
Control	20	25.5	1.41	0.315	

SD: standard deviation, SE: standard error

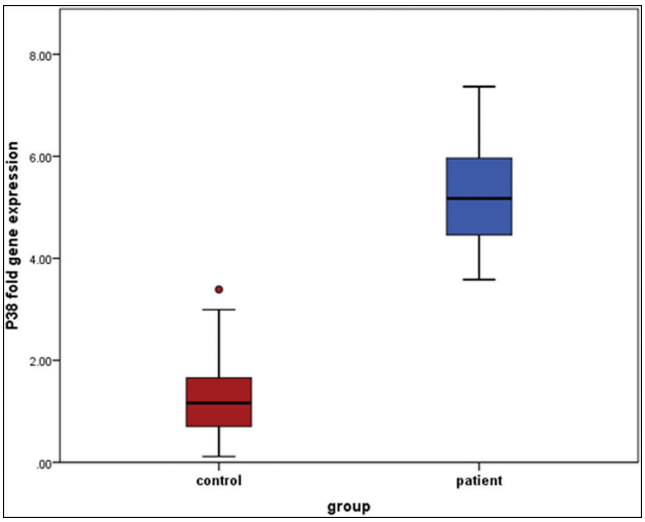

Figure 4: MAPK p38 gene expression fold among control and UTI patients versus the reference gene (SFRP-1)

The results described MAPK p38 gene fold using mean crossing point deviations between samples and controls and real-time PCR efficiencies and represent a significant difference at $P \leq 0.0001$ as shown in Tables 2 and 3. They showed the MAPK p38 gene (CT) expression in UTI patients associated with *Proteus mirabilis* infection and represent a significant difference at $P \leq 0.05$. The MAPK p38 gene (CT) expression in UTI patients is associated with *Proteus mirabilis* infection and represents a significant difference at $P \leq 0.05$ [Figure 4].

Discussion

UTI is one of the most common types of infections in the local community, and vulnerability of uropathogens to commonly applied antibiotics has decreased during recent years. The mean age of patients was 34.29 ± 13.27. The results indicated that the *Proteus mirabilis* resistance to some antibiotics has increased with the prescription of years, because of the random and wrong use of these antibiotics and increasing the rate of *Proteus* infections. On the other hand, the bacterium produces β-lactamases, especially extended-spectrum β-lactamases (ESBLs), by this bacteria as well as, transfer genetic elements carrying the genes of these enzymes, and number of mutations occur with these type of enzymes leading to increase resistance to antibiotic especially β-lactam, in addition to other mechanisms such as or alteration the access to the target site by modification of penicillin-binding proteins (PBPs) and alteration the target site.[11,12]

Penicillin group (ampicillin, amoxicillin, and piperacillin) in this study revealed a moderate resistance at 40%, 52%, and 4%, respectively; this result corresponds with other studies[13] from Russia and[14] Czech, as their results were 45.7% and 23%, respectively. Also, other studies such as[15] from Iraq and[16] KSA[17] found that resistance to ampicillin was 75%, 80%, 61.9%, and 71.4%, respectively. The results of the carbapenem antibiotic ertapenem show high sensitivity but imipenem showed high resistance of *Proteus* (92%).

The current study showed high resistance to both tigecycline and nitrofurantoin (100%), similar to a study[18]

that showed high resistance to nitrofurantoin (92.5%) and similar to a study[17] as most isolates were resistant to nitrofurantoin (88.9%); while Talebi *et al*. (2023) showed lowest resistance for nitrofurantoin (6.8%). Antibiotic medication is used for the treatment of uncomplicated lower UTIs. It is effective against most Gram-positive and Gram-negative organisms. Nitrofurantoin is also primary usage which has remained in prophylaxis and treatment of UTI.[19,20]

Gentamicin and amikacin (aminoglycosides) remain effective such as against *Proteus mirabilis* infections, similar to the study.[21,22] Moreover, high susceptibility to the fluoroquinolone antibiotic (ciprofloxacin) coincides with other studies[23] whose results noted higher level of sensitivity (60.23%)

Finally, about 60% of *Proteus* was resistant to ceftriaxone, consistent with the study[22] from Indonesia and with the study[24] from Iraq found 52.5% and 58%, respectively. *Proteus mirabilis* showed high sensitivity to ciprofloxacin, amikacin, cefepime, and piperacillin antibiotic; thus, these antibiotics could be the most effective and the drugs of choice for infections caused by *Proteus mirabilis.*

Gene expression of MAPK p38 by RT-PCR quantitation

The present study showed that MAPK p38 was upregulated and highly expressed among patients with UTI caused by *Proteus mirabilis* than the control. The expression of MAPK p38 is significant among UTIs due to its involvement in various aspects of UTI pathogenesis. Additionally, the present study found that the expression of MAPK p38 gene increased in UTI patients with *Proteus mirabilis* when compared with the control group, so the expression gene increased by more than 7% fold when compared with the control group.

In an organism, the expression of genes can be influenced by the environment, including the organism's internal as well as world external world in which the organism is located or developed, which includes such factors as its hormones and metabolism.

The expression of MAPK p38 has been investigated in various studies related to UTIs. The study[25] found that MAPK p38 plays an important role in lower urinary tract dysfunction in mice with spinal cord injury. Another study[8] showed that activated MAPK p38 by TcpC, a virulence factor of uropathogenic *Escherichia coli* (UPEC), contributes to the production of macrophage inflammatory protein-2 (MIP-2) in kidney cells.

Additionally,[26] the study observed that increased expression of MAPK p38 in the renal cortex of rats treated with gentamicin, suggesting its involvement in the pathogenesis of tubulointerstitial nephritis. Therefore, MAPK p38 expression is intricately linked to UTIs and associated complications. Moreover, the expression of P38 increases in disease due to disease-induced changes rather than being disease-causing.[27] The gene expression increases in disease due to a significant genetic component affecting transcriptional networks, particularly in adipose tissue, leading to conditions like obesity through inflammatory and immune response genes.[28]

The high expression of MAPK p38 is reported in the present study and others that activate the MAPK p38 pathway in these diseases may reflect the presence of cytokines or growth factors.[29]

Conclusion

High rates of antibiotic resistance were observed among most of the isolates, making them difficult to treat. Amikacin, cefepime, and piperacillin can be considered the top options for the treatment of MDR *Proteus mirabilis* UTI.

The significant increment in MAPK p38 gene expression among patients with *Proteus mirabilis* urinary tract infection makes it a good biomarker for UTIs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Dadzie I, Quansah E, Puopelle Dakorah M, Abiade V, Takyi-Amuah E, Adusei R. The effectiveness of dipstick for the detection of urinary tract infection. Can J Infect Dis Med Microbiol 2019;2019:1-6.
2. Dougnon V, Assogba P, Anago E, Déguénon E, Dapuliga C, Agbankpè J, *et al*. Enterobacteria responsible for urinary infections: A review about pathogenicity, virulence factors, and epidemiology. J Appl Biol Biotech 2020;8:117-24.
3. Dyar OJ, Pagani L, Pulcini C. Strategies and challenges of antimicrobial stewardship in long-term care facilities. Clin Microbiol Infect 2015;21:10-9.
4. Pulcini C, Clerc-Urmes I, Attinsounon CA, Fougnot S, Thilly N. Antibiotic resistance of Enterobacteriaceae causing urinary tract infections in elderly patients living in the community and in the nursing home: A retrospective observational study. J Antimicrob Chemother 2019;74:775-81.
5. Demirci M, Ünlü O, Tosun A. Detection of O25b-ST131 clone, CTX-M-1 and CTX-M-15 genes via real-time PCR in *Escherichia coli* strains in patients with UTIs obtained from a University Hospital in Istanbul. J Infect Public Health 2019;12:640-4.
6. Hasan TH, Alasedi KK, Jaloob AA. *Proteus mirabilis* virulence factors. Int J Pharm Res 2021;13:2145-9.
7. Gräb J, Rybniker J. The expanding role of p38 mitogen-activated protein kinase in programmed host cell death. Microbiol Insights 2019;12:1178636119864594.
8. He Y, Fang J, Zhang C, Pan J, Jin Q, Yang Y, *et al*. TcpC secreting uropathogenic *E. coli* promoted kidney cells to secrete MIP-2 via p38 MAPK pathway. Mol Med Rep 2017;16:3528-34.
9. Yue J, López JM. Understanding MAPK signaling pathways in apoptosis. Int J Mol Sci 2020;21:2346.

10. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protocols 2008;3:1101-8.
11. Rossolini GM, D'andrea MM, Mugnaioli C. The spread of CTX-M-type extended-spectrum β-lactamases. Clin Microbiol Infect 2008;14:33-41.
12. Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: Origin and diffusion. Front Microbiol 2012;3:20597.
13. Rafalskiy V, Pushkar D, Yakovlev S, Epstein O, Putilovskiy M, Tarasov S, *et al*. Distribution and antibiotic resistance profile of key gram-negative bacteria that cause community-onset urinary tract infections in the Russian Federation: Resource multicentre surveillance 2017 study. J Glob Antimicrob Resist 2020;21:188-94.
14. Cernohorská L, Chvílová E. *Proteus mirabilis* isolated from urine, resistance to antibiotics and biofilm formation. Klin Mikrobiol Infekc Lek 2011;17:81-5.
15. Al-Bassam WW, Al-Kazaz AK. The isolation and characterization of *Proteus mirabilis* from different clinical samples. J Biotechnol Res Center 2013;7:24-30.
16. Bahashwan SA, El Shafey HM. Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. Eur Sci J 2013;9:188-202.
17. Hussein EI, Al-Batayneh K, Masadeh MM, Dahadhah FW, Al Zoubi MS, Aljabali AA, *et al*. Assessment of pathogenic potential, virulent genes profile, and antibiotic susceptibility of *Proteus mirabilis* from urinary tract infection. Int J Microbiol 20202020;2020:1231807.
18. Mirzaei A, Nasr Esfahani B, Raz A, Ghanadian M, Moghim S. From the urinary catheter to the prevalence of three classes of integrons, β-lactamase genes, and differences in antimicrobial susceptibility of *Proteus mirabilis* and clonal relatedness with Rep-PCR. Biomed Res Int 2021;2021:1-10.
19. Raheema RH, Hadi SH, Chabuck ZA. Study of some virulence genes from uropathogenic *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated in Wasit Province, Iraq. MJBL 2024;21:207-13.
20. Raheema RH, Nasser DD, Chabuck ZAG. Molecular detection of SPA-type of methicillin-resistant *Staphylococcus aureus* from urinary tract infection patient in Wasit Province, Iraq. Med J Babylon 2023;20:619-25.
21. Zafar U, Taj MK, Nawaz I, Zafar A, Taj I. Characterization of *Proteus mirabilis* isolated from patient wounds at Bolan Medical Complex Hospital, Quetta. Jundishapur J Microbiol 2019;12:e87963.
22. Suhartono S, Mahdani W, Khalizazia K. Prevalence and antibiotic susceptibility of *Proteus mirabilis* isolated from clinical specimens in the Zainoel Abidin General Hospital, Banda Aceh, Indonesia. Open Access Maced J Med Sci 2022;10:1532-7.
23. Feglo P, Opoku S. AmpC beta-lactamase production among *Pseudomonas aeruginosa* and *Proteus mirabilis* isolates at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. J Microbiol Antimicrob 2014;6:13-20.
24. Al-Nabhani NA, Shami AM. Molecular study of carbapenem resistance genes in *Proteus mirabilis* isolated from clinical samples in Baghdad hospitals. Iraqi J Biotechnol 2023;22:154-162.
25. Shimizu N, Wada N, Shimizu T, Suzuki T, Kurobe M, Kanai AJ, *et al*. Role of p38 MAP kinase signaling pathways in storage and voiding dysfunction in mice with spinal cord injury. Neurourol Urodyn 2020;39:108-15.
26. Volpini RA, Balbi APC, Costa RS, Coimbra TM. Increased expression of p38 mitogen-activated protein kinase is related to the acute renal lesions induced by gentamicin. Braz J Med Biol Res. Rev Bras Pesq Med Biol 2006;39:817-23.
27. Porcu E, Sadler MC, Lepik K, Auwerx C, Wood AR, Weihs A, *et al*. Differentially expressed genes reflect disease-induced rather than disease-causing changes in the transcriptome. Nat Commun 2021;12:5647.
28. Emilsson V, Thorleifsson G, Zhang B, Leonardson AS, Zink F, Zhu J, *et al*. Genetics of gene expression and its effect on disease. Nature 2008;452:423-8.
29. Al-Khafaji NSK, Al-Dahmoshi HOM, Chabuck ZAG, Iftikhar M, Ozdemir FA. Microbiome and metabolomics. Med J Babylon 2023;20:236-9.