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Effect of Some Drugs (Painkiller and Anti-Inflammatory) on Antibiotic Resistance Genes(*vim,imp,ndm,oxa48* and *aac(6)*) in *Proteus Mirabilis*

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Abstract

Proteus mirabilis is gram-negative bacteria that is considered responsible for urinary tract infections (UTIs), especially catheter-associated urinary tract infections. The samples of urine (119) were collected from Baghdad hospitals. Proteus spp was recognized by morphology, the Vitek-2 compact system, and the 16SrRNA gene. Antibiotic susceptibility test was also done using the vitek-2 system. The antibiotic resistance genes *vim, imp, ndm, oxa48* and *aac(6)* were amplified using PCR. The Results were shown out of 35 isolates belonging to *P. mirabilis*, they appeared highest resistance against minocycline, ticarcillin, trimethoprim/sulfamethoxazole, and ticarcillin/ clavulanic with 71.40 %, 68.57%, 65.70% and 57.10% respectively; while, they were sensitive to meropenem and piperacillin/ tazobactam with 2.85 %and 5.57% respectively. Seven isolates were classified as XDR, and the two strongest isolates of them were selected (no 90 and no 99). Only The *aac(6)* gene appeared positive band at size (395bp) in both isolates while other genes(*vim,imp,ndm,oxa48*) were not detected in both of the isolates. RT-PCR revealed a down-regulation at 0.015 and 0.485 folding change in gene expression of the *aac(6)* gene under the effect of olfen and dexamethasone; while it showed Up-regulation at 3.245, 3.55 and 3.22 folding change in gene expression under the effect of paracetemol, piroxicam and nefopam respectively. From the above finding, concluded that each of these drugs a had different effect on the *aac(6)* gene whether it is up regulation or down regulation. wither it up regulation or down regulation.

Keywords: *Proteus mirabils*, *aac(6)*gene, antibiotic resistance, paracetemol, PCR .

1.Introduction

Proteus mirabilis is a gram-negative, facultative anaerobe rod-shaped bacterium most noted for its swarming motility in a specific bull's-eye pattern. and urease activity [1]. Additionally, *P. mirabilis* is a component of the microflora of the human gut. [2]. *P. mirabilis* is not often a pathogen, but when it comes into contact with urea in the urinary sys-tem, it can cause problems. Infection may then spread to different body sites from there [3]. Asymptomatic bacteriuria is common, especially in the elderly and those with type 2 diabetes, although *P. mirabilis* can cause symptomatic infections of the urinary tract, such as cystitis and pyelonephritis [4, 5]. Urosepsis,

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which develops from bacteremia, can be fatal in patients with these diseases. Further, urolithiasis (the production of urinary stones) can result from a *P. mirabilis* infection [6].
In addition to UTI, this species can also cause infection in the respiratory tract, eye, ear, nose, skin, throat, burns, and wounds and has been implicated in neonatal meningoencephalitis, empyema, and osteomyelitis [7, 8]. *Proteus mirabilis* possesses many virulence factors that cause UTI, including urease, motility and adhesion mediated by flagella and fimbria, toxins such as hemolysin and Proteus toxic agglutinin (Pta), lipopolysaccharide (LPS), and metal acquisition and biofilm development [9]. *P. mirabilis* may be resistant to benzyle penicillin, oxacillin, tetracycline, and macrolides due to its pathogenicity. Benzylpenicillin, oxacillin, tetracycline, and macrolides may not work on *P. mirabilis* [10]. *P. mirabilis* may develop ampicillin resistance through the synthesis of chromosomal beta-lactamases and plasmid-mediated beta-lactamases [11]. Due to their ability to impart resistance to third-generation cephalosporins including cefotaxime, ceftriaxone, and ceftazidime, as well as the monobactam aztreonam, Proteus' extended-spectrum beta-lactamases (ESBLs) raise more serious health issues. ESBLs do not hydrolyze cephamycins (cefoxitin, cefotetan, and cefmetazole) or carbapenems (imipenem, meropenem). Proteus is developing carbapenem resistance [12, 13]. *P. mirabilis* can obtain antibiotic resistance through horizontal gene transfer (HGT) and become MDR, such as the aac(6′)-Ib gene that causes aminoglycoside resistance and the OXA-48 gene that causes carbapenem resistance [14, 15]. The focus of this research is on finding ways to make bacteria more responsive to antibiotics. The aim of this study is to test the effect of different drugs on *P. mirabilis* antibiotic susceptibility and whether they can alter the state of the bacterium from resistance to being sensitive toward antibiotics, thus making antibiotics that are ineffective against *P. mirabilis* efficient again.

2. Materials And Methods

2.1 Isolation and identification

One hundred and nineteen sterile containers of urine were collected between October 2021 and February 2022 from facilities in Baghdad, Iraq, such as the Baghdad Teaching Hospital, the Ghazi Al-Hariri Hospital for Surgical Specialties, and the Teaching Laboratories at Medical City. The collected specimens were inoculated on MacConkey agar (Himedia/India) and incubated at 37ºCfor 24 hours. Morphological characteristics were used to identify the isolates. The specimens were grown on blood, and MacConkey agar was used for the cultivation and identification of the isolates as *P. mirabilis* or not, as well as molecular identification using specific *P. mirabilis* 16SrRNA primers (16SrRNA-F:5_AGAGTTTGATCCTGGCTCAG_3),
*16SrRNA*R:(5-CTACGGCTACCTTGTTACGA-3)[16]. To identify bacterial isolates.

2.2 Antibiotic susceptibility test

In this method, the test was conducted using the vitek-2 system to detect multidrug resistance (MDR) for 16 different antibiotics, including amikacin, aztreonam, cefalexin, cefazidime, cefepime, ciprofloxacin, gentamicin, imipenem, meropenem, minocycline, tazobactam, ticarcillin, clavulanic acid, tobramycin, trimethoprim, and piperacillin.

2.3 Molecular assay

All isolates' overnight cultures' genomic DNA was extracted using the ABIOpure Extraction Kit. Quantus Fluorometer measurements of extracted DNA concentration verified sample quality for subsequent usage.The primers listed in Table 1 were used to carry out conventional polymerase chain reaction (PCR) to amplify fragments of the genes *vim*, *imp*, *ndm*, *oxa48*, and *aac6* and to

detect antibiotic resistance genes. The PCR mixture was prepared in a total volume of 20 µl and consisted of 10 µl of FIREPol® Master Mix. To fill the remaining 20 µl, distilled water was used, which is devoid of cutting enzymes and ions. Two microliters of each primer and DNA template are included in the reaction mixture. A master cycler (Eppendorf, Germany) was used to optimize and run the thermal program, which included: initial denaturation at 95ºC for 5 minutes, denaturation at 95ºC for 30 seconds, annealing (oxa48 for 55 ºC, *imp* for 48 ºC, *vim* for 48 ºC, *ndm* for 58 ºC and *aac6* for 54 ºC) for 30 seconds, extension 72 ºC for 1 minute, final extension 72 ºC for 7 minutes, and hold 10 ºC for 4 minutes. The PCR products were verified by electrophoresis in agarose gel (1.5% in 1X TAE buffer) containing ethidium bromide (10 mg/1 ml). DNA ladder marker was applied in the control well.

2.4 Quantitative real-time polymerase chain reaction (qRT-PCR)

The TRIzolTM Reagent technique was used to isolate RNA from the sample.
Nuclease-free conditions are required for RT-PCR. The GoTaq® 1-Step RT-qPCR system separates RNA sample preparation, reaction mixture assembly, PCR, and reaction analysis. The gene expression was measured based on the Livak equation 2^ΔΔct, which is a quick and straightforward approach to analyzing gene expression levels in real-time PCR [20].

3. Results

3.1 Isolation and identification

Out of 119 samples that were collected from urine and cultivated, the results showed that 35 isolates were *P. mirabilis*. The isolates had pale colonies and swarming movement (bull's-eye pattern) on both blood and MacConkey agar **Figure 1**. Further identification using PCR, the positive results appeared on clear bands with a size of 1500 bp for the *16sr RNA* gene **Figure 2**.

3.2 Antibiotic susceptibility test

The susceptibility of 35 isolates of *P. mirabilis* against 16 antibiotics was tested using the Vitek-2 compact system. The results showed resistance up to 71.40% to minocycline, followed by 68.57% ticarcillin, (65.70%) trimethoprim/sulfamethoxazole, (57.10%) ticarcillin/clavulanic acid, and (54.28%) gentamicin, respectively. Only seven of the entire isolates (20%) contained extensively drug-resistant (XDR) bacterial isolates. The meropenem recorded the highest sensitivity (2.85%), followed by (5.70%) amikacin, (8.57%) piperacillin/tazobactam, (27.10%) imipenem, (31.40%) ciprofloxacin, and (34.20%) piperacillin, respectively shown in **Figure 3**. Two of these 7 isolates that showed more resistance to gentamicin than the other isolates selected to complete the current study were labeled as (NO.90 and NO.99).

3.3 Detection of antibiotic resistance genes

The resistance genes (*imp*,*vim*, *oxa48,* and *ndm*) were not detected among the tested isolates(Figure 4), but only 2 out of 35 bacterial isolates (5.71%) showed positive bands (395 bp) for the aminoglycoside resistant gene *aac(6)* **Figure 5.**

3.4 Gene expression of the *aac*(6) gene in *P. mirabilis* isolates

The gene expression of *aac(6)* has shown down regulation with 0.015 and 0.485 fold changes after being treated with olfen and dexamethasone, respectively, but up regulation was noted after being treated with other drugs **Table 2.** These results were compared to the control

Table 1. primers used for detection of antibiotic resistance genes.

Gene	Sequence 5-3	References
OXA48-F	5_ AGCTTGATCGCCCTCGATTT _3	[17]
OXA48-R	5_GAATACCACCGTCGAGCCAG_3	
VIM-F	5_GTACGCATCACCGTCGACAC_3	[18]
VIM-R	5_TGACGGGACGTATACAACCAGA_3	
IMP-F	5_AAGTTAGTCAMTTGGTTTGTGGAGC_3	[18]
IMP-R	5_CAAACCACTACGTTATCTKGAGTGTG_3	
NDM-F	5_TTTACTAGGCCTCGCATTTG_3	Designed
NDM-R	5_GCCCAGCTTCGCATAAA_3	
AAC(6)-F	5_TATGAGTGGCTAAATCGA_3	[19]
AAC(6)-R	5_CCCGCTTTCTCGTAGCG_3	

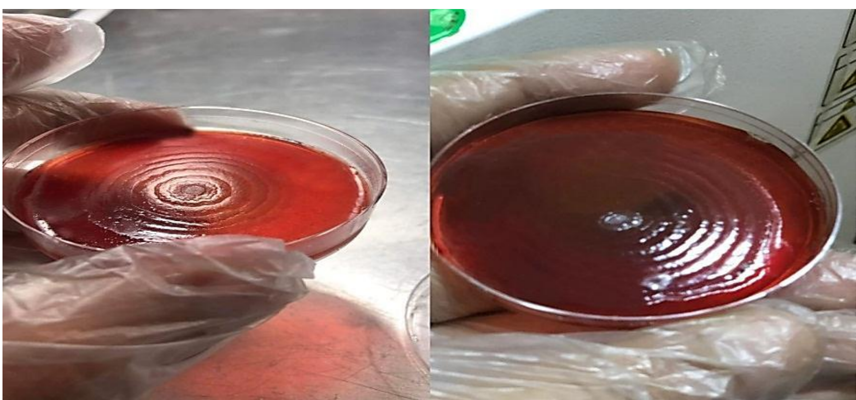

Figure 1 . Swarming motility(bull's-eye pattern) of *P. mirabilis*

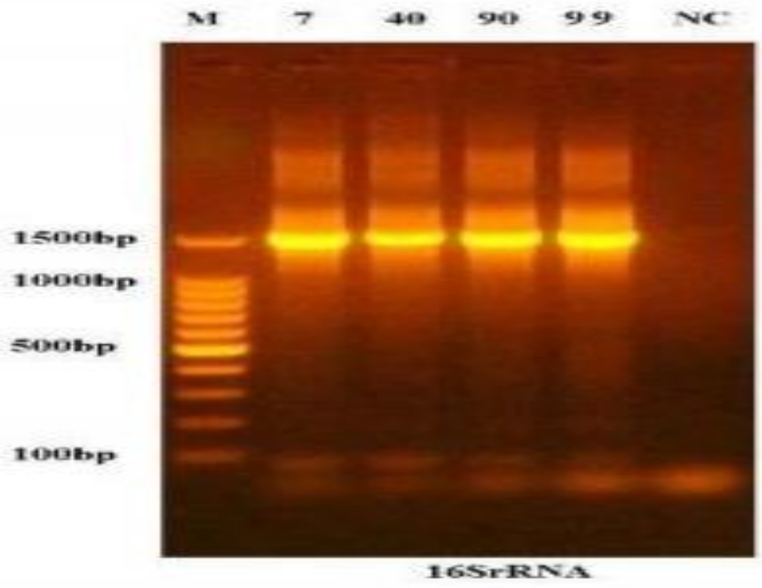

Figure 2. The findings of the 16srRNA gene amplification in P. mirabilis samples fractionated on 1.5% agarose gel electrophoresis labeled with 1500 bp ladder marker

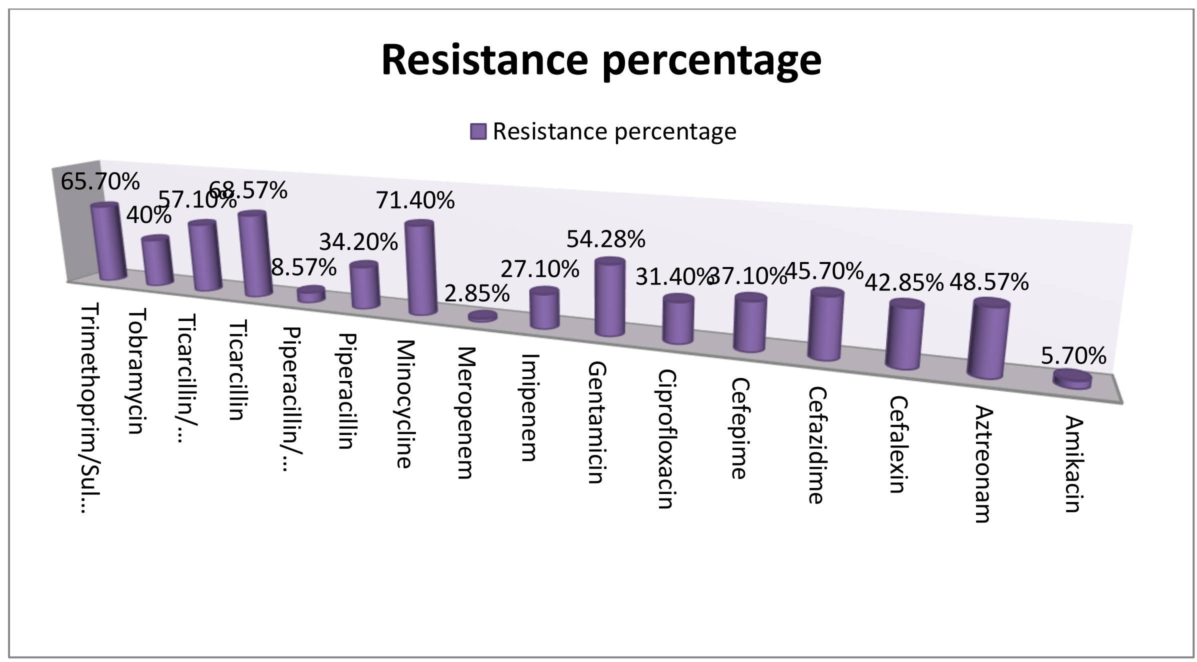

Figure 3. Antibiotics resistance percentage for *P. mirabilis* isolates

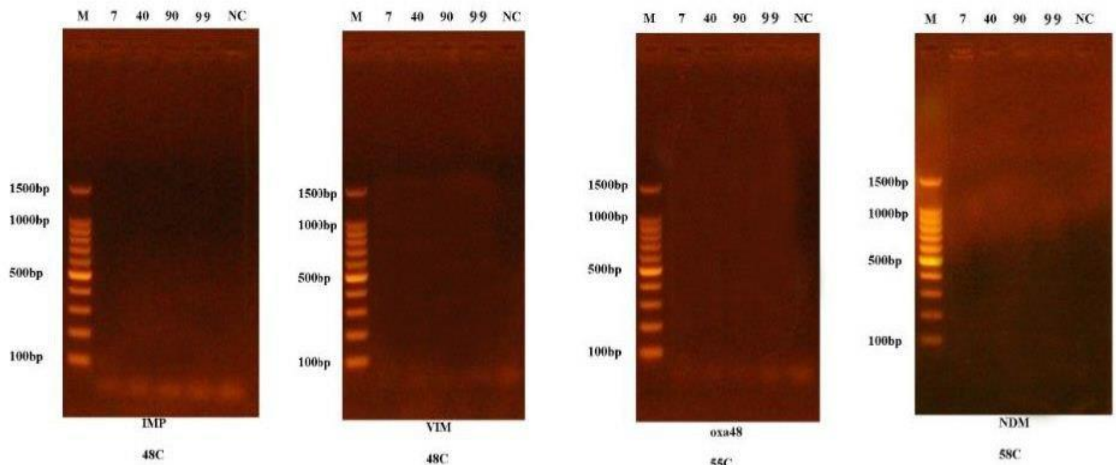

Figure 4. The negative amplification of genes(*iMP,VIM, oxa48 and NDM*) in *P. mirabilis* samples fractioned on 1.5% agarose gel electrophoresis stained with Eth.Br.M: 1500bp ladder marker, NC: negative control.

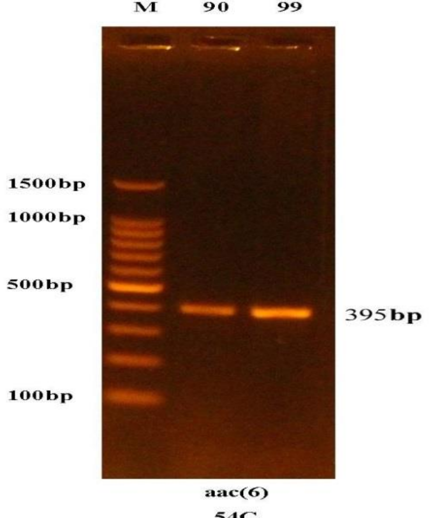

Figure 5. The amplification of *aac(6)* gene(395bp) in *P. mirabilis* samples fractioned on 1.5% agarose gel electrophoresis stained with Eth.Br.M: 1500bp ladder marker, NC: negative control.

Table 2 . Fold change of *aac(6)* gene expression of *P. mirabilis* isolates that were treated with different drugs

Sample	aac(6)	House keeping gene	DCT	DDCT	Folding
Control	26.56	8.48	18.09	0.00	1.00
Piroxicam	28.19	11.92	16.27	-1.82	3.55
Dexamethasone	27.30	7.95	19.35	1.265	0.485
Nefopam	29.05	12.43	16.61	-1.475	3.22
Olfen	31.77	7.325	17.84	6.34	0.015
Paracetemol	31.98	15.51	16.14	-1.615	3.245

4. Discussion

The leading results showed a higher incidence of *P. mirabilis* (29.4%) compared to Treska Dh. Kamil and Sanaria F. Jarjes, showed an incidence of 26% of all samples identified as *Proteus* (90% of these *Proteus* samples identified as *Proteus mirabilis).* [21]. Also, the incidence was about twice as high as reported by Dalia A. Ahmed, who stated that 19.7% of all collected samples were identified as *Proteus (66.6% of* samples were identified as *Proteus mirabilis).* [22]. *Proteus* of this increase in *Proteus* isolates is attributed to hygiene, lack of contamination, increased antibiotic resistance, and the emergence of *Proteus mirabilis*' extended-spectrum beta-lactamases (ESBLs), which may pass on resistance to the next generation via horizontal gene transfer (HGT), thus causing serious health problems. The highest resistance percentage was for minocycline (71.4%). 25 isolates were resistant to minocycline. Minocycline A semi-synthetic antibiotic derivative from tetracycline is presently used systemically to treat a variety of illnesses brought on by both Gram-positive and Gram-negative bacteria. Minocycline is largely bacteriostatic and works by limiting bacterial protein synthesis by attaching to the 30S ribosomal subunit and preventing the ligation of the aminoacyl-tRNA, a process shared by other tetracycline antibiotics. [23]. While other studies by Serry FM et al. showed that isolates were completely resistant to tetracycline (100%). The resistance percentage for gentamicin was (54.28%) 19 isolates showed resistance to gentamicin. This result is consistent with those of Essam F.A. Al-Jumaily and Sara Hussein Zgaer, who found that Gentamycin had a moderate effect (55.8%) on *P. mirabilis* isolates because it is an aminoglycoside, a class of potent, broad-spectrum antibiotics that inhibit prokaryote protein synthesis. [25]. These findings further demonstrate that imipenem, a kind of carbapenem, kills bacteria by piercing their cell walls, attaching to penicillin-binding proteins (PBPs), and inactivating their internal autolytic inhibitor enzymes [26]. It has a resistance percentage of (27.1%). This disagrees with results by Essam F.A. Al-Jumaily and Sara Hussein Zgaer that showed that imipenem was the most effective drug against P. mirabilis isolates (97.2%). [25]. The lowest resistance percentage (2.85%) was for meropenem, which also belongs to the class carbapenem, with only 1 isolate being resistant to meropenem. This agrees with results by Serry FM et al., who showed the lowest rate of resistance was also for meropenem (6.4%). [24]. Our results show that none of the tested isolates contained the antibiotic resistance genes (imp,vim, oxa48, and ndm), which leads us to explain that these genes may be carried on plasmids, might be lost, or that the isolates may resist antibiotics by another mechanism such as permeability of membrane or bypass. Nadezhda K. Fursova and co-authors showed that the oxa48 gene was detected in 18.75% of P. mirabilis isolates [27]. Additionally, S. Vourl discovered that clinical isolates of P. mirabilis from a hospital in Thessaloniki, Greece, that were genetically linked showed reduced sensitivity to imipenem and possessed the vim gene. [28]. The aac (6) gene is the most common gene that produces the aminoglycoside-modifying enzymes necessary to impart resistance to gentamicin, tobramycin, kanamycin, and amikacin. Resistance to both fluoroquinolones and amino-glycosides

may be brought on by the aac(6) mutant gene. A study by Piotr Wieczorek et al. also gave a positive result, as the aac(6) gene was detected in five isolates of P. mirabilis with a decreased sensitivity to aminoglycoside [29]. Other results by Najlaa Abdallah D. Al-Oqaili et al. showed that 40% of P. mirabilis isolates contained the aac (6) gene [30]. Based on the results in Table 4, we can observe the effect that each drug had on the expression of the acc (6) gene (increase or decrease). The most surprising result was that paracetamol showed a high increase in gene expression (upregulation) in both of the gentamicin-resistant isolates, although it had a different effect on bacterial susceptibility when combined with gentamicin by inhibiting bacterial growth phenotypically, which may lead us to believe that paracetamol has a contrast effect and requires further studies to fully understand and demonstrate its effect. Dexamethasone and olfen showed a matched effect on bacterial susceptibility with gentamicin since both of them showed a decrease in gene expression (downregulation) in both of the isolates, especially olfen, which showed a huge decrease in both of the isolates, which confirms its phenotypic impact that showed inhibition of bacterial growth when combined with gentamicin for both of the isolates. Nefopam and piroxicam enhance gene expression (upregulate), and they also exhibit a modest reduction in bacterial resistance to gentamicin when administered in conjunction with antibiotics, suggesting that their effects are complex. Other results by Hisham A. Abbas confirms the effect of diclofenac (olfen) and shows downregulation of virulence genes that ranged from the SarA gene, the AgrA gene, the HLA gene, the FnbA gene, the IcaA gene, the SigB gene, and the CrtM gene in Staphylococcus aureus [31].

5. Conclusion

Paracetamol, piroxicam, and nefopam have a positive effect on *the aac(6) gene inP. mirabilis* as they cause upregulation of the gene responsible for resistance toward aminoglycosides. Olfen and dexamethasone showed increased sensitivity toward gentamicin as they caused downregulation in the *aac(6) gene*. These two drugs are recommended to be used in combination with antibiotics to decrease bacterial resistance.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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References

1. Armbruster, C.E.; Mobley, H.L.; Pearson, M.M. Pathogenesis of *Proteus mirabilis* infection. *EcoSal Plus* **2018**, *8(1)* 10.1128/ecosalplus.ESP-0009-2017. https://doi.org/10.1128/ecosalplus.ESP-0009-2017.
2. Coker, C.; Poore, C.A.; Li, X.; Mobley, H.L. Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes and infection* **2000**, *2(12),* 1497-505. DOI: https://doi.org/10.1016/s1286-4579(00)01304-6.
3. Gonzales G. *Proteus Infections*. eMedicine from WebMD. Last edited. **2006**, 2.

4. Matthews, S.J.; Lancaster, J.W. Urinary tract infections in the elderly population. *The American Journal of Geriatric Pharmacotherapy* **2011**, *9(5),* 286-309. DOI: https://doi.org/10.1016/j.amjopharm.2011.07.002.
5. Papazafiropoulou, A.; Daniil, I.; Sotiropoulos, A.; Balampani, E.; Kokolaki, A.; Bousboulas, S.; Konstantopoulou, S.; Skliros, E.; Petropoulou, D.; Pappas, S. Prevalence of asymptomatic bacteriuria in type 2 diabetic subjects with and without microalbuminuria. *BMC Research Notes* **2010**, *3*, 1-5. DOI: https://doi.org/10.1186/1756-0500-3-169.
6. Schaffer, J.N.; Pearson, M.M. *Proteus mirabilis* and urinary tract infections. *Microbiology Spectrum* **2015**, *3(5),* 10.1128/microbiolspec.UTI-0017-2013. DOI: https://doi.org/10.1128/microbiolspec.UTI-0017-2013
7. Jacobsen, S.M.; Stickler, D.J.; Mobley, H.L.; Shirtliff, M.E. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clinical Microbiology Reviews* **2008**, *21(1),* 26-59. DOI: https://doi.org/10.1128/CMR.00019-07.
8. O'Hara, C.M.; Brenner, F.W.; Miller, J.M. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. Clinical Microbiology Reviews **2000**, *13(4),* 534-46. DOI: https://doi.org/10.1128/cmr.13.4.534-546.2000.
9. Norsworthy, A.N.; Pearson, M.M. From catheter to kidney stone: the uropathogenic lifestyle of *Proteus mirabilis. Trends in Microbiology* **2017**, *25(4),* 304-315. DOI: https://doi.org/10.1016/j.tim.2016.11.015.
10. Stock, I. Natural antibiotic susceptibility of *Proteus* spp., with special reference to *P. mirabilis* and *P. penneri* strains. *Journal of Chemotherapy* **2003**, *15(1),*12-26. DOI: https://doi.org/10.1179/joc.2003.15.1.12.
11. Song, W.; Kim, J.; Bae, I.K.; Jeong, S.H.; Seo, Y.H.; Shin, J.H.; Jang, S.J.; Uh, Y.; Shin, J.H.; Lee, M.K.; Lee, K. Chromosome-encoded AmpC and CTX-M extended-spectrum β-lactamases in clinical isolates of *Proteus mirabilis* from Korea. *Antimicrobial Agents and Chemotherapy* **2011**, *55(4),* 1414-9. DOI: https://doi.org/10.1128/AAC.01835-09.
12. Philippon, A.L.; Labia, R.O.; Jacoby, G.E. Extended-spectrum beta-lactamases. *Antimicrobial Agents and Chemotherapy* **1989**, *33(8),* 1131-1136. DOI: https://doi.org/10.1128/AAC.33.8.1131.
13. Jean, S.S.; Lee, W.S.; Yu, K.W.; Liao, C.H.; Hsu, C.W.; Chang, F.Y.; Ko, W.C.; Chen, R.J.; Wu, J.J.; Chen, Y.H.; Chen, Y.S. Rates of susceptibility of carbapenems, ceftobiprole, and colistin against clinically important bacteria collected from intensive care units in 2007: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). *Journal of Microbiology, Immunology and Infection* **2016**, *49(6),* 969-76. DOI: https://doi.org/10.1016/j.jmii.2014.12.008.
14. Mokracka, J.; Gruszczyńska, B.; Kaznowski, A. Integrons, β-lactamase and qnr genes in multidrug resistant clinical isolates of *Proteus mirabilis* and *P. vulgaris*. Apmis **2012**, *120(12),* 950-958. DOI: https://doi.org/10.1111/j.1600-0463.2012.02923.x
15. Ohno, Y.; Nakamura, A.; Hashimoto, E.; Matsutani, H.; Abe, N.; Fukuda, S.; Hisashi, K.; Komatsu, M.; Nakamura, F. Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in a primary care hospital in Japan, 2010–2013. *Journal of Infection and Chemotherapy* **2017**, *23(4),* 224-229. DOI: https://doi.org/10.1016/j.jiac.2016.12.013.
16. Al-Mudallal, N.; Khudair A.; Alsakini A.; Zidane, N. Molecular Detection And Phylogenetic Analysis Of 16s Rna Gene Of Proteus Mirabilis Isolated From Different Clinical Sources In Baghdad Hospitals. *Biochemical & Cellular Archives* **2021**, *21(2),* 471. https://connectjournals.com/03896.2021.21.4711.
17. Al-Shamarti, M.J. Molecular Detection of Oxa-48 β-lactamase in *Proteus* spp. causing UTI. *Journal of Pure and Applied Microbiology* **2019**, *13 (3),* 1467-1473. https://doi.org/10.22207/JPAM.13.3.17.
18. Bisiklis, A.; Papageorgiou, F.; Frantzidou, F.; Alexiou-Daniel, S. Specific detection of blaVIM and blaIMP metallo-β-lactamase genes in a single real-time PCR. *Clinical Microbiology and Infection* **2007**, *13(12),* 1201-1203. DOI: https://doi.org/10.1111/j.1469-0691.2007.01832.x.

19. Alsherees, H.A.; Abdzaid, A.J.; Talib, R. Molecular study of Proteus mirabilis bacteria isolated from urine and wounds in hospitals Al-Najaf province. *International Journal of Advanced Research in Bological Sciences* **2016**, *3(6),* 99-105.
20. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods* **2001**, *25(4),* 402-408. https://doi.org/10.1006/meth.2001.1262.
21. Kamil, T.D.; Jarjes, S.F. Molecular Characterization of *Proteus* spp. from Patients Admitted to Hospitals in Erbil City. *Polytechnic Journal* **2021**, *11(2),* 95-99. DOI: https://doi.org/10.25156/ptj.v11n2y2021.pp95-99.
22. Ahmed, D.A. Prevalence of *Proteus* spp. in some hospitals in Baghdad City. *Iraqi Journal of Science* **2015**, *56(1),* 6656-6672. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/10376.
23. Martins, A.M.; Marto, J.M.; Johnson, J.L.; Graber, E.M. A review of systemic minocycline side effects and topical minocycline as a safer alternative for treating acne and rosacea. *Antibiotics* **2021**, *10(7),* 757. DOI: https://doi.org/10.3390/antibiotics10070757.
24. Serry, F.M.; Gomaa, S.E.; Abbas, H.A. Antimicrobial resistance of clinical *Proteus mirabilis* isolated from different sources. *Zagazig Journal of Pharmaceutical Sciences* **2018**, *27(1),* 57-63. DOI: https://doi.org/10.21608/ZJPS.2018.38156.
25. Al-Jumaily, E.; Zgaer, S.H. Multidrug resistant Proteus mirabilis isolated from urinary tract infection from different hospitals in Baghdad City. *International Journal of Current Microbiology and Applied Sciences* **2016**, *5(9),* 390-399. http://dx.doi.org/10.20546/ijcmas.2016.509.041.
26. Aslam, B.; Rasool, M.; Muzammil, S.; Siddique, A.B.; Nawaz, Z.; Shafique, M.; Zahoor, M.A.; Binyamin, R.; Waseem, M.; Khurshid, M.; Arshad, M.I. *Carbapenem resistance: Mechanisms and drivers of global menace*. IntechOpen, **2020**. DOI: https://doi.org/10.5772/intechopen.90100.
27. Fursova, N.K.; Astashkin, E.I.; Knyazeva, A.I.; Kartsev, N.N.; Leonova, E.S.; Ershova, O.N.; Alexandrova, I.A.; Kurdyumova, N.V.; Sazikina, S.Y.; Volozhantsev, N.V.; Svetoch, E.A. The spread of bla OXA-48 and bla OXA-244 carbapenemase genes among *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter* spp. isolated in Moscow, Russia. *Annals of Clinical Microbiology and Antimicrobials*. **2015**, *14(1),* 1-9. DOI: https://doi.org/10.1186/s12941-015-0108-y.
28. Vourli, S.; Tsorlini, H.; Katsifa, H.; Polemis, M.; Tzouvelekis, L.S.; Kontodimou, A.; Vatopoulos, A.C. Emergence of Proteus mirabilis carrying the blaVIM-1 metallo-β-lactamase gene. *Clinical Microbiology and Infection* **2006**, *12(7),* 691-694. DOI: https://doi.org/10.1111/j.1469-0691.2006.01489.x.
29. Wieczorek, P.; Sacha, P.; Hauschild, T.; Ostas, A.; Kłosowska, W.; Ratajczak, J.; Tryniszewska, E. The aac (6') Ib gene in *Proteus mirabilis* strains resistant to aminoglycosides. *Folia histochemical et Cytobiologica* **2008**, *46(4),* 531-533. DOI: https://doi.org/10.2478/v10042-008-0068-6
30. l-Oqaili, N.A.; Al-Shebli, M.K.; Almousawi, A.N. Molecular Characterizations of Aminoglycoside Modifying Enzymes of *Proteus mirabilis* Isolated From Patients in Al-Qadisiyah Governorate. *Research Journal of Pharmacy and Technology* **2018**, *11(7),* 2809-2813. DOI: https://doi.org/10.5958/0974-360X.2018.00518.8.
31. Abbas, H.A.; Atallah, H.; El-Sayed, M.A.; El-Ganiny, A.M. Diclofenac mitigates virulence of multidrug-resistant *Staphylococcus aureus*. *Archives of Microbiology* **2020**, *202,* 2751-2760. DOI: https://doi.org/10.1007/s00203-020-01992-y.