Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 381 - 390
Received 05 March 2021; Accepted 01 April 2021.

# Molecular Investigation of Pyocyanin Biosynthesis genes Among Multidrug Resistance Clinical Pseudomonas Aeruginosa isolates

**Maytham H.J. Al-Thabhawee[1], Hussein O.M. AL-Dahmoshi[2*]**
[1,2]Biology Department, college of science, University of Babylon, Iraq
*Corresponding author Email:dr.dahmoshi83@gmail.com

## ABSTRACT:

Multidrug resistance Pseudomonas aeruginosa (MDRPA) is most important issue in healthcare setting. Pyocyanin production among MDRPA must increase the worseness of infection by side adverse like tissue damage subsequent harmful effects of pyocyanin for host. The killing of bacteria accomplished by inhibition of pyocyanin production may represent a promise treatment approach. The study include investigation of 50 isolates of MDRPA for pyocyanin biosynthesis and resistance for antibiotics. Molecular diagnosis using P. aeruginosa specific primer pairs, investigation of phzA, phzM and phzS using specific primer pairs by PCR were also performed. The results revealed high resistance to beta lactam antibiotics (78% for ceftazidime, 78% for cefepime and 46% for piperacillin) can indicate possessing of isolates for beta lactamases and this confirmed by dropping resistance to piperacillin to 16% when combined with tazobactam. Also the results shown the ability of MDRPA for pyocyanin production using the system of genes (phzA, phzM, phzS) among all isolates except (3 isolates for phzS). The current study conclude that all isolated of P. aeruginosa were highly virulent due to their possessing of pyocyanin biosynthesis system and beta lactamases make using of piperacillin-tazobactam and meropenem a good choice to kill bacteria along with impairment of pyocyanin production reducing the possible harmful effects of this pigment.

**KEYWORDS:** Pyocyanin, phzA, phzM, phzS, piperacillin-tazobactam

## Introduction

*Pseudomonas aeruginosa* is an opportunistic nosocomial pathogen which can dominant in all niches. Their adaptability to different conditions may come from possessing different virulence factors exploited for survival. One of the most important virulence factor is phanezines. P. aeruginosa produces redox-active pigments called phenazines that affect gene expression, metabolic flux, and redox balancing in their producers[1,2]. *P. aeruginosa* produce a single compound, phenazine-1-carboxylic acid (PCA). Subsequent conversion of PCA to pyocyanin is mediated in *P. aeruginosa* by two novel phenazine-modifying genes,*phzM* and *phzS*, which encode putative phenazine-specific methyltransferase and flavin-containing monooxygenase, respectively[3].

Pyocyanin regards as virulence factor and produced by about 95% of the P. aeruginosa. It cause pulmonary tissue damage. Pyocyanin interferes with the regulation of ion transport, ciliary beat frequency, and mucus secretion in airway epithelial cells by altering the cytosolic concentration of calcium[4,5]. Pyocyanin promotes virulence by interfering with several cellular functions in host cells including electron transport, cellular respiration, energy metabolism, gene expression, and innate immune mechanisms. promotes inflammatory responses by imposing oxidative stress on host cells[6,7]. It was found that pyocyanin production by *P. aeruginosa* suppresses the acute inflammatory response by pathogen-driven acceleration of neutrophil apoptosis and by reducing local inflammation, and that this is advantageous for bacterial survival Phenazines have a

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 381 - 390
Received 05 March 2021; Accepted 01 April 2021.

potential to alter antibiotic susceptibility[8,9]. Resistance to antibiotics may be either intrinsic or acquired[10,11]. The occurrence of multidrug resistant *P. aeruginosa* is growing in the world, limiting the therapeutic options[12,13]. Despite availability of newer antimicrobial agents possessing anti-Pseudomonal activities, P. aeruginosa is still responsible for causing life threatening infection in hospitals[14,15]. Even though development of multi-drug resistant Pseudomonas aeruginosa strains, which are difficult to be treated, some available antibiotics still able to dominate pseudomonal infections with a reasonable percentage of success, for example, colistin sulfate and quinolones (ciprofloxacin and levofloxacin)[16,17]. The current study was conducted to investigate Phenazine Biosynthesis genes among multidrug resistance P. aeruginosa isolates.

## Materials And Methods

### Bacterial isolates:

Fifty P. aeruginosa isolated were collected from different specimens and subjected for primary identification test using Pseudomonas chromogenic agar (Condalab/Spain) and confirmed using genus specific (for Pseudomonas spp.) and species specific (for P. aeruginosa) (Table 1).

### Antibiotic susceptibility Assay:

It was performed using 14 antibiotics agent according to CLSI-2019[18].

### Polymerase Chain Reaction

DNA was extracted according to manufactures instructions (IntronBio/Korea). The primers were dissolved according to manufacturer instructions (Macrogen/Korea). The primer pairs and PCR conditions were listed in (Table 1)

**Table 1.** Primer pairs and PCR conditions

| Primer name | 5' to 3' sequence | Product (bp) | Annaeling Temp. (°C) | Reference |
|---|---|---|---|---|
| ***Ps. Spp.*** | F:GACGGGTGAGTAATGCCTA<br>R:CACTGGTGTTCCTTCCTATA | 618 | 56 | [19] |
| ***Ps. aeru.*** | F:GGGGGATCTTCGGACCTCA<br>R:TCCTTAGAGTGCCCACCCG | 956 | 61 | [19] |
| ***phzA1*** | F:TCAGCGGTACAGGGAAACAC<br>R:GAAGTGGTTCGGATCCTCGG | 283 | 60.3 | This study |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 381 - 390
Received 05 March 2021; Accepted 01 April 2021.

| | | | | |
|---|---|---|---|---|
| ***phzA2*** | F:CGACAACCTGGAATTGCGTC<br>R:GTTTTATCCGGCCGTTCTCG | 368 | 59.3 | This study |
| ***phzS*** | F:CTGGTCGCCTATCCGATCTC<br>R:GCTCTTCTCGGTCTTCGGTC | 507 | 61.3 | This study |
| ***phzM*** | F:GGATGGCCTTGGTCAATTCG<br>R:GATCTTCCAGGGCGATACCC | 350 | 60.3 | This study |

## Results

The results of isolation revealed high percentage of *P. aeruginosa* among UTIs patients 18(36%), lower respiratory tract infection patients 13(26%) wounds and burn infections 9(18%), otitis media 5(10%), bacteremia 2(4%), vaginosis 2(4%) and 1(2%) for meningitis (table2). Results of resistance for 14 antibiotics according to CLSI revealed that 39(78%) of P. aeruginosa isolates were resistant to ceftazidime (CAZ) and cefepime (FEP), 23(46%) for piperacillin (PRL), 15(30%) for gentamycin (CN), 14(28%) for ciprofloxacin (CIP), 13(26%) for tobramycin (TOB), 12(24%) for Aztreonam (ATM),11(22%) for amikacin (AK), 10(20%) for ofloxacine (OFX), 9(18%) for levofloxacin (LEV), 8(16%) for piperacillin-tazobactam (PTZ), 7(14%) for netilmicine, imipenem (IPM) and meropenem (MEM) (Figure 1). Pyocunine biosynthesis was investigated via detection of Phenazine gene (*phzA*) which encode for phenazine-1-carboxylic acid (PCA) who subsequently converted to pyocyanin by two enzyme: Phenazine-1-carboxylate N-methyltransferase and Flavin-containing monooxygenase which endcoded by (*phzM*) and (*phzS*) genes respectively. The results revealed that all p. aeruginosa isolated have *phzA* gene and *phzM* while only 47(94%) have *phzS* gene (Table 3), (Figure 2-5).

**Table 2.** Distribution of P. aeruginosa isolates among Diseases

| **Disease** | **Specimen** | **Bacterial Isolate** | |
|---|---|---|---|
| | | **No.** | **%** |
| **UTIs** | midstream urine | 18 | 36% |
| **RTIs** | Broncoalveolar lavage | 13 | 26% |
| **Wound and burn infections** | Wound burn swab | 9 | 18% |
| **Otitis Media** | Ear swab | 5 | 10% |
| **Bacteremia** | Blood stream | 2 | 4% |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 381 - 390
Received 05 March 2021; Accepted 01 April 2021.

| | | | |
|---|---|---|---|
| **Vaginosis** | High vaginal swab | 2 | 4% |
| **Meningitis** | CSF | 1 | 2% |
| Total | | 50 | 100% |

**Table 3.** Distribution of Phenazine biosynthesis gene among P. aeruginosa isolates

| **Gene** | **P. aeruginosa isolates** | |
|---|---|---|
| | **No.** | **%** |
| **phzA1** | 50 | 100% |
| **phzA2** | 50 | 100% |
| **phzM** | 50 | 100% |
| **phzS** | 47 | 94% |

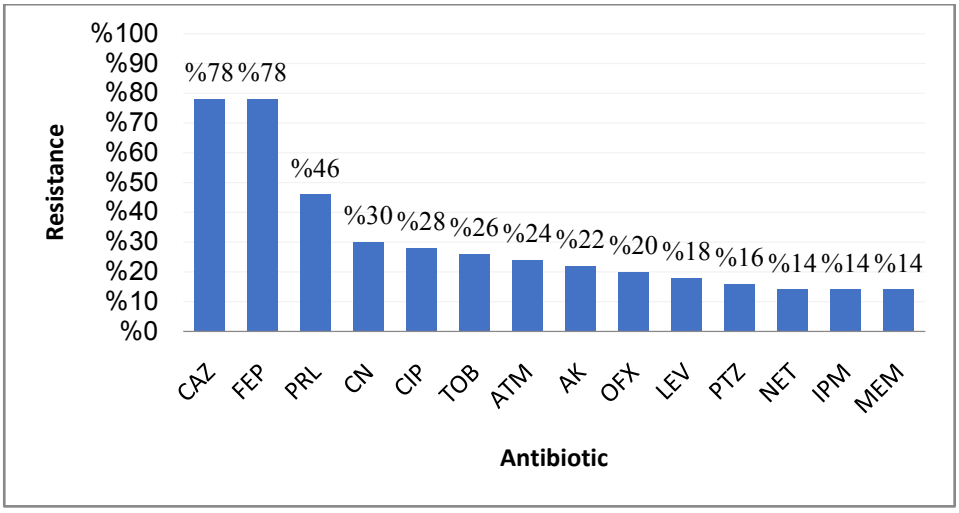



**Figure 1.** Antibiotic resistance percentage of P. aeruginosa to 14 antibiotics (ceftazidime (CAZ), cefepime (FEP), piperacillin (PRL), gentamycin (CN), ciprofloxacin (CIP), tobramycin (TOB), Aztreonam (ATM), amikacin (AK), ofloxacine (OFX), levofloxacin (LEV), piperacillin-tazobactam (PTZ), netilmicine, imipenem (IPM) and meropenem (MEM)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 381 - 390
Received 05 March 2021; Accepted 01 April 2021.

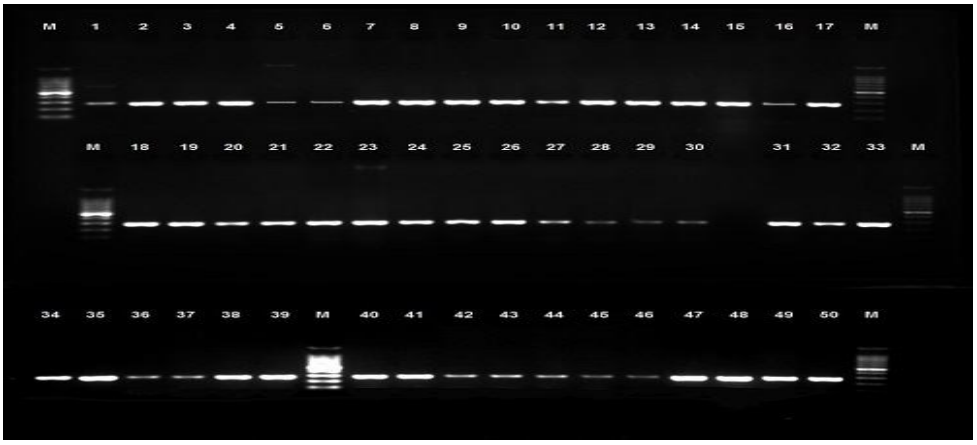



**Figure 2.** 1.5% agarose gel electrophoresis of phzA1 amplicon (283bp) among P. aeruginosa isolates. M represent 100bp DNA ladder , lane 1-50 represent the isolates ,TBE 1x,at Voltage 75volt for 60min.

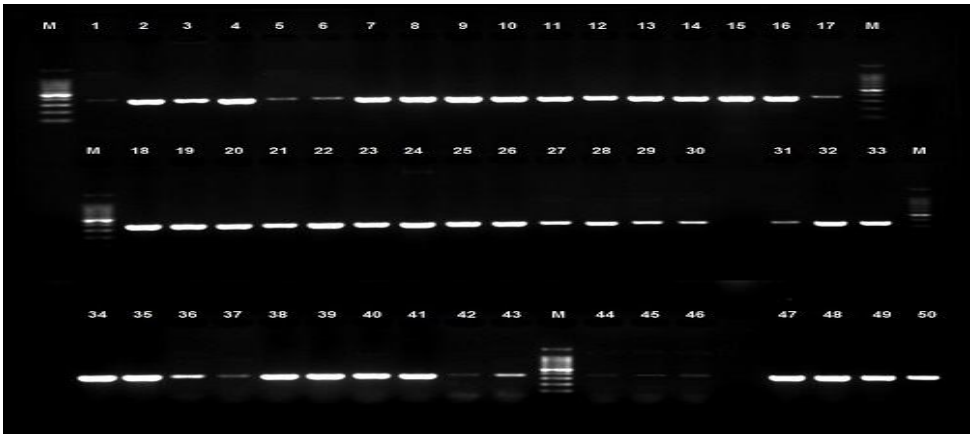
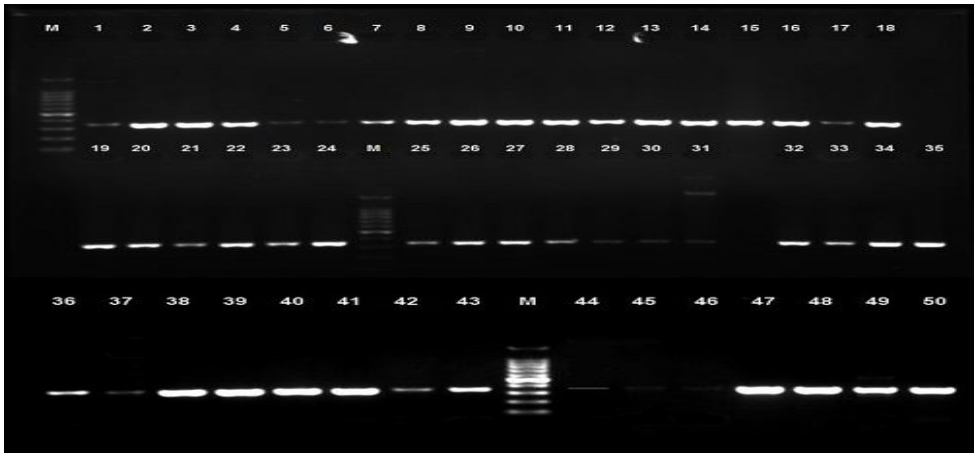



**Figure 3.** 1.5% agarose gel electrophoresis of phzA2 amplicon (368bp) among P. aeruginosa isolates. M represent 100bp DNA ladder, lane 1-50 represent the isolates,TBE 1x,at Voltage 75volt for 60min.



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 381 - 390
Received 05 March 2021; Accepted 01 April 2021.

**Figure 4.** 1.5% agarose gel electrophoresis of phzM amplicon (350bp) among P. aeruginosa isolates. M represent 100bp DNA ladder, lane 1-50 represent the isolates,TBE 1x,at Voltage 75volt for 90min

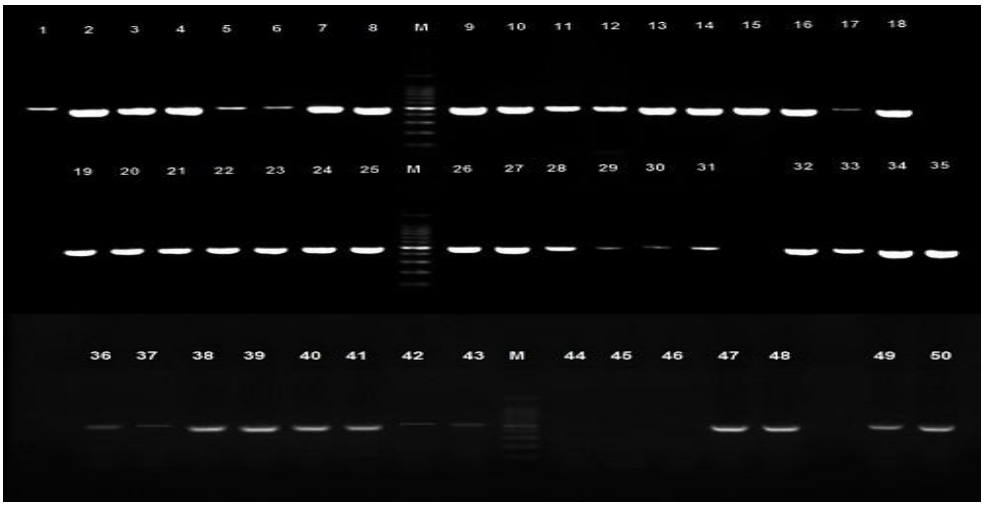



**Figure 5.** 1.5% agarose gel electrophoresis of phzS amplicon (507bp) among P. aeruginosa isolates. M represent 100bp DNA ladder, lane 1-50 represent the isolates,TBE 1x,at Voltage 75volt for 60min.

## Discussion

Our results may be totally agree with previous studies whose found that dominance of P. aeruginosa among UTIs, RTIs and wound-burn infections[20-22]. Implication of P. aeruginosa in UTIs may be as nosocomial pathogen resulted from placing and removing of indwelling urinary catheters[23]. Intensive care unit (ICU) admission is a risk for multidrug-resistant (MDR) P. aeruginosa to critically ill pneumonia patients[24]. In burn centers, P. aeruginosa acts as a major cause of nosocomial infections and this is may attributed to a high prevalence of the antibiotic resistance and biofilm formation ability[25].

*P. aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams. The resistance may be intrinsic (low outer membrane permeability, expression of efflux pumps and the production of antibiotic-inactivating enzymes), acquired (either horizontal transfer of resistance genes or mutational changes) and adaptive (involves formation of biofilm which serves as a diffusion barrier to limit antibiotic access to the bacterial cells) resistance[26,27].

The results shown high resistance to beta lactams (ceftazidime (CAZ), cefepime (FEP), piperacillin (PRL) and this is mainly mediated by beta lactamases due to that when use piperacillin-tazobactam the resistance was dropped from 46% to 16%. Beta lactamases regard as intrinsic mechanism of resistance leading to inactivating of beta lactam rendering them inactive. Beta lactamase inhibitor like tazobactam (An irreversible inhibitor of a wide variety of bacterial beta-lactamases) can improve many beta lactams like piperacillin once combined with them.

Piperacillin-tazobactam is the best β-lactam–β-lactamase inhibitor combination that is frequently used for the management of Pseudomonas aeruginosa infections[28-30]. The results of

current study revealed the ability of all P. aeruginosa isolated to produce pyocyanin making them more virulent and have great harmful consequences due to implication of pyocyanin in tissue damage, interfering with immune response and triggering proinflamattory responses[31,32]. Natural phenazines are versatile secondary metabolites that are mainly produced by Pseudomonas and Streptomyces. All phenazine-type metabolites originate from two precursors: phenazine-1-carboxylic acid (PCA) in Pseudomonas or phenazine-1,6-dicarboxylic acid (PDC) in Streptomyces and other bacteria[33].Pyocyanin has been shown to intercalate with extracellular DNA to promote cell-to-cell interactions between the P. aeruginosa cells by influencing their physicochemical interactions and the cell surface properties. It has been suggested that pyocyanin may also contribute to the biofilm formation by the promotion of extracellular DNA[34].

There is a need for therapeutics strategies to kill P. aeruginosa and at same time preventing the possible damages resulted from pyocyanin productions. Our results suggest Piperacillin-tazobactam, meropenem and combination as a perfect regimens to treat such bacterial isolates. Many results shown the inhibitory effect of Piperacillin-tazobactam and meropenem on biofilm formation and reduce the productions of pyocyanin[34-37].

## Conclusion

The current study conclude that all isolated of P. aeruginosa were highly virulent due to their possessing of pyocyanin biosynthesis system and beta lactamases make using of piperacillin-tazobactam and meropenem a good choice to kill bacteria along with impairment of pyocyanin production reducing the possible harmful effects of this pigment.

## References

[1] Price-Whelan, A., Dietrich, L. E., & Newman, D. K. (2007 September 1). Pyocyanin alters redox homeostasis and carbon flux through central metabolic pathways in Pseudomonas aeruginosa PA14. Journal of Bacteriology, 189(17), 6372–6381. doi:10.1128/JB.00505-07

[2] Dietrich, L. E., Okegbe, C., Price-Whelan, A., Sakhtah, H., Hunter, R. C., & Newman, D. K. (2013 April 1). Bacterial community morphogenesis is intimately linked to the intracellular redox state. Journal of Bacteriology, 195(7), 1371–1380. doi:10.1128/JB.02273-12

[3] Mavrodi, D. V., Bonsall, R. F., Delaney, S. M., Soule, M. J., Phillips, G., & Thomashow, L. S. (2001 November 1). Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from Pseudomonas aeruginosa PAO1. Journal of Bacteriology, 183(21), 6454–6465. doi:10.1128/JB.183.21.6454-6465.2001

[4] Denning, G. M., Railsback, M. A., Rasmussen, G. T., Cox, C. D., & Britigan, B. E. (1998 June 1). Pseudomonas pyocyanine alters calcium signaling in human airway epithelial cells. American Journal of Physiology, 274(6), L893–L900. doi:10.1152/ajplung.1998.274.6.L893

[5] Lau, G. W., Hassett, D. J., Ran, H., & Kong, F. (2004 December 1). The role of pyocyanin in Pseudomonas aeruginosa infection. Trends in Molecular Medicine, 10(12), 599–606. doi:10.1016/j.molmed.2004.10.002

[6] Rada, B., & Leto, T. L. (2013 February 1). Pyocyanin effects on respiratory epithelium: Relevance in Pseudomonas aeruginosa airway infections. Trends in Microbiology, 21(2), 73–81. doi:10.1016/j.tim.2012.10.004

[7] Vipin, C., Ashwini, P., Kavya, A. V., & Rekha, P. D. (2017 February 28). Overproduction of Pyocyanin in Pseudomonas aeruginosa by Supplementation of Pathway Precursor shikimic acid and Evaluation of its Activity. Research Journal of Pharmacy and Technology, 10(2), 533–536. doi:10.5958/0974-360X.2017.00106.8

[8] Allen, L., Dockrell, D. H., Pattery, T., Lee, D. G., Cornelis, P., Hellewell, P. G., & Whyte, M. K. (2005 March 15). Pyocyanin production by Pseudomonas aeruginosa induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. Journal of Immunology, 174(6), 3643–3649. doi:10.4049/jimmunol.174.6.3643

[9] Schiessl, K. T., Hu, F., Jo, J., Nazia, S. Z., Wang, B., Price-Whelan, A., . . . Dietrich, L. E. P. (2019 February 15). Phenazine production promotes antibiotic tolerance and metabolic heterogeneity in Pseudomonas aeruginosa biofilms. Nature Communications, 10(1), 762. doi:10.1038/s41467-019-08733-w

[10] Varshan, R., & Prakasam, G. (2016 September 1). Detection of bla VIM gene encoding Metallo Beta Lactamase resistance among clinical isolates of Pseudomonas aeruginosa. Research Journal of Pharmacy and Technology, 9(9), 1465. doi:10.5958/0974-360X.2016.00284.5

[11] Al-Byti, A. M., Chakmakchy, S. A., Waheeb, A. A., & Alazzawy, M. A. (2020). Multidrug-Resistant Pseudomonas aeruginosa Isolated from surgical sites after plastic surgery in Kirkuk city-Iraq. Research Journal of Pharmacy and Technology, 13(1), 335-338.

[12] Varshitha, A., & Gopinath, P. (2016 October 1). Detection of bla TEM-1 gene for ESBL production among clinical isolates of Pseudomonas aeruginosa. Research Journal of Pharmacy and Technology, 9(10), 1623. doi:10.5958/0974-360X.2016.00323.1

[13] Abbas, M. K., Kadhum, D. A., Shabeeb, A. K., & Mohammed, S. A. (2020 September 4). Combination effect of ciprofloxacin and streptomycin with cefotaxime against multi-drug resistant Pseudomonas aeruginosa from different clinical samples. Research Journal of Pharmacy and Technology, 13(9), 4403–4408. doi:10.5958/0974-360X.2020.00779.9

[14] Mahaseth, S. N., Chaurasia, L., Jha, B., & Sanjana, R. K. (2020 December 31). Prevalence and antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolated from various clinical samples in a tertiary Care Hospital. Janaki Medical College Journal of Medical Science, 8(2), 11–17. doi:10.3126/jmcjms.v8i2.33972

[15] Jyothi, P., Shahapur, P. R., & Metri, B. C. (2021). Comparison of various Phenotypic Tests for Detection of Metallo-beta-Lactamase in Pseudomonas aeruginosa isolates at a Tertiary Care Centre. Research Journal of Pharmacy and Technology, 14(2), 1022-1024.

[16] Saleh, M. M., Sadeq, R. A., Latif, H. K. A., Abbas, H. A., & Askoura, M. (2018). Antimicrobial susceptibility and resistance profile of Pseudomonas aeruginosa isolates from patients at an Egyptian hospital. Research Journal of Pharmacy and Technology, 11(8), 3268–3272. doi:10.5958/0974-360X.2018.00601.7

[17] Sreeja, M. K., Gowrishankar, N. L., Adisha, S., & Divya, K. C. (2017 June 28) Antibiotic resistance-reasons and the most common resistant pathogens - A review. Research Journal of Pharmacy and Technology, 10(6), 1886–1890. doi:10.5958/0974-360X.2017.00331.6

[18] Clinical and Laboratory Standards Institute. (2019). Performance standards for antimicrobial susceptibility testing (29th ed) CLSI Supplement M100.

[19] Spilker, T., Coenye, T., Vandamme, P., & LiPuma, J. J. (2004). PCR-based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered

from cystic fibrosis patients. Journal of Clinical Microbiology, 42(5), 2074–2079. doi:10.1128/jcm.42.5.2074-2079.2004

[20] Ozdemir, K., Dizbay, M., & UĞRAŞ DİKMEN, A. S. İ. Y. E. (2013). Incidence and risk factors of nosocomial infections in elderly and adult patients in intensive care units.

[21] Inci, A., Karabay, A., Erus, S., & Demiraran, Y. (2016 December 1). Nosocomial infections and associated risk factors in geriatric patients in the intensive care unit. Eurasian Journal of Emergency Medicine, 15(4), 177–180. doi:10.5152/eajem.2016.35744

[22] Al-Obaidi, R. D., & Al-Dahmoshi, H. O. (2020). Biofilm and antibiotic resistance profile among Pseudomonas aeruginosa isolated from clinical samples. Eurasia J Biosci, 14(1), 1135-1139.

[23] Li, Y., Ren, L., & Zou, J. (2019 January 1). Risk factors and prevention strategies of nosocomial infection in geriatric patients. Canadian Journal of Infectious Diseases and Medical Microbiology, 2019, 6417959. doi:10.1155/2019/6417959

[24] Trinh, T. D., Zasowski, E. J., Claeys, K. C., Lagnf, A. M., Kidambi, S., Davis, S. L., & Rybak, M. J. (2017 September 1). Multidrug-resistant Pseudomonas aeruginosa lower respiratory tract infections in the intensive care unit: Prevalence and risk factors. Diagnostic Microbiology and Infectious Disease, 89(1), 61–66. doi:10.1016/j.diagmicrobio.2017.06.009

[25] Karami, P., Mohajeri, P., Yousefi Mashouf, R. Y., Karami, M., Yaghoobi, M. H., Dastan, D., & Alikhani, M. Y. (2019 November 1). Molecular characterization of clinical and environmental Pseudomonas aeruginosa isolated in a burn center. Saudi Journal of Biological Sciences, 26(7), 1731–1736. doi:10.1016/j.sjbs.2018.07.009

[26] Mulcahy, L. R., Burns, J. L., Lory, S., & Lewis, K. (2010 December 1). Emergence of Pseudomonas aeruginosa strains producing high levels of persister cells in patients with cystic fibrosis. Journal of Bacteriology, 192(23), 6191–6199. doi:10.1128/JB.01651-09

[27] Breidenstein, E. B., de la Fuente-Núñez, C., & Hancock, R. E. (2011 August 1). Pseudomonas aeruginosa: All roads lead to resistance. Trends in Microbiology, 19(8), 419–426. doi:10.1016/j.tim.2011.04.005

[28] Tannous, E., Lipman, S., Tonna, A., Hector, E., Hussein, Z., Stein, M., & Reisfeld, S. (2020 July 22). Time above the MIC of piperacillin-tazobactam as a predictor of outcome in Pseudomonas aeruginosa bacteremia. Antimicrobial Agents and Chemotherapy, 64(8). doi:10.1128/AAC.02571-19

[29] Babich, T., Naucler, P., Valik, J. K., Giske, C. G., Benito, N., Cardona, R. (2020 May 23). Ceftazidime, carbapenems, or piperacillin-tazobactam as single definitive therapy for Pseudomonas aeruginosa bloodstream infection: A multisite retrospective study. Clinical Infectious Diseases, 70(11), 2270–2280. doi:10.1093/cid/ciz668

[30] Al Muqati, H., Al Turaiki, A., Al Dhahri, F., Al Enazi, H., & Althemery, A. (2021 March 1). Superinfection rate among the patients treated with carbapenem versus piperacillin/tazobactam: Retrospective observational study. Journal of Infection and Public Health, 14(3), 306–310. doi:10.1016/j.jiph.2020.11.015

[31] Winstanley, C., & Fothergill, J. L. (2009 January 1). The role of quorum sensing in chronic cystic fibrosis Pseudomonas aeruginosa infections. FEMS Microbiology Letters, 290(1), 1–9. doi:10.1111/j.1574-6968.2008.01394.x

[32] Hall, S., McDermott, C., Anoopkumar-Dukie, S., McFarland, A. J., Forbes, A., Perkins, A. V., . . . Grant, G. D. (2016 August). Cellular effects of pyocyanin, a secreted virulence factor of Pseudomonas aeruginosa. Toxins, 8(8), 236. doi:10.3390/toxins8080236
[33] Guo, S., Wang, Y., Dai, B., Wang, W., Hu, H., Huang, X., & Zhang, X. (2017 October). PhzA, the shunt switch of phenazine-1,6-dicarboxylic acid biosynthesis in Pseudomonas chlororaphis HT66. Applied Microbiology and Biotechnology, 101(19), 7165–7175. doi:10.1007/s00253-017-8474-3
[34] Das, T., & Manefield, M. (2012). Pyocyanin promotes extracellular DNA release in Pseudomonas aeruginosa. PLOS ONE, 7(10), e46718. doi:10.1371/journal.pone.0046718
[35] Fothergill, J. L., Panagea, S., Hart, C. A., Walshaw, M. J., Pitt, T. L., & Winstanley, C. (2007 December). Widespread pyocyanin over-production among isolates of a cystic fibrosis epidemic strain. BMC Microbiology, 7(1), 45. doi:10.1186/1471-2180-7-45
[36] Fuse, K., Fujimura, S., Kikuchi, T., Gomi, K., Iida, Y., Nukiwa, T., & Watanabe, A. (2013 January 1). Reduction of virulence factor pyocyanin production in multidrug-resistant Pseudomonas aeruginosa. Journal of Infection and Chemotherapy, 19(1), 82–88. doi:10.1007/s10156-012-0457-9
[37] Aleanizy, F. S., Alqahtani, F. Y., Eltayb, E. K., Alrumikan, N., Almebki, R., Alhossan, A., . . . AlQahtani, H. (2021 January 1). Evaluating the effect of antibiotics sub-inhibitory dose on Pseudomonas aeruginosa quorum sensing dependent virulence and its phenotypes. Saudi Journal of Biological Sciences, 28(1), 550–559. doi:10.1016/j.sjbs.2020.10.040