How to Cite:
Al-Absawe, S. A. A., & Tuwaij, N. S. S. (2022). Screening of AmpC-Family genes among Pseudomonas aeruginosa isolated from burn patients in Najaf City, Iraq. *International Journal of Health Sciences*, *6*(S7), 3547–3555. https://doi.org/10.53730/ijhs.v6nS7.12572

Screening of AmpC-family genes among Pseudomonas aeruginosa isolated from burn patients in Najaf city, Iraq

Salam Ali Abd Al-Absawe
Department of Biology, Faculty of Science, University of Kufa, Iraq

Nabil Salim Saaid Tuwaij
Department of Biology, Faculty of Science, University of Kufa, Iraq
Email: nabeel.tuwaij@uokufa.edu.iq

***Abstract*---**Burn patients are at risk of severe infections caused by *pseudomonas aeruginosa* due to their immunosuppressive conditions. A one mechanism of resistance in this pathogen by *AmpC* beta-lactamases, therefore this study included molecular screening AmpC family genes among isolates of *P. aeruginosa*. The present work was conducted in Al-Sadr Medical City, and burns unit center as well as some chief clinical laboratories in Al-Najaf City period December 2021 to March 2022. An entirety of 68 patients were checked to recognize *P. aeruginosa* isolates. Bacterial growth were 59 (86.76%), as following 12(17.64%), 47 (69.11%) and 9 (13.23%) for gram-positive bacteria, gram-negative and non- growth respectively .Results recorded 32(54.23%) of *P. aeruginosa* isolates. At same respect, the percentage of *P. aeruginosa* isolates in women were 20(33.89%) compared with 12(20.33%) in male. Results showed 20(62.5%) of isolates were resistance to piperacillin-tazobactam, , with a MIC of 128 µg/ml, Also data in table (2) showed that 32(100%) resistance to cefazoline antibiotic at MIC of 64 µg/ml. Whereas, the bacterial isolates showed less resistance to ceftazidime and cefepime, as the resistance ratios reached 21(65.62%) and 19(59.37%) respectively.the results showed that 20(62.5%) were resistant to imipenem while 19(59.37) and 18(56.25) were resistance gentamycin and amikacin respectively. Both ciprofloxacin and levofloxacin had resistance ratios involved 20(62.5) and 21(65.62%) respectively. Finally, the MIC of tigecycline showed that 32(100%) of *P. aeruginosa* isolates were resistant to this drug. Molecular screen of universal *AmpC* gene was 25(78.12%), while screening on *AmpC* –type family gene in current work was revealed high rate and frequency of *DHA* gene 30(93.75%), While *MOX, CIT, FOX, ACC* and *EBC* genes no detected in current study.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 9 May 2022, Manuscript revised: 18 July 2022, Accepted for publication: 27 August 2022

***Keywords*---**PCR, AmpC family, *Pseudomonas aeruginosa*, Burn patients.

Introduction

Important source of infection in humans, *Pseudomonas aeruginosa* is a Gram-negative bacterium that has been linked to severe morbidity and mortality (Spagnolo *et al.*,2021). Infectious disease produced by *P. aeruginosa* can be deadly to humans because they pose a risk to those with low immunity, such as patients suffering from skin burns, as well as patients staying in hospitals for prolonged periods of time and people undergoing organ transplants. (Mohanty *et al.*, 2020). Microbial infection of severe burn wounds is now a serious medical problem. *Pseudomonas aeruginosa* infections are among the most serious among bacteria capable of colonizing such burns, causing severe delays in burn patient recovery or leading to lethal effects. (Gonzalez *et al.*, 2016).

Antibiotics can be challenging to employ in the event of these disorders since *P. aeruginosa* has inherent resistance to several antibiotics. (Alvarez-Ortega *et al.,* 2011). Infection is responsible for 42 to 65 percent of all fatalities in burn victims. (Kelly *et al.*, 2022; Salih *et al.,* 2022) ,Additionally, *P. aeruginosa* has acquired a number of ways for resisting antibiotics either by horizontal acquisition of resistant determinants or through the identification of mutations in chromosomal genes. (Langendonk *et al.*, 2021; Medhat and Aljanabay, 2022a). However, because *P. aeruginosa* is able to withstand many of the currently available antibiotics, treating *P. aeruginosa* infections has grown to be quite difficult. *P. aeruginosa* has an inducible ampC gene, which encodes the hydrolytic enzyme -lactamase, similar to other Gram-negative bacteria. The amide bond of the b -lactam ring can be broken by this enzyme, rendering -lactam antibiotics inactive (Pang *et al.*, 2019).

Materials and Methods

Burn specimens, bacterial isolation and identification

A total of 68 clinical specimens were collected from patients admitted to Burn center and privates clinical laboratories in Al-Najaf City .Specimens collection following removal of old dressing of burns patients, the surface of the burns wound was cleaned with normal saline to prevent contamination , Each Specimen was collected by swabbing the wound with a sterile cotton- tip swab stick (Medhat and Aljanaby, 2022b). Whole burn specimens were cultured immediately on blood agar and MacConkey agar and incubated aerobically in 37oC for 24 hour (MacFaddin, 2000**). Morphological characters and main some biochemical test as well as oxidase test were used to diagnosis the bacteria and all suspected *P. aeruginosa isolate* was confirmed using Vitek-2 system**

Antibiotic susceptibility

All *P. aeruginosa* isolates were measurement the MICs using Vitek-2 compact system. The 0.5 McFarland bacterial suspension was prepared. Cards were

automatically filled, sealed, and loaded into the Vitek-2 compact system t for incubation and reading. The GN AST 76 card used for *P. aeruginosa* isolates contained piperacillin-tazobactam(PIP); cefazoline(CEZ); ceftazidime(CAZ); cefepime(FEP); gentamicin(CN); amikacin(AK); imipenem(IMI); ciprofloxacin(CIP); levofloxacin(LEV) and (TGC) tigecycline. MICs of each antimicrobial agent generated by the VITEK 2 system were compared with each MIC determined by guide of Clinical and Laboratory Standards Institute CLSI(2021)

Nucleic acid extraction and PCR assay

Using a genomic DNA extraction mini kit (Favorgen, Biotech, Corp. Korea), the whole nucleic acid of 32 isolates of *P. aeruginosa* were extracted. The extraction was carried out in accordance with the manufacturer's instructions. All of the genes listed in table(1) were examined and detected using the PCR technique after the nucleic acid was preserved at -20 ^{o}C using a deep freezing apparatus . After adding ethidium bromide dye at a concentration of 0.5 g/ml, the gel document (Cleaver, United Kingdom) was used to apply migration of PCR products at 1% agarose (iNtRoN, Biotech Inc., Korea) (Hasan et al., 2021; Al-Hamdani and Tuwaij, 2020).

Table (1)
Sequence of primers and annealing degree used in this study

Gene name	**Primer Sequence 5' to 3'**	**Annealing** (^{o}C**)**	**Product Size (bp)**	**Reference**
CIT-F	TGGCCAGAACTGACAGGCAAA	64	462	Kolar *et al.*, (2020)
CIT-R	TTTCTCCTGAACGTGGCTGGC			
MOX-F	GCTGCTCAAGGAGCACAGGAT	64	520	
MOX-R	CACATTGACATAGGTGTGC			
DHA-F	AACTTTCACAGGTGTGCTGGGT	64	405	
DHA-R	CCGTACGCATACTGGCTTTGC			
ACC-F	AACAGCCTCAGCAGCCGGTTA	64	346	
ACC-R	TTCGCCGCAATCATCCCTAGC			
EBC-F	TCGGTAAAGCCGATGTTGCGG	64	302	
EBC-R	CTTCCACTGCGGCTGCCAGTT			
FOX-F	AACATGGGGTATCAGGGAGAT	64	190	
FOX-R	CAAAGCGCGTAACCGGATTGG			
AmpC-F	TGCTCGGCATCTCTTGCTCT	60	581	Zhou *et al.* (2003)
AmpC-R	CAGCTTGAGCGGCTTAAGGA			

Results

Among 68 patients involved 40(58.82%) female and 28(41.17%) was males, the results of culture growth indicated that 47(69.11 %) gram negative bacteria and 12(17.64 %) gram positive bacteria while 9(13.23%) no bacterial growth. The results listed in table 2 indicated that the percentage of *P. aeruginosa* isolates

based on bacterial growth in females were 20(33.89%) compared with 12(20.33) in male from a total of 32(54.23%) *P. aeruginosa* isolates.

Table (2)
Distribution of *Pseudomonas aeroginosa* in positive culture bacterial growth according to the gender

gender	specimens growth		Gram-negative		*Pseudomonas aeruginosa*	
	number	%	number	%	number	%
Male	25	42.37	19	32.2	12	20.33
Female	34	57.62	28	47.54	20	33.89
Total	59	100	47	79.66	32	54.23

Measurement of MIC using Vitek-2 compact system

Results showed 20(62.5%) of isolates were resistance to piperacillin-tazobactam, , with a MIC of 128 µg/ml, Also data in table (3) showed that 32(100%) resistance to cefazoline antibiotic at MIC of 64 µg/ml. Whereas, the bacterial isolates showed less resistance to ceftazidime and cefepime, as the resistance ratios reached 21(65.62%) and 19(59.37%) respectively.the results showed that 20(62.5%) were resistant to imipenem while 19(59.37) and 18(56.25) were resistance gentamycin and amikacin respectively. Both ciprofloxacin and levofloxacin had resistance ratios involved 20(62.5) and 21(65.62%) respectively. Finally, the MIC of tigecycline showed that 32(100%) of *P. aeruginosa* isolates were resistant to this drug (table 3).

Table (3)
Antimicrobial agents susceptibility of *Pseudomonas aeroginosa* isolates according to Vitek-2 system (MIC)

Antibiotics	Resistance (%)	Intermediate (%)	Sensitive (%)
PIP	20(62.5)	0(0)	12(37.5)
CEZ	32(100)	0(0)	0(0)
CAZ	21(65.62)	1(3.12%)	10(31.25)
FEP	19(59.37)	3(9.37)	10(31.25)
CN	19(59.37)	1(3.12%)	12(37.5)
AK	18(56.25)	2(6.25)	12(37.5)
IMI	20(62.5)	0(0)	12(37.5)
CIP	20(62.5)	1(3.12%)	11(34.37)
LE	2165.62)	2(6.25)	9(28.12)
TGC	32(100)	0(0)	0(0)

However, according to data of PCR, the results revealed that 25(78%) and 30(93.75%) of *P. aeruginosa* isolates harbored both universal AmpC and DHA genes respectively (figure 1 and 2), While they did not show any positive band for *MOX, CIT, FOX, ACC* and *EBC* genes among 32 isolates of *P. aeruginosa* isolates.

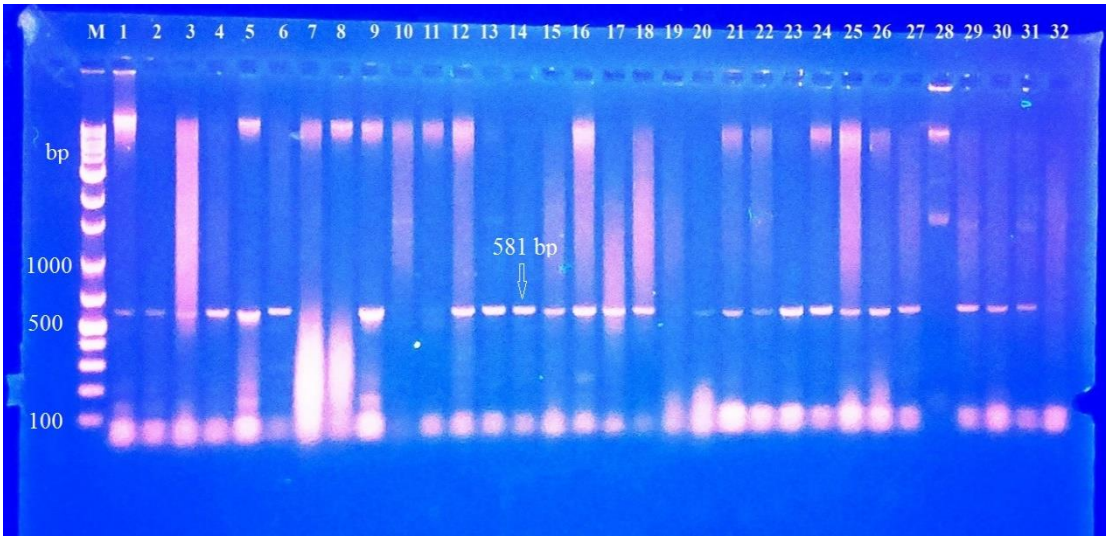

Figure (1): PCR amplification of Universal AmpC gene among *P. aeruginosa* isolates

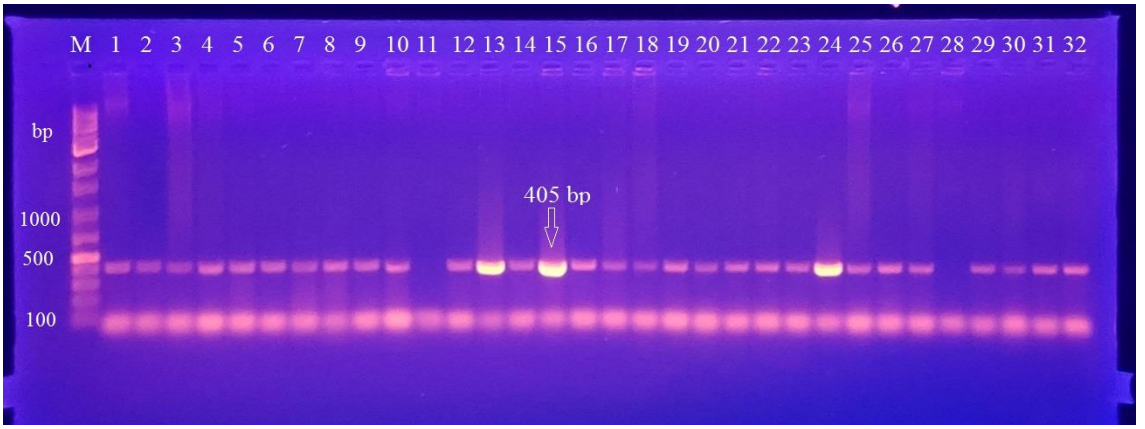

Figure (2): PCR amplification of DHA gene among *P. aeruginosa* isolates

Discussion

The results recorded high rate of *P. aeruginosa* isolates among burn patients However current results agree with several scientific studies indicate that bacterial infection occurs in burn patients immediately after the burn wound, as these burned areas are devoid of normal flora, as they acquire microorganisms, or from contact or from the external environment surrounding the patient, as well as an endogenous source (Church *et al.*, 2006). Antimicrobial agents are potent

medicines used in the management infections of bacteria. The unsuitable use of antibiotics has promoted the spread resistance in most pathogens, (Livermore, 2018; Hadi and Aljanaby, 2022). Results of current research revealed that 62.5% of *P. aeroginosa* isolates were resistance to combination drug of piperacillin-tazobactam, this rate above of rate that obtained by Rezaei, *et al.*, (2016) they recorde that 9% of *P. aeroginosa* isolates were resistance to piperacillin-tazobactam drug. At same respect, findings of antibiogram for current study were above from results that obtained by Siddiqua *et al.*, (2018) reported that Resistance rates to cefepime, ceftriaxone, cefotaxime, and gentamicin varied from 47% to 88%. All the isolates were comparatively better susceptible to meropenem, ciprofloxacin, amikacin and imipenem which range from 76 to 87%. Current works revealed high resistance to drugs of beta- lactams and imipenem, a work was done by Ameen *et al.*, (2015) In Pakistan they observed that 49.5% of the isolates of *P. aeruginosa* tested were imipenem resistant. The data of the current study about antibacterial agents revealed the high rates of antibiotic resistance in *P. aeruginosa* isolates in the hospital, which will pose a challenge to improve the experimental antimicrobial treatment of *P. aeruginosa* infection and therefore requires serious shedding and the establishment of an integrated program that supervises the antibiotic treatment of patients in Najaf city with antibiotics effectively. Production of *AmpC* beta-lactamase among Gram-negative pathogens involving isolates of *P. aeruginosa* is regarded as a universal threat and the development of these multidrug-resistant bacteria is a threat to commercial antibiotics, there were a few microorganisms involved *P. aeruginosa* which produced AmpC beta-lactamase make it resistance to some beta-lactam drugs as well as β-lactamase inhibitor-β-lactam combinations **(**Chika *et al.*, 2016; Kunz *et al.*, 2022**;** Hadi *et al.,* 2022**).** However, present study recorded high rate of *AmpC* gene in *P. aeruginosa* isolates and this may be reflect on phenotypic character through high resistance to beta-lactam drugs and this agree with Mirsalehian *et al.*, (2014) they observed that *AmpC* beta-lactamase was important reason of multiple resistance of beta-lactam drugs. At same respect, a recent study done by Nasser *et al.*,(2020) they recorded that among 65 mutidrug-rsistant *P. aeruginosa* isolates were 40(61.5%) of these harbored AmpC gene , and all isolates were isolated from burn and wound patients. Other studies recorded 68.6% and 36.8% of *P. aeruginosa* isolates which isolated from burn patients were contain AmpC gene (Rafiee, 2014; Rostami *et al.*, 2018). Present study recorded that *DHA* gene was exist in high rate (93.75%) among isolates of *P. aeruginosa* and this finding may be higher rate on what it observed in Iran by Fazeili and Momttaz *et al.*, (2014) they mention that *DHA* gene was obtained at rate 21.56% in *P. aeruginosa* isolates. However, the genes *MOX, CIT, FOX, ACC* and *EBC* were not detected in this study, while a study done by Tahmasebi *et al.*, (2018) they recorded different rate among *AmpC*-family genes in *P. aeruginosa* isolates included 11.57% , 22.1% , 36.6% for *AAC, FOX, MOX* genes respectively, and 2.1% for *CIT*, and *DHA* genes. PCR data of this study was closed to finding of a recent local study done by Al-Ghazaly and Tuwaij (2022) in Al-Najaf City/Iraq, they observed that *DHA* gene was found 100% in all 28 isolates of *Acinetobacter* spp. while *MOX, CIT, FOX, ACC* and *EBC* genes were not observed.

Reference

Al-Ghazaly, N. F. Z., & Tuwaij, N. S. S. (2022). Distribution of AmpC-Family genes among Acinetobacter spp isolates in Najaf City, Iraq. *International Journal of Health Sciences*, *6*(S1), 10152– 10161.

Al-Hamdani, Z. A. & Tuwaij, N. S.(2020).Molecular Screening of Virulence Genes among Uropathogenic *Enterococcus faecalis* Isolated from patients in Al-Najaf City, Iraq. *International Journal of Pharmaceutical Investigation 12*(4):331-341.

Alvarez-Ortega C, Wiegand I, Olivares J, Hancock RE, & Martínez JL. (2011). The intrinsic resistance of Pseudomonas aeruginosa to β-lactams. Virulence; 2 (2): 144-146.

Ameen N, Memon Z, Shaheen S, Fatima G, & Ahmed F. (2015). Imipenem Resistant Pseudomonas aeruginosa: The fall of the final quarterback. Pak J Med Sci.;31(3):561-5.

Chika, E., Charles, E., Ifeanyichukwu, I., Chigozie, U., Chika, E., Carissa, D., & Michael, A. (2016). Phenotypic detection of AmpC beta-lactamase among anal Pseudomonas aeruginosa isolates in a Nigerian abattoir. *Archives of Clinical Microbiology*, *7*(2), 1-5.

Church D, Elsayed S, Reid O, Winston B, & Lindsay R. (2006). Burn wound infections. Clin Microbiol Rev. 19(2):403-34.

Dorri, K., Modaresi, F., Shakibaie, M. R., & Moazamian, E. (2022). Effect of gold nanoparticles on the expression of efflux pump mexA and mexB genes of Pseudomonas aeruginosa strains by Quantitative real-time PCR. *Pharmacia*, *69*(1), 125-133.

Fazeli, N., & Momtaz, H. (2014). Virulence gene profiles of multidrug-resistant Pseudomonas aeruginosa isolated from Iranian hospital infections. *Iranian Red Crescent Medical Journal*, *16*(10).

Hadi, H. I., Enaya, M. A., Enaya, M. A., & Aljanaby, A. A. J. (2022). Duodenal ulcer and gastric cancer patients infected with Helicobacter Pylori in AL-Najaf City, Iraq. *International Journal of Health Sciences*, *6*(S5), 8789–8793.

Hadi, H. I. & Aljanaby, A. A. J. (2022). Helicobacter Pylori-oncogenic protein cytotoxin-associated gene A and assessment of CD14 and CD163 in duodenal ulcer and gastric cancer patients. International Journal of Health Sciences, 6(S2), 839–851.

Hasan TH, ALasedI KK, Aljanaby AAJ. A Comparative Study of Prevalence Antimicrobials Resistance Klebsiella pneumoniae among Different Pathogenic Bacteria Isolated from Patients with Urinary Tract Infection in Al-Najaf City, Iraq. Latin American Journal of Pharmacy, 2021, 40(Special Issue), pp. 174–178.

Gonzalez MR, Fleuchot B, Lauciello L, Jafari P, Applegate LA, Raffoul W, Que YA, & Perron K. (2016). Effect of Human Burn Wound Exudate on Pseudomonas aeruginosa Virulence. M Sphere. 27;1(2):e00111-15.

Kelly, E. J., Oliver, M. A., Carney, B. C., & Shupp, J. W. (2022). Infection and Burn Injury. European Burn Journal, 3(1), 165-179.

Kolar, M., Cermak, P., Hobzova, L., Bogdanova, K., Neradova, K., Mlynarcik, P., & Bostik, P. (2020). Antibiotic Resistance in Nosocomial Bacteria Isolated from Infected Wounds of Hospitalized Patients in Czech Republic. *Antibiotics*, *9*(6), 342.

Kunz Coyne, A. J., El Ghali, A., Holger, D., Rebold, N., & Rybak, M. J. (2022). Therapeutic strategies for emerging multidrug-resistant Pseudomonas aeruginosa. *Infectious Diseases and Therapy*, 1-22.

Langendonk, R. F., Neill, D. R., & Fothergill, J. L. (2021). The building blocks of antimicrobial resistance in Pseudomonas aeruginosa: implications for current resistance-breaking therapies. Frontiers in Cellular and Infection Microbiology, 11, 665759.

Livermore, D. M. (2012). Current Epidemiology and Growing Resistance of Gram-Negative Pathogens. Korean J.Intern Med.27(2): 128–142.

MacFaddin, J.F.(2000). Biochemical tests for identification of medical bacteria .(3rd edition).The Williams and Wilkins-Baltimor. USA.

Medhat, A. R., & Aljanabay, A. A. J. (2022a). Epidemiology of typhoid fever in Balad City, Iraq. International Journal of Health Sciences, 6(S1), 1049-1063

Medhat, A. R., & Aljanabay, A. A. J. (2022b). Assessment of some immune markers in typhoid-patients: A case control study . International Journal of Health Sciences, 6(S1), 4199–4210.

Mirsalehian, A., Kalantar-Neyestanaki, D., Nourijelyani, K., Asadollahi, K., Taherikalani, M., Emaneini, M., & Jabalameli, F. (2014). Detection of AmpC-β-lactamases producing isolates among carbapenem resistant P. aeruginosa isolated from burn patient. *Iranian journal of microbiology*, *6*(5), 306.

Mohanty, S., Baliyarsingh, B., & Nayak, S. K. (2020). Antimicrobial Resistance in Pseudomonas aeruginosa: A concise review. Antimicrobial Resistance-A One Health Perspective. 49.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. (2019). Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology advances, 37(1), 177-192.

Performance Standards for Antimicrobial Susceptibility Testing (CLSI). (2021): 30th ed supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. p: 316.

Pesingi, P. V., Singh, B. R., Pesingi, P. K., Bhardwaj, M., Singh, S. V., Kumawat, M., Sinha D.K., & Gandham R.K. (2019). MexAB-OprM efflux pump of Pseudomonas aeruginosa offers resistance to carvacrol: a herbal antimicrobial agent. Frontiers in microbiology. 10:2664.

Rafiee, R., Eftekhar, F., Tabatabaei, S. A., & Tehrani, D. M. (2014). Prevalence of extended-spectrum and metallo β-lactamase production in AmpC β-lactamase producing Pseudomonas aeruginosa isolates from burns. *Jundishapur journal of microbiology*, *7*(9).

Rezaei, F., Saderi, H., Boroumandi, S., & Faghihzadeh, S. (2016). Relation between Resistance to Antipseudomonal β-Lactams and ampC and mexC Genes of Pseudomonas aeruginosa. Iranian journal of pathology, 11(1), 47.

Rostami, S., Sheikh, A. F., Shoja, S., Farahani, A., Tabatabaiefar, M. A., Jolodar, A., & Sheikhi, R. (2018). Investigating of four main carbapenem-resistance mechanisms in high-level carbapenem resistant Pseudomonas aeruginosa isolated from burn patients. *Journal of the Chinese Medical Association*, *81*(2), 127-132.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Salih NS, Yahya WI, Al-Labban HMY, Aljanaby AAJ (2022). Schiff bases compounds prepared from Phenyl hydrazine as a starting material were Synthesized, Characterized, and their Biological activity was Investigated. Research J Pharm and Tech. 15(8):3595-3598.

Siddiqua, M., Alam, A. N., Akter, S., & Ferdousi, R. S. (2018). Antibiotic resistance pattern in pseudomonas aeruginosa isolated from a private Medical College Hospital. KYAMC Journal, 9(1), 16-19.

Spagnolo, A. M., Sartini, M., & Cristina, M. L. (2021). Pseudomonas aeruginosa in the healthcare facility setting. Reviews in Medical Microbiology, 32(3), 169-175.

Tahmasebi H, Alikhani M Y, Dehbashi S, Arabestani M R. Determination of Minimum Inhibitory Concentration of Different Antibiotic Groups in Clinical Isolates of *Pseudomonas aeruginosa* Containing p-AmpC and Their Relationship with Antibiotic Resistance Pattern. Avicenna J Clin Med. 2018; 24 (4) :277-284**.**

Zhou, Z., Li, L., Yu, Y., & Ma, Y. (2003). The status of drug resistance and ampC gene expression in Enterobacter cloacae. Chinese medical journal, 116(08), 1244-1247.