Iraqi Journal of Biotechnology, October 2020, Vol. 19, No. 2, 37-45

Isolation and Identification of Multidrug Resistance Among Clinical and Environmental *Pseudomonas aeruginosa* Isolates

Rami A. Kareem AL-Fridawy[1,2] ،Wathiq Abbas Hatite Al-Daraghi[2] , Marwa Hameed Alkhafaji[3]

[1]Baghdad Health Department \ Al-Karkh, Ministry of Health, Baghdad, Iraq.
[2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.
[3] University of Science Department of Biology.

Received: August 25, 2020 / **Accepted:** September 20, 2020 / **Published:** October 1, 2020

Abstract: *Pseudomonas aeruginosa* is the most common opportunistic pathogen causing morbidity and mortality in hospitalized patients due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a preference. So *P. aeruginosa* is an extremely versatile Gram-negative bacterium capable of thriving in a broad spectrum of environments, and this performs main problems to workers in the field of health. One hundred and fifty samples were collected from different sources from Baghdad hospitals, divided into two main groups: clinical (100) specimens and (50) samples as an environmental, collected from October 2019 to the March 2020. All of these samples were cultured by specific and differential media, Forty six isolates of *P. aeruginosa* bacteria were identified by using microscopic examination, biochemical tests and confirmed by VITEK-2 compact system. The antibiotic sensitivity test recognized for all bacterial isolates and the results showed high resistant to Amikacin, Cefepime, Ciprofloxacin, Gentamicin, Meropenem, Piperacilin, Ticarcillin, Ticarcillin/Clavulanic Acid and Tobramycin, and high sensitive to Ceftazidime, Colistin and Imipenem. Biofilm formation were detection by using Microtiter plate method, were results includes out of 23 isolates, three (13%) were formed as weak biofilm, seven (30.4 %) were developed as moderate biofilm, whereas eleven (47.8%) were constituted as strong biofilm, while only two (8.6%) was unable to form biofilm.

Keywords: *Pseudomonas aeruginosa*; multidrug Resistance; Hospitals.

Corresponding author: (Email: ramiabdalkareem85@gmail.com).

Introduction

Pseudomonas aeruginosa is Gram-negative bacterium, rod-shaped, an opportunistic pathogen responsible for serious infections and nosocomial outbreaks world-wide. Its produces an extracellular biofilm matrix that consists of nucleic acids, lipid vesicles, exopolysaccharides, and proteins (1). *P. aeruginosa* can develop antimicrobial resistance either through acquisition of resistance genes on the plasmid or mutational processes that alter the expression and/or function of chromosomally encoded mechanisms. Both strategies for developing drug resistance can severely limit the therapeutic options for serious infections treatment (2).

Infections caused by multi-drug resistance (MDR) *P. aeruginosa* are associated with significant increases in morbidity, mortality, need for surgical

intervention double the length of hospitalization and overall cost of patient care (3). *P. aeruginosa* signifies an unusual phenomenon of antimicrobial resistance among pathogens since practically all known mechanisms of resistance are found in this organism including decreased outer membrane permeability, penicillin binding protein modification, increased expression of efflux pumps system, alginate and enzymatic inactivation of antibiotics (4).

Pseudomonas aeruginosa have more than virulence factor one of the most dangerous is biofilm. The formation of biofilm begins with a reversible attachment of the planktonic cells followed by the adhesion to the surface, the bacteria then form a monolayer and irreversibly attach by producing an extracellular matrix. Next, a micro colony is formed where multilayers appear. During later stages, the biofilm is mature, forming characteristic "mushroom" structures due the polysaccharides. Finally, some cells start to detach and the biofilm will disperse (5,6).

The aim of study is isolation and identification of multi-drugs resistance of *P. aeruginosa* from clinical and environmental samples.

Materials and Methods

Specimens Collection

Through the period from October 2019 to March 2020, one hundred different clinical specimens were collected from patients referring several hospitals in Baghdad. Furthermore, fifty environmental samples were collected from the same hospitals.

The collected specimens were burn swab (n =41), wound swab (n =21), ear swab (n =10), mid-stream urine (n =12), Broncho alveolar lavage (BAL) (n =9), sputum (n =7), and environmental samples were operating room (n =8), emergency tools (n=7), hospital floor (n=13), patient bed (n=10), patient room (n=9), cleaning water (n=3).

Bacterial Isolation

All clinical and environmental samples were cultured by streaking on Blood agar, MacConkey agar and Cetrimide agar, incubated for 24 hours at 37ºC. This isolates may belong to *P. aeruginosa* growth on Cetrimide agar which is the selective media for *P. aeruginosa*.

Bacterial Identification

The colonies morphology on Blood agar, MacConkey agar and Cetrimide agar was depended initially to identify bacterial isolates, colony shape, texture, colour and edges were examined. In addition to macroscopic characteristics; microscopic examination of a gram-stained slide was examined under a light microscope with special regard towards cell shape and arrangement. Performing conventional biochemical tests and VITEK 2 compact system was depended to complete the identification.

Antibiotic susceptibility test

The susceptibility of *Pseudomonas aeruginosa* isolates to antibiotics (Amikacin, Cefepime, Ceftazidime, Ciprofloxacin, Colistin, Gentamicin, Imipenem, Meropenem, Piperacilin, Ticarcillin, Ticarcillin/Clavulanic Acid and Tobramycin) were tested by using

VITEK 2 Compact system Gram Negative Susceptibility using software version 5.01 and AST-GN76 (*Pseudomonas aeruginosa*) cards according to the manufacturer's instructions.

Microtiter plate method

The microtiter plate method is based on crystal violet staining and measuring of optical density (OD), to estimate biofilm intensity, absorbance was determined at 630nm by ELISA reader. The absorbance values represented the intensity of the biofilm thickness that formed by the studied isolates on the surface of microtiter well. The obtained results were categorized into four groups (non-biofilm producer, weak, moderate, and strong), after calculating the biofilm formation capacity for all tested isolates and negative controls, the cut-off value (ODc) was established. It is defined as three standard deviations (SD) above the mean OD of the negative control: ODc value was calculated for each microtiter plate separately. When a negative value was obtained, it presented as zero, while any positive value was an indicator for biofilm production (7).

Results and Discussion

Isolation and Identification of *Pseudomonas aeruginosa*

Out of One hundred and fifty clinical and environmental samples were analyzed for the presence of *P. aeruginosa*, the results of identification revealed the detection of fifty four isolates of *Pseudomonas* spp.

Only 54 isolates (36%) were able to grow on cetrimide agar at which is the selective media for *Pseudomonas* spp., the colony characters appears on cetrimide agar plates was mucoid, smooth in shape with flat edges and elevated center, have fruity odor and creamy color, as in Figure (1).

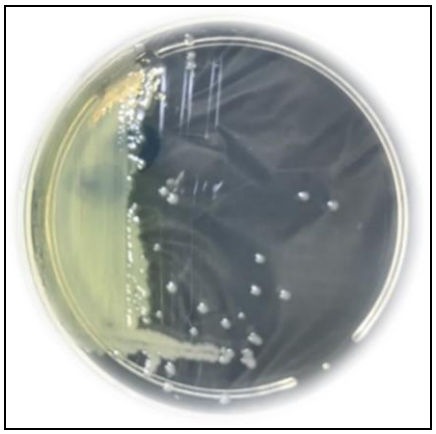

Figure (1): *Pseudomonas aeruginosa* colonies on Cetrimide agar.

Biochemical tests

Some biochemical tests were performed for more validation, showed 46 isolates of *P. aeruginosa* provided by some biochemical tests, results showed positive results for oxidase test, catalase test and production of B-hemolysis on blood agar, negative to Gram's stain and capable of growing on

cetrimide agar as blue greenish colonies (at 37˚C for 24 hrs.).

All isolates were positive for oxidase, which is considered as an important diagnostic test for *P. aeruginosa*. This enzyme activates an electron transport among donor bacteria and pigment, which is reduced to dark purple color due to the ability to produce cytochrome oxidase (that oxidase tetramethyl-p-phenylenediamine). Moreover, all isolates were positive for catalase test due to the ability to produce catalase enzyme (that reduce hydrogen peroxide to water and oxygen gas bubbles) (8).

Only 46 isolates were capable of growing on cetrimide agar (at 42˚C for 24 hrs.), the growth at 42C is considered an important diagnostic characteristic for *P. aeruginosa* among other species of *Pseudomonas* genus, like *P. putida* and *P. fluorescences* that grow at 4˚C, but not 42˚C (9).

Antibiotic susceptibility test

Antibiotic susceptibility test were performed to twenty *Pseudomonas aeruginosa* isolates by using VITEK 2 Compact system and the results reveled on figure (2).

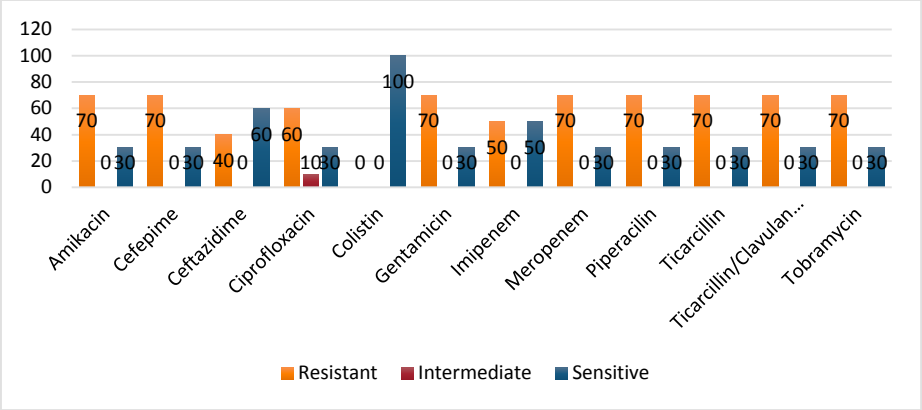

Figure (2): Multidrug Resistance Pattern of *P. aeruginosa* Isolates.

The result revealed the same susceptibility pattern of *P. aeruginosa* isolates toward Amikacin, Cefepime, Gentamicin, Meropenem, Piperacilin, Ticarcillin, Ticarcillin/Clavulanic Acid and Tobramycin with 70% and 30% of the isolates were resistant and sensitive respectively. The highest sensitivity percentage was found toward Colistin in which all the isolates were sensitive. Moreover, with Ceftazidime 60% of the isolates were sensitive while only 40% isolates were resistance. However, 50% of the tested isolates were resistance to Imipenem and the same percentage were sensitive to the same antibiotics.

The result of Ciprofloxacin resistance (60%) in current study, was in agreement with previous local study (61.3%) reported by Othman *et al.* (2014) (10). Likewise, agreement was achieved with another previous local study (58.13%) obtained by Abdulammer *et al.* (2018) (11). Also there was an agreement with international studies that showed higher resistant rates. Among them, by Javiya *et al.* (2008) (12) recorded (69.64%), and (64%) obtained by Nouri *et al.* (2016) (13). On contrary, the result of current study came to disagree with previous researchers who stated that

only 29 % Nader *et al*. (2017) (14) and 28.9% Al-Marjani *et al*. (2014) (15) of *P. aeruginosa* isolates were resistant to Ciprofloxacin. Moreover, (35%) were resistance in local study reported by Dakhl *et al*. (2016) (16). While, the resistance against aminoglycosides (Amikacin, Gentamicin and tobramycin) in current study (70%) was higher than those of a local study (46.96%) carried out by Nader *et al*. (2017) (14). Likewise, it goes along with a Brazilian study obtained by Araujo *et al*. (2016) (17). While, the result of Imipenem and Meropenem was 50% and 70% respectively in the present study is higher than the result of a local study 18.18% obtained by Nader *et al*. (2017) (14).

In regarding to cefepime and ceftazidime, the present findings had lower resistance (70%) and (40%) respectively than Nader *et al*. (2017) (14) percentage of (81.81%). In the current study Colistin therapy was highly effective with 100% sensitivity. On contrary, local study reported that 22.4% of *P. aeruginosa* isolates were resistance by Shehab *et al*. (2019) (18). *P. aeruginosa* is considered as a major cause of hospital-acquired infections due to its high antibacterial resistance by Emami *et al*. (2015) (19). This ability is either normal or acquired through mutations in their genetic material or through the horizontal transference of genes by Saderi *et al*. (2015) (20).

The results of present study were compatible with other local studies. A study reported by Shilba *et al*. (2015) (21) established that the highest resistance was against gentamicin; whereas another study found that the resistance of *P. aeruginosa* isolates to gentamicin was (100%) by Al-Daraghi *et al*. (2020) (22). Moreover, a percentage of (61.3%) was recorded towards ciprofloxacin by Othman *et al*. (2014) (10).

An agreement was achieved with other international studies as well. About (65%) of isolates were susceptible to ciprofloxacin by Grillon *et al*. (2016) (23). Also (46.96%) and (34.84%) were reached against gentamicin and ciprofloxacin, respectively by Nader *et al*. (2017) (14). Furthermore, The obtained Ceftazidime resistance in the current study was higher when compared to the results of Gailienė *et al*. (2007) (24). Also, the results of the present study disagree with a study done in Jordan by Al Dawodeyah *et al*. (2018) (1). Likewise, the results of resistance for amikacin antibiotics, which belong to aminoglycosides class, show that (70 %) of isolates were resistant. This result agrees with a study indicated that the resistance percentage to amikacin was (70.5%) by Al-Sheikhly *et al*. (2020) (25). And with another study indicated that the resistance percentage to amikacin was (75%) by Al-Salihi *et al*. (2014) (26). This result disagrees separately with two studies reported the similar percentage of resistance (18%) by Al-Taai *et al*. (2016) (27), Sharma *et al*. (2016) (28).

Aminoglycosides, such as gentamicin are multifunctional hydrophilic sugars that possess several amino and hydroxyl functionalities that are able to inhibit prokaryotic protein synthesis in bacterial cell by binding to 30S subunit of the ribosome, and changing the conformation of the A site to one that resembles the one induced by interaction between cognate tRNA and mRNA by Juayang *et al*. (2017) (29).

The primary resistance mechanism in *Pseudomonas* spp. against aminoglycoside antibiotics is enzymatic modification by aminoglycoside modifying enzymes that are divided into acetyl-transferases, phosphor transferases, and nucleotidyl transferases by Cox *et al*. (2015) (30). To overcome this problem, new semisynthetic aminoglycosides were developed in the 70s. The most widely used semi synthetic aminoglycoside is amikacin, which is refractory to most aminoglycoside modifying enzymes by Ramirez *et al*. (2017) (31). In addition, this resistance to amikacin is due to abundant secretion of alginate by bacteria, which are associated with the positive charge of antibiotics that belong to aminoglycosides class and prevents its spread within the cell by Lambert *et al*. (2002) (32).

On the other hand, the current results showed less resistance percentage, (53.34%) and (36.67%), against imipenem and ceftazidime, respectively. The result of imipenem in current study agree to some extent with a local study carried out in Karbala province by (21), she reported (60%) resistance. On contrary, by Al-Dulami *et al*. (2017) (33) demonstrated a much lower resistance (9.8%) against imipenem. While, in this study, *P. aeruginosa* isolates have revealed resistance (40%) for the third generation of cephalosporin, ceftazidime. This result was incompatible with the finding of local study (51.67%) by Shilba *et al*. (2015) (21). Similarly, by Al-Doory *et al*. (2012) (34) reported that (61.6%) of his isolates were resistant to ceftazidime, alongside with by Al-Dulami *et al*. (2017) (33) who reported a percentage of (68.6%). The reason behind the bacterial resistance to these antibiotics is due to the production of β-lactamase enzymes which act to destroyed β-lactams ring thereby leads to modification of antibiotics structure and spoilage their effects by Boehr *et al*. (2003) (35).

Microtiter plate method

In this study the absorbance values represented the intensity of the biofilm thickness that formed by the studied isolates on the surface of microtiter well. The obtained results were categorized into four groups (non-biofilm producer, weak, moderate, and strong). The results are summarized in Table (1).

Table (1): The percentage of *P. aeruginosa* biofilm intensity based on source of isolate.

Samples (No. of Isolates)	Non-biofilm producer	Weak (%)	Moderate (%)	Strong (%)
Burn (8)	0 (0%)	1 (12.5%)	2 (25%)	5 (62.5%)
Wound (5)	1 (20%)	1 (20%)	1 (20%)	2 (40%)
Mid-Stream Urine (3)	0 (0%)	1 (33.3%)	1 (33.3%)	1 (33.3%)
Bronchoalveolar lavage (2)	0 (0%)	0 (0%)	1 (50%)	1 (50%)
Ear (2)	1 (50%)	0 (0%)	1 (50%)	0 (0%)
Sputum (1)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Environmental (2)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Total (23)	2 (8.6%)	3 (13%)	7 (30.4%)	11 (47.8%)

According to the above results listed in Table (1), this study includes out of 23 isolates, three (13%) were formed as weak biofilm, seven (30.4 %) were developed as moderate biofilm, whereas eleven (47.8%) were

constituted as strong biofilm, while only two (8.6%) was unable to form biofilm.

The differences in biofilm formation among isolates might be owing to several reasons, differences of isolates capacity to form biofilm or perhaps differences in primary number of cells that succeeded in adherence and differences of quality and quantity of quorum sensing signaling molecules (auto inducers) that produced from each isolate to play important roles. A part form moderate biofilm, there is no specific pattern governs the distribution of biofilm intensity among specimens. I.e. each biofilm intensity is a specimen-specific. Perhaps the reason behind such findings is the variation in genetic makeup of each strain by EA Abd Al Rhman et al. (2018) (36).

In local study in Baghdad province\ Iraq Included that the differences in biofilm thickness, 25 isolates distributed as follows (burn 7, mid-Stream Urine 7, wound 5, bronchoalveolar lavage 2, sputum 3 and ear 1) the results showed three (12 %) as weak biofilm, fourteen (56%) as moderate biofilm, whereas seven (28%) as strong biofilm, while only one (4%) was unable to form biofilm by Al-Sheikhly et al. (2020) (25).

Conclusion

1. The high prevalence of *P. aeruginosa* was located in burn followed by wound.
2. The highest frequency resistance was against aminoglycosides (Amikacin & Gentamicin) and fluoroquinolones (Ciprofloxacin) while the less resistance was against Colistin followed by Ceftazidime, then Imipenem.
3. Biofilm formation is variable in terms of density, most of it is strong, followed by medium and then weak and not forming the biofilm.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both health and environmental and higher education and scientific research ministries in Iraq

Conflict of Interest

The author’s declare that they have no conflict of interest.

Funding: Self-funding.

References

1. Al Dawodeyah, H. Y.; Obeidat, N.; Abu-Qatouseh, L. F. and Shehabi, A. A. (2018). Antimicrobial resistance and putative virulence genes of *Pseudomonas aeruginosa* isolates from patients with respiratory tract infection. *Germs*. 8: 31.
2. Streeter, K. and Katouli, M. (2016). *Pseudomonas aeruginosa*: A review of their Pathogenesis and Prevalence in Clinical Settings and the Environment.
3. Bassetti, M.; Vena, A.; Croxatto, A.; Righi, E. and Guery, B. (2018). How to manage *Pseudomonas aeruginosa* infections. *Drugs in context*. 7: 1-18.
4. Hall, C. W.; Hinz, A. J.; Gagnon, L. B.P.; Zhang, L.; Nadeau, J.P.; Copeland, S., *et al.* (2018). *Pseudomonas aeruginosa* biofilm antibiotic resistance gene *ndvB* expression requires the RpoS stationary-phase sigma factor. *Applied and environmental microbiology*. 84: 1-18.
5. Kariminik, A.; B., Majid and Babak, K. (2017). *Pseudomonas aeruginosa* quorum sensing modulates immune responses: an updated review article. *Immunology letters*. 190: 1-6.
6. Negm, N. A.; Ismail, D. A.; Sahar, A. and Abou Kana, M. T. (2019). 7 Adhesion of

Bacteria to Solid Surfaces. *Surfactants in Tribology*, Volume 6.

7. Atshan, S. S.; Nor Shamsudin, M.; Sekawi, Z.; Than Thian Lung, L.; Hamat, R. A.; Karunanidhi, A., *et al*. (2012). Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. *Journal of Biomedicine and Biotechnology*. 2012: 1-12.
8. Shila, S.J.; Ahmed, N.N. and Dastogeer; K.M.G. (2013). Detection of *Pseudomonas syringae* pv. lachrymans associated with the seeds of cucurbits. *Universal Journal of Agricultural Research*. 1: 1-8.
9. Tang, Y. and Stratton, C.W. (2006). Advanced Techniques in Diagnostic Microbiology Springer Science and Business Media, LLC. *Printed in the United States of America*. (TB/EB). 9: 7.
10. Othman, N.; Babakir-Mina, M.; Noori, C. K. and Rashid, P. Y. (2014). *Pseudomonas aeruginosa* infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates. *The Journal of Infection in Developing Countries*. 8: 1498-1502.
11. Abdulammer, H. (2018). Detection of quinolones chromosomal resistance in *Pseudomonas aeruginosa* isolated from wounds infection. MSc. thesis in microbiology. Biology department. College of Science. Baghdad.
12. Javiya, V. A.; Ghatak, S. B; Patel, K. R. and Patel, J. A. (2008). Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology*. 40: 230.
13. Nouri, R.; Rezaee, M. A.; Hasani, A.; Aghazadeh, M. and Asgharzadeh, M. (2016). The role of *gyrA* and *parC* mutations in fluoroquinolones-*resistant Pseudomonas aeruginosa* isolates from Iran. *brazilian journal of microbiology*. 47: 925-930.
14. Nader, M. I.; Kareem, A. A.; Rasheed, M. N. and Issa, M. A. (2017). Biofilm Formation and Detection of *pslÁ* Gene in Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Thi-Qar, Iraq. *Iraqi journal of biotechnology*. 16: 89-103.
15. Al-Marjani, M. F. (2014). Presence of qnr gene in environmental and clinical *Pseudomonas aeruginosa* isolates in Baghdad. *International J Microbiology and Applied sci Issn*. 3: 853-857.
16. Dakhl, Z.F. and Alwan, S.K. (2016). Detection of some genes resistance to quinolones in *Pseudomonas aeruginosa* isolated from different clinical sources in Al-Diwaniya. *Ameri. J. BioMedicine. AJBM*, 4(10):427-434.
17. Araujo, B. F.; Ferreira, M. L.; de Campos, P. A.; Royer, S.; Deivid Batistão, W., *et al*. (2016). Clinical and molecular epidemiology of multidrug-resistant *P. aeruginosa* carrying aac (6')-Ib-cr, qnrS1 and blaSPM Genes in Brazil. *PloS one*. 11: 23-46.
18. Shehab, S. K. and Jassim, E. H. (2019). Impact of Mint oil and Colistin antibiotic on *pilB* gene of clinical *Pseudomonas aeruginosa* Isolates from Baghdad, Iraq. *Iraqi journal of biotechnology*. 18: 43-54.
19. Emami, S.; Nikokar, I.; Ghasemi, Y.; Ebrahimpour, M.; Sedigh, H.; Araghian, A., *et al*. (2015). Antibiotic resistance pattern and distribution of *pslA* gene among biofilm producing *Pseudomonas aeruginosa* isolated from waste water of a burn center. *Jundishapur journal of microbiology*. 8: 1-5.
20. Saderi, Horieh and Owlia, Parviz. (2015). Detection of multidrug resistant (MDR) and extremely drug resistant (XDR) *P. aeruginosa* isolated from patients in Tehran, Iran. *Iranian journal of pathology*. 10: 265.
21. Shilba, A. A.; Al-Azzawi, R. H. and Al-Awadi, S. J. (2015). Dissemination of Carbapenem Resistant Pseudomonas aeruginosa among Burn Patients in Karbala Province Iraq. *Iraqi Journal of Science*, 56: 1850-1857.
22. Al-Daraghi, W. A. H. and Al-Badrwi, M. S. A. (2020). Molecular Detection for Nosocomial Pseudomonas aeruginosa and its Relationship with multidrug Resistance, Isolated from Hospitals Environment. *Medico Legal Update*. 20: 631-636.
23. Grillon, A.; Schramm, F.; Kleinberg, M. and Jehl, F. (2016). Comparative activity of ciprofloxacin, levofloxacin and moxifloxacin against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* assessed by minimum inhibitory concentrations and time-kill studies. *PloS one*. 11: 1-10.

24. Gailienė, G.; Pavilonis, A. and Kareivienė, V. (2007). The peculiarities of *Pseudomonas aeruginosa* resistance to antibiotics and prevalence of serogroups. *Medicina*, 43: 36.
25. Al-Sheikhly, M. A.; Musleh, L. N. and Al-Mathkhury, H. J. (2020). Gene Expression of *pelA* and *pslA* in *Pseudomonas aeruginosa* under Gentamicin Stress. *Iraqi Journal of Science*. 295-305.
26. Al-Salihi, S. S.; Hameed, B. H. and Hameed, B. H. (2014). Antibiosis resistant of *Pseudomonas aeruginosa* isolated from different clinical specimens. *kirkuk university journal for scientific studies*. 9: 15-28.
27. Al-Taai, H. R. R. (2016). Bacteriological Comparison Between *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* Isolated from Different Infectious Sources. *Diyala Journal For Pure Science*. 12: 162-182.
28. Sharma, S. and Srivastava, P. (2016). Resistance of antimicrobial in *Pseudomonas aeruginosa*. *Int J Curr Microbiol App Sci*. 5: 121-128.
29. Juayang, A. C.; Lim, C. P. and Gallega, C. T. (2017). Five-year antimicrobial susceptibility of *Pseudomonas aeruginosa* from a local tertiary hospital in Bacolod City, Philippines. *Tropical medicine and infectious disease*. 2: 28.
30. Cox, G.; Stogios, P.J.; Savchenko; A. and Wright, G. D. (2015). Structural and molecular basis for resistance to aminoglycoside antibiotics by the adenylyltransferase ANT (2″)-Ia. *MBio*. 6: e02180-02114.
31. Ramirez, M. S. and Tolmasky, M. E. (2017). Amikacin: uses, resistance, and prospects for inhibition. *Molecules*, 22: 2267.
32. Lambert, P. (2002). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of the royal society of medicine*. 95: 22.
33. Al-Dulami, H.F.L. (2017). Assessment the Effect of Quorum Sensing genes (*lasI*, *rhlI*) and Some Plant Extracts on Some Virulence Factors of *Pseudomonas aeruginosa* Isolated from Different Clinical Sources. MSc. Thesis. Department of Biology. College of Science. University of Anbar, Iraq.
34. Al-Doory, I. A. H. (2012). A diagnostic study of *Pseudomonas aeruginosa* isolated from contaminated burns and wounds using cultural and molecular methods. M. Sc. Thesis. College of Science for Women, University of Baghdad, Iraq.
35. Boehr, D. D.; Jenkins, S. I. and Wright, G. D. (2003). The Molecular Basis of the Expansive Substrate Specificity of the Antibiotic Resistance Enzyme Aminoglycoside Acetyltransferase-6′-Aminoglycoside Phosphotransferase-2" The Role of ASP-99 as an Active Site Base Important for Acetyl Transfer. *Journal of Biological Chemistry*. 278: 12873-12880.
36. Abd Al Rhman E. A.; Abd El Haliem, N. F.; Hassan, E. M.; Al Seadawy, L. I.; El Fouhil, D. F. and Asheiba, Z. F. (2018). Phenotypic and Genotypic Characterization of *Staphlococcal* Biofilm Formation from Neonatal Infection Isolates. *The Egyption Journal of Medical Microbiology*. 2: 1-9.