

Phenotypic Discrimination of Antibiotic Resistance Among *P. Aeruginosa* Collected from Burn Sample

Raghad A. Ridin

1Department of Quality Assurance &Academic Performance.

2 Presidency /University of Thi Qar, Iraq.

adilraghad59@gmail.com

Abstract

Pseudomonas aeruginosa is characterized by that it is associated with many morbidities and deaths for people with immunodeficiency, as well as people who visit hospitals frequently and patients with burns, especially severe ones, because the bacteria are Gram-negative, opportunistic, and cause severe infections. The study included 45 samples that isolated the advanced *aeruginosa* bacteria from moderately burned men and women, their ages ranged between 12 to 60 years. The purpose of the study was to investigate the susceptibility of *P. aeruginosa* to resistance to some antibiotics for the purpose of controlling the incidence of wound infection in patients with burns. The phenotypic pattern of the bacteria under study shows that there is severe resistance to some antibiotics, while the bacteria are unable to resist some other antibiotics.

Introduction

Pseudomonas aeruginosa is characterized by that it is associated with many morbidities and deaths for people with immunodeficiency, as well as people who visit hospitals frequently and patients with burns, especially severe ones, because the bacteria are Gram-negative, opportunistic, and cause severe infections (1,2,3). This type of micro-organisms has the ability to withstand harsh growth conditions represented by few nutrients and high humidity, in addition to its ability to resist many antibiotics and sterilizers, and this explains the large number of its presence in medical equipment and devices and the keyboard of computers as well as in medicines and medical preparations (4,5). *Pseudomonas aeruginosa* in the burn center is considered one of the most common bacteria that causes transmissible diseases (nosocomial), and clinical isolates and isolates from the hospital environment contribute greatly to the spread of resistance against antibiotics as well as the ability to form biofilms (6). Therefore, it has become a source of great concern in all medical societies of the world, as it has caused the emergence of new resistant strains as a result

of the excessive use of antibiotic treatments in the burn halls. This is one of the reasons why the Nosocomial Infection made *Pseudomonas aeruginosa* use various means that make them able to resist the large variety of antibiotics, as they contain multiple enzymes that work to inhibit the action of antibiotics such as carbapenems and also beta-lactams (7,8). *P. aeruginosa* depends on several virulence factors for its pathogenesis (9,10,11). in addition to its ability to form biofilms. These biofilms reduce the efficiency of treatment against bacteria, as it helps in chronic infection (12). The presence of biofilms keeps these bacteria from drying out and helps them stick to the membranes, which reduces the effect of the body's immune defense mechanisms (13). There are several reasons for the spread of *P. aeruginosa*, including the contamination of the environment of the patient lying in the hospital, where he can become infected through the medical staff infected with this type of bacteria or through infected visitors, and the hospital environment may contain strains that are multiple drug resistance(MDR) and thus be a source of crosstalk for the spread of infection (14,15). The incidence of *P. aeruginosa* in patients with burns, especially severe ones, ranges from acute to chronic injury (16) and the percentage in which these bacteria spread ranges from 33% in all types of burns to 59% in burns of high severity and the difficulty and sensitivity of patients with severe burns makes the process of treating them difficult, which results in them having great complications (17). Therefore, *P. aeruginosa* infection has been reported in many hospitals (18,19). Patients with moderate and severe burns and those suffering from *P. aeruginosa* infection are considered a special cases illness because this type of bacteria has the ability to acquire resistance to many classes of drugs, although these antibiotics are effective against many other bacterial species (20) Because it has developed itself for high resistance against efficient classes of antibiotics (24,25). As they use defensive methods that include changing the target sites for drug action or lowering the concentration of the antibiotic or by applying the antibiotic by the action of the enzymes they use as virulence factors (23). Therefore, *P. aeruginosa* is known as an MDR for one or more types of antibiotics that are usually used against it (25). Moreover, the multiple drug resistance is caused by many factors, including the occurrence of genetic mutations in the genetic structure of bacteria or increased efflux, as well as horizontal gene transfer, which leads to limiting the action of the antigens used intravenously, such as imipenem and aminoglycosides (26,27).

Materials and Methods

Diagnosis of *P. aeruginosa*

The current study includes 60 samples of patients suffering from moderate and severe burns lying in the burn halls, and they have signs of sores and wounds. Swabs were taken from these prominent wounds using TSP transport medium, and then the nutrient media was inoculated such as blood agar and McConkey agar within two hours from taking swabs for the purpose of primary isolation, the *P. aeruginosa* bacteria were then placed in an incubator at a temperature of 37° C for 16 to 24 hours after the incubation process. Standard methods were used in bacteriology for the purpose of

distinguishing bacteria, then the bacteria were cultured in new cultures to obtain high purity of the bacterial culture, and then a test for sensitivity to antibiotics was conducted (22).

Susceptibility to antibiotics test

The antibiotic sensitivity test was conducted according to what was agreed upon in the Clinical Laboratory Standards Institute, (CLSI) (21). The use of antibiotic tablets is following a company.

Result

P. aeruginosa bacteria were isolated from 45 patients out of a total of 60 patients with moderate or severe burns lying in the burn hall. A sample consisted of 30 males and 15 females, their ages ranged from 12 to 60 years. Resistance to antibiotics ranged from highest to lowest resistance as follows: Timentin (ticarcillin/clavulanate), meropenem (MEM), ceftazidime (CAZ), imipenem (IMP), amikacin (AMK), Colistin and polymyxin B while the sensitivity to antibiotics was ranked from highest sensitivity to lowest sensitivity as follows: polymyxin B (PB), Colistin, amikacin, imipenem, ceftazidime, meropenem and Timentin as in table (1).

Table (1): resistance and sensitivity of *p. aeruginosa* to some antimicrobial agents.

Type Of Antibiotic	Number Of Resistant Samples / %	Number Of Sensitive Samples / %
Timentin	30 (66.6 %)	15 (33.3 %)
Meropenem	28 (62.2 %)	17 (37.7 %)
Ceftazidime	25 (55.5 %)	20 (44.4 %)
Imipenem	22 (48.8 %)	23 (51.1 %)
Amikacin	16 (35.5 %)	29 (64.4 %)
Colistin	2 (4.4%)	43 (95.5 %)
Polymyxin B	Zero(0 %)	45 (100 %)
Total	45 Samples	45 Samples

Discussion

Increased rates of mortality and health-acquired morbidity are strongly correlated with *P. aeruginosa* (28). This danger lies in the fact that this causative agent is the source of infection in patients with burns this is because of its effective ability to form a biofilm, which helps it to resist many antibiotics, which allows it to grow in very harsh conditions, despite sometimes the use of

antibiotics against it or its resistance to the surrounding conditions. The process of biofilm formation is one of the most important virulence factors possessed by *Pseudomonas aeruginosa* (29,30). There is a significant difference between the biofilm formation process and the MDR phenotype (31,32,33). Recent studies have recorded that the MDR phenotype of *P. aeruginosa* bacteria ranges from 4 to 60% of the infection rate that spreads in hospitals around the world (34,35). The current study is consistent with () which identified a group of antibiotics, including the study that *P. aeruginosa* is resistant to, as well as the high sensitivity of polymyxin, indicating that bacteria cannot resist this type of antibiotic (36). *P. aeruginosa* deactivates carbapenems and beta-lactams, by using ESBL (extended spectrum beta lactamases) and MBLs (metallo- β -lactamases), which are defense mechanisms used by bacteria that are resistant to many antibiotics that are considered to be one of the important means in the process of resistance against these antibiotics (8). Another innate defense used by *P. aeruginosa* is its expression of a multi-therapeutic efflux system due to reduced permeability of the outer membrane in bacteria. The chromosomal factors as well as the plasmid increase the resistance of *P. aeruginosa* to antibiotics with high efficiency, such as imipenem and ceftazidime, while it was found that the bacteria have low or may not be resistant to some antibiotics such as (37). The lower respiratory tract is one of the most common sources of *P. aeruginosa* infection, as well as the main reason for the phenomenon of multidrug resistance and recurrent bacteremia, especially when the source of infection is wounds. Thus, the incidence of resistance in some strains against the antibiotic ceftazidime is related to resistance to the antibiotic gentamicin, while resistance of some strains of *P. aeruginosa* to meropenem is associated with resistance to the antibiotic amikacin (38).

References

1. Maroui, I., Barguigua, A., Aboukacem, A., Elhafa, H., Ouarrak, K., Sbiti, M., ... & Belhaj, A. (2017). Clonal analysis of clinical and environmental *Pseudomonas aeruginosa* isolates from Meknes region, Morocco. *Polish Journal of Microbiology*, 66(3).
2. Schaber, J. A., Hammond, A., Carty, N. L., Williams, S. C., Colmer-Hamood, J. A., Burrowes, B. H., ... & Hamood, A. N. (2007). Diversity of biofilms produced by quorum-sensing-deficient clinical isolates of *Pseudomonas aeruginosa*. *Journal of medical microbiology*, 56(6), 738-748.
3. Alikhani, M. Y., Parsavash, S., Arabestani, M. R., & Hosseini, S. M. (2017). Prevalence of antibiotic resistance and class 1 integrons in clinical and environmental isolates of *Pseudomonas aeruginosa*. *Avicenna Journal of Clinical Microbiology and Infection*, 4(4), 12086-12086.
4. Davane, M., Suryawanshi, N., Pichare, A., & Nagoba, B. S. (2014). *Pseudomonas aeruginosa* from hospital environment. *Journal of Microbiology and Infectious Diseases*, 4(01).
5. de Abreu, P. M., Farias, P. G., Paiva, G. S., Almeida, A. M., & Morais, P. V. (2014). Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. *BMC microbiology*, 14(1), 1-10.

6. **Karami P., Mohajeri P., Mashouf R. Y., Karami M., Yaghoobi M. H., Dastan D., Alikhani M. Y. (2019).** Molecular characterization of clinical and environmental *Pseudomonas aeruginosa* isolated in a burn center, Saudi Journal of Biological Sciences, Volume 26, Issue 7, Pages 1731-1736.
7. **Hassuna, N. A., Mohamed, A. H. I., Abo-Eluoon, S. M., & Rizk, H. A. W. A. (2015).** High prevalence of multidrug resistant *Pseudomonas aeru.* Archives of Clinical Microbiology, 6(4).
8. **Karami, P., Bazmamoun, H., Sedighi, I., Nejad, A. S. M., Aslani, M. M., & Alikhani, M. Y. (2017).** Antibacterial resistance patterns of extended spectrum β -lactamase-producing enteropathogenic *Escherichia coli* strains isolated from children. Arab Journal of Gastroenterology, 18(4), 206-209.
9. **Schaber, J. A., Hammond, A., Carty, N. L., Williams, S. C., Colmer-Hamood, J. A., Burrowes, B. H., ... & Hamood, A. N. (2007).** Diversity of biofilms produced by quorum-sensing-deficient clinical isolates of *Pseudomonas aeruginosa*. Journal of medical microbiology, 56(6), 738-748.
10. **Alikhani, M. Y., Tabar, Z. K., Mihani, F., Kalantar, E., Karami, P., Sadeghi, M., ... & Farajnia, S. (2014).** Antimicrobial resistance patterns and prevalence of blaPER-1 and blaVEB-1 genes among ESBL-producing *Pseudomonas aeruginosa* isolates in West of Iran. Jundishapur Journal of Microbiology, 7(1).
11. **Safari, M., Alikhani, M. Y., Arabestani, M. R., Kakhki, R. K., & Jafari, R. (2014).** Prevalence of Metallo- β -lactamases encoding genes among *pseudomonas aeruginosa* strains isolated from the bedridden patients in the intensive care units. Avicenna Journal of Clinical Microbiology and Infection, 1(1), 19216-19216.
12. **Rasamiravaka, T., Labtani, Q., Duez, P., & El Jaziri, M. (2015).** The formation of biofilms by *Pseudomonas aeruginosa*: a review of the natural and synthetic compounds interfering with control mechanisms. BioMed research international, 2015.
13. **Rasamiravaka, T., Vandeputte, O. M., Pottier, L., Huet, J., Rabemanantsoa, C., Kiendrebeogo, M., ... & El Jaziri, M. (2015).** *Pseudomonas aeruginosa* biofilm formation and persistence, along with the production of quorum sensing-dependent virulence factors, are disrupted by a triterpenoid coumarate ester isolated from *Dalbergia trichocarpa*, a tropical legume. PloS one, 10(7), e0132791.
14. **Chemaly, R. F., Simmons, S., Dale Jr, C., Ghantaji, S. S., Rodriguez, M., Gubb, J., ... & Stibich, M. (2014).** The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment. Therapeutic advances in infectious disease, 2(3-4), 79-90.
15. **Oliveira, A. C. D., & Damasceno, Q. S. (2010).** Surfaces of the hospital environment as possible deposits of resistant bacteria: a review. Revista da Escola de Enfermagem da USP, 44(4), 1118-1123.
16. **Sadikot RT, Blackwell TS, Christman JW, Prince AS.(2005).** Pathogen–host interactions in *Pseudomonas aeruginosa* pneumonia. Am J Respir Crit Care Med.;171(11):1209–1223.
17. **Lister PD, Wolter DJ, Hanson ND. (2009).** Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev.;22(4):582–610.
18. **Pitten, F. A., Panzig, B., Schröder, G., Tietze, K., & Kramer, A. (2001).** Transmission of a multiresistant *Pseudomonas aeruginosa* strain at a German University Hospital. Journal of Hospital Infection, 47(2), 125-130.

19. **Climo, M. W., Pastor, A., & Wong, E. S. (1997).** An outbreak of *Pseudomonas aeruginosa* related to contaminated urodynamic equipment. *Infection Control & Hospital Epidemiology*, 18(7), 509-510.
20. **Moolenaar, R. L., Crutcher, J. M., San Joaquin, V. H., Sewell, L. V., Hutwagner, L. C., Carson, L. A., ... & Jarvis, W. R. (2000).** A prolonged outbreak of *Pseudomonas aeruginosa* in a neonatal intensive care unit did staff fingernails play a role in disease transmission?. *Infection Control & Hospital Epidemiology*, 21(2), 80-85.
21. **Hassuna, N. A., Mohamed, A. H. I., Abo-Eluoon, S. M., & Rizk, H. A. W. A. (2015).** High prevalence of multidrug resistant *Pseudomonas aeruginosa*. *Archives of Clinical Microbiology*, 6(4).
22. **luit, A. C., J. Verhoef, F. J. Schmitz, and The European SENTRY Participants (2001).** Frequency of isolation and antimicrobial resistance of gram-negative and gram-positive bacteria from patients in intensive care units of 25 European university hospitals participating in the European arm of the SENTRY Antimicrobial Surveillance Program 1997-1998. *Eur. J. Clin. Microbiol. Infect. Dis.*20:617-625.
23. **Livermore, D. M.2002.** Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin. Infect. Dis.*34:634-640.
24. **Stanković-Nedeljković, N., Todorović, B., Kocić, B., Ćirić, V., Milojković, M., & Waisi, H. (2015).** *Pseudomonas aeruginosa* serotypes and resistance to antibiotics from wound swabs. *Vojnosanitetski pregled*, 72(11), 996-1003.
25. **Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012).** Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, 18(3), 268-281.
26. **Jovicic, B., Lepsanovic, Z., Suljagic, V., Rackov, G., Begovic, J., Topisirovic, L., & Kojic, M. (2011).** Emergence of NDM-1 metallo- β -lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. *Antimicrobial agents and chemotherapy*, 55(8), 3929-3931.
27. **Sardelic, S., Bedenic, B., Colinson-Dupuich, C., Orhanovic, S., Bosnjak, Z., Plecko, V., ... & Rossolini, G. M. (2012).** Infrequent finding of metallo- β -lactamase VIM-2 in carbapenem-resistant *Pseudomonas aeruginosa* strains from Croatia. *Antimicrobial agents and chemotherapy*, 56(5), 2746-2749.
28. **Hassuna, N. A., Mohamed, A. H. I., Abo-Eluoon, S. M., & Rizk, H. A. W. A. (2015).** High prevalence of multidrug resistant *Pseudomonas aeruginosa*. *Archives of Clinical Microbiology*, 6(4).
29. **de Almeida, K. D. C. F., Calomino, M. A., Deutsch, G., de Castilho, S. R., de Paula, G. R., Esper, L. M. R., & Teixeira, L. A. (2017).** Molecular characterization of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated in a burn center. *Burns*, 43(1), 137-143.
30. **Taylor, P. K., Yeung, A. T., & Hancock, R. E. (2014).** Antibiotic resistance in *Pseudomonas aeruginosa* biofilms: towards the development of novel anti-biofilm therapies. *Journal of biotechnology*, 191, 121-130.
31. **Gurung, J., Khyriem, A. B., Banik, A., Lyngdoh, W. V., Choudhury, B., & Bhattacharyya, P. (2013).** Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. *Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine*, 17(4), 214.

- 32. Yekani, M., Memar, M. Y., Alizadeh, N., Safaei, N., & Ghotaslou, R. (2017).** Antibiotic resistance patterns of biofilm-forming *Pseudomonas aeruginosa* isolates from mechanically ventilated patients. *International Journal of Scientific Study*, 5(5), 1-5.
- 33. Karami, P., Mohajeri, P., Yousefi Mashouf, R., Karami, M., Yaghoobi, M. H., Dastan, D., & Alikhani, M. Y. (2019).** Molecular characterization of clinical and environmental *Pseudomonas aeruginosa* isolated in a burn center. *Saudi journal of biological sciences*, 26(7), 1731–1736.
- 34. Haeili, M., Ghodousi, A., Nomanpour, B., Omrani, M., & Feizabadi, M. M. (2013).** Drug resistance patterns of bacteria isolated from patients with nosocomial pneumonia at Tehran hospitals during 2009-2011. *The Journal of Infection in Developing Countries*, 7(04), 312-317.
- 35. Singh, A., Goering, R. V., Simjee, S., Foley, S. L., & Zervos, M. J. (2006).** Application of molecular techniques to the study of hospital infection. *Clinical microbiology reviews*, 19(3), 512-530.
- 36. Haeili, M., Ghodousi, A., Nomanpour, B., Omrani, M., & Feizabadi, M. M. (2013).** Drug resistance patterns of bacteria isolated from patients with nosocomial pneumonia at Tehran hospitals during 2009-2011. *The Journal of Infection in Developing Countries*, 7(04), 312-317.
- 37. Cunha BA. (2002).** *Pseudomonas aeruginosa*: resistance and therapy. *Semin Respir Infect*.
- 38. Dambrauskiene A, Adukauskiene D, Jeroch J, Vitkauskiene A. (2009).** *Pseudomonas aeruginosa* padermiu, sukelusiu bakteriemija, sasajos su infekcijos zidiniu ir ju atsparumas antibiotikams [Pseudomonas aeruginosa bacteremia: associations with a source of infection and antibiotic resistance]. *Medicina (Kaunas)*.;45(1):1-7. Lithuanian. PMID: 19223699.