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# Identification Of Metalloβ-Lactamses and Integron Genes In *Pseudomonas Aeruginosa* Isolated From Burn Injury Patients: Phenotype And Genotype

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***Abstract.****Pseudomonas aeruginosa, which generates metallo-β-lactamase (MBL), is the cause of infections linked to burn patients, and this is a growing global problem. The objectives of the recent study were to determine antibiotic susceptibility of P. aeruginosa isolates, the presence of MBLs genes, and Integron gene class-1 . One hundred burn patients at the Al-Hussein Teaching Hospital in Al Smawah, Iraq, were isolated clinically. Twenty (20%) of these were determined to be P. aeruginosa by 16sRNA and biochemical testing. To further identify the antibiotic sensitivity for each isolate was employed by Disc Diffusion Method . The phenotypic MBL production For detected isolates was evaluated by Combined Disc Diffusion Method (CDD). Integron gene class -1 (Int-1) and Genes encoding MBLs were identified by Polymerase Chain Reaction (PCR). P. aeruginosa was shown to be totally resistant to ceftriaxone, ampicillin, piperacillin, gentamicin, and ciprofloxacin in this investigation. 90% of the isolates were determined to be multidrug resistant, and different levels of moderate to lowest resistance were observed in Amikacin, Meropenem, Aztreonam, Cefixime, and Levofloxacin. While 90% of the isolates possessed the Int-1 gene, our recorded the absence of All MBLs genes (Bla $_{IMP}$, Bla $_{VIM}$, Bla $_{GIM}$ ) From All isolates under investigation. Illustrating for the first time how crucial it is to put in place appropriate infection control measures in order to give patients the best care possible and stop the development of these resistant microbes among burn patients.*

***Key words :****Multidrug resistant (MDR), Metallo-β lactamases genes , Integron class -1 , Burn infections .*

## 1. INTRODUCTION

Non-fermentative and gram-negative Particularly in moist environments, *Pseudomonas aeruginosa* has remarkable adaptability in settling in a variety of biological niches (1), It is a rod-shaped, 0.5 mm to1.0 mm broad, 0.5mm to 5 mm long, heterotrophic bacteria that doesn't produce spores. It can move because it has one or two polar flagella (2). It appears to be an elusive human pathogen that can cause a number of deadly illnesses. The greatest risk of morbidity and death associated with this nosocomial infection is for hospitalized patients. It poses a major risk to human health due of its capacity to adapt, endure, and build resistance to a range of antibiotics (3). Actually, *P. aeruginosa* is often seen in the respiratory tracts of people who have underlying medical conditions as well as injured body parts including burns, surgical wounds, or ocular traumas. Meningitis, septicemia, and necrosis can arise from it infiltrating the organism from these colonized sites (4). Because of their large surface area and prolonged hospital stays, burns are a more durable and plentiful source of infection than surgical wounds (5). They also provide an ideal habitat for bacterial multiplications. Although burn wound infections are no longer the leading reason of death for burn patients, burn wound

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sepsis is still a serious infectious consequence (6). Numerous antibiotics, including carbapenems, aminoglycosides, β-lactams, fluoroquinolones, and tetracyclines, are mostly used to treat infections. β-lactam antibiotics are essential in the treatment of infections *caused by P. aeruginosa* due to their proven safety and effectiveness. The most often utilized class of antibiotics globally for treating infections is the β-lactam antibiotics (7). Apart from its innate resistance to an extensive array of antimicrobial agents, this organism possesses the exceptional ability to develop resistance against widely used antimicrobial agents by the acquisition of resistant determinants horizontally or through the selection of chromosomal gene changes (9).

A different kinds of antibiotics, including β-lactams, aminoglycosides and quinolones have been found to be resistant to P. aeruginosa isolates, both clinically and epidemiologically (10). Enterobacteriaceae most frequently develop resistance by the hydrolytic , or "β-lactamases." production . These enzymes lysis the amide bond in β-lactam ring, which makes the drug inert so ends the treatment.

β-lactamases are categorized in a complex way depending upon their genetic composition, biochemical features , and substrate type β-lactamase inhibitor. β-lactamases are grouped depending upon molecular makeup into four classes i.e. A ,B,C and D. Class B β-lactamases are known as metallo—β-lactamases (MBLs)because they have one or two zinc ions in the active site which enhance the hydrolytic action (11). MBLs are inhibited by EDTA and thiolates (12). Several types of MβL genes have been found Based on amino acid sequence homology; among the most widely utilized and therapeutically significant class B enzymes are Imipenemase (IMP), New Delhi MBL (NDM), Verona integrin-encoded MBL (VIM),Germany Imipenemase (GIM) , and Sao Polo Imipenemase (SIM) (13). In order to confirm that clinical isolates have MBL genes, molecular methods are considered suitable screening tools. The situation is exacerbated by the broad dispersion of integrons and plasmids, which house the majority of MβL-encoding genes (14). Class-1(Int-1) is the most common class of integrons (15). It has been established that integrons and drug resistance are related, and integrons play a n important role in the transmission of antibiotics resistance among different species of bacteria. Higher death rates and less successful treatment outcomes have been associated with certain organism infections. Therefore, in order to stop nosocomial transmission, administer appropriate medication swiftly, and start stringent infection control measures, early diagnosis of MβL-producing organisms is necessary (16). PCR detection provides a high degree of reproducibility and sensitivity for identifying MBL genes in P.

aeruginosa isolates that generate MBL (17). Our study aimed to detect if P. aeruginosa burn isolates produced MBL and the frequency of BlaIMP, Bla VIM, Bla GIM, and Integron class-1 (Int-1) genes.

## 2. METHODS

### Bacterial Isolates

A total of 100 samples were collected from burn patients who were sent to the Al Hussein Teaching Hospital in Al-Smawah, Iraq, during the course of three months, from March 2024 to June 2024. Standard laboratory techniques were used to identify the different species of bacteria, such as the biochemical test (using API-20E assays from bioMérieux, https://www.biomerieux.com). and validated by Polymerase Chain Reaction (PCR) with 16sRNA. Before testing, the isolates were subcultured twice and kept at -70°C in trypticase soy broth with 20% glycerol

### Antimicrobial susceptibility testing

The antimicrobial susceptibility of Mueller Hinton agar plates (Merck Co., Germany) was assessed using the Standard disk diffusion method in compliance with the Clinical and Laboratory Standards Institute (CLSI) standards (18) Table-1 .

**Table-1 : Antibiotic Discs ,Concenteration and Company**

| No. | Disc Name and concentration(µg) | Company |
|---|---|---|
| **1.** | Ceftazidime (CAZ 30) | Bio-analyze (Turkey ) |
| **2.** | Cefixime (CFM 5) | |
| **3.** | Ceftriaxone (CRO10) | |
| **4.** | Aztreonam (ATM 30) | |
| **5.** | Ciprofloxacin (CIP 10) | |
| **6.** | Levofloxacin (LEV 5) | |
| **7.** | Ampicillin (AM 25) | |
| **8.** | Piperacillin (PRL 100) | |
| **9.** | Amikacin (AK 30) | |
| **10.** | Gentamicin (CN 10) | |

### MBL phenotypic detection

The combination disk diffusion test (CDDT) was employed to identify MBLs phenotypically. Test isolates were streaked over Muller Hinton agar (MH) to create a lawn culture (0.5 McFarland opacity standard), and the culture was left (3-5) minutes to dry. The imipenem and mixed imipenem/ EDTA (10 / 750) discs were placed on the surface of agar plate .After an overnight incubation at 37 oC , imipenem and Imipenem-EDTA inhibition zones

were measured and assessed . Approximately difference of more than 7 mm in the inhibition zone of the IMP-EDTA disc means MBLs Positive results (19).

**Polymerase Chain Reaction (PCR)**

The genetically identify of Metallo-β-lactamases, Integron class -1 genes, and P.aeruginosa strain, PCR was performed using particular primer pairs.

**Table 2 : Primers Sequences for 16sRNA for *P.aeruginosae* , Metallo Beta-lacatmases genes and Integron Gene Class-1 (*Int-1*) ,Gene Reference, and Product Size,**

| GENE | PRIMER SEQUENCES 5' ------'3 | PCR product | Reference |
|---|---|---|---|
| **16sRNA *P.aeruginosa*** | F: TGCCTGGTAGTGGGGGATAA<br>R: GGATGCAGTTCCCAGGTTGA ` | **505 bp** | **(20)** |
| ***Int 1*** | F: GGTGTGGCGGGCTTCGTG<br>R: GCATCCTCGGTTTTCTGG | **480 bp** | **(21)** |
| ***Bla*VIM** | F: GTTTGGTCGCATATCGCAAC<br>R: AATGCGCAGCACCAGGATAG | **382 bp** | **(22)** |
| ***Bla*IMP** | F GGAATAGAGTGGCTTAACTCTC<br>R CCAAACTACTAGGTTATCT | **232 bp** | **(23)** |
| ***Bla*GIM** | F: TGCCCTGCTGCGTAACATCG<br>R: GGCGGCTCCATCGGTGTG | **149bp** | **(24)** |

**Traditional PCR assay**

**Preparing a PCR master mix reaction**

This reaction was made in accordance with Transgen biotech Inc.'s (China) specifications. **Master mix contents : The content of Master Mix As in Table -3 .**

**Table -3 : PCR Master Mix content**

| An element | Reaction |
|---|---|
| **Forward Primer (10 µM )** | 1 µl |
| **Reverse Primer (10 µM )** | 1 µl |
| **DNA Template** | changeable |
| **Easy Taq 2X Master Mix with Standard Buffer** | 25 µl |
| **Nuclease-free water** | changeable |
| **Total** | 50 µl |

And the reaction components as in Table -4 below :-

| The Parts | Amounts |
|---|---|
| **DNA Polymerase** | 500 U×1 |
| **10×*EasyTaq*® Buffer and Mgcl2** | 1.2 ml×1 |
| **2.5 mM dNTPs** | 800 µl×1 |
| **6×DNA Loading Buffer** | 1 ml×1 |

**Thermocycling Conditions for PCR :**

Each gene's PCR thermocycler conditions were determined using a traditional PCR thermocycler apparatus, as indicated in Table 4:

**Table-5 : PCR Amplification Program**

| Step | Temperature(˚C) | Time | No.of cycles cycle |
|---|---|---|---|
| **Initial denaturation** | 94 | 3 min | **1** |
| **Denaturation** | 94 | 30**sec** | **35** |
| **Annealing** | Variables according to (primer's TM)<br>according to ((according to | 30 **sec** | |
| **Extension** | 72 | 1 min | |
| **Final extension** | 72 | 5 min | **1** |
| **Hold temperature** | **-4** | ∞ | **-** |

## 3. RESULTS AND DISCUSSION

One of the most important bacteria that cause nosocomial infections is *P.aeruginosa* because of its natural resistance to a wide range of antibiotic treatments. It has the rare capacity to become resistant to a wide range of antimicrobial medications via a variety of mechanisms (26). Twenty *P.aeruginosa* clinical isolates Figs. (1 and 2) were gathered over the course of the investigation. These results align with (27, 28, and 29). *P. aeruginosa* shown complete resistance to ampicillin, piperacillin, gentamicin, ciprofloxacin, and ceftriaxone in this study. There were different degrees of moderate to lowest resistance found for cefixime, levofloxacin, meropenem, amikacin, and aztreonam. The results were consistent with the work done by (30, 31). Antibiotic resistance may be attributed to a number of factors, including self-medication, treatment noncompliance, sales of faulty drugs, the spread of resistant isolates among patients, and—above all—the affordability and accessibility of antibiotics without a prescription. The primary cause of the formation of various antibiotic resistance strains, which in turn promotes

the selection and spread of antibiotic-resistant illnesses, is thought to be antibiotic abuse without antibiotic sensitivity testing (32). In a recent investigation, isolates that showed resistance more than three drugs were classified as multidrug resistant (MDR), accounting for 90% of the isolates. Among the mechanisms that have evolved in multi-resistant P. aeruginosa are modified target enzymes, reduced cell permeability, an efflux pump, and antibiotic inactivation (33). Due to their recent genetic structure acquisition, Gram-negative bacteria MBLs has developed into a strong resistance determinant that is dangerous to human health and is essential for drug resistance (34). The Imipenem-EDTA disc combo approach for phenotypic detection, A recent investigation (20) examined the isolates' capacity to generate MBLs. Our research revealed that there were 20 (100%) MBL producers overall, which is consistent with (28, 35); nonetheless, (29) showed a low rate. The current investigation's findings indicate that no Bla $_{IMP}$, Bla$_{VIM}$, or Bla$_{GIM}$ was found in any of the isolates (Figures 4,5,6).This result is in line with (11,29,36) and indicates that the research isolates that produced MBLs phenotypically did not possess the MBLs gene. Given that PCR is the gold standard method, it's plausible that the isolates had MBL variants or extra carbapenemase genes that the study's primers overlooked. This would explain the potentiality of a false positive phenotypic finding (37). Furthermore, because the bacterial membrane is fragile, the use of EDTA might produce false-positive results even in cases where phenotypic testing is straightforward and highly sensitive (38).In the present investigation, eighty percent of the isolates harbored class 1 integron gene. Figure (7).These findings are aline with (29, 39). Class I integrons are very common, perhaps because of their remarkable capacity to ensnare a great deal of drug resistance genes. Moreover, gene cassette arrays with a range of resistance genes that may provide resistance against various drugs have been developed (40). Prior research has indicated a connection between several forms of integrons, particularly class 1 integrons, and multiple resistance (41). Our findings show that integron-containing isolates had a significantly higher frequency of MDR isolates (about 90%), demonstrating the significance of these elements in the transmission of resistance genes between pathogens. Consistent with our results, (42) identified a significant percentage of MDR isolates from integron-positive patients.


**Figure -1 : Api 20 E for P.aeruginosa isolated form Burn Patients**

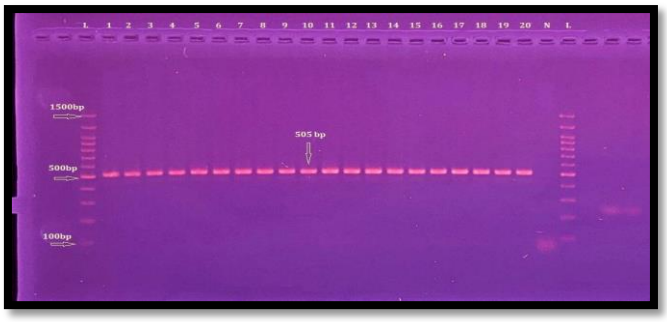


**Figure 2: The gel electrophoresis image for *P.aeruginosa* (16 sRNA Primer).**

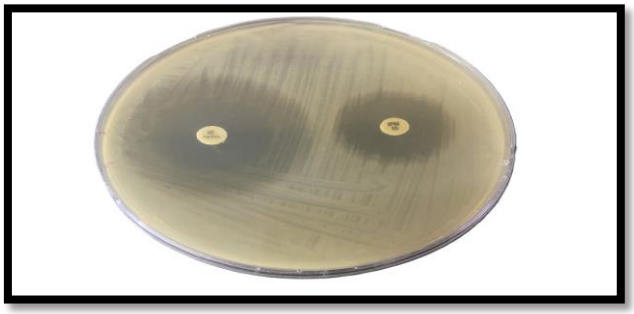

**Figure-3 : Metallo Beta-lactamases Producing From *P.aeruginosa* by CDD method .**

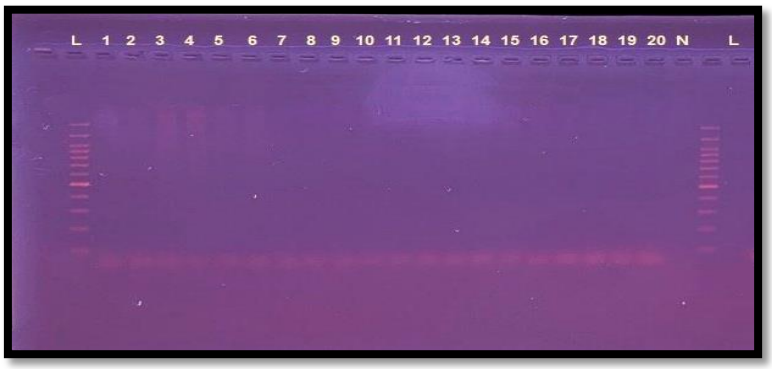


**Figure-4: The gel electrophoresis image for the ($Bla_{VIM}$ primer) .**

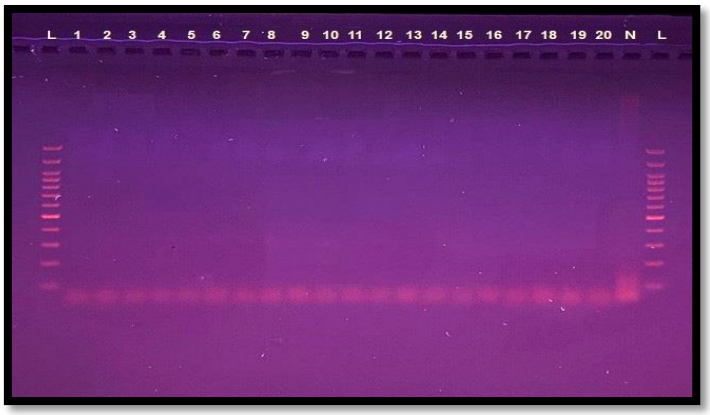


**Figure -5: The gel electrophoresis image for the( Bla $_{IMP}$ primer)**

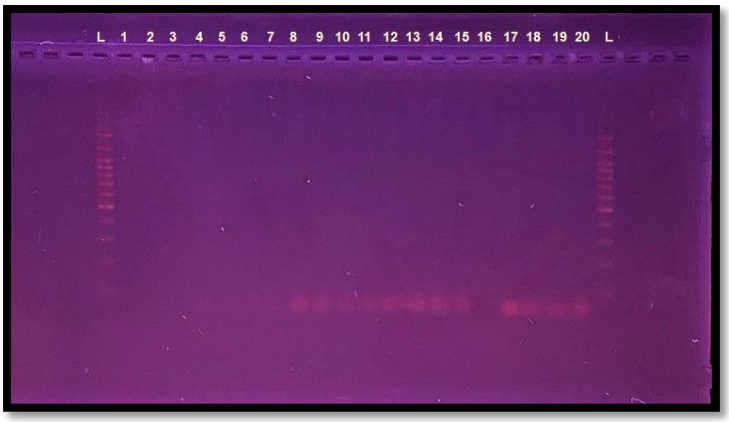


**Figure -6: Gel electrophoresis Image for PCR product of ( Bla *GIM* primer) .**

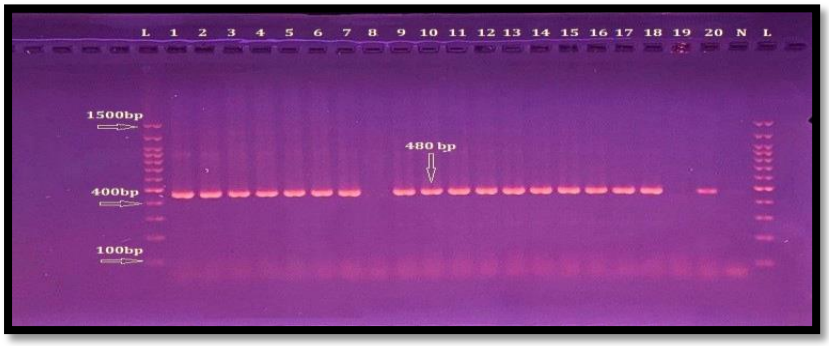


**Figure -7 :The PCR product image for the Int-1 primer**

## 4. CONCLUSIONS

The prevalence of P. aeruginosa MBLs is rising everywhere, including in Iraq. Because MβL producers are extremely resistant to the majority of antibiotics, they pose a considerable threat. Therefore, in order to identify MβL producers early and prevent the spread of such strains, a suitable and routine screening approach should be devised specifically for all resistant P. aeruginosa isolates. Most of the time, it was discovered that the isolates in this investigation were resistant to most antibiotics.

Since Class I integrons are frequently seen in *P. aeruginosa* isolated from clinical samples, it is necessary to assess integrons as contributing factors in antibiotic resistance because they are frequently responsible for antibiotic resistance gene transfer.

## 5. REFERENCES

Abdelaziz, S. M., Aboshanab, K. M., Yahia, I. S., Yassien, M. A., & Hassouna, N. A. (2021). Correlation between the antibiotic resistance genes and susceptibility to antibiotics among the carbapenem-resistant Gram-negative pathogens. Antibiotics, 10, 255. https://doi.org/10.3390/antibiotics10030255.

Al-Charrakh, A. H., Al-Awadi, S. J., & Mohammed, A. S. (2016). Detection of metallo-β-lactamase producing Pseudomonas aeruginosa isolated from public and private hospitals in Baghdad, Iraq. Acta Medica Iranica, 54(2).

Ali, M. G., Almoneim, Z. A., & Kareem, S. M. (2023). Evaluated gene expressions of metallo-beta-lactamase genes GIM, VIM, SPM in Pseudomonas aeruginosa clinical isolates. Molecular Biology Reports, 50, 10111-10120. https://doi.org/10.1007/s11033-023-08883-7

Alkhudhairy, M. K., & Al-Shammari, M. M. M. (2020). Prevalence of metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from diabetic foot infections in Iraq. New Microbes and New Infections, 35. https://doi.org/10.1016/j.nmni.2020.100661.

Al-Mohammed, T. A., & Mahmood, H. M. (2024). Carbapenem resistance related with biofilm formation and pilin genes in clinical Pseudomonas aeruginosa isolates. International Journal of Pharmaceutical Sciences, 33(1), 72-78. Available from: https://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/2272

Anoar, K. A., Ali, F. A., & Omer, S. A. (2014). Detection of metallo [beta]-lactamase enzyme in some Gram-negative bacteria isolated from burn patients in Sulaimani city, Iraq. European Scientific Journal, 10(3), 485-496.

Bahrami, M., Mohammadi-Sichani, M., & Karbasizadeh, V. (2018). Prevalence of SHV, TEM, CTX-M, and OXA-48 β-lactamase genes in clinical isolates of Pseudomonas aeruginosa in Bandar-Abbas, Iran. Avicenna Journal of Clinical Microbiology and Infection, 5(4), 86-90. https://doi.org/10.34172/ajcmi..18.

Bandekar, N., Vinodkuma, C. S., Basalarajapp, K. G., Prabhakar, P. J., & Nagaraj, P. (2011). Beta lactamase mediated resistance among Gram-negative bacilli in burn infections. International Journal of Biomedical Research, 2(3), 766-770.

Clinical and Laboratory Standards Institute (CLSI). (2022). Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement M100-S23. Wayne, PA: CLSI. 205 p.

Delarampour, A., Ghalehnoo, Z. R., Khademi, F., & Vaez, H. (2020). Antibiotic resistance patterns and prevalence of class I, II, and III integrons among clinical isolates of Klebsiella pneumoniae. Le Infezioni in Medicina, 8(1), 64-69.

Dent, L., Dana, R. M., & Siddharth, P. (2010). Multi-drug resistant Acinetobacter baumannii: A descriptive study in a city hospital. BMC Infectious Diseases, 10(1).

Drieux, L., Brossier, F., Sougakoff, W., & Jarlier, V. (2008). Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: Review and bench guide. Clinical Microbiology and Infection, 14(1), 90-103.

Ebrahimpour, M., Nikokar, I., Ghasemi, Y., Ebrahim-Saraie, H. S., Araghian, A., & Farahbakhsh, M. (2018). Antibiotic resistance and frequency of class 1 integrons among Pseudomonas aeruginosa isolates obtained from wastewaters of a burn center in Northern Iran. Annali di Igiene, 30, 112-119.

El-Mosallamy, W. A. E., Osman, A. S., Tabl, H. A. E., & AL-Tabbakh, A. M. (2015). Phenotypic and genotypic methods for detection of metallo-beta-lactamase (MβL) producing Pseudomonas aeruginosa. Egyptian Journal of Medical Microbiology, 24(3), 27-35.

European Centre for Disease Prevention and Control. (2013). Point prevalence survey of healthcare associated infections and antimicrobial use in European acute care hospitals. Stockholm: ECDC. Available at: http://ecdc.europa.eu/en/publications/Publications/healthcare-associated-infections-antimicrobial-use-PPS.pdf

Findlay, J., Raro, O. H. F., Poirel, L., et al. (2024). Molecular analysis of metallo-beta-lactamase-producing Pseudomonas aeruginosa in Switzerland 2022–2023. European Journal of Clinical Microbiology and Infectious Diseases, 43, 551–557. https://doi.org/10.1007/s10096-024-04752-8.

Fluit, A., Visser, M., & Schmitz, F. (2014). Molecular detection of antimicrobial resistance. Clinical Microbiology Reviews, 836-871.

Gupta, V., Datta, P., & Chander, J. (2006). Prevalence of metallo-beta-lactamase (MBL) producing Pseudomonas spp. and Acinetobacter spp. in a tertiary care hospital in India. Journal of Infection, 52(5), 311-314.

Heggers, J. P., Haydon, S., Ko, F., Hayward, P. G., Carp, S., & Robson, M. C. (1992). Pseudomonas aeruginosa exotoxin A: Its role in retardation of wound healing: The Lindberg Award. Journal of Burn Care & Rehabilitation, 13(5), 512-518.

Hocquet, D., Berthelot, P., Roussel-Delvallez, M., Favre, R., Jeannot, K., Bajolet, O., et al. (2007). P. aeruginosa may accumulate drug resistance mechanisms without losing its ability to cause bloodstream infections. Antimicrobial Agents and Chemotherapy, 51, 3531–3536.

Hosseinia, S. M. J., Naeinib, N. S. H., Khaledic, A., Daymadd, S. F., & Esmaeilid, D. (2016). Evaluate the relationship between class 1 integrons and drug resistance genes in clinical isolates of Pseudomonas aeruginosa. The Open Microbiology Journal, 10, 188-196. DOI: 10.2174/1874285801610010188.

Jafri, S. A., Qasim, M., Masoud, M. S., Ur-Rahman, M., Izhar, M., & Kazmi, S. (2014). Antibiotic resistance of E. coli isolates from urine samples of urinary tract infection (UTI) patients in Pakistan. Bioinformation, 10(7), 419-422.

Lin, H., Feng, C., Zhu, T., Li, A., Liu, S., Zhang, L., Li, Q., Zhang, X., Lin, L., Lu, J., Lin, X., Li, K., Zhang, H., Xu, T., Li, C., & Bao, Q. (2022). Molecular mechanism of the β-lactamase mediated β-lactam antibiotic resistance of Pseudomonas aeruginosa isolated from a Chinese teaching hospital. Frontiers in Microbiology, 13, 855961. doi: 10.3389/fmicb.2022.855961.

Lucena, A., Dalla Costa, L. M., Nogueira, K. da S., Matos, A. P., Gales, A. C., & Raboni, S. M. (2014). Comparison of phenotypic tests for the detection of metallo-beta-lactamases in clinical isolates of Pseudomonas aeruginosa. Enfermedades Infecciosas y Microbiología Clínica, 32(10), 625-630. https://doi.org/10.1016/j.eimc.2014.03.015.

McCarthy, K. (2015). Pseudomonas aeruginosa: Evolution of antimicrobial resistance and implications for therapy. Seminars in Respiratory and Critical Care Medicine, 36, 44-55.

Mohemmad, H. J., Abbas, Y. A., & Alkafaji, H. R. (2023). Assessment of beta-lactamases and integrons genes among bacteria isolated from bladder cancer patients with urinary tract infections. Journal of Population Therapy and Clinical Pharmacology, 30(8), e9-e23. DOI: 10.47750/jptcp.2023.30.08.002

Mojica, M. F., Bonomo, R. A., & Fast, W. B. (2016). Metallo-beta-lactamases: Where do we stand? Current Drug Targets, 17, 1029-1050. [CrossRef]

Pitout, J. D. D., Gregson, D. B., Poirel, L., McClure, J. A., Le, P., & Church, D. L. (2005). Detection of Pseudomonas aeruginosa producing metallo-β-lactamases.

Qu, T. T., Zhang, J., Wang, J., Tao, J., Yu, Y. S., et al. (2009). Evaluation of phenotypic tests for detection of MβL-producing P. aeruginosa strains in China. Journal of Clinical Microbiology, 47, 1136-1142.

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), e16436. DOI: 10.5812/jjm.16436.

Recio, R., Mancheño, M., Viedma, E., Villa, J., Orellana, M. A., Lora-Tamayo, J., & Chaves, F. (2020). Predictors of mortality in bloodstream infections caused by Pseudomonas aeruginosa and impact of antimicrobial resistance and bacterial virulence. Antimicrobial Agents and Chemotherapy, 64(2), e01759-19.

Sambrook, J., Fritsch, E., & Maniatis, T. (1989). Molecular cloning: A laboratory manual. Cold Spring Harbor, New York.

Santos, A. L., Santos, A. B. D., Ito, C. R. M., Queiroz, P. H. P. D., Almeida, J. A. D., et al. (2020). Profile of Enterobacteria resistant to beta-lactams. Antibiotics, 9, 410. http://dx.doi.org/10.3390/antibiotics9070410

Shaebth, L. J. (2018). Molecular identification and sequencing of Pseudomonas aeruginosa virulence genes among different isolates in Al-Diwaneyah hospital. Iraqi Journal of Veterinary Sciences, 32(2), 183-188.

Sharifi, H., Pouladfar, G. H., Shakibaie, M. R., Pourabbas, B., Mardaneh, J., & Mansouri, S. H. (2019). Prevalence of β-lactamase genes, class 1 integrons, major virulence factors, and clonal relationships of multidrug-resistant Pseudomonas aeruginosa isolated from hospitalized patients in southeast of Iran. Iranian Journal of Basic Medical Sciences, 22, 806-812. doi: 10.22038/ijbms.2019.35063.8340

Sid Ahmed, M. A., Khan, F. A., Sultan, A. A., et al. (2020). β-Lactamase-mediated resistance in MDR-Pseudomonas aeruginosa from Qatar. Antimicrobial Resistance and Infection Control, 9, 170. https://doi.org/10.1186/s13756-020-00838-y

Suryadevara, N., Ooi, Y. S., & Ponnaiah, P. (2017). A study on metallo-β-lactamases producing Pseudomonas aeruginosa in water samples from various parts of Malaysia. African Journal of Biotechnology, 16, 573.

Walsh, T. R., Tolemann, M. A., Poirel, L., & Nordman, P. (2005). MβL: The quiet before the storm? Clinical Microbiology Reviews, 18(2), 306-325.

Wood, S. J., Kuzel, T. M., & Shafkhani, S. H. (2023). Pseudomonas aeruginosa: Infections, animal modeling, and therapeutics. Cells, 12(1), 19910.

Yadav, B., & Tyagi, R. D. (2020). Development of molecular methods to detect and control emerging drug-resistance pathogens. In Current Developments in Biotechnology and Bioengineering (pp. 377-419). Netherlands: Elsevier.

Ye, Y., Xu, L., Han, Y., Chen, Z., Liu, C., & Ming, L. (2018). Mechanism for carbapenem resistance of clinical Enterobacteriaceae isolates. Experimental and Therapeutic Medicine, 15, 1143–1149.

Zarei-Yazdeli, M., Eslami, G., Zandi, H., Kiani, M., Barzegar, K., Alipanah, H., Mousavi, S. M., & Shukohifar, M. (2018). Prevalence of class 1, 2, and 3 integrons among multidrug-resistant Pseudomonas aeruginosa in Yazd, Iran. International Journal of Mycobacteriology, 10(5), 300-306.