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Analyzing Pseudomonas aeruginosa Isolates from Diwaniyah Hospitals, Iraq, for Beta-Lactamase Genetic and Behavioral Resistance Mechanisms Via Genetic Detection

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Abstract:

Hospital-related infections, particularly from intensive care units, are brought about by the bacteria *Pseudomonas aeruginosa*. Because it is resistant to drugs and may exist in hospitals, it can be hard to kill off. Because of this, it creates a significant risk and challenge in hospital situations. The chemical and genetic traits of *Pseudomonas aeruginosa*, most notably its beta-lactamase synthesis and antibiotic resistance, have been part of several studies performed in the Al-Diwaniyah Province. One of the more crucial phases when developing antimicrobial tactics is identifying the biological indicators associated with antibiotic-resistant bacteria.

While Pseudomonas aeruginosa had been isolated from a number of clinics in past studies, here remains not many assets available for its molecular characterization and resistance patterns. Finding out more on a generalization of genes associated with resistance like GES, blaOXA-48, and SPM can help build approaches for managing bacterial infections. Using traditional molecular techniques, this study sought to obtain and determine *Pseudomonas aeruginosa* from medical samples, investigate its capacity to resist drugs, and identify its presence of genes that create beta-lactamases. Findings: 15 out of 30 clinical specimens (50%) had *Pseudomonas aeruginosa* confirmed. Burns (60%), wounds (26.6%), and urine (13.4%) had the greatest isolation rates.

60% of the isolates produced beta-lactamase, suggesting that they were resistant to a lot of antibiotics with the notable exception of tigecycline, as determined by the Rapid Iodine Assay and the Vitec II 2 system. The PCR method revealed that the 16S rRNA gene was found in all isolates. In addition, 20% had the SPM gene, 40% had the blaGES gene, and 75% had the OXA-48 gene. This study is one of the first to explain *Pseudomonas aeruginosa's* molecules in the Diwaniyah Province focused on the genetic factors that lead to its heightened resistance and production of beta-lactamases. we are consequences, To avoid the development of drug-resistant Pseudomonas aeruginosa in healthcare environments, findings

show the urgent importance for ongoing surveillance, strict infection control procedures, and antibiotic control programs.

Keywords: *Pseudomonas aeruginosa*, Beta-Lactamase Production, Antibiotic Resistance, Molecular Characterization, PCR Detection, Nosocomial Infections

Introduction:

Pseudomonas aeruginosa is a major and widespread opportunistic pathogen that leads to serious hospital-acquired infections in individuals with weakened immune systems. Its remarkable ability to withstand various antibiotics, including beta-lactams, has complicated its use in clinical settings. One important aspect that contributes to a decreased vulnerability to beta-lactamases is the generation of these enzymes, especially ESBLs and MBLs. This has resulted in a lack of treatment options and an increase in hospital and overall mortality rates. [1].

In Iraq, the steigende Bekanntheit of multidrug-resistant (MDR) P. aeruginosa has aufgestiegen as an important public health Thema. Jedoch, there is keine records hinsichtlich the molecular characterization of beta-lactamase genes and their correlation with phenotypic resistance patterns in P. aeruginosa isolates from Diwaniyah hospitals. Es ist entscheidend to erfassen the genetic basis of resistance in order to devise effective strategies for managing infections and to recommend appropriate antibiotic therapies.. [2].

P. aeruginosa shows antibiotics resistance that can be either acquired or selbst hergeleitet from transferable genetic Faktoren such as plasmids and Erbgut. This resistance can be assigned to vielfältige Faktoren, such as alterations to the target sites on the bacterial cell wall, increased drug transport due to the aktivierung der efflux pumps, diminished antibiotic absorbtion because of a less permeable outer cell membrane, and die Bildung von carbapenemases that break down all beta-lactam antibiotics, including carbapenems. [3].[4].

1.2 Aim of the study

The aim of the study is to isolate and identify *Pseudomonas aeruginosa*, uncover antibiotic resistance mechanisms, and molecularly detect beta-lactamase genes present in this bacterium.

Materials and Methods

2.1. Sample collection

Various clinical samples were collected from different body sites for hospitalized patients of all ages and genders, including wounds, burns, and urine. From November 2024 to May 2025, a total of 30 samples were gathered from various hospitals and health centers in the Diwaniyah Governorate, including the Women's and Children's Hospital, Diwaniyah General Teaching Hospital, and Public Health Laboratory.

2.2 Isolation and identification

The swabs and samples were transferred directly to the laboratory for incubation at 37°C in an aerobic environment for 24 hours to determine their cultural, microscopic, and biochemical characteristics. Bacterial species were isolated using culture media and identified based on a series of biochemical tests (catalase, oxidase, IMVC, and TSI tests), as well as the morphological and microscopic characteristics of the cultured isolates. Additionally, using the Vitec 2 system, the isolate was identified as *Pseudomonas aeruginosa*.

2.3 Quick, standardized iodine method for identifying the capacity of bacteria to generate beta-lactamase enzymes

a. Several freshly developed colonies, aged 18 to 24 hours and grown on heart-brain infusion medium, were transferred to a microdilution plate containing 100 microliters of penicillin G solution in each well, using sterile wooden sticks. The colonies were combined thoroughly and then incubated for 30 minutes at a temperature of 37 °C.

b. To ensure the contents are uniform, incorporate 50 microliters of starch solution and 20 microliters of iodine solution, mixing them thoroughly with wooden sticks. The blue color is produced by the reaction of iodine and starch, and a rapid change from blue to white within approximately five minutes is a positive indication.

Table 1. Primers used in this study with their nucleotide sequence and amplification size.

Gene name	Oligo sequence (5'-3') (primer)	Product Size (bp)	Reference
16 srRNA	F TCAACCTGGGAACTGCATCC R CAGACTGCGATCCGGACTAC	688 bp	(Klindworth *et al*., 2013)
bla SPM	F AAAATCTGGGTACGCAAA CG R ACATTATCCGCTGGAACAGG	271 bp	(Moosavian *et al.*, 2015)
bla GES	F ATGCGCTTCATTCACGCAC R CTATTTGTCCGTGCTAAGG	860 bp	(Silva Júnior *et al.*, 2018)
bla OXA-48	F TTGGTGGCATCGATTATCGG R GAGCACTTCTTTTGTGATGGC	744 bp	(Hashemi *et al*., 2017)

2.4 Genomic DNA extraction

Nucleic Acid extraction from Pseudomonas aeruginosa was performed using Geneaid USA's pre-made Genomic DNA Mini Kit, following the provided instructions.

2.5 Preparation of Agarose Gel

It was prepared using the method developed by Sambrook and his group, as follows:

a. Utilizing a magnetic hot plate stirrer, mix 1.5 g of Agarose gel into 100 ml of TBE buffer solution at a concentration of (1X) for 15 minutes until dissolved.

b. After the gel cooled to 50°C, three microliters of the radioactive DNA stain ethidium bromide were incorporated into it and mixed thoroughly.

c. The acro gel was poured into the migration mold (the tray holding the comb) and allowed to solidify for fifteen minutes at room temperature. To create and define the holes (wells) in the gel needed for injecting the amplified samples, the comb was then carefully removed from the gel.

2.6 PCR Master Mix Preparation

1. In accordance with the instructions from the supplier of the Wizpure PCR FDmix and as shown in Table 2 below, prepare this blend in PCR tubes:

Table 2: Components of the PCR master mix.

Mixture Ingredients	Size
PCR FD mix	1Tube
10mM Forward Primer	1.5 µL
10mM Reverse Primer	1.5 µL
Template DNA	5 µL
PCR water	12 µL
Total	20 µL

2. The prepared tubes containing the mixture were placed in the Vortex machine for five seconds. The tubes were subsequently placed in the PCR thermocycler machine to perform the DNA amplification process under optimal thermal cycle conditions.

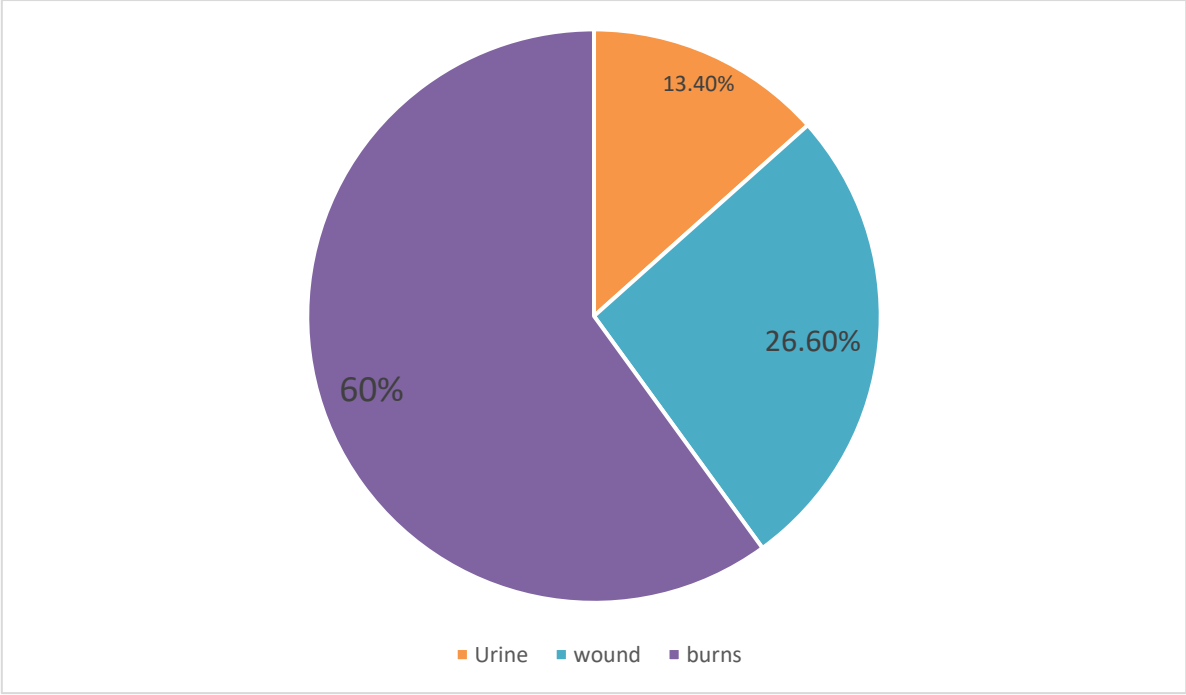
3. The software that employs thermal cycles to increase DNA quantity. The Thermocycler PCR apparatus was employed and configured for the genes under investigation, based on the reaction.

Results and Discussion

3.1 Numbers and percentages of *Pseudomonas aeruginosa* isolation

Out of 30, only 15 are isolated (Isolation rate: 50 %). *Pseudomonas aeruginosa* showed the highest isolation rate from burns at 60% and the second highest from wound infections at 26.6%. Urine samples, by contrast, exhibited the lowest isolation rate, 13.4%. The results align with those of the study by (6), which discovered that *P. aeruginosa* was the organism most often isolated from burn patients, accounting for 59.3% of all isolates. This supports the notion that burn wounds, being moist and high in protein content, provide an ideal breeding ground for bacteria. In the same way, the current findings align with those of (7), who observed that the rates of *P. aeruginosa* isolation from wound infections varied between 20% and 30%, which is in close agreement with the 26.6% found in this research. Die Resultate dieser research, however, vary from die von (8), who observed a deutlich erhohte rate of P. aeruginosa isolation from urine samples at 38%. This was associated with the routine application of urinary catheters, particularly in Intensive Care Units. To conclude, this study's findings highlight the importance of the clinical environment in finden the sources of infections caused by P. Furthermore, they indicate the Bedeutung of implementing strict infection control Schritte—besonders in surgical and burn units—to reduce the spread of resistant P. aeruginosa strains.

Figure 1. Distribution of *Pseudomonas aeruginosa* isolates according to sample source.



Nine of the 15 samples (60%) had been able to create beta-lactamase enzymes, per the results. Furthermore, these bacteria's resistance to certain antibiotics, including penicillins and cephalosporins with a beta-lactam ring in their structure, aligns with prior statements. [9]. According to the findings from the Vitek device, most of the isolates exhibited a resistance rate of 100% to the examined

antibiotics. The only exception was tigecycline, which proved effective in inhibiting bacteria, with its sensitivity percentage ranging between (0.3-1).

Table 3 shows the proportion of beta-lactamase production among Pseudomonas aeruginosa isolates from various sources. 40% of the isolates were non-producers, while 60% produced beta-lactamase. Among these isolates, one was from burns and two from urine, and they did not generate beta-lactamases.

Table 3: Ability of *P. aeruginosa* isolates to produce beta-lactamase enzymes according source isolated.

Ability to produce beta-lactamase enzymes	Burn	Wound	Urine	Percentage %
Producing isolate	6	3	0	60%
Non-producing isolate	3	1	2	40%

3.2. Molecular diagnosis of *P. aeruginosa* through polymerase chain reaction

In all 15 bacterial isolates studied, the 16SrRNA gene was tested and found to be present in every single one of them (100%). 20% of the isolates contained virulence genes such as *bla* SPM, 40% contained *bla* GES, and 75% contained *bla* OXA-48. This can be ascribed to the ability of these bacteria to produce beta-lactamase enzymes and their resistance to antibiotics in this class, which is achieved through changes to their outer membrane proteins and efflux pumps. [10],[11]. *Pseudomonas aeruginosa* is considered a multidrug-resistant bacterium, having multiple mechanisms that provide resistance to these drugs. These comprise the synthesis of beta-lactamase enzymes that neutralize antibiotics, the utilization of efflux pumps to expel antibiotics from the cell, and the existence of specific genes on its chromosomes or plasmids that promote antibiotic resistance, as depicted in Figure (3). This is one of the main factors that contribute to the resistance of the studied bacteria to antibiotics. [12],[13] [14].

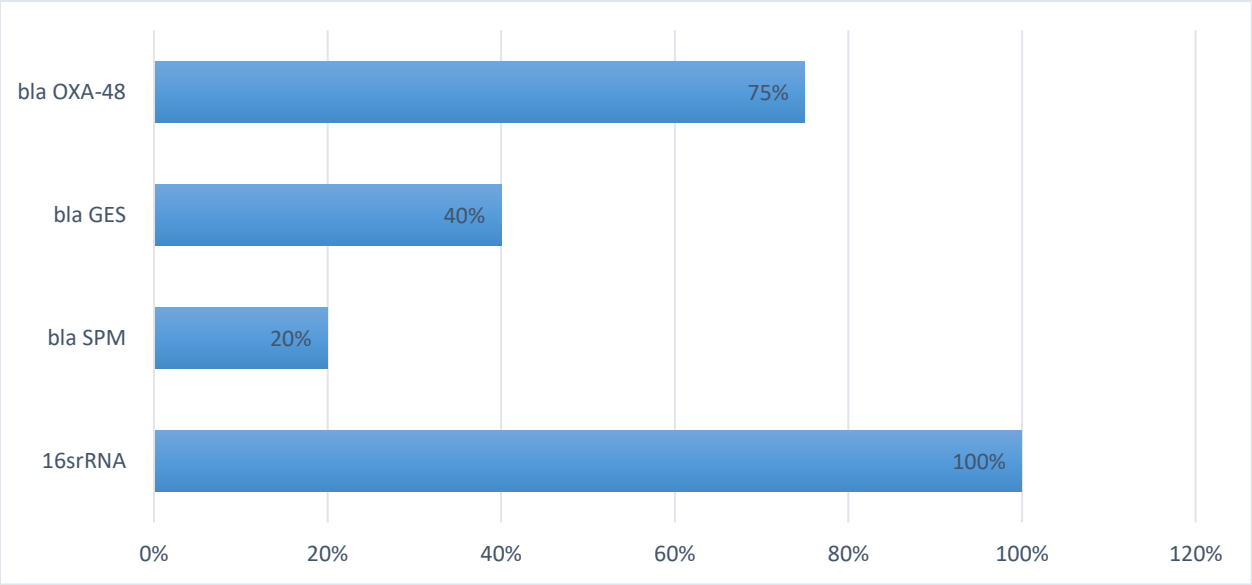


Figure (3) The percentage of gene prevalence in *Pseudomonas aeruginosa* bacteria under study.

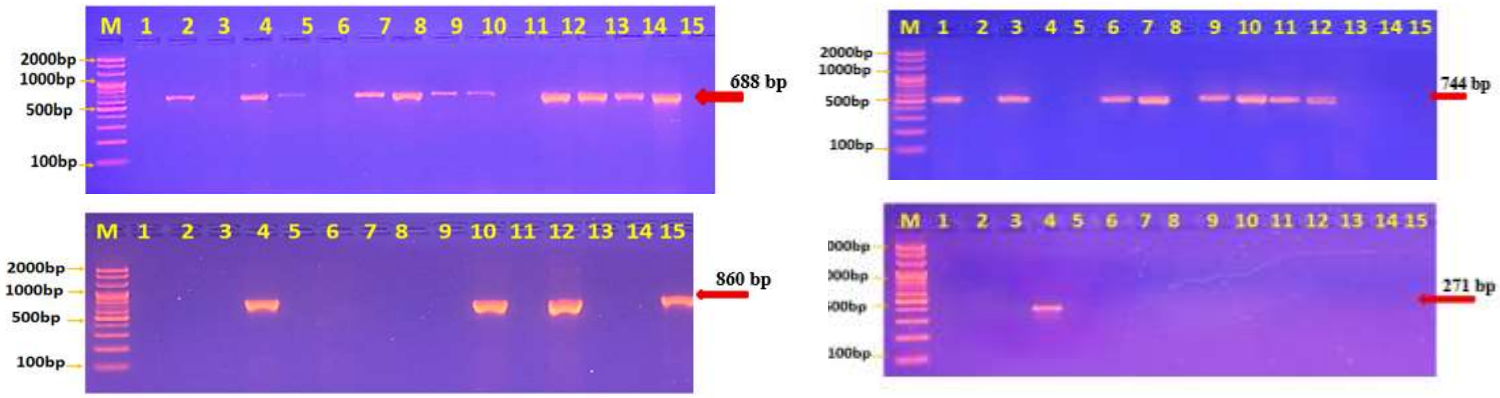


Figure (4) PCR result

Conclusion

This indicates that Pseudomonas aeruginosa is common in medical specimens from the Diwaniyah Governorate and that it is tolerant to tigecycline but significantly resistant to most medication. The 16S rRNA gene was found in all isolates, and 75% of them had the bla OXA-48 gene, 40% the bla GES gene, and 20% the bla SPM gene, suggesting a significant potential for the formation of beta-lactamases.

The findings show the dire need to improve managing antibiotic programs, strengthen infection control procedures, and continue molecular detection of the growth of multiple-drug-resistant Pseudomonas aeruginosa in hospitals. Further research should therefore identify treatment options beyond therapy, including strategies that emphasize novel antimicrobials or targeted gene inhibition methods to address this erheblicher pathogen.

References

[1]. 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. https://doi.org/10.1016/j.biotechadv.2018.11.013

[2]. Oliver, A., Mulet, X., López-Causapé, C., & Juan, C. (2015). The increasing threat of Pseudomonas aeruginosa high-risk clones. Drug Resistance Updates, 21–22, 41–59. https://doi.org/10.1016/j.drup.2015.08.002

[3]. Hussein, N. H., & Al-Kadmy, I. M. S. (2021). Detection of Metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from different clinical specimens in Iraq. Gene Reports, 23, 101082. https://doi.org/10.1016/j.generep.2021.101082

[4]. Al-Khudhairy, M. K., & Al-Shammari, H. M. (2022). Phenotypic and genotypic detection of extended-spectrum beta-lactamases among Pseudomonas aeruginosa clinical isolates in Iraq. Iranian Journal of Microbiology, 14(1), 46–54. https://doi.org/10.18502/ijm.v14i1.8795

[5]. Lee, K., Lim, Y. S., Yong, D., Yum, J. H., & Chong, Y. (2003). Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. Journal of Clinical Microbiology, 41(10), 4623–4629. https://doi.org/10.1128/JCM.41.10.4623-4629.2003

[6]. Ghasemian, S., Hosseinnia, H., Khazaie, S., Ghasemian, S., & Zarifi, M. K. (2023). Molecular characterizations of antibiotic resistance, biofilm formation, and virulence determinants of Pseudomonas aeruginosa isolated from burn wound infection. Journal of Clinical Laboratory Analysis. Advance online publication. https://doi.org/10.1002/jcla.24992

[7]. Hashemzadeh, R., Chen, H., Wang, C., & Peng, Y. (2022). The clinical characteristics and microbiological investigation of pediatric burn patients with wound infections in a tertiary hospital in Ningbo, China: A ten-year retrospective study. Frontiers in Microbiology, 13, 103409. https://doi.org/10.3389/fmicb.2022.1034909

[8]. Ahmed, M., Basha, S. J., Al-Mugheed, K., Alsenani, N. H., & Alshammari, F. D. (2023). The profile of bacterial infections in a burn unit during and after the COVID-19 pandemic period. Antibiotics, 13(9), 823. https://doi.org/10.3390/antibiotics13090823

[9]. Almzıl, N. R. H. (2023). Antibiotic resistance profiles of Pseudomonas aeruginosa isolated from clinical samples. Central Asian Journal of Medical and Natural Science, 4(6), 504–512. https://cajmns.centralasianstudies.org/index.php/CAJMNS/article/view/2017

[10]. El-Kholy, A., Saied, T., El-Mahallawy, H. A., Hashem, M., & Abdel Aziz, M. (2024). Emergence of OXA-48-producing Pseudomonas aeruginosa in Egypt: A rising threat. BMC Infectious Diseases, 24(1), 123. https://doi.org/10.1186/s12879-024-09012-w

[11]. Silva, K. C., Lincopan, N., Reinert, C., Menezes, L. C. B., Alves, L. R., & Moreno, A. M. (2012). Prevalence of bla_{SPM-1} among carbapenem-resistant Pseudomonas aeruginosa isolates in Brazil. BMC Infectious Diseases, 12, 176. https://doi.org/10.1186/1471-2334-12-176

[12]. Pérez-Varela, M., Corral, J., Aranda, J., & Barbé, J. (2021). Role of efflux pumps in the antibiotic resistance of Pseudomonas aeruginosa. New Microbes and New Infections, 44, 100930. https://doi.org/10.1016/j.nmni.2021.100930

[13]. Li, Y., Wang, Y., He, Z., Ma, L., & Xu, X. (2024). Prevalence and characterization of β-lactamase genes in multidrug-resistant Pseudomonas aeruginosa isolates from clinical samples. BMC Microbiology, 24, 258. https://doi.org/10.1186/s12866-024-03405-2

[14]. Tamber, S., & Hancock, R. E. W. (2006). Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. Journal of Bacteriology, 188(23), 8337–8342. https://doi.org/10.1128/JB.00859-06