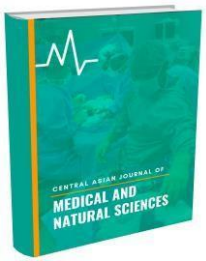

CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
https://cajmns.centralasianstudies.org/index.php/CAJMNS
Volume: 05 Issue: 04 | October 2024 ISSN: 2660-4159

Article

Study of the Sensitivity Patterns of Some Antibiotics to Pseudomonas Aeruginosa Isolated From Patients with Wounds in Nasiriyah City

Nasser Saleh Lhwak[1*]

1. Department of Pathological Analytics, College of Medical Applied Science, University of Shatra, Thi-Qar, Iraq

* Correspondence: nassersaleh@shu.edu.iq

Abstract: Pseudomonas aeruginosa is a Gram-negative pathogen known for causing infections in hospitalized patients, particularly due to its multidrug resistance mechanisms, which significantly increase morbidity and mortality. The growing prevalence of multidrug-resistant strains has severely limited treatment options, highlighting the urgent need for novel therapeutic approaches. This study aimed to evaluate the antibiotic susceptibility of P. aeruginosa isolates from wound infections in patients at Hussain Teaching Hospital, Nasiriyah, between February and September 2024. Out of 100 wound samples, 14 P. aeruginosa isolates were identified using biochemical, morphological, and culture analyses. Antibiotic susceptibility testing revealed that most isolates showed high resistance to trimethoprim/sulfamethoxazole and amoxicillin, while imipenem was the most effective antimicrobial agent. These findings underscore the importance of continuous monitoring of antibiotic resistance patterns and the need for alternative treatment strategies to combat drug-resistant infections.

Keywords: Antimicrobial resistance, Wound infection, Pseudomonas aeruginosa

Citation: Nasser Saleh Lhwak. Study of the Sensitivity Patterns of Some Antibiotics to Pseudomonas Aeruginosa Isolated From Patients with Wounds in Nasiriyah City. Central Asian Journal of Medical and Natural Science 2024, 5(4), 996-1001.

Received: 29th August 2024
Revised: 29th Sept 2024
Accepted: 6th Oct 2024
Published: 13th Oct 2024

1. Introduction

A Gram-negative bacterium that is a member of the proteobacteria phylum is called Pseudomonas aeruginosa [1]. This contributes to hospital infections and disease in individuals with compromised immune systems[2].one of the main reasons why burn victims have significant infections, which can have a 50% fatality rate [3]. The Centers to Disease Control and Prevention state that P.aeruginosa is largely a nosocomial infections pathogen, with an average annual incidence of 0.4 percent in hospitals across the United States.

The bacteria is the fourth most often identified nosocomial infection. Any illness that developed in a hospital according to estimates, P. aeruginosa is the source of around 10% of all hospital-acquired infections, and the death rate for patients with impaired immune systems can vary from 20% to 70% [4].Pathogenic bacteria emit a variety of chemicals known as virulence factors when they penetrate host tissue. Through their toxicity and invasiveness, virulence factors harm tissues and facilitate the growth of germs. Pseudomonas aeruginosa produces a wide range of extracellular and cell-associated virulence factors. [5]. P. aeruginosa primary phenazine pigment, pyocyanin, has been

demonstrated to have a role in the pathogenicity of the organism. Because pyocyanin is blue in color and causes P. aeruginosa stationary phase cultures to become green, it is easily recognized whether it is present in diseased tissues, pus, or dressings. [6].

Four out of seven wound dressing for burn patients with P.aeruginos infections were found for possess pyocyanin. When the invasive bacteria significantly hinder the healing process of a wound, the wound is considered infected. Toxic chemicals are created by pathogenic bacteria during their invasion of a host, resulting in tissue harm for host. These compounds, referred to as virulence factors, facilitate the establishment of bacteria within the host. In response for the bacterial invasion, the host attack inflammatory cells such as neutrophil, which emit inflammatory mediators, oxygen radical and cytotoxic enzymes that further harm the host's tissue. The infected wound's non-healing stage is likewise influenced by this host response mechanism [7].Because of biofilm development, once a bacteria is established in a wound, it is nearly tough to eradicate using antibiotics. [8].

Every wound has bacterial contamination, which mean that while germs are present, they are not reproduce. Endogenous sources, which include the gastrointestine system, skin around the wound, the environment, or the healthcare practitioner, can infect wounds[9].Classic indications of an infection in a wound include pain, fever, edema, redness, purulence, and decreased function. Additionally, low transcutaneous oxygen tension, the formation of necrotic tissue, an unpleasant odor, wound disintegration, granulation tissue degeneration and discolouration, and increased friability are all possible indications of chronic wounds [9].P.aeroginosa is one of the most prevalent pathogens in chronic wounds. a highly hazardous microorganism because it may create biofilms that are resistant to it [10].

However, burns or wounds compromise the immune system and damage the barrier, making it possible for opportunistic pathogens like P.aeroginosa . An opportunistic pathogen, P. aeruginosa is frequently obtained in hospital settings and is linked to respiratory, urinary, burn, and chronic wound infections [1]. P.aeruginosa has two different mechanisms of antibiotic resistance: mutationally acquired resistance and non-mutational intrinsic resistance.Aminoglycosides and fluoroquinolones are two significant antibiotic groups that are used to treat Pseudomonas infections. The efficacy of antibiotics is decreased when Pseudomonas quickly develops resistance to these agents [11].

From burns and wounds, 48 P. aeruginosa isolates were isolated by Haleem et al. [12]. They showed intermediate resistance for Amikacin 35.5%, Ciprofloxacin 31.26% , Polymyxine 40% and for Ampicillin, Cefotaxime, Chloramphenicol, Penicillin, Doxycycline, and Erythromycin 100% but were vulnerable for Pipracillin and Ticarcilline. [13] Mukerjee et al. P.aeruginosa isolates from Hospital were resistant for a wide range of antibiotics, including several B-lactams, cephalosporin, aminglycosidws, and fluoroquinolone,the only medications with significantly lower resistance levels were piperacillin, carbenicillin, amikacin, and ciprofloxacin. showed that a plasmid was found in P. aeroginosa and that it confers resistance to carbencillin, streptomycin, sulfanilamide, and gentamycin. The fact that it can replicate in both P. aeruginosa and E. coli has led to its classification as an overseas range plasmid.

2. Materials and Methods

Samples Collection

Samples were gathered during m0nths of February and September 2024.One hundred sample were collected from patients with wounds infection who were hospitalized to the Hussain Teaching Hospital in Nasiriyah City, located in the Thi-Qar region.

Phenotypic identifications of Pseudomnas aeruginosa

Samples were cultured on MacConkey and blood agar, a single clear colony that did not ferment lactose was chosen. The colony was then subcultured on the suitable medium. First, Gram-negative rods in an unarranged manner were used for a Gram-stain. Once more, the organism was cultivated in MacConkey agar plates for yield colorless colonies that were non-lactose fermenter. Pyocyanin, an expigment with a blue-green hue, is produced by P. aeruginosa, and the colonies were big, oval, and flat. It also has a distinct fruity scent. After being tentatively recognized as P.aeruginosa, it was subcultured in nutrient agar slant and incubated for 24 hours in 37 C. Then, it was refrigerated in 4 C. [14].

Antimicrobial Susceptibility Testing

Recommendations for the disc diffusion method, also known as the Kirby- Bauer method, were given by the Clinical and Laboratory Standard Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) [17]. Muller Hinton agar was sterilized, prepared, and the medium was refrigerated to 45°C before being placed into a sanitized Petri plate.The suspension of the inoculum was made using a colony. suspension standardized to satisfy turbidity standards set by McFarland 0.5. A swab of sterile cotton moistened in The Muller Hinton agar plate surface was evenly swabbed with bacterial solution to inoculate the plates. Using sterile forceps, the antibiotic disc was placed on the inoculums' surface.The infected plates were incubated in 37°C for 18 to 24 hours. The diameters of the inhibition zones produced through the antimicrobials' inhibitions of bacterial growth during incubation were measured using a ruler.Each table in the inhibitory zone interpretation chart that the NCCLS suggests

3. Results and Discussion

Frequency of Pseudomonas aeruginosa Isolation from Wounds Specimen.

From February to September 2024, all of one hundred samples were taken from patients visiting Hussain Hospital in Nasiriyah City, Iraq. Our findings indicated that 14 (or 14%) of the 100 samples had pseudomonas aeruginosa cultures that were positive.To establish that the recovered bacterial isolates are P.aeruginosa species, all of the isolates were put through a battery of confirmatory assays.These gram-negative rods of bacteria from the smear preparation are motile, do not produce spores, and are organized in single or short chains. Utilizing morphological, biochemical, and microscopical methods, all P. aerogenosa isolates and identifications are made.To study the generation of pigment, the isolates were cultivated on a range of mediums.

When the isolates were cultivated on selective medium at 37°C, the colors they created were observed. The isolates produced brown/blue, which is indicative of pyomelanin production, and yellow/green, which is indicative of pyocyanin production.Urease synthesis, citrate utilizations, and the Indole test for catalase, oxidase and nitrate were among the biochemical tests used. Of the isolates with positive cultures of P.aeruginosa, 14 (14%) were found. P.aeruginosa was found in 20% of the wounds by Kheder [18], which is greater than our findings. Additionally, our findings were in line with those of Salih [19], who isolated 50 samples out of 200 burns from hospitalized patients in Sulaimaniya.

Othman [20] found that 32% of 156 burn patients had P. aerogenosa, which is more than our findings. Antimicrobials sensitivity tested was make on all P.aeruogenosa isolate. The results were interpreted using the standard value established by the Clinical and Laboratory Standard Institute of Antimicrobial sensitivity testing (C.L.S.I.). As indicated in Table (1), the most resistant antibiotics were Trimeth/sulpha (21.42%) and amoxicillin (0%) while the most sensitive antibiotics were Imipenem (78.57%), Meropenem (71.42%), and Aimikacin (71.52%).

Table 1. Antibiotic susceptibility patterns in pseudomonas aeroginosa isolates (n=14).

Antibiotics	S	%	R	%
Amikacin	10	71.42%	4	28.57 %
Amoxicillin	0	0 %	0	100 %
Cefepime	9	64.28 %	5	% 35.71
Ceftazidime	9	64.28 %	5	% 35.71
Ciprofloxacin	9	64.28 %	5	% 35.71
Gentamicin	9	64.28 %	5	% 35.71
Imipenem	11	78.57%	3	21.42%
Meropenem	10	71.42 %	4	28.57 %
Norfloxacin	7	50%	7	50 %
Tobramycin	9	64.28 %	5	% 35.71
Trimeth /sulfa	3	21.42%	11	78.57%

In order to determine the pattern of antibiotic resistance in P.aeruginosa isolates, a total of fourteen isolates were subjected to a screening process to determine their resistance to eleven commonly used antibiotics, which included amoxicillin, ciftazidine, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, and trimeth/sulfa.The results show that Imipenem (78.57%) was the most effective antibiotic, followed by Meropenem (71.42%) and Amikacin (71.42%).

Table (1) demonstrated that the greatest proportion of resistant individuals was 100% for amoxicillin and 78.57%) for trimeth/sulfa. According to Henwood et al. [22], P.aeruginosa has a chromosomal AmpC Beta-lactamase and impermeability multidrug efflux pump that make it naturally resistant to several antimicrobial agents. Aminopenicillin, monobactam, carbapenem, the fourth and fifth generations of cephalpsporins, amino glycosides, and fluoroquinolones all shown beneficial action. In line with our findings, which showed that of the 14 isolates, 21.42% were resistant to imipenem, Olayinka et al., [23] reported that (20 %) of P.aeruginosa isolate from clinical samples obtained from the surgical units of Ahmadu Bello University Teaching Hospital in Nigeria were sensitive for imipenem.

Othman [20] reported that over 50 P.aeruginosa isolates from various clinical specimens are resistant to amikacin (98%) and cephotaxime (96%) as well as rifampicin (80%), ampicillin (70%), augment (70%), and doxycycline (60%) respectively. According to Haleem et al. [12], Pseudomonas aeruginosa isolated from several clinical patients shown 100% resistance. whereas they showed moderate resistance to Amikacin 39.5%, Ciprofloxacin 31.26%, and Polymyxin 40% and were sensitive for Pipracillin and Ticarciline in a percentage rate (20.08%) for each of the following antibiotics: ampicillin, Cefotaxine, Chloramphenicol, Penicillin, Doxycycline, and Erythromycin.. Due to the discovery of nine distinct enzymes in bacteria that catalyze the phosphorylation, acetylation, and coradenylylation of aminoglycoside antibiotics, the mechanism underlying bacterials resistance for these clinicals isolate are typically regulated by enzymatic antibiotic activation.

Additionally, doctors usually start antibiotic medication for patients before sending samples to the microbiology lab, which results in a high percentage of negative samples upon culture. Antibiotic resistance has been shown to be influenced by the overuse and abuse of antibiotics. Since nine distinct enzymes that catalyze the phosph0rylation, acetylation, and coradenylylation of aminoglycoside antibiotics have now been identified in bacteria, the mechanisms underlying bacterial resistance to clinical isolates of aminoglycoside antibiotics are typically controlled by enzymatic antibiotic activation [24].Additionally, doctors usually start antibiotic medication for patients before sending samples to the microbiology lab, which results in a high percentage of negative samples upon culture. Antibiotic resistance has been shown to be influenced by the overuse and abuse of antibiotics.

4. Conclusion

In conclusion, this study underscores the increasing threat posed by multidrug-resistant Pseudomonas aeruginosa in wound infections, with a notable resistance to common antibiotics such as trimeth/sulfa and amoxicillin, while imipenem proved to be the most effective. The high resistance rates observed emphasize the urgent need for novel therapeutic strategies and more judicious use of antibiotics to prevent further resistance development. The findings not only highlight the clinical challenge of treating P. aeruginosa infections but also raise concerns about the implications for public health, especially in healthcare settings with vulnerable patients. Further research should focus on understanding the molecular mechanisms underlying resistance and exploring alternative treatments, including the potential for novel antimicrobials or combination therapies to combat this formidable pathogen.

REFERENCES

[1] M. T. Madigan, J. M. Martinko, and Brock, Biology of Microorganisms, 11th ed. New Jersey, USA: Pearson Prentice Hall, 2006.

[2] J. Engel, "Pseudomonas aeruginosa Internalization by Non-Phagocytic Cells," Am. J. Med. Microbiol. Immunol., vol. 5, pp. 343-368, 2007.

[3] M. Mahboobi, F. Shahcheraghi, and M. M. Feizabadi, "Bactericidal Effects of Essential Oils from Clove, Lavender, and Geranium on Multidrug-Resistant Isolates from Pseudomonas aeruginosa," Iran. J. Biotechno., vol. 4, no. 2, pp. 137-140, 2006.

[4] J. F. Parsons, B. T. Greenhagen, K. Shi, K. Calabrese, H. Robinson, and J. E. Ladner, "Structural and Functional Analysis of the Pyocyanin Biosynthetic Protein PhzM from Pseudomonas aeruginosa," Biochemistry, vol. 469, no. 7, pp. 1821-1828, 2007.

[5] E. Kipnis and T. Sawa, "Targeting Mechanisms of Pseudomonas aeruginosa Pathogenesis," Med. Mal. Infect., vol. 36, pp. 78-91, 2006.

[6] M. H. Wilcox, T. G. Winstanley, and R. C. Spencer, "Epidemiology of Drug Resistance: Implications for a Post-Antimicrobial Era," J. Sci., vol. 257, pp. 1050-1055, 1994.

[7] T. Bjarnsholt, M. Kirketerp-Møller, P. Ø. Jensen, K. G. Madsen, and R. Phipps, "Why Chronic Wounds Will Not Heal: A Novel Hypothesis," Wound Repair Regen., vol. 16, no. 1, pp. 2-10, 2008.

[8] M. Kirketerp-Møller, P. Ø. Jensen, M. Fazli, K. G. Madsen, and J. T. Pedersen, "Distribution, Organization, and Ecology of Bacteria in Chronic Wounds," J. Clin. Microbiol., vol. 46, no. 8, pp. 2717-2722, 2008.

[9] R. G. Sibbald, H. Orsted, G. S. Schultz, P. Coutts, and D. Keast, "Preparing the Wound Bed: Focus on Infection and Inflammation," Ostomy Wound Manage., vol. 49, no. 11, 2003.

[10] T. R. Thomsen, M. S. Aasholm, V. B. Rudkjøbing, A. M. Saunders, T. Bjarnsholt, and M. Givskov, "The Bacteriology of Chronic Venous Leg Ulcers Examined by Culture-Independent Molecular Methods," Wound Repair Regen., vol. 18, no. 1, pp. 38-49, 2010.

[11] H. P. Schweizer, "Efflux as a Mechanism of Resistance to Antimicrobials in Pseudomonas aeruginosa and Related Bacteria," Genet. Mol. Res., vol. 2, pp. 48-62, 2003.

[12] H. Haleem, J. K. Tarrad, and I. A. Banyan, "Isolation of Pseudomonas aeruginosa from Clinical Cases and Environmental Samples, and Analysis of its Antibiotic Resistant Spectrum at Hilla Teaching Hospital," Med. J. Babylon, vol. 8, no. 4, pp. 45-52, 2011.

[13] S. Mukerjee, S. Chaki, S. Barman, S. Das, H. Koley, and G. Dasidar, "Effective Elimination of Drug Resistance Genes in Pseudomonas aeruginosa by Antipsychotic Agents Thioridazine," Curr. Res. Bacteriol., vol. 5, no. 1, pp. 36-41, 2012.

[14] B. Kleyn, Microbiology Experiments: A Health Science Perspective, 4th ed. New York, USA: McGraw-Hill, 2003.

[15] I. Nakasone, T. Kinjo, N. Yamane, K. Kisanuki, and C. M. Shiohira, "Laboratory-Based Evaluation of the Colorimetric VITEK 2 Compact System for Species Identification and the Advanced Expert System for Detection of Antimicrobial Resistances," Diagn. Microbiol. Infect. Dis., vol. 58, pp. 191-198, 2007.

[16] M. Kasse, B. Baars, S. Friedrich, F. Szabados, and S. G. Gatermann, "Performance of MicroScan Walkaway and Vitek 2 for Detection of Oxacillin Resistance in a Set of Methicillin-Resistant Staphylococcus aureus Isolates with Diverse Genetic Backgrounds," J. Clin. Microbiol., vol. 47, no. 8, pp. 2623-2625, 2009.

[17] P. A. Wayne, "Performance Standard for Antimicrobial Susceptibility Testing," Clin. Lab. Stand. Inst., 15th ed., CLSI/NCCLS M100-S15, 2005.

[18] A. K. Kheder, "Studies on Antibiotic Resistance by Plasmids of Pseudomonas aeruginosa," Ph.D. dissertation, Science Education College, Salahaddin University-Erbil, Kurdistan, Iraq, 2002.

[19] S. S. H. Salih, "Some Bacteriological and Molecular Genetic Studies of Pseudomonas aeruginosa Isolated from Different Environments," M.Sc. thesis, Higher Academy of Human and Scientific Studies, Iraq, 2008.

[20] A. Othman, "Antiplasmid (Curing) Effect of Alcoholic Extract of Rosmarinus Officinalis on Resistant Isolate of Pseudomonas aeruginosa," M.Sc. thesis, Medical Microbiology College of Medicine, Hawler Medical University, 2011.

[21] C. Langelotza, A. Mueller-Raua, S. Terziyskia, B. Raua, P. A. Gastmeierc, and J. Geffersc, "Gender-Specific Differences in Surgical Site Infections: An Analysis of 438,050 Surgical Procedures from the German National Nosocomial Infections Surveillance System," Viszeralmedizin, vol. 30, pp. 114-117, 2014.

[22] C. Henwood, D. Livermore, D. James, and M. Waner, "Antimicrobial Susceptibility of Pseudomonas aeruginosa: Results of a UK Survey and Evaluation," Br. J. Antimicrob. Chemother., vol. 47, pp. 789-799, 2001.

[23] A. T. Olayinka, B. O. Olayinka, and B. A. Onile, "Antibiotic Susceptibility and Plasmid Pattern of Pseudomonas aeruginosa from the Surgical Unit of a University Teaching Hospital in North Central Nigeria," J. Med. Med. Sci., vol. 1, no. 3, pp. 79-83, 2009.

[24] M. Pollack, G. L. Mandell, J. E. Bennett, and R. Dolin, "Pseudomonas aeruginosa," in Principles and Practice of Infectious Diseases, G. L. Mandell, J. E. Bennett, and R. Dolin, Eds. 5th ed., 2000.