THE HISTOPATHOLOGICAL CHANGES IN LIVER TISSUE FOLLOWING INJECTION WITH ANTIBIOTIC-RESISTANT *PSEUDOMONAS AERUGINOSA*

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Abstract
The study was aimed to investigated the e histopathological changes in liver tissue following infection with antibiotic-resistant Pseudomonas aeruginosa, material and method P_. aeruginosa was antibiotic-resistant P. aeruginosa from Al-Mustansiriyah University, College of Science, Microbiology Laboratory. The antibiotic susceptibilityfor pathogens was determined using the Kirby Bauer method , the used antibiotcs are Netilmicin (Net), Ofloxacin (OF), Meropenem (MEM), Chloramphenicol (C), Ciprofloxacin (CIP), Levofloxacin (LE), Tobramycin (TOB) , Amikacin (AK), Norfloxacin (NOR), Cefuroxime (CXM), Cefepime (CPM), Cefoxitin (FOX), Nalidixic acid (NA), and Gentamicin (CN) ة Tetracycline (TE) for in vivo study the study was included two groups each group contain 10 male rats Group 1: The animals were administered distilled water and served as control. Group 2 : all animal were injected by Intraperitoneal injection with 1×10^{8} CFU/mL of P. aeruginosa .Results Pseudomonas aeruginosa isolate demonstrated extensive resistance to entire used antibiotics the histological examination showed Group 1 the liver was normal as normally hepatocytes & normal sinusoid with normal central vein normal portal triad and normally arrangement of cords While the group 2 of liver severe necrosis with atrophy of hepatic cords and disaagrngment of hepatic cords & severe dilation of sinusoids & damage of vascular tunica and severe necrosis with atrophy of hepatic cords and disaagrngment of hepatic cords. Conclusion P. aeruginosa effects of on tissues are closely linked to their resistance to antibiotics. Based on the histological analysis of liver sections, this conclusion was reached in the current investigation.

Keywords: *P. aeruginosa, antibiotic-resistant, histopathological, liver*

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Introduction

Pseudomonas *aeruginosa* is the most common cause of hospital-acquired infections worldwide. The problem of multidrug resistance (MDR) is serious, increasing morbidity and mortality rates among patients, due to its ability to exhibit a variety of potent virulence traits and resistance to multiple antibiotics (Spagnolo et al., 2021). MDR-resistant Pseudomonas aeruginosa isolates are more likely to cause outbreaks because they can colonize and proliferate in environments where other bacteria cannot survive. For an infection to persist and increase the incidence of hospital-acquired infections, the bacteria must be both pathogenic and drug-resistant (Martínez and Baquero, 2002). The pathogen's virulence traits enable it to survive and withstand a variety of environmental stressors, such as the harsh conditions within the patient's body during medication. According to Beceiro et al. (2012), *P.aeruginosa* possesses several genes that enable it to adapt more effectively to its environment. The presence of expulsion systems, synthesis of antibiotic-degrading enzymes, reduced outer membrane permeability, and changes in target have contributed to the bacteria's high resistance to many antimicrobial drugs, although *P. aeruginosa* infection is rarely fatal. Multidrug-resistant have consequently proliferated (Bassetti et al., 2018). Most of these known resistance mechanisms are exhibited by P. aeruginosa through intrinsic chromosomally encoded or genetically acquired resistance features, impeding the main classes of antibiotics, such β-lactams as, aminoglycosides, quinolones, and polymyxins (Bassetti et al., 2018). When the immune response is compromised, a bacterial infection becomes successful. Furthermore, prolonged inflammation in wounds is known to be associated with an ineffective healing process (Thuenauer et al., 2020). By secreting pro apoptotic molecules, which are linked to an persistent inflammation, *P. aeruginosa* may modify the inflammatory response (Trøstrup et al., 2013). Exotoxin A, for instance, alters genes expression in mammalian cells and induces death in macrophages and polymorphonuclear neutrophils (PMNs) (Pena et al., 2019). The cellular components that are produced when PMNs die increase inflammation and cause collateral damage to the host. Similarly, the broken neutrophils will eventually release their pro-inflammatory substance in the absence of macrophages (Parameswaran & Patial, 2010).

which typically result in illness resolution and pathogen eradication. Ironically, tissue injury and bacterial persistence are frequently linked to the robust inflammatory reactions to *P. aeruginosa*. The purpose of the study was to examine the histological alterations in liver tissue after Pseudomonas aeruginosa infection that was resistant to antibiotics (Lin & Kazmierczak et al., 2017). The study's objective is to examine the histological alterations in liver tissue after antibiotic-resistant *P.aeruginosa* infection.

Material and Method

Microorganisms in study

The antibiotic-resistant *P.aeruginosa* was obtained from University of Al-Mustansiriyah University, College of Science, Microbiology Laboratory.

The antibiotic susceptibility

The antibiotic susceptibility for pathogens was determined using the Kirby Bauer method.This method was used to determine the susceptibility of bacteria to Netilmicin (Net), Ofloxacin (OF), Meropenem (MEM), Chloramphenicol (C), Ciprofloxacin (CIP), Cefepime (CPM), Levofloxacin (LE), , Tobramycin (TOB), Norfloxacin (NOR) ,Amikacin (AK), Cefoxitin (FOX), Nalidixic acid (NA), and Gentamicin (CN) Cefuroxime (CXM), Tetracycline (TE) and the result was interpreted according to the guideline of CLSI.

The process of infecting rats with *P. aeruginosa*

P. aeruginosa was grown in tryptic soy broth at 37°C for 24 hours to induce rat infection. The growth concentration was then adjusted to 0.5 by the optical density at 600 nm (OD600), follow centrifugation for 5 minutes at 8000 rpm.

After washing the bacterial cell pellet with a physiological solution and centrifuging it once more at 8000 rpm for five minutes, the pellet was suspended in 10 mL of physiological solution. The turbidity of the suspension was adjusted to 1×10^8 CFU/mL, and it was then injected intraperitoneally (ip) into the experimental animal.(Hasson Al-Husseini et al.,2020).

Experimental Design

The experiment was included 2groups (each group involved 10 Male rat) as following

Group 1: The animals were administered distilled water and served as control.

Group 2 : included (10 rats), 1×10^8 CFU/mL *of P. aeruginosa* was administered via intraperitoneal injection (ip).

Sacrifice animals

Following the conclusion of each seven-day experiment, the animals were dissected. Liver and kidney specimens were taken for the histological examination, fixed for 24 hours in a buffered 10% formaldehyde solution, and then processed into 5 micron-thick paraffin slices. The sections were viewed under a light microscope after being stained with hematoxylin and eosin (Bancroft&Gamble, 2008).

Results and Discussion

The resistance rates of Netilmicin (Net) 100%, Ofloxacin (OF) 100%, Meropenem (MEM) 100%, Chloramphenicol (C)100%, Ciprofloxacin (CIP) 100%, Levofloxacin (LE) 100%, Amikacin (AK) 100%, Tobramycin (TOB) 100%, Norfloxacin (NOR) 100%, Tetracycline (TE) 0%, Cefuroxime (CXM) 100%, Cefepime (CPM) 100%, Cefoxitin (FOX) 100%, 100 و%Nalidixic acid (NA) 100%, and Gentamicin (CN) 100%

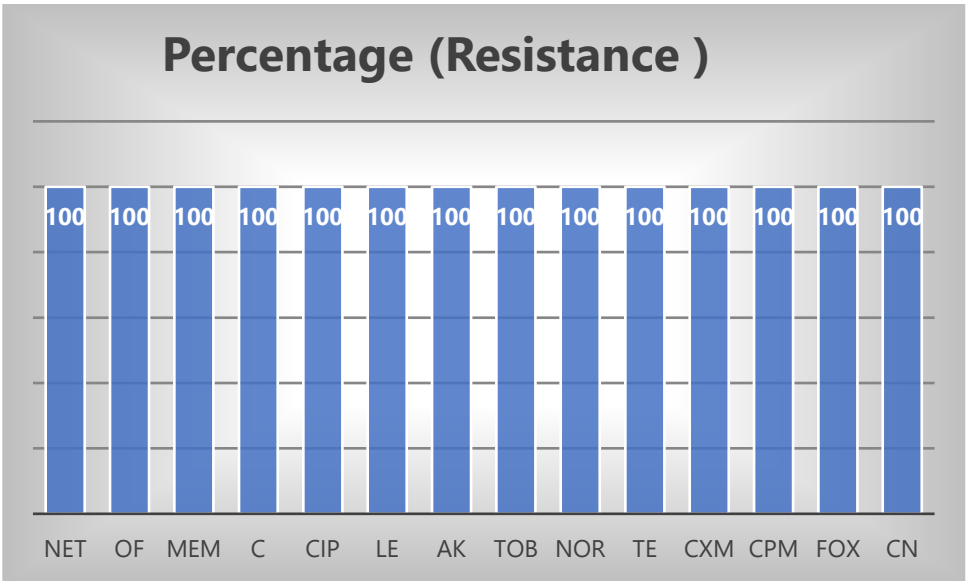

Figure (1): The antibiotic susceptibility of *Pseudomonas aeruginosa*

The bacterial outer membrane, which functions as an efficient barrier to stop antibiotics from reaching their targets in the cell wall, the cytoplasm and cell membrane, is responsible for the high degree of resistance to these antibiotics. Enzymes secreted from the inner membrane that are inactivated can also operate more effectively in the periplasmic region. Variations in outer membrane permeability, especially through modifications in porin channels, are important in many resistance mechanisms. Reduced absorption via the cytoplasmic membrane, reduced across the outer membrane, and active antibiotic efflux out of the cell are mechanisms that lower intracellular antibiotic concentrations (Henwood et al., 2001). Furthermore, the increasing resistance of Pseudomonas aeruginosa to many antibiotics is attributed to several factors, most notably genetic factors that increase its virulence and epidemic potential, making treatment with

conventional methods more challenging. The use of nanoparticles to enhance antimicrobial efficacy and overcome resistance mechanisms is a promising approach in this field, particularly when combined with other chemical agents (Jassem, 2021). This growing resistance underscores the urgent need to research and implement alternative treatment strategies. Interestingly, *P. aeruginosa* has also exhibited adaptive resistance, reacting dynamically to environmental stimuli (Elvadani et al., 2024). Mutational resistance in *P.aeruginosa* is a complex phenomenon that allows it to resist antimicrobial drugs through a variety of mechanisms. Different resistance tactics emerge as a result of these changes, affecting important aspects of bacterial biology (Cervicinska, 2020).

Histological changes

The liver in group 1 had normal hepatocytes and sinusoids as well as a normal central vein with normal portal triad, and a normal cord arrangement (figures 2 A and B). As shown in figure (3 A and B), group 2 of the liver exhibits significant necrosis with atrophy of hepatic cords and disaagrngment of hepatic cords (4) as well as extensive sinusoidal dilatation and vascular tunica damage.

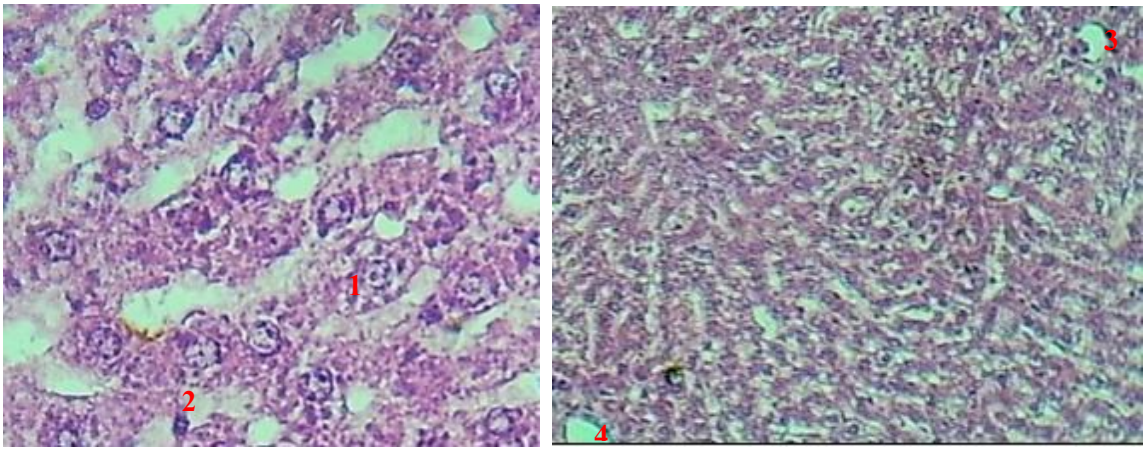

Figure 2: shows liver with normal sinusoid (2), normal hepatocytes (1), normal central vein (3), normal portaltriad (4), and normal cord arrangement (arrow). 100x H&E

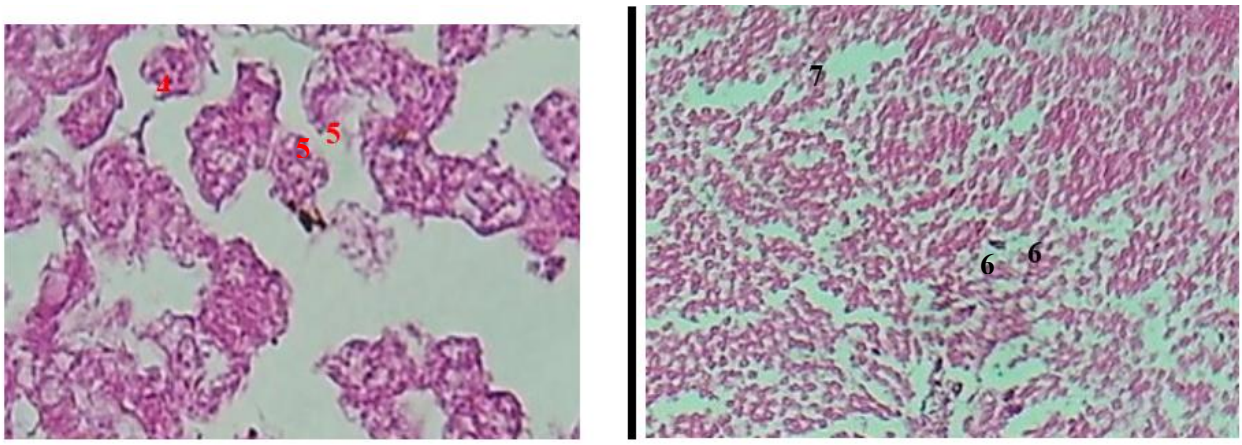

Figure (3) : section of liver severe necrosis with atrophy of hepatic cords and disaagrngment of hepatic cords (4)&severe dilation of sinusoids (5)&damage of vascular tunica (6) and severe necrosis with atrophy of hepatic cords and disaagrngment of hepatic cords (7). H&E.100x

As Chiu et al. (2017) found that *P. aeruginosa* exotoxin A (PEA) induces severe hepatotoxicity in experimental mouse and is helpful in evaluation of immune-mediated liver tissue injuries, the results were consistent with several studies regarding the histological abnormalities in liver. It is yet unclear how different strains of rats react to PEA-induced hepatotoxicity. In this investigation, we assessed the degree of hepatotoxicity caused by PEA in six genetically distinct rat strains. A single intravenous injection of PEA was given to male rats and reported treated rats' histologic liver sections displayed extensive, hepatocyte nuclear condensation , diffuse necrosis, bleeding, and hepatic cord dissociation and There were several mild hepatocyte necrosis in sections and apoptotic bodies.. Additionally, the results corroborated those of (Al-Jubury, et al., 2010), who investigated the toxic effects of *P. aeruginosa* metabolic products on histological aspects in rats. They discovered that the microscopic examination of the organs revealed numerous histopathological alterations brought on by the treatment, including hemorrhage, central vein extension, sinusoidal disarray, degeneration, and hepatocyte necrosis in the liver.

In contrast, Chuang et al. (2007) examined age-related variations in PEA-induced hepatotoxicity in rats and found that several histological changes in liver depending on the rat ages as mild apoptotic hepatocytes in the periportal area, diffuse necrosis, minimal apoptotic hepatocytes, cell swelling and single cell necrosis in the periportal to midzonal regions, numerous apoptotic and single cell necrotic hepatocytes.

Furthermore, Wang et al. (2022) revealed that liver cells in mice infected with *P.aeruginosa* exhibited significant vacuolar degeneration and edema, while Al-Mousawi and Ali (2019) reached similar conclusions. They examined the histological changes in the spleen and liver of carp (*Cyprinus carpio*) infected with *P.aeruginosa* and found that the livers of the antibiotic-treated group during the infection period showed abnormal accumulation of lymphoid follicles, enlarged bile ducts, hepatocyte edema, infiltration of inflammatory cells, and narrowing of the hepatic sinuses , the exotoxin A (PEA), which causes hepatocyte apoptosis, necrosis, bleeding, and sinusoidal disarrangement, is the main way that *P.aeruginosa* causes severe liver injury in rats. Immune responses, such as elevated TNF-alpha, IL-2, and IL-6, mediate this hepatotoxicity and frequently result in elevated serum AST/ALT levels. *P. aeruginosa* lacks several mechanisms that enable highly adapted pathogens to evade or postpone detection by the immune system. Rather, it causes strong inflammatory reactions during an acute infections, which typically result in the removal of the pathogen and the infection's resolution. Ironically, tissue injury and bacterial persistence are frequently linked to significant inflammatory reactions to *P. aeruginosa* (Lin & Kazmierczak, 2017).

Conclusion

The pathogenic effects of the *P. aeruginosa* on tissues are closely linked to their resistance to antibiotics. Based on the histological analysis of liver sections, this conclusion was reached in the current investigation.

References

1. Al-Jubury, N. O., Sahood, A. S.,&Hussein, O. R. (2010). Effectof P. aeruginosa metabolic products on some hematological, biochemical and histological parameters of male rats. *Al-Kufa University Journal for Biology*, *2*(2).
2. Al-Mossawai, O. F.,&Ali, A. H. (2019). Histopathological changes in the liver and spleen of common carp Cyprinus carpio L. challenge with Pseudomonas aeruginosa (Schroeter, 1872) fed with dietary chitosan and ciprofloxacin. *Basrah Journal of Agricultural Sciences*, *32*(2), 193-207.
3. Bancroft, J. D., & Gamble, M. (2008). *Bancroft's theory and practice of histological techniques*. Churchill Livingstone.
4. BassettiM.VenaA.CroxattoA.RighiE.GueryB. (2018). How to manage *Pseudomonas aeruginosa* infections. *Drugs*. *Context*7, 1–18. doi: 10.7573/dic.212527
5. BeceiroA.TomásM.BouG. (2012). Antimicrobial resistance and virulence: a beneficial relationship for the microbial world?*Enferm. Infecc. Microbiol. Clin.*30, 492–499. doi: 10.1016/j.eimc.2012.01.011
6. Chiu, C. C., Wang, Y. C., Huang, W. C., Chen, Y. H., Hung, S. W., Huang, Y. T., ...&Chang, Y. C. (2017). Differences in genetic background contribute to Pseudomonas exotoxin A-induced hepatotoxicity in rats. *Toxins*, *9*(7), 224.
7. Chuang, H. L., Huang, Y. T., Chiu, C. C., Chen, H. H. C., Chu, Y. Y.,&Chen, T. H. (2009). Influence of age on susceptibility to Pseudomonas aeruginosa exotoxin A-induced hepatotoxicity in Long-Evans rats. *Journal of Veterinary Medical Science*, *71*(2), 163-169.
8. Elfadadny, A., Ragab, R. F., AlHarbi, M., Badshah, F., Ibáñez-Arancibia, E., Farag, A., ...&Nageeb, W. M. (2024). Antimicrobial resistance of Pseudomonas aeruginosa: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Frontiers in microbiology*, *15*, 1374466.
9. Hasson Al-Husseini, A. M., Mohammed, G. J.,&Saba Falah, K. (2020, November). Study of the correlation between levels of TNF-α and MCP-1 in plasma and tissues of rats infected with pseudomonas aeruginosa. In *Journal of Physics: Conference Series* (Vol. 1664, No. 1, p. 012117). IOP Publishing.
10. Henwood, C. J., Livermore, D. M., James, D., Warner, M.,&Pseudomonas Study Group, T. (2001). Antimicrobial susceptibility of Pseudomonas aeruginosa: results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disc susceptibility test. *Journal of antimicrobial chemotherapy*, *47*(6), 789-799.
11. Jasim, R. A. (2021). Strategies for challenging development in antimicrobial resistance. *Medical Journal of Babylon*, *18*(3), 172-177.
12. Lin, C. K.,&Kazmierczak, B. I. (2017). Inflammation: a double-edged sword in the response to Pseudomonas aeruginosa infection. *Journal of innate immunity*, *9*(3), 250-261.
13. Lin, C. K.,&Kazmierczak, B. I. (2017). Inflammation: a double-edged sword in the response to Pseudomonas aeruginosa infection. *Journal of innate immunity*, *9*(3), 250-261.
14. MartínezJ. L.BaqueroF. (2002). Interactions among strategies associated with bacterial infection: pathogenicity, Epidemicity, and antibiotic resistance. *Clin. Microbiol. Rev.*15, 647–679. doi: 10.1128/CMR.15.4.647-679.2002
15. Parameswaran, N.,&Patial, S. (2010). Tumor necrosis factor-α signaling in macrophages. *Critical Reviews™ in Eukaryotic Gene Expression*, *20*(2).
16. Pena, R. T., Blasco, L., Ambroa, A., González-Pedrajo, B., Fernández-García, L., López, M., ...&Tomás, M. (2019). Relationship between quorum sensing and secretion systems. *Frontiers in microbiology*, *10*, 1100.
17. Serwecińska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. *Water*, *12*(12), 3313.

SpagnoloA. M.SartiniM.CristinaM. L. (2021). *Pseudomonas aeruginosa* in the healthcare facility setting. *Rev. Med. Microbiol.*32, 169–175. doi: 10.1097/MRM.0000000000000271

18. Thuenauer, R., Landi, A., Trefzer, A., Altmann, S., Wehrum, S., Eierhoff, T., ...&Römer, W. (2020). The Pseudomonas aeruginosa lectin LecB causes integrin internalization and inhibits epithelial wound healing. *MBio*, *11*(2), 10-1128.
19. Trøstrup, H., Thomsen, K., Christophersen, L. J., Hougen, H. P., Bjarnsholt, T., Jensen, P. Ø., ...&Moser, C. (2013). P seudomonas aeruginosa biofilm aggravates skin inflammatory response in BALB/c mice in a novel chronic wound model. *Wound Repair and Regeneration*, *21*(2), 292-299.
20. Wang, Y., Jian, S., Li, W., Zhao, L., Ye, G., Shi, F., ...&Tang, H. (2022). Epigallocatechin-3-gallate ameliorates liver injury secondary to Pseudomonas aeruginosa pneumonia. *International Immunopharmacology*, *112*, 109239.