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#### **Original Research Article**

# **Evaluation Effect on Lungs Tissue in Mice (Mus Masculus) of Infected with Klebsiella Pneumonia and Therapeutied by Azithromycin**

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**Abstract:** Klebsiella pneumoniae is a mainly cause of severe respiratory infections which may leading to a chronic inflammation or to death, Azithromycin has recently received a lot of scientific attention due to its outstanding effectiveness in treating Other bacterial and some parasitic infections, because it is widely and safe for human use and is believed to be associated with side effects fewer compared with the other macrolide. The current study aims to isolation K. pneumonia from respiratory infection and evaluation histological changes on lungs and possible protective role by azithromycin and of VIT. C, included isolation K.pneumenia from diagnosed patients with respiratory diseases from Samarra hospital at January to April 2024. All sputum samples were cultured by using selective media for K.pneumonia and Vitic device. In this study used 15 mice which randomly divided to three groups: The first group no treatment, second group treated with 30mg/kg of azithromycin, while third group treated with 30mg/kg from azithromycin plus 20mg/kg of vitamin C. In the results included morphologic the microscopic and biochemical examinations to identify of klebsiella and the histological changes of the lung tissue which included hemolysis in different area of tissue, thickening of the alveoli walls and infiltration of neutrophil and inflammation cells that showed in sections of groups which treated with k.pneumonia, while azithromycin and vitamin were worked on repair abnormalities and the pathologic changes on lung tissue.

**Keywords:** Lung, k. Pneumonia, Azithromycin, vitaminC.

### Introduction

Klebsiella pneumoniae (K. pneumoniae) is a gram negative and an life-threatening illnesses are often caused by opportunistic pathogens and nosocomial infections for example a pneumonia, infection of bloodstream, and the urinary tract infection, in the immune compromised patients so as in the healthy persons [1, 2]. The development of medical therapeutic strategies in so as to classical antibiotic therapies, Klebsiella pneumonia is becoming a more significant global health concern due to the emergence of resistant of drug by produces of carbapenemase and by  $\beta$ -lactamases enzymes [3–5]. Additionally, multidrug-resistant Klebsiella pneumonia strains are becoming with a limited clinical treatment option [6–8].

Acute lung diseases which caused by Gram-negative bacteria such as K.pneumoniae which has mortality with highly rates during absence of treatment [9]. But in the same time not provide protection against organs which may harmful side which may came from the active immunologic response [10]. Use antibiotics or anti-inflammatory may be the good options to containing the infection agents addition to working on the harmful of the over stimulated immune response [11, 12], but newly and because of the side damages of antibiotics and the problem of the antibiotic resistance, the medicine became toward complementary methods of the medicine [13 -15].

Azithromycin is a widely and effectively antibiotic, are the most common antibiotics used to treat bacterial infection [16]. Also it has over effects on some chronic diseases and on the host defense reactions [17]. Because has role

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in activite genes which virus recognition such as MDA5 and RIG-I, addition to improve immune response against viruses by increased regulating the production of type I and III interferons, therefore, it is a good choice to response in infectious agents such as in the last years used against COVID-19, and for respiratory support during hospitale treatment (hospitalization) [18, 19].

VIT. C plays the important role in the immune-regulating system, and plays important factor in protect body from any oxidant damage [20].

# MATERIAL AND METHODS

Collected 30 samples of sputum from patients with respiratory diseases after were diagnosed by a specialist doctor at Samarra hospital from January to April 2024. The sputum samples were then cultured on some the differential and selective media at 37° for 24 hours after growth were isolated and identified according to microscopic, biochemical examination and diagnosed by used vitech device.

In this study (15) mice Mus masculus were used with weight from 25 to 30 gram, the experimental mice divided into three groups, five mice in each all. The mice at all groups were given klebsiella pneumenia intranasally in a volume (50)  $\mu$ L and were kept in upright without anaesthesia, animals were stayed for at nearly two minutes to allow allow proper transfer of inoculum in to the respiratory passage until lungs. A The animals kept in free water and diet with temperature at (22-26)° during the experiment days. After 12 hours of the infection, the first group without any treated, the second group treated with (30 mv/kg) single dose of azithromycin orally daily for 10 days, and last group treated with same dose of azithromycin addition to vitamin C (20 mg/kg) orally daily [21]. After 24 hours of end the experiment mice anesthetized then sacrificed and lungs were taken. To quantitative the K.pneumonia count in this study, the lungs were serial dilutions of the lung tissue were made, then planted on Macconkey agar and incubated at 37°C for 24 hours.

The lung tissue was prepared for the histological and microscopical examination according to [22], then were stained by used hematoxyline and eosin (H&E) stain.

## **RESULTS AND DISCUSSION**

In this study, K. Pneumonia was isolated from sputum samples according to morphologic cultural which showed mucoid pink colored colonies in MacConkey agar media and showed blue colored colonies in hichrom agar (figure 1).

Bacterial count in lungs of the mice infected with K. pneumoniae and treated with azithromycin or/and vitaminC both second and third groups after 15 day infection day as in (figure 2) showed decrease in the counts of the klebsiella compared with the first group. That results agree with [23, 24]. That azithromycin and vitaminC working as factors to decreased of the lung inflammation in mice treated after infection.

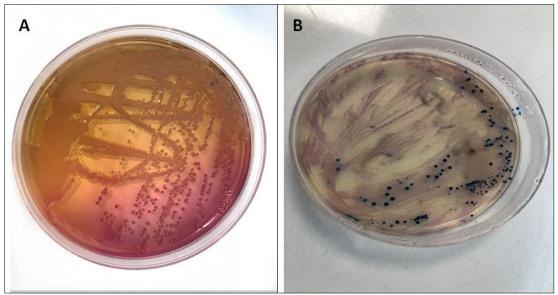


Figure 1A: Mucoud pink colonies in MacConkey agar, B: blue colonies in hichrom agar of the K. Pneumonia

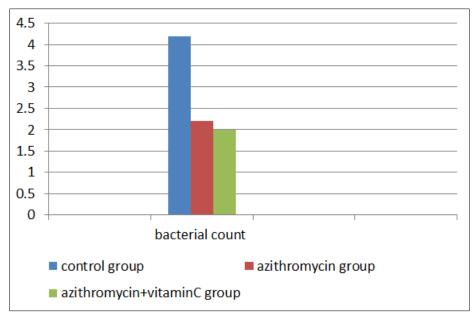


Figure 2: Bacterial count of the lungs infection with K.pneumonia.

The histopathological results of lungs tissue examination showed that the first group included hemolysis in different area of tissue addition to thickening in walls of alveoli, as founded alveoli filled with a lot of neutrophils and inflammation cells infiltration (Figure 3A), compared with the other groups. The second group which treated by azithromycin alone (figure 3B) showed inflammation cells fewer than first group. The third group showed improvement in the histological structure of lung (figure 3C).

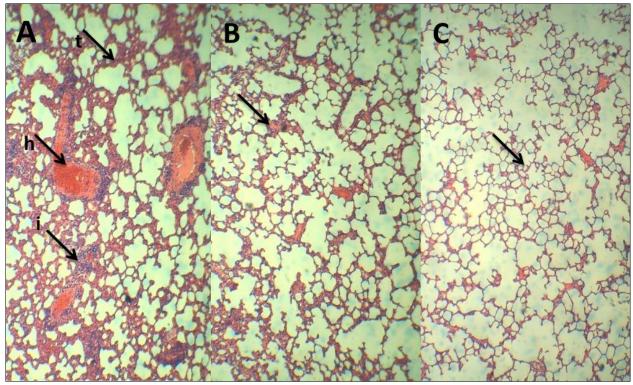


Figure 3A: showed inflammation cells infiltration (i), hemolysis (h), thickness in walls of alveoli (t). (B): inflammation cells infiltration fewer than first group (C): Walls of alveoli. H&E stain (10x)

The current study, mice infectioned with klebsiella showed some histopathological changes such as thickening of the alveolar wall in the lung and that consistent with [25], addition to hemolysis in different area of tissue and increase in neutrophil with inflammation cells, this may be caused by toxic enzymes of k.Pneumonia which increase formation of free

radical which damage proteins, cell membrane and damage of blood vessels, and caused by stimulates of the inflammatory response with chemoattractive factors which Initially related into neutrophil cells [26, 27].

The current results showed that the treatment with azithromycin and vitaminC helped greatly in reducing bacterial numbers in the lung and reducing the histopathological effects of the lung, because the protective effect of azithromycin and anti oxidant role for vitaminC.

Azithromycin used as the first choice to treatment for pneumonia over 15 years and it is recommended in a lot of countries as an important part of treatment [18].

# **CONCLUSION**

Azithromycin given a good effect on lung infected with K.pneumonia, additional to the positive role of vitaminC along with antibiotic against inflammation.

# REFERENCES

- 1. Ahmad, T. A., El-Sayed, L. H., Haroun, M., Hussein, A. A., & El Sayed, H. (2012). Development of immunization trials against Klebsiella pneumoniae. *Vaccine*, *30*(14), 2411-2420.
- 2. Rosen, D. A., Hilliard, J. K., Tiemann, K. M., Todd, E. M., Morley, S. C., & Hunstad, D. A. (2016). Klebsiella pneumoniae FimK promotes virulence in murine pneumonia. *The Journal of infectious diseases*, 213(4), 649-658.
- 3. Munoz-Price, L. S., Poirel, L., Bonomo, R. A., Schwaber, M. J., Daikos, G. L., Cormican, M., ... & Quinn, J. P. (2013). Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *The Lancet infectious diseases*, 13(9), 785-796.
- 4. Paterson, D. L., Ko, W. C., Von Gottberg, A., Mohapatra, S., Casellas, J. M., Goossens, H., ... & Yu, V. L. (2004). Antibiotic therapy for Klebsiella pneumoniae bacteremia: implications of production of extended-spectrum β-lactamases. *Clinical infectious diseases*, 39(1), 31-37.
- 5. Santino, I., Bono, S., Nuccitelli, A., Martinelli, D., Petrucci, C., & Alari, A. (2013). Microbiological and molecular characterization of extreme drug-resistant carbapenemase-producing Klebsiella pneumoniae isolates. *International Journal of Immunopathology and Pharmacology*, 26(3), 785-790.
- 6. Arnold, R. S., Thom, K. A., Sharma, S., Phillips, M., Johnson, J. K., & Morgan, D. J. (2011). Emergence of Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria. *Southern medical journal*, 104(1), 40.
- 7. Rapp, R. P., & Urban, C. (2012). Klebsiella pneumoniae carbapenemases in Enterobacteriaceae: history, evolution, and microbiology concerns. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 32(5), 399-407.
- 8. da Silva, R. M., Traebert, J., & Galato, D. (2012). Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae: a review of epidemiological and clinical aspects. *Expert opinion on biological therapy*, 12(6), 663-671.
- 9. Bhatia, M., & Moochhala, S. (2004). Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, 202(2), 145-156.
- 10. Woo, P. C., Lau, S. K., & Yuen, K. Y. (2002). Macrolides as immunomodulatory agents. *Current Medicinal Chemistry-Anti-Inflammatory & Anti-Allergy Agents*, 1(2), 131-141.
- 11. Hand, W. L., Hand, D. L., & King-Thompson, N. L. (1990). Antibiotic inhibition of the respiratory burst response in human polymorphonuclear leukocytes. *Antimicrobial agents and chemotherapy*, *34*(5), 863-870.
- 12. Mikasa, K., Kita, E., Sawaki, M., Kunimatsu, M., Hamada, K., Konishi, M., ... & Narita, N. (1992). The antiinflammatory effect of erythromycin in zymosan-induced peritonitis of mice. *Journal of Antimicrobial Chemotherapy*, 30(3), 339-348.
- 13. Huang, M. T., Lou, Y. R., Ma, W., Newmark, H. L., Reuhl, K. R., & Conney, A. H. (1994). Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer research*, *54*(22), 5841-5847.
- 14. Huang, M. T., Newmark, H. L., & Frenkel, K. (1997). Inhibitory effects of curcumin on tumorigenesis in mice. *Journal of Cellular Biochemistry*, 67(S27), 26-34.
- 15. Kawamori, T., Lubet, R., Steele, V. E., Kelloff, G. J., Kaskey, R. B., Rao, C. V., & Reddy, B. S. (1999). Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer research*, *59*(3), 597-601.
- 16. McMullan, B. J., & Mostaghim, M. (2015). Prescribing azithromycin. Australian prescriber, 38(3), 87.
- 17. Parnham, M. J., Haber, V. E., Giamarellos-Bourboulis, E. J., Perletti, G., Verleden, G. M., & Vos, R. (2014). Azithromycin: mechanisms of action and their relevance for clinical applications. *Pharmacology & therapeutics*, 143(2), 225-245.
- 18. Fohner, A. E., Sparreboom, A., Altman, R. B., & Klein, T. E. (2017). PharmGKB summary: macrolide antibiotic pathway, pharmacokinetics/pharmacodynamics. *Pharmacogenetics and genomics*, 27(4), 164-167.
- 19. Martín-Loeches, I., Bermejo-Martin, J. F., & Vallés, J. (2013). Macrolide-based regimens in absence of bacterial co-infection in critically ill H1N1 patients with primary viral pneumonia. *Intensive Care Med*, 39(4), 693–702.

- 20. Padhani, Z. A., Moazzam, Z., Ashraf, A., Bilal, H., Salam, R. A., Das, J. K., & Bhutta, Z. A. (2020). Vitamin C supplementation for prevention and treatment of pneumonia. *Cochrane Database of Systematic Reviews*, (4).
- 21. Tsubouchi, K., Araya, J., Minagawa, S., Hara, H., Ichikawa, A., Saito, N., ... & Kuwano, K. (2017). Azithromycin attenuates myofibroblast differentiation and lung fibrosis development through proteasomal degradation of NOX4. *Autophagy*, *13*(8), 1420-1434.
- 22. Bancroft, J. D., & Stevens, A. (1990). Theory and practice of histological techniques, n616.07583 T4.
- 23. Bansal, S., & Chhibber, S. (2014). Phytochemical-induced reduction of pulmonary inflammation during Klebsiella pneumoniae lung infection in mice. *The Journal of Infection in Developing Countries*, 8(07), 838-844.
- 24. El-Naeem, A., Abeer, F., Abouelella, A., & Baset, A. S. (2022). Evaluation of Effect of Azithromycin on The Heart of Adult Male Albino Rats and the Possible Protective role of VIT. C (Histological and Immune-histochemical Study). SVU-International Journal of Medical Sciences, 5(2), 518-532.
- 25. Zhou, H. X., Ning, G. Z., Feng, S. Q., Jia, H. W., Liu, Y., & Feng, H. Y. (2013). Cryptococcosis of lumbar vertebra in a patient with rheumatoid arthritis and scleroderma: case report and literature review. *BMC Infectious Diseases*, 13, 1-7.
- 26. El-Shitany, N. A., & El-Desoky, K. (2016). Protective effects of carvedilol and vitamin c against azithromycin-induced cardiotoxicity in rats via decreasing ROS, IL1-β, and TNF-α production and inhibiting NF-κB and caspase-3 expression. *Oxidative medicine and cellular longevity*, 2016(1), 1874762.
- 27. Mansour, B. S., Salem, N. A., Kader, G. A., Abdel-Alrahman, G., & Mahmoud, O. M. (2021). Protective effect of Rosuvastatin on Azithromycin induced cardiotoxicity in a rat model. *Life sciences*, 269, 119099.