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Original Research Article

Isolation and Identification *Klebsiella pneumoniae* and Use as Microbial Source for Extract Enzyme Arginine Deiminase from Respiratory Infections in Sheep at Babylon City, Iraq

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Abstract: This study was aimed to isolate and identify of *Klebsiella pneumonia* and testing the production of Arginine deiminase (ADI) purified from *Klebsiella pneumonia* in vitro, 30 samples were collected from sheep infected with respiratory infections from veterinary clinics and hospitals from October to April 2024 then transported by transport media to the laboratory for culture and microbial examine. Out of the 21 samples, 13 (61.9%) showed bacterial growth, with the majority being Gram-negative bacteria. The remaining 8 samples (38%) were gram-positive. The analysis revealed that *Klebsiella pneumoniae* was the most identified bacterial strain. Out of the total bacteria used, 7 (53.8%) were identified as gram-negative bacteria, while the remaining were of different types. This study identified the enzyme arginine deiminase from 7 clinical isolates of *Klebsiella pneumonia* whereby the enzyme was extracted and purified. The objective was to quantify K. pneumonia for this enzyme production capacity to measure its overall capability on subsequent phases via screening. This screening method was based on the measurement of the precise arginine deiminase activity using spectrophotometric assay. The specific activity of purified enzyme was 10 U/min with approximate molecular weight 120 kDa and the isolate K. pneumonia N7 was found most effective in production of the enzyme.

Keywords: Bacteria, *Klebsiella pneumonia*, arginine deiminase, spectrophotometer.

INTRODUCTION

Klebsiella spp., particularly K. pneumoniae, are of great importance as a cause of Respiratory Tract Infection especially the ones that are acquired in hospitals. *Klebsiella pneumoniae* is able to adhere to host cells due to its capsular polysaccharides that form its outer surface layer; also practical to note that capsular polysaccharide is one of the major factors that make K. pneumoniae to be virulent (Harada *et al.,* 2016). Further, it may result in infections in companions and humans, which are zoonotic, meaning they start within the community. And it is the second most common species of Enterobacteriaceae that causes respiratory and urinary tract infections (UTI) in mammals (Marques *et al.,* 2018, Mazinani and Rude. 2020, Berihulay *et al.,* 2019). Pneumonia is a persistent problem that affects the well-being of small ruminants and ultimately contributes to adverse, long-term effects in their overall quality of life. This phenomenon is a result of a multi-factorial process involving host mol biology, microbes, the environment, and deficient bio-social measures (Ghanem 2015). Klebsiella pneumoniae is a potent pathogen responsible for pneumatic infections in small ruminants especially in sheep and goats (Rajashekar *et al.,* 2023) Basing, as is gaining global recognition due to the rise of severe infections, antibiotic resistance, and forming of biofilms. Solbergs, Ragogning, and Hartz (2018) indicate that these factors make it difficult for effective treatment interventions to be established. The increased antibiotic inability to kill bacteria irrespective of being G+ or G- reduces the applicability of conventional therapeutic protocols (Wijesingh, *et al.,* 2021).

Renewable resources create enzymes that are both biocompatible and biodegradable. They serve as catalysts processes within the organism. Moreover, it is critical for the organism's survival, and enzymes play an important role in

facilitating life processes in all forms of life, ranging from viruses to humans (Sheldon *et al.*, 2013; Yalcin, 2014). Tumor cells have a high need for arginine, which promotes tumor development, leading to the exploration of arginine deiminase as a potential anticancer drug (Choi., 2012, Xiong, 2014). The objective of this study is to identify and diagnose Klebsiella pneumonia bacteria that contribute to respiratory inflammation in sheep. Additionally, we aim to characterize and extract the arginine deiminase enzyme in a laboratory setting by measuring the specific activity of the enzyme using spectrophotometry.

METHODOLOGY

Sample Collection

A total of 30 nasal swabs were collected from sheep infected with respiratory infections from veterinary clinics and hospitals in Babil /Iraq. Samples were collected from different age groups and sex from October to April 2024.

A Laboratory-Prepared Media:

This study utilized various agricultural media as per the instructions provided by their respective manufacturers. The culture media types were prepared by sterilizing them at a temperature of 121 °C for 15 minutes. Subsequently, they were incubated at 37 ℃ for 24 hr. The pH was then adjusted to a value of 7.

Laboratory Diagnosis of Isolates:

Once we obtained the bacterial isolates, we performed the diagnosis through microscopic examination and Gram-stain staining. This allowed for the observation of the specific shape and colors, as well as the identification of the bacteria using biochemical tests and the VITEK2 system (bioMerieux, France), following the instructions.

Examine the Capacity of K. pneumonia to Produce Arginine Deiminase:

Each isolate of K. pneumonia was streaked onto a nutrient agar medium and then incubated at 37 °C for 24 hrs. Subsequently, a solitary colony was selected and positioned the central region of the M-9 mm. Then, set the plate in an incubator at 37°C for 48 hours. Then used a precise technique to determine the activity of isolates that generate arginine deiminase. We used spectrophotometry to quantitatively screen the synthesis of L. citrulline from arginine.

Statistical Analysis:

The statistical analysis was performed using SPSS 23. The statistical differences across distinct groups were determined using the Pearson chi-square test (Team R C, R Foundation for Statistical Computing).

RESULTS

The results show in table (1), 21(70%) sample were positive bacterial growth, most of it 13 (61.9%) were Gram negative bacteria, while the remaining 8 (38%) were Gram positive. Results showed the most common bacterial isolates were Klebsiella pneumonia 7(53.8%) as Gram-negative bacteria, table (2), the colonies with pink color and mucous texture on MacConkey agar while on blood agar appears pale and gave gamma-Heamolysis result, figure (1A, B), and the others different type of bacteria, 7 Klebsiella pneumonia clinical isolate were used to extract and purify the enzyme arginine deiminase then screening the ability of K. pneumonie in production enzyme by quantitative screening which depends on the determination of specific activity of arginine deiminase by used spectrophotometer. Table (3) show that all isolates of K. pneumonia were arginine deiminase producers with variable degree. The specific activity of purified enzyme reached 10 U/mg and the isolate K. pneumonia N5 was the best isolate in production of enzyme as well as selected for further studies of arginine deiminase production and application.

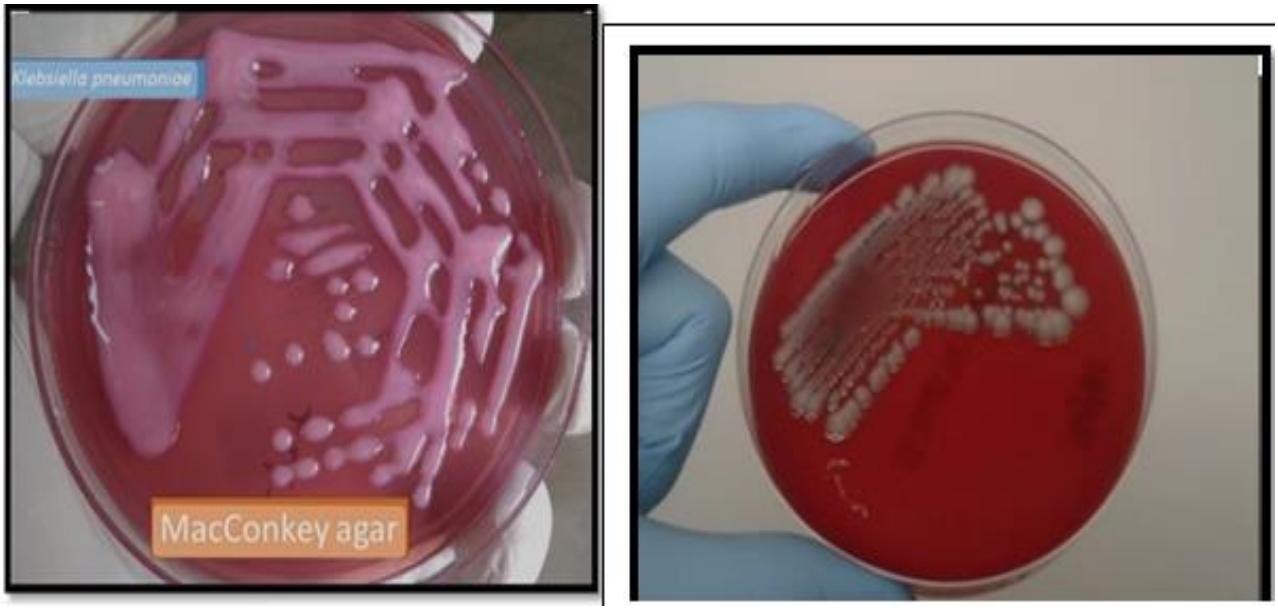
Table 1: Isolate distribution based on bacterial growth, categorized as positive or negative

No. of culture	G- culture	G+ culture
30	21	9
100 %	(70%)	(30%)

Table 2: Distribution of bacterial isolates

Type of bacterial isolates	Total
Gram negative bacteria	
klebsiella pneumoniae	7(53.8%)
Pseudomonase aeruginosa	3(23%)
Enterobacter aerugenos	2(15.3)
Serratia spp.	1 (7.6%)
Total	**13 (61.9%)**

Type of bacterial isolates	Total
Gram positive bacteria	
Streptococcus pneumonia	4 (50%)
Staphylococcus aureus	2 (25%)
haemophilus influenzae	1 (12.5%)
neisseria meningitidis	1 (12.5%)
Total	**8 (38.%)**



A B

Figure 1, A: Mucoid colonies of *K. pneumoniae*; B: Colonies of *K. pneumoniae* on blood agar at 37 C° for 24 hrs.

Table 3: Quantitative screening of isolates producing arginine deiminase

Isolate number	Enzyme specific activity(U/mg)
N1	1
N2	6.9
N3	3
N4	4
N5	5
N6	3.5
N7	10

DISCUSSION

The latest findings revealed that K. pneumoniae infected 53.8% of the examined animals. A prior study in Egypt revealed that K. pneumoniae infected 36% of pneumonic sheep and goats (Ali and Abu-Zaid 2019.) Nevertheless, small ruminants in Egypt exhibited infection rates of 27.15% and 13.39% specifically in cases when respiratory symptoms were present (Fouad *et al.*, 2022) and Nigeria (Ugochukwu *et al.*, 2017). Our study found a higher infection rate of K. pneumoniae in sheep. Nevertheless, these variations did not achieve statistical significance, aligning with the results documented by (Zaghawa and El-Sify, 2010; Kattimani *et al.*, 2020). The weak immune system, heightened vulnerability to transportation stress, abrupt environmental changes, and viral infections can link to the greater susceptibility of young animals. These variables all render them more susceptible to infection (Pavan *et al.*, 2021; Makani *et al.*, 2023). The findings of our study demonstrated that all isolates of Pneumonia exhibited the production of arginine deiminase, albeit with varying degrees. The isolate labeled as No.7 had the highest efficiency in producing arginine deiminase, as evidenced by a specific activity of 10 U/mg in the crude filtrate (Wijesinghe *et al.*, 2021). An example of a change is citrullination, which is facilitated by the protein arginine deiminases (PADs), a distinct group of enzymes (Wu *et al.*, 2011; Rafeeq and Sharba 2022).

CONCLUSION

Klebsiella pneumoniae isolates were cultured and isolated from clinical samples, and the identification of K. pneumoniae was performed by culture examination. Assessing the capacity of K. pneumonia to produce arginine deiminase and quantifying the specific activity and protein content of the enzyme using a spectrophotometer.

Acknowledgments: The authors declare that no competing exist**.**

Conflict of Interest: None.

REFERENCES

- Harada, K., Shimizu, T., Mukai, Y., Kuwajima, K., Sato, T., Usui, M., Tamura, Y., Kimura, Y., Miyamoto, T., Tsuyuki, Y., Ohki, A., & Kataoka, Y. (2016). Phenotypic and molecular characterization of antimicrobial resistance in Klebsiella spp. isolates from companion animals in Japan: clonal dissemination of multidrug-resistant extended-spectrum β-lactamase-producing Klebsiella pneumoniae. *Front Microbiol, 7*, 1021.
- Marques, C., Belas, A., Franco, A., Aboim, C., Gama, L. T., & Pomba, C. (2018). Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. *J Antimicrob Chemother, 73*, 377–384.
- Mazinani, M., & Rude, B. (2020). Population, world production and quality of sheep and goat products. *Am. J. Anim. Vet. Sci, 15*, 291–299. Berihulay, H., Abied, A., He, X., Jiang, L., & Ma, Y. (2019). Adaptation mechanisms of small ruminants to environmental heat stress. *Animals, 9*, 75.
- Ghanem, M., Yousif, H., Abd El-Ghany, A., Abd El-Raof, Y., & El-Attar, H. (2015). Evaluation of pulmonary function tests with hemato-biochemical alterations in Boer goats affected with Klebsiella pneumoniae. *Benha Vet. Med. J., 29*, 53–62.
- Franco, M. F., Gaeta, N. C., Alemán, M. A., Mellville, P. A., Timenetsky, J., Balaro, M. F., & Gregory, L. (2019). Bacteria isolated from the lower respiratory tract of sheep and their relationship to clinical signs of sheep respiratory disease. *Pesqui. Veterinária Bras, 39*, 796–801.
- Rajashekar, B., Shivajyothi, J., Reddy, Y. N., & Putty, K. (2023). Characterization of bacterial pathogens involved in pneumonia of sheep and Goats. *Indian. J. Small Rumin, 29*, 89–93.
- Wijesinghe, G. K., Feiria, S. B., Maia, F. C., Oliveira, T. R., Joia, F., Barbosa, J. P., Boni, G. C., & HÖfling, J. F. (2021). In-vitro antibacterial and antibiofilm activity of Cinnamomum verum leaf oil against Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae. *An. Da Acad. Bras. De Ciências, 93*, e20201507.
- Sheldon, R. A., & van Pelt, S. (2013). Enzyme immobilization in biocatalysis: Why, what and how. *Chemical Society Reviews, 42*, 6223-6235.
- Shibatani, T., Kakimoto, T., & Chibata, I. (1975). Crystallization and properties of L-arginine deiminase of Pseudomonas putida. *The Journal of Biological Chemistry, 250*, 4580–4583.
- YalcinCakir, F., Ergin, E., Gurgan, S., Sabuncuoglu, S., SahinArpa, C., Tokgoz, I., Ozgunes, H., & Kiremitci, A. (2014). Effect of Bleaching on Mercury Release from Amalgam Fillings and Antioxidant Enzyme Activities: A Pilot Study. *Journal of Esthetic and Restorative Dentistry. Iences, 369*, 4295–4315.
- Xiong, L., Teng, J. L., Watt, R. M., Kan, B., Lau, S. K., & Woo, P. C. (2014). Arginine deiminase pathway is far more important than urease for acid resistance and intracellular survival in Laribacter hongkongensis: A possible result of arc gene cassette duplication. *BMC Microbiology, 14*.
- Team, R. C. R. (2015). Foundation for Statistical Computing; Vienna, Austria: 2014. R: a language and environment for statistical computing, p. 2013.
- Ali, A., & Abu-Zaid, K. (2019). Study on Klebsiella pneumoniae causing respiratory infection in small ruminants. *Anim. Health Res. J., 7*, 57–67.
- Fouad, E. A., Khalaf, D. D., Farahat, E., & Hakim, A. S. (2022). Identification of predominant pathogenic bacteria isolated from respiratory manifested small ruminants in western north Egypt with regard to their susceptibility to antibiotics. *Int. J. Health Sci, 6*, 10818–10828
- Ugochukwu, I. C., Aneke, C. I., Ezeasor, C. K., Msheila, W. P., Idoko, S., Kwabugge, A., Shoyinka, S. V. O., Chineme, C. N., Chah, K. F., & Ugochukwu, E. I. (2017). Pathomorphology and aerobic bacteria associated with pneumonia in small ruminants slaughtered at the Nsukka abattoir. *Anim. Res. Int., 14*, 2644–2651.
- SifyZaghawa, A., & El-Sify, A. (2010). Epidemiological and clinical studies on respiratory affections of sheep. *Minufiya Vet. J., 7*, 93–103. Kattimani, T. S., Ravindra, B., Vivek, R., Halmandge, S., & Patil, N. (2020). Prevalence of pneumonia in goats in and around the Bidar. *Pharma Innov. J., 9*, 250–252.
- Pavan Kumar, C., Ramesh, P., Sundar, N., & Veere Gowda, B. (2021). Epidemiological studies on sheep bacterial respiratory tract infections in and around proddatur, YSR Kadapa, Andhra Pradesh. *Pharma Innov. J., 10*, 629–632.
- Makani, K. C., Kumar, V. A., Kumar, K. S., & Kumar, A. V. (2023). Prevalence of bacterial pneumonia in Sheep in and around Hyderabad, Telangana. *Pharma Innov. J, 12*, 1695–1697.

- Wijesinghe, G. K., Feiria, S. B., Maia, F. C., Oliveira, T. R., Joia, F., Barbosa, J. P., Boni, G. C., & HÖfling, J. F. (2021). In-vitro antibacterial and antibiofilm activity of Cinnamomum verum leaf oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. *An. Da Acad. Bras. De Ciências, 93,* e20201507.
- Wu, M.-C., Lin, T.-L., Hsieh, P.-F., Yang, H.-C., & Wang, J.-T. (2011). Isolation of genes involved in biofilm formation of a *Klebsiella pneumoniae* strain causing pyogenic liver abscess. *PLoS ONE, 6*, e23500. Rafeeq, H., & Sharba, Z. (2022). Study the effect of cinnamon and tea tree oils on biofilm formation of *Klebsiella pneumoniae*. *J. Appl. Sci. Nanotechnol, 2*, 16–26.