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 specifically used 7 clinical isolates of Klebsiella pneumonia to extract and purify the enzyme arginine deiminase. The goal was to assess K. pneumonia ability to produce this enzyme through quantitative screening. This screening method relied on determining the specific activity of arginine deiminase using a spectrophotometer. The specific activity of purified enzyme reached 10 U/mg and the isolate *K. pneumonia* N7 was the best isolate in production of enzyme.

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

INTRODUCTION

Klebsiella spp., namely Klebsiella pneumoniae, are significant contributors to respiratory inflammation and infections acquired in healthcare settings. The presence of capsular polysaccharide, a key virulence factor on the surface of Klebsiella pneumoniae, primarily contributes to the development of the disease (Harada *et al*., 2016). Additionally, it can lead to infections in both companion animals and people that originate within the community. It is the second most prevalent species of Enterobacteriaceae responsible for respiratory and urinary tract infections (UTI) in mammals (Marques *et al*., 2018, Mazinani and Rude, 2020, Berihulay *et al.,* 2019). Pneumonia is a chronic issue that impairs the welfare of small ruminants, resulting in lasting consequences and a general deterioration in their quality of life. This phenomenon is caused by an intricate interaction of elements, including host biology, diverse agents such as microbes, environmental conditions, and inadequate biosecurity (Ghanem 2015). Klebsiella pneumoniae is a highly pathogenic bacteria frequently linked to pneumonia in sheep and goats (Rajashekar *et al*., 2023). As well as is gaining global recognition because of the rising prevalence of severe infections, antibiotic resistance, and the creation of biofilms. These factors pose significant obstacles to the development of successful treatment strategies (Franco *et al.,* 2019). The escalating antibiotic resistance observed in both Gram-positive G+ and G- bacteria requires the use of alternate therapeutic techniques (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 °C 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 (1 A, 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. %)

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

Figure 1A: Mucoid colonies of *K. pneumoniae*

Figure 1B: Colonies of *K. pneumoniae* on blood agar at 37 Cº for 24hrs

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.

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