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Original Article

Immune Response to Hypervirulent *Klebsiella pneumoniae* Serotypes K1 and K2 in Animal Models

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ABSTRACT

Hypervirulent *Klebsiella pneumonia* (HvKp) is a globally emerging pathogen distinguished by severe clinical manifestations, including respiratory and systemic infections. Different classical *K. pneumoniae* (CKp),hvKp is associated with capsular serotypes K1 and K2, hypermucoviscosity, and enhanced virulence factors, posing significant public health risks. Considerate host immune responses to hvKp is critical for elucidating its pathogenicity. This study aimed to characterize the prevalence of K1/K2 serotypes in clinical isolates from humans and sheep, assess virulence phenotypes, and evaluate cytokine responses (IFN-γ and IL-10) ng/ml in rats infected with *HvK pneumonia*. A total of 300 samples (150 human sputum, 150 sheep nasal swabs) were collected. Isolates were identified using microbiological, biochemical (Vitek-2), and molecular methods (*16S rRNA* PCR). Hypermucoviscosity was confirmed via string test. Capsular serotyping (K1/K2) was performed using *magA and K2*-specific PCR. Forty rats were divided into four groups: control (PBS) and infected (HvKp ATCC® 43816, 1×10^8 CFU) via IN or SC routes. IFN-γ and IL-10 ng/ml levels were measured post-infection. Among 42 *K. pneumoniae* isolates (25 human17 sheep), 19.04% harbored K1/K2 serotypes. The string test identified isolates as hypermucoviscous. Infected via the IN route exhibited significantly elevated IFN-γ levels (2.48 ± 0.47 ng/mL vs. control 0.50 ± 0.23 ng/mL, P<0.01), while SC infection showed a moderate increase (1.89 ± 0.14 ng/mL, P<0.0001). IL-10 levels showed no significant differences across groups. hvKp serotypes K1/K2 trigger a robust pro-inflammatory immune response, evidenced by elevated IFN-γ, particularly following intranasal infection. The persistence of IL-10 suggests a regulatory mechanism to mitigate inflammation.

Keywords: Immune response, hypervirulent Klebsiella pneumoniae, Rat model, Hypermucoviscosity, Intranasal infection, Subcutaneous infection.

1 Introduction

Klebsiella pneumoniae is a Gram-negative, non-motile, rod-shaped bacterium known as an opportunistic pathogen responsible for various respiratory infections [1]. *Hypervirulent Klebsiella pneumoniae* (hvKp) is a globally widespread pathogen of concern. Hv *Klebsiella pneumoniae* is considered more virulent than classical *Klebsiella pneumoniae* (cKp). Pneumonia was first identified as a disease in Asia, where *Klebsiella pneumoniae* a key causative agent was historically linked to pyogenic liver abscesses. From its origins in Asia, the pathogen eventually disseminated globally, becoming a significant public health concern [2,3]. Virulence factors of hv *Klebsiella pneumoniae* encompass multiple siderophore systems for iron uptake, enhanced capsule production such as K1 or K2 serotypes colibactin toxin production, and the hypermucoviscosity phenotype observable on culture media [4,5]. *Klebsiella* species are widely found in various environments, including soil, water, and human and animal infections, as these are the natural habitats of human and animal intestinal organisms [6,7].

Virulence factors contribute contribute to the occurrence of the disease. These factors include capsular polysaccharides, plasmids, and fimbriae lipopolysaccharides (LPS) the most important virulence factors are siderophore [8]. Both classical and hypervirulent strains rely on virulence factors that mediate host adherence through two distinct types of fimbriae. This binding is critical for initial colonization and subsequent biofilm formation, facilitating persistent infection. There is also an outer layer in the bacteria called the polysaccharide capsule that interacts with the host. There is also lipopolysaccharide (LPS), an effective protector against many immune responses and the phagocytosis process [9]. The mechanical barriers and epithelial cells of the upper respiratory tract serve as critical components of innate immunity, providing defense against pathogenic bacterial invasion. In experimental rat models of pulmonary infection, these protective mechanisms trigger immune modifications that can lead to elevated tumor necrosis factor-alpha (TNF-α) production [10].

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There are several pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IFN-γ/γ, and TNF, which cause inflammation for the purpose of immune response against pathogens. Pro-inflammatory markers increase, including IL-1β, IL-6, and IL-8 [11]. Therefore, they are deemed anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, and TGF-β. They act as pro-inflammatory substances and at the same time their action depends on the amount of these cytokines produced [12]. Bacterial organisms can resist host immunity, causing various diseases, and induce virulence through the production of certain substances [13]. This study elucidates the immune response to hvKp serotypes K1/K2 in rats, highlighting elevated IFN-γ levels post-infection and limited IL-10. Addressing these gaps would enhance thoughtful of the *Klebsiella pneumoniae* pathogenesis and host adaptation.

2 Methods and Materials

2.1 Sample collection

Three hundred specimens were collected from Nasiriya Teaching Hospital and the veterinary hospital, and specimens included 150 nasal swabs from sheep and 150 sputum samples from humans. Information was also collected from patient records. Then, samples were taken and transferred to the laboratory in special containers to culture them on MacConkey agar and incubate them at 37°C for 24 hours. Gram staining was performed, and bacterial isolates were identified using the Vitek-2 system. Biochemical tests were subsequently employed to confirm the presence of *Klebsiella pneumoniae*, as described by [2]. Identification by Molecular methods included PCR amplification of 16S rRNA using universal primers for 16S rRNA of *K. pneumoniae.*

2.2 Specimen processing

Samples were collected from humans suffering from respiratory infections, where the sample was sputum and samples were collected from infected sheep by nasal swab. Then the samples were cultured after a period not exceeding an hour using a loop, the streaking method on MacConkey agar medium. After that, the plates were incubated at 37 ^{0}C for 24 hrs. with pink color mucoid colonies on MacConkey agar are indicative of *K. pneumonia***e.**

2.3 String test

This test is essential for identifying hypervirulent Klebsiella pneumoniae. Bacteria were cultured on MacConkey agar and incubated at 37°C for 24 hours. Hypermucoviscosity was assessed by lifting a colony with a loop; the formation of a string ≥5 mm indicated a positive result [15,16].

2.4 Molecular assay

Extracted genomic DNA from *Klebsiella pneumoniae* isolates by (Bromega/United States) according to the instructions and methodology of Gram-negative bacteria. Genomic DNA was extracted from *Klebsiella pneumoniae* isolates using a standard protocol for Gram-negative bacteria, followed by *Conventional PCR amplification was performed targeting the 16S rRNA gene, along with the* ***magA*** *(K1) and* ***K2*** *genes, using specific primers*. Primer sequences included AGAGTTTGATCCTGGCTCAG (forward) and TACGGTTACCTTGTTACGACTT (reverse) for 16sRNA (1500 bp) [17], GGTGCTCTTTACATCATTGC (forward) and GCAATGGCCATTTGCGTTTGCGTTAG (reverse) for *MagA-K1* (1283 bp) [18], and GGATTATGACAGCCTCTCCT (forward) with CGACTTGGTCCCAACAGTTT (reverse) for *K2* (908 bp) [19]. Amplification utilized a specialized thermal cycling program comprising initial denaturation (94°C, 5 min), thirty-five to forty cycles of denaturation at 94°C for 45 seconds, annealing at 53–57°C for 35–45 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 7 minutes.

2.5 Animals used in the study

Forty adult male laboratory albino rats (Rattus norvegicus), aged 10–12 weeks and weighing 250–300 grams, were utilized in this investigation. The animals were housed in standardized polypropylene cages (25 × 40 × 15 cm) fitted with stainless steel wire lids. Each cage contained autoclaved wood shavings as bedding, which was replaced regularly to maintain hygiene. Rats were provided ad libitum access to commercial rodent feed and filtered water. Environmental conditions were maintained at 25 ± 2°C with 55–60% relative humidity, 12-hour light/dark cycles, and adequate ventilation. Before experimentation, all animals underwent a 14-day acclimatization period under these conditions to minimize stress-related variability.

Following acclimatization, the rats were randomly allocated into four experimental groups (n = 10 per group) and inoculation according to [20].

• Group 1 served as the control group and received phosphate-buffered saline (PBS) via subcutaneous injection

• Group 2 served as the control group and received phosphate-buffered saline (PBS) via intranasal injection.

• Group 3 was inoculated with 1X10^8 CFU/ mL (0.5 McFarland turbidity) of viable hypervirulent *Klebsiella pneumoniae* subcutaneous

• Group4 was Inoculated with 1X10^8 CFU/ mL (0.5 McFarland turbidity) of viable hypervirulent *Klebsiella pneumoniae* intranasal

The bacterial inoculum was prepared from a clinical isolate of hv*Klebsiella pneumoniae* (ATCC® 43816), cultured and quantified according to established protocols [20,21]. All procedures adhered to institutional guidelines for animal welfare and were approved by the relevant ethics committee.

2.6 Levels of cytokine in serum

Blood was drawn directly from the heart using a sterile syringe then the samples were separated to obtain serum for measuring IFN-γ ng/ml and IL-10 ng/ml levels. This CLIA kit uses the Sandwich-CLIA principle based on coating a micro CLIA plate with an antibody specific for rat cytokines. The samples or standard samples are combined into the wells of the micro CLIA plate then a biotinylated antibody is added to detect cytokines.

3 Results

Of the three hundred samples, the results revealed 25 human *Klebsiella pneumoniae* isolates and 17 sheep isolates. There were no statistically significant differences between *Klebsiella* isolates from sheep and humans. At level P<0.01. All isolates were confirmed using Vitek and molecular methods through PCR amplification of 16S rRNA using universal primers for *16S rRNA* of *K. pneumoniae* Table 1.

Hypermucoviscosity was identified by culturing bacteria on MacConkey agar, incubating at 37°C for 24 hours, and assessing the formation of a ≥5 mm string when a colony was lifted with a loop. The study revealed that 8 of 42 (19.04%) *Klebsiella pneumoniae* isolates exhibited hypermucoviscosity (Figure 1, Table 1). These included five human-derived isolates and three isolates obtained from sheep.

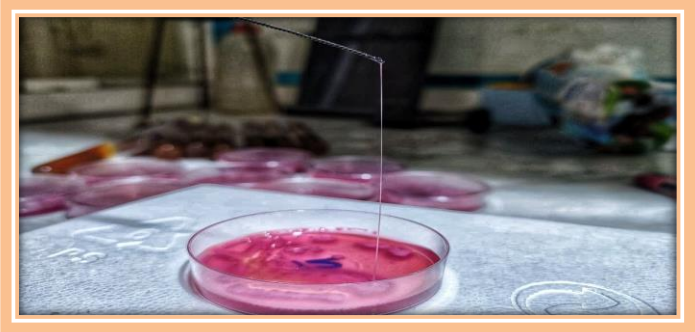

Figure 1: hypervirulent *Klebsella pneuoniae* on MacConkey agar where the hypermucoviscosity by lifting the colony with the loop as it forms, with a length of the string 5 mm.

Table 1: The number and percentage of classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumoniae* (hvKp) isolates from human and sheep samples.

Sample	No. of sample	C.Kp. (%)	Hv. Kp. (%)
Human	150	20 (13.33)	5 (3.33)
Sheep	150	14 (9.33)	3 (2)
Total	300	34 (11.33)	8 (2.66)
X^2		1.19	0.514
P value		0.274	0.474

The capsular serotypes of *K. pneumoniae* isolated from different samples (human and sheep) were detected by identifying the presence of specific PCR amplified genes, including the results showed that the k1 and k2 genes were present at approximately 8 / 19.04 % of the *K. pneumoniae* strains isolated from human and sheep sources as show in table 2, Figure 2,3.

Table 2: Detection of K1 and K2 genes in *K.pneumoniae.*

Sample	*K.pneumoniae* Total NO.	magA for K1 NO./%	K2 NO./%
Human	25	5 / 20	5 / 20
Sheep	17	3 / 17.64	3 / 17.64
Total	42	8 / 19.04	8 / 19.04

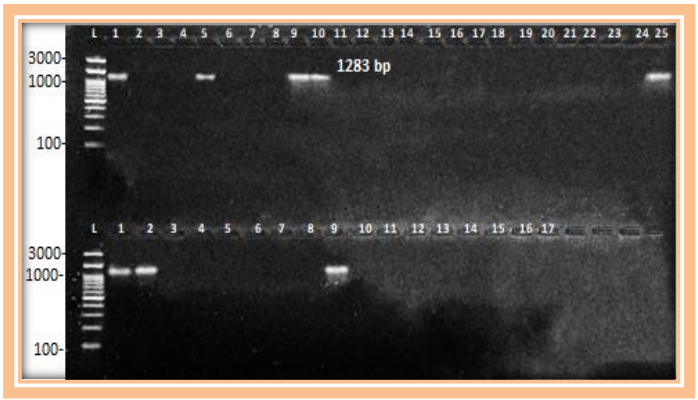

Figure 2: Electrophoresis of the polymerase chain reaction product of the *magA* gene for K1 (1283 bp) of *K. pneumoniae* isolates. Lane L.: lader (3000 bp), lanes (1-25): human samples and lanes (1-17) : represent sheep samples.

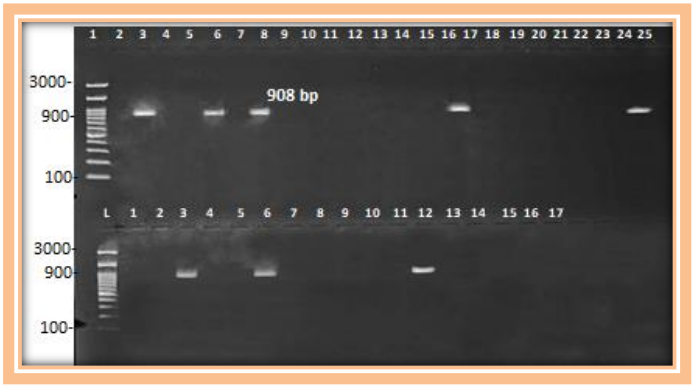

Figure 3: Electrophoresis of the polymerase chain reaction product of the *K2* gene (908 bp) of *K. pneumoniae* isolates. Lane L: lader (3000 bp), lanes (1-25): human samples and lanes (1-17) : represent sheep samples**.**

The findings of the present study indicated that the levels of IFN-γ ng/ml as Pro-inflammatory cytokines in the serum of rats

infected with *hypervirulentKlebsiella pneumoniae* bacteria compared to the control group were high, as shown in Table 4 Intranasal method The results recorded in Table 3showed that there was a significant difference in the level of IFN-γ ng\ml between experimental group and Control group using the intranasal method. However, using the SC. Method, the results showed a highly significant difference between the two groups at the level P<0.01. also showed that there was no significant difference between the two methods for the experimental group at the level P<0.05, as well as the control group (horizontal comparison). Which reached 0.531±0.18 ng/mL in the intranasal and 0.380±0.19 ng/mL in the subcutaneous, comparison to . The control group, the results indicated that the level of IFN-γ ng/mL in the intranasal was higher compared to the subcutaneous Table 3.

Table 3: Mean level of IFN-γ ng\ml in the serum for the experimental group compared with the control group.

Groups	Method		Calculated T value	Calculated P value
	intranasal	SC		
Control group	0. 500± 0.23ng/ml	0. 366± 0.22ng/ml	0.915	0.387 (NS)
Experimental group	2.48 ± 0.47ng/ml	1.89 ± 0.14ng/ml	1.17	0.275 (NS)
Calculated T value	4.07	8.51		
Calculated P value	0.013 (S)	<0.0001 (HS)		

NS: No significant difference at P<0.05, S: Significant difference at P<0.01: HS: Highly significant difference at P<0.01. SC: subcutaneous.

IL-10 levels measured at 10 ng/ml showed a slight increase in the experimental group compared to controls, but this difference was not statistically significant following intranasal administration. Similarly, SC administration revealed no significant difference in IL-10 levels between the experimental and control groups. Furthermore, horizontal comparison (between administration methods within each group) at p < 0.05 showed no significant difference in IL-10 levels between the intranasal and SC routes for either the experimental or control group (Table 4).

Table 4: Mean level of IL-10 ng\ml in the serum for the experimental group compared with the control group.

Groups	Method		Calculated T value	Calculated P value
	intranasal	SC		
Control group	0.677±0.17 ng/ml	0.926±0.16 ng/ml	1.03	0.331(NS)
Experimental group	1.046±0.29 ng/ml	0.923±0.38 ng/ml	0.252	0.807(NS)
Calculated T value	1.06	0.006		
Calculated P value	0.317(NS)	0.995(NS)		

NS: No significant difference at P<0.05 SC: subcutaneous**.**

4 Discussion

These results showed that colonies of *Klebsiella pneumoniae* isolated from a sample of patients with respiratory tract infection expanded to form a thread of more than 5 mm in length . The results of this study are similar to the results reached by[22] in Japan, which indicated that in order to distinguish between *cK. pneumoniae* and hv*K. pneumoniae* certain relevant factors are needed to confirm the presence of *hvK. pneumoniae* strains have their virulence capabilities, such as serotypes (K1, K2), as it is believed that the phenotypic appearance of mucus hyperviscosity (thread test) is a definitive and accurate diagnosis of *hvK. Pneumoniae* [23].

In these findings, PCR revealed capsular serotypes (K1, K2) of *Klebsiella pneumoniae*, 19.04% respectively. This result agreement with a study conducted by [24] in Al-Dewaniyah Teaching Hospital. There is a study that has indicated that the K1 and K2 serotypes are closely associated with the Hv. *Klebsiella pneumoniae* strain, while at the same time, there are studies to the contrary that Hv. *Klebsiella pneumoniae* are associated with non-K1/K2 capsular serotypes [23].

Many studies have indicated the immune response against serotypes K1 and K2 of the Hv. *Klebsiella pneumoniae*. Although some studies have not been conducted on experimental animals, especially rats, they provide valuable comparative insights into the host immune responses against these bacterial serotypes.

IFN-γ levels (ng/ml) differed significantly between the experimental and control groups following intranasal administration. A highly significant difference was observed between the groups after SC administration. However, statistical analysis revealed no significant difference in IFN-γ levels between the administration methods (intranasal vs. SC) for either the experimental or control group. These findings align with[25] in Kufa, Iraq. The current study findings validate the function of IFN-γ ng/ml in combating bacteria, particularly (hv) *K. pneumoniae*, and protecting the body through a variety of ways. There are study recorded by [26] which showed that inflammatory monocytes rapidly accumulate in the lungs of infected animals with *K. pneumoniae* and produce pro-inflammatory cytokines and increasing frequency of innate lymphocytes, and promotes the clearance of pneumonia. There is a study conducted by Dentice et.al., [27] on rats infected with pneumonia due *to K. pneumoniae*, and through an analysis of the gene transcription of the lungs, they found an increase in cytokines and chemokines, especially pro-inflammatory, in coordination with the active ribosome.

The results showed no significant difference in the level of interleukin 10 between the experimental group and the control group in the intranasal method of administration, as well as the subcutaneous injection method between the two groups (the experimental group and the control group). The results also showed that at the same time no significant difference between the two methods for the experimental group as well as the control group (horizontal comparison). Consistent with findings by [27], serum IL-10 levels increased significantly following respiratory pathogen infection compared to controls. This regulatory cytokine elevation was also associated with the presence of commensal bacteria in the respiratory tract, suggesting a role for IL-10 in differentially regulating inflammatory responses. Furthermore [28], demonstrated a statistically significant difference in IL-10 mediated immune responses between groups, specifically linking this effect to the 65.5 kDa pili protein of *K. pneumoniae*. Researchers by [29] have found that any defect in the gene expression or cellular signaling of interleukin-10 leads to a decrease in its levels. Some types of bacteria may also affect the production of interleukin 10, which leads to impaired control of inflammation

Conclusion

This study demonstrates that hypervirulent *K. pneumoniae* serotypes K1 and K2 induce a pronounced Th1-driven immune response in rats through elevated pro-inflammatory cytokines level (IFN-γ), particularly via the intranasal route. The slight, non-significant rise in IL-10 implies a compensatory anti-inflammatory mechanism, potentially moderating tissue damage. The prevalence of K1/K2 serotypes in both human and animal isolates underscores their zoonotic relevance and genetic adaptability. Future research should focus on host-pathogen interactions at mucosal sites and therapeutic modulation of cytokine pathways to effectively combat *HvK. pneumoniae* infection.

References

[1] Gorrie, C. L. *et al.* Genomic dissection of Klebsiella pneumoniae infections in hospital patients reveals insights into an opportunistic pathogen. *Nat. Commun.* **13**, 3017 (2022).

[2] Mario, E., Hamza, D. & Abdel-Moein, K. Hypervirulent Klebsiella pneumoniae among diarrheic farm animals: a serious public health concern. *Comp. Immunol. Microbiol. Infect. Dis.* **102**, 102077 (2023).

[3] Wyres, K. L. *et al.* Genomic surveillance for hypervirulence and multi-drug resistance in invasive Klebsiella pneumoniae from South and Southeast Asia. *Genome Med.* **12**, 1–16 (2020).

[4] Yang, Y. *et al.* Clinical and microbiological characteristics of hypervirulent Klebsiella pneumoniae (hvKp) in a hospital from North China. (2019).

[5] Choby, J. E., Howard-Anderson, J. & Weiss, D. S. Hypervirulent Klebsiella pneumoniae–clinical and molecular perspectives. *J. Intern. Med.* **287**, 283–300 (2020).

[6] Abdulkareem, M. M., Yassin, N. A. & Abdulrahman, M. A. Prevalence of Silent Plasmid-Mediated Quinolone Resistance (PMQR) Genes Among Clinical Isolates Fluoroquinolone-Sensitive Klebsiella pneumoniae. *Passer J. Basic Appl. Sci.* **5**, 207–212 (2023).

[7] Abdul-Wahab, D., Mahdi, S. & Alboslemy, T. Distribution of TEM and SHV Genes in ESBL-Producing Klebsiella pneumoniae Strains Isolated From Various Clinical Samples in Al-Basrah Province, Iraq. *South East. Eur. J. Public Heal.* 212–218 (2024) doi:10.70135/seejph.vi.976.

[8] Kadhum, A. A. & Khudor, M. H. Phenotypic and molecular characteristics of biofilm and other virulence genes in E. coli and K. pneumoniae isolates from healthy dairy cow, human and environmental sources. *Indian J. Forensic Med. Toxicol.* **15**, 2452–2458 (2021).

[9] Karampatakis, T., Tsergouli, K. & Behzadi, P. Carbapenem-resistant Klebsiella pneumoniae: virulence factors, molecular epidemiology and latest updates in treatment options. *Antibiotics* **12**, 234 (2023).

[10] Sequeira, R. P., McDonald, J. A. K., Marchesi, J. R. & Clarke, T. B. Commensal Bacteroidetes protect against Klebsiella pneumoniae colonization and transmission through IL-36 signalling. *Nat. Microbiol.* **5**, 304–313 (2020).

[11] Almuntashiri, S. *et al.* CC16 regulates inflammation, ROS generation and apoptosis in bronchial epithelial cells during Klebsiella pneumoniae infection. *Int. J. Mol. Sci.* **22**, 11459 (2021).

[12] Chen, Z., Bozec, A., Ramming, A. & Schett, G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **15**, 9–17 (2019).

[13] Abdulla, N. R., Abdullah, F. A., Kadhum, A. A., Ghanyem, H. S. & Abdulla, N. R. PCR detection of nontuberculous Mycobacteria 16s Rrna in cows and sheep subclinical mastitis. *Adv. Anim. Vet. Sci* **12**, 1969–1975 (2024).

[14] Madhi, K. S., Jasim, A. S., Nasear, H. A., Ibraheim, H. K. & Gharban, H. A. J. Phylogenetic analysis of Klebsiella pneumoniae isolates of respiratory tract infections in humans and sheep. *Open Vet. J.* **14**, 2325 (2024).

[15] A Zuber, S. & R Ganjo, A. Detection of hypervirulent and classical type of Klebsiella pneumoniae and screening their resistant properties in Erbil city. *Zanco J. Med. Sci.* **27**, (2023).

[16] Al-Fahdawi, A. M., Mutter, T. Y. & Zylstra, G. J. Genotypic identification of hypervirulent Klebsiella pneumoniae in Anbar, Iraq. *Malays. J. Microbiol.* **19**, (2023).

[17] Monawer, A. Molecular Analysis of Klebsiella Pneumoniae Isolates Collected from Sputum Samples in Duhok Iraq. *Al-Nahrain J. Sci.* **28**, (2025).

[18] Qasim, B. M. & Khalid, H. Microbiological and Molecular Study of K1, K2 Genes among Klebsiella pneumoniae isolated from Urine Specimens in Duhok city, Iraq. *J. Life Bio Sci. Res.* **3**, 12–16 (2022).

[19] Alsanie, W. F. Molecular diversity and profile analysis of virulence-associated genes in some Klebsiella pneumoniae isolates. *Pract. Lab. Med.* **19**, e00152 (2020).

[20] Mohammed, A. M. & jawed Al-jobbery, M. Comparison between efficiency of propolis extracts and antibiotic treatment of Klebsiella pneumonia in rats. *AL-Qadisiyah J. Vet. Med. Sci.* **18**, (2019).

[21] SADEQ, Z. E., LAFTA, I. J., AL-REKABI, F. M. K. & FARAJ, R. A. The immunomodulatory role of clove and cinnamon extracts on Klebsiella pneumoniae infected rats. *Bulg. J. Vet. Med.* (2025).

[22] Watanabe, N. *et al.* Evaluation of an optimal agar medium for detecting hypervirulent Klebsiella pneumoniae using string test. *Access Microbiol.* **6**, 000834-v3 (2024).

[23] Rastegar, S., Moradi, M., Kalantar-Neyestanaki, D. & Hosseini-Nave, H. Virulence factors, capsular serotypes and antimicrobial resistance of hypervirulent Klebsiella pneumoniae and classical Klebsiella pneumoniae in Southeast Iran. *Infect. Chemother.* **51**, (2019).

[24] 24. Hamza AL-Hamdawee, A. M. & Hassoon AL-Zeydi, A.-J. K. Diagnosis of Klebssiella pneumonia Isolated from Clinical Cases of Hospital-Acquired Infection in Al-Dewaniyah Teaching Hospital. *Al-Qadisiah Med. J.* **15**, (2019).

[25] Ali, A. J. M., Alkhudhairy, M. K. & Tobal, D. D. Immunological study of Klebsiella Pneumoniae isolated from pneumoniae infection patients. *Biomed. Chem. Sci.* **1**, 11–19 (2022).

[26] Xiong, H. *et al.* Innate lymphocyte/Ly6Chi monocyte crosstalk promotes Klebsiella pneumoniae clearance. *Cell* **165**, 679–689 (2016).

[27] Dentice Maidana, S. *et al.* Respiratory commensal bacteria increase protection against hypermucoviscous carbapenem-resistant Klebsiella pneumoniae ST25 infection. *Pathogens* **11**, 1063 (2022).

[28] Agustina, D. *et al.* Pili Protein 65.5 kDa of Klebsiella pneumoniae Induced a Decrease in IL-10 in Mice. (2022).

[29] Papoutsopoulou, S. *et al.* Interleukin-10 deficiency impacts on TNF-induced NFκB regulated responses in vivo. *Biology (Basel).* **11**, 1377 (2022).