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Research Article

Molecular and Immunological Characterization of Lipopolysacchaide in *Klebsiella pneumoniae*

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MFAA conducted the experimental work, data analysis and manuscript drafting. MKAH supervised the study and revised the manuscript.

Keywords
Lipopolysaccharide (LPS), *Klebsiella pneumoniae*, Interleukins, IL-1 alpha, IL-4, IL-10, *16S rRNA* gene

Abstract | The present study aims to addressing the prevalence of *Klebsiella pneumoniae* as a main causative agent of pneumonia in Iraq in Al-Muthanna governorate, and investigating the potential of the lipopolysaccharide (LPS) isolated from a randomly selected hypervirulence *K.pneumoniae* (hvKp) strain to reduce and increase the levels of pro-inflammatory cytokines (IL-1 alpha (α) and IL-4) and anti-inflammatory cytokines (IL-10), respectively in LPS immunized experimental mice. In this study, 100 bacterial isolates were isolated from lower respiratory tract clinical samples. These clinical isolates were identified using *16S rRNA* gene sequencing. Results revealed that most of the bacterial strains were *K. pneumoniae* sp. The potential the lipopolysaccharide (LPS) of one randomly selected hvKp clinical strain to immunize experimental mice prior artificial infection with *K. pneumoniae* was unraveled by estimating the level of the pro-inflammatory cytokines (IL-1α and IL-4) and the anti-inflammatory cytokines (IL-10) in the serum of experimental mice. The experimental mice groups included untreated LPS group prior artificial infection with *K. pneumoniae* and treated LPS group prior artificial infection with *K. pneumoniae*. Data revealed no significant difference (P>0.05) in the level of the pro-inflammatory cytokine IL-4 between the two experimental mice groups. In contrast, there was a significant difference (P<0.05) in the levels of the pro-inflammatory cytokine IL-1α cytokine and the anti-inflammatory cytokine IL-10 between two experimental mice groups. The IL-1α/IL-10 ratios in both groups were calculated to be 0.803 and 0.963 for the LPS untreated group and the LPS treated group, respectively. However, the IL-4/IL-10 ratios in both groups were calculated to be 40.13 and 26.02 for the control group and the LPS treated group, respectively.

Novelty Statement | This study offers new insights into the molecular and immunological features of lipopolysaccharide in *Klebsiella pneumoniae.* It identifies distinct LPS characteristics linked to virulence and resistance, highlighting its potential as a diagnostic or vaccine target.

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Introduction

K. pneumoniae is opportunistic rod-shaped Gram-negative bacteria. Normally, it is well known by physicians as hospitalized acquired pathogen and community acquired pathogen (also known as Friedländer's pneumoniae) (Lau, 2007). Also, *K. pneumoniae* is known as the causative agent of pneumoniae. *K. pneumoniae* is non-motile, lactose fermenting bacterium. *K. pneumoniae* inhabits the feces and the respiratory tract of approximately 5 % of normal persons and it is the etiological agent behind bacterial pneumoniae (1%).

K. pneumoniae is a well-recognized as an opportunistic pathogen that has great implication in a wide range of

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infections in human, which encompass wound infection, bloodstream infection, pneumonia, urinary tract infection, and gastrointestinal tract infections (Sandegren *et al.*, 2012). *K. pneumoniae* infections are associated with community acquired pneumonia and hospital acquired pneumonia.

LPS molecule, an outer membrane component of the cell, consists of three separate moieties: lipid A (phosphate amine glucose + fatty acids), a core polysaccharide, and O-antigen (O-Ag) polysaccharide side chain a side chain (Younes, 2024). Lipid A, an endotoxin, attaches the molecule of LPS into the outer membrane and does stimulate the immune system (Alexander and Rietschel, 2001). The core polysaccharide does link the O-Ag onto the lipid A moiety and normally harbors negative charges as a result of the phosphate substitutions (Abdulrazzaq *et al.*, 2024).

The clearance of the *K. pneumoniae* from the infection would necessitate certain robust host defense mechanisms, to which the bacterial surface does contribute. Three constituents of the outer wall of Gram-negative bacteria are supposed to be included in development of immunity are: LPS, membrane proteoglycans (Al-Sehlawi, 2012), (a 'core protein' with one or more covalently attached glycosaminoglycan (GAG) chains), and outer membrane proteins (OMPs) (Nucera *et al.*, 2006). OMP-A is regarded as one of the key proteins of the outer membrane of Gram-negative bacteria. This protein is highly preserved among the Enterobacteriaceae members and is supposed to contain two domains. While, the influences of LPS and membrane proteoglycans on immune cells are been extensively designated. Few studies assessed the OMP characteristics, chiefly stressed on its immunomodulatory function like macrophages (Kayaoglu and Qrstavik, 2004).

K. pneumoniae was identified based on the *16S-23S* internal transcribed spacer (Liu *et al.*, 2008). The rRNA sequences have a crucial role in the evolutionary microbial studies, especially, the *16S rRNA* gene is the gold standard for identification the bacterial isolate from scratch on both genus and species levels (Hansen *et al.*, 2004). Reportedly, the 16S rRNA gene exists as one gene on the rRNA operon. Additionally, each bacterial strain has at least two copies of this operon. On top and above, the heterogenous nature of the rRNA operons within the same strain increases the potential of this operon in the identification scenario.

Materials and Methods

Sample collection

In this study, twenty clinical samples were enrolled. These clinical samples were taken from hospitalized patients suffering from respiratory tract infection in Al-Rumaytha hospital. All the samples were collected by sterile cotton swab after that immediate transfer to the laboratory for culturing the bacterial pathogens.

Preservation and maintenance of bacterial isolates

Bacterial strains enrolled in this study were preserved at two levels: short and long-terms preservation. The short-term preservation was accomplished by preparing nutrient agar in slants. The slants were stored at 4°C for four weeks. The renewal of theses slants was conducted monthly. On the other hand, the long-term preservation was conducted by preparing overnight broth cultures from each bacterial strain. Then sterile glycerol at a final concentration of 50% was added to these cultures aseptically. The glycerol stocks were stored at -80 °C (Mahon and Lehman, 2022).

Culture media

According to Forbes *et al.* (2007) culture media used in this study were listed in (Table 1). All media were prepared according to the manufacturer's instructions (Shilpa *et al.*, 2016).

Table 1: Physiological tests profile for twenty *K. pneumoniae* strains.

Test	*Klebsiella pneumoniae*
Motility	–
Urease	+
Indole production	–
Methyl red test	V
Voges-proskauer test	+
Citrate utilization	+
Oxidase production	–
Catalase production	V
Live at 10 °C	–
Gas production from lactose at 44.5° C	+
Gas production from glucose (acid butt/ acid slant), and no H_2S production	+

Cell morphology

Gram stain

A smear of each tested bacterial strain was prepared on a glass slide. Then the protocol of Gram stain was performed. Then, the stained bacterial smears were visualized under the light microscope using the oil immersion lens of 100 X magnification (Collee *et al.*, 1996). A Gram-ve and Gram+ve bacterial cells stained in red (safranine as counter stain) and violet (crystal violet stain), respectively.

Capsule Stain

To distinguish between the capsulated and the non-capsulated bacterial strains, capsule staining was performed. The capsulated bacterial cells showed clear halo zone against dark background. Whereas, the non-

capsulated cells showed no clear zone against dark background (Collee *et al.*,1996).

Physiological tests

The bacterial strains were subjected to some physiological tests such as enzyme production (catalase, oxidase, and urease), motility, growth at 10 °C, production of gas from lactose after growth at 44.5 °C, IMVC test (indole production, methyl red test, Voges Proskauer test, and citrate utilization), glucose fermentation with gas production and without H_2S production (Collee *et al.*,1996).

Genomic and molecular protocols

Genomic bacterial DNA extraction

The 20 *K. pneumonia*e clinical strains, assigned to the genus and specie levels based on morphological and biochemical identification, were subjected to genomic DNA isolation using Anatolia Genomic DNA Mini Kit as per manufacturer's instructions.

Evaluation of extracted genomic DNA: Quality and quantity

The extracted genomic DNA from the 20 tested bacterial strains were evaluated from quality and quantity using Nano Drop spectrophotometer (Thermofisher Scientific Co., USA) (Krebs *et al.*, 2010). Shortly, a drop of the genomic DNA in quest was applied to the probe sensor of the Nano Drop Spectrophotometer. Then, the sample DNA was measured at the following wavelengths: 260 nm, 260/280 ratio, and 260/230 ratio. Moreover, a scan for each genomic DNA was performed from 200-399 nm to unravel the purity and the concentration of the tested genomic DNA. The ratio 260/280 value of >1.8 indicates pure DNA that is free from protein and phenolic compounds contamination. Conversely, the ratio 260/280 value of ≥ 2.0 indicates genomic DNA contaminated with RNA. However, the ratio 260/230 value of 2.0 evidences that the genomic DNA sample is free from contamination with humic acid and carbohydrates. The readings of Ab 260 nm, reflecting the concentration of genomic DNA in the sample, is expressed in terms of ng/µl.

Amplification and analysis of the 16S rRNA gene

The entire 16S rRNA housekeeping gene (~1500 bp) was amplified for all bacterial strains investigated as part of this study in PCR reactions with F8-27(5′-AGAGTTT TGATCCTGGCTCAG-3′) and R1510-1492(5′-GGTTACCTTGTTACGACTT-3′) as primers established by Eden *et al.* (1991). PCR was performed in a total volume of 50 µL reaction mixture that included 50 ng of genomic DNA, 25 µL of 2X Taq PCR Master Mix (Abm Co., Korea), 2 pmol of each primer, and nuclease-free water. The thermal cycler (AnalytikJena) program was 5 min at 94°C of initial denaturation, 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 1.5 min extension at 72°C, and a final extension step at 72°C for 7 min. Reactions were incubated at 4°C until use. The products of PCR were observed by agarose gel electrophoresis (1%) and the positive amplicons were purified with Wizard SV Gel and PCR Clean-Up System (Promega, USA). The same primer pair was used for sequencing. The resulting nucleotide sequences were compared with the most similar sequences for homology with the NCBI BLASTN tool. Ten best hits of each sequence were aligned with MEGA 11.0 and genetic relationships were examined using the Neighbor-Joining method to determine the relatedness of the isolates with other bacterial strains.

Isolation and purification of lipopolysaccharides (LPS) from K. pneumoniae

The LPS from *K. pneumoniae* cells were extracted using the hot aqueous phenol method as reported by Westphal (1965) after performing slight modifications (Schmidt, 1973). Shortly, the bacterial suspensions were centrifuged at 10,000 rpm for 5 min. Then, proteinase K (100 µg/ml) was added to the cell mixture and the tubes were kept at 65°C for an hour to get rid of protein contamination. The mixture was treated with RNase (40 µg/ml) and DNase (20 µg/ml) to get rid of RNA and DNA contamination, respectively. However, phospholipids were eliminated, by stirring the cells in a mixture of chloroform/methanol at a ratio of 2:1 for 24 h, in a screw cap bottle, at 4 °C. Then, few drops of methanol were added until noting the appearance of an emulsion. The treated mixture was centrifuged at 3,000 rpm for 10 min to remove the supernatant layer containing the phospholipids. After that, fresh chloroform/methanol was added once again, vortexed followed by centrifugation at 3,000 rpm in glass tubes for 10 min. The supernatant layer containing the phospholipids was collected again. The last washing step was repeated twice (Schmidt, 1973). Then, cells were dried by acetone. The phospholipids-free dry-cells were suspended in distilled water (6%), then placed in a water bath at 68 °C, together with a bottle containing 90% (w/v) phenol; when both bottles reach the water bath temperature, equal volume of the phenol solution was poured to the bacterial suspension (i.e., to reach a final concentration of 45% phenol). Then the mixture was stirred vigorously for 20 min, then the extraction bottles were transferred to an ice bath for 10 min, and centrifuged (3,000 rpm, 20 min, 4 °C) in transparent phenol resistant plastic tubes. According to the method, the following layers appeared from the bottom to the top: A-insoluble material, phenolic layer (yellow to brown), that proteins and nucleic acid, B-Inter-phase layer (insoluble material), and C-Aqueous layer (opalescent to white) of the LPS. The aqueous layer was carefully taken from each tube. Equal volume of distilled water was added (i.e. the same volume of C layer). The mixture was vigorously stirred for 20 min at 68-°C, as previously described, cooled on ice, centrifuged and the aqueous layer was collected once again. The gathered aqueous layers from all steps

mentioned above were subjected to dialysis in a dialysis tube for three days against distilled water, with routine water change every 12 h to remove phenol. Finally, the extracted LPS was lyophilized for further uses.

Sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS- PAGE (10%) was used to check the purity of LPS and the gel was stained with silver staining according to the following protocol (Merril *et al*., 1981).

Assay of human IL-1α (Interleukin 1 Alpha) ELISA kit

Human interleukin 1 alpha (IL-1 α) was detected by ELISA kit (E-EL-H0088, Elabscience USA) as per procedure stated in the instruction manual of the kit.

Assay of rat interleukin 4 (IL-4) ELISA kit

Human interleukin 1 alpha (IL-1 α) was detected by ELISA kit (E-EL-R0014, Elabscience USA) as per procedure stated in the instruction manual of the kit.

Assay of HS human IL-10 (Interleukin 10) ELISA kit

Human interleukin 1 alpha (IL-1 α) was detected by ELISA kit (E-HSEL-H0005, Elabscience USA) as per procedure stated in the instruction manual of the kit.

Results and Discussion

Klebsiella pneumoniae *strains in clinical samples*

One hundred clinical isolates, collected from hospitalized patients suffering from respiratory tract infection (RTI) in Al-Rumaytha hospital in Samawa/ Iraq, were recruited to conduct this study. Twenty out of one hundred (20 out of 100) clinical isolates (Figure 1) were assigned to the pathogenic bacterium *Klebsiella pneumoniae*, a pathogen a global threat on the horizon as reported by CDC (Center for Disease Control and Prevention) and WHO (World Health Organization). *Klebsiella pneumoniae* is one of the main causative agents of healthcare-related infections involving nosocomial pneumonia, urinary tract infection, and bacteremia (Russo and Marr, 2019). Increasingly, this pathogen has classified as one of the top ten multidrug-resistant pathogens encountering hypervirulent strains and problematic Gram-negative bacteria according to the statistics derived from World Health Organization (WHO), published in 2024 on WHO web site hosted on the server: https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON527.

The community associated hypervirulent strains of *K. pneumoniae* have assigned to sequence types ST23/65/66/86. However, 16 countries (e.g., Algeria, Argentina, Australia, Canada, India, Iran, Japan, Oman, Philippines, Switzerland, Thailand and the United Kingdom), provided responses about the prevalence of ST23 hvKb (hypervirulent *Klebsiella pneumoniae*) strain (WHO data). Regarding the situation of hvKp strains prevalence in the East Mediterranean region, the freely accessible data is rare and is reported only through laboratory surveillance for AMR within healthcare services or surveying epidemiological reports in just a few countries. Subsequently, the present study had aimed to unravel the molecular epidemiology of *K. pneumoniae* strains among respiratory tract infected samples gathered from hospitalized patients in Iraq. The aim was extended to search for the hvKp strains among the isolated *K. pneumoniae* strains.

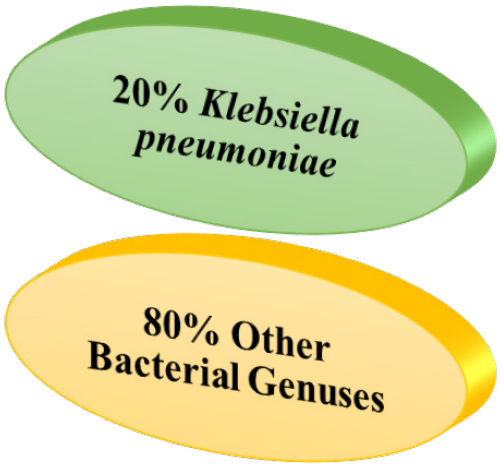

Figure 1: Percentage of *K. pneumoniae* existed in the clinical samples enrolled in this study.

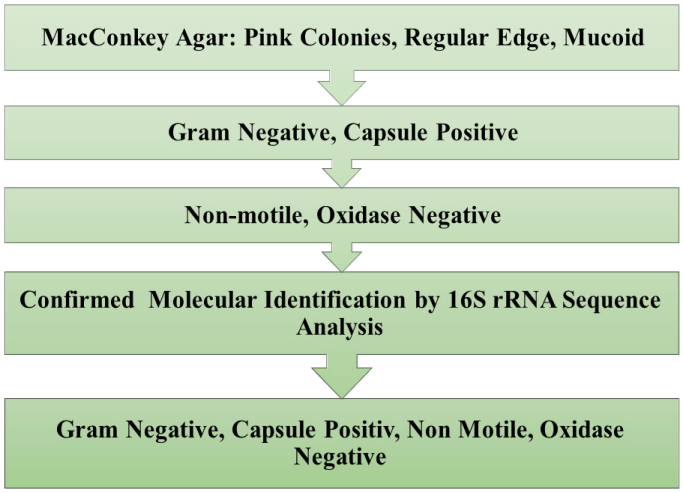

Figure 2: A chart deciphering the five-step identification scheme employed in this study to help verify the identity of the bacterial strains, isolated from clinical samples, as *Klebsiella pneumoniae*.

The affiliation of these twenty bacterial isolates to be *K. pneumoniae* was realized after passing five-step protocol for identification. This six-step identification scheme included colonial morphology, microscopical cell morphology, physiological test, *16S rRNA* gene sequencing analysis, and molecular detection of some virulence encoding genes (Figure 2). The next sections would display

the results of every step in the five-step identification scheme. With regard to the frequency of occurrence of *K. pneumoniae* strains in the infected respiratory tract gathered samples, 20% of the respiratory tract infections isolates were assigned to *K. pneumoniae*, deduced from a five-step identification scheme as mentioned previously in the results section (in this study). The present percentage is considered somehow high percentage compared to those previously reported. Previous retrospective studies conducted in Makkah and Bisha, Saudi Arabi revealed 14.7 and 18.6% frequency of occurrence of *K. pneumoniae*, respectively (Jalal *et al*., 2023). Two previous studies conducted in Iran (Rahimi and Vesel, 2017) and in Greece (Maraki *et al*., 2024) reported 7.4 and 6.8% frequency of occurrence for *K. pneumoniae* in lower respiratory tract infections samples collected from hospitalized patients, respectively. Another previous study conducted in South Africa in 2022 reported 15.5% frequency of occurrence of *K. pneumoniae* in lower respiratory tract infections samples collected from infants (Zar *et al*., 2022). A previous study conducted in Italy, showed 10.9% frequency of occurrence for *K. pneumoniae* (Santella *et al*., 2021). Kaseb *et al*. (2023) reported a high frequency of occurrence for *K. pneumoniae* in respiratory tract infections samples (Kaseb *et al*., 2023) in Iran compared to our finding in the present study. A recent study, conducted in Indonesia, has reported a high frequency of occurrence (39.96%) of *K. pneumoniae* in respiratory tract infections samples (Prastiyanto *et al*., 2024) compared to our present finding. Two previous studies had reported 37.5 (Behera *et al*., 2020) and 27.5% (Duan *et al*., 2020) frequency of occurrence of *K. pneumoniae* in respiratory tract infections samples, collected from patients in intensive care unit. A previous study conducted in Egypt revealed that *Klebsiella* spp. encountered 34.7% of the collected isolates from clinical samples in Menoufia University Hospitals and the prevalent spp. was *K. pneumoniae* (91.3%) (Elbrolosy *et al*., 2020). El-Badawy *et al*. (2017) found that *Klebsiella* spp. were the most frequent pathogens (38%) among the collected nosocomial isolates. A previous study conducted in Nepal in 2022 revealed 37.93% frequency of occurrence for *K. pneumoniae* in lower respiratory tract specimens (Connor, 2013). A study conducted in Iraq revealed 54.84% frequency of occurrence for *K. pneumoniae* in lower respiratory tract infections specimens (Madhi *et al*., 2024). On the other hand, a low percentage (12%) of *K. pneumoniae* was reported in respiratory tract specimens gathered from Iraq (Shakir *et al*., 2022). The discrepancy in the frequency of occurrence of *K. pneumoniae* among various studies could be mainly attributed to the sample size, epidemiological differences, regional varaitions in the hygienic status, and criteria of the involved patients. This would necessitate and encourage enlarging our sample size in prospective studies to cope with other studies.

Colonial morphology of K. pneumoniae *strains*

Only twenty out of one hundred bacterial strains, isolated on MacConkey agar at 37 °C after 24 h. The typical *K. pneumoniae* colonial morphological features on MacConkey agar had pink, regular edge, mucoid lactose fermenting colonies (Figure 3A). The co-selective and differential MacConkey agar medium helps prevent the growth of the Gram-+ve bacteria and differentiate between the lactose fermenting isolates from the non-lactose fermenting ones. The selective and the differential agents encountered in MacConkey agar were crystal violet and lactose, respectively. The crystal violet is a Gram-+ve growth inhibitor. Whereas lactose helps differentiate between the non-lactose fermenting bacterial strains (with yellow colonies appearance) from the lactose fermenting bacterial strains (with pink colonies appearance). The colonial morphological features of the isolated clinical strains underpinned greatly the presumptive identity of these strains as *K. pneumoniae*. In addition, the colonial morphology output greatly supported the transfer to the next identification step (cell morphological features) in the scheme mentioned above.

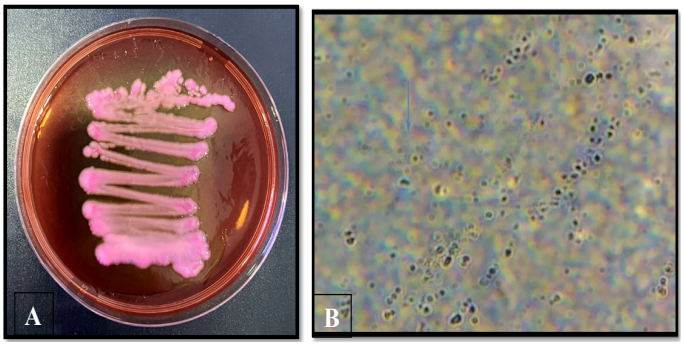

Figure 3: *K. pneumoniae* colonies showed pink, mucoid, regular edge appearance on MacConkey agar plate incubated at 37 °C for 24 h (A), Capsule staining of *K. pneumoniae* (B).

Cell morphological features of K. pneumoniae *strains*

The twenty bacterial strains presumptively assigned to *K. pneumoniae* based on colonial morphological features were subjected to the second step in the five-step identification scheme: examining the cell morphological features under the light microscope. The tested morphological features were the category of Gram type using Gram stain and the presence or absence of capsule based on capsule stain (Figure 3B). The twenty bacterial strains proved to be Gram -ve based the output obtained from gram stain. Moreover, all twenty bacterial strains exhibited capsule based on the output derived from capsule stain. The output derived from the examined morphological features using Gram -stain and capsule stain added additional evidence concerning the identity of the twenty tested bacterial strains as *K. pneumoniae*. Additionally, this finding supported the transfer to the third step in the five-step identification scheme; conducting physiological tests.

Physiological tests of K. pneumoniae strains

A set of physiological tests: Enzyme production (catalase, urease, and oxidase), motility, growth at 10 °C, production of gas from lactose after growth at 44.5 °C, IMVC test (indole production, methyl red test, Voges-Proskauer test, and citrate utilization), and glucose fermentation with gas production and without H_2S production. The obtained results from this set of physiological tests were illustrated in (Table 1). The obtained profile of physiological tests was typical *K. pneumoniae* profile. The finding of this set of physiological tests added further evidence concerning the identity of the tested twenty bacterial strains as *K. pneumoniae*. This finding greatly underpinned the transfer to the next step in the five-step identification scheme; *16S rRNA* sequence analysis.

16S rRNA *gene sequence analysis of* K. pneumoniae *strains*

The full length of *16S rRNA* gene (1500 bp) was successfully amplified from the twenty tested clinical strains of *K. pneumoniae* enrolled in this study (Figure 4) amplified by PCR The molecular identification of *16S rRNA* gene sequencing for the twenty tested bacterial strains, affiliated as *K. pneumoniae* based on colony morphology, cell morphology, and physiological tests evidenced the identification of all twenty tested bacterial strains as *K. pneumoniae*.

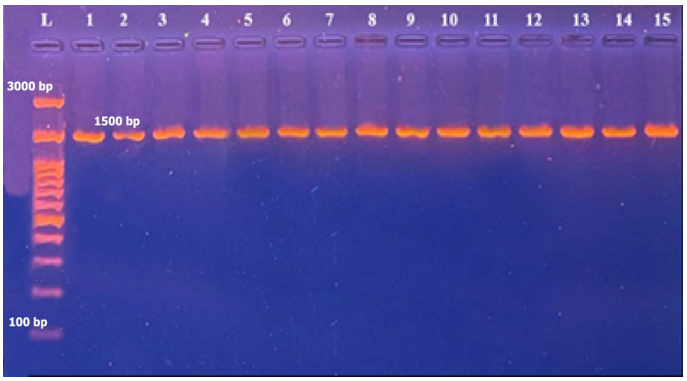

Figure 4: Agarose gel electrophoresis 1% showing amplification of full size 16S rRNA gene (~1500 bp) from clinical isolates of Klebsiella pneumoniae. Lane L: DNA ladder (100-3000 bp); Lanes 1 to 15: positive amplification products of clinical strains.

Lipopolysaccharides of K. pneumoniae

The LPS of one clinical strain of *K. pneumoniae* randomly selected, evaluated, and detected on SDS-PAGE after staining with silver stain (Figure 5). In the context of tailoring of a vaccine in prospective studies against *K. pneumoniae* as a prophylactic agent antagonizing the *K. pneumoniae* infections, the lipopolysaccharide of a randomly selected hvKp *K. pneumoniae* strain was given for a group of animals for immunization purposes in this study. However, it was reported that protective effect of the prophylactic agent can somewhat be elucidated by modulation of pro-inflammatory cytokine response (Rukavina *et al*., 2005). Whereas, the secretion of pro-inflammatory cytokines during the infection's course could be destructive and might result to shock, various-organ dysfunction and fatality (Pinsky *et al*., 1993; Marty *et al*., 1994). The anti-inflammatory cytokines, for example IL-10, which are mandatory to help downregulate the inflammatory process and sustain homeostasis with guaranteed appropriate functionality of host organs (Gerard *et al*., 1993; Howard *et al*., 1993).

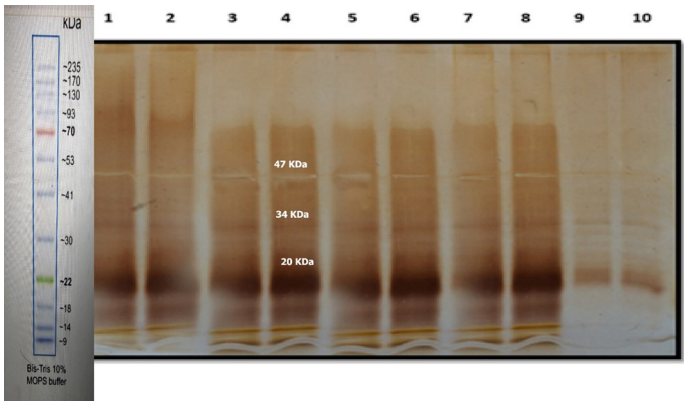

Figure 5: SDS-PAGE (10%) profile of lipopolysaccharides (LPS) extracted from *Klebsiella pneumoniae* clinical isolate. Lanes 1–10 show LPS bands at different molecular weights, notably at ~20 kDa (lipid A core), ~34 kDa, and ~47 kDa (polysaccharide components), indicating variation in LPS composition across samples.

Evaluation of cytokines in study cohorts

The estimated levels of IL-10, IL-4, and IL-1a in sera of both animals groups: Lipopolysaccharide untreated and lipopolysaccharide treated ones after immunization with LPS of one strain of *K. pneumoniae* (providing that this strain harbored the three virulence genes mentioned above) were displayed in (Table 2; Figure 5). No significant difference (P>0.05) could be traced in the serum level of IL-4 between the two groups. On the other hand, a significant difference at P<0.05 was noted in the level of IL-1α between the two groups. Similarly, significant difference at P<0.05 was noted in the level of IL-10 between the two groups.

Table 2: Interleukins (IL) levels in cohorts groups (Mean ±SE).

Group	IL-4	IL-1 alpha (α)	IL-10
Untreated with LPS	979.88±164.2	19.61±7.06	24.42±5.06
Treated with LPS	936.44±109.9	34.68±3.78	35.98±2.32
Calculated T- value	0.227	2.069	2.374
Calculated P-value	0.822(NS)	0.048(S)	0.026(S)

*NS, No significant difference at P>0.05; *S, Significant difference at P<0.05

Lipopolysaccharide is established as a stimulator of IL-10 secretion and IL-10 was recognized to result in beneficial

influences in numerous experimental models (Yoshizawa *et al*., 1996). Our results do support such previously reported findings. Our finding revealed that the lipopolysaccharide of the *K. pneumoniae* hvKp strain, randomly selected, did succeed to initiate an immune response, in the level of the anti-inflammatory cytokine IL-10 (Table 2, Figure 6), in lipopolysaccharide treated animals group compared to the untreated lipopolysaccharide group (control group). Unlike our IL-10 finding, the pro-inflammatory cytokine IL-4 level did not show significant differences between the lipopolysaccharide treated group and the lipopolysaccharide untreated group (Table 2, Figure 6). Similarly, to IL-10 present finding, IL-1α level exhibited significant differences between the lipopolysaccharide treated group and the control group (Table 2, Figure 6). Likewise, our IL-10 finding, Rukavina *et al*. (2004) found a discriminative pattern of the cytokine IL-10 between the two experimental animals groups protected previously immunized with anti-polysaccharide antibody (Ru-O1) against lipopolysaccharide of hvKp strain of *K. pneumoniae*) and unprotected (control). In addition, numerous studies addressed the issue of the homeostasis between anti- and pro-inflammatory cytokines for the output of systemic infection (Calandra *et al*., 1990; Cannon *et al*., 1990). For instance, the pro-inflammatory cytokine IL-6 is able to provoke or elicit a robust inflammatory effect that would end up with hypotension, organs dysfunction, and mortality (Calandra *et al*., 1990; Cannon *et al*., 1990). Such response eventually does activate a compensatory anti-inflammatory reaction including antagonist mediators encompassing IL-10, which is capable for suppressing the synthesis of pro-inflammatory cytokines and efficiently helps down-regulate the pro-inflammatory reaction (Cassatella *et al*., 1993; Fiorentino *et al*., 1991).

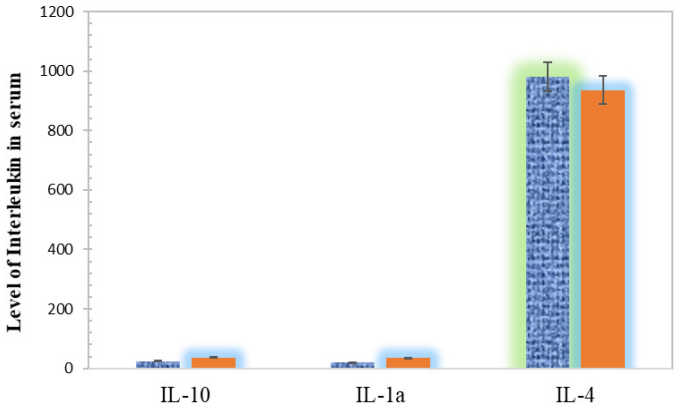

Figure 6: Levels of IL-4, IL-1a, and IL-10 in sera of study animals cohorts: Lipopolysaccharide untreated and lipopolysaccharide treated ones. Blue bar: lipopolysaccharide untreated group. Orange bar: lipopolysaccharide treated group. Levels of interleukins are expressed in terms of mean values ± SE.

The levels of the pro-inflammatory cytokine IL-1α and the level of anti-inflammatory IL-10 were significantly higher in the lipopolysaccharide treated group compared to the lipopolysaccharide untreated group (Table 2, Figure 6). However, to get a deep insight about the levels of pro-inflammatory cytokines IL-4 and IL-1α and their correlation with the level of the anti-inflammatory cytokine IL-10, the ratios IL-1α/IL-10 and IL-4/IL-10 were considered herein in the present study (Table 2, Figure 6). The IL-1α/IL-10 ratios in both groups were calculated to be 0.803 and 0.963 for the lipopolysaccharide untreated group and the lipopolysaccharide treated group, respectively. However, the IL-4/IL-10 ratios in both groups were calculated to be 40.13 and 26.02 for the control group and the lipopolysaccharide treated group, respectively. This would greatly help outline certain conclusions regarding the levels of pro-inflammatory and the anti-inflammatory cytokines in both groups. Comparing the magnitude values of the pro-inflammatory cytokines and anti-inflammatory cytokines in both groups are mis-leading. As shown, the calculated ratios IL-1α/IL-10 and IL-4/IL-10 could predict robust and poor sepsis outcome for the lipopolysaccharide treated group, respectively. The poor and robust sepsis outcomes are wanted and unwanted outcomes of sepsis, respectively. This would entitle conducting further studies to help enlarge the number of estimated pro-inflammatory and anti-inflammatory cytokines in both groups. Perhaps, the whole picture differs. Definitely, we could not judge the performance of lipopolysaccharides and its influences in provoking different levels of pro-inflammatory and anti-inflammatory cytokines from a small number. From another side, the commercially available ELISA kits could play a major role in estimating the accurate levels of the tested pro-inflammatory and anti-inflammatory cytokines in the serum samples. In other words, the sensitivity of each kit should be considered very well.

Conclusions and Recommendations

This work established the immunomodulatory action of lipopolysaccharides (LPSs) of Klebsiella pneumoniae, in particular their influence on cytokines, IL-1α and IL-10. The distinct differences between LPS-treated and untreated groups indicate a potential protective effect of LPS on the host immune recovery. Larger and more comprehensive studies are warranted to confirm these findings and to consider how they may be applied to the study of vaccine or immunotherapy development.

Declarations

Acknowledgement

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IRB approval

This study was approved by the Institutional Review Board (IRB) of the University of Al-Qadisiyah, College of Education, under reference number 13234, dated December 16, 2024.

Ethical statement

All procedures involving animals were conducted in accordance with institutional and national ethical guidelines.

Declaration of generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies wer used in the writing process.

Statement of conflict of interest

The authors have declared no conflict of interest.

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