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Molecular characterization of some genes in *Klebsiella pneumoniae* isolates from different clinical cases

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Abstract

Objective

This investigation aimed to investigate the prevalence of *Klebsiella pneumoniae* as a primary causative agent of pneumonia in the Al-Muthanna governorate, Iraq, and to describe the molecular profile of virulence genes related to hypermucoviscosity (e.g., *magA* and *rmpA*) in epidemic strains extracted from lower respiratory tract samples of hospitalized patients with pneumonia at Al-Rumaytha Hospital. Additionally, we estimated the prevalence of hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp) strains.

Materials and methods

A total of 100 bacterial isolates were gathered from lower respiratory tract samples of patients at Al-Rumaytha Hospital between April 18, 2024, and September 18, 2024. Isolates were identified through below steps: colonial morphology on MacConkey agar, cell morphology via Gram and capsule staining observed under a light microscope, physiological experiments, biochemical experiments, molecular identification applying 16S rRNA gene sequencing, and diagnosis of virulence genes (*magA* and *rmpA*) via polymerase chain reaction (PCR) with gene-specific primers amplifying fragments of 1283 bp for *magA* and 409 bp and 340 bp for *rmpA*.

Results

Of the 100 isolates, 20 exhibited a typical *K. pneumoniae* profile, with identity affirmed by 16S rRNA gene sequencing. Sequences from 15 of these strains were deposited in GenBank under

accession numbers PQ814166 to PQ814180. Phylogenetic analysis grouped these 15 strains into 10 clades: Clade A (PQ814167, PQ814177), Clade B (PQ814171), Clade C (PQ814166, PQ814176), Clade D (PQ814179, PQ814180), Clades E, F, and G (PQ814169, PQ814173, PQ814174, respectively), Clade H (PQ814170, PQ814175), Clade I (PQ814172), and Clade J (PQ814168, PQ814178). The hypermucoviscosity genes *magA* and *rmpA* were detected in 55% and 50% of the 20 *K. pneumoniae* isolates, respectively. The prevalence of hypervirulent *K. pneumoniae* (hvKp) strains was 50%.

Conclusions

This investigation affirms *K. pneumoniae* as a meaningful cause of pneumonia in the Al-Muthanna governorate, with a notable presence of hypervirulent strains (50%) described by *magA* and *rmpA* genes. These results highlight the importance of molecular characterization in understanding the pathogenicity and epidemiology of *K. pneumoniae* in clinical settings.

Keywords: 16S rRNA, *Klebsiella pneumoniae*, *magA* gene, *rmpA* gene

Paper Type: Research Paper.

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Introduction

The identification of bacterial isolates from scratch is an issue of paramount importance in the diagnostics field. Accuracy, sensitivity, and speed are three critical aspects for selecting the appropriate method for validated bacterial identification to help reduce mortality and morbidity in this context. Rapid and precise diagnosis enables timely treatment, curbing the spread of infections and improving patient outcomes, exclusively in regions with high disease burden. The genus *Klebsiella* was first discovered by Edwin Klebs, a German bacteriologist, in 1834. The

nomenclature of *Klebsiella* was derived from its discoverer, Edwin Klebs. Later, in 2006, *Klebsiella* was also designated as Friedländer's bacilli, as proposed by Brisse et al. (2006), in recognition of Carl Friedländer's contributions to understanding its role in pneumonia. Previously, the genus *Klebsiella* was divided into three species based on the profile of biochemical reactions. However, it was subsequently stratified into six main species based on DNA homology: *K. pneumoniae*, *K. oxytoca*, *K. planticola*, *K. terrigena*, *K. ornithinolytica*, and *K. mobilis* (Holt et al., 1994). Among these, *K. pneumoniae* is further classified into three subspecies based on biochemical reaction profiles: *K. ozaenae*, *K. rhinoscleromatis*, and *K. pneumoniae*. These classifications highlight the diversity within the genus and its adaptability to numerous environments and hosts. *K. pneumoniae* is distinguished as a rod-shaped, Gram-negative, opportunistic pathogen. It is well-known to physicians as both a hospital-acquired and community-acquired pathogen, often referred to as Friedländer's pneumonia (Lau, 2007). This bacterium is a leading causative agent of pneumonia, exclusively in immunocompromised individuals. *K. pneumoniae* is non-motile and lactose-fermenting, with a polysaccharide capsule that enhances its pathogenicity by shielding it from host immune responses. *Klebsiella* is announced to be an opportunistic bacterium inhabiting the intestine, where it is regarded as part of the normal intestinal flora. Enteric bacteria typically do not create disease in the gut but can modulate functional and nutritional processes. However, these bacteria can become pathogenic when they reach tissues outside the intestinal core, like the respiratory tract or bloodstream (Brooks et al., 2007). *K. pneumoniae* is exist in the feces and respiratory tract of approximately 5% of healthy individuals and is the etiological agent of bacterial pneumonia in about 1% of cases, with higher prevalence in clinical settings. *K. pneumoniae* is well-distinguished as an opportunistic pathogen with meaningful implications in a broad range of human infections, encompassing bloodstream infections, pneumonia, wound infections, urinary tract infections, and gastrointestinal tract infections (Sandegren et al., 2012). Its infections are related to both community-acquired and hospital-acquired pneumonia, posing challenges in treatment due to increasing antimicrobial resistance. The capsule polysaccharide (CPS) is designated as one of the most meaningful virulence factors in *K. pneumoniae*, with a thickness of approximately 160 nm. The CPS exhibits a complex acidic polysaccharide structure composed of repeating subunits of 4–6 sugars (e.g., glucose, galactose, mannose, fructose, and rhamnose) and frequent acids like uronic acid, glucuronic acid, galacturonic acid, and pyruvic acid as negatively charged components (Podschun and Ullmann, 1998). This capsule protects the bacterium from phagocytosis and contributes to its survival in hostile environments. *K. pneumoniae* virulence parameters vary based on infection sites, reflecting local variations in host defense mechanisms (Highsmith and Jarvis, 1985). For instance, the profile of virulence factors in *K. pneumoniae*

strains creating urinary tract infections likely differs from those creating respiratory tract infections observed in the lungs of patients with bacterial pneumonia (Turton et al., 2010). This site-specific variation underscores the need for targeted molecular investigations to understand pathogenicity. Reports indicate that hypervirulent *K. pneumoniae* (hvKp) strains are prevalent in the East Mediterranean region; however, freely accessible data stay scarce and are announced only through laboratory surveillance for antimicrobial resistance (AMR) within healthcare services or epidemiological surveys in a few countries (WHO, 2024). The emergence of hvKp, described by enhanced virulence, poses a growing public health threat, exclusively in regions with restricted surveillance infrastructure. Capsular polysaccharide genes consist of gene clusters responsible for the synthesis of numerous CPS serotypes (Kawai, 2006). Different genes have been distinguished as markers of virulence in *K. pneumoniae*, containing *magA*, *rmpA*, and 16S rRNA genes. Mucoviscosity-related gene A (*magA*), a chromosomal gene, was originally identified. Hypermucoviscosity and *magA* are more prevalent in invasive *K. pneumoniae* strains. *magA*-negative *K. pneumoniae* strains lack their exopolysaccharide layer and become susceptible to phagocytosis. Notably, *magA* is confined to the gene cluster of *K. pneumoniae* capsule serotype K1 (Lin et al., 2012). The regulator of mucoid phenotype A gene (*rmpA*), typically harbored on a plasmid, confers a highly mucoviscous phenotype that enhances and regulates capsular polysaccharide synthesis (Rivero et al., 2010). This trait is critical for the bacterium's capability to evade host immune defenses and persist in infected tissues. The 16S ribosomal RNA (16S rRNA) gene has been pivotal in bacterial identification. Previously, *K. pneumoniae* subsp. *pneumoniae* was identified based on the 16S-23S internal transcribed spacer. The 16S rRNA gene is the gold standard for identifying bacterial isolates at both genus and species levels due to its unique characteristics: universal distribution among bacterial species, a strong foundation for comparative phylogenetic investigations, status as a core housekeeping gene, provision of evolutionary data, and a structure of conserved regions interspersed with variable regions (Park et al., 2011). Furthermore, the epigenome, encompassing mechanisms like DNA methylation, chromatin remodeling, histone tail modifications, microRNAs, and long non-coding RNAs, interacts with environmental factors like nutrition, pathogens, and climate to influence gene expression profiles and the emergence of specific phenotypes (Mousavizadeh et al., 2009; Mohammadabadi et al., 2021). Multi-level interactions between the genome, epigenome, and environmental factors may occur, shaping bacterial adaptability and virulence (Sulimova et al., 2007; Mohammadabadi et al., 2024b). Furthermore, evidence suggests that epigenome variation influences health and productivity outcomes (Mohammadabadi, 2016). In eukaryotes, gene expression is temporally and multidimensionally controlled, with only a small subset of the

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genome expressed in each tissue type, depending on the developmental stage (Shahsavari et al., 2023; Mohammadabadi et al., 2024a). Thus, gene expression in eukaryotes is tissue-specific (Mohammadabadi et al., 2025). The quantity of gene products in a given tissue, as well as in other tissues, regulates gene expression. A fundamental activity in molecular biology is studying genes and proteins related to traits at the cellular or chromosomal level (Mohammadinejad et al., 2022; Mohammadabadi et al., 2024c). Therefore, the aim of this investigation was to address the prevalence of *Klebsiella pneumoniae* as a primary causative agent of pneumonia in the Al-Muthanna governorate, Iraq. We sought to describe the molecular fingerprint of virulence genes related to hypermucoviscosity (e.g., *magA* and *rmpA*) in epidemic strains extracted from lower respiratory tract samples of hospitalized patients with pneumonia at Al-Rumaytha Hospital. Additionally, we estimated the prevalence of hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp) strains to better understand their distribution and impact in this region.

Materials and methods

Ethics statement: This investigation was managed in strict accordance with the ethical principles outlined in the Declaration of Helsinki. The research protocol was reviewed and approved by the Institutional Review Board and the Research Ethics Committee of the University of Al-Qadisiyah, Iraq (IQ-UAQ.RES.2023.727).

Sample collection: In this investigation, 100 clinical samples were gathered from hospitalized patients diagnosed with respiratory tract infections at Al-Rumaytha Hospital. Samples were achieved applying sterile cotton swabs and immediately transferred to the laboratory for bacterial pathogen culturing.

Preservation and maintenance of bacterial isolates: Bacterial strains applied in this investigation were preserved at two levels: short-term and long-term. For short-term preservation, nutrient agar slants were prepared and stored at 4°C for four weeks, with renewal managed monthly. For long-term preservation, overnight broth cultures were prepared for each bacterial strain, and sterile glycerol was added aseptically to a final concentration of 50% (v/v). The glycerol stocks were stored at -80°C (Mahon and Lehman, 2022).

Culturing: The purity of each selected single colony was affirmed by successive streaking on nutrient agar, incubated at 37°C for 24 hours, until single colonies with consistent color, appearance, and texture (pure cultures) were achieved. Pure colonies were then sub-cultured on MacConkey agar and incubated at 37°C for 24 hours to differentiate lactose fermenters from non-lactose fermenters (Collee et al., 1996).

Colonial morphology: A single pure colony from each extracted bacterial strain was cultured on MacConkey agar to evaluate lactose fermentation capability. Lactose-fermenting

colonies appeared pink, while non-lactose-fermenting colonies appeared yellow on MacConkey agar (Collee et al., 1996).

Cell morphology-gram stain: Cell morphology and Gram staining characteristics of each bacterial strain were examined under a light microscope after staining with Gram stain. Gram-negative bacterial cells stained red with safranin as the counterstain (Collee et al., 1996).

Capsule stain: Capsule staining was carried out to differentiate between capsulated and non-capsulated bacterial strains (Collee et al., 1996).

Physiological experiments: Bacterial strains were subjected to physiological experiments, containing enzyme production (catalase, urease, and oxidase), motility, growth at 10°C, gas production from lactose after growth at 44.5°C, IMViC experiments (indole production, methyl red experiment, Voges-Proskauer experiment, and citrate utilization), and glucose fermentation with gas production but without H_2S production (Collee et al., 1996).

Genomic bacterial DNA extraction and PCR: An overnight broth culture of each clinical bacterial strain was prepared at 37°C with an agitation speed of 150 rpm. Five milliliters of each culture were transferred to 15 mL Falcon tubes, and bacterial cells were harvested by centrifugation at 8,000 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in 200 µL of lysozyme. The mixture was incubated at 37°C for 30 minutes in a water bath, followed by the addition of 20 µL of proteinase K and 200 µL of LB1 buffer to each sample. The mixture was then incubated at 60°C for 15 minutes. Subsequently, 200 µL of 100% ethanol was added and mixed by vortexing. Each sample mixture was applied to a silica spin column (Qiagen, Germany) and centrifuged at 8,000 rpm for 1 minute. The flow-through was discarded, and the spin columns were placed in new collection tubes. Five hundred microliters of buffer W2 (washing buffer) were added to each spin column, followed by centrifugation at 8,000 rpm for 1 minute, and the flow-through was discarded. This washing step was repeated with an additional 500 µL of buffer W2, followed by centrifugation at 8,000 rpm for 1 minute, and the flow-through was discarded. The empty columns were centrifuged at 8,000 rpm for 1 minute to remove residual ethanol. Each spin column was then placed in a new 1.5 mL Eppendorf tube and left at room temperature for 20 minutes to evaporate any staying ethanol. Subsequently, 50–100 µL of buffer EL3 (elution buffer) was added to each spin column, and the columns were incubated at room temperature for 5 minutes to facilitate DNA elution. The columns were then centrifuged at 12,000 rpm for 1 minute. The eluted genomic DNA samples were stored at -20°C for further apply. The primer sets applied in this investigation are listed in Table 1.

Results and discussion

Klebsiella pneumoniae strains in clinical samples: One hundred clinical isolates, gathered from hospitalized patients with respiratory tract infections (RTI) at Al-Rumaytha Hospital in Samawa, Iraq, were included in this investigation. Twenty of the 100 clinical isolates (20%) were identified as the pathogenic bacterium *Klebsiella pneumoniae*, a global threat as announced by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO, 2024). *Klebsiella pneumoniae* is a major causative agent of healthcare-related infections, containing nosocomial pneumonia, urinary tract infections, and bacteremia (Russo and Marr, 2019). Increasingly, this pathogen has been classified among the top ten multidrug-resistant pathogens, with hypervirulent strains and problematic Gram-negative bacteria noted in WHO statistics (WHO, 2024).

Table 1. Sequence of primer sets applied in this investigation

Target gene	Primer sequence (5'–3')	Product size (bp)	Reference
16S rRNA	F8-27: AGAGTTTGATCCTGGCTCAG	1500	Eden et al., 1991
	R151-1492: CTACGGCTACCTTGTTACGA		
magA	F: GGTGCTCTTTACATCATTGC	1283	Yu et al., 2006
	R: GCAATGGCCATTTGCGTTAG		
rmpA	F: ACTGGGCTACCTCTGCTTCA	409	
	R: CTTGCATGAGCCATCTTTCA		

Community-related hypervirulent strains of *K. pneumoniae* have been assigned to sequence types ST23, ST65, ST66, and ST86. Sixteen countries (e.g., Algeria, Argentina, Australia, Canada, India, Iran, Japan, Oman, Philippines, Switzerland, Thailand, and the United Kingdom) announced the prevalence of the ST23 hvKp (hypervirulent *Klebsiella pneumoniae*) strain (WHO, 2024). In the East Mediterranean region, freely accessible data on hvKp strain prevalence are scarce and announced only through laboratory surveillance for antimicrobial resistance (AMR) within healthcare services or epidemiological surveys in a few countries. Consequently, this investigation aimed to elucidate the molecular epidemiology of *K. pneumoniae* strains in respiratory tract infection samples from hospitalized patients in Iraq, with an additional centralize on identifying hvKp strains among the extracted *K. pneumoniae* strains. The identification of these 20 bacterial isolates as *K. pneumoniae* was affirmed through a five-step protocol. This identification scheme included colonial morphology, microscopic cell morphology, physiological experiments, 16S rRNA gene sequencing analysis, and molecular diagnosis of virulence-encoding

genes (Figure 1). The following sections exist the results of each step in the five-step identification scheme. Regarding the frequency of *K. pneumoniae* in respiratory tract infection samples, 20% of the isolates were identified as *K. pneumoniae* based on the five-step identification scheme described earlier. This percentage is relatively high compared to previous reports. Retrospective investigations in Makkah and Bisha, Saudi Arabia, announced frequencies of 14.7% and 18.6%, respectively (Jalal et al., 2023; Ibrahim et al., 2018). Investigations in Iran (Rahimi and Vesal, 2017) and Greece (Maraki et al., 2024) announced frequencies of 7.4% and 6.8%, respectively, for *K. pneumoniae* in lower respiratory tract infection samples from hospitalized patients. An investigation in South Africa in 2022 announced a 15.5% frequency in lower respiratory tract infection samples from infants (Zar et al., 2022). In Italy, a frequency of 10.9% was observed (Santella et al., 2021). Kaseb et al. (2023) announced a higher frequency of *K. pneumoniae* in respiratory tract infection samples in Iran compared to our results. A recent investigation in Indonesia announced a notably higher frequency of 39.96% (Prastiyanto et al., 2024). Two investigations announced frequencies of 37.5% (Behera et al., 2020) and 27.5% (Duan et al., 2020) in respiratory tract infection samples from intensive care unit patients. In Egypt, *Klebsiella* spp. accounted for 34.7% of clinical isolates from Menoufia University Hospitals, with *K. pneumoniae* being the predominant species (91.3%) (Elbrolosy et al., 2020). El-Badawy et al. (2017) found *Klebsiella* spp. to be the most frequent pathogens (38%) among nosocomial isolates. It was announced a 37.93% frequency for *K. pneumoniae* in lower respiratory tract specimens. In Iraq, an investigation revealed a 54.84% frequency for *K. pneumoniae* in lower respiratory tract infection specimens (Madhi et al., 2024), while a lower percentage (12%) was announced in respiratory tract specimens from Iraq (Almjalawi et al., 2022). The variation in *K. pneumoniae* frequency across investigations may be attributed to differences in sample size, epidemiological factors, regional variations in hygiene status, and patient inclusion criteria. This highlights the need for larger sample sizes in future investigations to align with another research.

Colonial morphology of *K. pneumoniae* strains: Twenty out of 100 bacterial strains were extracted on MacConkey agar at 37°C after 24 hours. The typical colonial morphology of *K. pneumoniae* on MacConkey agar showed pink, regular-edged, mucoid, lactose-fermenting colonies (Figure 2). The selective and differential MacConkey agar medium inhibits the growth of Gram-positive bacteria and distinguishes lactose-fermenting isolates (pink colonies) from non-lactose-fermenting ones (yellow colonies). The medium contains crystal violet as a Gram-positive growth inhibitor and lactose as the differential agent. The colonial morphology strongly supported the presumptive identification of these strains as *K. pneumoniae* and facilitated progression to the next identification step (cell morphological features) in the scheme.

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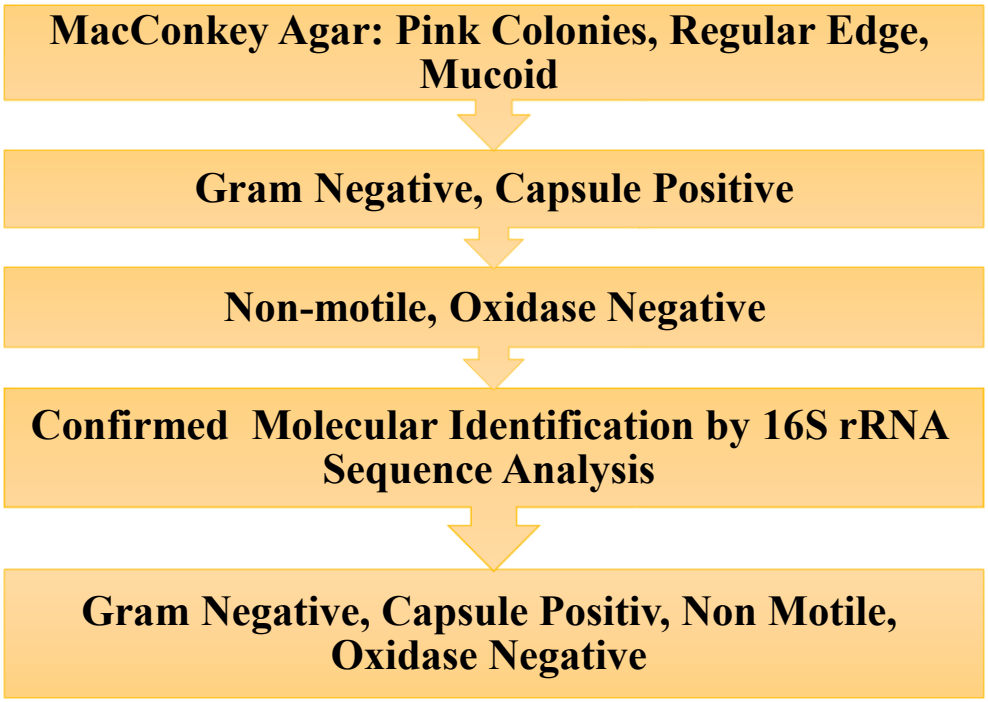

Figure 1. A chart deciphering the five-step identification scheme employed in this investigation to verify the identity of bacterial strains extracted from clinical samples as *Klebsiella pneumoniae*

Figure 2. *K. pneumoniae* colonies showed pink, mucoid, regular-edged appearance on a MacConkey agar plate incubated at 37°C for 24 hours

Cell morphological features of *K. pneumoniae* strains: The 20 bacterial strains presumptively identified as *K. pneumoniae* based on colonial morphology were subjected to the

second step of the five-step identification scheme: examination of cell morphological features under a light microscope. The experimented features included Gram type, evaluateed by Gram staining, and the presence or absence of a capsule, identified by capsule staining. All 20 strains were affirmed as Gram-negative based on Gram staining results. Additionally, all 20 strains exhibited a capsule, as evidenced by capsule staining (Figure 3). These morphological results prepared further evidence supporting the identification of the 20 strains as *K. pneumoniae* and justified progression to the third step: physiological experiments.

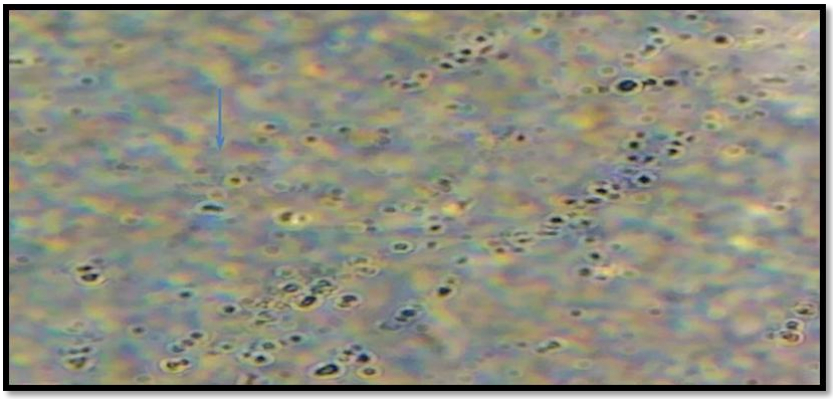

Figure 3. A capsule stain photo illustrating the presence of a capsule in one of 20 experimented bacterial strains, as a representative example

Physiological experiments of *K. pneumoniae* strains: A series of physiological experiments was managed, containing enzyme production (catalase, urease, and oxidase), motility, growth at 10°C, gas production from lactose at 44.5°C, the IMViC experiment (indole production, methyl red experiment, Voges-Proskauer experiment, and citrate utilization), and glucose fermentation with gas production and without H_2S production. The results are presented in Table 2. The physiological experiment profile was consistent with that of *K. pneumoniae*, providing additional evidence for the identification of the 20 experimented strains as *K. pneumoniae*.

16S ribosomal RNA (16S rRNA) gene sequence analysis of *K. pneumoniae* strains: The full length of the 16S rRNA gene (1500 bp) was successfully amplified by PCR from the 20 experimented clinical strains of *K. pneumoniae* in this investigation (Figure 4). Molecular identification via 16S rRNA gene sequencing, combined with colony morphology, cell morphology, and physiological experiments, affirmed all 20 strains as *K. pneumoniae*. A BLASTN (Basic Local Alignment Search Tool) similarity search revealed sequence similarity ranging from 98% to 99.8% with other *K. pneumoniae* 16S rRNA gene sequences deposited in NCBI. Based on molecular identification guidelines, a sequence similarity >98% affirms species-

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level identification. The 16S rRNA gene sequences for 15 of the experimented strains were deposited in the GenBank database under accession numbers: PQ814166, PQ814167, PQ814168, PQ814169, PQ814170, PQ814171, PQ814172, PQ814173, PQ814174, PQ814175, PQ814176, PQ814177, PQ814178, PQ814179, and PQ814180.

Table 2. Physiological experiments profile for 20 *K. pneumoniae* strains

Experiment	*K. pneumoniae*
Motility	–
Urease	+
Indole production	–
Methyl Red experiment	V
Voges-Proskauer experiment	+
Citrate utilization	+
Oxidase production	–
Catalase production	V
Growth at 10°C	–
Gas production from lactose at 44.5°C	+
Gas production from glucose (acid butt/acid slant), no H_2S production	+

***(+): positive result, (–): negative result, (V): variable result.**

A multiple sequence alignment, managed applying the MUSCLE algorithm in MEGA 11, compared the 15 nucleotide sequences of the 16S rRNA gene from the experimented *K. pneumoniae* isolates with eight sequences from closely related species deposited in GenBank, as identified by BLASTN analysis (Figure 5). The alignment revealed near-complete matching among the 23 sequences, with single nucleotide polymorphisms (SNPs) at specific positions across the gene. In spite of these variations, all 15 experimented isolates were affirmed as *K. pneumoniae*. However, these nucleotide differences highlighted variability among the 15 strains, which was further elucidated in the phylogenetic tree (Figure 6). The SNPs, randomly distributed along the 16S rRNA gene, may reflect the polymorphic nature of the gene, with multiple copies of the rRNA operon (16S rRNA gene, internal transcribed spacer, tRNA gene, 23S rRNA gene, and 5S rRNA gene) exist within the same strain. The genetic relatedness of these 15 strains to other closely related species was examined through a phylogenetic tree constructed applying MEGA 11 (Figure 6). In spite of the restricted capability of 16S rRNA gene sequencing to differentiate strains within the same species, the tree revealed that the *K. pneumoniae* isolates from this investigation formed 10 clades. Clade A included PQ814167 and PQ814177; Clade B included PQ814171; Clade C comprised PQ814166 and PQ814176; Clade D involved PQ814179 and PQ814180; Clades E, F, and G included PQ814169, PQ814173, and PQ814174, respectively;

Clade H included PQ814170 and PQ814175; Clade I included PQ814172; and Clade J comprised PQ814168 and PQ814178. Other closely related *K. pneumoniae* strains from countries like Mexico, China, Hungary, Germany, Saudi Arabia, India, and Greece showed distinct genetic affiliations, more distant than the local clinical strains from this investigation. This suggests that the Iraqi *K. pneumoniae* clinical strains investigated here exhibit a unique pattern, except for Clade G. Thus, further genotyping investigations applying methods like multi-locus sequence typing (MLST), core-genome MLST, rRNA-MLST, and random amplified polymorphic DNA (RAPD) are warranted to describe these strains at the strain level. The 16S rRNA gene sequence analysis strongly verified the identity of 15 out of 20 clinical *K. pneumoniae* strains, supporting progression to the final step: diagnosis of predominant virulence genes.

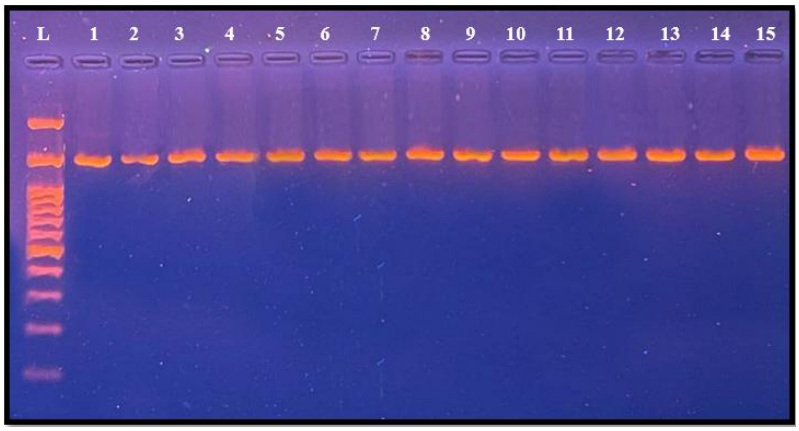

Figure 4. 1% agarose gel electrophoresis showing the amplification of the full length of the 16S rRNA gene from a *K. pneumoniae* clinical strain in this investigation as a representative example

Conventional identification of *K. pneumoniae* has relied on phenotypic biomarkers, like biochemical profiles (Podschun and Ullmann, 1998), which often fail to prepare accurate identification. In spite of limitations, biochemical profile-based methods stay routine tools for rapid bacterial identification. Polyphasic identification protocols have long been recommended for bacterial identification. Thus, confirmation of conventional methods via molecular approaches is essential. Our polyphasic identification protocol included five steps, culminating in 16S rRNA gene sequence analysis. Our results showed 100% concordance between conventional phenotypic methods and molecular identification for all 10 isolates submitted for sequencing. In contrast, He et al. (2016) announced 86% (13/15) concordance between phenotypic and molecular approaches. Our molecular identification successfully affirmed the isolates at both genus and species levels, unlike He et al. (2016), where identification was restricted to the genus level. Similarly, Abbas et al. (2020) announced 100% concordance between phenotypic and molecular approaches for *K.*

pneumoniae strains from wound and burn infections. Discrepancies in 16S rRNA-based trees across investigations suggest the need for additional genes, like *khe* and *rpoB*, to ensure identification at both genus and species levels. Although 16S rRNA gene sequencing is attended the gold standard for bacterial identification, it struggles to distinguish closely related species, like *Klebsiella* and *Shigella* (He et al., 2016).

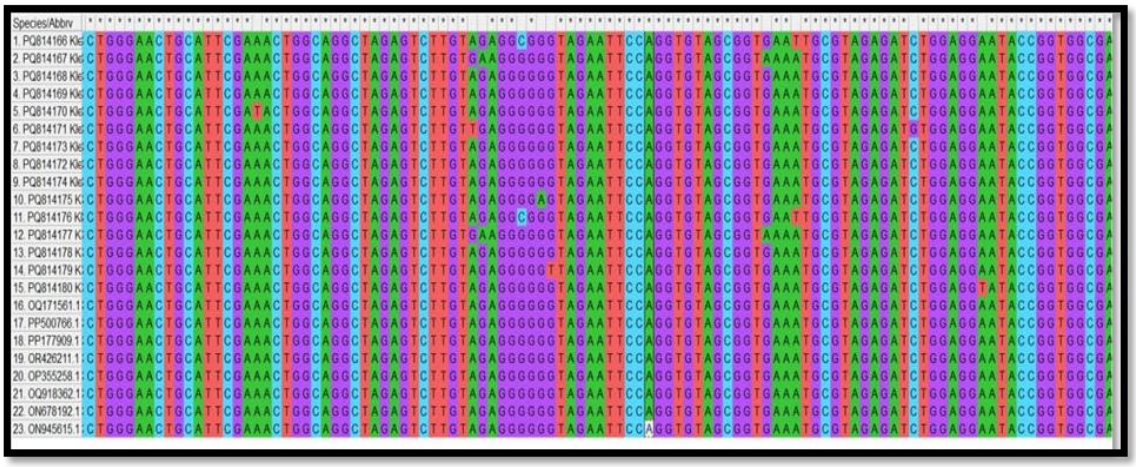

Figure 5. A screenshot of a multiple sequence alignment, carried out by MEGA 11, comparing 15 nucleotide sequences of the 16S rRNA gene (PQ814166, PQ814167, PQ814168, PQ814169, PQ814170, PQ814171, PQ814172, PQ814173, PQ814174, PQ814175, PQ814176, PQ814177, PQ814178, PQ814179, and PQ814180) from *K. pneumoniae* strains in this investigation with eight sequences (OQ171561.1, PP500766.1, PP177909.1, OR426211.1, OP355258.1, OQ918362.1, ON78192.1, and ON945615.1) of closely related species deposited in GenBank, selected based on BLASTN analysis. Identity (100%) at the nucleotide level was evident at most positions, with some bases showing variation along the gene

Additionally, 16S rRNA-based trees in Enterobacteriaceae are inconsistent across publications regarding the relative positions of genera (Granier et al., 2003). This variability may stem from the polymorphic nature of the 16S rRNA gene, with multiple copies announced across bacterial species and strains (Hashimoto et al., 2003). Our 16S rRNA-based tree revealed variations among the *K. pneumoniae* isolates, supporting their grouping into different clades (Figure 6). This underscores the polymorphic nature of the gene. However, further discrimination needs molecular approaches like multi-locus sequence typing (MLST).

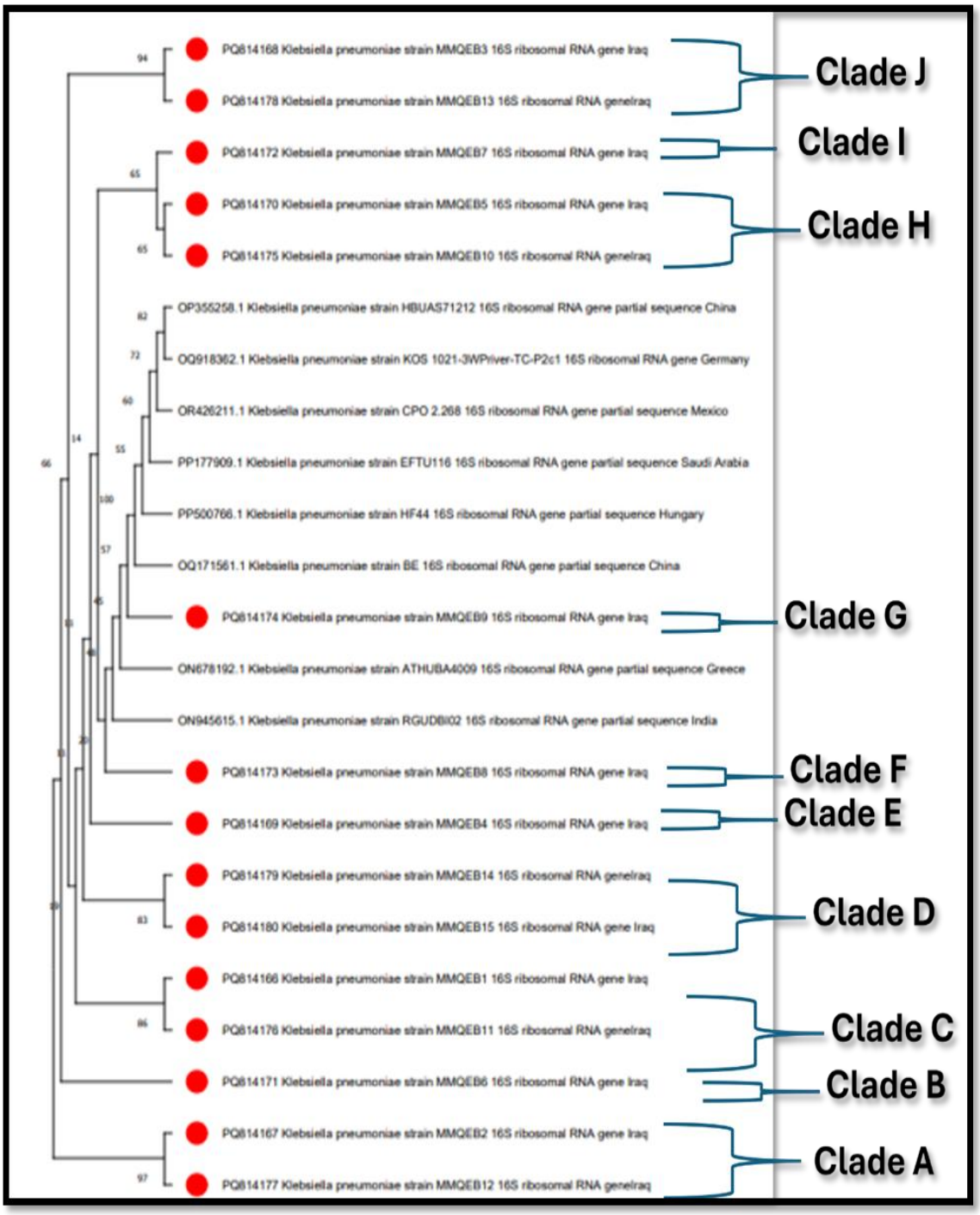

Figure 6. A phylogenetic tree constructed with MEGA 11 illustrating the genetic relatedness of 15 *K. pneumoniae* clinical strains extracted in this investigation with other closely related *K. pneumoniae* strains from numerous countries. Clades represent groups of closely related *K. pneumoniae* strains based on 16S rRNA gene sequences. Local *K. pneumoniae* clinical strains are highlighted with filled red circles. Numbers on branches represent bootstrap values from 1000 resamplings

Diagnosis of virulence genes in *K. pneumoniae* strains-diagnosis of Mucoviscosity-related gene A (*magA*): PCR amplification of a 1283 bp fragment of the capsular virulence factor *magA* gene yielded varied results among the 20 experimented clinical *K. pneumoniae* strains (Figure 7). Data showed that genomic DNA from 11 of 20 strains (55%) successfully amplified the *magA* fragment, while 45% were negative.

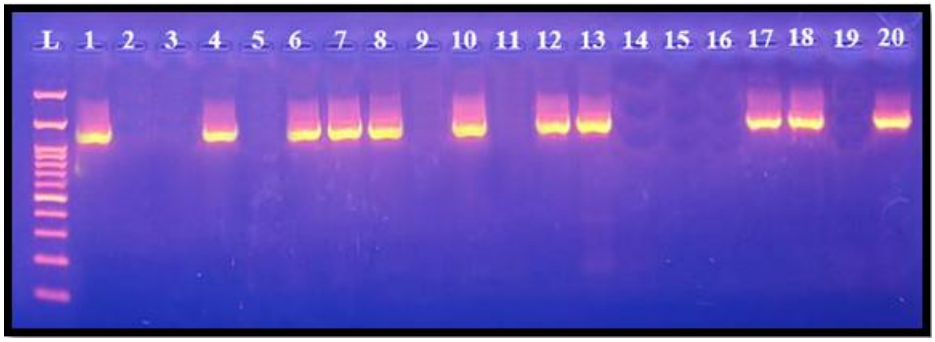

Figure 7. 1% agarose gel electrophoresis showing the amplification of a 1283 bp fragment of the *magA* gene from 20 clinical *K. pneumoniae* strains. L: DNA ladder. Lanes 1-20: PCR products from *magA* gene amplification applying genomic DNA from the 20 strains

The *magA* and *rmpA* genes are implicated in *K. pneumoniae* virulence, exclusively mucoviscosity (Lin et al., 2012). Our data revealed 55% and 50% occurrence of *magA* and *rmpA* genes, respectively, among the 20 experimented isolates (Figures 7 and 8). In contrast, Zamani et al. (2013) announced a lower 3.8% occurrence of *magA* in *K. pneumoniae* from clinical samples. However, researchers found a 98% occurrence of *magA* in *K. pneumoniae* from liver abscesses.

Diagnosis of regulator of mucoid phenotype A gene (*rmpA*): PCR amplification of a 409 bp fragment of the capsular virulence factor *rmpA* gene showed varied results among the 20 experimented *K. pneumoniae* strains (Figure 8). Data indicated that genomic DNA from 10 of 20 strains (50%) successfully amplified the *rmpA* fragment, with 50% negative. The *rmpA* gene exhibited a 58% occurrence in *K. pneumoniae* from urine samples in Egypt and 16.5% in Poland (Kot et al., 2023). In India, occurrence rates were 22% for *rmpA*, 11% for *magA*, and 7% for co-occurrence of both genes (Devanathan et al., 2024). Ali et al. (2020) announced a 7.34% occurrence of *magA* in *K. pneumoniae* from ICU and infectious disease ward patients in Bangladesh. Amraie et al. (2014) observed a 2.3% occurrence of *magA* in 173 *K. pneumoniae* strains from numerous clinical samples in Iran. Liu et al. (2018) announced 43.53% and 62.87% occurrence of *rmpA* and *magA*, respectively, in *K. pneumoniae* from elderly hospitalized patients in China. Another investigation in China in 2016 showed a 40% occurrence of *rmpA* (Zhang et al., 2016). In Iraq's Najaf Governorate, *magA* and *rmpA* exhibited 58% and 62% occurrence, respectively (Al-Kamoosi and Al-Azawi, 2021). Another Iraqi investigation announced 45.65%

and 23.91% occurrence of *rmpA* and *magA*, respectively, in *K. pneumoniae* from Babylon Hospital (Abdul-Razzaq et al., 2014). In Najaf City, Iraq, occurrence rates were 7% for *magA* and 27.7% for *rmpA* (AL-Aammar et al., 2023). Bakhtiari et al. (2021) announced a 27.07% occurrence of *rmpA* in *K. pneumoniae* from hospitalized patients in Tehran, Iran.

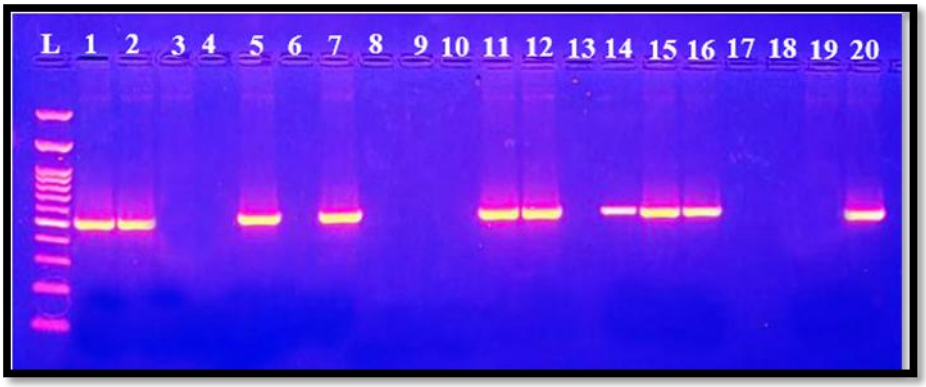

Figure 8. 1% agarose gel electrophoresis showing the amplification of a 409 bp fragment of the *rmpA* gene from 20 clinical *K. pneumoniae* strains. L: DNA ladder. Lanes 1-20: PCR products from *rmpA* gene amplification applying genomic DNA from the 20 strains

Ratio of hvKp to cKp: The ratio of hypervirulent *K. pneumoniae* (hvKp) to classical *K. pneumoniae* (cKp) strains was estimated. Data revealed that 10 of 20 experimented strains (50%) harbored both *magA* and *rmpA* virulence genes, denoting 50% were hvKp and 50% were cKp. Hypervirulent *K. pneumoniae* (hvKp) is an emerging pathotype with greater virulence than classical *K. pneumoniae* (cKp). The hvKp pathotype is often described by multidrug resistance. Distinguishing between cKp and hvKp is critical for diagnostics, predicting patient outcomes after antibiotic treatment, and controlling prevalence through epidemics. Knowledge gaps regarding hvKp include: (a) incidence across nations, (b) rising antibiotic resistance profiles, (c) mechanisms of pathogen entry, (d) delineation of hvKp virulence genes, (e) effective control strategies, (f) treatment duration, (g) mechanisms of metastatic spread, (h) benefits of prophylaxis for close contacts, and (i) infection control in intensive care units through hvKp infections. Our results revealed that 50% of the 20 experimented isolates were hvKp, raising concerns about the prevalence of hvKp in Iraq, especially given the scarcity of data in the East Mediterranean region, where information is restricted to laboratory surveillance for AMR in a few countries. Our data align with an investigation in China reporting a 47.52% (96/202) occurrence of hvKp among *K. pneumoniae* from elderly hospitalized patients (Liu et al., 2018). Another investigation in China in 2016 announced a 37.8% occurrence (Zhang et al., 2016). In Najaf City, Iraq, a 2023

investigation announced an 80% (80/100) occurrence of hvKp in *K. pneumoniae* from numerous clinical samples (AL-Aammar et al., 2023). Variations in the occurrence of *magA* and *rmpA* across investigations may be attributed to factors like sample size, diagnosis method sensitivity, source of clinical samples, antibiotic apply, geographical distribution, and the number of genes evaluateed for hypermucoviscosity and biofilm formation in *K. pneumoniae* isolates.

Conclusions: Our results underscore the critical role of molecular characterization in elucidating the pathogenicity and epidemiological patterns of *Klebsiella pneumoniae* in clinical settings. This investigation highlights distinct molecular features that contribute to the virulence and spread of this pathogen, emphasizing the need for targeted research to further investigate these mechanisms. Specifically, our data strongly advocate for additional researches to investigate the molecular differences between hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp) pathotypes, as these distinctions could inform clinical management and infection control strategies. Furthermore, we recommend the apply of the *rpoB* gene for phylogenetic tree construction to accurately differentiate closely related *Klebsiella* species, containing *K. pneumoniae*, *K. quasipneumoniae*, and *K. oxytoca*, thereby enhancing taxonomic resolution and diagnostic precision. Additionally, our results robustly support the application of multilocus sequence typing (MLST) as a reliable method to distinguish strains within *K. pneumoniae*, facilitating a deeper understanding of strain diversity and transmission dynamics. These insights pave the way for improved therapeutic approaches, surveillance programs, and public health interventions to mitigate the impact of *K. pneumoniae* infections.

Author contributions

Conceptualization and investigation design: A.M., Data acquisition: A.A.M., Data analysis and interpretation: A.A.M. and A.M., Manuscript drafting: A.A.M., Critical revision of the manuscript for intellectual content: A.M., Writing—original draft preparation: A.A.M., Writing—review and editing: A.M., Supervision and project administration: A.M., Final approval of the manuscript: A.M. and A.A.M. All authors have thoroughly reviewed and approved the final version of the manuscript for submission and agree to be accountable for all aspects of the work, ensuring its accuracy and integrity.

Data availability statement

The datasets generated and analyzed through the current investigation are not publicly available due to privacy and ethical restrictions but can be achieved from the corresponding author upon reasonable request, subject to approval by the relevant institutional authorities.

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Ethical considerations

This investigation was managed in strict accordance with the ethical principles outlined in the Declaration of Helsinki. The research protocol was reviewed and approved by the Institutional Review Board and the Research Ethics Committee of the University of Al-Qadisiyah, Iraq (IQ-UAQ.RES.2023.727). Informed consent was achieved from all participants prior to their involvement. Participation was entirely voluntary, and all subjects were fully informed about the investigation's purpose, procedures, potential risks, and benefits. They were also assured of their right to withdraw from the investigation at any time without any consequences. The confidentiality of all personal and sensitive data was rigorously maintained throughout the investigation, with data anonymized to protect participant privacy.

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Conflict of interest

The authors declare no conflicts of interest related to this investigation. No financial or personal relationships exist that could have influenced the design, execution, or reporting of this investigation.

References

Abbas, O. N., Mhawesh, A. A., & Al-Shaibani, A. B. (2020). Molecular identification of pathogenic *Klebsiella pneumoniae* strains producing biofilm. *Medico-Legal Update, 20*(3), 1068–1074. https://doi.org/10.37506/MLU.V20I3.1544

Abdul-Razzaq, M. S., Al-Khafaji, J. K. T., & Al-Maamory, E. H. K. A. (2014). Molecular characterization of capsular polysaccharide genes of *Klebsiella pneumoniae* in Iraq. *International Journal of Current Microbiology and Applied Sciences, 3*(7), 224–234.

Al-Aammar, M. H. (2023). Molecular detection of some virulence factors of hypervirulent *Klebsiella pneumoniae* that are associated with pathogenicity. *Al-Kufa University Journal for Biology, 15*(2), 73–80. https://doi.org/10.36320/ajb/v15.i2.12008

Ali, M. H., Anwar, S., Toma, N. J., Rafid, I., Hasan, M. K., & Foysal, M. J. (2020). Molecular detection and PCR-RFLP analysis of Mucoviscosity-associated gene A (*magA*) in clinical isolates of multidrug-resistant *Klebsiella pneumoniae* in Bangladesh. *The Open Microbiology Journal, 14*(1), 196–204. https://doi.org/10.2174/1874285802014010196

Al-Kamoosi, A., & Al-Azawi, I. (2021). Detection of capsular polysaccharide virulence genes *rmpA* and *magA* of *Klebsiella pneumoniae* isolate from diabetic foot ulcer patient in Najaf Governorate in Iraq. *Indian Journal of Forensic Medicine & Toxicology, 15*(2), 3061–3067. https://doi.org/10.37506/ijfmt.v15i2.14841

Almjalawi, B. S. A., Al-Awade, H. A. R., Al-Mafragy, H. S., & AL Masaoodi, N. (2022). Antibacterial activity of *Capsicum annuum* L. juice against *Klebsiella pneumoniae* isolated from respiratory tract infections. *Iranian Journal of War and Public Health, 14*(2), 139–146. http://ijwph.ir/article-1-1146-en.html

Amraie, H., Shakib, P., Rouhi, S., Bakhshandeh, N., & Zamanzad, B. (2014). Prevalence assessment of *magA* gene and antimicrobial susceptibility of *Klebsiella pneumoniae* isolated from clinical specimens in Shahrekord, Iran. *Iranian Journal of Microbiology, 6*(5), 311–316. https://ijm.tums.ac.ir/index.php/ijm/article/view/379

Bakhtiari, R., Javadi, A., Aminzadeh, M., Molaee-Aghaee, E., & Shaffaghat, Z. (2021). Association between presence of *rmpA*, *mrkA* and *mrkD* genes and antibiotic resistance in clinical *Klebsiella pneumoniae* isolates from hospitals in Tehran, Iran. *Iranian Journal of Public Health, 50*(5), 1009–1016. https://doi.org/10.18502/ijph.v50i5.6118

Behera, B., Sahu, K. K., Bhoi, P., & Mohanty, J. N. (2020). Prevalence and antimicrobial susceptibility patterns of bacteria in ICU patients with lower respiratory tract infection: A cross-sectional study. *Journal of Acute Disease, 9*(4), 157–160. https://doi.org/10.4103/2221-6189.288593

Brisse, S., Grimont, F., & Grimont, P. A. D. (2006). The Genus *Klebsiella*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. (eds) The Prokaryotes. Springer, New York, NY. https://doi.org/10.1007/0-387-30746-X_8

Brooks, G. F., Carrol, K. C., Butel, J. S., & Morse, S.A. (2007) Jawetz, Melnick and Adelber's Medical Microbiology. 24th Edition, McGraw Hill Companies, New York, 44. https://www.scirp.org/reference/referencespapers?referenceid=3405269

Collee, J., Fraser, A., Marmion, B., & Simons, A. (1996). *Mackie and McCartney's practical medical microbiology* (14th ed., p. 561). Churchill Livingstone.

Devanathan, K., Sivaraman, U., Chinnadurai, R., Easow, J. M., & Vinayagam, V. (2024). Prevalence of hypervirulent rmpA and magA genes in clinical isolates of *Klebsiella pneumoniae* and their association with drug resistant pattern: A cross-sectional study. *Journal of Clinical & Diagnostic Research, 18*(7), DC20–DC24. https://doi.org/10.7860/JCDR/2024/69730.19668

Duan, N., Du, J., Huang, C., & Li, H. (2020). Microbial distribution and antibiotic susceptibility of lower respiratory tract infections patients from pediatric ward, adult respiratory ward, and respiratory intensive care unit. *Frontiers in Microbiology*, *11*, Article 1480. https://doi.org/10.3389/fmicb.2020.01480

El-Badawy, M. F., Tawakol, W. M., El-Far, S. W., Maghrabi, I. A., Al-Ghamdi, S. A., Mansy, M. S., Ashour, M. S., & Shohayeb, M. M. (2017). Molecular identification of aminoglycoside-modifying enzymes and plasmid-mediated quinolone resistance genes among *Klebsiella pneumoniae* clinical isolates recovered from Egyptian patients. *International Journal of Microbiology, 2017*, 8050432. https://doi.org/10.1155/2017/8050432

Elbrolosy, A., Eissa, N., Al-Rajhy, N., El-Mahdy, E., & Mostafa, R. (2020). *MrkD* gene as a regulator of biofilm formation with correlation to antibiotic resistance among clinical *Klebsiella pneumoniae* isolates from Menoufia University Hospitals. *Egyptian Journal of Medical Microbiology, 29*(3), 137–144. https://doi.org/10.51429/EJMM29318

Granier, S. A., Plaisance, L., Leflon-Guibout, V., Lagier, E., Morand, S., Goldstein, F. W., & Nicolas-Chanoine, M. H. (2003). Recognition of two genetic groups in the *Klebsiella oxytoca* taxon on the basis of chromosomal beta-lactamase and housekeeping gene sequences as well as ERIC-1R PCR typing. *International Journal of Systematic and Evolutionary Microbiology, 53*(Pt 3), 661–668. https://doi.org/10.1099/ijs.0.02408-0

Hashimoto, J. G., Stevenson, B. S., & Schmidt, T. M. (2003). Rates and consequences of recombination between rRNA operons. *Journal of Bacteriology, 185*(3), 966–972. https://doi.org/10.1128/JB.185.3.966-972.2003

He, Y., Guo, X., Xiang, S., Li, J., Li, X., Xiang, H., He, J., Chen, D., & Chen, J. (2016). Comparative analyses of phenotypic methods and 16S rRNA, *khe*, *rpoB* genes sequencing

for identification of clinical isolates of *Klebsiella pneumoniae*. *Antonie van Leeuwenhoek, 109*(7), 1029–1040. https://doi.org/10.1007/s10482-016-0702-9

Highsmith, A. K., & Jarvis, W. R. (1985). Klebsiella pneumoniae: Selected virulence factors that contribute to pathogenicity. Infection Control, 6(2), 75–77. https://doi.org/10.1017/s0195941700062640

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's manual of determinative bacteriology* (9th ed., pp. 786–788). Williams & Wilkins. Baltimore, 786-788. https://www.scirp.org/reference/referencespapers?referenceid=1838672

Ibrahim, M. E. (2018). High antimicrobial resistant rates among Gram-negative pathogens in intensive care units: A retrospective study at a tertiary care hospital in Southwest Saudi Arabia. *Saudi Medical Journal, 39*(10), 1035–1043. https://doi.org/10.15537/smj.2018.10.22944

Jalal, N. A., Al-Ghamdi, A. M., Momenah, A. M., Ashgar, S. S., Bantun, F., Bahwerth, F. S., Hariri, S. H., Johargy, A. K., Barhameen, A. A., Al-Said, H. M., & Faidah, H. (2023). Prevalence and antibiogram pattern of *Klebsiella pneumoniae* in a tertiary care hospital in Makkah, Saudi Arabia: An 11-year experience. *Antibiotics, 12*(1), 164. https://doi.org/10.3390/antibiotics12010164

Kaseb, Z., Hassanzadeh, S., Mehri, A., Khosravi, M., Ganjloo, S., & Ghazvini, K. (2023). The prevalence of respiratory tract infections in the Ghaem Hospital of Mashhad. *African Journal of Biotechnology Research, 15*(2), 31–35. https://academicjournals.org/journal/JBR/article-abstract/5C3A8A770925

Kawai, T. (2006). Hypermucoviscosity: An extremely sticky phenotype of Klebsiella pneumoniae associated with emerging destructive tissue abscess syndrome. *Clinical Infectious Diseases, 42*(10), 1359–1361. https://doi.org/10.1086/503429

Kot, B., Piechota, M., Szweda, P., Mitrus, J., Wicha, J., Grużewska, A., & Witeska, M. (2023). Virulence analysis and antibiotic resistance of *Klebsiella pneumoniae* isolates from hospitalised patients in Poland. *Scientific Reports, 13*, 4448. https://doi.org/10.1038/s41598-023-31086-w

Lau, H. Y. F. (2007). *Identification of Endogenous Mechanisms That Affect Klebsiella pneumoniae Growth in the Murine Host* (Doctoral dissertation). A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Microbiology and Immunology) in the University of Michigan 2007.

Lin, T. L., Yang, F. L., Yang, A. S., Peng, H. P., Li, T. L., Tsai, M. D., Wu, S. H., & Wang, J. T. (2012). Amino acid substitutions of MagA in Klebsiella pneumoniae affect the biosynthesis

of the capsular polysaccharide. *PLoS ONE, 7*(10), e46783. https://doi.org/10.1371/journal.pone.0046783

Liu, C., Shi, J., & Guo, J. (2018). High prevalence of hypervirulent *Klebsiella pneumoniae* infection in the genetic background of elderly patients in two teaching hospitals in China. *Infection and Drug Resistance, 11*, 1031–1041. https://doi.org/10.2147/IDR.S161075

Madhi, K. S., Jasim, A. S., Nasear, H. A., Ibraheim, H. K., & Gharban, H. A. J. (2024). Phylogenetic analysis of *Klebsiella pneumoniae* isolates of respiratory tract infections in humans and sheep. *Open Veterinary Journal, 14*(9), 2325–2333. https://doi.org/10.5455/OVJ.2024.v14.i9.21

Mahon, C. R., & Lehman, D. C. (2022). *Textbook of diagnostic microbiology* (7th ed.). Elsevier.

Maraki, S., Mavromanolaki, V. E., Kasimati, A., Iliaki-Giannakoudaki, E., & Stafylaki, D. (2024). Prevalence and antimicrobial resistance trends among lower respiratory tract pathogens in Crete, Greece, 2017–2022. *Infection & Chemotherapy, 56*(4), 492–501. https://doi.org/10.3947/ic.2024.0060

Mohammadabadi, M. (2016). Inter-simple sequence repeat loci associations with predicted breeding values of body weight in kermani sheep. *Genetics in the Third Millennium, 14*(4), 4386-4393. https://sciexplore.ir/Documents/Details/472-591-539-924

Mohammadabadi, M. and Asadollahpour Nanaei, H. (2021). Leptin gene expression in Raini Cashmere goat using Real Time PCR. *Agricultural Biotechnology Journal*, *13*(1), 197-214. https://doi.org/10.22103/jab.2021.17334.1305

Mohammadabadi, M., Afsharmanesh, M., Khezri, A., Kheyrodin, H., Babenko Ivanivna, O., Borshch, O., Kalashnyk, O., Nechyporenko, O., Afanasenko, V., Slynko, V. and Usenko, S. (2025). Effect of Mealworm on GBP4L Gene Expression in the Spleen Tissue of Ross Broiler Chickens. *Agricultural Biotechnology Journal*, *17*(2), 343-360. doi: 10.22103/jab.2025.25277.1714

Mohammadabadi, M., Akhtarpoor, A., Khezri, A., Babenko, O., Stavetska, R. V., Tytarenko, I., Ievstafiieva, Y., Buchkovska, V., Slynko, V. and Afanasenko, V. (2024a). The role and diverse applications of machine learning in genetics, breeding, and biotechnology of livestock and poultry. *Agricultural Biotechnology Journal*, *16*(4), 413-442. https://doi.org/10.22103/jab.2025.24662.1644

Mohammadabadi, M., Babenko Ivanivna, O., Borshch, O., Kalashnyk, O., Ievstafiieva, Y. and Buchkovska, V. (2024c). Measuring the relative expression pattern of the UCP2 gene in different tissues of the Raini Cashmere goat. *Agricultural Biotechnology Journal*, *16*(3), 317-332. https://doi.org/10.22103/jab.2024.24337.1627

Mohammadabadi, M., Kheyrodin, H., Afanasenko, V., Babenko Ivanivna, O., Klopenko, N., Kalashnyk, O., Ievstafiieva, Y., & Buchkovska, V. (2024b). The role of artificial intelligence in genomics. *Agricultural Biotechnology Journal, 16*(2), 195-279. https://doi.org/10.22103/jab.2024.23558.1575

Mohammadinejad, F., Mohammadabadi, M., Roudbari, Z., & Sadkowski, T. (2022). Identification of Key Genes and Biological Pathways Associated with Skeletal Muscle Maturation and Hypertrophy in *Bos taurus*, *Ovis aries,* and *Sus scrofa*. *Animals*, *12*(24), 3471. https://doi.org/10.3390/ani12243471

Mousavizadeh, A., Mohammad Abadi, M., Torabi, A., Nassiry, M. R., Ghiasi, H. and AliEsmailizadeh Koshkoieh, A. (2009). Genetic Polymorphism at the Growth Hormone Locus in Iranian Talli Goats by Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP). *Iranian Journal of Biotechnology*, *7*(1), 51-53. https://www.ijbiotech.com/article_7064.html

Park, J. S., Hong, K. H., Lee, H. J., Choi, S. H., Song, S. H., Song, K. H., Kim, H. B., Park, K. U., Song, J., & Kim, E. C. (2011). Evaluation of three phenotypic identification systems for clinical isolates of *Raoultella ornithinolytica*. *Journal of Medical Microbiology, 60*(Pt 4), 492–499. https://doi.org/10.1099/jmm.0.020768-0

Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews, 11*(4), 589–603. https://doi.org/10.1128/CMR.11.4.589

Prastiyanto, M., Rahmah, A., Punjungsari, T., Harianie, L., Rukmana, R., & Chairunnisa, A. (2024). Prevalence of multi-drug-resistant bacteria from sputum isolates of respiratory infections from Indonesian pneumonia patients. *Microbes and Infectious Diseases*. Advance online publication. https://doi.org/10.21608/mid.2024.341022.2377

Rahimi, B., & Vesal, A. (2017). Prevalence study of multi-drug resistant klebsiella pneumoniae strains isolated from respiratory tract infections. *Journal of Pure and Applied Microbiology*, *11*(1), 181-186. https://doi.org/10.22207/JPAM.11.1.23

Rivero, A., Gomez, E., Alland, D., Huang, D. B., & Chiang, T. (2010). K2 serotype *Klebsiella pneumoniae* causing a liver abscess associated with infective endocarditis. *Journal of Clinical Microbiology, 48*(2), 639–641. https://doi.org/10.1128/JCM.01779-09

Russo, T. A., & Marr, C. M. (2019). Hypervirulent *Klebsiella pneumoniae*. *Clinical Microbiology Reviews, 32*(3), e00001-19. https://doi.org/10.1128/CMR.00001-19

Sandegren, L., Linkevicius, M., Lytsy, B., Melhus, Å., & Andersson, D. I. (2012). Transfer of an Escherichia coli ST131 multiresistance cassette has created a Klebsiella pneumoniae-

specific plasmid associated with a major nosocomial outbreak. *Journal of Antimicrobial Chemotherapy, 67*(1), 74–83. https://doi.org/10.1093/jac/dkr405

Santella, B., Serretiello, E., De Filippis, A., Veronica, F., Iervolino, D., Dell'Annunziata, F., Manente, R., Valitutti, F., Santoro, E., Pagliano, P., Galdiero, M., Boccia, G., & Franci, G. (2021). Lower respiratory tract pathogens and their antimicrobial susceptibility pattern: A 5-year study. *Antibiotics, 10*(7), 851. https://doi.org/10.3390/antibiotics10070851

Shahsavari, M., Mohammadabadi, M., Khezri, A., Asadi Fozi, M., Babenko, O., Kalashnyk, O., Oleshko, V., & Tkachenko, S. (2023). Correlation between insulin-like growth factor 1 gene expression and fennel (*Foeniculum vulgare*) seed powder consumption in muscle of sheep. *Animal Biotechnology*, *34*(4), 882–892. https://doi.org/10.1080/10495398.2021.2000997

Sulimova, G. E., Azari, M. A., Rostamzadeh, J., Mohammad Abadi M.R., & Lazebny O.E. (2007). κ-casein gene (*CSN3*) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker. *Russian Journal of Genetics 43*, 73–79. https://doi.org/10.1134/S1022795407010115

Turton, J. F., Perry, C., Elgohari, S., & Hampton, C. V. (2010). PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. Journal of Medical Microbiology, 59(5), 541–547. https://doi.org/10.1099/jmm.0.015198-0

WHO (2024). Antimicrobial Resistance, Hypervirulent Klebsiella pneumoniae - Global situation. https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON527

Zamani, A., Yousefi Mashouf, R., Ebrahimzadeh Namvar, A. M., & Alikhani, M. Y. (2013). Detection of *magA* gene in *Klebsiella* spp. isolated from clinical samples. *Iranian Journal of Basic Medical Sciences, 16*(2), 173–176. https://pubmed.ncbi.nlm.nih.gov/24298386/

Zar, H. J., MacGinty, R., Workman, L., Burd, T., Smith, G., Myer, L., Häggström, J., & Nicol, M. P. (2022). *Klebsiella pneumoniae* lower respiratory tract infection in a South African birth cohort: A longitudinal study. *International Journal of Infectious Diseases, 121*, 31–38. https://doi.org/10.1016/j.ijid.2022.04.043

Zhang, Y., Zhao, C., Wang, Q., Wang, X., Chen, H., Li, H., Zhang, F., Li, S., Wang, R., & Wang, H. (2016). High prevalence of hypervirulent *Klebsiella pneumoniae* infection in China: Geographic distribution, clinical characteristics, and antimicrobial resistance. *Antimicrobial Agents and Chemotherapy, 60*(10), 6115–6120. https://doi.org/10.1128/AAC.01127-16

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بررسی مولکولی برخی ژن‌ها در ایزوله‌های کلبسیلا پنومونیه از موارد بالینی مختلف

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چکیده

هدف: این مطالعه با هدف بررسی شیوع کلبسیلا پنومونیه به‌عنوان عامل اصلی ذات‌الریه در استان المثنی، عراق، و بررسی پروفایل مولکولی ژن‌های بیماری‌زایی مرتبط با هایپرموکوویسکوزیته (مانند magA و rmpA) در سویه‌های اپیدمیک جدا شده از نمونه‌های دستگاه تنفسی تحتانی بیماران بستری مبتلا به ذات‌الریه در بیمارستان الرمیثه انجام شد. علاوه بر این، شیوع سویه‌های هایپرویرولنت کلبسیلا پنومونیه (hvKp) و سویه‌های کلاسیک کلبسیلا پنومونیه (cKp) نیز برآورد گردید.

مواد و روش‌ها: در مجموع ۱۰۰ ایزوله باکتریایی از نمونه‌های دستگاه تنفسی تحتانی بیماران در بیمارستان الرمیثه بین ۱۸ آوریل ۲۰۲۴ و ۱۸ سپتامبر ۲۰۲۴ جمع‌آوری شد. ایزوله‌ها از طریق گام‌های زیر شناسایی شدند: مورفولوژی کلونی روی آگار مَک‌کانکی، مورفولوژی سلولی از طریق رنگ‌آمیزی گرم و کپسول و مشاهده زیر میکروسکوپ نوری، آزمایش‌های فیزیولوژیکی، آزمایش‌های بیوشیمیایی، شناسایی مولکولی با استفاده از توالی‌یابی ژن S rRNA۱۶، و تشخیص ژن‌های بیماری‌زایی magA) و rmpA) از طریق واکنش زنجیره‌ای پلیمراز (PCR) با استفاده از پرایمرهای اختصاصی ژن که قطعاتی به طول ۱۲۸۳ جفت‌باز برای magA و ۴۰۹ و ۳۴۰ جفت‌باز برای rmpA را تکثیر می‌کردند.

نتایج: از ۱۰۰ ایزوله، ۲۰ مورد پروفایل معمولی کلبسیلا پنومونیه را نشان دادند که هویت آن‌ها با توالی‌یابی ژن 16SrRNA تأیید شد. توالی‌های ۱۵ سویه از این ایزوله‌ها در GenBank تحت شماره‌های دسترسی PQ814166 تا PQ814180 ثبت شدند. تحلیل فیلوژنتیک این ۱۵ سویه را به ۱۰ کلاد تقسیم کرد: کلاد A (PQ814167, PQ814177)، کلاد B (PQ814171)، کلاد C (PQ814166, PQ814176)، کلاد D (PQ814179, PQ814180)، کلادهای E، F، و G (به ترتیب PQ814169،

PQ814173، وPQ814174)، کلاد H (PQ814170, PQ814175)، کلاد I (PQ814172) و کلاد J (PQ814168, PQ814178). ژن‌های هایپرموکوویسکوزیته magA و rmpA به‌ترتیب در ۵۵٪ و ۵۰٪ از ۲۰ ایزوله کلبسیلا پنومونیه شناسایی شدند. شیوع سویه‌های هایپرویرولنت کلبسیلا پنومونیه 50% (hvKp) بود.

نتیجه‌گیری: این مطالعه تأیید می‌کند که کلبسیلا پنومونیه یک عامل مهم ذات‌الریه در استان المثنی، با حضور قابل‌توجه سویه‌های هایپرویرولنت (۵۰٪) که با ژن‌های magA و rmpA مشخص می‌شوند است. این یافته‌ها اهمیت بررسی مولکولی در درک بیماری‌زایی و اپیدمیولوژی کلبسیلا پنومونیه در محیط‌های بالینی را برجسته می‌کند.

کلمات کلیدی: ژن magA، ژن rmpA، کلبسیلا پنومونیه، 16SrRNA

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