Research Article

Molecular Detection of K1 and K2 Serotypes of Hypervirulent *Klebsiella pneumoniae* in Minced Beef

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Abstract | Capsular polysaccharides (CPS) of hypervirulent *Klebsiella pneumoniae* (hvKp), particularly serotypes K1 and K2, are important virulence factors. To assess the occurrence of K1 and K2 serotypes of hvKp in food sources, a total of 100 minced meat samples (50 minced beef and 50 minced chicken) were collected from local markets in Baghdad and screened for the presence of these serotypes. Initial bacterial identification was performed based on morphological characteristics, selective culture media, and the indole test. The isolates were subsequently confirmed using the Vitek® 2 Compact system and amplification of the 16S rRNA gene followed by nucleotide sequencing. The presence of the regulator of mucoid phenotype A (*rmpA*) gene was also investigated. Furthermore, the isolates were subjected to the string test, biofilm formation assay, and hemolytic activity testing. Overall, *K. pneumoniae* was identified in 18 (18%) of the samples. PCR analysis revealed that 3 (27.2%) isolates from minced beef belonged to the K1 and K2 serotypes and harbored the *rmpA* gene. These isolates were classified as hypervirulent *K. pneumoniae* (hvKp), including one K1 isolate (9.09%) and two K2 isolates (18.18%). The string test demonstrated a predictive value of 66.6% for detecting hvKp strains. The hvKp isolates exhibited moderate biofilm-forming ability. However, only one isolate showed hemolytic activity and multidrug resistance to ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/clavulanic acid, ceftazidime, cefepime, aztreonam, amikacin, gentamicin, tobramycin, nalidixic acid, moxifloxacin, ciprofloxacin, levofloxacin, minocycline, and tetracycline. These findings suggest that minced beef may serve as a potential reservoir for multidrug-resistant hvKp strains, highlighting the need for increased attention to food safety and public health concerns associated with contaminated meat products.

Keywords | Hypervirulent *Klebsiella pneumoniae*, K1and K2 serotypes, Minced chicken meat, *rmpA*

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INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, encapsulated bacterium responsible for a wide range of infections, including pneumonia, meningitis, and urinary tract infections (Guerra *et al*., 2022). In the 1980s, a unique case was first documented in Taiwan involving a pyogenic liver abscess accompanied by endophthalmitis, caused by hypervirulent *K. pneumoniae* (hvKp) (Zhu *et al*., 2021). The pathogenicity and virulence of *K. pneumoniae* are largely attributed to its capsule and other associated virulence factors. Hypervirulent strains produce a thick capsule with a high concentration of exopolysaccharides, which plays a major role in bacterial virulence (Al-Busaidi *et al*., 2024).

To date, more than 79 capsular serotypes have been identified in *K. pneumoniae*. Certain serotypes are more frequently associated with hvKp strains, including K1, K2, K5, K16, K20, K54, K57, and KN1. Among these, serotypes K1 and K2 are particularly notable for their high virulence

and account for approximately 70% of hvKp strains (Russo *et al*., 2018). The regulator of mucoid phenotype A (*rmpA*) gene is a key virulence determinant in hvKp strains, as it enhances capsule production (Cheng *et al*., 2010). Additionally, the string test is a semi-quantitative assay widely used to detect the hypermucoviscous phenotype of hvKp strains (Tan *et al*., 2014).

Since its initial description, the terms hypermucoviscosity and hypervirulence have often been used interchangeably (Fank et al., 2005). However, Catalán-Nájera *et al*. (2017) clarified that these represent distinct phenotypes and should not be considered synonymous, as the detection of hypermucoviscosity by the string test alone is insufficient to confirm hypervirulence. Therefore, the rapid identification of genetic markers associated with the hypervirulent phenotype is essential for the development of accurate diagnostic tools.

K. pneumoniae can be found in various environments, including soil, the gastrointestinal tract, the skin of mammals, and fecal matter. Although it is commonly associated with hospital-acquired infections, several studies suggest that food may serve as a potential route of transmission. However, limited studies have reported the presence of hvKp capsular genes in food sources (Hartantyo *et al*., 2020; Bedair et al., 2016). Due to the inappropriate and excessive use of antibiotics in clinical settings, *K. pneumoniae* has developed significant multidrug resistance, posing a serious public health concern (Shadkam *et al*., 2021). In this context, hvKp strains are particularly alarming because of their high virulence and their ability to acquire antimicrobial resistance. The convergence of hypervirulence and multidrug resistance further complicates clinical management and requires urgent attention (Wang *et al*., 2025).

Moreover, *K. pneumoniae* is capable of forming biofilms, which are extracellular polysaccharide matrices that enhance antibiotic resistance and protect bacteria from phagocytic clearance (Shadkam *et al*., 2021). Biofilms can develop on medical devices, water systems, food-processing surfaces, and other biotic and abiotic environments. Biofilm-forming bacteria significantly impact the food industry by contributing to food spoilage, outbreaks of foodborne illness, and increased mortality (Kot and Witeska, 2024).

Although *K. pneumoniae* represents a potential threat to consumers due to possible transmission during food production and processing, data regarding antibiotic resistance profiles of isolates from non-human sources remain limited (Junaid *et al*., 2022). Additionally, hemolysin has been identified as a potential virulence factor associated with the pathogenicity of *K. pneumoniae* (Gundogan *et al*., 2011). Therefore, the present study aimed to evaluate the prevalence, antibiotic susceptibility, biofilm-forming ability, and hemolytic activity of capsular K1 and K2 serotypes of hvKp isolated from minced meat samples collected from local markets in Baghdad.

MATERIALS AND METHODS

Sample collection

A total of 100 minced meat samples (50 minced beef and 50 minced chicken) were collected from local markets in Baghdad. The samples, weighing 250–300 g each, were placed in an insulated icebox and immediately transported to the Meat Hygiene Laboratory, Department of Public Health, College of Veterinary Medicine, University of Baghdad, Iraq.

Sample processing and bacterial isolation

For each sample, 25 g of meat was aseptically mixed with 225 mL of peptone water and homogenized using a laboratory stomacher (Lab Blender, Circulator-400, UK) for approximately 10 minutes. Subsequently, 1 mL of the homogenized suspension was inoculated into Tryptone Soy Broth (TSB) (HiMedia, India) and incubated overnight at 37°C. After incubation, 0.1 mL of the enriched culture was evenly spread onto HiCrome™ Klebsiella Selective Agar Base (HiMedia, India) and incubated at 35–37°C for 24 hours (Gundogan *et al*., 2011; Aslam *et al*., 2022).

The following day, magenta-colored colonies were subcultured into fresh TSB and incubated overnight at 35–37 °C. The refreshed cultures were then streaked onto MacConkey agar plates (HiMedia, India) and incubated overnight at 37 °C. Large, mucoid, pink-colored colonies were selected and preserved as pure isolates for further analysis.

Bacterial identification by indole test

Tryptone broth was inoculated with a single pure colony and incubated at 35°C (±2°C) for 24–48 hours. Following incubation, five drops of Kovac's reagent were added to the broth. The appearance of a pink ring at the surface indicated a positive indole test (MacWilliams, 2009).

Molecular detection of *K. pneumoniae* and virulence genes

DNA extraction of *K. pneumoniae* isolates

Genomic DNA of the eighteen *K. pneumoniae* isolates was extracted using the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corporation, Taiwan). This kit is specifically designed for genomic DNA extraction from Gram-negative bacteria.

Primers and PCR reaction

The partial 16S ribosomal RNA gene sequence of *K. pneumoniae* strain 2484 (GenBank accession no.

MT634697.1) was used to validate the molecular detection of the isolates. PCR primers were designed in this study using NCBI GenBank sequences and synthesized by Macrogen, Korea. Three sets of primers were employed to detect the K1 and K2 serotypes as well as the *rmpA* gene (Pinpimai *et al*., 2022).

Each PCR reaction (25 µL) contained 12.5 µL of GoTaq® Green PCR Master Mix (Promega, USA), 5 µL of DNA template, 1.25 µL of forward primer, 1.25 µL of reverse primer, and 5 µL of PCR-grade distilled water. PCR amplification was performed using a thermocycler (Promega, USA) following the manufacturer's instructions for GoTaq® G2 Master Mix. The detailed thermocycling conditions for each primer set are presented in Table 1. 3µl of ethidium bromide was used to stain the amplified PCR results after they were placed onto a 1% agarose gel. The PCR results were later seen by using automated UV Gel Documentation (Analytic, Germany).

DNA sequencing

The PCR positive products (K1 and K2 serotypes) of *K. pneumoniae* were sent to Macrogen Corporation Company/ South Korea for obtaining the nucleotides sequencing. Then, sequence data were registered at NCBI under certain accession numbers.

Phenotypic detection of virulence factors

String test

The string test was performed to assess the hypermucoviscous phenotype of *K. pneumoniae*. All isolates were streaked onto HiCrome™ Klebsiella Selective Agar Base and MacConkey agar plates and incubated overnight at 37˚C. A positive result was indicated by the formation of a viscous string ≥5 mm in length when a colony was touched with an inoculation loop (Gundogan *et al*., 2011).

Production of biofilms

The ability of *K. pneumoniae* to form biofilms was assessed using the Congo Red Agar (CRA) method. The medium was prepared by dissolving 37 g/L of Brain Heart Infusion agar, 50 g/L of supplemental sucrose (Accusmix, India), and 10 g/L of agar-agar in 900 mL of distilled water, which was then autoclaved at 121˚C for 15 minutes. Separately, 0.8 g of Congo red dye was dissolved in 100 mL of distilled water and autoclaved. After cooling both solutions to 50˚C, they were combined and poured into Petri plates.

The 18 *K. pneumoniae* isolates were streaked onto the prepared CRA plates and incubated aerobically overnight at 37˚C. Positive biofilm formation was indicated by the appearance of black colonies with a dry, crystalline texture (Al-Dujaily and Mahmood, 2022; Jasim and Hayyawi, 2025).

Hemolysin test

All *K. pneumoniae* isolates were streaked on a blood agar plat with 5% (vol/vol) of human blood and incubated overnight at a temperature 37˚C. Hemolysis was seen around colonies deemed a positive result (Buxton, 2016; Hashim and Nema, 2018).

Antibiotic susceptibility testing and automated biochemical analysis using the Vitek 2 compact

Gram-negative kits were used with the Vitek® 2 Compact system (BioMérieux, France). A sterile solution of physiological sodium chloride was combined with 2 to 3 new fresh colonies. DensiChekTM was used to modify McFarland turbidity to an optical density of 0.50–0.63. Each Vitek 2 ID-GN and 2 AST-N222 card was then filled with 5 mL of the suspension. Lastly, the Vitek 2 cassette was filled with the cards and suspended. The symmetric reference strains stored in the Vitek 2 Compact software were used to compare the *K. pneumoniae* identification. While the antibiotic resistance and susceptibility were evaluated according to the minimal inhibitory concentrations (MICs).

Table 1: Targets, primer sequences, amplicon sizes (bp), and PCR Thermocycling conditions for all primers used in this study.

Target	Sequences (5'- 3')	Amplicon size (bp)	Pre-denaturation (1 cycle)	Amplification			Final extension	No. of cycles
				Secondary denaturation	Annealing	Extension		
Capsular type K1	F:GGTGCTCTTTACATCATTGC R:GCAATGGCCATTTGCGTTAG	1283 bp	95 °C for 2 min	95°C for 30 sec	50 °C for 45 sec	72 °C for 1.5 min	72°C for 5 min	35 cycles
Capsular type K2	F:GACCCGATATTCATACTTGACAGAG R:CCTGAAGTAAAATCGTAAATAGATGGC	641 bp	95°C for 2 min	95°C for 30 sec	52°C for 30 sec	72°C for 45 sec	72°C for 5 min	35 cycles
RmpA	F:ACTGGGCTACCTCTGCTTCA R:CTTGCATGAGCCATCTTTCA	516 bp	95°C for 2 min	95°C for 30 sec	59°C for 30 sec	72°C for 45 sec	72°C for 5 min	35 cycles
16S rRNA	F:CCTGGACAAAGACTGACGCT R:AGTTGCAGACTCCAATCCGG	584 bp	95°C for 2 min	95°C for 30 sec	58°C for 30 sec	72°C for 45 sec	72°C for 5 min	35 cycles

BP: Base pair, F: Forward, R: Reverse.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, 2019) to evaluate the effects of different factors on the study parameters. The Chi-square test was used to compare percentages, with significance levels set at $p \leq 0.05$ and $p \leq 0.01$. For small sample sizes, Fisher's Exact Test (FET) was applied, as it is valid for all sample sizes.

RESULTS AND DISCUSSION

Bacterial diagnosis

The suspected *K. pneumoniae* isolates detected on HiCrome™ Klebsiella Selective Agar Base and MacConkey agar plates (Figure 1), as well as by the indole test, were further confirmed using 16S rRNA gene amplification (Figure 2) and the Vitek® 2 Compact system. Out of 100 minced meat samples, 18 (18%) isolates of *K. pneumoniae* were recovered, including 11 from minced beef and 7 from minced chicken (Table 2). These results indicate that minced meat may serve as a significant reservoir of *K. pneumoniae*.

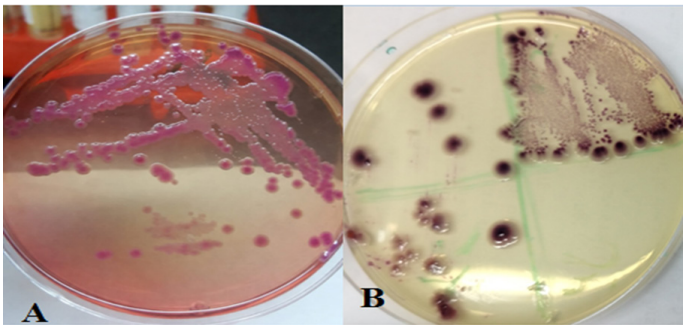

Figure 1: Colony morphology of *Klebsiella pneumoniae*. (A) Large, mucoid, pink colonies on MacConkey agar. (B) Purple-magenta colonies on HiCrome™ Klebsiella Selective Agar Base.

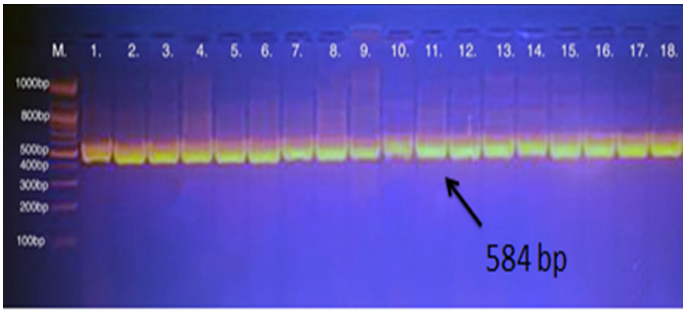

Figure 2: Electrophoresis of a 2% agarose gel stained with 3 µL ethidium bromide showing PCR-amplified 16S rRNA gene of *Klebsiella pneumoniae*. PCR products were visualized under UV light. M: DNA ladder (100–1500 bp); lanes 1–18: positive *K. pneumoniae* isolates (~548 bp).

Although *K. pneumoniae* is not classified as a primary foodborne pathogen (Guo *et al.*, 2016), the findings of this study indicate that it can be transmitted through minced meat. Al-Khafaji *et al.* (2024) reported varying prevalence rates of *K. pneumoniae* in minced meat (10/28; 35.71%) and chicken samples (8/28; 28.57%). Similarly, Theocharidi *et al.* (2022) assessed Greek meat products in Athens and found higher contamination in bovine meat (35.6%) compared to chicken meat (25.6%). In contrast, Al-Dabbagh (2022) reported a high prevalence of *K. pneumoniae* in beef (56%). The contamination of minced meat may occur at various stages of food processing, from production to marketing, or may originate from infected animals. These findings highlight the need for further research to better understand the sources and routes of contamination.

Table 2: Isolation percentage of *K. pneumoniae* from beef and chicken minced meat.

Type of samples	Total samples	No. of positive samples (%)	χ2	p-Value
Beef minced meat	50	11(22%)	1.34	0.247
Chicken minced meat	50	7 (14%)		
Total	100	18 (18%)		

Fisher's exact test; Statistically non-significant at ($p > 0.05$).

Molecular detection of *rmpA* gene and K1/K2 serotypes of hvKp

There are a few investigations that have revealed the presence of *K. pneumoniae* having capsular genes in food (Bedair et al., 2016). This study results revealed that 3 (27.2%) isolates from beef minced meat, which included one K1 and two K2 serotypes, had the *rmpA* gene and were designated as K1/K2 (hvKp) (Figures 3, 4 and 5). While the remaining isolates 8(72.7%) from minced beef and 7(100%) from chicken minced meat were designated as non K1/K2 (untypable). The data are represented in Table 3. The current results indicate that beef minced meat is an important source of hvKp.

Table 3: Molecular detection of K1, K2 serotypes with the *rmpA* gene in minced meat samples.

Serotypes	Beef minced meat (No=11)	Chicken minced meat (No=7)	p-value (Fisher's test)
K1	1(9.09%)	Non	1.000
K2	2 (18.18%)	Non	0.505
K1/K2 possessing the *rmpA*	3(27.2%)	Non	0.262
Non K1/K2 (untypable)	8(72.7%)	7(100%)	0.262

Fisher's exact test was used. Statistically non-significant at ($p > 0.05$).

Hypervirulent *K. pneumoniae* strains produce large amounts of a protective exopolysaccharide capsule (Al-Busaidi *et al.*, 2024). In this study, three (16.6%) hvKp isolates were

identified from beef mincemeat, including one K1 and two K2 isolates, all harboring the *rmpA* gene. Similar findings were reported in Singapore, where K1 and K2 serotypes were detected in only 5 out of 147 raw and ready-to-eat retail food samples (Hartantyo *et al*., 2020). In China, only two K1 and one K2 isolate were recovered from 1,200 retail food samples (Zhang *et al*., 2018). Conversely, in Egypt, Abdel-Rhman (2020) identified 26 K1 and K2 isolates from 44 ready-to-eat processed meat samples from cows and/or chickens. These differences highlight the influence of sample origin and geographical variation on hvKp prevalence (Zakaria *et al*., 2024).

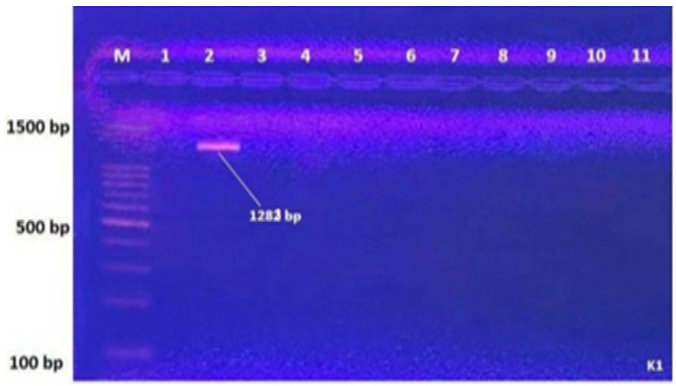

Figure 3: Electrophoresis of a 2% agarose gel stained with 3 µL ethidium bromide showing PCR-amplified capsular gene of K1 *Klebsiella pneumoniae*. PCR products were visualized under UV light. M: DNA ladder (100–1500 bp); lane 2: K1 serotype of hvKp (~1283 bp).

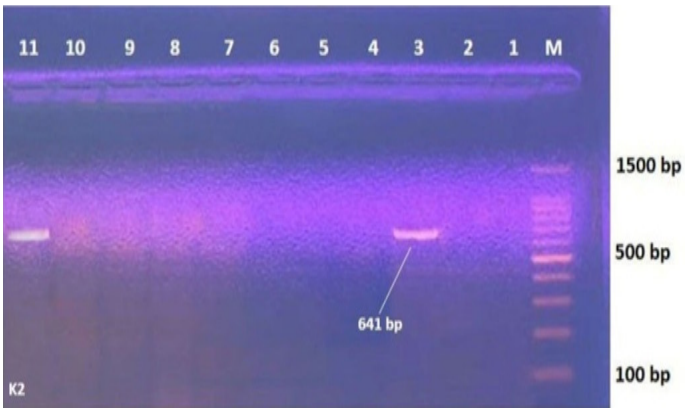

Figure 4: Electrophoresis of a 2% agarose gel stained with 3 µL ethidium bromide showing PCR-amplified capsular gene of K2 *Klebsiella pneumoniae*. PCR products were visualized under UV light. M: DNA ladder (100–1500 bp); lanes 3 and 11: K2 serotype of hvKp (~641 bp).

The *rmpA* gene is a key virulence factor of *K. pneumoniae* and is strongly associated with hvKp strains (Zhang *et al*., 2018). In this study, the *rmpA* gene was detected in K1 and K2 hvKp isolates, consistent with Shoja *et al*. (2022), who also reported its coexistence with these serotypes. In contrast, Hartantyo *et al*. (2020) did not detect the *rmpA* gene in food-derived *K. pneumoniae*. In Iraq, three *K. pneumoniae* isolates from soil were found to harbor the *rmpA* gene (Mohamed-Jawad, 2019), and K2/*rmpA*-positive hvKp has also been documented in local cheese from Baghdad (Rahma and Jasim, 2025). The detection of this pathogen in soil and dairy products suggests potential contamination of other food items and a significant risk to human health.

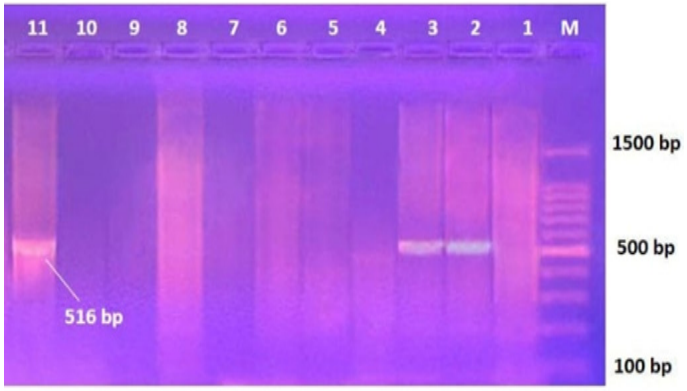

Figure 5: Electrophoresis of a 2% agarose gel stained with 3 µL ethidium bromide showing PCR-amplified *rmpA* gene of hypervirulent *Klebsiella pneumoniae*. PCR products were visualized under UV light. M: DNA ladder (100–1500 bp); lanes 2, 3, and 11: *rmpA* gene (~516 bp).

Phenotypic characteristics of different K1/K2 (hvKp) and non K1/K2 (untypable) isolates

Three of the eighteen *K. pneumoniae* isolates had the hypermucoviscous phenotype, according to the string test. The string test yielded a positive result in 2(66.6%) of K1/K2 (hvKp) isolates and negative in 1(33.3%), while it was positive in 1 (6.6%) non K1/K2 (untypable) isolates and negative in 14(93.3%), with non-significant value. In addition, in this study the biofilm formation strongly linked to the K1/K2 (hvKp) isolates (100%) than non K1/K2 (untypable) isolates 3 (20%). On the other hand, non-biofilm formation strongly linked to 12 (80%) of non K1/K2 (untypable) isolates than K1/K2 (hvKp) isolates (0%), with a significant difference ($p < 0.05$). There was no significant difference between hemolysis of blood between K1/K2 (hvKp) and non K1/K2 (untypable) isolates. Only 1 (33.3%) isolate of K1/K2 (hvKp) was positive to hemolysis (Table 4, Figure 6).

MacConkey's agar is still widely used in clinical laboratories for differentiating and isolating bacterial species from various samples. Encapsulated bacteria, including *K. pneumoniae*, utilize lactose and produce capsules, giving rise to mucoid or sticky wet colonies (Jung et al., 2024).

In this study, 3 of 18 (16.6%) *K. pneumoniae* isolates produced large, mucoid, sticky, wet, pink colonies on MacConkey's agar, regardless of whether the strain was

Table 4: Phenotypic characteristics of different K1/K2 (hvKp) and non-K1/K2 (Untypable) isolates.

Type of test	Group K1/K2 (hvKp) (n = 3) Non K1/K2 (untypable) (n = 15)	Positive No (%)	Negative No (%)	Total positive isolation No (%)	p-value (Fisher's test)	Significant
String test	K1/K2 (hvKp)	2 (66.6 %)	1(33.3%)	3(16.6%)	0.142	Non-significant
	Non-K1/K2 (untypable)	1 (6.6%)	14(93.3%)			
Biofilm	K1/K2 (hvKp)	3 (100%)	0 (0%)	6(33.3%)	0.010	Significant (p < 0.05)
	Non-K1/K2 (untypable)	3 (20%)	12 (80%)			
Hemolysis	K1/K2 (hvKp)	1 (33.3%)	2 (66.6%)	1(5.5%)	0.083	Non-significant
	Non-K1/K2 (untypable)	0 (0%)	15 (100%)			

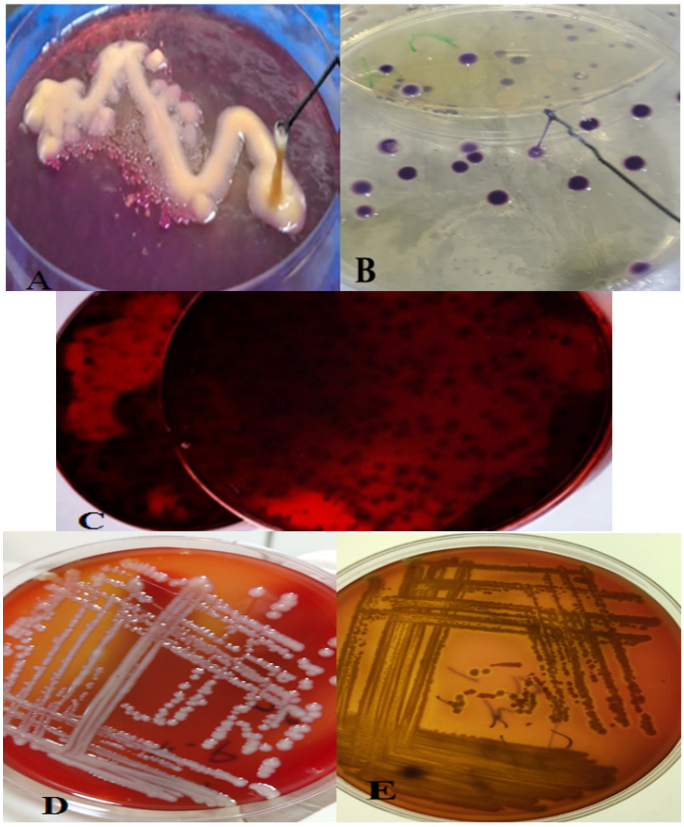

Figure 6: Phenotypic detection of virulence factors of hvKp isolates. (A) Positive string test on MacConkey agar. (B) Positive string test on HiCrome™ Klebsiella Selective Agar Base. (C) Moderate biofilm formation indicated by black, dry, crystalline colonies on Congo Red Agar (CRA). (D) Mucoid colonies of hvKp on blood agar. (E) Beta-hemolysis surrounding colonies on blood agar.

hypervirulent or not (Figure 6). This finding aligns with Al-Khafaji *et al.* (2024). However, the string test, which is semi-qualitative, is highly sensitive to user technique and colony conditions (Tan et al., 2014; Zhu et al., 2021).

The results of this study indicate that hypermucoviscosity alone does not reliably predict hypervirulence. One (33.3%) of the K1/K2 (hvKp) isolates lacked this trait, while one (6.6%) of the non K1/K2 (untypable) isolates exhibited it. These findings are consistent with an Egyptian study in which 21/57 isolates showed the hypermucoviscous phenotype, but PCR-confirmed virulent genes were only present in 18/21 isolates (Taha *et al.*, 2024). Another study conducted in Baghdad, Iraq, found that 57.14% of isolates exhibited the hypermucoviscous phenotype using the string test (Al-Khafaji *et al.*, 2024). Additionally, Abdel-Rhman (2020) reported that 59% of isolates from bovine and/or chicken processed meat were hypermucoviscous.

All three K1/K2 (hvKp) isolates (100%) and three (20%) of the non K1/K2 (untypable) isolates in the current study exhibited moderate biofilm formation, with a significant difference ($P < 0.05$). Non K1/K2 isolates were more frequently associated with non-biofilm formation (12/15; 80%) compared to K1/K2 (hvKp) isolates (0%). Positive biofilm formation was indicated by dark gray colonies spreading over the surface of the medium, consistent with Sadeq *et al.* (2025). Silva *et al.* (2024) reported that food isolates formed slightly less biofilm than human-origin isolates, though this was not statistically significant. Beckman *et al.* (2024) also found that the biofilm-forming ability of hvKp isolates was comparable to non-hvKp isolates. Other studies by Yao *et al.* (2025) and Song *et al.* (2024) reported that hypermucoviscous hvKp strains often produce abundant capsular polysaccharides, which can inversely affect biofilm formation.

Hemolysin is a well-recognized virulence factor and may indicate intestinal toxin-producing potential in Gram-negative bacteria (Gundogan *et al.*, 2011). In this study, only one K1/K2 (hvKp) isolate tested positive for hemolysin, a lower level than previously reported by Gundogan *et al.* (2011) and El-Sukhon (2023).

Antimicrobial resistance profile of K1/K2 (hvKp) isolates

In the present investigation, all *K. pneumoniae* isolates were resistant to ticarcillin and piperacillin (100%) and fully sensitive (100%) to imipenem and meropenem. The isolates, however, exhibited variable susceptibility to other antibiotics, as summarized in Table 5.

Previous studies have generally shown that hvKp strains are sensitive to common antimicrobial agents. However, recent reports have highlighted the emergence of

Table 5: Antimicrobial susceptibility pattern of hvKp isolates recovered from minced beef (sensitive, intermediate, and resistant).

Results of AST cards				Types of antibiotics	Class of antibiotic
P-value	Resistant	Intermediate	Susceptible		
0.0262 *	3 (100%)	0 (0%)	0 (0%)	Ticarcillin	Penicillin
0.0498 *	2 (66.6%)	0 (0%)	1 (33.3%)	Ticarcillin/clavulanic acid	
0.0262 *	3 (100%)	0 (0%)	0 (0%)	Piperacillin	
0.0498 *	1(33.3%)	0 (0%)	2 (66.6%)	Piperacillin/ clavulanic acid	
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Ceftazidime	Cephalosporin III/IV
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Cefepime	
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Aztreonam	Monobactam
0.0262 *	0 (0%)	0 (0%)	3 (100%)	Imipenem	Carbapenem
0.0262 *	0 (0%)	0 (0%)	3 (100%)	Meropenem	
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Amikacin	Aminoglycosides
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Gentamicin	
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Tobramycin	
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Nalidixic acid	Quinolone
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Moxifloxacin	
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Ciprofloxacin	Fluroquinolones
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Levofloxacin	
1.00 NS	1 (33.3%)	1 (33.3%)	1 (33.3%)	Minocycline	Tetracycline
0.0498 *	1 (33.3%)	0 (0%)	2 (66.6%)	Tetracycline	
0.0498 *	2 (66.6%)	0 (0%)	1 (33.3%)	Trimethoprim/Sulfamethoxazole	Sulfonamides
---	0.0419 *	0.7813 NS	0.0447 *	P-value	

* (P≤0.05), NS: Non-Significant difference was observed among the tested antibiotics.

multidrug-resistant hvKp strains (Al-Ismail *et al.*, 2025). In this study, only 1 of 3 K1/K2 (hvKp) isolates was multidrug-resistant, which aligns with Bedair et al. (2016), who found that approximately 27.3% of hvKp isolates displayed resistance to three or more antibiotic classes. The multidrug-resistant isolate in the current study was resistant to trimethoprim/sulfamethoxazole, nalidixic acid, amikacin, and gentamicin (Table 5).

All K1/K2 (hvKp) isolates were fully sensitive (100%) to imipenem and meropenem, making these the most effective antibiotics, consistent with the findings of Hartantyo *et al.* (2020) but differing from those reported by Bedair et al. (2016) and Abdel-Rhman (2020). The study also revealed that K1/K2 (hvKp) isolates were resistant to penicillin, a resistance attributed to beta-lactamase enzyme production, which renders penicillin ineffective (Kot *et al.*, 2023). Resistance to ceftazidime, aztreonam, levofloxacin, and ciprofloxacin observed in this study is consistent with Junaid *et al.* (2022). In contrast, Tang *et al.* (2024) reported that meat-derived isolates exhibited resistance to tetracycline and gentamicin.

The presence of multidrug-resistant hvKp strains may be linked to the lack of strict regulations governing antibiotic use in Iraq, emphasizing the importance of monitoring foodborne hvKp as a potential public health risk.

Registration of K1/K2 (hvKp) Strains of *K. pneumoniae* in GenBank

The sequences of the three isolates of hvKp were listed under the name of *K. pneumoniae* strain Rahma IQ, at (NCBI), as exhibited in Table 6.

Table 6: K1 and K2 (hvKp) isolates registered in GenBank.

No	Types of serotypes	Name of isolates in GenBank	Accession numbers
1	K1	*Klebsiella pneumoniae* strain Rahma IQ1 1282 genomic sequence	PX055692.1
2	K2	*Klebsiella pneumoniae* strain Rahma IQ2 1776 genomic sequence	PX055693.1
3	K2	*Klebsiella pneumoniae* strain Rahma IQ4 1621 genomic sequence	PX055695.1

Characteristics of different K1 and K2 (hvKp) isolates from minced meat

The K1/K2 (hvKp) isolates were confirmed by the presence of the *rmpA* gene. In this study, 2 of 3 isolates were positive in the string test, 1 of 3 exhibited hemolytic activity, and 1 of 3 was multidrug-resistant, while all isolates were capable of biofilm formation. The phenotypic characteristics of the K1/K2 (hvKp) isolates are summarized in Table 7.

Table 7: Characteristics of different K1 and K2 (hvKp) isolates.

No.	Serotypes	accession numbers	rmpA	String test	Biofilm	Hemolysis	Antibiotic resistance
1	K1	PX055692.1	Positive	Positive	Positive	Negative	Non-multidrug resistant
2	K2	PX055693.1	Positive	Positive	Positive	Positive	Multidrug resistant
3	K2	PX055695.1	Positive	Negative	Positive	Negative	Non-multidrug resistant

Among the hvKp isolates, capsular type K2 was the predominant serotype (2/3). This finding aligns with the study by Mohammed and Mahmood (2024), which reported that 85.24% of isolates were K2-positive, while only 4.91% were K1-positive. Notably, all K1/K2 hvKp isolates in this study carried the *rmpA* gene alongside biofilm-forming ability. These results suggest that the *rmpA* gene not only regulates the mucoid phenotype but also plays a crucial role in promoting the structural stability of bacteria within biofilms, thereby enhancing the pathogenicity and adaptability of hvKp.

This observation is consistent with Yao *et al.* (2025), who reported that the *rmpA* gene is vital for controlling the phenotypic switch between hypermucoviscosity and biofilm formation, highlighting its broad regulatory role across different bacterial species, including hvKp. Furthermore, the identification of a multidrug-resistant K2 hvKp isolate represents a significant clinical concern, as the combination of hypervirulence and antibiotic resistance severely restricts treatment options and increases mortality associated with hvKp infections.

CONCLUSION

The present study revealed that *K. pneumoniae* can be transmitted by minced meat, and minced beef represent an important source of multidrug resistant hvKp isolates consisting of K1 and K2 serotypes harboring a *rmpA* genes. Moreover, hvKp isolates formed a distinctive colony on a special culture medium with a hypermucoviscous phenotype and capabilities to form biofilm and hemolysin production. These findings underscore the potential health hazards associated with such isolates and highlight the importance of addressing the associated food safety risks.

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NOVELTY STATEMENT

This study reports the molecular detection of hypervirulent Klebsiella pneumoniae (hvKp) serotypes K1 and K2 carrying the rmpA gene in retail minced beef in Baghdad, Iraq. The findings highlight minced beef as a potential reservoir of multidrug-resistant hvKp with implications for food safety and public health.

AUTHOR'S CONTRIBUTION

MIR and HNJ conceived the ideas and designed the study. MIR performed a collected samples and laboratory analyses. MIR and HNJ performed data analyses. MIR writing the original of the manuscript. All authors read, reviewed and approved the final manuscript for publication.

Ethical approval

All experimental design and procedures conducted in this study were reviewed and approved by the Committee for the Care and Use of Animals in Research at the College of Veterinary Medicine, University of Baghdad (Ethics Approval No. 1205/P.G., dated 19/05/2025).

Data availability

The data used in this study are available from corresponding author upon request.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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