Iraqi Journal of Biotechnology, 2026, Vol. 25, No. 2, 291-303

A Study of the virulence genes of hypermucoviscous *Klebsiella pneumoniae* and their comparison with classic *Klebsiella pneumoniae*

[1]Tabarek Ghanim Ibrahim, [1]Luma Abdulhady Zwain[2]
[1]Department of Biology, Collage of Education Pure Science Ibn Al-Haitham, University of Baghdad, Iraq.

Abstract

Background. *Klebsiella pneumoniae* is an important opportunistic pathogen responsible for a wide range of healthcare- and community-associated infections. Hypermucoviscous *K. pneumoniae* exhibits enhanced virulence due to the presence of specific virulence-associated genes that contribute to bacterial pathogenicity and disease severity. **Aim.** To determine the prevalence of hypermucoviscous *Klebsiella pneumoniae* isolates and investigate the distribution of selected virulence genes (*k2, kfu, entB, hcp, rmpA,* and *magA*) in comparison with classical *K. pneumoniae* isolates. **Methods.** Fifty-seven clinical isolates were collected from different clinical specimens, including urine, blood, sputum, cerebrospinal fluid, tissue artificial bone, burns, and wound samples, from several hospitals in Baghdad between August and November 2024. Bacterial isolates were identified using microscopic examination, cultural characteristics, biochemical tests, and the Vitek-2 Compact system. Hypermucoviscosity was evaluated using the string test. Polymerase chain reaction (PCR) was performed to detect the presence of the virulence genes (*k2, kfu, entB, hcp, rmpA,* and *magA*), and statistical analysis was conducted using the Pearson Chi-square test. **Results.** Of the 57 collected isolates, 55 were confirmed as *K. pneumoniae*, with urinary tract infections representing the highest proportion (58.18%). Only five isolates (9%) exhibited the hypermucoviscous phenotype. PCR analysis of 35 representative isolates demonstrated that the *entB* gene was detected in 100% of isolates, followed by *kfu* and *hcp* genes (97.14% each), while the *k2* gene was detected in 88.57% of isolates. Neither *rmpA* nor *magA* genes were detected in any isolate. Significant differences were observed in the distribution of virulence genes ($p \leq 0.005$). **Conclusion.** The study demonstrated that both hypermucoviscous and classical *K. pneumoniae* isolates harbor multiple virulence genes, with *entB* being the most prevalent. The absence of *rmpA* and *magA* genes indicates that hypermucoviscosity is likely influenced by additional virulence determinants rather than these genes alone.

Keywords: Hypermucoviscous *Klebsiella pneumoniae*, Virulence genes.
Corresponding author: (Email: Tabarak.Ghanem2202@ihcoedu.uobaghdad.edu.iq)

Introduction

Klebsiella pneumoniae is an important intestinal bacterium and is one of the opportunistic pathogens that cause a wide range of diseases and show an increasingly frequent acquisition of antibiotic resistance (1, 2). Opportunistic diseases are caused by such as, urinary tract infections, pneumonia, sepsis, meningitis, soft tissue infections, diarrhea, and wound infections (3,4). *K.pneumoniae* bacteria are Gram-negative bacilli, immobile, anaerobic facultative (5,6). The rapid spread of multidrug-resistant *K. pneumoniae* strains poses a major global health threat as these strains are responsible for a large number of infections in hospitals with high infection and mortality rates, these bacteria are present in the environment (soil and surface water) and on abiotic surfaces such as medical equipment (7,8,9). *K. pneumoniae* has a range of factors that contribute to virulence and pathogenicity including capsule (especially capsule serotypes k1 and k2), lipopolysaccharide, ferric carriers siderophores, fimbriae, outer membrane proteins, and type VI secretion system (T6ss) (10). In addition, a distinctive, hypervirulent strain of *K.pneumoniae* has emerged with a hypermucoviscous phenotype and has appeared as a pathogen responsible for invasive infections (11). It is an invasive variant that differs from the classic *K.pneumoniae* bacteria in it is characterized by hypermucoviscosity and hypervirulence, which causes community-acquired infections such as pyogenic liver abscess, pneumonia, meningitis, and endophthalmitis (10). All virulence genes, individually or in combination, contribute to varying degrees to the initiation, invasion, spread and severity of *K.pneumoniae* (12). Many Gram-negative bacteria encode a molecular machine called the Type VI secretion system (T6SS) which is a tube connected to a membrane that looks like systolic bacteriophages physically and mechanically, and various substances such as antibacterial and anti-eukaryotic active substances are injected through this system (13).

The *rmpA* gene (Regulator of the mucoid phenotype) provides a hypermucoviscous phenotype of *K.pneumoniae*, *magA* (Mucoviscosity–associated gene A) is responsible for capsule polysaccharide production in the K1 serotype (14). The *Kfu* gene is an ABC ferric transport system that promotes and regulates capsule formation, hypermucoviscosity and ferric iron uptake (15). The *k2* gene of the capsule type k2 is the most common virulence factor in the bacteria *K.pneumoniae* (16), as well as the *entB* gene that codes for siderophore, which *entß* enhances the virulence of *K.pneumoniae* due to the excessive encoded of enterobactin and thus promotes ferric absorption and the formation of the biofilm (17,18). *hcp* gene is a key gene for the secretory system of type VI and in the bacteria *K. pneumoniae* is essential for competition between bacteria, and affects drug resistance. Deleting this gene further regulates resistance genes and changes physiological pathways, highlighting its role in pathogenicity and competitiveness (19). This study aimed to determine the virulence genes of hypermucoviscous *K.pneumoniae.*

Material and Methods

Isolation and diagnosis of bacteria

Fifty seven samples were collected from different clinical sources from Baghdad hospitals belonging to *K.penumoniae* at the period August to November 2024, eighteen isolates from Ibn Al-Baladi Hospital for Children and Gynecology, three from Imam Ali Hospital, two from Al-Yarmouk Teaching Hospital, three from the Central Child Teaching Hospital, fifteen from Imamian Al-Kadhimin Medical Hospital, fourteen from Al-Shaheed Ghazi Al-Hariri Hospital for Specialized Surgery, two from the Specialized Wounds and Burns Hospital. These isolates including 34 isolates from urinary tract infection patients, 10 isolates from blood, 6 from sputum, 2 from spinal fluid, Tissue artificial bone and burns, and 1 from wounds.

All of them were cultured on the of MacConkey agar and their fermentation of lactose was observed and then examined under a microscope and all isolates were tested using biochemical tests for the purpose of diagnosing bacteria, which include; Catalase, oxidase, Simmons citrate, Voges Proskauer, Indol and Methyl red tests followed by confirmation of diagnosis using Vitek-2 compact system.

Hypermucoviscosity assay

The hypermucoviscosity of bacterial isolates was detected using the String test. In this test, one of the single colonies growing on MacConkey agar was taken and stretched using the loop and formed a sticky chain longer than 5 mm, indicating positive testing (20).

DNA extraction and PCR reaction

DNA was extracted from *K. pneumoniae* using the extraction kit prepared by (ABIO pure,USA) according to the company's instructions and the extracted DNA was stored at a temperature of -20 for subsequent use, polymerase chain reaction was used to detect virulence genes and the primers sequences and amplification size were summarized in (Table 1) and according to (13, 21) Each reaction from the polymerase chain reaction was adjusted to a total volume of 20 µl of the main polymerase chain reaction mixture 10 µl Master MiX, 1 µl from the forward primer, 1 µl from the reverse primer, 3 µl from the DNA and then completely 5 of Nuclease-free water (Table 2). After amplification 5 µl of the PCR mixture was analyzed by electrophoresis of the agarose gel (1.5% agarose in the TAE buffer with ethidium bromide satin). 100 base pair ladder was used as a marker of DNA size ,and photographes of ethidium bromide stain lines in the gel was used using the gel imaging system under condition (Table 3).

Table (1): Primer used in the study

Gene	Primer Sequence (3'-5')	Product size pb	Reference
hcp	F: TCCCGACCGATAACAACAACACC R: GATGTCGTGCATCAGGGGAT	242	13
entB	F-GTCAACTGGGCCTTTGAGCCGTC R-TATGGGCGTAAACGCCGGTGAT	400	21
rmpA	F-CATAAGAGTATTGGTTGACAG R- CTTGCATGAGCCATCTTTCA	461	21
k2	F-CAACCATGGTGGTCGATTAG R-TGGTAGCCATATCCCTTTGG	531	21
kfu	F-GGCCTTTGTCCAGAGCTACG R-GGGTCTGGCGCAGAGTATGC	638	21
magA	F-GGTGCTCTTTACATCATTGC R-GCAATGGCCATTTGCGTTAG	1283	21

Table (2): Contents of the polymerase chain reaction mix k2, rmpA, magA, kfu, entB, hcp.

Contents of the reaction mix	Size in microliters
Master Mix	10
Forward primer	1
Reverse primer	1
Nuclease Free Water	5
DNA	3
Total volume	20

Table (3): Optimal conditions for gene detection.

PCR phase	Temperature	Time	Cycle number
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	35
Annealing	42 OR 60	00:45	
Extension	72	00:45	
Final extension	72	07:00	1
Hold	10	10:00	

Statistical analysis

The Pearson-chi square was utilized to determine the significant between different percentage of isolates and genes under study. Statistical significance was defined as a probability value ($p \leq 0.05$) (22).

Results

Diagnosis of bacteria

Klebsiella pneumoniae isolates were diagnosed by microscopic, cultural characteristics and biochemical tests. All isolates were Gram-positive and gave growth on MacConkey agar. They were large and round pink colonies in the form of a shiny dome with a mucous texture. Also, the results of the biochemical tests showed that all the isolates examined were positive for the Catalase test, negative for the oxidase test and positive for both the Simmons citrate and Voges Proskauer tests, while negative for the indol and methyl red tests (Table 4). The diagnosis was confirmed using the ViTek 2 compact system which was recorded 87-99% (Table 5). Fifty five isolates were identified as *Klebsiella pneumoniae*. Urinary tract infection (UTI) specimens represented the highest proportion of isolates (58.18%), followed by blood (18.18%), sputum (10.90%), burns (3.63%), cerebrospinal fluid (3.63%), tissue artificial bone (3.63%), and wounds (1.81%). A statistically significant difference was observed among the different clinical sources ($p \leq 0.001$) (Table 5).

Table (4): Biochemical tests

Test	Result
Growth on MacConkey agar	+
Oxidase	-
Catalase	+
Simon citrate media	+
Voges Proskauer	+
Methyl red	-
Indol	-

Table (5): Number and percentage of *K.pneumoniae* according of source

Source	No. of clinical sample	No. of *K.pneumoniae*	Percentage
UTI	34	32	58.18%[a]
Blood	10	10	18.18%[b]
Sputum	6	6	10.90%[c]
Burns	2	2	3.63%[d]
Wounds	1	1	1.81%[d]
Spinal cord fluid	2	2	3.63%[d]
Tissue artificial bone	2	2	3.63%[d]
p-value			0.001*

*Significant differences at p≤0.001, Similar latters indicated that non-significant differences, Different latters indicated that significant differences.

Hypermucoviscosity test

The results of the detection of hypermucoviscosity in *K.pneumoniae* showed that among the selected isolates were five isolates with a rate of 9% hypermucoviscosity. They formed a viscosity chain of more than 5 mm long (Figure 1), which was considered positive for the chain test on the formation of hypermucoviscosity (Table 6).

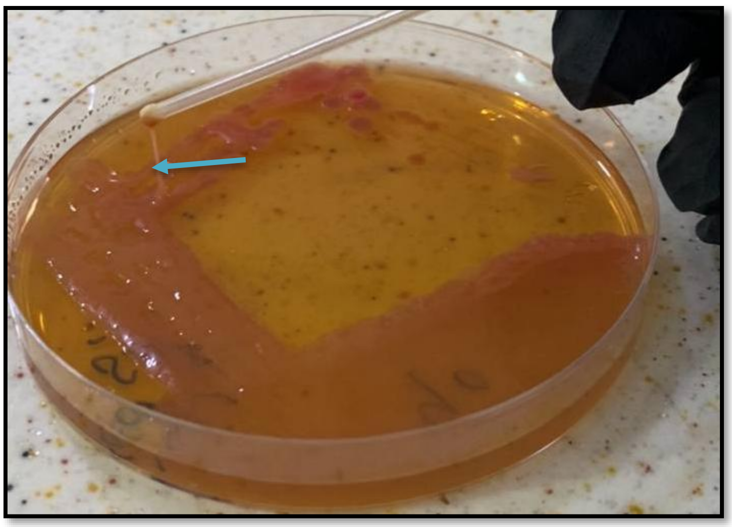


Figure (1): Hypermucoviscosity of *Klebsiella pneumoniae* on MacConkey agar

Table (6): Number and percentage of *K.pneumoniae* with hypermucoviscosity.

No. of isoaltes	Hypermucoviscosity> 5mm		Hypermucoviscosity< 5mm	
	Number	Percentage	Number	Percentage
55	5	%9	50	%.90.90

Molecular detection of virulence genes

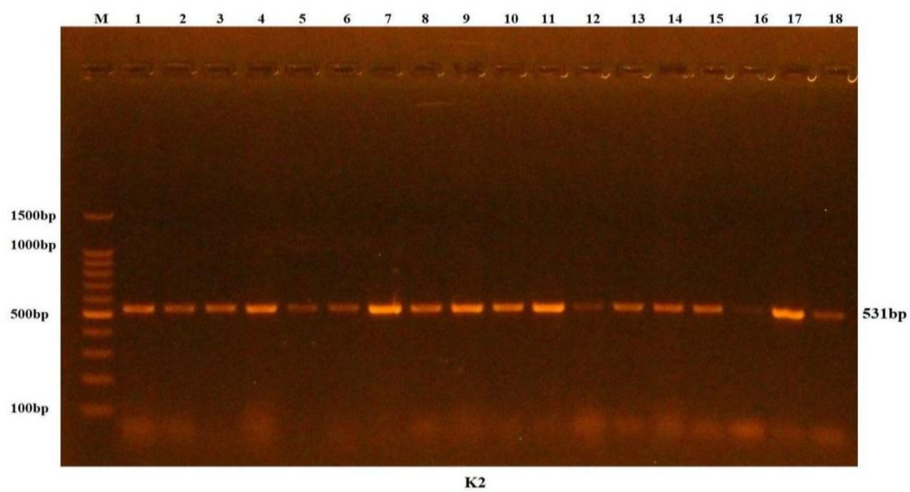
After the DNA extraction of the isolates of the bacterium *K.pneumoniae* using the extraction kit prepared by (ABIO pure, USA) using a special initiator for the bacteria *K.pneumoniae*, which targets a specific sequence of genes (*hcp*, *entB*, *k2*, *Kfu*, *magA*, *rmpA*) for the purpose of knowing the isolate had these genes, the genes were detected for clinical isolations.

Thirty-five antibiotic-resistant clinical isolates (22 urine, 6 blood, 4 sputum, 2 tissue artificial bone and 1 burn) were tested and it was noted that the isolates possess, the *k2* gene by 88.57%, the *kfu* gene by 97.14%, and the *entB* gene by 100%, 97.14% *hcp* gene, and isolates were not observed to have any of the *magA* and *rmpA* genes (Table 7 and Figure 2).

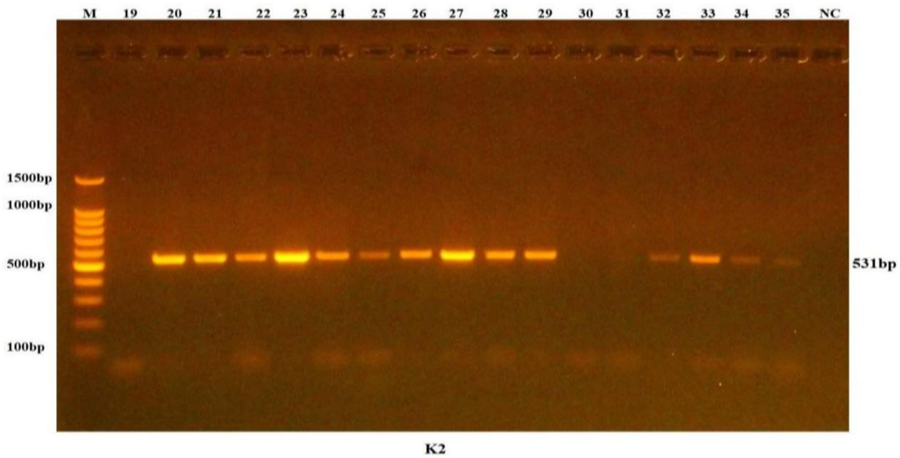
Table (7): Detection of some virulence genes of *K.pneumoniae* bacteria.

Gene	Total number of isolates	Number of gene-containing isolates	Percentage
k2	35	31	88.57%[a]
Kfu	35	34	97.14%[b]
entB	35	35	100%[b]
hcp	35	34	97.14%[b]
rmpA	35	0	0.00%[c]
magA	35	0	0.00%[c]
p-value			0.005*

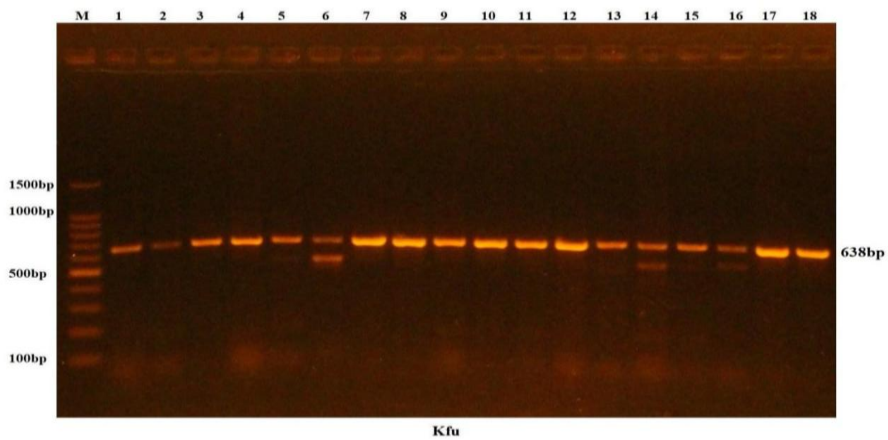
*Significant differences at p≤0.005.
-Similar latters indicated that non-significant differences.
-Different latters indicated that significant differences.



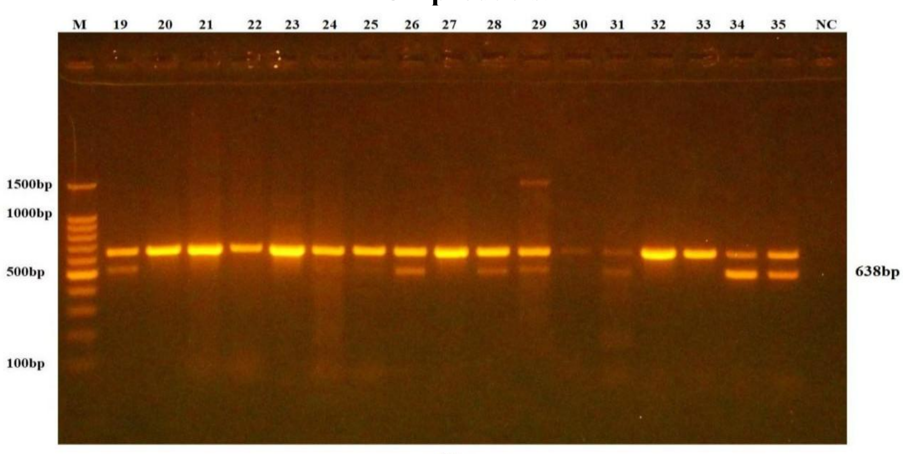
A. The amplification of *k2* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 531 bp PCR products.



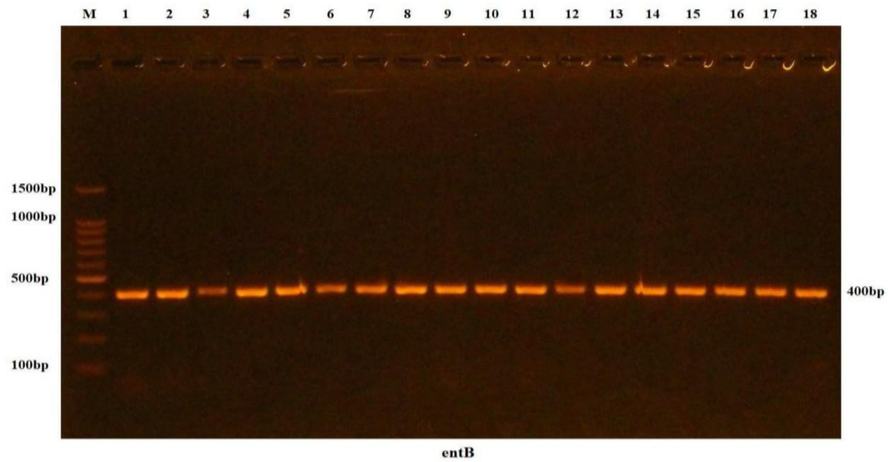
B. The amplification of *k2* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 19-35 resemble 531 bp PCR products.



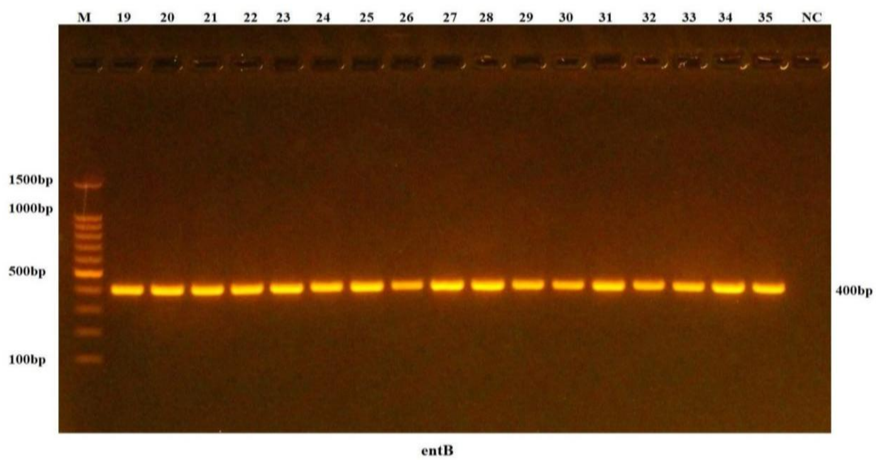
C. The amplification of *kfa* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 638 bp PCR products.



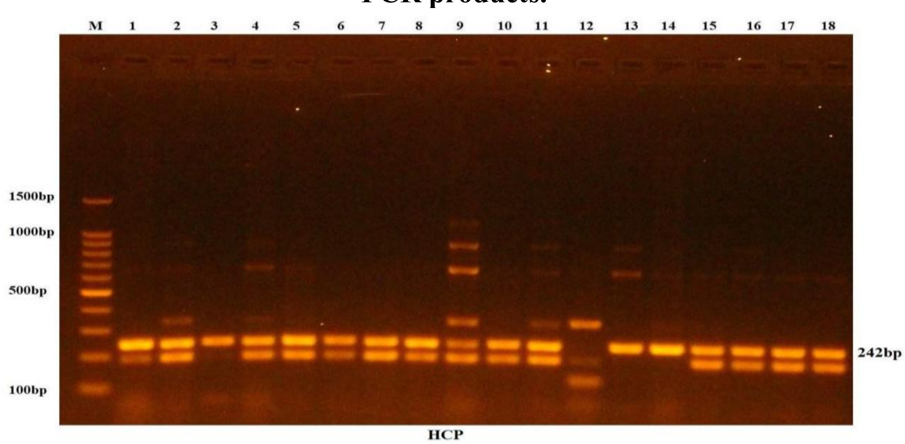
D. The amplification of *kfu* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 19-35 resemble 638 bp PCR products.



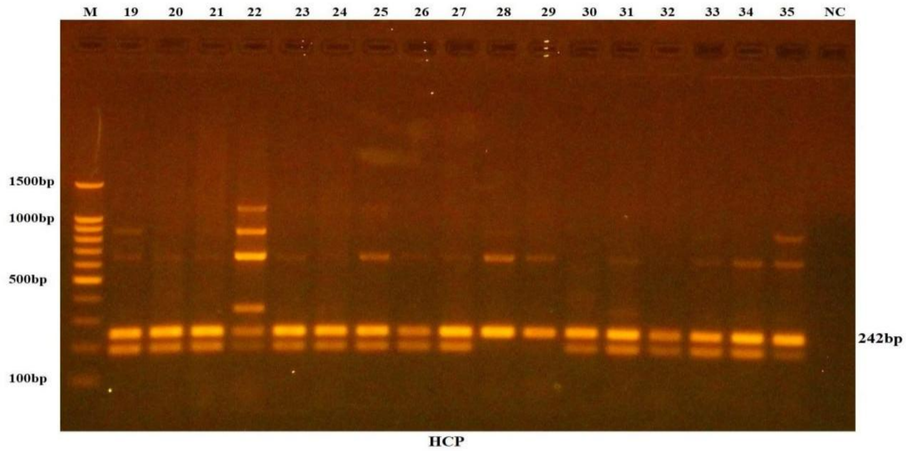
E. The amplification of *entB* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 400 bp PCR products.



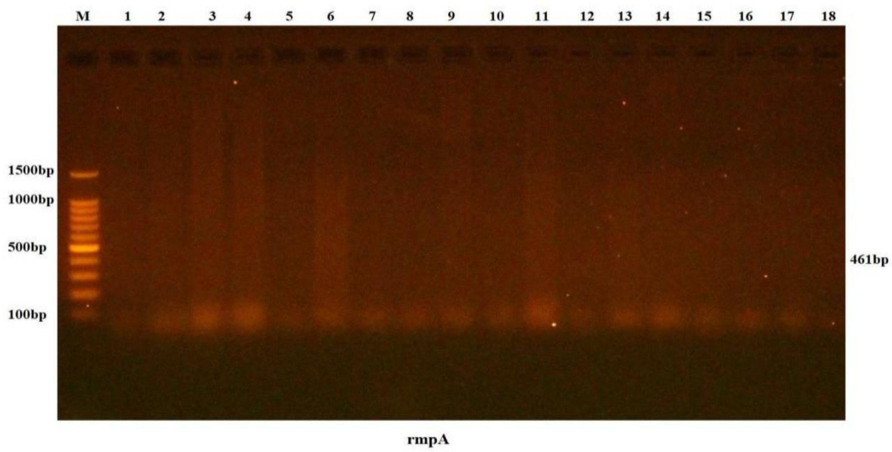
F. The amplification of *entB* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 19-35 resemble 400 bp PCR products.



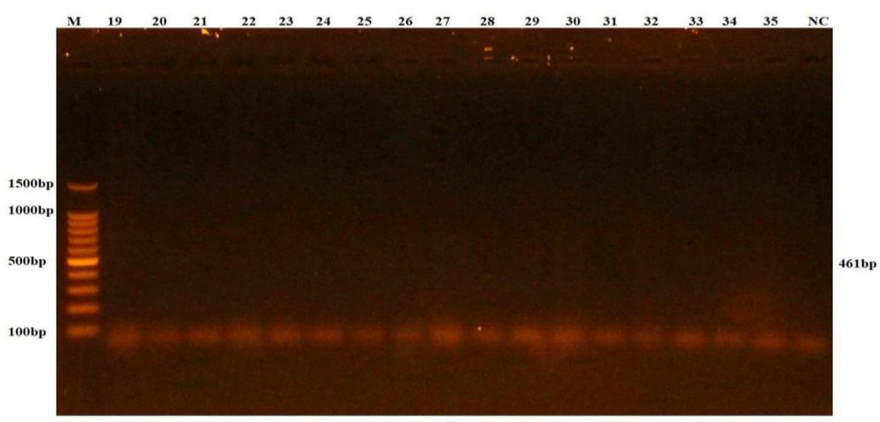
G. The amplification of *hep* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 242 bp PCR products.



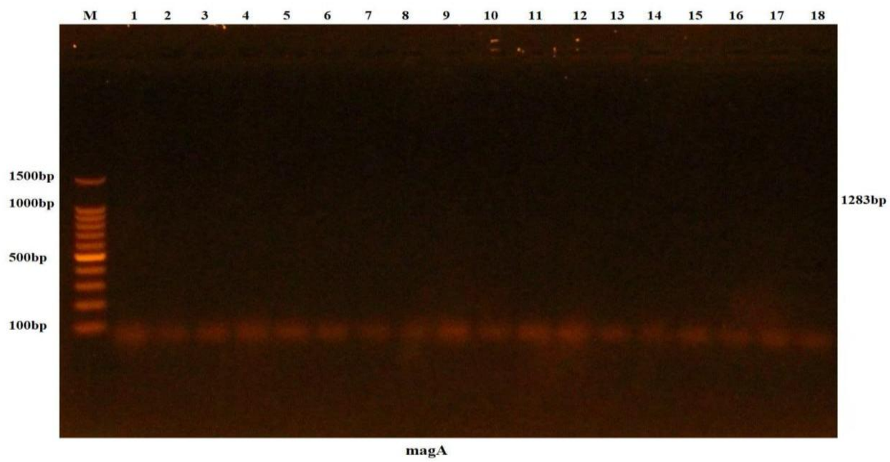
H. The amplification of *hep* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 19-35 resemble 242 bp PCR products.



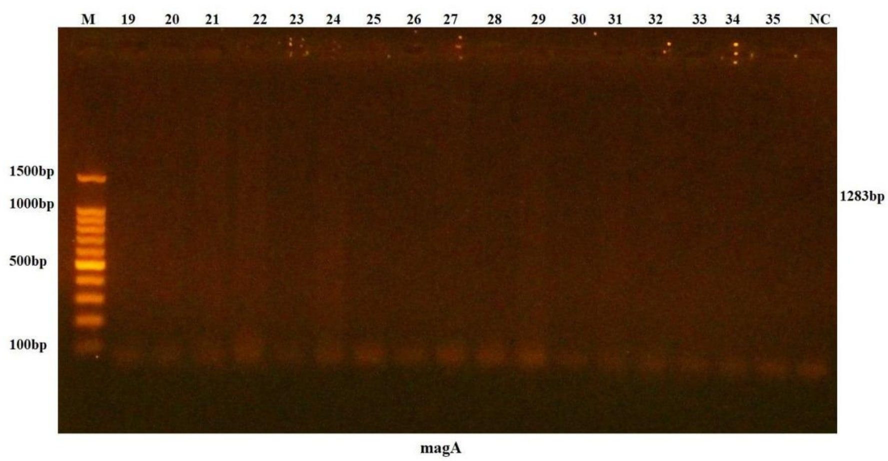
K. The amplification of *rmpA* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 461 bp PCR products.



I. The amplification of *rmpA* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 19-35 resemble 461 bp PCR products.



J. The amplification of *magA* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 1283 bp PCR products.



M. The amplification of *magA* gene of *K.pneumoniae* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100-1500 bp ladder marker. Lanes 1-18 resemble 1283 bp PCR products.

Figure (2): Virulence genes amplification assay of *K.pneumoniae*

Discussion

The predominance of *K. pneumoniae* isolates recovered from urinary tract infections observed in the present study is consistent with previous reports. It was reported that 30% of *K. pneumoniae* isolates originated from urine, followed by blood (24%), sputum and wounds (18%), and burns (10%) (23). Similarly, it was found that urinary tract infections accounted for 66% of *K. pneumoniae* infections, whereas wounds and tissues represented 10%, blood and cerebrospinal fluid infections were less frequent, and pneumonia accounted for 15% (7). Likewise, it was reported that urine represented the most common source of isolation (29.88%), followed by blood (28.73%), while burns, sputum, and wounds showed lower prevalence (18). These findings support the present results and confirm that the urinary tract remains the primary source of *K. pneumoniae* clinical isolates.

Only 9% of the isolates exhibited the hypermucoviscous phenotype in the present study. This prevalence was lower than that reported by (24), who detected hypermucoviscosity in 35.9% of isolates, but was comparable to the findings of (25), who reported a prevalence of 13.39%. It was suggested that hypermucoviscosity is closely associated with bacterial hypervirulence and is influenced by mutations and genes involved in metabolism and transport pathways, which may regulate biofilm formation (26). It was also reported that hypermucoviscous isolates are commonly associated with capsule serotypes K1 and K2 because of enhanced iron acquisition and increased capsule production (27).

The molecular analysis demonstrated that *entB* was the most prevalent virulence gene, followed by *kfu*, *hcp*, and *k2*. Similar findings were reported by (21), who observed that *entB* was the predominant virulence gene among *K. pneumoniae* isolates. It was also demonstrated a high prevalence of the *hcp* gene, supporting its important role in bacterial virulence and interbacterial competition (13, 28).

Neither *rmpA* nor *magA* genes were detected in the present isolates. Similar findings were reported by (14), who also failed to detect these genes. It was suggested that *rmpA* may be lost during bacterial adaptation associated with antimicrobial resistance (29). It was proposed that hypermucoviscous isolates lacking *rmpA* may rely on other virulence-associated genes (12). Likewise, it was reported the absence of both *rmpA* and *magA*, indicating that these genes are not the only determinants of hypermucoviscosity (30). It was further demonstrated that hypermucoviscous isolates belonging to capsule serotype K2 lacked both *rmpA* and *magA*, suggesting that hypervirulence is a multifactorial trait (31). It was identified K2 as the predominant capsule serotype among hypermucoviscous isolates (32), whereas (33) demonstrated that hypermucoviscosity may occur independently of *rmpA* due to the presence of alternative genetic determinants regulating capsule production and mucoviscosity.

Conclusion

From the study, it was concluded that hypermucoviscous *K.pneumoniae* and classic *K. pneumoniae* contain virulence genes.

Ethical Approval

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments. Ethical approval for the study was obtained from the College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, under official approval No. 5487, dated 4 August 2024. Clinical samples were collected after obtaining the necessary permissions from the participating hospitals, and all procedures were performed in accordance with the approved institutional ethical guidelines.

References

1. Effah, C.Y.; Sun, T.; Liu, S. and Wu, Y. (2020). Klebsiella pneumoniae: an increasing threat to public health. Annals of Clinical Microbiology and Antimicrobials, 19: 1-9.
2. Hasan, S.A.R; Al-Jubori, S.S. and Salman, J. A.S. (2021). Molecular Analysis of fimA

Operon Genes among UPEC Local Isolates in Baghdad City. Archives of Razi Institute, 76(4): 829-840.

3. Kadum, S. M. and Al Rubaeye, D.S. (2020). Colistin Susceptibility in Carbapenem Resistant Klebsiella Pneumoniae and their Ability of Biofilm Formation. Iraqi Journal of Science: 517-527.
4. Rahal, B.S.; Abdulha, A.A.; Salman, N.Y.; Kais Kassim Ghaima, K.K. and Mohamed, M.M. (2021). The Role of EDTA in Biofilm Eradication of Klebsiella pneumoniae Isolated from Wound Infections. Iraqi Journal of Biotechnology, 20(1): 96-102.
5. Ali, S. Y. and Malik, T. (2020). 44. Detection of carbapenem resistant Klebsiella species isolated from clinical samples. Pure and Applied Biology (PAB), 9(2): 1662-1666.
6. Mustafa, M.S. and Abdullah, R.M. (2020). Role of oqxA and oqxB Genes in the Development of Multidrug Resistant Phenotype among Clinical Klebsiella pneumoniae Isolates from Various Cases. Iraqi Journal of Science, 61(8): 1902-1912.
7. Guerra, M.E.S.; Destro, G.; Vieira, B.; Lima, A. S., Ferraz, L.F.C.; Hakansson, A. P. and Converso, T.R. (2022). Klebsiella pneumoniae biofilms and their role in disease pathogenesis. Frontiers in Cellular and Infection Microbiology, 12: 877995.
8. Khalid, M.Y. and Ghareeb, A.M. (2022). Colistin resistant and biofilm formation among multi-drug resistant Klebsiella pneumoniae isolated from different clinical samples. Iraqi Journal of Biotechnology, 21(2): 457-464.
9. Hamady, D.R. and Ibrahim, S.K. (2020). The study on ability of Escherichia coli isolated from different clinical cases to biofilm formation and detection of csgD gene responsible for produce Curli (Fimbriae). Biochemical and Cellular Archives, 20(2).
10. Zhu, J.; Wang, T.; Chen, L. and Du, H. (2021). Virulence factors in hypervirulent Klebsiella pneumoniae. Frontiers in Microbiology, 12: 642484
11. Prokesch, B.C.; TeKippe, M.; Kim, J.; Raj, P.; TeKippe, E.M. and Greenberg, D.E. (2016). Primary osteomyelitis caused by hypervirulent Klebsiella pneumoniae. The Lancet Infectious Diseases, 16(9): e190-e195.
12. Remya, P. A.; Shanthi, M. and Sekar, U. (2019). Characterisation of virulence genes associated with pathogenicity in Klebsiella pneumoniae. Indian Journal of Medical Microbiology, 37(2): 210-218.
13. Mohamed, N.A.; Alrawy, M. H., Makbol, R.M.; Mohamed, A.M.; Hemdan, S.B. and Shafik, N. S. (2023). Type VI secretion system (T6SS) in Klebsiella pneumoniae, relation to antibiotic resistance and biofilm formation. Iranian Journal of Microbiology, 15(5): 601.
14. Al-Aajem B.M.R.; Jasim, H.M.; Saleem, A.J. (2021). Detection of Virulent genes Khe, iuc, rmp, magA in Klebsiella pneumoniae isolated from urinary tract infection. International Journal of Drug Delivery Technology, 11(4): 1470-1473.
15. Booq, R.Y.; Abutarboush, M.H.; Alolayan, M.A.; Huraysi, A.A.; Alotaibi, A.N.; Alturki, M.I. and Sendy, B.K. (2022). Identification and characterization of plasmids and genes from carbapenemase-producing Klebsiella pneumoniae in Makkah Province, Saudi Arabia. Antibiotics, 11(11): 1627.
16. Abd Al-Rhman, R.M. and Al-Aubydi, M. A. (2015). Determination the relationship between some genetic aspects with the capsule formation for pathogenic Klebsiella pneumoniae serotypes k1 and k2. Iraqi Journal of Science: 1385-1393.
17. Ali, Z. S., & Shami, A. M. (2023). Molecular Study of Siderophore Genes in Carbapenems Resistant Klebsiella pneumoniae Isolated from Urinary Tract Infection Patients. Iraqi journal of Biotechnology, 22(1).
18. Ajeel, E.A. and Mohammed, R.K. (2022). Genotype and Phenotype Investigation of CTX-M Gene among Multidrug Resistant Klebsiella pneumoniae Isolates. Iraqi Journal of Biotechnology, 21(2).
19. Li, W.; Huang, X.; Li, D.; Liu, X.; Jiang, X.; Bian, X. and Zhang, J. (2023). Genomic and transcriptomic analysis revealed new insights into the influence of key T6SS genes hcp and vgrG on drug resistance and interbacterial competition in Klebsiella pneumoniae. bioRxiv, 2023: 05.
20. Hyun, M.; Lee, J.Y. and Kim, H.A. (2024). Clinical and Microbiologic Analysis of Klebsiella pneumoniae Infection: Hypermucoviscosity, Virulence Factor, Genotype, and Antimicrobial Susceptibility. Diagnostics, 14(8): 792
21. Ali, A. M. and Al-Kakei, S.N. (2019). Virulence factors genotyping of Klebsiella pneumoniae clinical isolates from Baghdad.

Reviews and Research in Medical Microbiology, 30(1), 36-46.

22. SAS, (2012). Statistical Analysis System, User's Guide. Statistical Version 9.1th ed. SAS. Inst, Inc. Cary, N.C, USA.
23. Mustafa, M. (2020). Prevalence of Quinolones Resistance Proteins Encoding Genes (qnr genes) and Co-Resistance with β-lactams among Klebsiella pneumoniae Isolates from Iraqi Patients. Baghdad Science Journal, 17(2): 0406-0406.
24. Assefa, M.; Amare, A.; Belachew, T. and Tigabu, A. (2024). Hypermucoviscous Klebsiella Pneumoniae Caused Community-Acquired Pneumonia in Gondar, Northwest Ethiopia. Clinical Laboratory, 70(9).
25. Ballén, V.; Gabasa, Y.; Ratia, C.; Ortega, R.; Tejero, M. and Soto, S. (2021). Antibiotic resistance and virulence profiles of Klebsiella pneumoniae strains isolated from different clinical sources. Frontiers in Cellular and Infection Microbiology, 11: 738223.
26. Liang, Q.; Chen, N.; Wang, W.; Zhang, B.; Luo, J.; Zhong, Y. and Zhou, Y. (2024). Co-occurrence of ST412 Klebsiella pneumoniae isolates with hypermucoviscous and non-mucoviscous phenotypes in a short-term hospitalized patient. Msystems, 9(7): e00262-24.
27. Sánchez-López, J.; García-Caballero, A.; Navarro-San Francisco, C.; Quereda, C.; Ruiz-Garbajosa, P.; Navas, E., and Diez-Aguilar, M. (2019). Hypermucoviscous Klebsiella pneumoniae: a challenge in community acquired infection. IDCases, 17: e00547.
28. Liao, W.; Huang, H.H.; Huang, Q. S.; Fang-Ling, D.; Wei, D.D.; La-Gen, W. and Liu, Y. (2022). Distribution of type VI secretion system (T6SS) in clinical Klebsiella pneumoniae strains from a Chinese hospital and its potential relationship with virulence and drug resistance. Microbial Pathogenesis, 162: 105085..
29. Shankar, C.; Basu, S.; Lal, B.; Shanmugam, S.; Vasudevan, K.; Mathur, P. and Veeraraghavan, B. (2021). Aerobactin seems to be a promising marker compared with unstable RmpA2 for the identification of hypervirulent carbapenem-resistant Klebsiella pneumoniae: in silico and in vitro evidence. Frontiers in Cellular and Infection Microbiology, 11: 709681.
30. Ragheb, S. M., and Osei Sekyere, J. (2024). Molecular characterization of hypermucoviscous carbapenemase-encoding Klebsiella pneumoniae isolates from an Egyptian hospital. Annals of the New York Academy of Sciences, 1535(1): 109-120
31. Osama, D.M.; Zaki, B.M.; Khalaf, W.S.; Mohamed, M.Y.A.; Tawfick, M.M. and Amin, H.M. (2023). Occurrence and molecular study of hypermucoviscous/hypervirulence trait in gut commensal K. pneumoniae from healthy subjects. Microorganisms, 11(3): 704.
32. Guo, Y.; Wang, S.; Zhan, L.; Jin, Y.; Duan, J.; Hao, Z. and Yu, F. (2017). Microbiological and clinical characteristics of hypermucoviscous Klebsiella pneumoniae isolates associated with invasive infections in China. Frontiers in cellular and infection microbiology, 7: 24.
33. Beckman, I.V.; Cella, R.L.; Azarian, E.; Rendueles, O. and Fleeman, R.M. (2024). Diverse polysaccharide production and biofilm formation abilities of clinical Klebsiella pneumoniae. npj Biofilms and Microbiomes, 10(1): 151.