Al-Qadisiyah Journal of Pure Science

ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514

DOI: 10.29350/jops

Al-Qadisiyah Journal of Pure Science

Phenotypic and Molecular Detection of Biofilm Formation in *Klebsiella pneumoniae* Isolated from Different Clinical Cases in Al-Hillah City

Fadel A. M. Al-Shareefi[1], , Hadaf Mahdi Kadhim[2]

[1]Department of Biology, College of Education, University of Al-Qadisiyah, Iraq.

[2]Department of Biology, College of Education, University of Al-Qadisiyah, Iraq.

*Corresponding author email: bio.edu.posta24.47@qu.edu.iq

Received: 14/03/2026; Accepted: 29/03/2026; Published Online: 29/03/2026.

Abstract

Klebsiella pneumoniae is an important opportunistic pathogen associated with a wide range of hospital-acquired infections. One of the major virulence factors of this bacterium is its ability to form biofilms, which enhance bacterial persistence and resistance to environmental stress. The present study aimed to investigate the phenotypic and molecular detection of biofilm formation in *Klebsiella pneumoniae* isolates obtained from different clinical cases in Al-Hillah city, Iraq. A total of 200 clinical samples, including sputum and urine specimens, were collected from hospitalized patients. The samples were cultured on Blood agar, MacConkey agar, and EMB agar for bacterial isolation and identification. Out of the total samples, 20 isolates were identified as *Klebsiella pneumoniae* using conventional biochemical tests and the VITEK automated identification system. The phenotypic detection of biofilm formation was performed using the microtiter plate (MTP) method with crystal violet staining. The results showed that 17 isolates (85%) were strong biofilm producers, while 3 isolates (15%) showed moderate biofilm formation. Molecular detection of biofilm-related genes was carried out using PCR. The results demonstrated that the treC gene (302 bp) was present in all isolates (100%), whereas the cpsD gene (386 bp) was detected in 9 isolates (45%). These findings indicate that *Klebsiella pneumoniae* isolates possess a strong ability to produce biofilms and harbor important genes associated with biofilm formation.

Keywords: *Klebsiella pneumoniae*; Biofilm formation; PCR; treC; cpsD

1. Introduction

Klebsiella pneumoniae is a Gram-negative, encapsulated, non-motile bacterium that belongs to the family Enterobacteriaceae. It is widely distributed in the environment and can be found in soil, water, plants, and on mucosal surfaces of humans, particularly in the gastrointestinal tract. Although it may exist as part of the normal microbiota, *Klebsiella pneumoniae* is considered an important opportunistic pathogen responsible for a wide range of community-acquired and hospital-acquired infections [1][2]. These infections include pneumonia, urinary tract infections, septicemia, meningitis, liver abscesses, and wound infections. The clinical importance of this bacterium has increased significantly over recent decades due to the emergence of multidrug-resistant strains (MDR) and hypervirulent lineages capable of causing severe and invasive infections [3].

Globally, infections caused by *Klebsiella pneumoniae* represent a major public health concern. Epidemiological studies have indicated that this pathogen contributes to a substantial proportion of hospital-acquired infections and is associated with high morbidity and mortality rates worldwide. Lower respiratory tract infections, bloodstream infections, and intra-abdominal infections represent the most common clinical manifestations associated with this bacterium. Due to the increasing prevalence of antimicrobial resistance, the World Health Organization (WHO) has classified carbapenem-resistant *Klebsiella pneumoniae* among the highest priority pathogens requiring urgent development of new antimicrobial therapies [4][5][6].

One of the most important virulence factors contributing to the pathogenicity of *Klebsiella pneumoniae* is its ability to form biofilms. Biofilms are structured microbial communities that adhere to biotic or abiotic surfaces and are embedded within a self-produced extracellular polymeric matrix composed mainly of polysaccharides, proteins, and extracellular DNA. Biofilm formation enables bacteria to survive under unfavorable environmental conditions and enhances their persistence within host tissues and on medical devices. It has been estimated that approximately 65–80% of bacterial infections are associated with biofilm formation, highlighting the clinical importance of this microbial lifestyle [7][3].

Bacteria embedded within biofilms exhibit significantly higher resistance to antimicrobial agents compared with planktonic cells. Studies have shown that microorganisms within biofilms may tolerate antibiotic concentrations up to 1000 times higher than those required to inhibit free-living bacterial cells. This increased resistance is attributed to several mechanisms including limited penetration of antimicrobial agents through the biofilm matrix, reduced metabolic activity of bacterial cells, the presence of persister cells, and activation of efflux pump systems. In addition, biofilms provide protection against host immune responses, thereby facilitating chronic and recurrent infections [8][9].

The formation of biofilms in *Klebsiella pneumoniae* is regulated by several genetic determinants that control bacterial adhesion, colonization, and extracellular matrix production. Numerous virulence genes have been identified as contributors to biofilm formation, including genes encoding fimbrial adhesins, capsular polysaccharides, and regulatory proteins. Fimbrial genes such as fimH, mrkA, and mrkD are involved in bacterial adhesion to epithelial cells and abiotic surfaces, representing the initial step in biofilm development [10][11][12].

Capsular polysaccharides also play a crucial role in the virulence of *Klebsiella pneumoniae*. Capsule-associated genes, including regulatory genes responsible for capsule synthesis, contribute to bacterial resistance against phagocytosis and complement-mediated killing. In addition to their protective function, capsular polysaccharides enhance bacterial aggregation and stability within the biofilm matrix, thereby strengthening the structural integrity of biofilm communities [13].

Among the genes associated with biofilm formation, the treC gene has attracted increasing attention due to its role in carbohydrate metabolism. The treC gene encodes trehalose-6-phosphate hydrolase, an enzyme involved in the metabolism of trehalose. This metabolic pathway contributes to the regulation of capsular polysaccharide production and influences bacterial surface structures that facilitate adhesion and biofilm maturation. Several studies have suggested that the treC gene may enhance the ability of *Klebsiella pneumoniae* to establish stable biofilms and persist in clinical environments [3].

Another important gene associated with biofilm formation is the cpsD gene, which plays a key role in the regulation of capsular polysaccharide synthesis. The capsule represents one of the major virulence factors of *Klebsiella pneumoniae*, as it protects bacterial cells from host immune defenses and contributes to bacterial aggregation within biofilm structures. The cps gene cluster, including cpsD, regulates the synthesis and export of capsular polysaccharides that form an important component of the extracellular matrix surrounding bacterial cells in biofilms [2][13].

The presence of biofilm-associated genes enhances the ability of *Klebsiella pneumoniae* to colonize both biological tissues and artificial surfaces such as urinary catheters and mechanical ventilation devices. Biofilm

formation on these surfaces plays a significant role in the development of device-associated infections, which are among the most common hospital-acquired infections worldwide [14].

In recent years, several studies conducted in Iraq have reported an increasing prevalence of multidrug-resistant *Klebsiella pneumoniae* isolates in clinical settings. Environmental investigations have also detected resistant strains of this bacterium in water sources such as the Al-Hillah River in Babylon Province, suggesting the potential circulation of resistant strains between environmental reservoirs and healthcare environments [15].

Given the increasing clinical significance of *Klebsiella pneumoniae* infections and the important role of biofilm formation in bacterial persistence and antimicrobial resistance, studying the phenotypic and molecular characteristics of biofilm formation among clinical isolates has become essential. Understanding the distribution of biofilm-associated genes and their relationship with bacterial virulence may provide valuable insights into the mechanisms underlying persistent infections and may contribute to the development of improved infection control strategies. Therefore, the present study focuses on the phenotypic and molecular detection of biofilm formation in *Klebsiella pneumoniae* isolates obtained from different clinical cases in Al-Hillah city, with the aim of providing a better understanding of the virulence characteristics of local clinical isolates and their potential role in hospital-associated infections.

2. Materials and Methods

Collection of Clinical Samples

Clinical samples were collected from hospitalized patients in hospitals of Al-Hillah city, Iraq, including Marjan Teaching Hospital and Al-Hillah Teaching Hospital. The collection of samples and laboratory work were carried out from 19 November 2025 to the end of December 2025. A total of 200 clinical samples were collected from different clinical cases including sputum and urine specimens.

Isolation and Cultivation of Bacteria

The collected specimens were cultured on Blood agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar for primary isolation of bacterial pathogens. The inoculated plates were incubated at 37°C for 24 hours, after which bacterial colonies were examined according to their morphological characteristics such as colony size, shape, color, and mucoid appearance.

Morphological and Biochemical Identification

Suspected colonies were subjected to morphological examination and conventional biochemical tests commonly used for the identification of Klebsiella pneumoniae. These tests included Catalase, Oxidase, Indole, Urease, Citrate utilization, and Triple Sugar Iron (TSI) tests. The obtained results were compared with the standard biochemical characteristics of *Klebsiella pneumoniae*.

Automated Identification (VITEK System)

The identification of bacterial isolates was further confirmed using the VITEK 2 Compact automated identification system (bioMérieux, France). This system identifies bacteria based on their biochemical profiles and provides the probability of identification. The results obtained from the VITEK system confirmed that all examined isolates were identified as Klebsiella pneumoniae.

Phenotypic Detection of Biofilm Formation

Biofilm formation among *Klebsiella pneumoniae* isolates was determined using the microtiter plate method (ELISA method). Bacterial isolates were inoculated into nutrient broth and incubated overnight at 37°C. The

bacterial suspension was transferred into 96-well microtiter plates and incubated for 24 hours. After incubation, the wells were washed with phosphate buffered saline (PBS) to remove non-adherent cells. The attached biofilm was stained using crystal violet, and the optical density (OD) was measured using an ELISA microplate reader (BioTek ELx800, BioTek Instruments Inc., USA) to determine the biofilm production ability of each isolate.

Molecular Detection of Biofilm Genes

DNA Extraction

Genomic DNA was extracted from bacterial isolates using a commercial bacterial genomic DNA extraction kit following the manufacturer's protocol (Geneaid Biotech Ltd., Taiwan). The purified DNA was stored at −20 °C and subsequently used as a template for PCR amplification.

PCR Amplification

PCR was performed to detect the biofilm-related genes treC and cpsD using specific primers (Table 1-3).

Table 1. Primers used for amplification of biofilm genes

Gene	Primer	Sequence (5'–3')	Product size
treC	F	GGTGCAGCGCTATCTGGCTAA	302 bp
	R	CGCCAAATTTAGAGCGCCAG	
cpsD	F	TGCGGGTTTTTGTGCTGATG	386 bp
	R	TCCCCACAGCATAATCGGTG	

Table 2. PCR Master Mix

Component	Volume (µL)
2X PCR Master Mix	12.5
Forward primer	1
Reverse primer	1
DNA template	2
Nuclease-free water	8.5
Total volume	25 µL

Table 3. PCR Cycling Conditions

Step	Temperature	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	
Annealing	53°C for treC and 57°C for cpsD	30 sec	35
Extension	72°C	45 sec	
Final extension	72°C	5 min	1

Agarose Gel Electrophoresis

PCR products were analyzed using agarose gel electrophoresis. The amplified DNA fragments were separated on 1.5% agarose gel prepared in TAE buffer and stained with ethidium bromide. The gel was run at 80–100 V for 45 minutes, and DNA bands were visualized using a UV transilluminator. The size of amplified products was determined by comparison with a DNA ladder marker.

3. Results

Distribution of Bacterial Isolates from Clinical Samples

Clinical samples were collected from hospitalized patients in hospitals of Al-Hillah city, Iraq, including Marjan Teaching Hospital and Al-Hillah Teaching Hospital. The process of sample collection and laboratory work started on 19 November 2025 and continued until the end of December 2025.

A total of 200 clinical samples were obtained, including sputum and urine specimens. The collected samples were cultured on Blood agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar for the isolation and identification of bacterial pathogens.

After incubation at 37°C for 24 hours, the results showed that 100 samples were negative for bacterial growth, while 20 isolates were identified as *Klebsiella pneumoniae* and 80 isolates belonged to other bacterial species, as shown in Table 4.

Table 4. Distribution of bacterial isolates from clinical samples

Culture Result	Number of Isolates
No bacterial growth (Negative)	100
Klebsiella pneumoniae	20
Other bacterial species	80
Total	200

Furthermore, the distribution of *Klebsiella pneumoniae* isolates according to the source of the specimen revealed that 17 isolates were obtained from sputum samples, while 3 isolates were obtained from urine samples, as shown in Table 5.

Table 5. Distribution of *Klebsiella pneumoniae* isolates according to sample source

Sample Source	Number of Isolates
Sputum	17
Urine	3
Total	20

Growth Characteristics of *Klebsiella pneumoniae* on Culture Media

After incubation of the cultured samples at 37°C for 24 hours, the bacterial isolates showed characteristic growth on the different culture media used for primary isolation and identification. The isolates demonstrated typical colonial morphology on Blood agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar.

On Blood agar, the colonies of *Klebsiella pneumoniae* appeared as large, smooth, round, and mucoid colonies with grayish coloration, which is characteristic of encapsulated bacteria. The mucoid appearance is mainly attributed to the presence of a polysaccharide capsule surrounding the bacterial cells.

On MacConkey agar, the isolates produced large pink mucoid colonies, indicating the ability of the organism to ferment lactose. This feature is considered an important characteristic in differentiating *Klebsiella pneumoniae* from other non-lactose fermenting Gram-negative bacteria.

Similarly, growth on Eosin Methylene Blue (EMB) agar appeared as large, mucoid colonies with a pink to purple coloration, confirming the lactose-fermenting nature of the organism.

The colonial morphology of *Klebsiella pneumoniae* on the different culture media is illustrated in Figures (1–3).

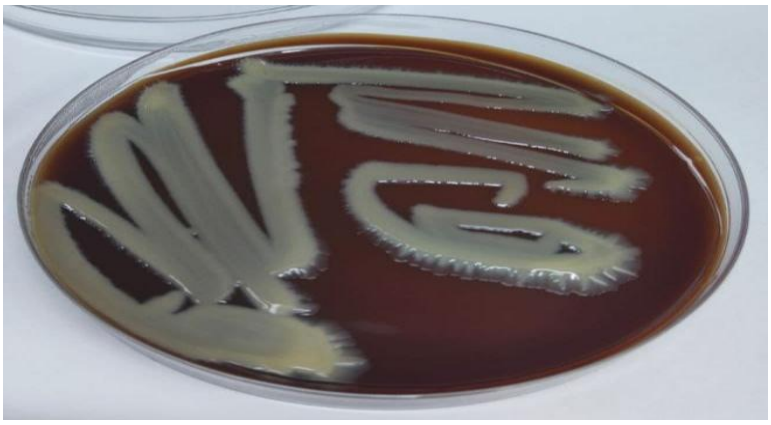

Figure 1: Growth of *Klebsiella pneumoniae* on Blood agar showing large mucoid colonies.

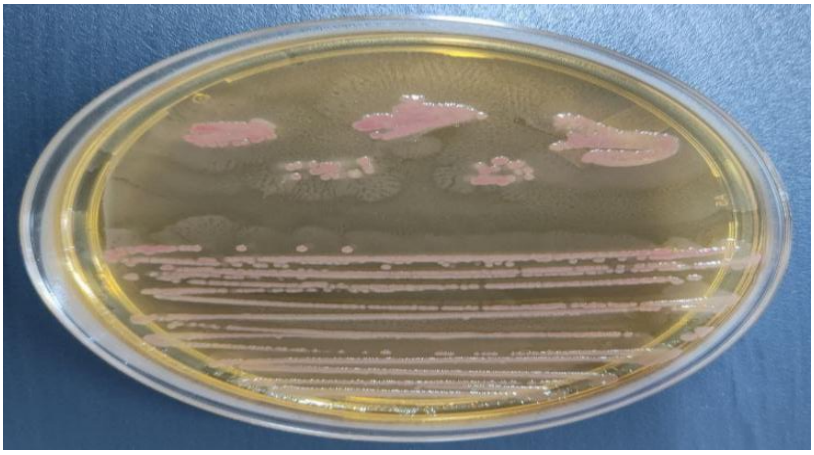

Figure 2: Growth of *Klebsiella pneumoniae* on MacConkey agar showing pink lactose-fermenting mucoid colonies.

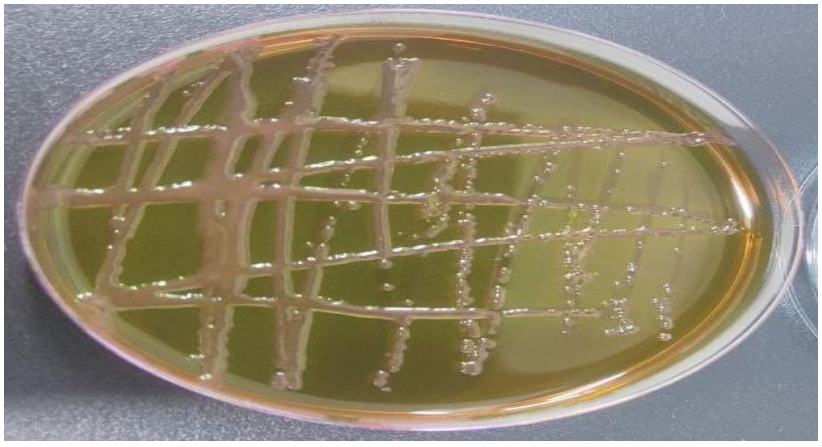

Figure 3: Growth of *Klebsiella pneumoniae* on EMB agar showing typical mucoid colonies.

Biochemical Tests for Identification of *Klebsiella pneumoniae*

The identification of *Klebsiella pneumoniae* isolates was further confirmed using conventional biochemical tests commonly used in bacterial diagnosis. The results of these tests showed typical biochemical reactions

consistent with the characteristics of *Klebsiella pneumoniae*. The biochemical reactions of the isolates are presented in Table 6.

Table 6. Conventional biochemical tests of *Klebsiella pneumoniae* isolates

Biochemical Test	Result
Catalase test	Positive (+)
Oxidase test	Negative (−)
Indole test	Negative (−)
Urease test	Positive (+)
Citrate utilization test	Positive (+)
Triple Sugar Iron (TSI) test	A/A, Gas production

Identification of *Klebsiella pneumoniae* Isolates

The identification of the bacterial isolates was confirmed using an automated identification system. The results demonstrated that all examined isolates (n = 20) were identified as *Klebsiella pneumoniae* with a probability of 99%, indicating a high level of accuracy and confidence in the identification process.
The identification results of the isolates included in the present study are summarized in Table 7.

Table 7. Identification of *Klebsiella pneumoniae* isolates

Isolate No.	Identified Organism	Probability
1	*Klebsiella pneumoniae*	99%
2	*Klebsiella pneumoniae*	99%
3	*Klebsiella pneumoniae*	99%
4	*Klebsiella pneumoniae*	99%
5	*Klebsiella pneumoniae*	99%
6	*Klebsiella pneumoniae*	99%
7	*Klebsiella pneumoniae*	99%
8	*Klebsiella pneumoniae*	99%
9	*Klebsiella pneumoniae*	99%
10	*Klebsiella pneumoniae*	99%
11	*Klebsiella pneumoniae*	99%
12	*Klebsiella pneumoniae*	99%
13	*Klebsiella pneumoniae*	99%
14	*Klebsiella pneumoniae*	99%
15	*Klebsiella pneumoniae*	99%
16	*Klebsiella pneumoniae*	99%
17	*Klebsiella pneumoniae*	99%
18	*Klebsiella pneumoniae*	99%
19	*Klebsiella pneumoniae*	99%
20	*Klebsiella pneumoniae*	99%

Phenotypic Detection of Biofilm Formation in *Klebsiella pneumoniae*

The phenotypic detection of biofilm formation among Klebsiella pneumoniae isolates was performed using the microtiter plate method with crystal violet staining. The optical density (OD) values were measured using an ELISA microplate reader to evaluate the biofilm production ability of each isolate.
Biofilm production was classified according to optical density (OD) values as follows: OD ≤ 0.12 was considered weak biofilm production, OD between 0.12–0.24 was considered moderate biofilm production, and OD ≥ 0.24 was considered strong biofilm production.
The results showed that most isolates were strong biofilm producers, while a few isolates demonstrated moderate biofilm production. The optical density values and the corresponding biofilm formation strength of the tested isolates are presented in Table 8.

Table 8. Phenotypic detection of biofilm formation in *Klebsiella pneumoniae* isolates using ELISA method

Isolate No.	OD Value	Biofilm Production
1	0.366	Strong
2	0.300	Strong
3	0.266	Strong
4	0.270	Strong
5	0.286	Strong
6	0.298	Strong
7	0.342	Strong
8	0.275	Strong
9	0.305	Strong
10	0.294	Strong
11	0.304	Strong
12	0.396	Strong
13	0.268	Strong
14	0.250	Strong
15	0.234	Moderate
16	0.216	Moderate
17	0.199	Moderate
18	0.245	Strong
19	0.260	Strong
20	0.257	Strong

The results indicated that 17 isolates (85%) were strong biofilm producers, whereas 3 isolates (15%) showed moderate biofilm formation. No weak or non-biofilm producing isolates were observed in the present study.

Molecular Detection of Biofilm-Related Genes in *Klebsiella pneumoniae*

The presence of biofilm-related genes in *Klebsiella pneumoniae* isolates was investigated using PCR amplification, and the amplified products were analyzed by agarose gel electrophoresis.
The results showed that the treC gene (302 bp) was detected in all examined isolates (20/20, 100%), indicating that this gene is highly prevalent among the studied *Klebsiella pneumoniae* isolates. The electrophoresis pattern of the amplified treC gene in all isolates is illustrated in Figure (4).
In contrast, the cpsD gene (386 bp) was detected in 9 isolates (45%), while the remaining 11 isolates (55%) were negative for this gene. The agarose gel electrophoresis pattern of the amplified cpsD gene is shown in Figure (5).

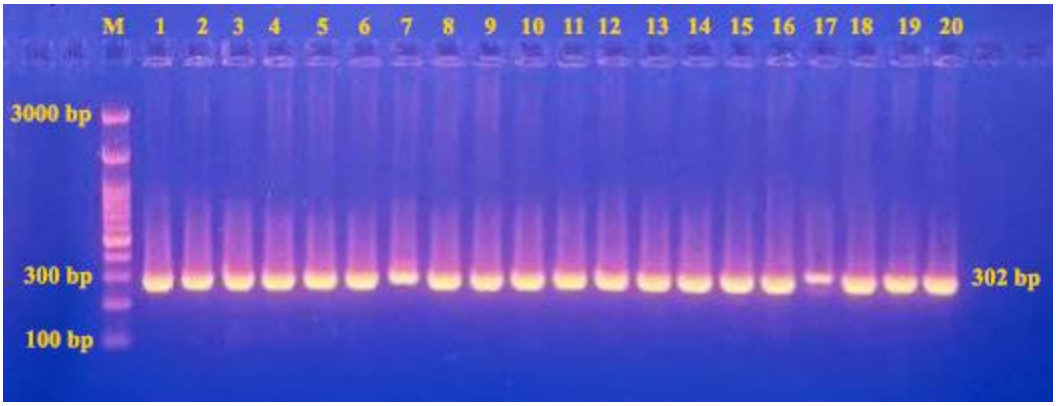

Figure 4. Agarose gel electrophoresis showing PCR amplification of the treC gene (302 bp) in *Klebsiella pneumoniae* isolates. Lane M represents the DNA marker, while all isolates (1–20) show positive amplification of the gene.

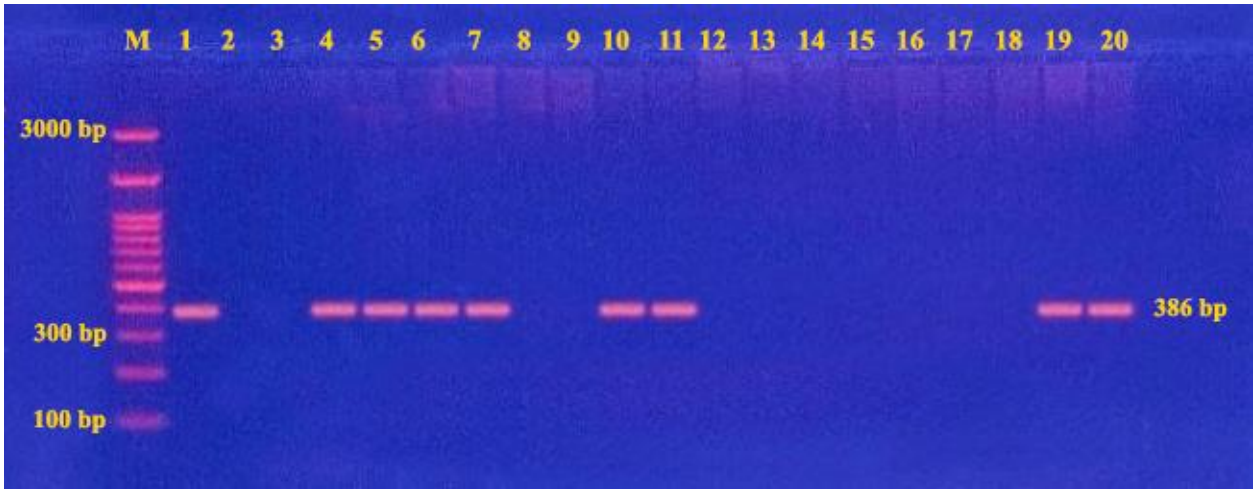

Figure 5. Agarose gel electrophoresis showing PCR amplification of the cpsD gene (386 bp) in *Klebsiella pneumoniae* isolates. Lane M represents the DNA marker, while positive bands are observed in lanes 1, 4, 5, 6, 7, 10, 11, 19, and 20.

4. Discussion

The present study investigated the phenotypic and molecular characteristics of biofilm formation in *Klebsiella pneumoniae* isolated from different clinical specimens collected from hospitalized patients in Al-Hillah city. The findings of the current study demonstrated that *K. pneumoniae* was isolated from a proportion of the examined clinical samples, indicating that this bacterium remains an important opportunistic pathogen responsible for a variety of hospital-associated infections. Similar findings have been reported in several epidemiological studies which identified *K. pneumoniae* as one of the most frequently isolated Gram-negative bacteria in clinical settings, particularly in respiratory and urinary tract infections [16][17].

The distribution of isolates according to sample source revealed that the majority of isolates were obtained from sputum samples compared with urine specimens. This observation is consistent with several previous studies that reported respiratory samples as one of the main clinical sources for *K. pneumoniae* isolation. For instance, a molecular study conducted in Iraq on sputum samples demonstrated that *K. pneumoniae* represented a significant proportion of respiratory bacterial isolates, highlighting its important role in respiratory infections among hospitalized patients [18]. In addition, other studies have reported that

respiratory tract infections caused by *K. pneumoniae* are particularly common among patients admitted to intensive care units and those undergoing mechanical ventilation [19].

The cultural and biochemical characteristics observed in the present study were consistent with the typical microbiological profile of *Klebsiella pneumoniae*. The appearance of large mucoid colonies on blood agar and lactose-fermenting colonies on MacConkey and EMB agar reflects the presence of a thick polysaccharide capsule surrounding the bacterial cells. The capsule represents one of the most important virulence factors of *K. pneumoniae*, as it contributes to bacterial resistance against host immune responses and enhances bacterial survival within host tissues [7][20].

One of the most significant findings in this study was the high ability of the isolates to produce biofilm structures. The phenotypic detection of biofilm formation revealed that a high percentage of the isolates were strong biofilm producers. This observation is consistent with several previous studies which reported that a large proportion of *K. pneumoniae* clinical isolates are capable of producing biofilms. For example, Karimi et al. reported that approximately 75% of clinical isolates were able to form biofilms using the microtiter plate method [16]. Similarly, an Iraqi study conducted in Wasit province reported that nearly all examined isolates were capable of producing biofilms, with the majority showing moderate to strong biofilm formation [21].

The high prevalence of biofilm formation observed in the present study may be explained by the adaptive advantages provided by biofilm growth. Biofilm formation allows bacterial cells to adhere to both biological and abiotic surfaces and facilitates bacterial persistence within hospital environments. Once established, biofilms act as protective barriers that limit the penetration of antimicrobial agents and shield bacterial cells from host immune responses. Consequently, bacteria embedded within biofilms may exhibit resistance levels up to one thousand times higher than planktonic bacterial cells [7][8].

The clinical significance of biofilm formation has been extensively documented in previous investigations. Biofilms formed on medical devices such as urinary catheters, endotracheal tubes, and other implanted materials can serve as reservoirs for persistent infections. Studies have shown that device-associated infections caused by *K. pneumoniae* are frequently linked to biofilm formation on plastic surfaces and medical equipment used in hospital settings [14]. These findings support the results of the present study, which demonstrated a high proportion of biofilm-producing isolates among clinical samples.

At the molecular level, the PCR analysis revealed that the treC gene was detected in all examined isolates. The universal presence of this gene among the studied isolates suggests that it may play an important role in biofilm formation in *K. pneumoniae*. The treC gene encodes trehalose-6-phosphate hydrolase, an enzyme involved in trehalose metabolism. This metabolic pathway has been associated with the regulation of carbohydrate metabolism and extracellular polysaccharide production, which are essential components of the biofilm matrix [3][22].

The detection of treC in all isolates in the present study may explain the high proportion of strong biofilm-producing isolates. Similar observations have been reported in molecular studies investigating biofilm-associated genes in *K. pneumoniae*, which demonstrated that genes involved in carbohydrate metabolism and polysaccharide synthesis contribute significantly to biofilm stability and development [9][23].

In contrast, the cpsD gene was detected in only a portion of the isolates, suggesting genetic variability among the studied strains. The cpsD gene is part of the capsular polysaccharide synthesis gene cluster and plays a crucial role in capsule production. The bacterial capsule contributes to virulence by protecting bacterial cells from phagocytosis and complement-mediated killing, in addition to facilitating bacterial aggregation during biofilm development [13].

The variation in cpsD distribution observed in the present study is consistent with previous molecular studies that reported differences in the distribution of virulence genes among clinical isolates of *K. pneumoniae*. Such variations may reflect genetic diversity among bacterial strains and may also be influenced by environmental conditions and infection sources [12][23].

Another possible explanation for the variation in cpsD distribution is the complex relationship between capsule production and biofilm formation. While capsule production may enhance bacterial aggregation and biofilm stability, excessive capsule production in hypervirulent strains may interfere with the early stages of bacterial adhesion by masking fimbrial structures required for surface attachment [24][25].

The high frequency of strong biofilm-forming isolates combined with the presence of biofilm-associated genes highlights the pathogenic potential of *Klebsiella pneumoniae* isolates circulating in hospital environments in Al-Hillah city. Similar concerns have been raised in other Iraqi studies which reported increasing rates of multidrug-resistant *K. pneumoniae* isolates associated with biofilm formation [26][27].

Furthermore, environmental investigations conducted in Babylon province have detected carbapenem-resistant *K. pneumoniae* strains in water sources such as the Al-Hillah River, suggesting that environmental reservoirs may contribute to the spread of resistant strains in clinical settings [15]. The circulation of such strains between environmental and hospital environments may play an important role in the epidemiology of *K. pneumoniae* infections in the region.

Overall, the findings of the present study emphasize the important role of biofilm formation and biofilm-associated genes in the pathogenicity of *Klebsiella pneumoniae*. Understanding the phenotypic and molecular characteristics of biofilm formation among clinical isolates may contribute to improving infection control strategies and developing more effective therapeutic approaches for managing infections caused by this opportunistic pathogen.

Conclusion

The results of the present study demonstrated that *Klebsiella pneumoniae* is commonly isolated from clinical samples of hospitalized patients in Al-Hillah city, particularly from sputum specimens. Most of the isolates exhibited a strong ability to form biofilms as determined by the phenotypic microtiter plate method. Molecular analysis revealed the presence of biofilm-related genes among the studied isolates, with the treC gene detected in all isolates, suggesting its important role in biofilm formation. In contrast, the cpsD gene was detected in a smaller proportion of isolates, indicating variability in the distribution of this gene among *Klebsiella pneumoniae* strains. Overall, the study confirms the significant role of biofilm formation and its associated genes in the pathogenicity of *Klebsiella pneumoniae*.

Acknowledgment

The author would like to express sincere gratitude to the staff of Marjan Teaching Hospital and Al-Hillah Teaching Hospital for their assistance in sample collection and laboratory work.

References

1. Monteiro, A. D. S. S., Cordeiro, S. M., & Reis, J. N. (2024). Virulence factors in Klebsiella pneumoniae: a literature review. *Indian journal of microbiology*, *64*(2), 389-401.
2. Dehbanipour, R., & Ghalavand, Z. (2022). Anti-virulence therapeutic strategies against bacterial infections: recent advances. *Germs*, *12*(2), 262.
3. Guerra, M. E. S., Destro, G., Vieira, B., Lima, A. S., Ferraz, L. F. C., Hakansson, A. P., & Converso, T. R. (2022). Klebsiella pneumoniae biofilms and their role in disease pathogenesis. *Frontiers in cellular and infection microbiology*, *12*, 877995.
4. Song, H. W., Lin, Y., Wan, T. E., Yang, G. H., Jiang, M. T., Fu, B. H., & Pan, J. S. (2025). Global burden of Klebsiella pneumoniae infections and antimicrobial resistance in 2019. *BMC Infectious Diseases*, *25*(1), 1773.
5. Luo, S., & Wang, Y. T. (2025). First-ever ASM Global Research Symposium on Microbes in Human Health in Tsinghua, Beijing, China. *Msphere*, *10*(3), e01092-24.

6. Crestani, C., Wyres, K. L., Abdulahi, J., Angrup, A., Boateng, W., Brand, C., ... & Brisse, S. (2026). Global perspectives on Klebsiella epidemiology and biology: conference report on the KLEBS 2024 symposium. *npj Antimicrobials and Resistance*, *4*(1), 4.
7. Vuotto, C., Longo, F., Balice, M. P., Donelli, G., & Varaldo, P. E. (2014). Antibiotic resistance related to biofilm formation in Klebsiella pneumoniae. *Pathogens*, *3*(3), 743-758.
8. Devanga Ragupathi, N. K., Muthuirulandi Sethuvel, D. P., Triplicane Dwarakanathan, H., Murugan, D., Umashankar, Y., Monk, P. N., ... & Veeraraghavan, B. (2020). The influence of biofilms on carbapenem susceptibility and patient outcome in device associated K. pneumoniae infections: insights into phenotype vs genome-wide analysis and correlation. *Frontiers in microbiology*, *11*, 591679.
9. Li, L., Gao, X., Li, M., Liu, Y., Ma, J., Wang, X., ... & Wang, Z. (2024). Relationship between biofilm formation and antibiotic resistance of Klebsiella pneumoniae and updates on antibiofilm therapeutic strategies. *Frontiers in Cellular and Infection Microbiology*, *14*, 1324895.
10. 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.
11. Alwan, A. M., Abdallah, H. N., & Hamzah, A. S. (2024). Molecular Analysis of FimH Gene in Biofilm-Producing and Multidrug Resistant Klebsiella pneumoniae. *Hilla University College Journal For Medical Science*, *2*(3), 25-31.
12. Makhrmash, J. H., Al-Aidy, S. R., & Qaddoori, B. H. (2022). Investigation of biofilm virulence genes prevalence in Klebsiella pneumoniae isolated from the urinary tract infections. *Archives of Razi Institute*, *77*(4), 1421.
13. Tiria, F., Odoyo, E., Georges, M., Nyerere, A., & Musila, L. (2023). Molecular Detection of Key Virulence-associated Genes and Phenotypic Analysis of Virulence Traits of Klebsiella pneumoniae Clinical Isolates from Kenya. *Journal of Pure & Applied Microbiology*, *17*(4).
14. Ochońska, D., Ścibik, Ł., & Brzychczy-Włoch, M. (2021). Biofilm formation of clinical Klebsiella pneumoniae strains isolated from tracheostomy tubes and their association with antimicrobial resistance, virulence and genetic diversity. *Pathogens*, *10*(10), 1345.
15. Abbas, F. M. (2022). Prevalence of Waterborne blaNDM-1 Gene Producing Carbapenem-resistant Klebsiella pneumoniae from Al-Hillah River Water, Babylon Province, Iraq. *J. Pure Appl. Microbiol*, *16*, 1873-1877.
16. Karimi, K., Zarei, O., Sedighi, P., Taheri, M., Doosti-Irani, A., & Shokoohizadeh, L. (2021). Investigation of antibiotic resistance and biofilm formation in clinical isolates of Klebsiella pneumoniae. *International journal of microbiology*, *2021*(1), 5573388.
17. Mohamed, I. Q., & Al-Taai, H. R. R. (2023). Phylogenetic analysis of Klebsiella pneumoniae isolated from nosocomial and community infection in Diyala, Iraq. *Iraqi Journal of Science*, 2726-2740.
18. Monawer, A. T. (2025). Molecular Analysis of Klebsiella Pneumoniae Isolates Collected from Sputum Samples in Duhok Iraq. *Al-Nahrain Journal of Science*, *28*(1).
19. Ferreira, R. L., Da Silva, B. C., Rezende, G. S., Nakamura-Silva, R., Pitondo-Silva, A., Campanini, E. B., ... & Pranchevicius, M. C. D. S. (2019). High prevalence of multidrug-resistant Klebsiella pneumoniae harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. *Frontiers in microbiology*, *9*, 3198.
20. Hullur, M. S., Natarajan, A., & Sreeramulu, P. N. (2022). Phenotypic characterization of virulence factors and antibiogram of klebsiella pneumoniae isolates from various clinical samples—a cross sectional study. *J Pure Appl Microbiol*, *16*(3), 1783-1791.
21. Husseina, H. H., Mahdib, H. T., Abedc, H. A. A. R., Hasanc, M. A., Hameedb, M. A. K., & Shakirb, A. F. (2025). Therapeutic efficacy of olive leave extract against Klebsiella pneumonia isolated from

diabetic foot patients in vitro. Journal of Medicinal and Pharmaceutical Chemistry Research. 7(3), 561-569.

22. Li, Y., & Ni, M. (2023). Regulation of biofilm formation in Klebsiella pneumoniae. *Frontiers in microbiology*, *14*, 1238482.
23. Nazari, M., Hemmati, J., & Asghari, B. (2024). Comprehensive analysis of virulence genes, antibiotic resistance, biofilm formation, and sequence types in clinical isolates of Klebsiella pneumoniae. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2024*(1), 1403019.
24. Nguyen, T. N. T., Howells, G., & Short, F. L. (2025). How Klebsiella pneumoniae controls its virulence. *PLoS Pathogens*, *21*(9), e1013499.
25. Yan, R., Lu, Y., Zhu, Y., Lan, P., Jiang, S., Lu, J., ... & Jiang, Y. (2021). A sequence type 23 hypervirulent Klebsiella pneumoniae strain presenting carbapenem resistance by acquiring an IncP1 bla KPC-2 plasmid. *Frontiers in Cellular and Infection Microbiology*, *11*, 641830.
26. Abdullah, I. T., Hasib, F. A., & Mohammad, F. I. (2023). Studying the prevalence of multidrug resistant Klebsiella pneumoniae in Kirkuk city. *NTU Journal of Agriculture and Veterinary Science*, *3*(4).
27. Alyaseri, M. A. A., Al-Jelehawy, A. H., & Al-Fatlawi, A. A. (2025). Prevalence and Antimicrobial Resistance Profiles of Klebsiella Pneumoniae in Karbala, Iraq Hospitals. *Karbala Journal of Pharmaceutical Sciences*, *16*(27), 12-24.