Research Article

Al-Iraqia Medical College Journal (AIMCJ)
ISSN (Online): 3104-4565
ISSN (Print): 3104-4557

Detection of the Grade of Biofilm Formation and Its Correlation with Antibiotic Resistance Among *Klebsiella Pneumoniae* Isolated from Iraqi Patients with Urinary Tract Infections

Ghada S. Rzwky[1], Sanaa Khudhur Jameell[1], Ban H. Adil[2*]

[1] Department of Medical Microbiology College of Medicine, Al-Iraqia University, Baghdad/Iraq.
[2] Department of Physics, College of Science for Women, University of Baghdad, Baghdad, Iraq.

ARTICLE INFO
Received: 20 / 05 / 2026
Revised: 55/ 06/ 2026
Accepted: 26/06/ 2026
Publish online: 15 /8 / 2026

*Corresponding Author: Ban H. Adil
Email: banha_phys@csw.uobaghdad.edu.iq

CITATION
Ghada S. Rzwky, Sanaa Khudhur Jameell, Ban H. Adil. Detection of the Grade of Biofilm Formation and Its Correlation with Antibiotic Resistance Among *Klebsiella Pneumoniae* Isolated from Iraqi Patients with Urinary Tract Infections. *AIMCJ*. 2026;3(2): 91-102.

https://doi.org/10.58564/AIMCJ3.2.2026.291

Abstract

Urinary tract infections are a serious public health problem that can be caused by a variety of organisms, particularly Gram-negative bacteria.

Multidrug-resistant *Klebsiella pneumonia* infections acquired in hospitals present a significant challenge due to their high virulence, to form biofilms. This increases their resistance to various antibiotics particularly in immunocompromised patients and those undergoing surgery.

This study was conducted to assess the biofilm-forming capacity of *K. pneumonia* isolates and its correlation to the antibiotic resistance of these biofilm-forming isolates in patients with Urinary tract infection.

This cross-sectional study included 300 patients with urinary tract infections at Imam Al-Kadhimia Medical Hospital and Al-Karama Teaching Hospital in Baghdad. Just 93 out of 300 individuals with urinary tract infection had positive *Klebsiella pneumoniae* growth. The Vitek 2 Compact System was used for sensitivity testing, and a microtiter plate was used to measure biofilm formation.

The study results showed that *K. pneumoniae* isolates were capable of forming robust biofilms in patients with UTIs. Other isolates showed moderate to weak biofilm formation capabilities.

In conclusion, the majority of the bacterial isolates of *K. pneumoniae* are strong biofilm-forming and exhibit broad-spectrum resistance to a range of antibiotics. This opens new avenues for research and development of non-traditional antibiotics capable of overcoming this multi-drug resistance bacteria.

Keywords: Urinary tract infection; Biofilm formation; Multidrug resistance (MDR); *Klebsiella pneumonia*; Microtiter plate Method.

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

Introduction

The disruption of physiological balance brought on by a pathogenic invasion is known as a urinary tract infection (UTI). Depending on the categorization of the microorganism which could be caused by bacteria (1), fungi (2), or viruses (3) different pathogenic mechanisms and virulence factors apply. While women have a 55% lifetime chance of UTIs, men still have a considerable risk, particularly as they age (4).

Antibiotic resistance continues to be a major global health issue with detrimental social and economic effects. One of the most important public health infections is exogenously resistant, broad-spectrum betalactamase producing enterobacteria (ESBLE), which calls for immediate investigation and the creation of novel medications (5).

Multidrug resistance (MDR), which is resistance to one antibiotic in three or more treatment classes; extensively widespread resistance (XDR), which is resistance to most classes; and pervasive resistance (PDR), which is resistance to all available antibiotics, are the three standard terms that the CDC and ECDC have agreed upon for categorizing bacterial severity. These descriptions outline the remaining therapeutic options and categorize the pathogenicity of superbugs (6).

Prominent infections with MDR of *Kl. pneumoniae*, require ongoing observation to guarantee the efficacy of empirical treatment (7). A biofilm is a complex assembly of microbial cells (such as bacteria or fungi) that adhere irreversibly to living or inanimate surfaces and are completely immersed within a self-produced sticky matrix of extracellular polymer (EPS) composed of polysaccharides, proteins, and DNA. A biofilm is a complex assembly of microbial cells (such as bacteria or fungi) that adhere irreversibly to living or inanimate surfaces and are completely immersed within a self-produced sticky matrix of extracellular polymer (EPS) composed of polysaccharides, proteins, and DNA (8). MDR of Klebsiella pneumoniae, are dangerous because of their virulence traits, particularly their ability to form biofilms that invade the host's mucosal surfaces (9).

The quorum sensing (QS) system, which controls microbial communication, creates these biofilms (10). Proteins and polysaccharides make up 90% of the extracellular matrix (EPS) that makes up these membranes (11). Antibiotics are trapped and kept from reaching their target by this matrix, which also serves as a physical barrier and chemical shield (12). Additionally, the membrane prevents white blood cells from absorbing germs by acting as immunological camouflage (13).

Material and Methods:

Collection of the urine sample

Between November 2025 and April 2026, 300 urine samples were taken from inpatients suffering from urinary tract infections (UTIs) at Al-Imamain Al-Kadhimain Medical Hospital and Al-Karama Teaching Hospital. The patient's name, age, sex, and date of UTI diagnosis were all included in a questionnaire used in this investigation. Hospitalized individuals had a general urinalysis after their urine was collected in sterile pee cups. Age range of the patients was between 18 and 85 years old.

Exclusion criteria

(A) include patients receiving therapy within the last two weeks, pregnant women, children under the age of eighteen,

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

(B) Every patient with kidney abnormalities, congenital issues, or severe autoimmune or infectious disorders.

Bacterial isolate reservation

1. Temporary preservation Once the identity of *K. pneumoniae* isolates was confirmed, a single pure isolated colony was transferred to Brain Heart Infusion Agar and Nutrient Agar slant, incubated overnight at 37°C, and then stored at 4°C for a brief amount of time (a few weeks). (14).

2. Extended preservation: After diagnosis, *K. pneumoniae* isolates were stored at -20°C for extended periods of time by moving a single pure isolated colony to 20% glycerol with brain heart infusion broth medium (14).

K. pneumoniae confirmation using the vitek-2 compact system:

The VITEK 2 device's antibiotic susceptibility test (AST) of *K. pneumoniae* against. It is effective against ten antibiotics, which are as follows: Ampicillin, Amoxicillin Piperacillin, Ceftriaxone, Ciprofloxacin, Gentamicin, Amikacin, Imipenem, Tigecycline, and Colistin; based on the "Kinetic Photometry" technology to ascertain the minimum inhibitory concentration (MIC). In compliance with standard operating procedures, the following standards and procedures were adhered to (15).

1. Essential Conditions

- For Gram-negative bacteria, the VITEK 2 AST Card was utilized.
- 0.45% to 0.50% NaCl is the sterile saline solution.
- Densitometer is the measurement tool.

2. Protocol

- In order to prepare the sample, pure colonies of *K. pneumoniae* are grown for 18 to 24 hours on unrestricted medium (MacConkey Agar).
- Turbidity Adjustment: After the colonies are moved to sterile saline, a DensiCheck device is used to modify the density to fit the McFarland scale (0.50 to 0.63 McFarland).
- Card Inoculation: Insert the sample tube and card into the cassette holder of the device. The bacterial suspension, which contains diluted amounts of antibiotics, is automatically drawn into the card channels by the device.
- Incubation and Reading: After inserting the card into the apparatus, it is incubated at 37°C. In order to track bacterial development, the device automatically reads the data every fifteen minutes.

3. Interpretation of Findings the Clinical Laboratory Standards Institute (CLSI) or the European Committee for Testing and Analysis's standards are compared to the results using the Advanced Expert System (AES) software. It is established what antibiotic concentration is necessary to totally stop bacterial growth. The outcome is categorized by the device as (S) sensitive, (I) intermediate, or (R) resistant. It is known that ESBL (broad-spectrum beta-lactamase) enzymes are produced by *K. pneumoniae*. The AES system in the VITEK instrument will notify the VITEK 2 of the possible presence of ESBL enzymes if the instrument displays antibiotic resistance (MIC ≥ 2 µg/mL according to (CLSI). The VITEK 2 instrument is automatically configured to these values in accordance with the Clinical Laboratory Standards Institute's (CLSI) requirements (16).

1. Where it is Sensitivity Classification For antibiotic (at a concentration of 30 micrograms per tablet or its equivalent on the VITEK card), the following classifications apply:

- Susceptible (S): If the MIC is equal to or less than 1 microgram/mL (≤ 1 µg/mL).

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

- Intermediate (I): If the MIC is equal to or greater than 2 micrograms/mL (2 µg/mL).
- Resistant (R): If the MIC is equal to or greater than 4 micrograms/mL (≥ 4 µg/mL).

2. Significance of the result in the VITEK instrument (AES system): When a result of (R) or even (I) appears for antibiotic, the VITEK instrument, using the Advanced Expert System,
3. performs a phenotype analysis: ESBL enzyme detection: If the MIC for antibiotic is high (≥ 2 (µg/mL), the device immediately suspects that bacteria are producing ESBL

K. pneumoniae biofilm development assay

- Initial Culture: Every isolate is cultivated in brain heart infusion (BHI) broth and cultured for a full day at 37°C. figure. 1. A.
- Turbidity Adjustment: To match the 0.5 McFarland turbidity, 100 µL of the overnight culture is added to 2 mL of regular saline.
- Plate Preparation: Fill each well of a sterile 96-well polystyrene microtiter plate with 180 µL of BHI broth supplemented with 1% glucose for each isolate, three replicate wells are inoculated with 20 µL of the bacterial suspension; wells containing only BHI-glucose broth are used as negative controls.
- Biofilm Formation: Plates are covered and incubated statically at 37°C for 24 hours.
- Washing and Fixation: Planktonic cells are aspirated, and wells are washed three times with distilled water. Biofilms are then fixed with
- 200L of absolute methanol for 10 minutes and air-dried.
- Staining: After 10 minutes of staining with 200 µL of 0.1% crystal violet (17) the adhering biofilm is rinsed three times with distilled water and allowed to air dry for 30 minutes at 37°C, figure 1. B.
- Measurement and Solubilization: 200 µL of a glacial acetic acid (18) and 100% ethanol mixture (1:1, v/v) are used to dissolve the bound dye. At 630 nm the optical density (OD) is determined.
- Classification Based on the determined cut-off value (ODc), isolates are categorized as weak, moderate, strong, or non-biofilm makers. Table1 (19).

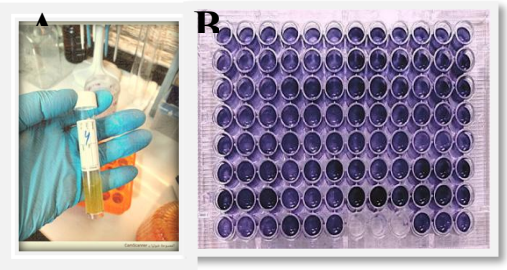

Figure 1. A . Cultivation of *K. pneumonia* in the growing medium of brain heart infusion broth (BHI) B. Staining: with Crystal Violet.

Table 1: Classification of *K. pneumonia* isolates according to the adherence.

OD values	Adherence	Biofilm intense
≤ 0.120	Non- adherent	Non-weak biofilm producer
0.120 - 0.240	Moderately adherent	Moderate
> 0.240	Strong adherent	High

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

Results:

Identification of K. pneumoniae Isolation

Three hundred bacterial isolates from urinary tract infection (UTI) clinical samples were analyzed and identified. The VITEK-2 system, which is very effective at precisely identifying the bacterial species, was used to evaluate the isolates. *K. pneumoniae* was found to be present in 93 isolates, or 31% of all isolates examined *K. pneumoniae* bacteria showed big, mucous, pink colonies typical of lactose fermentation when cultured on MacConkey agar Figure 2. The proportion of women was 55% (51.15) higher than that of men 45 % (41.85) Figure 3. This percentage shows how important this bacterium is as a primary cause of complicated UTIs in the research region.

Biofilm formation detection using micro titer plate method

The current research noted the following based on the "strength" of biofilm formation as categorized by Optical Density (OD) 93 isolates or 31% of all isolates examined *K. pneumoniae:*
These strains cannot form a complete or solid biofilm and only produce trace amounts of EPS.38 (40.86%) Weak Producers: The EPS produced by these strains is extremely low and they are frequently less resilient to severe environments and antibiotics than their counterparts that do 25(26.88%), figure 4. The variety of ciliates used in extraction, the accumulation of isolation-specific mutations within the biological matrix, the variation in growth-specific iodine, and the differences in the preservation of the bacteria's sugar capsule are some of the causes of this bio-oxygen diversity in *K. pneumoniae* bacteria.

Drug Resistance in Isolates That Form Biofilms

As Table 2. illustrates, our study's findings showed a connection between biofilm production and antibiotic resistance. The results demonstrated that biofilm-forming isolates differed in antibiotic resistance, ranging from resistant to sensitive to intermediate, based on the VITEK instrument employed, MIC values, and their interpretation as resistant, sensitive, and intermediate (using VITEK-specific tables). Colistin and Tigecycline was the most successful antibiotic against isolates that formed (moderat, weak) biofilms.

According to the table 3. of antibiotic susceptibility test findings against 30 isolates of multidrug-resistant *K. pneumoniae*, the remaining 93 isolates in this study were the 30 isolates with the strongest biofilm formation. In the VITEK 2 system, these isolates showed different levels of multidrug resistance. The current study's findings revealed that the bacterial isolates' resistance levels to 10 distinct antibiotics differed significantly according to Table 3 and Figure 5, the isolates were divided into three groups sensitivity (S), intermediate (I), and resistant (R). Piperacillin and Ceftriaxone (28 isolates) and Ampicillin and Amoxicillin (30 isolates showed the greatest rates of full resistance among the isolates.

On the other hand, the isolates' resistance to the other antibiotics gradually declined. Colistin showed the resistance (12 isolates) the lowest rate of intermediate (only 10 isolates), and (only 8 isolates) is sensitivity.

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

The data were statistically examined using GraphPad Prism software in order to verify the scientific relevance of these variations, with a significance level of ($P < 0.001$). The full comparison test revealed highly significant differences in the effects of various antibiotics on the bacterial isolates. On the other hand, the isolates' resistance to the other antibiotics gradually declined. Colistin showed the resistance (12 isolates) the lowest rate of intermediate (only 10 isolates), and (only 8 isolates)is sensitivity. The data were statistically examined using GraphPad Prism software in order to verify the scientific relevance of these variations, with a significance level of ($P < 0.001$). The full comparison test revealed highly significant differences in the effects of various antibiotics on the bacterial isolates.

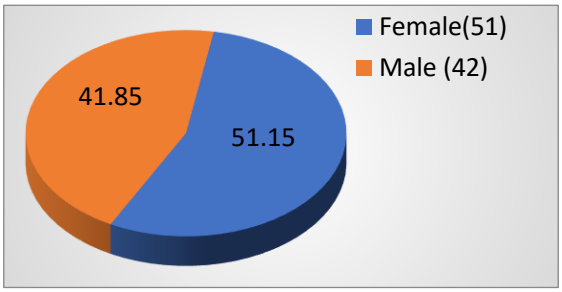

Figure 2: *K. pneumonia* positive cases by sex

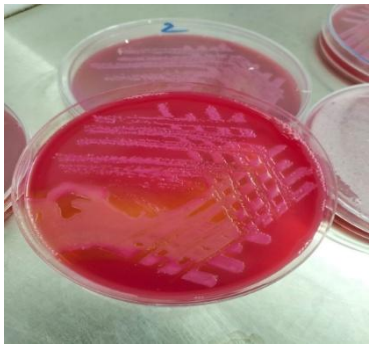

Figure 3. Growth of multidrug-resistant (MDR) *K. pneumoniae* on MacConkey agar, showing characteristic large, mucoid, pink colonies due to lactose fermentation.

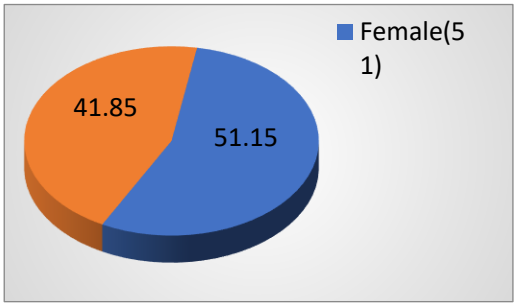

Figure 3. Growth of multidrug-resistant (MDR) *K. pneumoniae* on MacConkey agar, showing characteristic large, mucoid, pink colonies due to lactose fermentation.

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

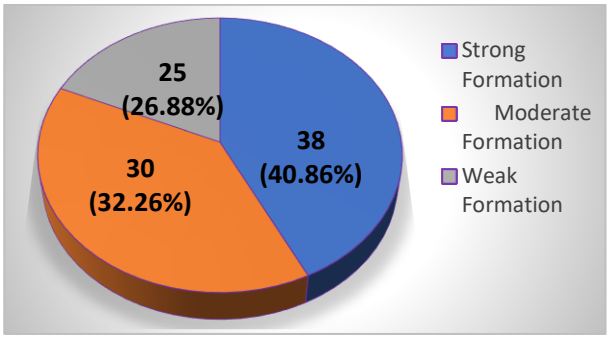

Figure 4: percentage of biofilm production *k. pneumonia* isolations

Table 2: The relationship between biofilm formation and antimicrobial resistance of *K. pneumoniae*

Antibiotic	Total	Number	Strong	Moderate	Weak
Ampicillin	93(100%)	90(96.77%)	30(32.26%)	38(40.86%)	22(23.66%)
Amoxicillin	93(100%)	85(91.40%)	30(32.26%)	38(40.86%)	17(18.28%)
Piperacillin	93(100%)	80(86.02%)	30(32.26%)	38(40.86%)	12(12.90)
Ceftriaxone	93(100%)	80(86.02%)	30(32.26%)	38(40.86%)	12(12.90)
Ciprofloxacin	93(100%)	65(69.89%)	30(32.26%)	34(36.56%)	1(1.08%)
Gentamicin	93(100%)	70(75.27%)	30(32.26%)	38(40.86%)	2(2.86%)
Amikacin	93(100%)	50(53.76%)	30(32.26%)	19(20.43%)	1(1.08%)
Imipenem	93(100%)	40(43.01%)	30(32.26%)	9(9.68%)	1(1.08%)
Tigecycline	93(100%)	30(32.26%)	30(32.26%)	0(0.00%)	0(0.00%)
Colistin	93(100%)	20(21.51%)	20(21.51%)	0(0.00%)	0(0.00%)

Table 3: Antibiotic susceptibility test findings against 30 isolates of MDR *K. pneumoniae*

Antibiotics	Resistant	Intermediate	Sensitive
	NO (%)	NO (%)	NO (%)
Ampicillin	30(100%)	0	0
Amoxicillin	30(100%)	0	0
Piperacillin	28 (93.33%)	1 (3.33%)	1(3.33%)
Ceftriaxone	28 (93.33%)	1(3.33%)	1(3.33%)
Ciprofloxacin	25(83.33%)	2(6.66%)	3(10.0%)
Gentamicin	24(80.0%)	4(13.33%)	2 (6.66%)
Amikacin	23(76.7%)	5(16.7%)	2(6.7%)
Imipenem	19(63.3%)	7(23.3%)	4(13.3%)
Tigecycline	18(60.0%)	8(26.66%)	4(13.33%)
Colistin	12(40.0%)	10(33.33%)	8(26.66%)

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

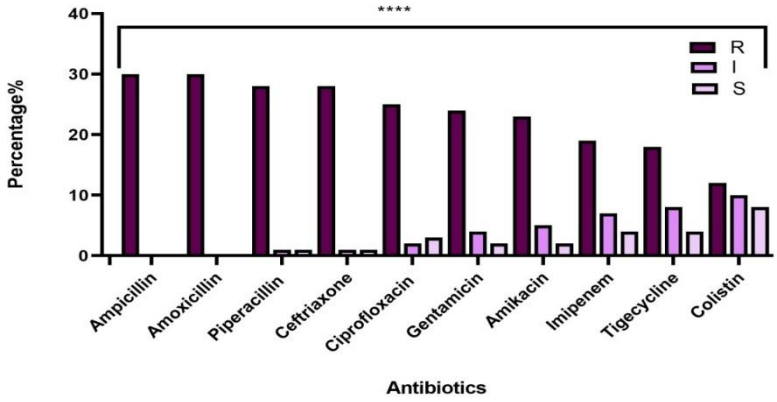

Figure 5: Antibiotic susceptibility test results against *K. pneumoniae*

Discussion

Distribution of the UTIs caused by K. pneumoniae

According to the current study's findings, women are more prone than men to urinary tract infections (55%,45%), respectively which is in line with the research done by (21). Physical characteristics like the shorter urethra and close closeness of the anus, as well as biochemical changes like decreasing estrogen levels, are responsible for the increased frequency in women (22) and since UTIs are linked to prostate issues in older men (23). increased disease virulence. We observe an increased incidence of resistant bacteria in women of reproductive age and postmenopausal women for several reasons, including hormonal changes, weakened imunity, and the shortened distance between the urethra and bladder. We also note that the 51-85 age group and older exhibited increased virulence of MDR bacteria. It is well known that the immune system is less efficient at these ages, which aligns with clinical studies indicating weakened immunity of the urinary tract at this age (24). Similarly, we generally over 60 years, compared to younger men, due to the increased prevalence of prostate problems and the strength and timing of biofilm formation. Biofilm formation and the frequent failure of conventional antibiotics to penetrate the biofilm in bedridden elderly patients are significant challenges. *Klebsiella'*s ability to produce a biofilm in urine is the key to its difficulty in eradication: After 24 hours: Microcolonies begin to settle and form a primary protective layer. After 48 hours: The biofilm reaches full stability, becoming 1000 times more resistant to antibiotics than normal bacteria (25). It is crucial to note that modern treatment protocols no longer focus solely on killing the bacteria, but also on using biofilm dissolvers or diuretics to prevent initial settlement within the first 24 hours. This underscores the importance of initiating treatment early to prevent disease progression and increased bacterial virulence. Additionally, out of 93 *K. pneumoniae* isolates, the clinical isolates that caused recurrent urinary tract infections were the most virulent and resistant to antibiotics, with a resistance rate of 88.17%. This is in line with the research done by (26). showed a clear ability to form weak biofilms in vitro, this does not preclude them from possessing the genetic content necessary for

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

strong binding and robust biofilm formation under suitable environmental conditions or selective pressures within the host. This is what we uncovered during research, where observed a significant change in the biological behavior of *K. pneumoniae* isolates in a group of long-term hospitalized patients through routine follow-up and field visits. One of the most important findings of this study is *Klebsiella*.

Detection of biofilm formation by using microtiter plate method

88.17% of *K. pneumoniae* isolates formed biofilms, where it was found that [32.26%] was highly biofilm-forming, [40.86%] was moderately biofilm-forming, and [26.88%] was weakly biofilm-forming them highly resistant to antibiotics. are consistent with the study (27). However, our results revealed that the majority of *K. pneumoniae* isolates formed biofilms strongly and moderately, which is inconsistent with the study by Karimi K et al. (28), they discovered that only 20% of *K. pneumoniae* isolates were strongly biofilm-forming. Although a small proportion of the clinical isolates studied showed a clear ability to form weak biofilms in vitro, this does not preclude them from possessing the genetic content necessary for strong binding and robust biofilm formation under suitable environmental conditions or selective pressures within the host. This is what we uncovered during research, where observed a significant change in the biological behavior of *K. pneumoniae* isolates in a group of long-term hospitalized patients through routine follow-up and field visits. One of the most important findings of this study is *K. pneumoniae's* remarkable ability to adapt to the host environment. Through clinical monitoring of urine samples, we found that isolates that initially exhibited limited biofilm formation and high sensitivity to treatment eventually acquired sophisticated defense mechanisms, rendering them resistant to multiple drugs and capable of forming highly cohesive biofilms after several months. This leads us to a crucial conclusion: all *K. pneumoniae* strains possess the latent genetic capacity to produce biofilms as a survival strategy, which only manifests in response to specific environmental and clinical stressors these result of present study are consistent with the study This is consistent with the previous study (29), which found that all *K. pneumoniae* isolates have the ability to form biofilms. According to the studies Modern bacteria have the ability to penetrate and bind to urinary epithelial cells, which promotes the proliferation of bacterial communities within the cells of the bladder (30).

Drug resistance of biofilm formation by bacterial isolates

According to results, all biofilm-forming *K. pneumoniae* isolates were 96.77% resistant to ampicillin, amoxicillin 91.4%, 86.02% Piperacillin Ceftriaxone 86.02%, ciprofloxacin 69.89%, Gentamicin 75.27%, Amikacin 53.76%, Imipenem 43.01%, Tigecycline 32.26%, and Colistin 21.51%. These results are consistent with those of previous research by Nerwati et al. (31), which found a high percentage of resistance to aminoglycosides, fluoroquinolones, and cephalosporins (32).

The excessive and careless use of beta-lactam antibiotics (like ampicillin and amoxicillin) in clinical and community settings is thought to be the cause of the high resistance rates found in the current study's results (30 isolates of *K. pneumoniae* multidrug-resistant (MDR)). This hypothesis was conclusively supported by statistical analysis using GraphPad Prism software; the extremely significant statistical value ($P < 0.001$) shows a genuine and significant difference in the effectiveness of the investigated antibiotics.

This significant statistical difference shows that, in comparison to conventional antibiotics, the isolates still show comparatively strong sensitivity to more recent or powerful antibiotics like

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

colistin, tigecycline, and imipenem. The limited use of an antibiotic like colistin as a first-line treatment due to its possible nephrotoxicity, which has maintained its biological efficacy against the examined isolates, is responsible for its statistical and clinical superiority in displaying the lowest resistance rates (33). These findings are in line with the statistical variations found in many domestic and international studies (34). The declining effectiveness of earlier antibiotics and the emergence of multidrug resistance (MDR).

Conclusion

The vast majority of *K. pneumoniae* isolates responsible for urinary tract infections are capable of forming biofilms and have demonstrated resistance to a wide range of antibiotics, indicating the existence of multidrug-resistant bacteria. This opens promising avenues for reformulating strategies to combat bacteria by introducing unconventional biological and chemical agents with attack mechanisms entirely different from traditional antibiotics, or that enhance their effectiveness when combined with them. The modern approach focuses on using natural, bioactive compounds and precise chemical inhibitors that are low or no toxicity to human cells but highly toxic to bacteria. These agents not only kill the bacterial cell but also disrupt virulence factors, such as targeting the structure of biofilms and disrupting cell-to-cell communication (Quorum Sensing). This shift towards complementary and alternative medicine in clinical laboratories represents the greatest hope for breaking the cycle of drug resistance and providing effective and safe treatment options capable of controlling urinary tract infections. So more studied should be done to prevent the development of multi-drug-resistant bacteria.

Conflict of Interest: Nil.

Funding: Nil.

Ethical considerations:

Ethical approval was obtained from the Ethics Committee of the Iraqi University/College of Medicine, Iraq (March 15, 2026).

Acknowledgment:

The authors would like to thank the staff and participants of the Diabetes and Endocrinology Center in Baghdad and Al-Kindi Teaching Hospital for their cooperation and contributions to this research.

References:

1. Filev R, Lyubomirova M, Bogov B, et al. Urinary tract infections caused by *Klebsiella pneumoniae* and prolonged treatment with trimethoprim/sulfamethoxazole. *Microorganisms*. 2025 Feb 14;13(2):422. https://doi.org/10.3390/microorganisms13020422
2. Khateb AM, Alamri LS, Alseyoufi WA, et al. Epidemiology of fungal urinary tract infections: A retrospective case series. *New Microbes and New Infections*. 2025 Dec 1;68:101657. https://doi.org/10.1016/j.nmni.2025.101657
3. Paduch DA. Viral lower urinary tract infections. *Current urology reports*. 2007 Jul;8(4):324-35. https://doi.org/10.1007/s11934-007-0080-y
4. Cox SM, van Hoof MW, Lo-A-Foe K, et al. Cross-sectional internet survey exploring women's knowledge, attitudes and practice regarding urinary tract infection-related symptoms in the Netherlands. *BMJ open*. 2022 May 1;12(5):e059978. https://doi.org/10.1136/bmjopen-2021-059978
5. Husna A, Rahman MM, Badruzzaman AT, et al. Extended-spectrum β-lactamases (ESBL): challenges and opportunities. *Biomedicines*. 2023 Oct 30;11(11):2937. https://doi.org/10.3390/biomedicines11112937
6. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology*

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

and infection. 2012 Mar 1;18(3):268-81. https://doi.org/10.1111/j.1469-0691.2011.03570.x

7. Mihai S, Vintila BI, Bereanu AS, Fratila AM, Codru IR. Lessons from Four Years (2021–2024) of *Klebsiella Pneumoniae* Resistance Surveillance Epidemiological Trends in a Romanian Intensive Care Unit. *Antibiotics*. 2025;14(8):825. https://doi.org/10.3390/antibiotics14080825
8. Kaleta MF, Petrova OE, Zampaloni C, et al. A previously uncharacterized gene, PA2146, contributes to biofilm formation and drug tolerance across the ɣ-Proteobacteria. *npj Biofilms and Microbiomes*. 2022 Jul 7;8(1):54. https://doi.org/10.1038/s41522-022-00314-y
9. Cruz A, Condinho M, Carvalho B, et al. The two weapons against bacterial biofilms: Detection and treatment. *Antibiotics*. 2021 Dec 3;10(12):1482. https://doi.org/10.3390/antibiotics10121482
10. Li Y, Ni M. Regulation of biofilm formation in *Klebsiella pneumoniae*. *Frontiers in microbiology*. 2023 Sep 7;14:1238482. https://doi.org/10.3389/fmicb.2023.1238482
11. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical microbiology reviews*. 2002 Apr;15(2):167-93. https://doi.org/10.1128/cmr.15.2.167-193.2002
12. Ochońska D, Ścibik Ł, Brzychczy-Włoch M. Biofilm formation of clinical *Klebsiella pneumoniae* strains isolated from tracheostomy tubes and their association with antimicrobial resistance, virulence and genetic diversity. *Pathogens*. 2021 Oct 18;10(10):1345. https://doi.org/10.3390/pathogens10101345
13. Almatroudi A. Biofilm resilience: molecular mechanisms driving antibiotic resistance in clinical contexts. *Biology*. 2025 Feb 6;14(2):165. https://doi.org/10.3390/biology14020165
14. Liu X, Xu Q, Yang X, et al. Capsular polysaccharide enables *Klebsiella pneumoniae* to evade phagocytosis by blocking host-bacteria interactions. *MBio*. 2025 Mar 12;16(3):e03838-24. https://doi.org/10.1128/mbio.03838-24
15. World Health Organization. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world: *Haemophilus influenzae*, *Neisseria meningitidis, Streptococcus pneumoniae, Neisseria gonorrhoea, Salmonella serotype Typhi, Shigella*, and *Vibrio cholerae*. *World Health Organization*; 2003.
16. Eigner U, Schmid A, Wild U, et al. Analysis of the comparative workflow and performance characteristics of the VITEK 2 and Phoenix systems. *Journal of Clinical Microbiology*. 2005 Aug;43(8):3829-34. https://doi.org/10.1128/jcm.43.8.3829-3834.2005
17. CLSI M100 (Clinical and Laboratory Standards Institute): Specifically, recent editions such as M100-Ed34 (for 2024). This guide is the world's premier reference for identifying bacterial breakpoints, including those in the Enterobacterales family, to which *Klebsiella pneumoniae* belongs.
18. Unubol N, Ayaş M, Uyar NY, et al. Inconsistent Findings Between Crystal Violet and Congo Red Methods on Biofilms with Comparative Sugar Supplementation. *Microorganisms*. 2025 Dec 21;14(1):21. https://doi.org/10.3390/microorganisms14010021
19. Seifi K, Kazemian H, Heidari H, et al. Evaluation of biofilm formation among *Klebsiella pneumoniae* isolates and molecular characterization by ERIC-PCR. *Jundishapur journal of microbiology*. 2016 Jan 2;9(1):e30682. https://doi.org/10.5812/jjm.30682
20. Stepanović S, Vuković D, Hola V, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by *staphylococci. Apmis*. 2007 Aug;115(8):891-9. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x
21. Soni S, Salhotra A, Suar M, editors. Handbook of research on diverse applications of nanotechnology in biomedicine, chemistry, and engineering. *IGI Global*; 2014 Aug 31.
22. Guerra ME, Destro G, Vieira B, et al. *Klebsiella pneumoniae* biofilms and their role in disease pathogenesis. *Frontiers in cellular and infection microbiology*. 2022 May 11;12:877995. https://doi.org/10.3389/fcimb.2022.877995
23. Trautner BW, Cortés-Penfield NW, Gupta K, Hirsch EB, et al. Clinical practice guideline by Infectious Diseases Society of America (IDSA): 2025 guideline on management and

AIMCJ. (2026),3; (2): 91-102
doi.org/10.58564/AIMCJ3.2.2026.291

treatment of complicated urinary tract infections: selection of antibiotic therapy for complicated UTI. *Clinical Infectious Diseases*. 2025 Dec 19:ciaf460. https://doi.org/10.1093/cid/ciaf460

24. Ayoub NS, Abdallah K, AL-Khazraji SK. Bacterial profile of urinary tract infections in Diabetic postmenopausal women. *Journal of the Faculty of Medicine Baghdad*. 2014 Apr 1;56(1):79-84. https://doi.org/10.32007/jfacmedbagdad.561435
25. Nayak DS, Das R, Sahoo SK, et al. ARGai 1.0: A GAN augmented in silico approach for identifying resistant genes and strains in E. coli using vision transformer. *Computational Biology and Chemistry*. 2025 Apr 1;115:108342. https://doi.org/10.1016/j.compbiolchem.2025.108342
26. Barman S, Kurnaz LB, Leighton R, et al. Intrinsic antimicrobial resistance: molecular biomaterials to combat microbial biofilms and bacterial persisters. *Biomaterials.* 2024 Dec 1;311:122690. https://doi.org/10.1016/j.biomaterials.2024.122690
27. Odewale G, Makanjuola OB, Ojedele RO, et al. Characterization of *Klebsiella pneumoniae* Virulence and Biofilm Formation Patterns in Southwestern Nigeria. *Frontiers in Bioscience-Elite*. 2025 Sep 26;17(3):37263. https://doi.org/10.31083/FBE37263
28. Karimi K, Zarei O, Sedighi P, et al. Research Article Investigation of Antibiotic Resistance and Biofilm Formation in Clinical Isolates of *Klebsiella pneumoniae*. https://doi.org/10.1155/2021/5573388
29. Kadhim RA, Jameel SK. Detection the phenotypic of biofilm formation and its correlation with antibiotic resistant among uropathogenic bacteria isolated from Iraqi children. *Ginekologia i Położnictwo*. 2024;1(68):1–4
30. Guerra M, Destro G, Cezar R, et al. Capsule Regulation Shapes *Klebsiella pneumoniae* Pathogenesis by Balancing Adhesion, Biofilm Formation, and Intracellular Survival. *International Journal of Molecular Sciences*. 2026 Feb 25;27(5). https://doi.org/10.3390/ijms27052169
31. Nirwati H, Sinanjung K, Fahrunissa F, et al. Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. *InBMC proceedings* 2019 Dec 16 (Vol. 13, No. Suppl 11, p. 20). London: BioMed Central. https://doi.org/10.1186/s12919-019-0176-7
32. Nazari M, Hemmati J, Asghari B. 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;2024(1):1403019. https://doi.org/10.1155/cjid/1403019
33. Gai Z, Samodelov SL, Kullak-Ublick GA, et al. Molecular mechanisms of colistin-induced nephrotoxicity. *Molecules*. 2019 Feb 12;24(3):653. https://doi.org/10.3390/molecules24030653
34. El-Sayed Ahmed MA, Zhong LL, Shen C, et al. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). *Emerging microbes & infections*. 2020 Jan 1;9(1):868-85. https://doi.org/10.1080/22221751.2020.1754133