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Investigation of the Resistance of Klebsiella Bacteria to Antibiotics and the Synthesis of Virulence Factors Isolated from Various Pathogenic Infections

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Abstract

The purpose of the study is to establish the level of antibiotic resistance in Klebsiella pneumoniae and to evaluate the production of virulence factors of the isolates originating from patients with diverse infections in Baghdad. In this study,75 clinical samples, including 32 samples of wounds, fifteen burn samples, 17 sputum samples and eleven samples of urine were collected from patients afflicted with different infections from some hospitals in Baghdad city, during the period from October to March 2023. It was found that 22 of the isolates were of Klebsiella pneumonia isolates after culturing the clinical samples on MacConkey agar, blood agar, and Eosin methylene blue medium, in addition to studying the morphologic appearance, biochemical tests, and confirmation of diagnosis using the VITEKA2 system. The antibiotic sensitivity test showed that all isolates showed (100%) multi-drug resistance to 7 antibiotics including Cefotaxime, Ampicillin, Chloramphenicol, Gentamicin, Rifampicin, while most of the isolates were sensitive to Azithromycin. The investigation of certain virulence factors of the Klebsiella pneumonia bacteria revealed that all of the isolates were encased in a capsule and were incapable of producing the hemolysin enzyme, but they were all able to produce the urease enzyme and form biofilm. The isolates produced bacteriocin at a rate of 12%.

Introduction

Klebsiella pneumoniae is a prominent member of the Enterobacteriaceae family, characterized by its Gram-negative, rod-shaped structure and facultatively anaerobic nature. The bacterium's dimensions typically range from 0.6 to 6 micrometers in length and 0.3 to 1 micrometer in width (Abbas et al., 2023). *Klebsiella pneumoniae* is non-motile, non-hemolytic, and does not produce hydrogen sulfide gas. Notably, it is encapsulated by a thick polysaccharide layer that confers significant protection against the host's immune system, particularly by resisting phagocytosis. When cultured on MacConkey agar, *Klebsiella pneumoniae* forms pink, mucoid, and smooth colonies, which is indicative of its ability to ferment lactose, sucrose, and glucose. These characteristics are not just taxonomic markers but are also directly related to the bacterium's role as an opportunistic pathogen. It is particularly adept at causing hospital-acquired infections such as respiratory tract infections, urinary tract infections, and infections in surgical wounds, making it a significant concern in healthcare settings (Puljko et al., 2024).

The pathogenic potential of *Klebsiella pneumoniae* is largely driven by its arsenal of virulence factors. These include the production of capsular polysaccharides, which enhance its ability to evade the host immune response, and fimbrial adhesins, which facilitate adherence to host tissues. Additionally, *Klebsiella pneumoniae* produces endotoxins like lipopolysaccharides, which can trigger severe inflammatory responses in the host (Huang et al., 2024). Of particular concern is the bacterium's ability to resist a wide range of antibiotics, a trait that has been

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increasingly observed in clinical isolates worldwide. This resistance is often mediated by the production of extended-spectrum beta-lactamases (ESBLs), enzymes that degrade beta-lactam antibiotics, rendering them ineffective. As a result, *Klebsiella pneumoniae* has become resistant to many advanced cephalosporins and other critical antibiotics, which are typically used as last-resort treatments for severe infections. The emergence of these multidrug-resistant (MDR) strains represents a significant challenge to public health, as they are associated with higher rates of morbidity and mortality, particularly in vulnerable populations such as hospitalized patients (Malik et al., 2024).

The global spread of *Klebsiella pneumoniae* infections, particularly those caused by MDR strains, has heightened concerns across the medical community. These strains, often referred to as "superbugs," are capable of withstanding nearly all available antibiotics, making infections exceedingly difficult to treat. Such strains have been identified in multiple regions, including India, Pakistan, the United Kingdom, the United States, Canada, and Japan. The rapid dissemination of these resistant strains is often linked to international travel and the global movement of patients, healthcare workers, and medical equipment, which facilitates the cross-border transmission of these pathogens (Morgado et al., 2024). The genetic mechanisms underlying this resistance are frequently associated with extrachromosomal elements such as plasmids. These plasmids carry resistance genes and can be transferred between bacterial cells via horizontal gene transfer mechanisms like conjugation. This ability to share genetic material between bacteria not only accelerates the spread of resistance within a population but also complicates efforts to control these infections (Zhao et al., 2024).

This study aims to address the pressing need for a deeper understanding of *Klebsiella pneumoniae* by isolating and identifying strains from patients with various infections in Baghdad, a region where antibiotic resistance is increasingly problematic. By conducting a thorough investigation of the antibiotic resistance profiles and virulence factors of these isolates, the study seeks to provide critical insights into the mechanisms that underlie the pathogen's ability to persist in the human host and resist treatment. This research is particularly timely given the rising incidence of MDR *Klebsiella pneumoniae* infections globally. Understanding the specific resistance patterns in local contexts, such as Baghdad, is essential for developing targeted and effective treatment strategies. Moreover, this study aims to inform the development of enhanced infection control measures that could help curb the spread of these dangerous pathogens within healthcare settings. Ultimately, the findings of this research have the potential to contribute significantly to the global effort to combat antibiotic-resistant infections and improve patient outcomes.

Methods

The study involved collecting 75 clinical samples from patients with various infections at hospitals and health centers in Baghdad, Iraq, between May and November 2022. These samples comprised 32 wound swabs, 15 burn swabs, 17 sputum samples, and 11 urine samples. To ensure sample integrity, sterile techniques were employed during collection, with immediate transportation to the laboratory. For example, sputum samples were obtained using sterile, marked cotton swabs, while urine samples were collected in sterile containers.

Bacterial Isolation and Identification

Upon arrival at the laboratory, the collected samples were cultured on blood agar, MacConkey agar, and nutrient agar using the streak plate method. This method was performed under sterile conditions near a flame to minimize contamination. The plates were incubated at 37°C for 24 hours, allowing bacterial colonies to develop. Post-incubation, the colonies were examined for

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

morphological characteristics such as color, shape, and texture. The colonies were further subjected to Gram staining for microscopic examination, helping differentiate Gram-positive from Gram-negative bacteria based on cell wall properties. To accurately identify the bacterial species, a series of biochemical tests were performed, including the Gram stain, motility test, oxidase test, catalase test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, hydrogen sulfide production test, urease test, gelatin liquefaction test, lactose fermentation test, and growth in KCN media. Each test contributed to building a comprehensive biochemical profile of the isolates, aiding in their accurate identification. To further validate the identification, the IMVIC test was used, along with advanced diagnostic systems like the Vitek-2 and API 20E systems, known for their rapid and reliable confirmation of biochemical results.

Antibiotic Resistance Testing

The antibiotic resistance of the Klebsiella pneumoniae isolates was assessed using the Kirby-Bauer disk diffusion method. This method is a standard approach for evaluating the effectiveness of antibiotics against bacteria. The study tested the isolates' sensitivity to seven antibiotics: Ampicillin, Chloramphenicol, Gentamicin, Rifampicin, Ciprofloxacin, Cefotaxime, and Azithromycin. A bacterial suspension was prepared to match the 0.5 McFarland standard, which was then spread uniformly on Mueller-Hinton agar plates. Antibiotic disks were placed on the agar, and the plates were incubated at 37°C for 24 hours. The results were determined by measuring the inhibition zones around the disks and comparing them to standard charts, classifying the isolates as resistant, intermediate, or sensitive to each antibiotic.

Detection of Virulence Factors

The study also investigated several virulence factors produced by Klebsiella pneumoniae. The presence of a capsule, a known virulence factor, was detected using the negative staining method with negrocin dye, which made the capsule visible as a clear halo around the bacterial cell under a microscope. Hemolysin production was tested by culturing the isolates on blood agar plates; the presence of a clear hemolysis zone indicated positive hemolysin production. Urease production was evaluated by inoculating the bacteria into urea broth and incubating it to observe any color change, which would indicate the enzyme's activity.Bacteriocin production, another virulence factor, was assessed using the cup-disc method. The isolates were cultured in brain-heart infusion broth and then spread onto TSA agar plates, where bacteriocin production was indicated by the inhibition zones around the discs. Finally, biofilm formation, which contributes to the bacteria's resistance to antibiotics and environmental stress, was measured using the 96-well microtiter plate method. This involved staining the adherent bacteria with crystal violet and measuring the absorbance at 570 nm to quantify biofilm production.

Results and Discussion

In this study, 75 bacterial samples were collected from patients of both sexes and different ages who suffered from different infections from some hospitals in Baghdad city. The microscopic examination revealed that 22 isolates were rod-shaped and capsulated, appeared in single or in pairs or in short chains. The cultural characteristics and biochemical tests were used to distinguish these bacteria from other species.

The biochemical tests were conducted on local isolates belonging to the genus *Klebsiella spp*. The *K. pneumonia* was identified based on its cultural and microscopic characteristics as well as the biochemical tests as shown in table (1) and figure (1).

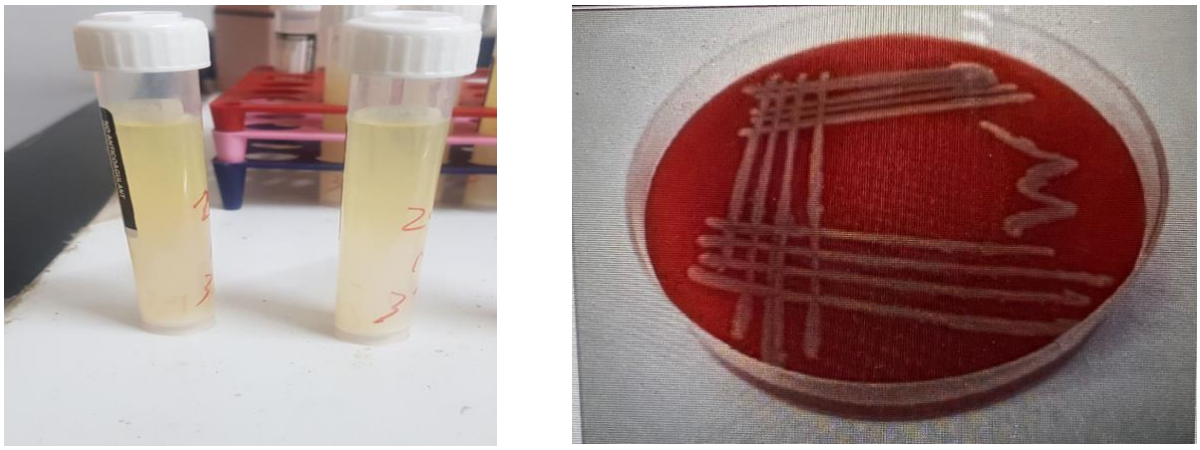
ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

To confirm the diagnosis of the isolates, the IMVIC test was performed, and the results were negative for indole test, negative for methyl red, but positive for both the voges proskauer and citrate utilization tests. Based on the results of microscopic, cultural, and biochemical tests, and according to (Kumar et al., 2011; Sharmeen et al., 2012), the *K. pneumoniae* isolates are of the positive type, and to confirm the diagnosis, the 200 API diagnostic kit was used, which is characterized by its easy and rapid confirmation of the results of biochemical examination and the Vitek system.

Table 1. Diagnostics for bacteria using culture, biochemistry, and microscopy

Test	K.pneumoniae
Gram stain.	-
Motility test	-
Oxidase test	-
Catalase test	+
Indol production test	-
Methyl red test	-
Voges proskauer test	+
Citrate utilization test	+
H_25 production test	-
Urea hydrolysis (urease test)	+
Gelatin laquification test	-
Lactose fermentation test	+
Growth in KCN media	+

Table 1 presents the results of various diagnostic tests used to identify *Klebsiella pneumoniae* based on its cultural, biochemical, and microscopic characteristics. The table shows that *Klebsiella pneumoniae* tested positive for several key biochemical tests, including catalase, Voges-Proskauer, citrate utilization, urease, and lactose fermentation, while it was negative for others, such as Gram stain, motility, oxidase, indole, and hydrogen sulfide production. These results provide a comprehensive diagnostic profile that confirms the identity of the isolates as *Klebsiella pneumoniae* and supports the accuracy of the identification process.



A B

Figure 1. K.pneumoniae bacteria colonies A- on nutrient broth medium B- on blood agar medium

Figure 1 illustrates the morphological characteristics of *Klebsiella pneumoniae* colonies grown on different culture media. In Panel A, the colonies on blood agar appear large, mucoid, and

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

dome-shaped, which is indicative of the presence of a polysaccharide capsule—a key virulence factor that enhances the bacteria's ability to evade host immune responses. Panel B shows the bacteria grown in nutrient broth, where the uniform turbidity suggests a high concentration of bacterial cells, indicating that *Klebsiella pneumoniae* is well-adapted to the nutrient conditions provided by the broth. These visual observations are critical in the preliminary identification of *Klebsiella pneumoniae* and highlight the bacterium's versatility in thriving under various environmental conditions.

Distribution of the isolates according to the infection site

The 75 isolates were classified according to the sites of infection after their identification. The highest rate of positive isolates was in urine samples (45.4%), while the lowest rate was in wound samples (9.3%). The results showed that the number of isolates of *K. pneumoniae* in urine samples were 5 (45.4%) isolates, and these results were consistent with the findings of (Sharma et al., 2016), who found that the rate of *K. pneumoniae* in urine samples was (40%).

In one study, it was reported that the burn swab isolate rate was (20%), which was similar to the findings of a study performed by (Aljanaby et al., 2016). The rate of *K. pneumoniae* reached 28%, and this bacterium is considered the second largest cause of burn infections after *Pseudomonas* referred to in that study. In the sputum samples, the rate of *K. pneumoniae* isolates in the study was (23.5%), which is close to the finding of (Mohamed et al.,2023), as the highest rate of *K. pneumoniae* (22%) was detected in sputum samples.

The results of wound samples recorded (9.3%), which is consistent with the findings of (Pattolath et al., 2024), who found that the rate of *K. pneumoniae* in wounds was (9%). In a study conducted by (Aiza et al.,2023), on isolates of the Enterobacteriaceae family, *K. pneumoniae* isolates were shown to be dominant in wound samples (24%).

Table 2. Number and type of isolates according to site of infection

Isolation type	Number of isolates	Positive isolates	% percentage
Urine	11	5	45.4
Burns	15	3	20
sputum	17	4	23.5
Wounds	32	3	9.3
the total	75	15	98.2

Table 2 categorizes the *Klebsiella pneumoniae* isolates based on their infection sites, revealing that the highest number of positive isolates was found in urine samples (45.4%), followed by sputum (23.5%), burns (20%), and wounds (9.3%). This distribution indicates that *Klebsiella pneumoniae* is particularly prevalent in urinary tract infections but also plays a significant role in respiratory infections, burn wound infections, and wound infections. The data suggest that *Klebsiella pneumoniae* has a strong affinity for various tissue types, contributing to its versatility as a pathogen.

Bacterial resistance to antibiotics

The resistance of isolates in this study was determined against 7 different antibiotics, and it was based on measuring the diameter of the inhibition zone and comparing it with what was stated in NCCLs, 2023. A group of beta-lactam antibiotics, aminoglycoside antibiotics, and quinolone antibiotics were used in addition to other antibiotics as shown in table (3).

All the isolates in our study showed complete (100%) resistance to the antibiotics Ampicillin and Chloramphenicol, and these results were in agreement with (Younus et al., 2024) as their

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

isolates also showed (100%) resistance to the two mentioned antibiotics, and this result is also consistent with the findings of (Lubwama et al., 2024) whose isolates of K. pneumoniae showed (100%) resistance.

The isolates in the present study showed high resistance to the antibiotic Gentamicin, which is to the findings of (odríguez et al., 2024), who reported a resistance rate of 94%, but this result was different from those of (Zaidan, 2021), who recorded a resistance rate of 81%. The antibiotic rate indicates that the bacteria possess a resistance plasmid that increases the resistance properties of the bacteria to Gentamicin, and the resistance properties can be transferred from resistant strains to sensitive strains to become resistant to one or more antibiotics (odríguez et al., 2024) All *Klebsiella pneumoniae* isolates showed complete (100%) resistance to the antibiotic Rifampicin, and this result is consistent with the findings of (Tuncer et al., 2024) who also found 100% resistance. This resistance is attributed to the RNA polymerase enzyme as a result of a chromosomal mutation that changed its genetic makeup, thus it prevents the binding of the antibiotic and does not inhibit the action of the enzyme (Chirabhundhu et al., 2024).

Klebsiella pneumonia isolates showed a complete (100%) resistance to the antibiotic Ciprofloxacin, a result which is not in agreement with that of (Younus et al., 2024). who found (72.3%) resistance to Ciprofloxacin. This antibiotic interferes with the enzymes responsible for DNA replication, as it stops the protein synthesis process (Sagheer et al., 2024). All isolates showed highly resistance (100%) to the antibiotic Cefotaxime, and this result is close to what was revealed by (Tetteh et al., 2024) who found that the resistance rate to the antibiotic Cefotaxime was (95.4%), while differs from the findings of (Ibrahim *et al*., 2024) who detected only (81.8%) resistance. The antibiotic Cefotaxime has the ability to penetrate the outer membrane of Gram-negative bacteria (Zhang et al., 2024).

In addition, *Klebsiella pneumoniae* isolates showed marked variation against the antibiotic Azithromycin, which showed (49.3%) sensitivity, and this result is not consistent with (Khairani et al., 2024) who recorded a sensitivity rate of (8.3%).

Table 3. Percentages of sensitivity and resistance of isolates to antibiotics

Antibiotic	Isolate Resistance	%
Ampicillin	75	100
Chloramphenicol	75	100
Gentamicin	75	100
Rifampicin	75	100
Ciprofloxacin	75	100
Cefotaxime	75	100
Azithromycin	37	49.3

Table 3 outlines the antibiotic resistance profiles of *Klebsiella pneumoniae* isolates, showing that all isolates were completely resistant (100%) to multiple antibiotics, including Ampicillin, Chloramphenicol, Gentamicin, Rifampicin, Ciprofloxacin, and Cefotaxime. Interestingly, 49.3% of the isolates were sensitive to Azithromycin. These findings underscore the multi-drug resistance (MDR) of *Klebsiella pneumoniae*, which poses a significant challenge for treatment, particularly in clinical settings where these antibiotics are commonly used. The high level of resistance observed in this study highlights the urgent need for alternative therapeutic strategies.

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

The current study included investigation of a number of virulence factors associated Because *K. pneumoniae* is pathogenic. The microscopic examination and using the negrocin stain were used to detect the presence of capsules in the bacterial isolates in this study, as the cells as bacilli surrounded by a halo, and laboratory examination showed that all isolates contained the capsule.

It was observed by the researcher (AL-Busaidi et al., 2024) that the K1 serotype found in the K. pneumoniae capsule is responsible for (14-23.4%) hospital-acquired infections. He also noted that the K1 serotype confers a survival characteristic to the bacteria and is an important marker for the occurrence of (63.4%) of liver abscesses caused by K. pneumoniae. Firstly, capsule production is a well-known virulence factor in K. pneumoniae. Capsules are outer layers of polysaccharides that surround bacterial cells, providing protection against host immune responses and facilitating bacterial colonization and survival in various host environments. In the case of K. pneumoniae, the presence of capsules has been associated with increased virulence and the ability to cause severe infections such as pneumonia, urinary tract infections (UTIs), and bloodstream infections. Therefore, in the context of the study, confirming the presence of capsules in all K. pneumoniae isolates underscores their potential to cause serious diseases and highlights the importance of understanding capsule-associated pathogenicity mechanisms.

Hemolysin production

The ability of the isolates in our study to produce hemolysin was investigated, and the outcomes demonstrated that none of the *K. pneumoniae* isolates could produce hemolysin on blood agar. Hemolysin is an important virulence factor for a wide range of gram-negative and gram-positive bacteria and contributes to their pathogenicity. The inability of these bacteria to produce hemolysin is attributed to several possibilities, such as that the effectiveness of hemolysin is controlled by an operon consisting of 4 genes, and these genes share together the expression of hemolysin production, and there may be one gene among them that is ineffective in expressing this production, which consequently leads to the inability of the bacteria to produce hemolysin (Chakkour et al., 2024). The study carried out by (Zhang et al., 2023) proved that a mutation in the hns gene in the *K. pneumoniae* strain exhibited the ability of the bacteria to show the effectiveness of hemolysin production.

Urease production

After urease production capacity was examined, it was discovered that all K. pneumoniae isolates were capable of producing this enzyme. This enzyme's capacity to hydrolyze urea into ammonia (4NH) and carbonic acid (H2CO3) highlights its significance. which is a nickel-containing crystalline enzyme that causes kidney stones, pyelonephritis, ammonia-induced encephalopathy, and liver coma. It also serves as a virulence factor for numerous pathogenic microorganisms (Fitzgerald etal., 2024).

The study of (Deutch, 2024) confirmed that the urease enzyme formation by *K. pneumoniae* is responsibility of an operon located on a chromosome, and that the formation of this enzyme is about 75 times higher than its abundance under nitrogen-limiting conditions. Accordingly, the expression of urease enzyme production efficiency is increased by the limited availability of nitrogen. Similarly, the detection of urease enzyme production by all K. pneumoniae isolates is significant due to its role in pathogenesis. Urease is an enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide. In the context of K. pneumoniae infections, urease production can lead to the alkalization of the urinary tract, which can contribute to the formation of kidney stones and exacerbate urinary tract infections. Moreover, ammonia

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

produced by urease activity can cause tissue damage and inflammation, further enhancing the pathogenic potential of K. pneumoniae.

Bacteriocin production

The results of bacteriocin-producing bacterial isolates showed that 9 (12%) isolates produced bacteriocins, as seen in tables (4,5) and figure (2), and the isolates were from multiple sources. These results were close to a study conducted by (Alattar et al., 2024) who also found that the bacteriocin production rate by *Klebsiella* was 12%, and these results do not mean that other bacterial isolates do not produce bacteriocin as (Gu et al., 2024) indicated that a modification of the colicin receptors occurred as a result of mutations that lead to the formation of resistant isolates.

It was indicated by (Gu, Q., et al.2024) that the presence of Cidrophorase in the medium inhibits the action of colicin by competing with it to bind to receptors on the surface of the sensitive cell, thus preventing it from showing its effectiveness. In the current study, TSA solid medium was used with the addition of 1% yeast, because the study of (Sunaryanto et al., 2024) indicated that adding yeast extract increases the production of bacteriocins.

The study performed by (Miller et al., 2024) reported that the solid medium is the best medium for bacteriocin production, due to the presence of bacterial cell receptors in the solid medium and their absence in the liquid medium. Bacteriocins are not direct virulence factors, but rather support the growth and reproduction of bacteria in the environments in which they are found, such as the intestines (Mfon et al., 2024).

By linking these virulence factors to disease severity, researchers can gain insights into the mechanisms underlying K. pneumoniae infections. For example, understanding how capsule production and urease activity contribute to the ability of K. pneumoniae to establish infections in specific host niches, evade immune responses, and cause tissue damage can inform the development of targeted therapies and interventions. Additionally, correlating virulence factor expression with clinical outcomes, such as disease progression, treatment response, and patient outcomes, can help identify biomarkers for disease severity and guide personalized treatment strategies.

Figure 2. Test to detect bacteriocin production by K.pneumoniae bacteria

Figure 2 demonstrates the results of the bacteriocin production test, which shows zones of inhibition around discs placed on TSA agar. The presence of these inhibition zones indicates that some *Klebsiella pneumoniae* isolates produce bacteriocins, which are antimicrobial peptides that inhibit the growth of closely related bacterial species. The varying sizes of these

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

inhibition zones suggest differences in the potency or concentration of bacteriocins produced by different isolates. This ability to produce bacteriocins provides *Klebsiella pneumoniae* with a competitive advantage in microbial communities, allowing it to dominate other species in the same environment.

Table 4. Ability of K.pneumoniae isolates to produce bacteriocins

Isolation number	Source of isolation	Inhibition zone/mm
1	Burns	8
3	Urine	25
5	Sputum	18
8	Burns	20
9	Urine	20
11	Urine	18
13	Wounds	-
14	Sputum	25
16	Burns	20
20	Wounds	-
22	Urine	25
23	Sputum	18
26	Urine	20
28	Wounds	-
31	Urine	-
34	Sputum	25
45	Wounds	20
55	Wounds	-
67	Wounds	-
70	Urine	-
72	Burns	25

Table 4 provides data on the bacteriocin production capabilities of *Klebsiella pneumoniae* isolates, showing the diameter of inhibition zones in millimeters. The table reveals that bacteriocin production varies among isolates, with inhibition zones ranging from 8 mm to 25 mm, indicating differences in the antimicrobial potency of the bacteriocins produced. This variability suggests that while some *Klebsiella pneumoniae* isolates are potent producers of bacteriocins, others are not, reflecting diversity in their antimicrobial capabilities and their ability to compete with other microbial species in the host environment.

Biofilm production

The results in the current study showed that all isolates (100%) had the ability to produce biofilms. The ability of *Klebsiella* to form biofilm was detected using the 96-well microtiter plate method. The results showed that all *Klebsiella* isolates were biofilm producers.

As shown in table (6) and figure (3), the biofilm production rate was consistent with (Pham et al., 2024), who revealed that *K. pneumoniae* produced a lot of biofilms (97.3%), but disagreed with the results of (Araújo etal.,2024), who found that the isolates produced only (52%) biofilm in large quantities. Also, this result was relatively close to what was found by (Abdulla et al., 2024) using the Congo red method, as their biofilm production rate was (83%). The Congo red method is one of the preferred methods for detecting bacterial production of the sticky layer, which is responsible for staining the polysaccharide layer which is considered as the basic

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

material of the biofilm. There are many environmental factors that may affect bacterial production of the mucous layer by using this method such as oxygen, temperature, and other conditions that can give different results (Rojas et al., 2023).

Bacteria that grow in biofilms are tolerant to many antibiotics and resistant to opsonization and phagocytosis and to various environmental conditions and also resistant to selective pressures (Datta et al., 2024).

The production of biofilm by *K. pneumoniae* in urinary tract devices is strong, and the microorganisms that form biofilm are approximately 1,000 times more resistant to antibiotics than those that do not. Also, the biofilm-producing bacteria are responsible for infections and diseases that are difficult to treat due to the difficulty and restriction of antibiotic penetration into the biofilm (de Sousa et al., 2024).

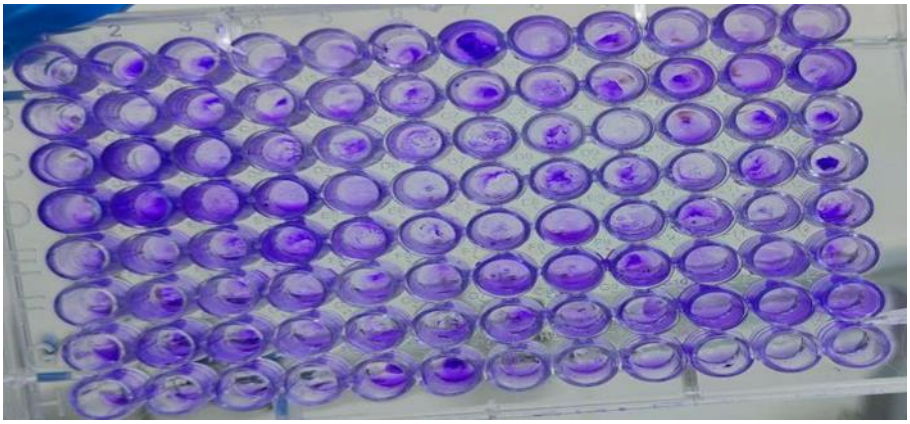


Figure 3. Test to detect biofilm production of Klebsiella bacteria

Figure 3 highlights the biofilm production capabilities of *Klebsiella pneumoniae* isolates using the 96-well microtiter plate method. The figure shows wells stained with crystal violet, where the intensity of the staining correlates with the level of biofilm production. Darker wells indicate higher levels of biofilm formation, which is quantified by measuring absorbance at 570 nm. The ability to form biofilms is a significant virulence factor for *Klebsiella pneumoniae*, as it protects the bacterial community from antibiotics and the host immune system, making infections particularly persistent and difficult to treat.

Table 6. Virulence factors found in the isolates under study

Isolation Number	Present Capsule	Hemolysin Production	Production Bacteriocin	Biofilm Production	Urease Production
7	+	-	+	+	+
9	+	-	+	+	+
11	+	-	-	+	+
13	+	-	-	+	+
17	+	-	-	+	+
21	+	-	+	+	+
23	+	-	-	+	+
34	+	-	-	+	+
37	+	-	+	+	+
39	+	-	-	+	+
41	+	-	-	+	+
43	+	-	+	+	+
49	+	-	+	+	+

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

51	+	-	-	+	+
53	+	-	-	+	+
57	+	-	-	+	+
59	+	-	+	+	+
61	+	-	-	+	+
63	+	-	+	+	+
67	+	-	-	+	+
71	+	-	-	+	+
75	+	-	+	+	+

Table 6 summarizes the detection of various virulence factors in *Klebsiella pneumoniae* isolates, including capsule formation, urease production, biofilm formation, bacteriocin production, and hemolysin production. The table shows that all isolates tested positive for capsule formation, urease production, and biofilm production, while a subset (12%) produced bacteriocins. However, none of the isolates produced hemolysin. This comprehensive overview of virulence factors underscores the pathogenic potential of *Klebsiella pneumoniae*, highlighting its ability to persist in the host environment and cause severe infections through multiple virulence mechanisms.

The findings of this study provide a comprehensive analysis of the antibiotic resistance and virulence factors exhibited by *Klebsiella pneumoniae* isolates collected from various clinical samples in Baghdad. The high prevalence of antibiotic resistance among these isolates, particularly to commonly used antibiotics such as Ampicillin, Chloramphenicol, Gentamicin, and Ciprofloxacin, underscores the growing challenge of treating infections caused by *Klebsiella pneumoniae*. This resistance is not only alarming but also indicative of the bacteria's adaptive mechanisms, which may be driven by both genetic mutations and the acquisition of resistance genes through horizontal gene transfer.

One of the most striking results of this study is the complete resistance observed across all isolates to multiple antibiotics. This multi-drug resistance (MDR) aligns with global trends, where *Klebsiella pneumoniae* has increasingly been recognized as a formidable pathogen in healthcare settings, particularly in causing nosocomial infections (Lowe et al., 2023). The resistance to Cefotaxime, a third-generation cephalosporin, is particularly concerning, as this antibiotic is often used as a treatment of last resort for severe bacterial infections. The ability of *Klebsiella pneumoniae* to resist such potent antibiotics suggests the presence of extended-spectrum beta-lactamases (ESBLs) or other resistance mechanisms that degrade the efficacy of these drugs (Rodriguez-Medina et al., 2024).

The resistance to Ciprofloxacin, a fluoroquinolone, is another critical finding, as it highlights the potential for *Klebsiella pneumoniae* to evade treatment even with broad-spectrum antibiotics. The mechanism of resistance in this context likely involves mutations in the bacterial DNA gyrase or topoisomerase IV, enzymes targeted by fluoroquinolones, as well as the presence of plasmid-mediated resistance genes (Zhang et al., 2024). The high level of resistance observed in this study necessitates a reconsideration of current antibiotic use policies and the implementation of stringent antibiotic stewardship programs to mitigate the spread of resistance.

The study also reveals significant insights into the virulence factors of *Klebsiella pneumoniae*. The universal presence of a capsule in all isolates is consistent with the pathogen's known virulence, as the capsule serves as a protective barrier against phagocytosis and contributes to the bacterium's ability to evade the host immune response (Gu et al., 2024). The production of biofilms by all isolates further enhances this virulence, as biofilms protect the bacteria from

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

both the host's immune system and antibiotic treatment, making infections particularly difficult to eradicate (Datta et al., 2024). The formation of biofilms on medical devices, such as catheters and ventilators, is a well-documented risk factor for persistent infections, and this study's findings reinforce the need for novel therapeutic strategies to disrupt biofilm formation.

Interestingly, while the study detected urease production in all isolates, hemolysin production was absent. The presence of urease is significant because it plays a role in the pathogenesis of urinary tract infections, by hydrolyzing urea to produce ammonia, which can damage host tissues and contribute to the formation of kidney stones (Fitzgerald et al., 2024). The absence of hemolysin production, however, suggests that not all virulence factors are uniformly expressed across *Klebsiella pneumoniae* strains, which may reflect the pathogen's ability to adapt to different host environments or the selective pressures of specific infections.

Moreover, the production of bacteriocins by a subset of isolates highlights the complex interactions between *Klebsiella pneumoniae* and other microbial species within the host. Bacteriocins can provide a competitive advantage by inhibiting the growth of closely related species, thus facilitating colonization and infection (Miller et al., 2024). This finding suggests that *Klebsiella pneumoniae* may not only be equipped to resist antibiotics but also to dominate microbial communities within the host, further complicating treatment strategies.

In light of these findings, it is evident that *Klebsiella pneumoniae* represents a significant public health challenge, particularly in the context of its MDR and virulence capabilities. The study's results underscore the urgency of developing new antimicrobial agents and alternative therapeutic approaches, such as bacteriophage therapy, to combat infections caused by this pathogen. Additionally, the implementation of robust infection control measures and continuous surveillance of antibiotic resistance patterns are critical to preventing the further spread of MDR *Klebsiella pneumoniae*.

Future research should focus on elucidating the genetic basis of the observed resistance and virulence traits, particularly through whole-genome sequencing and transcriptomic analysis. Such studies could provide deeper insights into the molecular mechanisms driving resistance and virulence in *Klebsiella pneumoniae*, potentially leading to the identification of novel targets for therapeutic intervention. Furthermore, exploring the environmental reservoirs of *Klebsiella pneumoniae* and its transmission dynamics within healthcare settings could inform more effective strategies for controlling outbreaks and reducing the burden of infections caused by this formidable pathogen.

Conclusion

This study provides a critical examination of the antibiotic resistance and virulence factors exhibited by Klebsiella pneumoniae isolates from various clinical samples collected in Baghdad. The alarming prevalence of multi-drug resistant (MDR) strains highlights the pressing challenges faced in treating infections caused by this pathogen. The isolates demonstrated complete resistance to several commonly used antibiotics, including Cefotaxime and Ciprofloxacin, underscoring the urgent need for alternative therapeutic approaches and the revision of current antibiotic use policies. This resistance pattern suggests the widespread presence of extended-spectrum beta-lactamases (ESBLs) and other resistance mechanisms within the local Klebsiella pneumoniae population. The study also reveals that Klebsiella pneumoniae possesses several virulence factors that significantly contribute to its pathogenicity. The universal presence of a capsule and the ability of all isolates to form biofilms are particularly concerning, as these factors enhance the bacteria's ability to evade the host immune system and resist antibiotic treatment. The production of urease by all isolates further

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

complicates treatment, particularly in urinary tract infections, where urease activity can lead to tissue damage and the formation of kidney stones. Interestingly, while none of the isolates produced hemolysin, the presence of bacteriocin production in some isolates suggests that Klebsiella pneumoniae can dominate microbial communities within the host by inhibiting the growth of competing bacterial species. This capability further enhances its survival and persistence in infected environments.

In light of these findings, it is evident that Klebsiella pneumoniae represents a significant public health threat, particularly in healthcare settings where antibiotic resistance is prevalent. The study emphasizes the need for robust infection control measures, continuous surveillance of antibiotic resistance patterns, and the development of new antimicrobial agents and alternative therapies, such as bacteriophage therapy and biofilm-disrupting agents. Future research should focus on elucidating the genetic mechanisms underlying antibiotic resistance and virulence in Klebsiella pneumoniae, as well as exploring environmental reservoirs and transmission dynamics. Such research could lead to more effective strategies for preventing and treating infections caused by this formidable pathogen, ultimately improving patient outcomes and safeguarding public health.

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