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# Molecular study of pneumonia infection caused by *Klebsiella pneumoniae*from Kut City

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## Abstract

To investigate the prevalence and antibiotic resistance patterns of *Klebsiellapneumoniae* causing pneumonia in Kut City, Iraq. Sputum, bronchoalveolar lavage, and blood samples were collected from 50 patients with pneumonia. Samples were cultured on selective media, and *K. pneumoniae* was identified using PCR targeting the *16S rRNA* gene.Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method.The Resultsshowed that 12 out of 50 (24%) patients were positive for *K. pneumoniae*. A higher prevalence was observed in the city (30%) compared to the village (15%). A male-to-female ratio of 2:1 was observed among positive cases. High resistance rates were observed to multiple antibiotics, including carbapenems (100% resistance). Lower resistance was found for trimethoprim-sulfamethoxazole (83.33%). The study highlights the significant public health threat posed by multi-drug resistant *K. pneumoniae* and in the city compared to the village suggests an association with population density and environmental factors. The gender disparity in infection rates requires further investigation. The study confirms a substantial prevalence of antibiotic-resistant *K.pneumoniae* in Kut City, emphasizing the need for enhanced infection control, surveillance, and development of new therapeutic strategies to combat this growing threat. Further research is needed to clarify the observed urban-rural and gender differences in infection rates. The study recommends using whole-genome sequencing for more accurate species identification in future studies

**Keywords**: Pneumonia, Fever, Polymerase chain reaction, Iraq

## Introduction

The growing prevalence of hospital-acquired infections, especially in immunocompromised patients, has drawn attention to *Klebsiella pneumoniae* in recent years. The bacterium is becoming a greater public health concern due to its capacity to develop hypervirulent characteristics and drug resistance. Although *K. pneumoniae* is a typical component of the human gut microbiota, some strains can cause serious infections such as meningitis, pneumonia, bloodstream infections, and urinary tract infections. The rise of antibiotic-resistant strains, such as carbapenem-resistant *K. pneumoniae* (CRKP), is especially concerning because it reduces the number of available treatments. Hypervirulent*K. pneumoniae* (hvKP) strains are also a significant concern. These strains can cause severe community-acquired infections, including endophthalmitis, necrotising fasciitis, and liver abscesses, even in healthy individuals. The rapid evolution of hypervirulent and antibiotic-resistant strains is alarming [1].

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*Klebsiella pneumoniae* is a non-motile, encapsulated, Gram-negative bacterium. Its capsule contributes to its virulence and protects it from the host immune system. Unlike many bacteria, it lacks flagella, which are necessary for motility. This organism can form biofilms on medical equipment and is found in various environmental conditions, including soil and surface waters. Biofilm formation on medical devices (such as IV lines, ventilators, and catheters) can lead to hospital-acquired infections, increasing the devices' resistance to treatment and disinfection. This is particularly problematic for immunocompromised patients. Its environmental adaptability, ability to colonise medical equipment, and capacity to acquire antibiotic resistance genes have made it a major concern in hospital settings [2].

The medical and scientific community widely agrees that nosocomial infections caused by multi-drug resistant (MDR) bacteria constitute a silent pandemic. The World Health Organization (WHO) estimates that these infections may cause 10 million deaths annually by 2050 [3]. *Klebsiella pneumoniae*, a Gram-negative bacterium, is one of the most common and important pathogens in nosocomial infections globally, and is highly susceptible to antibiotic resistance (AMR). This bacterium can cause a range of infections, including liver abscesses, pneumonia, sepsis, and urinary tract infections [4]. A decade ago, *K. pneumoniae* was estimated to be responsible for approximately 2 million severe infections in healthcare settings, including 351,000 bloodstream infections [5]. Estimates suggest *K. pneumoniae* caused 790,000 deaths globally, with neonatal sepsis accounting for 214,000 of these deaths.

## Materials and Methods

### Samples

This study included 50 patients admitted to Al-Zahraa Teaching Hospital between May and August 2024 with fever and cough. Each patient provided sputum and bronchoalveolar lavage samples. 5 ml venous blood samples were collected aseptically into labelled EDTA-plastic tubes and stored frozen at -20°C until testing.

### Molecular examination

*Klebsiellapneumoniae*cultures were grown on nutrient agar in the laboratory. Samples were inoculated onto selective media supporting *K. pneumoniae* growth, including MacConkey agar and Mueller-Hinton agar. Cells were harvested after 18–24 hours. DNA extraction (method A) was performed according to the manufacturer's instructions (Intron, Korea). The extracted DNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific, USA), following the manufacturer's instructions. A 2 µl volume of chromosomal DNA was measured at 260 nm to determine the concentration (ng/µl); purity was assessed using the A260/A280 ratio. Specific primers targeting the *16S rRNA* gene (F: 5'-ATGTTCAGGAAGGATGTCGTCG-3', R: 5'-AACCGACTTTGTCCAGCACAAAC-3') were used for rapid identification and amplification from clinical samples. Antibiotic susceptibility testing of positive *K. pneumoniae* isolates was performed using the disk diffusion method (Kirby-Bauer test), a standard method for determining antibiotic susceptibility. Bacterial isolates were spread on Mueller-Hinton agar plates, antibiotic disks were applied, and after incubation, zone diameters were measured and interpreted according

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to Clinical and Laboratory Standards Institute (CLSI) criteria to determine susceptibility (sensitive, intermediate, or resistant).

**Result**

The frequency of *K. pneumoniae* positive patients varied geographically, with the greatest difference observed between the city and village (Table 1). In the city, 8 of 8 tested cases were positive for *K. pneumoniae*. In the village, 3 of 4 cases were positive. This higher prevalence in the village may be related to environmental factors, population density, or occupational exposures. Conversely, the lower apparent prevalence in the urban community may reflect differences in transmission dynamics, possibly linked to agricultural practices or other exposure sources. These findings highlight the importance of considering regional characteristics and specific geographical locations when assessing risk and implementing preventative measures for *K. pneumoniae* infections.

**Table 1:Distribution of patient numbers regarding area and positive cases**

| **Area** | **Numbers** | **Positive** | % |
|---|---|---|---|
| **City** | 30 | 9 | 30% |
| **Village** | 20 | 3 | 15% |
| **Total** | 50 | 12 | 24% |

This study included 50 individuals from Wasit province who had undergone pneumonia testing; 12 tested positive for *K. pneumoniae*. The male-to-female ratio was 2:1 (table 2). This suggests a potential gender difference in pneumonia diagnosis or incidence within this cohort. Further investigation is needed to explore possible underlying causes, including biological factors, access to healthcare, and environmental influences.

**Table 2:Distribution of patient numbers regarding gender and positive cases**

| **Variables** | **Frequency** | **Percentage %** |
|---|---|---|
| **Sex** | | |
| Male | 8 | 66.66 |
| Female | 4 | 33.33 |
| Total | 12 | 100 |

Antibiotic disks, impregnated with specific antibiotics, were placed onto the agar surface, ensuring sufficient spacing to prevent overlapping zones of inhibition. Plates were then incubated at 35–37°C for 18–24 hours, allowing the antibiotics to diffuse into the agar, creating a concentration gradient. Following incubation, zones of inhibition (areas of no bacterial growth) surrounding each disk were measured in millimetres.

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**Table 3: table of antibiotic resistance for *K.pneumoniae*(*1-12 number of samples)**

| Antibiotic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Imipenem | R | R | R | R | R | R | R | R | R | R | R | R |
| Ampicillin | R | R | R | R | R | R | R | R | R | R | R | R |
| Cefepime | R | R | R | R | R | R | R | R | R | R | R | R |
| Cefoxitin | R | R | R | R | R | R | R | R | R | R | R | R |
| Amikacin | R | R | R | R | R | R | R | R | R | R | R | R |
| Gentamicin | R | R | R | R | R | R | R | R | R | R | R | R |
| Ciprofloxacin | R | R | R | R | R | R | R | R | R | R | R | R |
| Trimethoprim-Sulfamethoxazole | R | R | R | R | R | S | R | R | R | S | R | R |
| Levofloxacin | R | R | R | R | R | R | R | R | R | R | R | R |
| Cefazolin | R | R | R | R | R | R | R | R | R | R | R | R |

**Table 4:Total of antibiotic resistance for *Klebsiella pneumoniae***

| **Antibiotic** | **Resistance Rate (%)** | **Note** |
|---|---|---|
| Imipenem | 100 | Highest resistance observed |
| Ampicillin | 100 | Highest resistance observed |
| Cefepime | 100 | Highest resistance observed |
| Cefoxitin | 100 | Highest resistance observed |
| Amikacin | 100 | Highest resistance observed |
| Gentamicin | 100 | Highest resistance observed |
| Ciprofloxacin | 100 | Highest resistance observed |
| Trimethoprim-Sulfamethoxazole | 83.33 | Least resistance among tested antibiotics |
| Levofloxacin | 100 | Highest resistance observed |
| Cefazolin | 100 | Highest resistance observed |

According to the results, 12 cases of *K. pneumoniae* were identified from 50 samples submitted to Al-Zahraa Teaching Hospital using specific primers targeting the 16S rRNA gene and DNA extraction. Agarose gel electrophoresis of the PCR products showed a specific band at 798 base pairs (bp) (Figure 1), confirmed using a 250–10,000 bp ladder marker (M).

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Twelve (24%) of the samples tested positive for *K. pneumoniae* (lanes 1–12 in Figure 1), indicating the presence of *K. pneumoniae* in these patients.

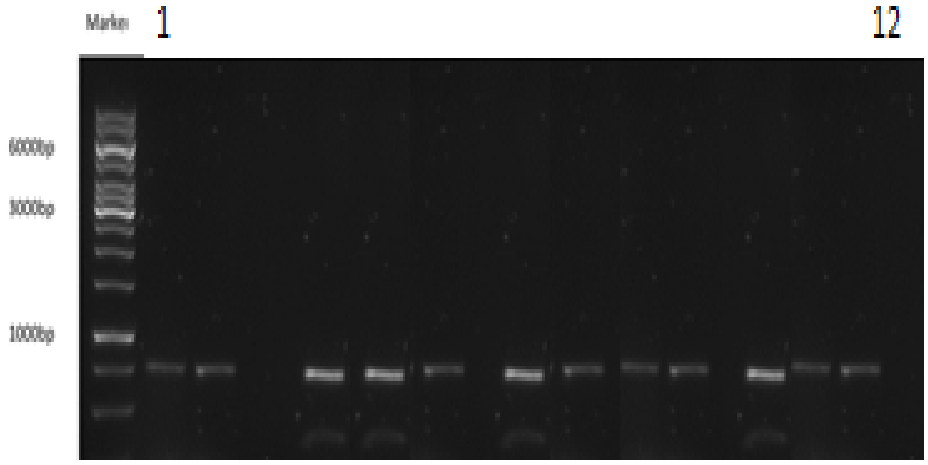


**Figure 1: Representative image for agarose gel electrophoresis of PCR products targeting 16S rRNA gene at 798 bp**

**Discussion**

The study by Alali. (2024) investigated the prevalence and antibiotic resistance patterns of *Klebsiella pneumoniae* in Kut City, Iraq, revealing a concerning level of infection and multi-drug resistance. The identification of 12 *K. pneumoniae*-positive cases among 50 samples (24%) highlights a significant public health threat in the region, consistent with the growing global concern regarding the rise of antibiotic-resistant Gram-negative bacteria [3, 6]. The higher prevalence observed in the city (30%) compared to the village (15%) suggests an association with population density and environmental factors characteristic of urban settings [7]. This urban-rural disparity aligns with other studies examining the spread of nosocomial infections, often facilitated by biofilm formation on medical equipment [8, 9]. The study's findings highlight the crucial need for further research to determine the specific environmental factors and occupational exposures driving this difference [10].

The observed male-to-female ratio of 2:1 among positive cases requires further investigation to determine if underlying biological factors, differential access to healthcare, or specific environmental exposures contribute to this gender disparity in infection rates [11]. This necessitates a more nuanced understanding of the interplay between social determinants of health and the epidemiology of *K. pneumoniae* infections.

The alarmingly high resistance rates to multiple antibiotic classes, including carbapenems (100% resistance in this study), reflect the global trend of increasing multi-drug resistance (MDR) in *K. pneumoniae* [4]. The high resistance to carbapenems, often the last-resort antibiotics for treating serious MDR Gram-negative bacterial infections, is especially worrisome and underscores the urgent need for improved infection control measures and the development of novel therapeutic strategies to combat this escalating threat [5, 12]. The relatively lower resistance to trimethoprim-sulfamethoxazole (83.33%) suggests a potential

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alternative treatment option, but further investigation is warranted before widespread clinical application [13].

The consistent amplification of a 798 bp band using 16S rRNA gene PCR confirmed the accuracy of this technique for *K. pneumoniae* identification and supports its application in future epidemiological studies. However, the limitations of relying solely on 16S rRNA gene sequencing for accurate identification of *K. pneumoniae* should be considered, particularly given the potential for misidentification of closely related species [14]. Future studies should incorporate whole-genome sequencing (WGS) to enhance the accuracy of species identification and resolve potential ambiguities.

## Conclusion

This study confirms a substantial prevalence of antibiotic-resistant *K. pneumoniae* in Kut City, Iraq, a finding that aligns with global trends and underscores a critical public health concern. The alarmingly high rates of resistance to multiple antibiotic classes, including carbapenems, necessitate the immediate implementation of enhanced infection control measures and the promotion of judicious antibiotic stewardship. Future research should investigate environmental factors contributing to the observed urban-rural and gender disparities in infection rates. Continuous monitoring of antibiotic resistance patterns and the development of novel therapeutic strategies are essential to effectively combat the escalating threat posed by MDR KP. This study's findings emphasize the need for robust public health surveillance and the adoption of evidence-based infection control practices to mitigate the burden of MDR KP infections in this region and globally. The observed high resistance underscores the limitations of current therapeutic options and highlights the urgent need for new approaches to combat this growing threat.

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