# Virulence Characteristics and Antibiotic Resistance of *Klebsiella pneumoniae* Isolated from Clinical Sources

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**Abstract** Klebsiella pneumoniae (K. pneumoniae) is a Gram-negative capsulated bacterium that causes a variety of human diseases. This study was to investigate the prevalence and antibiotic susceptibility pattern of K. pneumoniae isolated from diverse clinical samples, as well as to discover some of the bacteria's virulence components using a molecular approach. One hundred and eighty patients suffering from respiratory, urinary, wounds and burns infections were enrolled in this study and evaluated for the presence of K. pneumoniae infection via culturing, microscopical, motility and biochemical characteristics as well as API 20E and VITEK 2 systems. Molecular technology was used for detection the presence of K1 and K2 genes as well as the presence and sequencing 16S ribosomal ribonucleic acid (16S rRNA) gene. The results showed that a low prevalence of K. pneumoniae (27.8%) in Iraqi patients with higher positivity rate in sputum samples (42%) compared to other clinical samples.. According to the results of phylogenetic tree construction of 16S rRNA gene, we identified six mutated clinical isolates of K. pneumoniae that were deposited in GenBank under the accession numbers of MW642198, MW642199, MW642200, MW642201, MW642202 and MW642203. The mutated 16S rRNA gene stains exhibited a significantly higher resistance to all antibiotics types that used in this study.

**Key Words:** *Klebsiella pneumoniae*, Antibiotics Resistance, *K1* and *K2* Genes.

## 1. Introduction

Klebsiella pneumoniae is a Gram-negative capsulated bacterium that is abundant in the environment and causes a variety of human infections including bloodstream infection (BSI), urinary tract infection (UTI), surgical-site infection, and pneumonia. It is a major pathogen for nosocomial pneumonia and a major source of sepsis [1]. In the healthcare setting, these infections are particularly problematic in infants, the elderly, and immunocompromised persons [2]. These infections are distinguished by their tendency to spread metastatically and their high morbidity and mortality [3].

The hypermucoviscosity trait [4] appears to be associated with the invasiveness of K. pneumoniae strains, as evidenced by the excessive "stickiness" of their colonies on agar plates. Due to an increase in the number of illnesses and an increase in the number of strains resistant to antibiotics, K. pneumoniae is receiving attention. More than a third of K. pneumoniae isolates reported to the European Centre for Disease Prevention and Control were resistant to at least one antimicrobial class, with the most common resistance phenotype being combined resistance to fluoroquinolones, third-generation cephalosporins, and aminoglycosides [5]. Furthermore, antibiotic-resistant genes have been found in Klebsiella species, which can spread to other gram-negative bacteria. In fact, Klebsiella was the first organism to discover many of the antibiotic-resistant genes presently found in multidrug-

IMDC-IST 2021, September 07-09, Sakarya, Turkey

DOI 10.4108/eai.7-9-2021.2315374

resistant organisms [6]. Smooth lipopolysaccharide (LPS with O antigen), pili for adherence to host cells, anti-phagocytic capsules (K antigen), and siderophores that aid the bacterium in its competition with the host are all produced by K. pneumonia [7]. The capsule serotypes K1 and K2, which are the most virulent to humans, have been the focus of research into the virulence determinants of K. pneumoniae [8]. As a result, determining medication resistance in two key pathogenicity serotypes, K1 and K2, requires studying the resistance of this gram-negative bacterium isolated from clinical specimens. The most common method for characterising K. pneumoniae isolates is molecular capsular typing, which has great repeatability in differentiating clinical isolates [9].

Many sequenced virulence genes have been discovered in K. pneumonia, including 16S RNA analysis and sequencing of sections within the 16S rRNA gene, which can increase the speed and effectiveness of estimating the variety of bacteria helpful for pathogen and identification [10]. The purpose of this work is to assess the prevalence and antibiotic susceptibility pattern of K. pneumoniae isolated from clinical samples (urine, sputum, wound smears, and burn smears), as well as to identify some virulence factors of K. pneumoniae using a molecular technique.

## 2. Materials and Methods

One hundred and eighty patients with age ranged from 16-55 years whom attending Al-Hussein Teaching, Mohammed Al-Mosawy and Al-Habbobi Hospitals during the period from beginning of October 2020 to end of March 2021. A written consent was obtained from each patient to fulfill the international research ethical criteria. The patients were classified according their suffering into; 42 patients with UTI, 33 patients with wounds, 15 patients with burn and 90 patients with respiratory tract infection (RTIs). From each subject with UTI or RTI; a fresh urine and sputum samples were obtained, respectively, and placed in the sterile suitable containers, whereas from each subject with wound or burn; a fresh swabs were taken from the sites of injuries. All collected samples were quickly transported to laboratory for culturing.

*Klebsiella pneumoniae* isolates were performed using blood and MacConkey agar as the method that illustrated via [11]. The primary detection of *K. pneumoniae* was assesses via culturing, microscopical, motility, and biochemical tests (catalase, indole, oxidase, citrate utilization, urease and glucose) characteristics according to the methods that mentioned in [11, 12]. The confirmatory diagnosis of this bacterium was done using API 20E system and VITEK 2 system (Bio-Merieux, France). Antimicrobial susceptibility test was done using disc diffusion method according to [13] with following antibiotics; doxycycline, rifampicin, ciprofloxacin, gentamycin, cefuroxime, ceftazidime, amikacin, imipenem, ceftriaxone and levofloxacin. The inhibition zone sizes were measured with calipers and the findings were calculated in reference to the clinical and laboratory standards institute guidelines [13].

Deoxyribonucleic acid (DNA) was extracted from bacterial isolates using bacterial DNA extraction kit (Anatolia, Turkey). The genes 16*S rRNA*, *K1* and *K2* were detected by polymerase chain reaction (PCR) technique using specific primers (Table 1) with using PCR thermo cycler system (Eppendrof, UAS). The method was preceded according to instructions of the company. After PCR amplification, the reaction product was separated by electrophoresis on agarose gel (1%) and then the PCR product image was taken [14].

**Table 1.** The primers that used in the study

| Genes | | Primer Sequences (5′————3′) | Product Size | References |
|---|---|---|---|---|
| 16S rRNA | F | AGAGTTTGATCCTGGCTCAG | 1500 bp | [15] |
| | R | GGTTACCTTGTTACGACTT | | |
| K1 | F | GGTGCTCTTTACATCATTGC | 1283 bp | [16] |
| | R | GCAATGGCCATTTGCGTTAG | | |
| K1 | F | CAACCATGGTGGTCGATTAG | 531 bp | [17] |
| | R | TGGTAGCCATATCCCTTTGG | | |

**16*S rRNA:*** *16S ribosomal ribonucleic acid*, **F:** Forward, **R:** Reverse and **bp:** base pair

The 16*S rRNA* gene was subjected to nucleotide sequencing with an automated DNA sequencing machine at Bio-Service unit (National Science and Technology Development Agency). The 16*S rRNA* gene sequences determined were aligned along with the sequences of type strains obtained from Multiple Sequence Alignment program CLUSTAL X (version 1.82) [18, 19].

Frequency distribution of categorical variables within the groups of the study was performed using Chi-Square method. Statistics were regarded as statistically significance at p-value <0.05.

## 3. Results

The results showed that out of 180 clinical samples, only 50 (27.8%) samples were detected as *K. pneumoniae* infections. In Figure 1, the results of *K. pneumoniae* were reported in sputum 21/50 (42%) followed in urine 14/50 (28%) and then wounds 8/50 (16%).

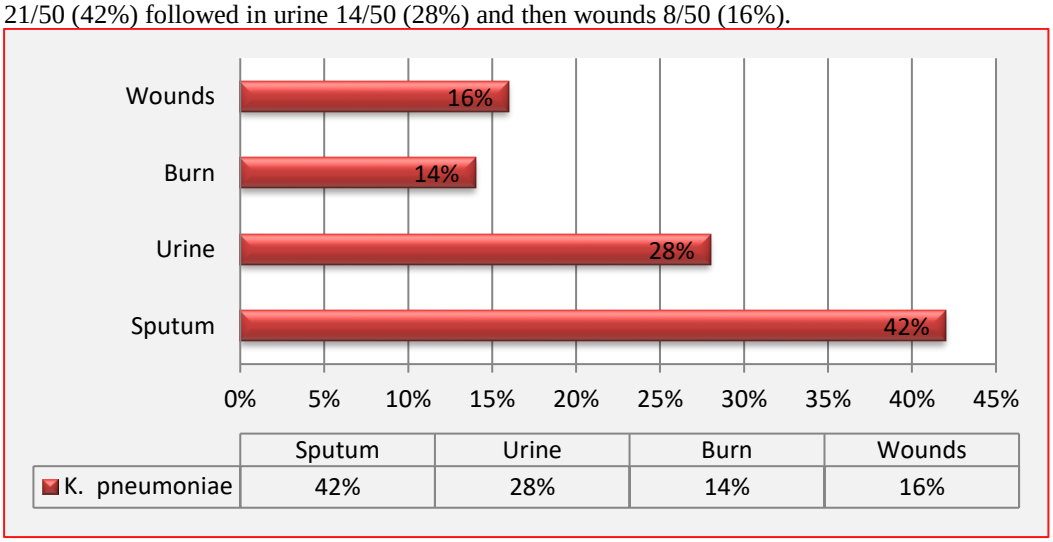

Figure 1: Prevalence of *K. pneumoniae* according to clinical samples

*Klebsiella pneumoniae* isolates susceptibility to antibiotics was illustrated in Table (2). For Imipenem, ciprofloxacin, levofloxacin, doxycycline and rifampicin antibiotics, the results of antibiotics assay of *K. pneumoniae* isolates was revealed a significantly ($P<0.05$) high frequency % of resistance (96%, 82%, 76%, 72% and 70%, respectively). In the same table, the results of *K. pneumoniae* isolates susceptibility to ceftazidime, ceftriaxone, and cefuroxime, showed that 33/50 (66%), 29/50 (58%) and 23/50 (46%) were found to be resistant to these antibiotics, respectively. For antibiotics susceptibility to gentamycin and amikacin, the results showed that 29/50 (58%) and 22/50 (44%) of isolates were sensitive to former two antibiotics, respectively.

Table 2: Antibiotics susceptibility pattern of *K. pneumoniae* isolates

| Antibiotics/ Isolates | Resistance | | Intermediate | | Sensitive | |
|---|---|---|---|---|---|---|
| | NO | % | NO | % | NO | % |
| Doxycycline | 36 | 72 | 5 | 10 | 9 | 18 |
| Rifampicin | 35 | 70 | 4 | 8 | 11 | 22 |
| Ciprofloxacin | 41 | 82 | 6 | 12 | 3 | 6 |
| Gentamycin | 10 | 20 | 11 | 22 | 29 | 58 |
| Cefuroxime | 23 | 46 | 16 | 32 | 11 | 22 |
| Ceftazidime | 33 | 66 | 9 | 18 | 8 | 16 |
| Amikacin | 22 | 44 | 6 | 12 | 22 | 44 |
| Imipenem | 48 | 96 | 1 | 2 | 1 | 2 |
| Ceftriaxone | 29 | 58 | 4 | 8 | 17 | 34 |
| Levofloxacin | 38 | 76 | 8 | 16 | 4 | 8 |

P.Value <0.05 For all except amikacin , N=Number and %=Percentage.

Regarded to molecular characterization, the current study results showed that out of 19 *K. pneumoniae* isolates, 7 isolates (36.8%) were positive for *K1* gene (Figure 2) and 6 isolates (31.6%) were positive for *K2* gene (Figure 3) ,whereas 6 isolates (31.6%) were neither *K1* nor *K2*.

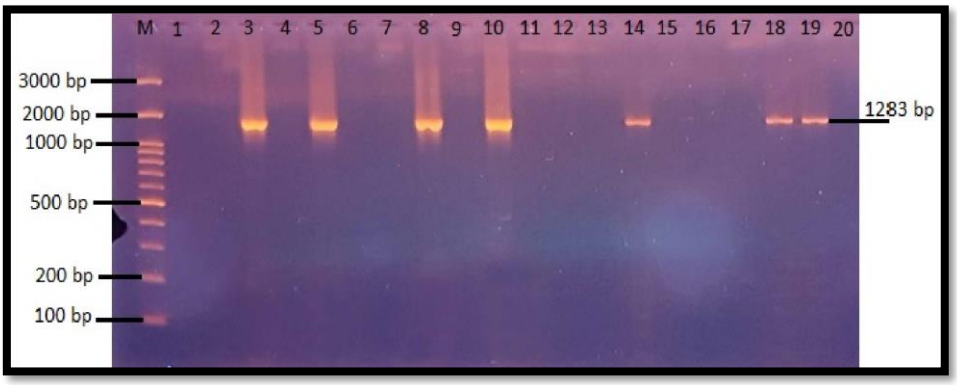


Figure 2: Polymerase chain reaction product (1283 base pair) of *K. pneumoniae K1* gene


Figure 3: Polymerase chain reaction product (531 base pair) of *K. pneumoniae K2* gene

The correlation between the antibiotics resistance and the present of *K1* and *K2* genes in 19 bacterial isolates is illustrated in Table (3). The results of gentamycin and amikacin revealed a significantly ($p<0.05$) high frequency % of antibiotics resistance among *K1* (71.4% for both) and *K2* (50% and 83.3%, respectively) positive isolates compared to *K1* and *K2* negative isolates (16.7% and 33.4%, respectively), whereas other antibiotics types revealed higher resistance rate, but without significant differences ($P>0.05$).

**Table 3.** Association between antibiotics resistance and the present of *K1* or *K2 K. pneumoniae* isolates

| Antibiotics/ Isolates | | *K1* positive (N=7 | | *K2* positive (N=6) | | Non-*K1* and *K2* (N=6) | | P.value |
|---|---|---|---|---|---|---|---|---|
| | | NO | % | NO | % | NO | % | |
| Doxycycline | R | 7 | 100 | 6 | 100 | 6 | 100 | >0.05 |
| Rifampicin | R | 7 | 100 | 5 | 83.3 | 5 | 83.3 | >0.05 |
| | I | 0 | 0 | 1 | 16.7 | 0 | 0 | |
| | S | 0 | 0 | 0 | 0 | 1 | 16.7 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ciprofloxacin | R | 6 | 85.7 | 5 | 83.3 | 5 | 83.3 | >0.05 |
| | I | 1 | 14.3 | 1 | 16.7 | 1 | 16.7 | |
| Gentamycin | R | 5 | 71.4 | 3 | 50 | 1 | 16.7 | <0.05 |
| | I | 0 | 0 | 3 | 50 | 5 | 83.3 | |
| | S | 2 | 28.6 | 0 | 0 | 0 | 0 | |
| Cefuroxime | R | 6 | 85.7 | 5 | 83.3 | 4 | 66.7 | >0.05 |
| | I | 1 | 14.3 | 1 | 16.7 | 1 | 16.7 | |
| | S | 0 | 0 | 0 | 0 | 1 | 16.7 | |
| Ceftazidime | R | 6 | 85.7 | 6 | 100 | 6 | 100 | >0.05 |
| | S | 1 | 14.3 | 0 | 0 | 0 | 0 | |
| Amikacin | R | 5 | 71.4 | 5 | 83.3 | 2 | 33.4 | <0.05 |
| | I | 1 | 14.3 | 0 | 0 | 2 | 33.3 | |
| | S | 1 | 14.3 | 1 | 16.7 | 2 | 33.3 | |
| Imipenem | R | 7 | 100 | 6 | 100 | 6 | 100 | >0.05 |
| Ceftriaxone | R | 6 | 85.7 | 4 | 66.7 | 5 | 83.3 | >0.05 |
| | I | 0 | 0 | 1 | 16.7 | 0 | 0 | |
| | S | 1 | 14.3 | 1 | 16.7 | 1 | 16.7 | |
| Levofloxacin | R | 6 | 85.7 | 4 | 66.7 | 5 | 83.3 | >0.05 |
| | I | 1 | 14.3 | 1 | 16.7 | 1 | 16.7 | |
| | S | 0 | 0 | 1 | 16.7 | 0 | 0 | |

R: Resistance, I: Intermediate, S: Sensitive and N: Number.

According to the results of the PCR and gene sequencing (phylogenetic tree construction) of *16S rRNA* gene of 19 *K. pneumoniae* isolates (data not shown), we identified six mutated clinical strains of *K. pneumoniae* that were deposited in GenBank under the accession numbers of MW642198, MW642199, MW642200, MW642201, MW642202 and MW642203. Therefore Table 4 shows the antibiotics susceptibility findings of these isolates, which revealed that the majority of the isolates 6 (100%) were resistance for all antibiotics, except gentamycin and amikacin were 5/6 (83.3%). The differences were significant ($p<0.05$) for all mentioned antibiotics.

**Table 4.** Antibiotics susceptibility pattern of 16*S rRNA K. pneumoniae* isolates.

(6S rRNA Positive Mutated Isolates (N=6))

| Antibiotics/ Isolates | Resistance | | Intermediate | | Sensitive | |
|---|---|---|---|---|---|---|
| | NO | % | NO | % | NO | % |
| Doxycycline | 6 | 100 | 0 | 0 | 0 | 0.0 |
| Rifampicin | 6 | 100 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 6 | 100 | 0 | 0 | 0 | 0 |
| Gentamycin | 5 | 83.3 | 0 | 0 | 1 | 16.7 |
| Cefuroxime | 6 | 100 | 0 | 0 | 0 | 0 |
| Ceftazidime | 6 | 100 | 0 | 0 | 0 | 0 |
| Amikacin | 5 | 83.3 | 1 | 16.7 | 0 | 0 |
| Imipenem | 6 | 100 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 6 | 100 | 0 | 0 | 0 | 0 |
| Levofloxacin | 6 | 100 | 0 | 0.0 | 0 | 0 |

P. Value <0.05 For all **, N=**Number, **%=**Percentage and **16S rRNA=**16S ribosomal ribonucleic acid.

## 4. Discussion

Klebsiella pneumoniae is one of the most common enteric bacteria, accounting for up to 10% of all nosocomial infections and causing severe morbidity and death in pneumonia and urinary tract infections [20]. The present study was found a low prevalence of *K. pneumoniae* (27.8%) in total included clinical samples. The result was in consistent with 19.6% and 24% as estimated respectively, via [21] along with [22] and seems to be lower than that reported by [23] in clinical samples, who reported a frequency % of *K. pneumoniae* positivity is 73.3%. The discrepancies in prevalence rates could be accounted in part by changes in the study population, sample collecting method, and isolation and diagnosis methodologies.

Klebsiella pneumoniae is a common cause of pneumonia, urinary tract infections, skin infections, soft tissue infections, bacteremia, and septicemia, all of which have significant morbidity and death rates [24]. In this investigation, 50 isolates of K. pneumoniae were gathered from several clinical sample sources. Among the patients, *K. pneumoniae* infection was the higher microorganism isolated from the sputum samples (42%), followed by the urine samples (28%) and wound swabs (16%), whereas the lowest prevalence was reported in burns swabs (14%) (Figure 1) which was in line with above mentioned findings. Our results were closer to a previous studies carried out by [25] who reported that the *K. pneumoniae* was the predominant gram negative bacilli (60%) in RTIs, [26] who recorded a 26.79% of UTIs were

*K. pneumoniae* [27], and [28] whom reported that 23.3% of wounds and 16% burns swabs were diagnose as *K. pneumoniae* infection, respectively. The varied frequencies of K. pneumoniae isolation from different samples could be attributable to sample size differences, as well as different geographical and clinical situations with associated risk factors.

Drug resistance in human pathogenic bacteria like K. pneumoniae is a common occurrence around the world. Because of the widespread use of antibiotics in medical practise, this is considered a serious problem in both poor and developed countries [29]. We studied K. pneumoniae isolated from various clinical specimens in Al-Nasiriyah City to see if there was a variation in medication resistance (Iraq). The present study was reported a significantly higher frequency % of imipenem resistance (96%) among *K. pneumoniae* isolates (Table 2), a result with high consistency with the [17] who reported that 93.3% of *K. pneumoniae* isolates were resistant to imipenem. Result of this study revealed that *K. pneumoniae* had higher resistance to ciprofloxacin (82%). In consistent with this finding, another study [30] observed that *K. pneumoniae* bacterial types are generally insensitive to ciprofloxacin (88%). High resistance rate to imipenem and ciprofloxacin in the present study might be due to uncontrolled consumption of these antibiotics in Iraq. Antibiotic resistance in K. pneumoniae has numerous mechanisms, including quinolone drugs like ciprofloxacin and levofloxacin. These techniques involved modulating the target sites that bind with antibiotics by causing chromosomal genetic mutations in the genes encoding for DNA grease or resulting in the development of antibiotic-resistant enzymes [31]. Coinciding with these evidences this study results (Table 2) exhibited high resistance for ciprofloxacin and levofloxacin (82% and 76%, respectively). In this study, doxycycline antibiotic had bad activity against *K. pneumoniae* isolates (Table 2). Matching to our findings, another study [32] verified that only 6% of *K. pneumoniae* isolates were sensitive to this antibiotic. Rifampin was found to be effective against gram-negative bacteria in the majority of cases. Rifampin monotherapy, on the other hand, is not recommended in clinical practise since rifampin-resistant mutations arose soon after treatment began [33]. In compatibility with these findings, current study revealed (Table 2) a low activity of this antibiotic against *K. pneumoniae* isolates. Previous findings have found an elevated level of resistant of *K. pneumoniae* isolates to third generation cephalosporin such as ceftazidime and ceftriaxone [34]. A previous study had reported that the rate of *K. pneumoniae* isolates insensitive to ceftazidime and ceftriaxone is 52% and 65%, sequently [35].

In harmony with current study findings, another study verified that 74.3% of *K. pneumoniae* isolates were resist to ceftazidime drug [36] and 65.8% were resist to ceftriaxone drug [29]. In the present research, the results of susceptibility of *K. pneumoniae* isolates to cefuroxime antibiotic showed that 46% of the isolates were resistance to this drug (Table 2). Consistent with this finding, previous studies showed that a 37.5% [37] and 89.47% [38]. These findings concluded that there is urgent need to test the antibiotics activity against patient's isolates before therapy initiation. Regarded to aminoglycosides antibiotics, results of present study confirmed that *K. pneumoniae* isolates were more sensitive to gentamycin (58%) and amikacin (44%) compared to other mentioned above antibiotics. In agreement with our findings, another study observed that *K. pneumoniae* bacterial types had low resistance to gentamycin (15%) and amikacin (33.3%) antibiotics [17].

The findings revealed that 36.8% and 31.6% of the *K. pneumoniae* isolates had *K1* and *K2* genes, respectively (Figures 2 and 3) and 31.6% of the remaining isolates were not carry either of them. In line with our results, another previous study [39] recorded an elevated prevalence of *K1* gene compared to *K2* gene or absent of both genes. According to this study, all bacterial isolates that positive for *K1* and/or *K2* genes were found to be resistance to all current study used antibiotics (Table 3). According to these findings, isolates carrying these

genes become more virulent than others, and the presence of these virulence factors combined with a high level of drug resistance should make bacteria a highly infectious agent and result in treatment failure [40].

The bacterial 16S rRNA gene has nine "hypervariable regions" (V1-V9), which show significant sequence variability among bacteria [41]. When individual antibiotics target a shared or partially overlapping binding site on the ribosome, simultaneous loss of antibiotic sensitivity after acquisition of a single rRNA nucleotide mutation to combinations of structurally separate antibiotics may occur. When the mutated nucleotide is directly involved in the antibiotic binding site, minor structural changes may suffice to yield resistance; however, resistance mutations can also occur at sites that are not involved in antibiotic binding but where mutation causes structural perturbations that propagate toward the antibiotic binding site [42]. The findings of the present study (Table 4) confirmed the evidence of impaired antibiotics activity against a mutated identified clinical *16S rRNA* gene *K. pneumoniae* strains. A better explanation for these findings is that methylation of nucleotides implicated in the binding of 16S rRNA to antibiotics causes a decrease of affinity, leading in high-level antibiotic resistance [43]. In clinical isolates of gram-negative bacteria, several plasmid-encoded 16S rRNA methylases have been discovered [44].

The limited sample size (n=50) was the primary limitation of this study. Furthermore, the study was mostly conducted in Al-Nasiriyah City, and patients under the age of 16 were not evaluated. Finally, the molecular tests were performed for 19 isolates only which may be increase the bias of sample selection.

**In conclusions**, the prevalence of *K. pneumoniae* was higher among patients with respiratory system diseases compared to other. The presence of *K1*, *K2* and mutated *16S rRNA* genes consistent with elevation the rate of *K. pneumoniae* isolates resistance to antibiotics. As a result, continuing antimicrobial resistance monitoring, cautious antimicrobial agent use, and the construction of a surveillance system are all critical for preventing the spread of antibiotic resistance strains in Iraq.

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