JAUA
Journal of Academic Union Association

ANTIBIOTIC SUSCEPTIBILITY OF CLINICAL *KLEBSIELLA PNEUMONIAE* ISOLATES OBTAINED FROM URINE OF HOSPITALIZED PATIENTS WITH URINARY TRACT INFECTIONS

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ABSTRACT

Klebsiella pneumoniae is a clinically important opportunistic pathogen responsible for several infections, including urinary tract infections (UTIs). The emergence and rising prevalence of multidrug-resistant *K. pneumoniae* strains poses serious challenges to global public health and complicates treatment options. In this study, the antibiotic susceptibility of *K. pneumoniae* strains isolated from urine samples of hospitalized patients with UTIs in Duhok, Iraq was investigated against the most commonly used antibiotics in the region using the disc diffusion method (also known as Kirby-Bauer method). In a total of 170 urine samples collected, 25 isolates were identified as *K. pneumoniae* using morphological, biochemical, VITEK 2, and molecular methods. All the isolates tested were completely resistant to cephalothin, cephradine, and piperacillin. The resistance rate was found as 96% against rifampin, amoxicillin/clavulanic acid, and ampicillin, followed by cefotaxime and amikacin (92%), and trimethoprim/sulfamethoxazole and ceftriaxone (88%). A moderate level of resistance was detected against ciprofloxacin and doxycycline, with a resistance rate of 60 and 52%, respectively. The lowest resistance was observed against gentamicin (20%), tetracycline (16%), and imipenem (4%). Most of the strains (88%) were multidrug-resistant (MDR) and 12% were extensively drug-resistant (XDR). These findings indicate a significantly high prevalence of multidrug resistance among clinical *K. pneumoniae* strains from UTI patients. Imipenem was the most effective drug among the tested agents, suggesting that carbapenems may still serve as a reliable treatment option.

Keywords: Antibiotics, Pathogen, Multidrug resistance, Urinary tract infection, VITEK 2

İDRAR YOLU ENFENSIYONU OLAN HASTANEDE YATAN HASTALARIN İDRARINDAN ELDE EDİLEN KLİNİK *KLEBSİELLA PNEUMONİAE* İZOLATLARININ ANTİBİOTİK DUYARLILIĞI

ÖZET

Klebsiella pneumoniae, idrar yolu enfeksiyonları dâhil olmak üzere çeşitli enfeksiyonlardan sorumlu klinik olarak önemli bir fırsatçı patojendir. Çoklu ilaca dirençli *K. pneumoniae* suşlarının ortaya çıkışı ve artan yaygınlığı, küresel halk sağlığı için ciddi zorluklar oluşturmakta ve tedavi seçeneklerini karmaşıklaştırmaktadır. Bu çalışmada, Irak'ın Duhok kentinde idrar yolu enfeksiyonları nedeniyle hastaneye kaldırılan hastaların idrar örneklerinden

izole edilen *K. pneumoniae* suşlarının, bölgede en sık kullanılan antibiyotiklere karşı antibiyotik duyarlılığı, disk difüzyon yöntemi (Kirby-Bauer yöntemi olarak da bilinir) kullanılarak araştırılmıştır. Toplanan toplam 170 idrar örneğinde, 25 izolat morfolojik, biyokimyasal, VITEK 2 ve moleküler yöntemler kullanılarak *K. pneumoniae* olarak tanımlanmıştır. Test edilen tüm izolatlar sefalotin, sefradin ve piperasiline tamamen dirençli bulunmuştur. Rifampin, amoksisilin/klavulanik asit ve ampisiline karşı %96 direnç oranı bulunurken, bunu sefotaksim ve amikasin (%92) ve trimetoprim/sülfametoksazol ve seftriakson (%88) takip etti. Siprofloksasin ve doksisikline karşı sırasıyla %60 ve %52 oranında orta düzeyde direnç saptandı. En düşük direnç ise gentamisin (%20), tetrasiklin (%16) ve imipenem (%4) karşısında gözlendi. Suşların çoğu (%88) çoklu ilaca dirençli (MDR) ve %12'si yaygın ilaca dirençliydi (XDR). Bu bulgular, idrar yolu enfeksiyon olan hastalarından alınan klinik *K. pneumoniae* suşları arasında çoklu ilaca direncin önemli ölçüde yüksek bir yaygınlığa sahip olduğunu göstermektedir. İmipenem test edilen ajanlar arasında en etkili ilaçtı ve bu da karbapenemlerin hâlâ güvenilir bir tedavi seçeneği olabileceğini göstermektedir.

Anahtar Kelimeler: Antibiyotikler, Patojen, Çoklu ilaç direnci, İdrar yolu enfeksiyonu, VITEK 2

1. INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen that asymptomatically colonizes the mucosal surfaces of the human body (Martin & Bachman, 2018). However, in individuals with an underlying disease, it is more likely to cause various infections, including respiratory, digestive, and urinary tract infections, as well as sepsis, endocarditis, meningitis, liver abscesses, and septicemia (Navon-Venezia et al., 2017; Chang et al., 2021). This pathogenic bacterium accounts for almost one-third of all Gram-negative-associated infections (Navon-Venezia et al., 2017). Therefore, significant precautions are required to reduce the incidence and spread of multidrug-resistant *K. pneumoniae* infections (Chang et al., 2021).

Extensive and inappropriate use of antibiotics has accelerated the emergence of antibiotic-resistant bacterial strains worldwide (Navon-Venezia et al., 2017). The increasing emergence of hypervirulent and carbapenem-resistant *K. pneumoniae* strains makes this species a major pathogen in both hospital and community settings, posing a serious threat to public health (Chang et al., 2021). The occurrence of resistant strains has made antibiotic-based treatments increasingly challenging to implement, leading to higher morbidity and mortality rates, prolonged hospitalization, and high medical costs (Navon-Venezia et al., 2017). According to the World Health Organization, increased antibiotic resistance is considered one of the top three health threats (Wise et al., 2011). A modeling study estimated that, in 2014, third-generation cephalosporin-resistant Escherichia coli and K. pneumoniae caused approximately 50 million severe infections worldwide (Temkin et al., 2018). The frequency of antibiotic resistance in *K. pneumoniae* strains has steadily increased over time since the investigations between 2005 and 2015 showed that this pathogen had become resistant to the four major antibiotic classes such as carbapenems, aminoglycosides, third-generation cephalosporins, and fluoroquinolones (Navon-Venezia et al., 2017).

According to that study, the resistance rate of *K. pneumoniae* strains to these classes varies considerably among countries.

Although antibiotic resistance in *K. pneumoniae* is a complex phenomenon, increases in the rate of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains can be attributed to the evolution of this pathogen upon continuous exposure to antibiotics because selective pressures lead to the emergence of various genetic mechanisms (Navon-Venezia et al., 2017). The incidence of these resistant strains, especially to β-lactams and carbapenems, significantly challenges the clinical treatments (Li et al., 2025). Bacterial resistance against antimicrobials can be acquired through chromosomal mutations and/or horizontal gene transfer by mobile genetic elements such as plasmids and transposons (Foster, 2017). *K. pneumoniae* harbors many chromosomal and plasmid-encoded genes responsible for antibiotic resistance such as β-lactam-, quinolone-, and aminoglycoside-resistance genes, and extended-spectrum β-lactamase (ESBL) genes (Navon-Venezia et al., 2017; Li et al., 2025). The dissemination of these genes among microorganisms contributes to the emergence of new resistant strains and species, especially under antibiotic pressure. UTIs (urinary tract infections) are one of the most common infectious diseases, responsible for approximately 150 million cases annually worldwide, and is associated with high morbidity and mortality in humans (Totsika et al., 2012). UTIs cause major problems on the economy of healthcare systems, costing billions of dollars annually in the United States alone (Litwin et al., 2005). The urinary tract system can be infected by various bacterial species but *K. pneumoniae* is considered the second most common uropathogenic bacterium in patients with UTI and chronic kidney disease (Cristea et al., 2017) and is one of the most concerning pathogens associated with antibiotic resistance (Navon-Venezia et al., 2017). Its multidrug resistance was also correlated with chronic renal failure (Cristea et al., 2017). However, in the literature, the antibiotic susceptibility of *K. pneumoniae* from UTI patients has not been studied in Duhok, Iraq. Therefore, the present study aimed to determine the antibiotic susceptibility patterns of clinical *K. pneumoniae* isolates obtained from hospitalized patients with UTIs in order to evaluate the current antibiotic resistance trends and guide appropriate therapeutic strategies.

2. MATERIALS AND METHODS

2.1. Sample Collection

Urine samples were collected from hospitalized patients with UTIs (n=170) at Azadi and Duhok Burn Hospital, Iraq between 03/01/2021 and 10/10/2021. Patients' gender and age (10-70 years) were recorded as demographic data. Samples were collected aseptically using sterile swabs by swabbing then immediately placed into sterile containers and transported to the laboratory under appropriate conditions for further analysis.

2.2. Morphological and Biochemical Characterization

Colony morphology, staining reactions, and biochemical testing were used to help identify the isolates from human urine (Atlas et al., 1995; MacFaddin, 2000). MacConkey agar was used as a selective and differential culture medium for *K.*

pneumoniae (Boll et al., 2012). Of the 170 samples tested, 25 were positive for *K. pneumoniae* based on the results of growth on the MacConkey agar (Lab, UK) at 37°C (pH 7.2). Gram-negative and enteric bacteria can be identified using lactose fermentation. Non-fermenters do not change color on MacConkey agar, but lactose fermenters do. In this study, bacteria fermenting lactose were distinguished from those that do not ferment lactose by subculturing pink, mucoid colonies on MacConkey agar (Holt et al., 1994).

2.3. VITEK 2 System for Identification of *K. pneumoniae* Isolates

K. pneumoniae isolates were confirmed in the VITEK 2 system as follow: First, a pure colony of bacterial isolates was placed into a sterile tube containing 3 mL of normal saline. The suspension was adjusted to the standard turbidity according to the manufacturer's instructions. The final turbidity was adjusted to 0.5-0.63 McFarland units. The inoculated tubes were placed into the VITEK 2 cassette. Each sample's data were transmitted to a computer connected to the VITEK 2 system. The tubes were incubated at 37°C for 24 hours to determine bacterial presence. Tests were carried out on VITEK cards (catalogue #418590) with 64 wells in this study. Inoculated bacteria on the card showed a pattern of positive or negative reactions as the microorganisms interacted with the card. This pattern was compared to a reference library for accurate identification and characterization. An organism in the samples must show an at least 85% match to the microorganisms in the library.

2.4. Molecular Characterization

A DNA isolation kit was used to extract the genomic bacterial DNA of the urine samples (Addbio, Korea). Briefly, 0.5 mL of overnight culture incubated at 37°C with shaking was taken into 1.5 mL Eppendorf tubes and then centrifuged at 14 000 rpm for 1 minute. Proteinase K solution (20 mg mL^{-1}) was added to each pellet. The mixture was re-suspended in 200 µL lysis solution by pipetting or vortexing. The solutions were incubated at 56°C for 10 minutes in a heat block. After a 15-second pulse vortex, 100 µL of binding-solution and ethanol were added to the tubes. Afterwards, the samples were centrifuged for 3 minutes at 13 000 rpm. In the final step, the supernatant was transferred to the upper reservoir of the collection tubes and then centrifuged at 13 000 rpm for 1 minute. The pellet was washed with the washing-solution (500 µL) and then briefly centrifuged. Genomic DNA was eluted in elution buffer and kept at -20°C.

The molecular characterization of *K. pneumoniae* isolates was confirmed by PCR method using the forward and reverse primers Pf: 5-ATTTGAAGAGGTTGCAAACGAT-3 and Pr1: 5-TTCACTCTGAAGTTTTCTTGTGTTC-3 (Liu et al., 2008). The primers were designed to amplify the 16S-23S rDNA internal transcribed spacer region. The PCR mixture (25 µL) consisted of 12.5 µL of hot-start premix (AddBio, Korea), 1 µL of each primer (10 pmol), 4 µL of template DNA (30-100 ng μL^{-1}), and nuclease-free water (Qiagen, Germany). PCR was conducted in GeneAmp 9700 thermal cycler (Applied Biosystems, USA) with the following protocol: Initial denaturation was at 95°C for 5 minutes, followed by 35 cycles of 1 minute at 94°C for denaturation, 1 minute at 58°C for annealing, and 1 minute at 72°C for extension. An additional 10-minute was run at 72°C for final extension. PCR products were visualized in a 2% agarose gel stained

with a red-safe DNA-dying solution (GeNetBio, Korea). Positive control was obtained from College of Veterinary Medicine, University of Duhok, Iraq.

2.5. Antibiotic Susceptibility Test

Disc diffusion method was used to investigate the antibiotic susceptibility of *K. pneumoniae*-positive isolates (Hudzicki, 2009). The isolates were kept in 5 mL of autoclaved 0.85% NaCl and their densities were adjusted to about 1.5 x 10^8 CFU mL^{-1} (McFarland standards). The list of the antibiotics and their concentrations were given in Table 1. Sterile 6 mm filter paper disk impregnated with defined concentrations of antibiotics was placed on the agar plates inoculated with the bacterial suspension. The agar surface was evenly inoculated using sterile swabs to ensure confluent growth. The disks were placed in an even array on the plate with a disk dispenser at well-spaced intervals from each other. The plates were kept at 37°C for between 18 and 24 hours. Following the incubation, the plates were examined for the presence of inhibition zones (clear rings) around the antimicrobial disks. The inhibition zones were measured (mm) and interpreted according to CLSI 2021 guidelines (Hudzicki, 2009) with the quality control strain *K. pneumoniae* ATCC 25922. Multidrug resistance was evaluated as follows: MDR resistant to ≥ 3 antibiotic classes; XDR resistant to all but ≤ 2 antibiotic classes (Khdhir et al., 2026).

Table 1. Antibiotics and concentrations used in the study.

No	Antibiotics	Abbreviation	Disc load (µg)
1	Cephalothin	KF	30
2	Cephradine	CE	30
3	Piperacillin	PRL	100
4	Rifampin	RA	30
5	Amoxicillin/Clavulanic acid	AMC	20/10
6	Ampicillin	AM	25
7	Cefotaxime	CTX	30
8	Amikacin	AK	30
9	Trimethoprim/Sulfamethoxazole	SXT	1.25/23.75
10	Ceftriaxone	CRO	30
11	Ciprofloxacin	CIP	5
12	Doxycycline	DO	30
13	Gentamicin	CN	10
14	Tetracycline	TE	30
15	Imipenem	IPM	10

3. RESULTS and DISCUSSION

UTIs are among the most common infectious diseases in humans (Totsika et al., 2012). *K. pneumoniae* is one of the most important causes of UTIs (Cristea et al., 2017) because its multidrug resistance is associated with severe infections and responsible for high morbidity and mortality worldwide (Cristea et al., 2017; Navon-Venezia et al., 2017). The antibiotic sensitivity patterns of *K. pneumoniae* strains also vary with the widespread use of antimicrobial agents globally (Navon-Venezia et al.,

2017). Such circumstances make the antibiotic-based treatments against *K. pneumoniae* infections more difficult in the urinary tract system. In this study, a total of 25 *K. pneumoniae* strains were isolated from urine of 170 hospitalized patients with UTIs. Following species identification with VITEK 2 system, molecular characterization of *K. pneumoniae* strains was confirmed with a species-specific primer pair developed based on 16S-23S rDNA gene region, yielding a 130-bp DNA fragment (Liu et al., 2008), as shown in Figure 1.

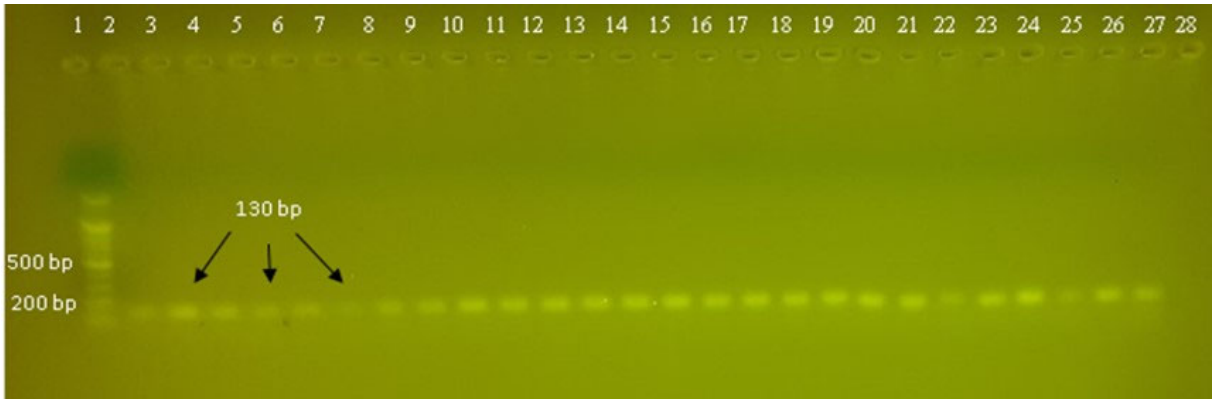

Figure 1. PCR confirmation of *Klebsiella pneumoniae* isolates. Line 1: DNA marker (100 bp); Line 2-26 are the samples of this study; Line 27 and 28 are positive and negative control, respectively.

The antibiotic susceptibility patterns of *K. pneumoniae*-positive isolates were evaluated against 15 antibiotics from different classes (Table 2). Resistance rates were markedly high among the antibiotics tested (Figure 2). All the tested isolates (100%) showed resistance to cephalothin, cephradine, and piperacillin. Similarly, a high level of resistance (96%) was also observed against rifampin, amoxicillin/clavulanic acid, and ampicillin. Resistance rate to cefotaxime and amikacin was 92%, which was followed by trimethoprim/sulfamethoxazole and ceftriaxone with an 88% of resistance rate. A moderate level of resistance was exhibited against ciprofloxacin and doxycycline with rates of 60 and 52%, respectively. On the other hand, low resistance rates were found for gentamicin, tetracycline, and imipenem with a resistance rate of 20, 16, and 4%, respectively (Figure 2 and Table 2). Notably, imipenem demonstrated the highest sensitivity in 96% (24/25) of the isolates. Intermediate resistance was found in only one isolate (K13) against rifampin.

Our results indicated a 96% resistance against both β-lactam antibiotics tested (amoxicillin/clavulanic acid and ampicillin). This finding is in good agreement with the results of a meta-analysis conducted in China in 2009-2013 with clinical *K. pneumoniae* strains, indicating a ~96% resistance for ampicillin (Wei et al., 2025). This ratio was almost the same in hypervirulent *K. pneumoniae* strains, with a resistance rate of 95.5% to ampicillin in another meta-analysis (Beig et al., 2024). The high resistance in *K. pneumoniae* against β-lactam antibiotics is due to the widespread production of the enzyme called β-lactamase that hydrolyzes the β-lactam ring of this class of antibiotics (Martin and Bachman, 2018). β-lactamases are

encoded by *TEM-1*, *TEM-2*, and *SHV-1* genes (Li et al., 2025). Mutations on these genes change the configuration of specific amino acids at the active sites of the β-lactamases, considerably reducing the affinity for β-lactam antibiotic groups, extending the antibiotic spectrum, therefore readily conferring resistance to enzyme inhibitors (Paterson & Bonomo, 2005; Li et al., 2025).

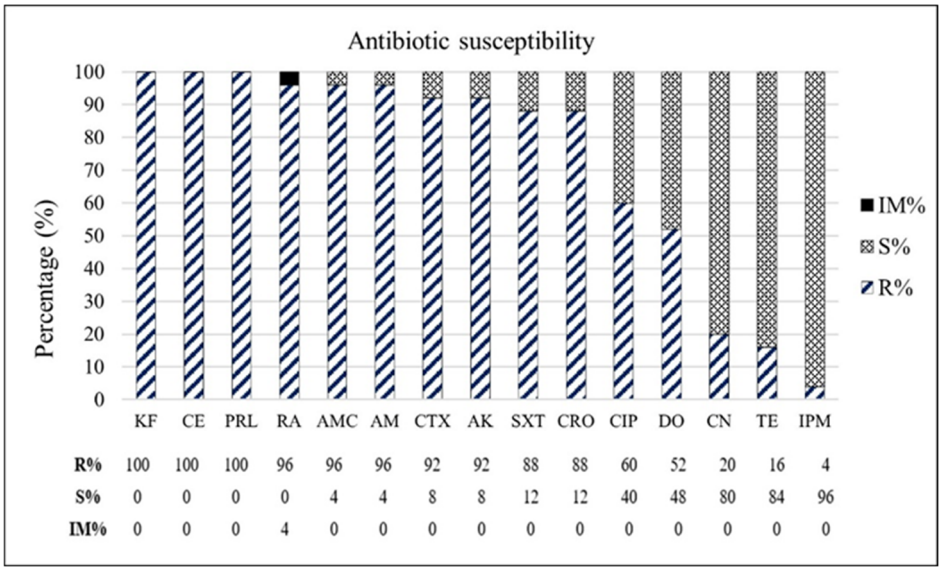

Figure 2. Antibiotic susceptibility of *Klebsiella pneumoniae* isolates (n=25) against 15 antibiotics tested. Cephalothin (KF), Cephradine (CE), Piperacillin (PRL), Rifampin (RA), Amoxicillin/Clavulanic acid (AMC), Ampicillin (AM), Cefotaxime (CTX), Amikacin (AK), Trimethoprim/Sulfamethoxazole (SXT), Ceftriaxone (CRO), Ciprofloxacin (CIP), Doxycycline (DO), Gentamicin (CN), Tetracycline (TE), and Imipenem (IPM). R, S, and IM mean resistant, sensitive, and intermediate, respectively.

In the current study, the resistance rate against third-generation cephalosporins (cefotaxime and ceftriaxone) was 92 and 88%, respectively. However, a retrospective analysis with *K. pneumoniae* isolates obtained between 1999 and 2010 years in United States indicated a ~30 and 89% resistance rate for cefotaxime and ceftriaxone, respectively (Braykov et al., 2013). The findings of the present work are highly consistent with that study for ceftriaxone but not for cefotaxime. Furthermore, a systematic review from China showed a 47% resistance to ceftriaxone (Wei et al., 2025). These different results may be explained by strain-to-strain variations in different sources (Alsarhan & Çam, 2023). In our study, a complete resistance to first-generation (cephalothin and cephradine) and a remarkably high resistance to third-generation cephalosporins may be attributed to the emergence and rapid dissemination of ESBL-encoding genes among Enterobacteriaceae (Pfeifer et al., 2010; Bush & Bradford, 2020). In a recent study, it was emphasized that plasmid-encoded ESBLs have now appeared worldwide with geographic preference for specific resistant phenotypes (Bush & Bradford, 2020). The enzyme ESBL is a rapidly evolving subclass of β-lactamases, which hydrolyzes extended-spectrum

cephalosporins and then makes the antibiotics largely ineffective (Paterson & Bonomo, 2005). The increasing prevalence of ESBL-producing *K. pneumoniae* strains over years explains their high resistance to both first- and third-generation cephalosporins in the present study (Wei et al., 2025).

A comprehensive meta-analysis on antibiotic susceptibility of *K. pneumoniae* strains from 17 countries demonstrated that the resistance rates to piperacillin, amikacin, trimethoprim/sulfamethoxazole, ciprofloxacin, doxycycline, gentamicin, and tetracycline were about 25, 41, 39, 46, 46, 36, and 59%, respectively (Beig et al., 2024). These rates in the current work are relatively higher as following; 100, 92, 88, 60, 52, 20, and 16%, respectively except gentamicin and tetracycline (Figure 2). Likewise, resistance to piperacillin was very high (~90%) in India as in the case of our finding but the reports in China showed a ~36% resistance to this antibiotic (Beig et al., 2024). Similar to our result (20%), gentamicin resistance was low (~30%) in a systematic review in China but amikacin resistance was remarkably low (~11%) in that study compared to our result (Wei et al., 2025). Similar scenarios apply to the trimethoprim/sulfamethoxazole, doxycycline, and tetracycline antibiotics (Beig et al., 2024). Country- and continent-based subgroup analysis indicated remarkable variations in the resistance patterns of multiple antibiotics in hypervirulent *K. pneumoniae* isolates across the different countries and continents (Beig et al., 2024). These differences may be because of the collection of *K. pneumoniae* isolates originating from different sources (Alsarhan & Çam, 2023). In that study, they emphasized that clinical and environmental strains can respond to antibiotic-based treatments differently since the co- and cross-selection of antimicrobial resistance genes among microorganisms depends on the source of pollution in environmental and clinical settings (Buelow et al., 2021). A recent meta-analysis conducted on a total of 177 studies reported that the highest antibiotic resistance rate for *K. pneumoniae* strains was observed in UTIs (Wei et al., 2025), which may explain the comparatively higher resistance rates of our clinical isolates against most antibiotics tested in this study.

Table 2. Antibiotic resistance profiles of 25 *Klebsiella pneumoniae*-positive samples against 15 antibiotics. Cephalothin (KF), Cephradine (CE), Piperacillin (PRL), Rifampin (RA), Amoxicillin/Clavulanic acid (AMC), Ampicillin (AM), Cefotaxime (CTX), Amikacin (AK), Trimethoprim/Sulfamethoxazole (SXT), Ceftriaxone (CRO), Ciprofloxacin (CIP), Doxycycline (DO), Gentamicin (CN), Tetracycline (TE), and Imipenem (IPM).

	Antibiotics	KF	CE	PRL	RA	AMC	AM	CTX	AK	SXT	CRO	CIP	DO	CN	TE	IPM	
Sample ID	K01	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	MDR
	K02	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	MDR
	K03	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	MDR
	K04	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	MDR
	K05	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	MDR
	K06	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	MDR
	K07	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S	MDR
	K08	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	MDR
	K09	R	R	R	R	S	S	R	S	S	R	S	S	R	R	S	MDR
	K10	R	R	R	R	R	R	R	R	S	R	R	R	S	R	S	MDR
	K11	R	R	R	R	R	R	R	R	S	R	R	S	S	R	S	MDR

K12	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	MDR
K13	R	R	R	IM	R	R	R	R	R	R	S	R	S	S	S	MDR
K14	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	XDR
K15	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	MDR
K16	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	MDR
K17	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	MDR
K18	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	XDR
K19	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	MDR
K20	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	MDR
K21	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	MDR
K22	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	MDR
K23	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	XDR
K24	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	MDR
K25	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S	MDR

R value	25	25	25	24	24	24	23	23	22	22	15	13	5	4	1
S value	0	0	0	0	1	1	2	2	3	3	10	12	20	21	24
IM value	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
R%	100	100	100	96	96	96	92	92	88	88	60	52	20	16	4
S%	0	0	0	0	4	4	8	8	12	12	40	48	80	84	96
IM%	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0

R, S, and IM mean resistant, sensitive, and intermediate, respectively. MDR and XDR indicate multidrug-resistant and extensively drug-resistant, respectively.

On the other hand, among *K. pneumoniae* strains, the highest sensitivity was found for imipenem, a class of carbapenems, with a 4% resistance rate (Figure 2). This is highly consistent with the finding of a meta-analysis covering the last 20 years in China, reporting a ~5% resistance rate (Wei et al., 2025). Low resistance to imipenem indicates that carbapenems are still among the most reliable treatment options in *K. pneumoniae*. Carbapenems are the last-resort β-lactams (Navon-Venezia et al., 2017) but imipenem resistance was found as ~45% in hypervirulent *K. pneumoniae* strains in the study conducted on 77 studies from 17 countries (Beig et al., 2024). Previously, the emergence of carbapenem-resistant *K. pneumoniae* strains has been reported globally, mainly due to the production of carbapenemase enzyme (Navon-Venezia et al., 2017; Chang et al., 2021; Li et al., 2023). The different types of carbapenemase are encoded, mostly, by some genes such as *blaKPC*, *blaNDM*, and *blaOXA* genes in *K. pneumoniae*, which was not studied in the present work, but they may be disseminated between the strains through carbapenemase-encoding plasmids and/or transposons (Navon-Venezia et al., 2017; Chang et al., 2021). The acquisition and upregulation of these genes, as well as mutations in them, contribute to carbapenem resistance in *K. pneumoniae* through the enhanced hydrolysis of carbapenems (Li et al., 2023).

In the current study, 88% of the isolates were MDR and 12% were XDR (Table 2), indicating that multidrug resistance is increasing significantly among clinical *K. pneumoniae* strains. Under antibiotic stress, *K. pneumoniae* strains accumulate antibiotic resistance genes by mutations or through acquisition of resistance gene-carrying plasmids or transposons, disseminating these genes through horizontal gene transfer mechanisms, leading to the incidence of multidrug-resistant variants, and being a health threat worldwide (Navon-Venezia et al., 2017). According to that study, the continuous global dissemination of these new phenotypes is a major source of human infections because of complicated treatment options in the

healthcare settings. The emergence of such resistant variants is expected to rise in the future around the globe due to the widespread use of antibiotics (Mohd Asri et al., 2021).

4. CONCLUSION

The present study revealed that the majority of clinical *K. pneumoniae* strains from urine of hospitalized patients with UTIs conferred an alarmingly high level of resistance against a wide range of antibiotics. Complete resistance was observed against first-generation cephalosporins and piperacillin. High resistance rates were also found for rifampin, β-lactams, third-generation cephalosporins, and amikacin. In contrast, the lowest resistance was against gentamicin, tetracycline, and imipenem. The resistance rate to imipenem was 4%, indicating that this carbapenem can be used as the most effective treatment option in *K. pneumoniae*-caused UTIs. All of our clinical strains were multidrug resistant. Multidrug resistance of this pathogen is highly correlated with severe infections, posing serious challenges to public health.

Conflicts of Interest

The authors declare no competing interests.

Ethical Approval

This study was approved with a reference #11112020-5-4 on 11/11/2020 by Research Ethics Committee of Ministry of Higher Education University of Duhok, Iraq.

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