REVIEW

Mechanisms of Antimicrobial Resistance in *Klebsiella*: Advances in Detection Methods and Clinical Implications

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Abstract: *Klebsiella spp.* are ubiquitous gram-negative bacteria, commonly present in natural environments and as part of the human microbiota. *Klebsiella* is involved in the occurrence and development of many diseases, and effective antibiotics for it have attracted the attention of researchers. In recent years, its multi-drug resistance, particularly to carbapenems and β-lactam antibiotics, has posed significant challenges to clinical treatment. Therefore, a comprehensive understanding of the resistance mechanisms of *Klebsiella*, along with the enhancement of detection methods, is crucial for effectively controlling the spread of drug-resistant strains and guiding individualized clinical treatment. This paper systematically reviews the epidemiological characteristics, resistance mechanisms, detection methods, and treatment strategies of *Klebsiella*, aiming to provide new insights for the clinical management of this pathogen.

Keywords: *Klebsiella*, drug resistance, detection method, β-lactam, carbapenems

Introduction

Klebsiella pneumoniae (KP) is a gram-negative, non-motile, capsulated opportunistic bacterium belonging to the *Enterobacteriaceae* family.[1] It is widely distributed in the environment, including soil, water, and plant surfaces, and is also commonly found on the skin, in the respiratory tract, and within the intestines of humans and animals.[2] *Klebsiella* was originally identified by German pathologist Carl Friedländer, who first described it in 1882. During his research on lung tissue samples from patients with pneumonia, he discovered a Gram-negative, short rod-shaped bacterium. Due to its strong association with certain cases of pneumonia, this bacterium is also referred to as "Friedlander pneumococcus". In 1896, the microbiology community named the genus *Klebsiella* in honor of Edwin Klebs, recognizing his significant contributions to medical microbiology. Klebs was a pioneer in the field of pathology and was among the first to apply bacteriology to the study of disease, and his research provided a theoretical foundation for the integration of bacterial taxonomy and pathology.[3,4] Subsequently, as medical research advanced, *K. pneumoniae* was recognized for its high pathogenicity and diverse infection routes, leading to a range of serious infections, including community-acquired pneumonia, urinary tract infections, gastroenteritis, septicemia, and liver abscesses, particularly in immunocompromised individuals,[5–8] this expanded understanding of its pathogenic scope suggested that *K. pneumoniae* is a pathogen with significant clinical importance.

In recent years, the widespread use of antibiotics has exacerbated the issue of *Klebsiella* resistance, and its antibiotic resistance has evolved from Multidrug Resistance (MDR) to Extensively Drug Resistance (XDR) and Pan-Drug Resistance (PDR).[9] Notably, hypervirulent *Klebsiella pneumoniae* (hvKP) not only possesses heightened virulence factors but also demonstrates increased drug resistance, complicating clinical treatment.[10] According to data from the Chinese Bacterial Resistance Surveillance (CHINET), the resistance rate of *Klebsiella* to carbapenem antibiotics, such as imipenem and meropenem, significantly increased between 2015 and 2019, although a decrease was observed in 2021.[11] These fluctuations indicate that resistance surveillance and management measures for *Klebsiella* require optimization and enhancement.

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Countries have developed their own policies for managing antimicrobial resistance in accordance with the WHO action plan. China's policy, known as the National Action Plan on Antimicrobial Resistance (2016–2020), includes several key initiatives: strengthening the surveillance of antimicrobial resistance, implementing a classification management system for antibiotics to standardize clinical use; restricting the use of growth-promoting antibiotics in aquaculture; and raising public awareness to reduce the self-purchase and use of antibiotics. In terms of medical institution management, the plan involves the formulation of an antibacterial drug prescription list and the implementation of special rectification actions to ensure the rational clinical use of antibiotics. Additionally, the management of resistance in the environment focuses on strengthening the monitoring and control of antibiotic pollution. Given that *Klebsiella* drug resistance poses a major threat to global public health, international health organizations and national centers for disease control have focused considerable attention on this issue. This paper reviews the classification, resistance mechanisms, virulence factors, and current treatment strategies for *Klebsiella*, aiming to provide valuable references for its prevention, control, and clinical application. By strengthening drug property monitoring, optimizing antibiotic usage, and developing new therapeutic methods, it is anticipated that the clinical challenges posed by *Klebsiella* can be more effectively addressed. This paper systematically reviews the epidemiological characteristics, resistance mechanisms, detection methods, and treatment strategies of *Klebsiella*, aiming to provide new insights for the clinical management of this pathogen.

Classification and Epidemiology of Klebsiella

Klebsiella pneumoniae (KP) can be classified into two pathogenic categories: classic *Klebsiella pneumoniae* (cKP) and hypervirulent *Klebsiella pneumoniae* (hvKP).[12] cKP is a prevalent pathogen in hospital-acquired infections, ranking second in infection rates only to *E. coli*, with a clinical detection rate of 6.69%. This pathogen is commonly found in the mouth, skin, digestive tract mucosa, and on the surfaces of medical equipment. It poses a significant risk to individuals with compromised immune systems, such as those with diabetes or malignant tumors, leading to severe pneumonia, urinary tract infections, respiratory infections, and other complications.[13,14] cKP exhibits high levels of drug resistance, particularly with respect to carbapenem antibiotics, where resistance rates exceed 10%.[9,15] Since the 1990s, hvKP has garnered increased attention as a notable cause of community-acquired infections, with a case fatality rate ranging from 3% to 32%.[16] Infections caused by hvKP are more frequently observed in otherwise healthy individuals and often result in invasive conditions, such as liver abscesses, endophthalmitis, and brain abscesses. Its high viscosity contributes to its propensity for causing such invasive infections. The identification criteria for hvKP include the wire drawing test,[17] capsule serotyping (eg, K1, K2),[18,19] and detection of virulence genes (eg *rmpA*/*rmpA2*). An hvKP infection is confirmed if two or more of these criteria are met.[20,21] hvKP infections tend to develop rapidly, exhibit stronger transmission, and result in more severe disease, thereby becoming a focal point of clinical concern.[22] Routine surveillance data revealed increasing rates of invasive extended-spectrum cephalosporin-resistant *Klebsiella pneumoniae* (ESCR-KP) in Switzerland, from 1.3% in 2004 to 8.5% in 2019.[23] A global exploration of 13,178 KP strains revealed that 7.8% of them were CR-hvKP (carbapenem-resistant hypervirulent *Klebsiella pneumoniae*).[24] In China, a significant study indicated that among the screened carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), as much as 36% carried hypervirulence factors.[25] This shows a significant risk to public health since these strains integrate resistance to potent antibiotics and increased pathogenicity.

Mechanism of Drug Resistance in Klebsiella

In recent years, the drug - resistance problem of *Klebsiella* has become increasingly severe. This is manifested in an increasing number of types of drugs that can be tolerated, and different species of *Klebsiella* exhibit species-specific resistance, for example, in the case of KP, genes like *blaKPC* and *blaNDM* confer resistance to β-lactams including carbapenems; *mcr-1* leads to colistin resistance; In *Klebsiella oxytoca, blaCTX-M* causes resistance to multiple β-lactams, and *aac(3)-II* results in aminoglycoside resistance; *Klebsiella granulomatis* may have *tet* genes, making it resistant to tetracycline.[1,26] An in - depth understanding of the drug - resistance mechanisms of *Klebsiella* is conducive to controlling its drug resistance.

The Production of Beta-Lactamase

β-lactam antibiotics encompass a range of compounds, including penicillin and its derivatives, cephalosporins, mono-amide rings, carbapenems, and penem enzyme inhibitors. These antibiotics function by binding to penicillin-binding proteins (PBPs), which disrupt the synthesis of bacterial cell walls.[27] However, Gram-negative bacteria can develop resistance by hydrolyzing these antibiotics through the production of ultra-broad spectrum beta-lactamases (ESBLs).[28,29] ESBLs are primarily transmitted via plasmids and are capable of hydrolyzing cephalosporins, monoamides, and penicillin antibiotics. They can be inhibited by clavulanate, which is classified as group 2be in functional classification and class A in molecular classification.[30] ZHANG et al found that 12.6% of the 230 strains of hvKP isolated were ESBL positive, with the *blaCTX-M* gene being the predominant genetic determinant. ESBL-positive strains carrying the *blaCTX-M* gene exhibit increased susceptibility in patients with a history of steroid therapy, neutropenia, or combination therapy.[31] SHANKAR et al and Qiu et al reported that the hvKP strains producing ESBL were predominantly of the serotype K54, with sequence types ST29 and ST2318.[32,33] Additionally, another study indicated that 56% of cKP strains and 17% of hvKP strains carried ESBLs.[34]

β-lactamase is a significant mechanism of bacterial drug resistance. Conventional β-lactamases can be categorized into chromosome-mediated and plasmid-mediated enzymes, and further classified based on their hydrolytic activity into penicillinases, cephalosporinases, broad-spectrum enzymes, and ultra-broad-spectrum enzymes.[35] Ultra-broad-spectrum β-lactamases (ESBLs) represent a critical resistance mechanism in *Klebsiella*; these plasmid-mediated enzymes, composed of serine protein derivatives, are capable of hydrolyzing penicillins, broad-spectrum and ultra-broad-spectrum cephalosporins, as well as monocyclic β-lactam antibiotics. The genotypes of ESBLs include TEM, SHV, OXA, CTX-M, PER, VEB, GES, IBC, among others. Notably, TEM and SHV are the most prevalent genotypes, with significant regional variations observed in different countries. For instance, SHV and CTX-M types are predominant in Italy, while SHV types are more common in Canada.[36] In China, CTX-M is the primary genotype, with CTX-M-3 and SHV-12 being dominant in Shanghai, whereas CTX-M types are predominant in Hangzhou. TEM-type ESBLs primarily derive from TEM-1, SHV-type ESBLs are predominantly found in *K. pneumoniae*, and CTX-M ESBLs exhibit resistance to ceftazidime while having a weaker hydrolytic ability against ceftaxime. The main drugs used to treat ESBL-producing bacteria are carbapenems and cephamycin antibiotics, with imipenem being the preferred choice due to its broad spectrum and high antibacterial activity.[37] In recent years, cases of ESBL-producing hypervirulent *Klebsiella pneumoniae* (hvKP) infections have been reported globally, indicating a growing concern regarding resistance to ESBLs.

Plasmid Mediated AmpC Enzyme

Plasmid-mediated resistance is a primary mechanism through which bacteria acquire antibiotic resistance. This resistance propagates among bacterial populations via plasmids—circular DNA molecules that carry resistance genes, thereby facilitating the rapid dissemination and long-term survival of resistant strains. Plasmids can spread among bacteria through various mechanisms, including conjugation, transformation, transduction, integrons, and transposons. Several factors contribute to the ability of plasmid-mediated resistance to promote the swift spread and persistence of drug-resistant strains: efficient transmission, strong adaptability, genetic stability, multi-resistance, and environmental transmission. The plasmids associated with *K. pneumoniae* drug resistance mainly include IncFII, IncL, IncX3, IncC and ColKP3. In addition, insertion sequences such as IS26 and transposon like Tn1546 also play important roles in the dissemination of *K. pneumoniae* drug resistance.[38–40]

Cephalosporinase (AmpC) is a significant β-lactamase produced by gram-negative bacilli, which can be mediated by both chromosomes and plasmids.[41] The plasmid-mediated expression of the AmpC enzyme in bacteria can result in resistance to first- to third-generation cephalosporins, cephalomycin, aztreonam, and other antibiotics; moreover, β-lactamase inhibitors are not effective against this enzyme.[42] AmpC enzymes are classified as Group 1 in the functional classification and Class C in the molecular classification. They can hydrolyze third-generation cephalosporins, cephalomycin (with the exception of ACC-1), and monocyclic β-lactam antibiotics, yet they remain sensitive to fourth-generation cephalosporins and carbapenems.

Resistance in *K. pneumoniae* is frequently linked to multiple germplasm and mobile genetic elements capable of carrying and transmitting antibiotic resistance genes. AmpC enzymes are commonly found in bacteria that do not

possess the inducible chromosomal genes encoding these enzymes, including *Escherichia coli, K. pneumoniae, Klebsiella acidogenes*, and *Enterobacter aerogenes*. The expression of the *AmpC* gene is regulated by various genes. *AmpD* belongs to the *ampC* gene cluster located on chromosomes. This cluster is often associated with induced drug resistance. *AmpR*, a promoter region about 100bp upstream of the *ampC* gene, controls the expression of the *AmpC* gene. *AmpG*, which is located in other regions of the chromosome, is responsible for transporting peptidoglycan fragments.[43] Research has demonstrated that certain strains, such as DHA-1- producing *Klebsiella pneumoniae*, exhibit reduced sensitivity to carbapenem drugs following the loss of outer membrane proteins.[44] Since the initial discovery of plasmid-mediated AmpC enzymes, such as MIR-1, in 1988, over 40 different genotypes have been reported. Studies indicate that plasmid-mediated enzymatic hydrolysis by AmpC enzymes has a broader spectrum, contributing to increased bacterial resistance to cefepime and other antibiotics.[45] Furthermore, plasmids encoding AmpC enzymes often coexist with other antibiotic resistance genes, including those conferring resistance to aminoglycosides, chloramphenicol, and sulfonamides, reflecting a pattern of multidrug resistance. Some bacteria are capable of simultaneously producing extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC enzymes, a phenomenon referred to as "super-broad spectrum β-lactamase".[46] This type of enzyme exhibits a wide range of degradation substrates and enhanced hydrolysis efficiency, which has significant epidemiological implications.

Carbapenemases

Carbapenems are atypical β-lactam antibiotics characterized by their broad antibacterial spectrum and potent antibacterial activity. Due to their stability against β-lactamases and low toxicity, carbapenems have emerged as one of the primary antibacterial agents for treating severe bacterial infections.[19] The production of *Klebsiella pneumoniae* carbapenemase (KPC) represents the principal mechanism by which *K. pneumoniae* develops resistance to carbapenem antibiotics.[47] These enzymes not only confer resistance to a wide array of β-lactam antibiotics but also exhibit resistance to β-lactam inhibitors.

Carbapenemase refers to a class of β-lactamases capable of hydrolyzing imipenem or meropenem. According to the Ambler classification (Table 1), it is primarily divided into Class A, Class B (which includes metallo-β-lactamases, or MBLs), and Class D enzymes. The most significant enzyme in Class A is KPC, which was first identified in North Carolina in 1996 and named KPC.[48] KPC-positive *Klebsiella pneumoniae* (KP) was first detected in China in 2004 in a 75-year-old ICU patient in Zhejiang Province. KPC-2 is the most prevalent carbapenemase type in China.[49] Class B metalloenzymes include NDM-1, IMP, VIM, among others, while Class D is primarily represented by OXA-48 enzymes. Studies have shown that resistance of carbapenemase - producing KP to carbapenem antibiotics has increased over the years, which is primarily because carbapenemases are generally plasmid - encoded enzymes, and the IncL/M plasmid is related to plasmids that carry the KPC-type carbapenemase gene (such as *bla_KPC*), which confers resistance to carbapenem antibiotics, and the types of plasmids involved in the propagation of the *bla_KPC* gene typically include the widely distributed IncF, IncI2, IncN, IncA/C, and pKpQIL-like plasmids, especially important in spreading *bla_KPC*

Table 1 Ambler Classification of Beta-Lactamases

Ambler Class	Enzyme Type	Key Active-Site Component	Common Representative Enzymes	Resistance Characteristics
Class A	Serine-beta-lactamases	Serine residue	KPC, ESBLs, TEM −1, SHV-1, CTX-M	Resistance to penicillins and early -generation cephalosporins. ESBLs can confer resistance to third-generation cephalosporins
Class B	Metallo-beta-lactamases	Zinc ion	NDM, IMP, VIM	Resistance to a wide range of beta -lactam antibiotics, including carbapenems
Class C	Serine-beta-lactamases	Serine residue	AmpC enzymes	Resistance to cephamycins and third-generation cephalosporins
Class D	OXA-type beta-lactamases	Serine residue	OXA-48-like enzymes	Resistance to oxacillin and some other beta-lactam antibiotics. Some can hydrolyze carbapenems

in *K. pneumoniae*.[38,39] According to the 2017 CHINET Bacterial Resistance Surveillance data, the resistance rates of KP to imipenem and meropenem rose from 3.0% and 2.9% in 2005 to 20.9% and 24.0% in 2017, respectively. Carbapenemase-producing strains exhibit resistance not only to carbapenem antibiotics, but also to penicillins, cephalosporins, and monobactams.

Beta-Lactamase Resistant to Enzyme Inhibitors

Enzyme-resistant beta-lactamases (IRBLs) are classified as Class A in molecular classification and group 2br in functional classification. These enzymes primarily arise from 1–3 mutations in the *TEM-1, TEM-2*, and *SHV-1* genes at specific amino acid sites. Other variants include SHV-10, SHV-49, OXY-2, and OXA-type β-lactamases. The amino acid replacement sites of IRBLs are commonly found at positions 69, 130, 244, 275, and 276 of TEM-1 and TEM-2. Mutations at these sites can significantly decrease the affinity for β-lactam antibiotics and alter the interaction with enzyme inhibitors, particularly at sites 69 and 244, which are located at or near the active site of TEM enzymes. Such mutations can confer resistance to enzyme inhibitors.[50,51] IRBLs can readily induce resistance in *Klebsiella pneumoniae* to amoxicillin, ticarcillin, and various enzyme inhibitors (such as clavulanate and sulbactam); however, they remain sensitive to narrow-spectrum cephalosporins, 7-alpha-methylcephalosporins, and oxyiminocephalosporins.

Gene Mutation and Horizontal Gene Transfer

The Role and Change of Membrane Porins

The extracellular lipid layers of Gram-negative bacteria, such as KP, contain numerous pores formed by various microporins, including OmpK35, OmpK36, and OmpK37. These pores serve as water-soluble diffusion channels that are non-specific and facilitate the crossing of the cell membrane.[52] Some beta-lactamases can penetrate the bacterial interior through these channels, thereby exerting their effects. However, alterations or the absence of microporous proteins can impede the penetration of antibacterial drugs into the bacterial cell, leading to drug resistance. OmpK35 is identified as the primary membrane porin associated with bacterial drug resistance, while OmpK37 is typically expressed at low levels or not at all. Changes in porin structure enhance bacterial resistance, and this resistance is more pronounced when inactivated enzymes coexist with altered porins, compared to the effects of inactivated enzymes alone. Webster et al demonstrated that the absence of OmpK35 and OmpK36 can result in meropenem resistance in KPN.[53] Conversely, Yang et al indicated that while the loss of membrane porins elevates the minimum inhibitory concentration (MIC) of carbapenem antibiotics, it does not necessarily confer imipenem resistance.[54]

Carbapenems represent the "last line of defense" among beta-lactam antibiotics and are utilized to treat Gram-negative bacterial infections that exhibit resistance to other antibiotic classes. However, inappropriate or excessive use of these agents can accelerate the emergence and dissemination of resistance. Many Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, inherently possess low levels of beta-lactamase genes, including *AmpC*. Under the selective pressure of carbapenems, these genes may undergo mutations that enhance their efficacy, leading to the production of ultra-broad spectrum β-lactamases or carbapenemases. Carbapenemase production is the primary mechanism by which bacteria develop resistance to carbapenem antibiotics. The genes encoding these enzymes, including *bla_NDM-1, bla_KPC*, and *bla_OXA-48*, are typically found on plasmids, facilitating their rapid spread through horizontal gene transfer.

Additionally, bacteria may diminish the permeability of carbapenems by down-regulating the expression or mutating porins. This mechanism often co-occurs with carbapenemase production, thereby further augmenting drug resistance. Mutations in porin genes may manifest as point mutations, small fragment deletions, whole gene deletions, or insertions. The most prevalent mutation involves insertions that disrupt porin synthesis, leading to reduced or absent porin levels.[52,55] The combination of porin deletion and β-lactamase activity can contribute to carbapenem resistance. Research has shown that *K. pneumoniae* strains with a deletion of OmpK36 expression, coupled with the production of SHV-2 extended-spectrum beta-lactamases (ESBLs), exhibit resistance to ketomycin, β-lactams, enzyme inhibitors, and carbapenems.[55]

Efflux Pump and Gene Mutation

The efflux pump is a significant mechanism of resistance in KP.[56,57] This organism possesses an energy-dependent protein efflux system located in its inner membrane, which actively expels drugs that infiltrate the bacteria. When specific regulatory mechanisms within the bacteria are altered, leading to enhanced expression of the active efflux system, the concentration of drugs within the bacteria may become insufficient to exert an antibacterial effect, thereby resulting in drug resistance.

The OqxAB efflux pump in KP is a key part of the multidrug - resistance mechanism. It reduces the intracellular concentration of β - lactam antibiotics, boosting the efficiency of β - lactam enzymes in degrading these drugs. When bacteria have both the OqxAB pump and β - lactam resistance genes, their resistance to β - lactam antibiotics rises significantly. This is often seen when the OqxAB pump works with down - regulated or mutated membrane porin expression, thereby reducing antibiotic penetration. OqxAB efflux pumps often co - exist on the same plasmid as other resistance genes like *bla_CTX - M, bla_KPC*, and *aac(6') - Ib - cr*. This not only lowers antibiotic levels by efflux but also heightens resistance through other means such as enzymatic degradation.[56] Furthermore, the OqxAB efflux pump can decrease quinolone concentrations by expelling them, thus acting synergistically with mutations in DNA cyclotase. Such synergistic mechanisms are particularly prevalent in drug-resistant strains of *Salmonella* and *K. pneumoniae*.

The expression of the OqxAB efflux pump is often upregulated during biofilm formation, which further enhances bacterial tolerance to antibiotics. The interplay between the biofilm's barrier function and the efflux pump complicates the treatment of bacterial infections.[58] Additionally, OqxAB efflux pumps may share substrates or energy sources with other toxic secretion systems, thereby bolstering bacterial viability. For instance, efflux pumps can diminish bacterial sensitivity to environmental toxins, such as bile salts, and enhance their capacity for colonization within the host. Due to its non-specific nature, this system encompasses a broad range of efflux substrates, including beta-lactams, quinolones, macrolides, and other antibacterial agents. Consequently, multiple active efflux systems often coexist within the same bacterial strain, contributing to resistance against various antibacterial drugs with distinct structures.

Research has identified AcrAB-TolC as the primary efflux system in bacteria, which is prevalent in KP and is a major contributor to its multidrug resistance.[59] Furthermore, studies indicate that mutations in the negative regulatory *ramR* gene, along with overexpression of the positive regulatory *ramA* gene—both of which are associated with the expression of the AcrAB efflux pump—can lead to increased expression of the AcrAB efflux pump, thereby enhancing the resistance of beta-lactam producing bacteria to beta-lactam antibiotics.[60,61] Additionally, the OqxAB efflux pump gene was initially believed to reside on the *K. pneumoniae* chromosome; however, it has since been recognized as a plasmid-mediated quinolone resistance gene capable of transferring between different bacterial species.

Topoisomerase and Gene Variation

In KP, the structural conformation of the *parC* gene, which encodes topoisomerase IV, and the *gyrA* gene, which encodes DNA gyrase, is susceptible to alteration when the targets of these two enzymes are mutated.[62] This alteration leads to the destabilization of the enzyme-DNA complex with antibiotics, ultimately resulting in quinolone resistance in KPN. Variation in the *gyrA* gene plays a predominant role in target gene variation, with common loci of variation including Ser83 and Asp87 of *gyrA*, as well as Ser80 and Glu84 of *parC*. Additionally, the plasmid-mediated drug resistance gene *qnr* specifically interacts with topoisomerase IV by encoding a protein that diminishes the efficacy of quinolones, thereby contributing to bacterial drug resistance.

Mechanism of Polymyxin Resistance

In polymyxin antibiotics, the primary mechanism of KP resistance is attributed to chromosomal mutations that facilitate target modification via the lipopolysaccharide modification system.[63] In strains possessing this system, alterations in the lipopolysaccharide structure result in a reduction of anionic charge, effectively impeding the binding of polymyxins. The typical modifications observed in lipopolysaccharides primarily stem from mutations in core genes, which lead to lipid A maturation, lipid A neutralization, and reactions involving phosphoethanolamine or palmitolipid binding.[64] Furthermore, the enhanced activity of lipopolysaccharide-modifying gene regulators, along with other mutations, can directly cause an abnormal increase in *pmrB* expression or a loss of activity in *mgrB*, both of which may contribute to polymyxin resistance in these strains. See Table 2 for details.

Table 2 Klebsiella Specific Drug Resistance Phenotypes/Genotypes

Category	Phenotype	Genotype
β-lactam antibiotics	Resistant to penicillin, cephalosporins and carbapenems	ESBL, KPC, NDM, OXA-48
Aminoglycosides	Amikacin resistant, gentamicin	AMEs, aac(6')-Ib
Polymyxins	Polycolistin B and polycolistin E	mcr
Sulfonamides and tetracyclines	Sulfamethoxazole and tetracycline	Sul, tet

Klebsiella Resistance Detection Method

Klebsiella resistance detection is an important link in clinical microbiology. It is often used to assess the susceptibility of the bacteria to different antibiotics, thereby helping to guide clinical treatment. The following are several commonly used detection methods (Table 3):

Traditional Drug Susceptibility Test

The traditional testing methods for KP primarily include the disk diffusion method, agar dilution method, broth dilution method, and concentration gradient method, among other classical approaches. These methods typically require a detection time of 16 to 24 hours and are commonly employed for the phenotypic screening of clinical drug-resistant strains. Their advantages include convenience, sensitivity, reproducibility, and low cost. However, these classical methods are limited to the phenotypic detection of bacterial resistance and do not allow for the identification of drug-resistance genes. The disk diffusion method, also known as the Kirby-Bauer (K-B) antibiotic test, involves placing a paper disc containing a specific concentration of antimicrobial agents on an MHA agar plate inoculated with KP.[65] The antimicrobial agents diffuse into the surrounding medium, creating one dimension, while the bacteria on the surface of the agar plate grow, produce resistant enzymes, and spread into the medium, forming a second dimension; hence, it is referred to as a two-dimensional test.[66] The plates are incubated at 37°C for 16 to 18 hours. Following incubation, the diameter of the antibacterial zone surrounding the paper disc is measured, allowing for the determination of bacterial sensitivity to the drug based on the size of this zone.

Table 3 Klebsiella Resistance Detection Method

Method	Process/Principle	Advantages
Broth Dilution Method	Various concentrations of antibiotics are prepared, following the addition of a standardized KP suspension, then incubated at 37 °C for 16 to 20 hours	Global standardization, quantitative and sensitive for detection the lowest inhibitory concentration (MIC) value of KP.
AGAR Dilution Method	Adding different concentrations of antibiotics to AGAR, followed by the inoculation of KP.	Simple, low cost, anaerobe-friendly,
Polymerase Chain Reaction	CRKP DNA was extracted by boiling method, PCR products were sequenced and compared with GenBank database	High-sensitivity, high-specificity, rapid, contribute to the development of effective clinical treatments
Pulsed Field Gel Electrophoresis	Bacterial genomic DNA is embedded in an AGAR block, enzymically cut it with appropriate endonuclides, and then the fragments are separated using a specialized electrophoresis system	For epidemiological investigation
Multilocus Sequence Typing	Sequence analysis involving conserved genes	Improve the accuracy of CRKP strain identification and classification
The Carba NP assay	Imipenem was reacted with the crude enzyme extract obtained after bacterial cell lysis	Detection time reduction
Mass spectrometry	Bacteria was identified by characteristic set of proteins with specific masses and charges	Fast, high sensitivity and specificity for detection CRKP

The broth dilution method is a traditional technique for detecting bacterial resistance. In this method, various concentrations of antibiotics are added to broth containing KP, which is then incubated at 37 °C for 16 to 20 hours following the addition of a standardized bacterial suspension. The minimum inhibitory concentration (MIC) is determined by observing bacterial growth. The microbroth dilution method is currently recognized as the international reference method for drug susceptibility testing, as established by the Clinical and Laboratory Standards Institute (CLSI). Its primary advantages include the global standardization of antimicrobial susceptibility tests, the straightforward process of inoculation and interpretation, and the ability to test multiple antimicrobial agents simultaneously against a single strain. However, a notable disadvantage is the significant variability in MIC values, which can result from technical discrepancies; thus, it requires operators to possess high experimental skills and a strong awareness of aseptic techniques. Conversely, the broth macro dilution method is an easily standardized and reliable reference method, offering substantial value and significance for interpreting the MIC values of drugs against specific bacterial strains in research and testing contexts.

The AGAR dilution method involves adding different concentrations of antibiotics to AGAR, followed by the inoculation of KP. After 48 hours of culture, colony-forming units (CFU) are counted.[66,67] Additionally, KP can be inoculated onto chromogenic AGAR medium, which allows for faster detection and screening of relevant drug-resistant bacteria, as sensitive strains exhibit a color response within 18 to 24 hours. Such methods are well-suited for multi-bacterial cultures and can effectively distinguish between strains and species. The AGAR dilution method is advantageous due to its simplicity, low cost, and the stability and reliability of the minimum inhibitory concentration (MIC) values. Epsilometer testing (E-test) employs a series of pre-prepared antibacterial drugs with a continuous exponential increase in concentration dilution on one side, while the other side features a plastic strip marked with MIC values on the concentration scale.[66] This strip is placed on an MHA AGAR plate inoculated with KP, and the MIC values on the strip are read after the culture period. The stable antibiotic concentration gradient on the E-test strip renders this method simple, sensitive, and accurate, allowing for precise reading of specific MIC values.

Application of Molecular Biology Techniques

Polymerase Chain Reaction (PCR) is a highly efficient and sensitive molecular biological technique used for amplifying specific DNA fragments.[68] In this study, CRKP DNA was extracted using the boiling method, followed by the application of specific primers to amplify gene fragments associated with drug resistance, including TEM, SHV, CTX-M, PER, VEB, DHA, MIR/ACT, KPC, IMP, VIM, SPM, GIM, and NDM-1. Subsequently, the PCR products underwent DNA sequencing and were compared against the GenBank database to identify specific resistance genotypes. This process provides a precise molecular basis for identifying drug resistance in bacteria, which can aid in the development of effective clinical treatments. Furthermore, through sequence analysis and the judicious design of PCR primers, the distribution and evolution of drug-resistant genes can be further investigated, enhancing the ability to monitor and control multi-drug-resistant strains.

Pulsed Field Gel Electrophoresis (PFGE) is a high-resolution molecular typing technique commonly employed for the analysis of bacterial genomes, particularly for the separation of large DNA fragments ranging from 50 kb to 10 Mb.[69] The fundamental principle involves embedding bacterial genomic DNA in an agar block, enzymatically cleaving it with appropriate endonucleases, and subsequently separating the fragments using a specialized electrophoresis system. In contrast to conventional gel electrophoresis, PFGE enhances the separation of large DNA fragments by alternating the direction of the electric field and varying the pulse time. According to the typing criteria proposed by Tenover et al,[70] PFGE maps can facilitate the typing analysis of strains. The specific criteria are as follows: if the PFGE maps of different strains exhibit the same number and size of bands, they are classified as belonging to the same type; if the differences in the map result in a change of 1 to 3 bands, this is regarded as a change in subtype. Conversely, if 4 to 6 bands differ in the map, the strains are considered to belong to different types. If seven or more bands differ, these strains are typically regarded as uncorrelated. PFGE is frequently utilized in epidemiological investigations, particularly in tracking outbreaks of pathogens and studying the relationships between different strains.[71] For instance, in the context of bacterial food poisoning or nosocomial infection outbreaks, PFGE can aid in tracing the source of the infection and determining whether different pathogens originate from the same strain or from distinct strains. In studies of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), PFGE is extensively employed to monitor the transmission pathways of various strains and to elucidate the genetic

variation among these strains. The integration of PFGE with Multi-Locus Sequence Typing (MLST) can provide a comprehensive scientific foundation for the prevention and control of the spread of drug-resistant strains.

Multilocus Sequence Typing (MLST) is a primary method in bacterial molecular epidemiology that involves the sequence analysis of conserved genes.[72] In the molecular epidemiological study of carbapenem-resistant CRKP, seven steward genes of *K. pneumoniae* (*gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB*) were amplified and sequentially analyzed. The sequence results of each gene's fixed region were then compared with sequences in the database to identify the Sequence Type (ST). The predominant genotypes identified were ST11 and ST15, which exhibited high frequency. Drug resistance genes encoded by ST11 include KPC-2, TEM-1, DHA-1, and SHV-11, among others. These genes are significant contributors to carbapenem resistance in K. pneumoniae, complicating the treatment of such bacterial infections. In addition to ST11 and ST15, other sequence types, such as ST395 and ST273, also hold important clinical and epidemiological significance. Different transmission pathways and drug resistance mechanisms may exist among these sequence types, highlighting the importance of studying them. Moreover, combining the MLST method with other molecular biology techniques, such as whole genome sequencing (WGS) and pulsed-field gel electrophoresis (PFGE), can enhance the accuracy of identification and classification of CRKP strains. This multi-technology approach not only aids in a comprehensive understanding of the genetic background and evolutionary relationships of resistant strains but also provides critical information for developing new diagnostic tools and treatment options. Overall, the application of MLST in the molecular epidemiology of CRKP offers robust technical support and a scientific foundation for addressing the challenges posed by drug resistance.

Emerging Rapid Detection Methods

The Carba NP assay utilizes a crude extract of enzymes obtained after bacterial cell lysis to react with imipenem.[73] Carbapenemases can hydrolyze carbapenem antibacterial agents, leading to ring-opening and the subsequent production of hydrogen ions (H+). A specific concentration of H+ can alter the color of the phenol red indicator in the detection solution, allowing for the determination of carbapenemase production based on the observed color change. The types of carbapenemase-producing strains are further identified using various enzyme inhibitors. This detection method is characterized by its simplicity, rapidity, sensitivity, and high specificity, particularly for the detection of KPC and metalloenzymes; however, it may yield false negative results for OXA-48 carbapenemase.[74] Commercially available kits can significantly reduce the testing time from 2 hours to 15 minutes.[75]

Mass spectrometry (MALDI-TOF) employs laser desorption ionization technology to swiftly identify carbapenem-resistant gram-negative bacteria by detecting specific protein signals.[76] The detection time ranges from 30 minutes to 2 hours, and this technology has been implemented in numerous hospitals to rapidly screen for pathogens associated with nosocomial infections, facilitating timely treatment interventions. The enzyme-linked immunosorbent assay (ELISA) detects carbapenemase within minutes through an antigen-monoclonal antibody reaction, achieving sensitivity and specificity rates exceeding 90%.[55] This method enables the simultaneous detection of KPC, NDM, VIM, IMP, and OXA-48 carbapenemases. Although the ELISA method is straightforward and easy to interpret, it is associated with a high cost, which may limit its accessibility for early and rapid diagnosis and treatment of high-risk patients.

Comparison and Evaluation of Detection Methods

In gene detection and phenotype assessment, various methods possess distinct characteristics that render them suitable for different application scenarios.[77] As a phenotypic detection approach, the traditional drug sensitivity test is limited to detecting a small number of antibacterial drugs, and it is both time-consuming and labor-intensive, particularly for certain bacteria that necessitate specialized growth environments. The MILST method demonstrates exceptional performance in genetic analysis, enabling the detection of multiple genotypes (eg ST11, ST15). Its advantages include good repeatability, high resolution, stable results, and ease of standardization; however, its high cost and limited applicability are notable drawbacks. FOUNOU et al utilized whole-genome sequencing (WGS) to analyze an ultra-broad-spectrum β-lactamase-producing K. pneumoniae strain isolated from a South African patient.[77] The whole-genome sequencing analysis confirmed the observed resistance, revealing that the strain harbored multiple beta-lactamase genes along with various other resistance genes. All *K. pneumoniae* isolates contained multiple multidrug-resistant (MDR) efflux pump genes,

including CmeA, CmeB, MATE, MFS, MacA, MarcB, MarA, OML, RND, AcrB, and AcrAB. These MDR efflux pumps confer resistance to several antibiotic families, including tetracyclines, fluoroquinolones, macrolides, tigecyclines, and beta-lactam antibiotics.

Due to its strong specificity and high sensitivity, the PCR method is widely employed to amplify drug-resistant genes and determine genotypes, such as TEM, SHV, and CTX-M. Despite its complexity and high cost, researchers favor this technique because of its robust specificity, high sensitivity, and low purity requirements for specimens. The PFGE method is primarily utilized for homology analysis and is regarded as the "gold standard" in bacterial molecular biotyping; however, improvements in its stability and standardization are still necessary. The RT-PCR method is used to analyze transcription products and drug resistance mechanisms of genes, demonstrating high sensitivity and broad applicability, although it has limitations when processing certain biological samples.[78]

Both the double-disk method and the three-dimensional test are employed to detect lactamase. The double-disk method is straightforward yet sensitive, whereas the three-dimensional test, while more reliable, is also more complex. The Carba NP test is rapid and easy to interpret, allowing for the detection of various carbapenemase enzymes; however, some strains yield ineffective results and are unable to detect the chromosomally encoded OXA carbapenemase.

Integration of Resistance Mechanisms and Detection Methods

Influence of Drug Resistance Mechanisms on Detection Strategies

The known resistance mechanisms of KP primarily include β-lactam enzymes, such as extended-spectrum β-lactamases (ESBLs) and carbapenemases, which hydrolyze and inactivate β-lactam antibiotics.[79] Additionally, alterations in membrane porins decrease drug entry channels into the cells, thereby reducing the intracellular concentration of the drugs. Enhanced efflux pumps further diminish drug effectiveness by expelling the drugs from the cell. Furthermore, genetic mutations in genes such as *gyrA* and *parC* lead to variations in the antibiotic target sites, preventing effective binding of the antibiotics.

Traditional methods for detecting bacterial resistance can provide phenotypic information; however, they require extended periods for culture and identification and are unable to detect resistance genes. In contrast, new technologies utilizing physical, chemical, and molecular biological methods offer shorter detection cycles and higher sensitivity, yet they cannot identify unknown resistance genes or mutated genes. High-throughput sequencing technology enhances the phenotypic detection capabilities of classical methods by accurately identifying bacterial drug resistance genes and discovering novel drug resistance genes and mutation sites.

PCR and RT-PCR assays were employed to detect specific resistance genes, including extended-spectrum beta-lactamases (ESBLs) and carbapenemase genes such as KPC, NDM, and VIM. The high specificity and sensitivity of PCR make it the preferred method for identifying drug-resistant genes.[80] Pulsed-field gel electrophoresis (PFGE) is utilized for homology analysis, which helps determine the homology and transmission routes of *K. pneumoniae*. This analysis is crucial for tracing the source of infections in hospitals and for developing effective prevention and control measures. The Carba NP test is used to identify the phenotype of carbapenemases. Additionally, two-disc and three-dimensional tests are employed to detect AmpC and ESBL enzymes. Although the three-dimensional test yields more reliable results, it is more complex to perform. Conversely, while the two-disc method is easier to operate, it exhibits lower sensitivity.

Optimize Clinical Treatment Strategies Based on Test Results

Understanding the resistance mechanisms of *K. pneumoniae* is crucial for selecting appropriate detection methods that can facilitate the rapid and accurate diagnosis of drug-resistant strains, thereby providing essential guidance for clinical treatment. Initially, therapeutic drugs should be selected based on the results of drug sensitivity tests to prevent the misuse of antimicrobials, particularly broad-spectrum agents, which can accelerate the development of bacterial resistance. Techniques such as PCR and RT-PCR enable the prompt identification of specific resistance genes in strains, allowing for the avoidance of ineffective medications. The choice of antibiotics should be informed by the identified resistance mechanisms (eg extended-spectrum beta-lactamases (ESBLs), carbapenemases). For instance, in the case of ESBL-producing KP, carbapenem antibiotics may be appropriate, while for carbapenemase-producing strains, drugs such as tigecycline or polymyxins should be considered.[81,82] Furthermore, homology analysis using pulsed-field gel

electrophoresis (PFGE) and other methodologies can be employed to trace the transmission pathways of drug-resistant bacteria, necessitating isolation measures to curb the spread of nosocomial infections. Regular monitoring of drug resistance patterns in pathogenic bacteria during treatment is essential, allowing for timely adjustments to treatment plans to prevent the further dissemination of drug-resistant strains.

The combination drug strategy, which represents the latest approach to treating patients with carbapenem-resistant Enterobacteriaceae (CRE) infections, advocates for the use of combination therapy to prevent or delay the emergence of resistant bacteria during antimicrobial treatment.[83] This strategy leverages the synergistic action between drugs to enhance therapeutic efficacy and mitigate the selection pressure for drug-resistant bacteria. In an experiment conducted by Ji et al[84] the results of drug sensitivity tests indicated that the combination of cefepime with amoxicillin and clavulanate achieved a significant synergistic effect in vitro, thereby reducing the minimum inhibitory concentration (MIC) of the respective antibacterial agents when the pathogens exhibited high resistance to carbapenems and cephalosporins. Additionally, other studies have demonstrated that tigecycline and polymyxins also exhibit synergistic effects in vitro.[85]

Phage therapy can be classified into two categories: lytic phages and lysogenic phages, based on their distinct life cycles. Lytic phages, in particular, are promising candidates for clinical treatment.[86] Research indicates that the lytic phage KPO1K2 effectively eradicates biofilms produced by *K. pneumoniae*, and when administered in conjunction with ciprofloxacin, it can significantly reduce the emergence of drug-resistant mutant strains.[66] In Western countries, phage therapy has re-emerged as a viable alternative to antibiotics for managing complex infections.[87,88] A notable case report documented the successful treatment of a patient with a prosthetic infection caused by KP.[89] pneumoniae using personalized intravenous phage therapy, which ultimately prevented the need for amputation. Furthermore, bacteriophage PSD has the ability to degrade bacterial polysaccharides externally; however, its application in vivo has thus far been restricted to animal studies. Tu et al demonstrated that this enzyme can markedly enhance the survival rate of mice infected with high doses of *K. pneumoniae* by analyzing the molecular structure and mechanism of PSD within the K1 type capsule.[90]

The emergence of resistance to *K. pneumoniae* presents a significant challenge in clinical treatment. The substantial financial investment and lengthy research and development cycle associated with new antimicrobial drugs have resulted in the rate of drug resistance outpacing the development of new antibiotics.[91] Currently, the predominant approach in the research and development of new antibacterial agents involves the extraction of natural products or the artificial synthesis of novel chemical structures targeting bacterial infections. Subsequent development is guided by the antibacterial efficacy of these compounds. Overcoming bacterial resistance is critical for drug development, and progress has been made in identifying potential metallo-beta-lactamase inhibitors, including phthalic acid, mitoxantrone, and sulfonyl-triazole analogues.[92] Furthermore, some studies have suggested that amlodipine and pantoprazole may partially reverse the resistance of Acinetobacter baumannii to imipenem; however, further investigation is required to determine whether amlodipine and pantoprazole are effective as efflux pump inhibitors (EPIs) for KP.[93]

Antibody therapy, it has been mentioned in studies that by inhibiting anti-alphagal antibodies in human serum, it can increase the bactericidal ability against KP and multi-drug resistant strains.[94] This suggests that by modulating the interaction of antibodies with bacteria, it may help to improve the effectiveness of treatment. Another study conducted the development of a monoclonal IgG3 antibody 8E3 against *K. pneumoniae* K2 capsular polysaccharide.[95] This antibody is specific and is able to recognize and bind to the bacteria's capsule polysaccharide, which may help neutralize the bacteria and prevent infection. Monoclonal antibodies that act directly on the K1 serotype or the LPS-O antigen portion have been successfully used to prevent and treat hvKP infection.[96]

Advantages and Challenges

Klebsiella, a prevalent pathogen, exhibits a variety of drug resistance mechanisms, including the production of β-lactamases, alterations in outer membrane proteins, and the activation of drug efflux pumps. These mechanisms provide substantial material for the study of microbial resistance. In terms of detection methods, molecular biology technologies have made significant advancements in recent years. The application of polymerase chain reaction (PCR), gene sequencing, and mass spectrometry has enhanced the sensitivity and accuracy of drug resistance gene detection. This not only improves the efficiency of clinical diagnosis but also provides a foundation for the formulation of individualized

treatment plans. In clinical applications, accurate detection methods enable physicians to swiftly identify drug-resistant strains, allowing for the selection of appropriate antibiotics for treatment. This approach reduces the unnecessary use of broad-spectrum antibiotics and mitigates the risk of further dissemination of drug-resistant strains. Furthermore, understanding the resistance mechanisms of Klebsiella contributes to the development of new antimicrobial agents and treatment strategies, thereby advancing the fields of medicine and pharmacy.

Despite numerous advancements in research and practice, the application of Klebsiella resistance mechanisms and their detection methods continues to encounter several challenges. First, the diversity and complexity of drug-resistant strains complicate research efforts, as different strains may develop resistance through various mechanisms, thereby complicating detection and diagnosis. Second, while current detection methods exhibit sensitivity, considerations regarding cost and time remain critical in clinical applications, particularly in medical settings with limited resources. Striking a balance between accuracy and cost-effectiveness in detection is an urgent issue that requires resolution. Furthermore, as resistant strains evolve, new resistance mechanisms may emerge, necessitating continuous updates to detection methods and diagnostic criteria to address these evolving challenges. To effectively curb the spread of drug-resistant strains, it is essential to enhance multidisciplinary cooperation and raise awareness among both the public and medical personnel.

Conclusion and Prospect

The issue of multidrug resistance in *K. pneumoniae* presents a significant challenge to global public health and clinical treatment. This paper provides an in-depth exploration of the epidemiological characteristics and complex resistance mechanisms associated with KP, highlighting the crucial roles of drug sensitivity testing and PCR technology in resistance detection. Although current treatment strategies, such as combination therapies, phage therapy, novel antimicrobial agents, and antibody therapies, have shown promise in mitigating KP resistance to some extent, further research and clinical validation are essential. In the long term, as the efficacy of existing antibiotics diminishes, the development of new antibacterial agents becomes increasingly vital. This development should not only aim to overcome the limitations of traditional antibiotics but also consider their environmental and ecological impacts. Bacteriophage therapy, recognized for its targeted approach and minimal side effects, has demonstrated considerable potential for application. However, despite encouraging results from laboratory studies, its safety and efficacy in clinical settings require validation through extensive clinical trials. Antibody therapy has also revealed distinct advantages in combating bacterial infections. Future research should focus on enhancing the development of anti-KP specific antibodies and optimizing their production and application strategies to improve clinical outcomes. Addressing the issue of resistance in KP necessitates close collaboration among clinical medicine, microbiology, pharmacy, and public health, alongside policy support from governments and relevant institutions to facilitate research advancements and effective clinical applications.

Future research must focus on multiple intertwined aspects to comprehensively address the challenges posed by KP. Firstly, efforts should be dedicated to enhancing the development of anti - KP specific antibodies and refining their production and application strategies. This is crucial as it directly contributes to improving clinical outcomes. Simultaneously, combating KP resistance demands a collaborative approach involving clinical medicine, microbiology, pharmacy, and public health. This multi - disciplinary cooperation, complemented by strong policy support from governments and relevant institutions, is essential for driving research progress and ensuring that new findings are effectively translated into clinical applications. In addition to antibody - related research and resistance management, future research will zero in on two other key areas. One is to conduct an in - depth and comprehensive analysis of the *Klebsiella* resistance genome through large - scale genomic studies. This endeavor will help unearth additional potential resistance mechanisms, which in turn will serve as the cornerstone for the development of innovative detection methods. The other area of focus is the advancement of new rapid detection technologies, especially those that can provide instant results at the bedside. By significantly reducing the time from sample collection to result delivery, these technologies will greatly enhance the timeliness of clinical decision - making, ultimately leading to more effective treatment strategies.

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Disclosure

The authors report no conflicts of interest in this work.

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