

# Major Predominant Serotypes and Virulence Genes and Antibiotic Resistance Characteristics of *Klebsiella pneumoniae* Clinical Isolates in Middle and East China

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**Background:** *Klebsiella pneumoniae* is a common opportunistic pathogen. Predominant serotypes, virulence genes, and resistance characteristics of *K. pneumoniae* isolates from patients in different regions of China require further investigation.

**Methods:** *K. pneumoniae* isolates from patients and healthy individuals in middle and east China were identified using an auto-bacterial detector. Major serotypes and virulence genes in the isolates were detected by polymerase chain reaction, while drug resistance of the isolates was determined using broth microdilution assays.

**Results:** Respiratory *K. pneumoniae* infection was observed in 70.0% of the patients. Of the *K. pneumoniae* isolates from patients, 42.3% were hypervirulent K (hvKp) serotypes, of which 30.1% and 37.0% belonged to K1 and K2 serotypes with 78.6–87.8% positive rates of *rmpA* and *rmpA2* virulence genes. The isolates from healthy individuals had fewer hvKp serotypes and *rmpA/rmpA2* genes (7.2% and 22.9%/26.5%). Resistance rates (38.6–79.5%) of the isolates from healthy individuals against 14 antibiotics were higher than those from patients (16.4–40.8%). The isolates from patients were sensitive to amikacin (83.6%) and polymyxin-B (93.9%) but presented 20.3% and 26.6% resistance rates to imipenem and meropenem, respectively. The isolates from patients with urinary infections exhibited higher resistances (42.1–52%) to cefoxitin, cefuroxime, ceftriaxone, ciprofloxacin, and levofloxacin than those from patients with respiratory or blood infections (22.4–39.3%). In the isolates from patients, the K47 and K64 serotypes exhibited multiple drug resistance (65–90%) against 14 antibiotics but all the hvKp serotypes displayed much lower antibiotic resistance (1.9–26.0%).

**Conclusion:** K1/K2 were the major predominant hvKp serotypes with *rmpA/rmpA2* virulence genes and carbapenem-resistant *K. pneumoniae* strains were prevalent in patients from middle and east China. The hvKp serotypes have low antibiotic resistance, but *K. pneumoniae* isolates from patients with urinary infections resist the cephalosporin/quinolone antibiotics for treatment of bacterial urinary infections. Amikacin and polymyxin-B can be used to treat drug-resistant *K. pneumoniae* infections.

**Keywords:** *Klebsiella pneumoniae*, extraintestinal infection, capsular serotypes, virulence genes, antibiotic resistance, multicenter study

## Introduction

*Klebsiella pneumoniae*, a member of the Gram-negative Enterobacteriaceae family, is a typical component of the healthy intestinal microbiota. However, this bacterium can act as an opportunistic pathogen that causes various extraintestinal infectious diseases, including pneumonia, urinary infection, liver abscesses, sepsis, and meningitis, due to bacterial translocation.<sup>1–3</sup> Although *K. pneumoniae* rarely causes intractable infections, the emergence of strains with enhanced antibiotic resistance, including carbapenem resistance and production of multiple extended-spectrum  $\beta$ -lactamases, has become a significant global public health concern.<sup>4,5</sup>

Based on the diversity of capsular polysaccharide-based animal toxicity, *K. pneumoniae* strains can be classified into hypovirulent classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumoniae* (hvKp).<sup>6,7</sup> Due to the lack of rapid and convenient methods for identifying hvKp in clinical practice, the string test has been widely used in clinical laboratories. However, recent studies have reported that the string test can only be used to identify hypermucoviscous *K. pneumoniae* (hmKp), as hypermucoviscosity and hypervirulence are the two complementary but distinct phenotypes of *K. pneumoniae* species.<sup>8,9</sup> Numerous previous studies have demonstrated that geographic differences in the prevalent hvKp serotypes and both virulence genes and drug resistance in different *K. pneumoniae* strains played important roles in the pathogenicity and prevalence of this bacterial pathogen.<sup>10–15</sup> Therefore, investigating the epidemiological characteristics of hvKp capsular serotypes, virulence genes, and antibiotic resistance of *K. pneumoniae* isolates from patients in different countries and regions has significant clinical importance.

All *K. pneumoniae* strains possessed capsules composed of different capsular polysaccharides. Based on the variations in K antigens within the CPS molecules, *K. pneumoniae* strains can be classified into numerous capsular serotypes.<sup>16</sup> Notably, the capsule is a key virulence factor of *K. pneumoniae*, enabling it to resist macrophage phagocytosis, block exogenous harmful substances, and mediate bacterial adhesion to host cells. Some capsular serotypes of *K. pneumoniae* have also been shown to be closely related to drug resistance.<sup>16–18</sup> For example, K1, K2, K5, K20, K54, and K57 have been identified as the major hvKp serotypes in many countries in the world including China,<sup>16–20</sup> whereas K47 and K64 serotypes are more prevalent in the multidrug-resistant (MDR) *K. pneumoniae* strains.<sup>18,21</sup>

*K. pneumoniae* can also produce various virulence factors. Previous studies have revealed that the products of the *rmpA* and *rmpA2* genes promote CPS synthesis of *K. pneumoniae*. Inactivation of either the *rmpA* or *rmpA2* gene leads to a significant decrease in the virulence of *K. pneumoniae* strains.<sup>21,22</sup> The *wcaG* gene product catalyzes the synthesis of fucose, a virulent component in the CPS of *K. pneumoniae*, which enables the bacterium to resist phagocytosis by host macrophages.<sup>23</sup> Adhesion and subsequent colonization of host cells play critical roles in the process of bacterial infection. Type I and III pili of *K. pneumoniae* have been confirmed to mediate the adhesion and colonization of host epithelial cells.<sup>24</sup> The *fimH* and *mrkD* genes are responsible for encoding type I and III pilins of *K. pneumoniae*.<sup>24,25</sup> In addition to the major virulence factors mentioned above, *K. pneumoniae* can also produce some other virulence-related factors, such as yersiniabactin, colibactin, aerobactin, and salmochelin responsible for iron ion uptake and subsequent transport from hosts.<sup>15</sup> However, the characteristic correlations between these major virulence genes and capsular serotypes in *K. pneumoniae* clinical isolates remain poorly understood.

Drug resistance in pathogenic bacteria, including *K. pneumoniae*, has always been a significant medical challenge.<sup>26–28</sup> Antibiotics are commonly used to treat various *K. pneumoniae*-infected diseases. However, the World Health Organization (WHO) reported that the antibiotic resistance of *K. pneumoniae* clinical isolates from different countries and regions has been continuously increasing in recent years.<sup>29</sup> In China, antibiotic-resistant *K. pneumoniae* strains are becoming increasingly prevalent.<sup>30</sup> Previous views suggested that the virulence of *K. pneumoniae* is negatively correlated with drug resistance. Nevertheless, recent studies have found that some *K. pneumoniae* plasmids can carry both multidrug resistance and virulence genes, resulting in the emergence of multidrug-resistant hvKp (MDR-hvKp).<sup>31,32</sup> Therefore, the correlation between antibiotic resistance and virulence in *K. pneumoniae* clinical isolates is an interesting research topic.

In the present study, 710 *K. pneumoniae* isolates were obtained from six hospitals in different regions of middle and east China between 2022 and 2024 to determine the major predominant hvKp serotypes (K1, K2, K5, K20, K54, and K57) and MDR-related serotypes (K47 and K64),<sup>16–20,31–33</sup> virulence genes (*rmpA*, *rmpA2*, *wcaG*, *fimH*, and *mrkD*),<sup>21–25</sup> and resistance to commonly used antibiotics.<sup>26–30</sup> This study aimed to identify the common *K. pneumoniae*-infected disease types, major predominant hvKp serotypes and virulence genes, and the correlation between serotypes and antibiotic resistance in *K. pneumoniae* isolates from patients in middle and east China. These results will help improve the clinical therapy and control of *K. pneumoniae*-infected diseases.

## Materials and Methods

### Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all patients and healthy individuals for routine physical examinations to collect clinical specimens to isolate *K. pneumoniae* strains. The study protocol was approved by the Ethics Committee of Hangzhou Medical College (permission No. 2021–111).

## Separation and Identification of *K. pneumoniae* Isolates

Sputum, bronchoalveolar lavage fluid, urine, urinary tract secretions, and peripheral blood samples from 627 patients, and stool samples from 83 healthy individuals for routine physical examinations were collected from six hospitals in Zhejiang (n = 194), Jiangsu (n = 164), Anhui (n = 169), and Henan (n = 100) provincial regions in middle and east China between 2022 and 2024 to isolate *K. pneumoniae* strains. Each sample was streaked for inoculation on a Columbia blood agar plate (BioMérieux, France) and cultured at 37°C for 16–18 h. Colonies on the plates were selected for bacterial enrichment by cultivation on Columbia blood agar plates and subsequent bacterial identification using a VITEK2-Compact Auto-Bacterial Detector (bioMérieux).

## String Test

Each of the 710 *K. pneumoniae* isolates was inoculated onto Columbia blood agar plates and cultured at 37°C with 5% CO<sub>2</sub> for 14–16 h. A bacterial inoculation loop was used to stretch a single colony of *K. pneumoniae* in the plates. A colonial mucoviscous string length  $\geq 5$  mm was considered as the hypermucoviscous phenotype *K. pneumoniae* (hmKp).<sup>34</sup>

## Determination of Major Capsular Serotypes in *K. pneumoniae* Isolates

Polymerase chain reaction (PCR) was used to determine the capsular serotypes (K1, K2, K5, K20, K47, K54, K57, and K64) in *K. pneumoniae* isolates.<sup>35</sup> Briefly, genomic DNAs of the 710 *K. pneumoniae* isolates were extracted using a Bacterial Genomic DNA MiniPrep Kit (Axygen, USA) and then quantified using ultraviolet spectrophotometry.<sup>36</sup> Using 100 ng of each genomic DNA as a template, separate PCRs were performed using a High-Fidelity Ex-Taq PCR Kit (TaKaRa-Bio, China) and different pairs of serotyping primers (Table 1). The PCRs were initiated by incubation at 95 °C for 3 min for DNA denaturation, followed by 35 cycles at 95 °C for 30s, 53–55°C for 30s and 72°C for 30–60 s to amplify the target gene fragments, followed by incubation at 72°C for 7 min for extension. For serotyping of each *K. pneumoniae* isolate, three independent PCRs were repeated for detection. The PCR products were examined by 1.5% agarose gel electrophoresis at 110V for 40 min.

## Detection of Major Virulence Genes in *K. pneumoniae* Isolates by PCR

PCR was also used to detect the major virulence genes of *K. pneumoniae* (*rmpA*, *rmpA2*, *wcaG*, *fimH*, and *mrkD*).<sup>37</sup> Genomic DNAs of the 710 *K. pneumoniae* isolates were extracted using a Bacterial Genomic DNA MiniPrep Kit (Axygen, USA) and then quantified using ultraviolet spectrophotometry as previously described.<sup>36</sup> Subsequently, separate PCRs were performed to detect the five virulence genes from the DNA templates as described earlier. The primers used in the PCR for detecting the virulence genes of *K. pneumoniae* are shown in Table 1. The PCR products were examined by 1.5% agarose gel electrophoresis.

## Drug Susceptibility Test

Broth microdilution assays in vitro were performed to determine the minimum inhibitory concentration (MIC) of ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, ceftazidime, ceftriaxone, cefotaxime, cefoperazone/sulbactam, meropenem, imipenem, amikacin, ciprofloxacin, levofloxacin, and polymyxin-B in 710 *K. pneumoniae* isolates as previously reported.<sup>38</sup> The obtained results were interpreted and determined as sensitive, intermediate, and resistant according to the 2021 Clinical and Laboratory Standards Institute (CLSI) M100-Ed31.<sup>38</sup> The sensitive, intermediate, or resistant result indicated that the MIC of an antibiotic was below, close to, or above its clinically common blood drug concentration. *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC25922 were used as controls.

## Data Analysis

The obtained data were statistically analyzed using SPSS software (version 29.0). The chi-squared test ( $\chi^2$ -test) and Fisher's exact probability test were used to determine significant differences. Statistical significance was defined as  $p < 0.05$ .

**Table 1** Primers Used in the PCRs of This Study

| Primer | Sequence (5'to 3')   | Product (bp) |
|--------|--|--------------|
| K1     | F:GGTGCTCTTTACATCATTGC<br>R: GCAATGGCCATTTGCGTTAG            | 1283         |
| K2     | F:GACCCGATATTCATACTTGACAGAG<br>R:CCTGAAGTAAAATCGTAAATAGATGGC | 641          |
| K5     | F:TGGTAGTGATGCTCGCGA<br>R:CCTGAACCCACCCCAATC                 | 280          |
| K20    | F:CGGTGCTACAGTGCATCATT<br>R:GTTATACGATGCTCAGTCGC             | 741          |
| K47    | F:GGACGCACAGTTTCCCAATTCGC<br>R:GCCCACATGAACCCACTTGGCA        | 392          |
| K54    | F:CATTAGCTCAGTGGTTGGCT<br>R:GCTTGACAAACACCATAGCAG            | 881          |
| K57    | F:CTCAGGGCTAGAAGTGTCAT<br>R:CACTAACCCAGAAAGTCGAG             | 1037         |
| K64    | F:TCAGTTCCGACCCTGATGCAGGTA<br>R:GCCAGAGCAACTATCATCCAAGCCA    | 268          |
| rmpA   | F:ACTGGGCTACCTCTGCTTCA<br>R:CTTGCATGAGCCATCTTTCA             | 516          |
| rmpA2  | F:GTGCAATAAGGATGTTACATTA<br>R:GGATGCCCTCCTCCTG               | 430          |
| wcaG   | F:GGTTGGGTCAGCAATCGTA<br>R:ACTATTCGCGCAACTTTTGC              | 169          |
| fimH   | F:ATGAACGCCTGGTCCTTTGC<br>R:GCTGAACGCCTATCCCCTGC             | 688          |
| mrkD   | F:AAGCTATCGCTGTACTTCCGGCA<br>R:GGCGTTGGCGCTCAGATAGG          | 340          |

**Abbreviations:** F, forward primer; R, reverse primer.

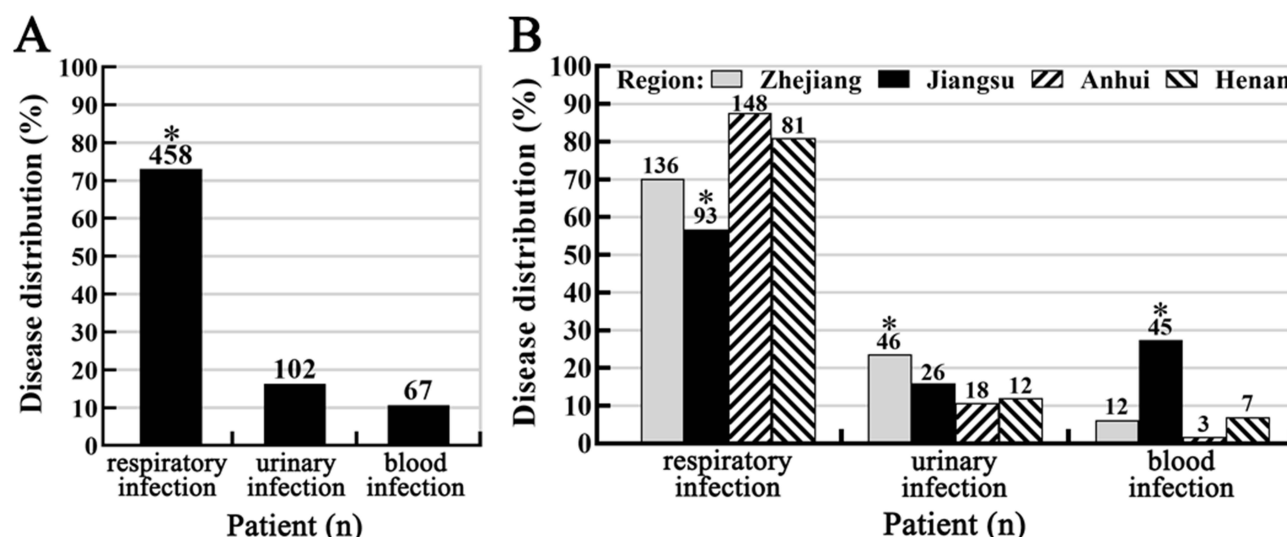
## Results

### Types and Distribution of *K. pneumoniae*-Infected Diseases in Patients

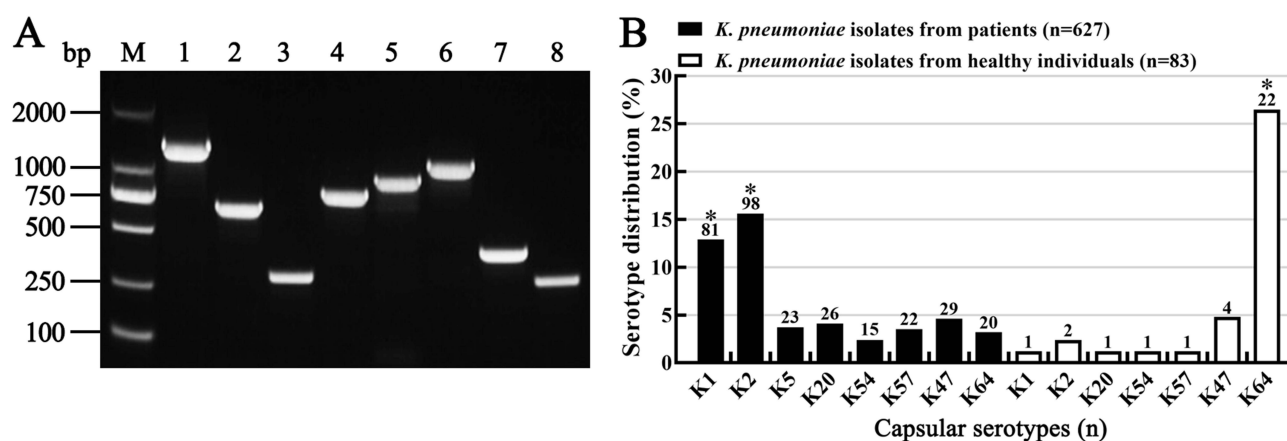
Among the 627 *K. pneumoniae*-infected patients, 73.0% (458/627), 16.3% (102/627), and 10.7% (67/627) of the patients were diagnosed with respiratory, urinary, and blood infections, respectively, and respiratory infection was the most common disease type (Figure 1A). The percentage of patients with respiratory or blood infections from the Jiangsu provincial region was significantly lower or higher (56.7% or 27.4%) than those from the Zhejiang, Anhui and Henan provincial regions (70.1–87.6% or 1.8–7.0%) (Figure 1B). Additionally, the percentage of patients with urinary infections from the Zhejiang provincial region was significantly higher (23.7%) than that from the other three provincial regions (10.6–15.8%) (Figure 1B). These data suggest that respiratory infections are a major *K. pneumoniae*-infected disease in patients from different provincial regions of middle and east China. However, there are regional or geographic differences in the predominant disease types caused by *K. pneumoniae*.

### Predominant Capsular Serotypes in the *K. pneumoniae* Isolates

Among the 627 *K. pneumoniae* isolates from patients with respiratory, urinary, or blood infections, 12.9% (81/627), 15.6% (98/627), 3.7% (23/627), 4.2% (26/627), 2.4% (15/627), and 3.5% (22/627) were determined by PCR as K1, K2, K5, K20, K54, and K57 hvKp serotypes (42.3% in total from 265/627), respectively. In contrast, 4.6% (29/627) and 3.2% (20/627) of the isolates (7.8% in total) were identified as K47 and K64 MDR-related serotypes (7.8% in total from 49/627) (Figure 2A and B). However, in the 83 *K. pneumoniae* isolates from the intestines of healthy individuals, only 1.2% (1/83), 2.4% (2/83), 1.2% (1/83), 1.2% (1/83), and 1.2% (1/83) of the isolates (7.2% in total from 6/83) were determined by PCR as K1, K2, K20, K54, and K57 hvKp serotypes, respectively (Figure 2B). Notably, the K5 hvKp serotype was undetectable in the isolates from the healthy individuals.



**Figure 1** Disease and regional distribution of *K. pneumoniae*-infected patients. **(A)**. Disease distribution in *K. pneumoniae*-infected patients. \* $p < 0.05$  vs the percentages of patients with urinary or blood infections. **(B)**. Regional distribution of different diseases in *K. pneumoniae*-infected patients. \* $p < 0.01$  vs the percentages of patients with respiratory, urinary, or blood infections from the other three provincial areas.



**Figure 2** Capsular serotypes in *K. pneumoniae* isolates. **(A)**. *K. pneumoniae* serotypes detected by PCR. Lane (M) DNA marker. Lanes 1–8: amplification fragments of K1 (1283 bp), K2 (641 bp), K5 (280 bp), K20 (741 bp), K54 (881 bp), and K57 (1037bp) hvKp, and K47 (392 bp) and K64 (268 bp) MDR-related serotypes of *K. pneumoniae* isolates, respectively. **(B)**. Distribution of hvKp and MDR-related serotypes in *K. pneumoniae* isolates. \* $p < 0.01$  vs the positive rates of K5, K20, K54, K57 hvKp, and K47 MDR-related serotypes in the isolates from patients and healthy individuals.

Moreover, 4.8% (4/83) and 26.5% (22/83) of the 83 *K. pneumoniae* isolates from healthy individuals (31.3% in total from 26/83) were identified as the K47 and K64 MDR-related serotypes, respectively (Figure 2B). These data suggest that K1 and K2 are the major predominant hvKp serotypes prevalent in patients in middle and east China, whereas the K47 and K64 MDR-related serotypes are more common in *K. pneumoniae* isolates from healthy individuals.

## Distribution Differences in Serotypes of the *K. pneumoniae* Isolates from Different Patients and Provincial Regions

Only the positive rate of the K2 hvKp serotype in *K. pneumoniae* isolates from patients with respiratory infections was significantly higher than that in patients with urinary or blood infections. However, neither the K1, K5, K20, K54, and K57 hvKp serotypes nor the K47 and K64 MDR-related serotypes in *K. pneumoniae* isolates from patients with different diseases displayed significant differences (Table 2). Moreover, there was no significant difference in the distribution of hvKp serotypes among *K. pneumoniae* isolates from patients in the different provincial regions (Table 3). However, the



**Table 2** Capsular Serotypes of *K. pneumoniae* Isolates from Different Patients

| Serotype* | Case (n) | Serotypes in the <i>K. pneumoniae</i> Isolates from Different Patients (n/%) |                           |                        |
|-----------|----------|--|---------------------------|------------------------|
|           |          | Respiratory infection (n=458)  | Urinary infection (n=102) | Blood infection (n=67) |
| K1        | 81       | 59/12.9  | 12/11.8                   | 10/14.9                |
| K2        | 98       | 84/18.3 <sup>Δ</sup>   | 9/8.8                     | 5/7.5                  |
| K5        | 23       | 19/4.2   | 2/2.0                     | 2/3.0                  |
| K20       | 26       | 20/4.4   | 4/3.9                     | 2/3.0                  |
| K54       | 15       | 10/2.2   | 2/2.0                     | 3/4.5                  |
| K57       | 22       | 18/3.9   | 2/2.0                     | 2/3.0                  |
| K47       | 29       | 20/4.4   | 6/5.9                     | 3/4.5                  |
| K64       | 20       | 14/3.1   | 5/4.9                     | 1/1.5                  |

**Notes:** \*indicates that K1, K2, K5, K20, K54, and K57 are hvKp serotypes while K47 and K64 are the MDR-related serotypes. <sup>Δ</sup>indicates  $p < 0.05$  vs the positive rate of K2 hvKp serotype in the *K. pneumoniae* isolates from patients with urinary or blood infections.

**Table 3** Capsular Serotypes in *K. pneumoniae* Isolates from Patients in Different Regions

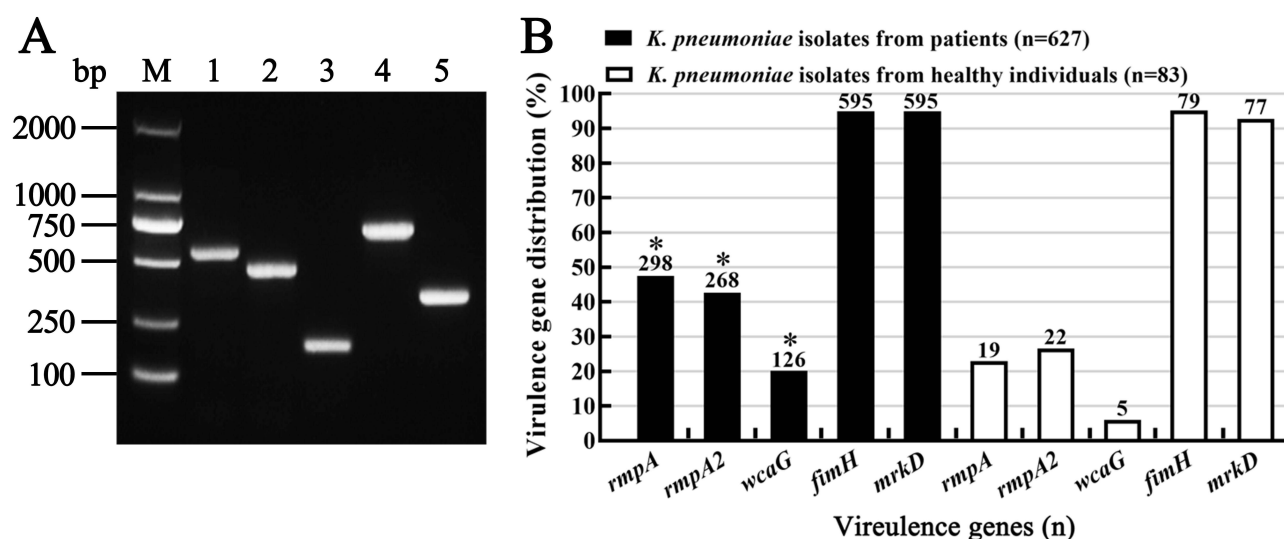
| Serotype* | Case (n) | Serotypes in the <i>K. pneumoniae</i> Isolates from Patients in Different Regions (n/%) |                 |               |                      |
|-----------|----------|---|-----------------|---------------|----------------------|
|           |          | Zhejiang (n=194)  | Jiangsu (n=164) | Anhui (n=169) | Henan (n=100)        |
| K1        | 81       | 26/13.4   | 17/10.4         | 23/13.6       | 15/15.0              |
| K2        | 98       | 37/19.1   | 21/12.8         | 22/13.0       | 18/18.0              |
| K5        | 23       | 6/3.1   | 4/2.4           | 9/5.3         | 4/4.0                |
| K20       | 26       | 6/3.1   | 7/4.3           | 6/3.6         | 7/7.0                |
| K54       | 15       | 5/2.6   | 4/2.4           | 4/2.4         | 2/2.0                |
| K57       | 22       | 5/2.6   | 5/3.1           | 6/3.6         | 6/6.0                |
| K47       | 29       | 0/0   | 7/4.3           | 7/4.1         | 15/15.0 <sup>Δ</sup> |
| K64       | 20       | 16/8.3 <sup>Δ</sup>   | 0/0             | 4/2.4         | 0/0                  |

**Notes:** \*indicates that K1, K2, K5, K20, K54 and K57 are hvKp serotypes while K47 and K64 are MDR-related serotypes. <sup>Δ</sup>indicates  $p < 0.05$  vs the positive rate of K47 or K64 MDR-related serotype in the *K. pneumoniae* isolates from patients in the other three provincial regions.

positive rate of K47 or K64 MDR-related serotypes in the isolates from patients in the Henan or Zhejiang provincial regions (8.3% or 15.0%) was significantly higher than that of patients in the other three provincial regions (0–4.3%) (Table 3). These data suggest that the K2 hvKp serotype is more common in *K. pneumoniae* isolates from patients with respiratory infections and that the prevalence of *K. pneumoniae* K47 and K64 MDR-related serotypes in patients has significant regional differences in middle and east China.

## Predominant Virulence Genes in the *K. pneumoniae* Isolates

In the 627 *K. pneumoniae* isolates from patients, 30.3% (190/627) of the isolates were positive in the string test, which was significantly higher than that (10.8%, 9/83) in the 83 *K. pneumoniae* isolates from the intestines of healthy individuals ( $p < 0.01$ ). The positive rates of the *rmpA*, *rmpA2*, and *wcaG* virulence genes in the 627 *K. pneumoniae* isolates from patients were 47.5% (298/627), 42.7% (268/627), and 20.1% (126/627), respectively, which were significantly higher than those of 22.9% (19/83), 26.5% (22/83), and 6.0% (5/83) of the 83 *K. pneumoniae* isolates from healthy individuals (Figure 3A and B). Furthermore, the *rmpA* and *rmpA2* virulence genes demonstrated significantly higher positive detection rates than the *wcaG* virulence gene in the *K. pneumoniae* isolates from patients ( $p < 0.01$ ). However, the 94.9% (595/627) and 94.9% (595/627) positive rates of the *fimH* and *mrkD* virulence genes in the 627



**Figure 3** Virulence genes in *K. pneumoniae* isolates. (A). *K. pneumoniae* virulence genes detected by PCR. Lane (M) DNA marker. Lanes 1–5: amplification fragments of *rmpA* (516 bp), *rmpA2* (430 bp), *wcaG* (169 bp), *fimH* (688 bp), and *mrkD* (340 bp) genes from *K. pneumoniae* isolates, respectively. (B). Distribution of virulence genes in *K. pneumoniae* isolates. \* $p < 0.01$  vs the positive rates of *rmpA*, *rmpA2*, and *wcaG* virulence genes in the isolates from healthy individuals.

*K. pneumoniae* isolates from patients showed no significant differences compared to the 95.2% (79/83) and 92.8% (77/83) of the 83 *K. pneumoniae* isolates from healthy individuals (Figure 3B). These data suggest that the *rmpA*, *rmpA2*, and *wcaG*, but not *fimH* and *mrkD* genes, play important roles in the virulence of *K. pneumoniae* isolates from patients in middle and east China

## Distribution Differences in Virulence Genes of the *K. pneumoniae* Isolates from Different Patients and Regions

The *rmpA* and *rmpA2* virulence genes were more common in the 627 *K. pneumoniae* isolates from patients with respiratory infections (52.0% and 46.5%, respectively) than in those from the patients with urinary or blood infections (34.3% and 32.4%, or 37.3% and 32.8%, respectively) (Table 4). Moreover, the positive rates (30.3% and 30.0%) of the *wcaG* virulence gene in *K. pneumoniae* isolates from patients in the Anhui and Henan provincial regions were significantly higher than those from the patients in the Zhejiang and Jiangsu provincial regions (14.4% and 10.4%, respectively) (Table 5). These data suggest that the *rmpA* and *rmpA2* virulence genes play important roles in the pathogenesis of *K. pneumoniae*-caused respiratory infections and that the distribution of *wcaG* virulence genes in *K. pneumoniae* isolates has regional or geographic differences in middle and east China.

**Table 4** Virulence Genes in *K. pneumoniae* Isolates from Different Patients

| Gene         | Case (n) | Virulence Genes in the <i>K. pneumoniae</i> Isolates from Patients (n/%) |                           |                        |
|--------------|----------|--|---------------------------|------------------------|
|              |          | Respiratory Infection (n=458)  | Urinary Infection (n=102) | Blood Infection (n=67) |
| <i>rmpA</i>  | 298      | 238/52.0*  | 35/34.3                   | 25/37.3                |
| <i>rmpA2</i> | 268      | 213/46.5*  | 33/32.4                   | 22/32.8                |
| <i>wcaG</i>  | 126      | 96/21.0  | 19/18.6                   | 11/16.4                |
| <i>fimH</i>  | 595      | 434/94.8   | 96/94.1                   | 65/97.0                |
| <i>mrkD</i>  | 595      | 435/95.0   | 96/94.1                   | 64/95.5                |

**Note:** \*indicates  $p < 0.05$  vs the positive rate of *rmpA* or *rmpA2* gene in the *K. pneumoniae* isolates from patients with urinary or blood infections.

**Table 5** Virulence Genes in the *K. pneumoniae* Isolates from Patients in Different Regions

| Gene         | Case (n) | Virulence Genes in the <i>K. pneumoniae</i> Isolates from Patients in Different Regions (n/%) |                 |               |               |
|--------------|----------|---|-----------------|---------------|---------------|
|              |          | Zhejiang (n=194)  | Jiangsu (n=164) | Anhui (n=169) | Henan (n=100) |
| <i>rmpA</i>  | 298      | 86/44.3   | 85/51.8         | 79/46.7       | 48/48.0       |
| <i>rmpA2</i> | 268      | 74/38.1   | 76/46.3         | 73/43.2       | 45/45.0       |
| <i>wcaG</i>  | 126      | 28/14.4   | 17/10.4         | 51/30.2*      | 30/30.0*      |
| <i>fimH</i>  | 595      | 189/97.4  | 155/94.5        | 158/93.5      | 93/93.0       |
| <i>mrkD</i>  | 595      | 184/94.8  | 154/93.9        | 159/94.1      | 98/98.0       |

**Note:** \*indicates  $p < 0.05$  vs the positive rate of *wcaG* gene in the *K. pneumoniae* isolates from patients in the Zhejiang or Jiangsu provincial regions.

## Correlation of hvKp Serotypes With Virulence Genes in the *K. pneumoniae* Isolates

In the 265 hvKp serotypes from 627 *K. pneumoniae* isolates, both *fimH* and *mrkD* virulence genes displayed similar high positive rates (92.3–98.0%) in the six different hvKp serotypes (Table 6). However, the positive rates (78.6–87.8%) of *rmpA* and *rmpA2* genes in the K1 and K2 hvKp serotypes were significantly higher than those (39.1–60.9%) in the other four hvKp serotypes (Table 6). In addition, the *wcaG* gene in the K1 and K54 hvKp serotypes also had significantly higher positive rates (63.0% and 73.3%) than those (11.5%–18.2%) in the other four hvKp serotypes (Table 6). These data suggest that the *rmpA* and *rmpA2* genes are related to the virulence of the predominant K1 and K2 hvKp serotypes in *K. pneumoniae* isolates from patients in middle and east China.

## Extensive Antibiotic Resistance of the *K. pneumoniae* Isolates

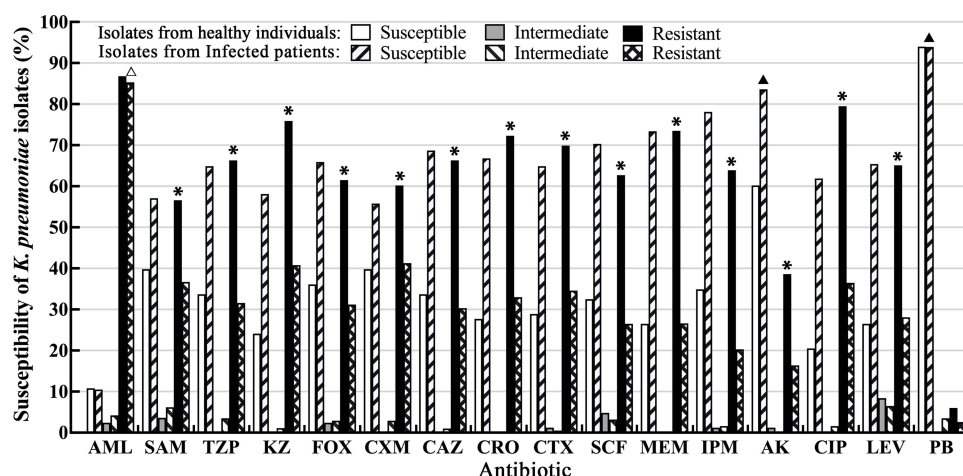
The 627 *K. pneumoniae* isolates from patients exhibited a much higher resistance rate (85.3%) against ampicillin but presented significantly higher sensitivity rates to amikacin (83.6%) and polymyxin-B (93.9%) among the 16 tested antibiotics (Figure 4 and Table S1). In particular, the resistance rates of the isolates against meropenem and imipenem, the two commonly used carbapenem antibiotics, reached 26.6% and 20.3%, respectively. Furthermore, the isolates exhibited relatively higher levels of resistance against the other 11 antibiotics (26.5–40.8%) belonging to either  $\beta$ -lactams or quinolones. Interestingly, except for ampicillin and polymyxin-B, the resistance rates (38.6–79.5%) of the 83 *K. pneumoniae* isolates from the intestines of healthy individuals against the other 14 tested antibiotics were significantly higher than those (16.4–40.8%) of the *K. pneumoniae* isolates from different clinical samples of the patients (Figure 4 and Table S1). These data suggest that *K. pneumoniae* isolates prevalent in patients in middle and east China exhibit extensive and relatively high resistance to the most commonly used antibiotics, including over 20% resistance rates against imipenem and meropenem, but display high sensitivity to amikacin and polymyxin-B.

**Table 6** Correlation of hvKp Serotypes with Virulence Genes from Patients

| Serotype* | Case (n) | Virulence Genes in the hvKp Serotypes from Patients (n/%) |                      |                      |                  |                  |
|-----------|----------|---|----------------------|----------------------|------------------|------------------|
|           |          | <i>rmpA</i> gene  | <i>rmpA2</i> gene    | <i>wcaG</i> gene     | <i>fimH</i> gene | <i>mrkD</i> gene |
| K1        | 81       | 68/84.0 <sup>Δ</sup>                                      | 63/77.8 <sup>Δ</sup> | 51/63.0 <sup>Δ</sup> | 79/97.5          | 78/96.3          |
| K2        | 98       | 86/87.8 <sup>Δ</sup>                                      | 77/78.6 <sup>Δ</sup> | 17/17.3              | 95/96.9          | 96/98.0          |
| K5        | 23       | 14/60.9   | 9/39.1               | 4/17.4               | 22/95.7          | 22/95.7          |
| K20       | 26       | 15/57.7   | 12/46.2              | 3/11.5               | 25/96.2          | 24/92.3          |
| K54       | 15       | 9/60.0  | 8/46.7               | 11/73.3 <sup>Δ</sup> | 14/93.3          | 14/93.3          |
| K57       | 22       | 12/54.5   | 11/50.0              | 4/18.2               | 21/95.5          | 21/95.5          |
| Total     | 265      | 204/77.0  | 180/67.9             | 90/34.0              | 256/96.6         | 255/96.2         |

**Notes:** \*: K1, K2, K5, K20, K54, and K57 are hvKp serotypes. <sup>Δ</sup> indicates  $p < 0.05$  vs the positive rates of the other hvKp serotypes.

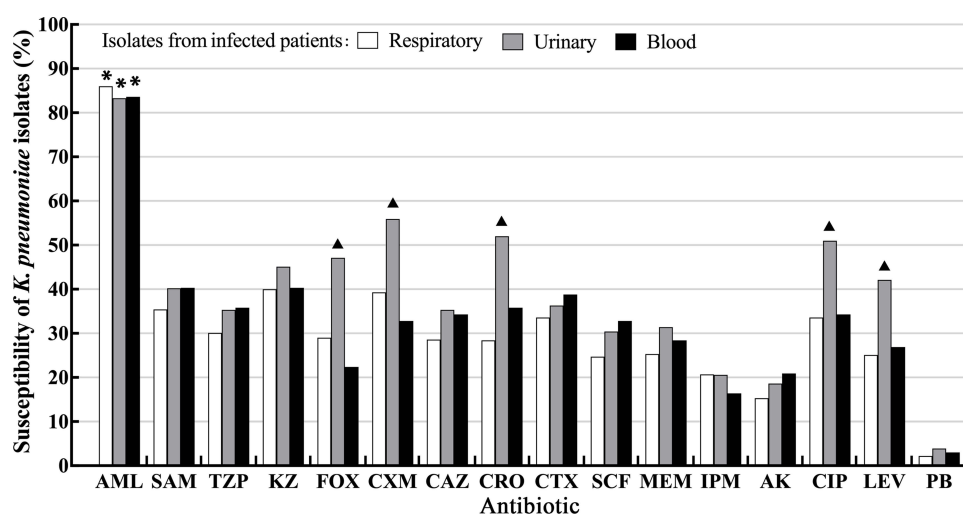




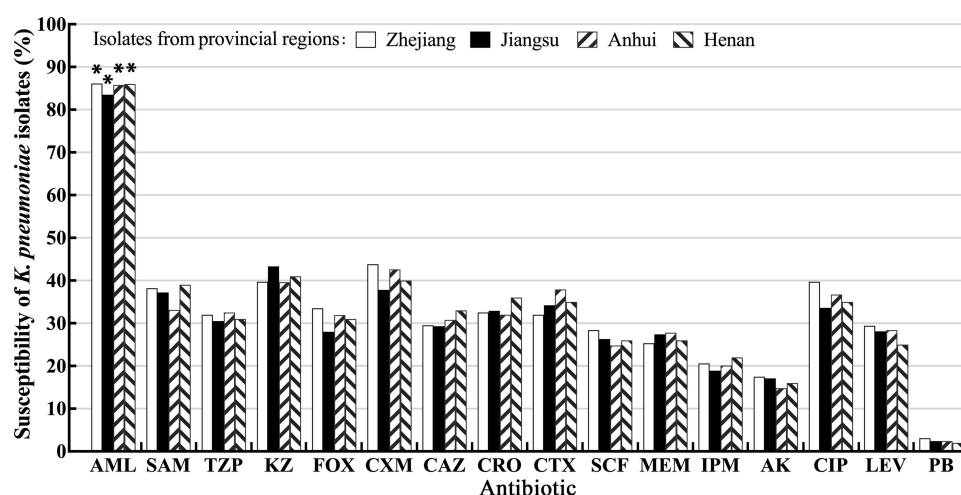
**Figure 4** Results of drug susceptibility tests of *K. pneumoniae* isolates. The abbreviations of 16 tested antibiotics: ampicillin (AML), ampicillin/sulbactam (SAM), piperacillin/tazobactam (TZP), ceftazidime (KZ), cefoxitin (FOX), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefotaxime (CTX), cefoperazone/sulbactam (SCF), meropenem (MEM), imipenem (IPM), amikacin (AK), ciprofloxacin (CIP), levofloxacin (LEV), and polymyxin-B (PB). \*indicates  $p < 0.01$  vs the resistance rates in the isolates from patients.  $\Delta$  indicates  $p < 0.01$  vs the resistance rates of the isolates from patients to the other 15 antibiotics.  $\Delta$  indicates  $p < 0.05$  vs the sensitivity rates of the isolates from patients to the other 14 antibiotics.

## Diversity of Antibiotic Resistance in the *K. pneumoniae* Isolates From Different Patients and Provincial Regions

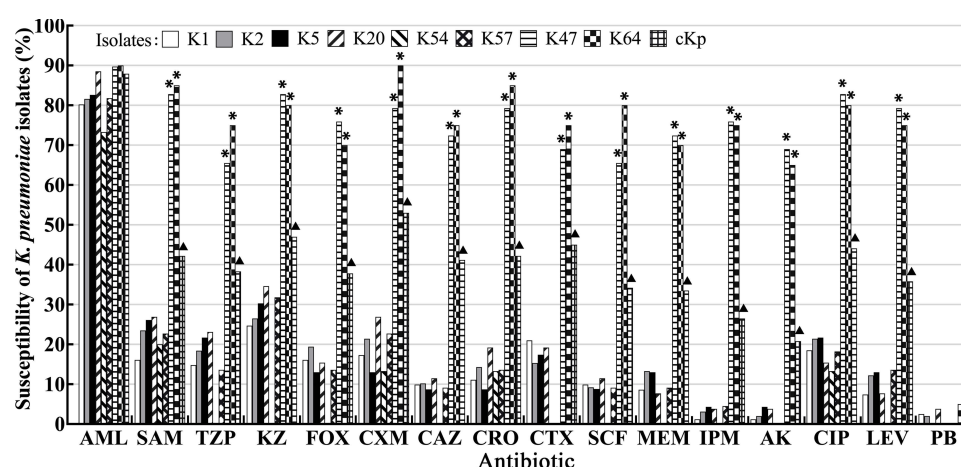
Except for the much higher resistance rates (83.3%, 83.6%, and 86.0%) against ampicillin in the 627 *K. pneumoniae* isolates from the patients with different diseases, the resistance rates (42.1–55.9%) of the isolates from patients with urinary infections to cefoxitin, cefuroxime, ceftriaxone, ciprofloxacin, and levofloxacin were significantly higher than those (22.4–39.3%) from patients with respiratory or blood infections (Figure 5 and Table S2). However, there were no significant differences in the resistance rates against any of the 16 tested antibiotics among the isolates from patients in different provincial regions (Figure 6 and Table S3). These data suggest that *K. pneumoniae* isolates from patients with urinary infections in middle and east China exhibit selective resistance to some specific antibiotics.



**Figure 5** Antibiotic resistance of *K. pneumoniae* isolates from different patients. The abbreviations of 16 tested antibiotics were the same as shown in Figure 4. \*indicates  $p < 0.01$  vs the resistance rates in the isolates against all the other tested antibiotics.  $\Delta$  indicates  $p < 0.05$  vs the resistance rates in the isolates from patients with respiratory or blood infections.



**Figure 6** Antibiotic resistance of *K. pneumoniae* isolates from patients in different regions. Antibiotic resistance of *K. pneumoniae* isolates from different patients. The abbreviations of 16 tested antibiotics were the same as shown in Figure 4. \*indicates  $p < 0.01$  vs the resistance rates in the isolates against all the other 15 antibiotics.



**Figure 7** Correlation between *K. pneumoniae* serotypes in patients with antibiotic resistance. Antibiotic resistance of *K. pneumoniae* isolates from different patients. The abbreviations of 16 tested antibiotics were the same as shown in Figure 4. \*indicates  $p < 0.05$  vs the resistance rates in the cKp isolates.  $\blacktriangle$  indicates  $p < 0.01$  vs the resistance rates in the K1, K2, K5, K20, K54, and K57 hvKp isolates, as well as in the K47 and K64 MDR-related isolates.

## Correlation of Serotypes With Antibiotic Resistance in the *K. pneumoniae* Isolates

Except for ampicillin and polymyxin-B, the K47 and K64 MDR-related serotypes in the 627 *K. pneumoniae* isolates from patients displayed significantly higher resistance rates (65.0–90.0%) to the other 14 tested antibiotics (Figure 7 and Table S4). In contrast, the 265 *K. pneumoniae* isolates belonging to six different major hvKp serotypes showed much lower resistance rates (1.9–26.0%) to these 14 antibiotics. The 313 non hvKp/MDR-related classical *K. pneumoniae* (cKp) isolates displayed significantly higher resistance rates (20.8–53.0%) than those of the hvKp isolates (Figure 7 and Table S4). These data suggest that the K47 and K64 MDR-related serotypes in *K. pneumoniae* isolates from patients possess multiple resistances to the commonly used antibiotics and the hvKp serotypes, including K1 and K2 prevailing in patients in Middle and East China, are not related to antibiotic resistance.

## Discussion

*K. pneumoniae* is a common opportunistic bacterial pathogen that can cause various diseases in nearly all human tissues and organs.<sup>1–3</sup> However, respiratory infection, including bronchitis and pneumonia, is the most common *K. pneumoniae*-infected disease.<sup>39</sup> In the present study, 627 *K. pneumoniae*-infected patients from four provincial regions (Zhejiang,

Jiangsu, Anhui, and Henan) in Middle and East China were clinically diagnosed with respiratory, urinary, or blood infection, with over 70% of the patients suffering from *K. pneumoniae* respiratory infection. These data indicate that the respiratory tract is the most susceptible site for *K. pneumoniae* isolates in middle and east China.

*K. pneumoniae* is a typical Gram-negative bacterium with a polysaccharide capsule. In addition to lipopolysaccharide endotoxin, the capsule is another key virulence factor of *K. pneumoniae*.<sup>24,40</sup> Some special capsular serotypes of *K. pneumoniae*, such as K1, K2, K5, K20, K54, and K57, have been confirmed as the major hvKp serotypes.<sup>16–20</sup> Previous studies have reported that K1 and K2 are the most common hvKp capsular serotypes in Asia,<sup>7,41,42</sup> but are rare in Europe and North America.<sup>43,44</sup> The present study also revealed that K1 and K2 are the predominant hvKp serotypes in the patients in Middle and East China. *rmpA*, *rmpA2*, and *wcaG* have been reported as important virulence genes of *K. pneumoniae*.<sup>21–23</sup> The present study found that the *rmpA* and *rmpA2* genes displayed significantly higher positive rates (47.5% and 42.7%) than that the *wcaG* gene (20.1%) in the *K. pneumoniae* isolates from patients. However, all the three virulence genes had significantly higher carrying rates in the isolates from patients than those from healthy individuals. The positive rates of both *rmpA* and *rmpA2* genes in the *K. pneumoniae* isolates from patients in middle and east China were also notably higher than those in northern Japan (22.2% and 19.8%) and New York of America (4.16% and 2.81%), but similar to those in eastern India (48.4% and 57.5%).<sup>45–47</sup> In particular, the K1 and K2 hvKp serotypes in the *K. pneumoniae* isolates from patients in this study exhibited significantly higher positive rates (78.6–87.8%) for *rmpA* and *rmpA2* genes than the K5, K20, K54, and K57 hvKp serotypes (39.1–60.9%). These data indicate that K1, K2, *rmpA*, and *rmpA2* are the predominant hvKp serotypes and virulence genes in *K. pneumoniae* isolates from patients in middle and east China, respectively, and that both *rmpA* and *rmpA2* genes are closely related to the virulence of K1 and K2 hvKp serotypes. In addition, *K. pneumoniae* isolates from patients with respiratory infections had the significantly higher proportions of *rmpA* and *rmpA2* genes (52.0% and 46.5%, respectively) than those from patients with urinary or blood infections (32.4–37.3%), suggesting a possible reason for the higher incidence rate of *K. pneumoniae* respiratory infections in this study.

Pili composed of pilins are surface structures in many bacteria that act as important virulence factors by mediating bacterial adhesion and colonization in hosts.<sup>48</sup> Most *K. pneumoniae* strains have type I pilin-encoding *fimH* gene and type III pilin-encoding *mrkD* gene.<sup>49</sup> Our study showed that 92.8–95.2% of the *K. pneumoniae* isolates from patients and healthy individuals were positive for *fimH* and *mrkD* genes. A recent study also reported that the positive rates of the two pilin-encoding genes in the *K. pneumoniae* isolates from a central European region were as high as 91.7% and 96.3%, respectively.<sup>50</sup> Therefore, *fimH* and *mrkD* genes are required for adhesion and colonization of *K. pneumoniae* in healthy individuals and patients, but not suitable for determining the virulence of *K. pneumoniae* isolates.

In clinical practice,  $\beta$ -lactam and quinolone antibiotics are usually selected to treat *K. pneumoniae*-infected diseases.<sup>51</sup>  $\beta$ -lactam antibiotics can be classified into penicillins, cephalosporins, and carbapenems based on their structural differences in  $\beta$ -lactam rings and their side chains in molecules.<sup>52</sup> Among the 16 antibiotics used in the drug susceptibility test of this study, 14 are the commonly used types of penicillins, cephalosporins, carbapenems, and quinolones, while the other two belong to aminoglycoside (amikacin) or polymyxin antibiotics. A previous study reported that *K. pneumoniae* is naturally resistant to ampicillin.<sup>4</sup> Our drug susceptibility test also showed that the ampicillin resistance rate was as high as over 85% in the 627 *K. pneumoniae* isolates from patients. More importantly, the six common cephalosporin antibiotics (cefazolin, cefoxitin, cefuroxime, ceftazidime, ceftriaxone, and cefotaxime) tested in this study had 30.3–41.3% resistance rates in the 627 isolates, which were similar to the resistance rates of cephalosporin antibiotics (20.8%–42.1%) in the *K. pneumoniae* isolates from patients in southwest China but notably lower than those in many other different countries of Asia (65.4%–87.2%) and southern Europe (50%–60%).<sup>4,5,30</sup> Compared to penicillin and cephalosporin antibiotics, carbapenem antibiotics exhibit many advantages, such as a wider antibacterial spectrum, strong antibacterial activity, and high  $\beta$ -lactase stability.<sup>53</sup> However, the *K. pneumoniae* isolates from patients in middle and east China also exhibited 26.6% and 20.3% resistance rates against meropenem and imipenem, the two commonly used carbapenem antibiotics, which were obviously higher than those in southwest and north China (7.0%–14.1%) but remarkably lower than those in several other countries of Asia (30.8%–89.0%) and southern Europe (50%–60%).<sup>4,5,30,54</sup> Fortunately, the 627 *K. pneumoniae* isolates from patients in this study displayed high sensitivity to amikacin (83.6%) and polymyxin-B (93.9%). These data indicate that the *K. pneumoniae* isolates prevailing in middle and east China have higher resistance to most cephalosporin antibiotics and ampicillin cannot be used to treat *K. pneumoniae*-infected diseases. In addition, these data also indicate the prevalence of carbapenem-resistant *K. pneumoniae* strains in patients in Middle and East China.

The continuous and widespread use of antibiotics can induce bacteria to develop antibiotic resistance.<sup>55</sup> Except for ampicillin and polymyxin-B, the 83 *K. pneumoniae* isolates from the intestines of the healthy individuals in this study presented much higher resistance rates against the other 14 tested antibiotics than the *K. pneumoniae* isolates from the patients, which may be due to intestinal bacteria, including *K. pneumoniae*, often stimulated by oral antibiotics. Moreover, the *K. pneumoniae* isolates from patients with urinary infections showed significantly higher resistance rates (42.1–55.9%) against cefoxitin, cefuroxime, ceftriaxone, ciprofloxacin, and ciprofloxacin than those (22.4–39.3%) from the patients with respiratory or blood infections. These five antibiotics are commonly used to treat bacterial urinary infections in clinical practice.<sup>56,57</sup> Previous studies reported that K47 and K64 are the major MDR-related serotypes, and hvKp serotypes had higher resistance rates against many different antibiotics.<sup>15,58–60</sup> In the present study, except for ampicillin and polymyxin-B, the K47 and K64 serotypes in the *K. pneumoniae* isolates from patients presented much higher resistance against the other 14 tested antibiotics; however, the hvKp serotypes in the isolates were unexpectedly found to have very low resistance to these antibiotics compared to the non-hvKp/MDR-related *K. pneumoniae* isolates. *K. pneumoniae* strains have been reported to evolve in two different directions: high toxicity-low drug resistance and low toxicity-high drug resistance.<sup>61,62</sup> Therefore, the correlation between hvKp serotypes and antibiotic resistance in *K. pneumoniae* isolates from different countries and regions requires further investigation. In addition, compared to *K. pneumoniae* isolates from the intestines of healthy individuals containing much fewer hvKp serotypes and *rmpA/rmpA2/wcaG* virulence genes but exhibiting significantly higher antibiotic resistance, *K. pneumoniae* isolates from different patients containing significantly more hvKp serotypes and virulence genes but displaying relatively lower antibiotic resistance seem to be exogenous strains. However, no differences were found in the disease types between *K. pneumoniae*-infected inpatients and outpatients and in the major hvKp serotypes, virulence genes, and antibiotic resistance characteristics between the *K. pneumoniae* isolates from the two different types of patients.

## Conclusion

*K. pneumoniae* infection is a common disease in China. This study revealed the major predominant hvKp serotypes, virulence genes, and antibiotic resistance characteristics of *K. pneumoniae* isolates from patients in middle and east China. Respiratory infections are the main *K. pneumoniae*-induced diseases. K1 and K2 were the major predominant hvKp serotypes in *K. pneumoniae* isolates from the patients. *rmpA* and *rmpA2* are the main virulence-determining genes that are closely related to the K1 and K2 hvKp serotypes. The *K. pneumoniae* isolates exhibited extensive and relatively high resistance to the most commonly used antibiotics, including over 20% resistance rates against meropenem and imipenem. *K. pneumoniae* isolates from patients with urinary infections displayed significantly higher resistance to cefoxitin, cefuroxime, ceftriaxone, ciprofloxacin, and levofloxacin than those from patients with respiratory or blood infections. In addition to ampicillin and polymyxin-B, the K47 and K64 MDR-related serotypes in *K. pneumoniae* isolates exhibited high resistance to all the other tested 14  $\beta$ -lactam and quinolone antibiotics. However, the major hvKp serotypes in the isolates showed much lower resistance rates to these 14 antibiotics, indicating that the high virulence of *K. pneumoniae* is not related to the antibiotic resistance.

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## Disclosure

The authors declare that they have no conflicts of interest.

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