


Molecular Characterization of *Klebsiella pneumoniae* Isolated from Sputum in a Tertiary Hospital in Xinxiang, China

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Background: In clinical practice, *Klebsiella pneumoniae* (*K. pneumoniae*) is a common opportunistic pathogen responsible for nosocomial infection. This study aimed to analyze the trend of antimicrobial susceptibility and virulent characteristics of *K. pneumoniae* isolated from sputum. In clinics, data of the current study will help in the clinical treatment of *K. pneumoniae* infection.

Results: The current research showed the resistance rates of the 20 *K. pneumoniae* isolates against 13 antibiotics ranged from 15.0% to 80.0%. The detection rate of extended spectrum β -lactamases (ESBLs) was up to 55%, while *bla_{SHV}* was the most prevalent ESBLs genes. Four strains (25.0%) of *K. pneumoniae* presented hypermucoviscous phenotype (HMV). Moreover, 18 strains (90.0%) showed the stronger biofilm-forming ability. *wzi*, *wab_G*, *fim_H*, *mrk_D* were the most prevalent virulence genes in current research. Ten strains were found capsule typing and the higher genetic diversity of colonizing *K. pneumoniae* in this region. K₁₉ exhibited a strong positive correlation with imipenem resistance, while K₁ showed strong correlations with *mag_A*. Furthermore, HMV phenotype showed significantly negative correlations with multidrug-resistant.

Conclusion: In the hospital, the antibiotic resistance of *K. pneumoniae* (isolated from sputum samples) has a serious concern. Additionally, strains of *K. pneumoniae* show the higher genetic diversity.

Keywords: *Klebsiella pneumoniae*, antimicrobial resistance, resistant genes, virulent genes, biofilm-forming

Introduction

K. pneumoniae (*Klebsiella pneumoniae*) is a common clinical conditional pathogen. It is an important etiological agent of nosocomial infections. It may cause many infectious diseases, such as pneumonia, bloodstream infections, urinary tract infections, and osteomyelitis.¹ It is second to *Escherichia coli* in terms of detection rate among gram-negative bacteria.² With the widespread use and sometimes abuse of antibiotics in the clinic, resistant strains increase each year. In particular, the emergence of multidrug-resistant (MDR) strains led to the failure of clinical antibacterial treatment and prolong the course of the disease.³ This situation may increase the medical costs of patients and the mortality of inpatients, which has emerged as an urgent threat to public health. Various virulence factors are utilized in the survival and immune escape of *K. pneumoniae* infection,⁴ such as capsular polysaccharide (CPS), lipopolysaccharide (LPS), fimbriae, iron acquisition and biofilm, etc. *K. pneumoniae* carrying different virulence factors that show the different pathogenic and clinical characteristics.⁵ The number of studies of clinically isolated *K. pneumoniae* has been increased dramatically in recent years.^{6–8} The emergence of MDR *K. pneumoniae* especially ESBLs-producing strains and carbapenem-resistant strains (CRKP) has brought more difficult for the treatment of *K. pneumoniae* infections in clinics.^{9,10} Moreover, *K. pneumoniae* is thought as an important vehicle for reservoir and transmission resistant and virulent genes,¹¹ Thus, a better understanding and monitoring of these isolates could help limit the spread of antimicrobial resistance. However, the study on antibiotic resistance and molecular characteristics of

K. pneumoniae isolated from Xinxiang city, was scarce. Here, we identify the antibiotic resistance and molecular characteristics of *K. pneumoniae* isolated from clinical sputum samples. This study aimed to better comprehend the molecular epidemiological characteristics of *K. pneumoniae* in this region, which has great clinical significance for preventing and controlling the *K. pneumoniae* infection and transmission.

Methods

Bacterial Isolates

Twenty strains of *K. pneumoniae* (19 from sputum and 1 from alveolar lavage fluid) were collected between July and November 2020 from the Affiliated People's Hospital of Xinxiang Medical University, Henan Province, China. The patients had been suffering from common underlying diseases, including chronic obstruction, pneumonia, septic chest, lung abscess, craniocerebral injury, coronary artery disease, gastrointestinal bleeding, cerebral infarction, and cirrhosis. The strains were part of the routine laboratory procedures in the hospital. All isolates were identified by staining, biochemical tests and the VITEK-2 compact system (bioMérieux, Craponne, France). *K. pneumoniae* ATCC 700603 was used as a standard control strain for species identification. The isolates were marked numerically as KP1-KP20.

Antibiotics and Reagents

Following antibiotics were used in this study: Ceftazidime (CAZ, 30 μ g), Ceftazidime-clavulanic acid (CAC, 30 μ g/10 μ g), Cefotaxime (CTX, 30 μ g), Ceftazidime-clavulanic acid (CTC, 30 μ g/10 μ g), Cefoxitin (CFX, 30 μ g), Aztreonam (AZT, 30 μ g), Piperacillin-tazobactam (PIT, 100 μ g/10 μ g), Piperacillin (PIP, 100 μ g), Imipenem (IPN, 10 μ g), Doxycycline (DOX, 30 μ g), Chloramphenicol (CHL, 30 μ g), Ciprofloxacin (CIP, 5 μ g), Levofloxacin (LVF, 5 μ g), Gatifloxacin (GAT, 5 μ g), Kanamycin (KAN, 30 μ g). Luria-Bertani (LB) agar plates and Columbia blood agar plates were purchased from Guangzhou Huankai Microbiology Technology Limited Company. The reagents of polymerase chain reaction (PCR) were purchased from Kangwei Century Co., Ltd. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The primer sequences are shown in Tables 1 and 2.

ESBLs Phenotype Confirmation Tests

ESBLs phenotypes of 20 Strains of *K. pneumoniae* were detected by the combined disc test (CDT) recommended by the Clinical & Laboratory Standards Institute (CLSI) 2020.¹² Briefly, the bacteria with turbidity equivalent to 0.5 McFarland standards were swabbed onto Mueller-Hinton (MH) agar plates containing 30 μ g of CTX and CAZ, with and without 10 μ g of clavulanic acid, and were placed independently, 25 mm apart (center to center) on a lawn culture. However, *K. pneumoniae* ATCC 700603 was used as a standard control strain. The plates were incubated at 37°C for 16–18 h. Isolates were considered ESBL positive if the inhibition zone measured around one of the combination discs was at least 5 mm larger than the corresponding cephalosporin disc.

Antimicrobial Susceptibility Testing

The sensitivity of *K. pneumoniae* isolates against 13 kinds of antibiotics was determined by the disc diffusion method recommended by CLSI.¹² Antibiotic susceptibility results were determined according to the CLSI 2020 standard. Moreover, *Escherichia coli* ATCC 35218 was used as a standard control strain. We considered strains resistant to at least three antimicrobial classes as MDR strains.¹³

Detection of Antimicrobial Resistance Genes

Bacterial DNA template was extracted by using the water boiling method. Briefly, bacteria were inoculated, and cultured overnight in LB liquid medium at 37°C. There was 1 mL bacterial culture added into the 1.5 mL EP tube, centrifuged at 12,000 RPM for 2 min, and discarded the supernatant. Then, the sediments were resuspended in 500 μ L water, centrifuged again under the aforementioned conditions, and discarded the supernatant. After Adding 200 μ L water, boiled at 100°C, 20–30 min to lysate thallus. Freezing for 10–15 min and centrifuged at 12,000 rpm at 4°C for 15 min. The supernatant (genomic DNA) was extracted into the new EP tube and stored at –20°C. Sterile water was used as blank control, and the following resistant genes of *K. pneumoniae* were amplified by PCR, including encode ESBLs (*bla*_{CTX-M},

Table 1 PCR Primer Sequence Information of Antimicrobial Resistance Genes

| Gene | Primer Sequence | Product Size (bp) | Ta Opt (°C) | References |
|-----------------------------|---|-------------------|-------------|------------|
| <i>bla_{CTX-M}</i> | P1: TCGGGAGGCAGACTGGGTGT P2: CCTTAGGTTGAGGCTGGGTGA | 688 | 57.7 | |
| <i>bla_{TEM}</i> | P1: TCGCCGCATACACTATTCTCAG P2: ACGCTCACCGGCTCCAGATTAT | 445 | 55.1 | [42] |
| <i>bla_{SHV}</i> | P1: ATGCGTTATATTCGCTGTG P2: TGCTTTGTTATTCGGGCCAA | 753 | 58.4 | [42] |
| <i>bla_{KPC}</i> | P1: ATGTCACTGTATCGCCGTC P2: TTAAGTCCCGTTGACGCC | 882 | 57.0 | |
| <i>bla_{IMP}</i> | P1: CTTGATGAAGGCGTTTATGT P2: GCCAAGCTTCTATATTTGCGT | 496 | 50.9 | |
| <i>bla_{NDM}</i> | P1: CCAGCTCGCACCGAATG P2: AACGCCGCACCAAACG | 564 | 58.8 | |
| <i>bla_{OXA-48}</i> | P1: ACATAAATCACAGGGCGTAG P2: TATAGTCACCATTGGCTTCG | 500 | 51.4 | |
| <i>qnr_A</i> | P1: CAAGAGGATTTCTACGCCAGGAT P2: TCGGCAAAGGTCAGGTCACAGC | 521 | 58.9 | |
| <i>qnr_B</i> | P1: GCACTGAATTTATCGGCTGTC P2: CAACGATGCCTGGTAGTTGT | 500 | 53.4 | |
| <i>oqx_A</i> | P1: TCCATACCAACCTCGTCTCCC P2: AGCGTGGCTTTGAACTCTGC | 529 | 59.8 | |
| <i>oqx_B</i> | P1: TGGTGGAGAACGTCGAGCGTAA P2: TCGGCGTGTGGTGAACCTGC | 648 | 60.4 | |
| <i>aac</i> | P1: AGTACAGCATCGTGACCAACA P2: CTCGAATGCCTGGCGTGTTC | 545 | 55.8 | [43] |

Abbreviations: PS, primer sequences without reference were obtained by primer 5.0 design (same below); bp, base pair; Ta opt, the optimal annealing temperature.

Table 2 PCR Primers Sequence Information of Virulence Genes

| Gene | Primer Sequence | Product Size (bp) | Ta Opt (°C) | References |
|-------------------------|---|-------------------|-------------|------------|
| <i>wzi</i> | P1: GTGCCGCGAGCGCTTTCTATC TTG GTATTCC P2: GAGAGCCACTGGTCCAGAA[CorT]TT[CorG]ACCGC | 580 | 55 | [16] |
| <i>mag_A</i> | P1: GGTGCTCTTTACATCATTGC P2: GCAATGGCCATTTGCGTTAG | 1282 | 58 | [44] |
| <i>omp_A</i> | P1: ACTGGGCTACCTCTGCTTCA P2: CTTGCATGAGCCATCTTTCA | 516 | 58 | [36] |
| <i>iuc_A</i> | P1: CCCGCTTCCCTACTTT P2: ATTCGCTTCGCTGTCC | 575 | 54.8 | |
| <i>iut_A</i> | P1: GGCTGGACATCATGGGAACCTGG P2: CGTCGGGAACGGGTAGAATCG | 300 | 55 | [36] |
| <i>wab_G</i> | P1: ACCATCGGCCATTTGATAGA P2: CGGACTGGCAGATCCATATC | 683 | 49 | [36] |
| <i>fim_H</i> | P1: TGCTGCTGGGCTGGTGCATG P2: GGGAGGGTGACGGTGACATC | 688 | 49 | [36] |
| <i>mark_D</i> | P1: TTCTGCACAGCGGTCCC P2: GATACCCGGCGTTTTTCGTTAC | 480 | 49 | [36] |

Abbreviations: bp, base pair; Ta opt, the optimal annealing temperature.

bla_{TEM}, *bla_{SHV}*), carbapenemase genes (*bla_{KPC}*, *bla_{NDM}*, *bla_{OXA-48}*, *bla_{IMP}*), quinolones resistance genes (*qnr_A*, *qnr_B*, *oqx_A*, *oqx_B*), and aminoglycoside resistance gene (*aac*). The corresponding gene sequences were taken from NCBI and completed primers designed as primer 5.0. The primer sequences of the resistant genes are shown in Table 1.

Determination of Mucinous Phenotype

The mucinous phenotype of *K. pneumoniae* was analyzed by “String-forming test” according to the previous method.¹⁴ Briefly, *K. pneumoniae* was transferred to Columbia blood plate for overnight culture and incubated at 37°C. Dip the colony with the inoculum ring, then lift the inoculum ring. If the adhesive wire formed larger than 0.5 cm, it was considered positive; otherwise, it was negative. The strain with high mucilage phenotype was considered positive in the “String-forming test”.

Analysis of Biofilm Formation Ability

Biofilm-forming ability was measured by determining adhesion to flat-bottomed microtiter plates (96-well).¹⁵ Briefly, each well of the 96-well microtitration plates was filled with 200 µL sterile broth liquid medium. During biofilm formation (18 h incubation), bacterial cultures were added to each well (1:100 in liquid medium, 200 µL liquid medium). Sterile LB liquid medium was used as a standard blank control. After incubation at 37°C for 48 h, total cell mass was measured as absorbance at 570 nm (OD₁) while the blank was OD₁₀. Each well was washed 3 times with phosphate buffer saline (PBS), dried for 1 h at 60°C and stained for 20 min with 200 µL of 1% crystal violet. After removing the crystal violet solution, each well was washed with PBS 4 times to remove the remaining stain. Air-dried was done in the aseptic processing table for 30 mins, and each well was added with 200 µL of 95% ethanol. After vibrating for 30 min, the absorbance was measured at 570 nm (OD₂), while the blank was OD₂₀. The biofilm formation capacity was calculated by using $B = (OD_2 - OD_{20}) / (OD_1 - OD_{10})$. All the strains were classified based on the adherence capabilities into the following categories: nonbiofilm producers ($B < 0.1$), weak biofilm producers ($B \geq 0.1$), moderate biofilm producers ($0.1 < B \leq 1.0$), strong biofilm producers ($B > 1.0$).

Analysis of Capsular Serotypes and Virulence Genes

Amplifying *wzi* genes for detecting capsular polysaccharide (antigen K) serotype is a new method for capsular serotyping, which has been used in the laboratory. In this study, *wzi* primers were provided for expanded PCR amplification according to the previous study,¹⁶ and the products were sent to Bioengineering (Shanghai) incorporated company for sequencing. Sequencing results were submitted Institut Pasteur website¹⁷ for comparative analysis, obtained strain *wzi* parting and part of capsular serotyping. Virulence genes were detected by PCR, including encoding capsular polysaccharide (*wzi*), adhesin (*fimH*, *markD*), lipopolysaccharide (*wabG*), mucous phenotypic related genes (*rmpA*, *magA*), and ferritin genes (*iucA*, *iutA*). PCR primer sequence of virulence genes has been shown in Table 2.

Statistical Analysis

Clustal W 2.1 was used to complete the alignment of *wzi* gene sequence. Initial phylogenetic trees were constructed using MEGA 7 based on the neighbor-joining method (500 bootstrap replicates) and Jukes-Cantor distance. Pearson's correlation coefficient was calculated by applying bivariate correlation analysis with SPSS software version 19.0. The correlation in the heat map of the generated data was computed by using Hplot.

Results

Antimicrobial Susceptibility Testing

The resistance of 20 strains of *K. pneumoniae* to 13 commonly used antibiotics has been shown in Figure 1. Overall, *K. pneumoniae* isolated from various underlying diseases showed high antibiotic resistance rates ranging from 15.0% to 80.0%. However, multi-drug resistance rates were upto 70.0%. The strains showed the highest resistance to PIP (80.0%), CTX (65.0%) and CIP (65.0%) respectively. The lowest resistance was to PIT (15.0%), followed by IPN (20.0%) and CFX (25.0%). The resistance rate of other antibiotics was about 50.0%.

ESBL Phenotype and Resistance Genes of *K. pneumoniae*

Table 3 describes the distribution of antibiotic resistance genes in clinical isolated *K. pneumoniae*. Strains producing ESBLs were common in the region with a detection rate of 55.0%, while *bla_{SHV}* was the most widely distributed gene, but the other two genes were less frequently detected. The *bla_{KPC}* type carbapenemase gene was detected in only three strains. Furthermore, the other types of carbapenemase genes were not detected in the samples. Among plasmid-mediated

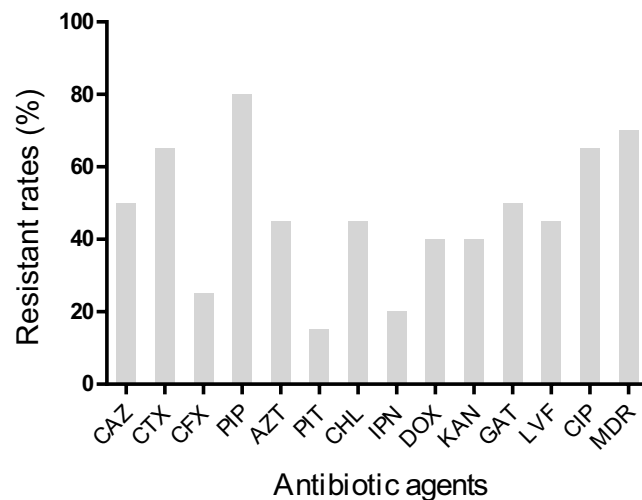


Figure 1 The resistant rates of 20 *K. pneumoniae* isolated from sputum against 13 antibiotics.

Abbreviations: CAZ, Ceftazidime; CTX, Cefotaxime; CFX, Cefoxitin; PIP, Piperacillin; AZT, Aztreonam; PIT, Piperacillin-tazobactam; CHL, Chloramphenicol; IPN, Imipenem; DOX, Doxycycline; KAN, Kanamycin; GAT, Gatifloxacin; LVF, Levofloxacin; CIP, Ciprofloxacin; MDR, multidrug-resistant.

quinolone resistance (*PMQR*) genes, *qnr_A* was not detected, while *oqx_{AB}* was present in almost all strains. Genes *aac* and *qnr_B* were scattered, with detection rates of 40.0% and 25.0%, respectively, shown in [Table S1](#). *bla_{CTX-M}+bla_{TEM}+aac+oqx_{AB}* gene strains were widely distributed about (30.0%), which possesses multidrug-resistant strains. The 2/3 strains showed co-production of *bla_{KPC}+bla_{SHV}+oqx_{AB}* among *bla_{KPC}*-producing strains. Overall, the detection multidrug-resistant rate was higher in strains isolated from pulmonary infectious diseases, which account for 87.5% (7/8).

Virulence Phenotypes and Genes of *K. pneumoniae*

The results of the crystal violet staining showed that all 20 *K. pneumoniae* could form biofilms, while 18 strains (90.0%) showed strong biofilms. String test showed that 5 strains (25.0%) presented the HMV phenotype. [Table 4](#) characterizes the distribution of the virulence genes of *K. pneumoniae* strains isolated from various underlying diseases. The *wab_G* gene encoding with LPS and

Table 3 Distribution of Antibiotic Resistance Genes and ESBL Phenotype in 20 Strains of *K. pneumoniae*

| Resistance Genes | Distribution | |
|---|--------------------------|---------------------|
| | Number of Strains (n=20) | Resistant Rates (%) |
| ESBL phenotype | 11 | 55.0 |
| <i>bla_{SHV}</i> | 12 | 60.0 |
| <i>bla_{CTX-M}</i> | 7 | 35.0 |
| <i>bla_{TEM}</i> | 8 | 40.0 |
| <i>bla_{KPC}</i> | 3 | 15.0 |
| <i>bla_{NDM}</i> | 0 | 0.0 |
| <i>bla_{OXA-48}</i> | 0 | 0.0 |
| <i>bla_{IMP}</i> | 0 | 0.0 |
| <i>qnr_A</i> | 0 | 0. |
| <i>qnr_B</i> | 5 | 25.0 |
| <i>oqx_A</i> | 18 | 90.0 |
| <i>oqx_B</i> | 19 | 95.0 |
| <i>aac</i> | 8 | 40.0 |
| <i>bla_{KPC}+bla_{SHV}+oqx_{AB}</i> | 2 | 10.0 |
| <i>bla_{CTX-M}+bla_{TEM}+aac+oqx_{AB}</i> | 6 | 30.0 |

Table 4 Distribution of Virulence Genes of *K. pneumoniae* Isolated from Sputum

| Distribution | Virulence Phenotypes | | Virulence Genes | | | | | | | |
|----------------------------|----------------------|-----|-----------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | BF | HMV | <i>wzi</i> | <i>rmp_A</i> | <i>mag_A</i> | <i>wab_G</i> | <i>fim_H</i> | <i>mrk_D</i> | <i>iuc_A</i> | <i>iut_A</i> |
| Number of positive strains | 20 | 5 | 20 | 5 | 2 | 20 | 18 | 17 | 7 | 6 |
| Positive rates (%) | 100 | 25 | 100.0 | 25.0 | 10.0 | 100.0 | 90.0 | 85.0 | 35.0 | 30.0 |

Abbreviations: BF, biofilm; HMV, hypermucoviscous phenotype.

wzi encoding with CPS were found in all strains. The genes *fim_H* and *mrk_D* encoding with pili were detected at 90% and 85%, respectively. The most prevalent virulence genes *iuc_A* and *iut_A* (encoding and regulating with aerobactin)¹⁸ were detected at 35.0% and 30.0%, respectively. Overall, the strains extracted from patients with craniosynostosis were more virulent and had a higher level of all virulence gene detection rate, accounting for 50% (3/6).

Capsular Serotypes and *wzi* Phylogenetic Tree

Among 20 *K. pneumoniae* isolates, 10 strains were identified as K1 (2 strains), K19 (2 strains), K17, K23, K24, K46.61, K14.64, and K43 (1 strain). In contrast, the other 10 strains were unknown capsular serotyping, which belonged to *wzi* 168, *wzi*84, *wzi*679, *wzi*206, *wzi*187, *wzi*209, *wzi*150, *wzi*516, *wzi*275, and *wzi*401. Each sequence corresponds indicated a distinct *wzi* allele. The corresponding capsular types followed the allele number. The phylogenetic tree showed a high genetic diversity with more branches of the sample *wzi* gene and significant differences were observed between strains isolated from the samples and the same families (Figure 2).

Correlation Analysis

Pearson's correlation analysis was performed to assess the existence of the potential correlation between antibiotic-resistant phenotypes and molecular characteristics of *K. pneumoniae* strains. Moreover, Figure 3 suggests that the MDR is positively

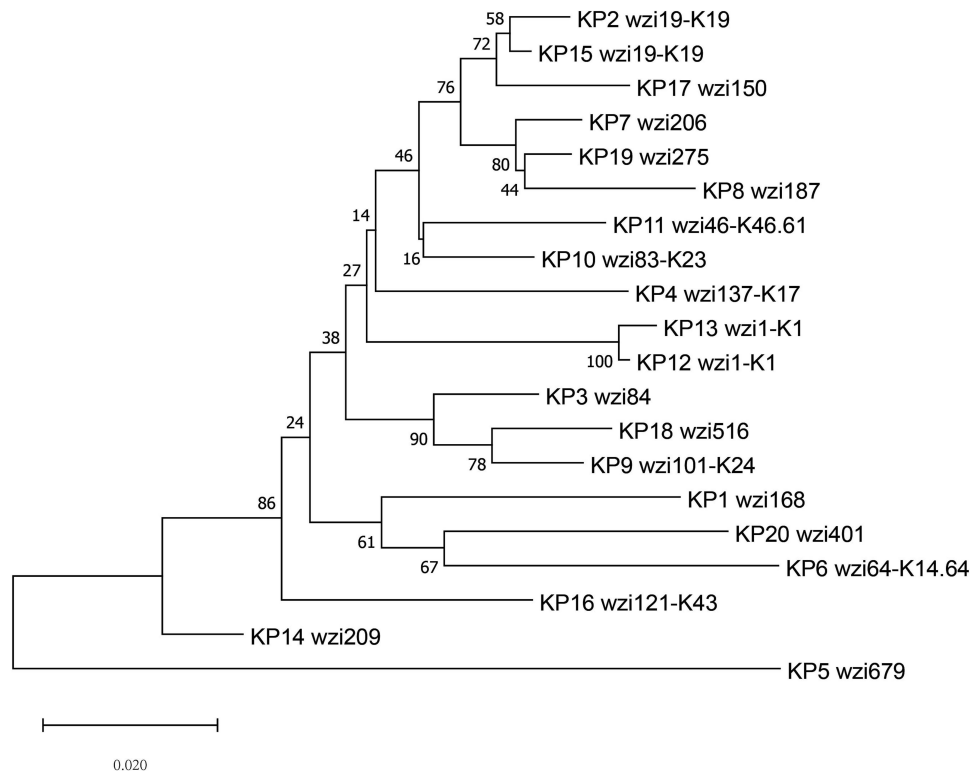


Figure 2 Phylogenetic tree of 20 *K. pneumoniae* isolated from sputum base on *wzi* gene sequence. Phylogenetic trees were constructed using MEGA 7 based on the neighbor-joining method (500 bootstrap replicates) and Jukes-Cantor distance. Each sequence corresponds indicated a distinct *wzi* allele. The corresponding capsular types followed the allele number.

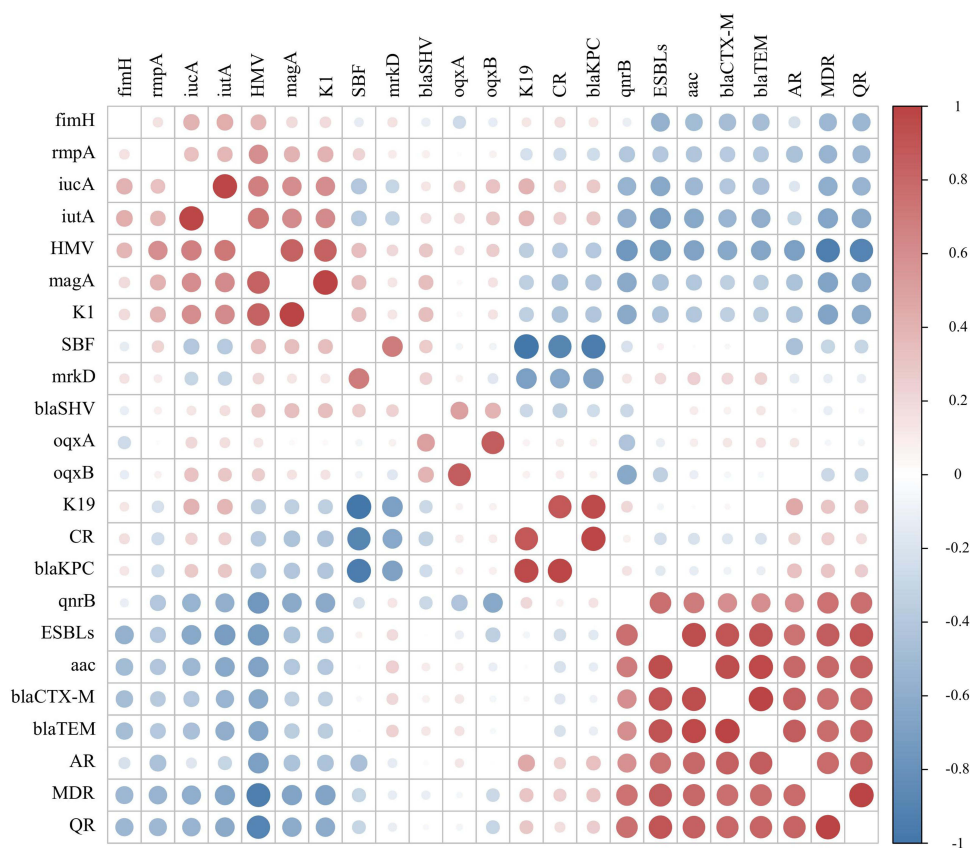


Figure 3 Pearson's correlation analysis between phenotypes and genotypes of 20 *K. pneumoniae* isolated from sputum. Red is positive correlations and blue is negative correlations between different parameters.

Abbreviations: CR, Carbapenem-like antibiotic resistance phenotype; QR, Quinolone antibiotic resistance phenotype; AR, Aminoglycoside antibiotic resistance phenotype; SBF, Strong biofilm.

significant correlated with *bla_{TEM}* ($r = 0.535$, $p < 0.05$), *bla_{CTX-M}* ($r = 0.480$, $p < 0.05$) and *aac* ($r = 0.535$, $p < 0.05$), while negative correlations with HMV ($r = -0.630$, $p < 0.01$) and *rmp_A* ($r = -0.630$, $p < 0.01$). HMV exhibited positive correlations with *rmp_A* ($r = 1.000$, $p < 0.01$), *iuc_A* ($r = 0.545$, $p < 0.05$), *iut_A* ($r = 0.630$, $p < 0.01$) and *mag_A* ($r = 0.577$, $p < 0.01$). SBF was positively correlated with *mrk_D* ($r = 0.327$). Some molecular types displayed positive or negative correlations with several resistance and virulence genes. K19 and CR ($r = 0.667$, $p < 0.01$), *bla_{KPC}* ($r = 0.793$, $p < 0.01$) displayed a strong positive correlation with SBF ($r = -1.000$, $P < 0.01$), and a negative correlation with *mrk_D* ($r = -0.327$). K1 was completely correlated with *mag_A* ($r = 1.000$, $p < 0.01$). According to [Figure 3](#), virulence genes *rmp_A* and *iut_A* ($r = 0.630$, $p < 0.01$) shows significantly positive correlation, and all antibiotic resistance phenotypes have a huge correlation with their genes.

Discussion

It has been reported that the antibiotic resistance of *K. pneumoniae* isolated from sputum has increased year by year.^{6–8} But molecular characteristics studies are still very rare. In the current study, 20 strains of *K. pneumoniae* (19 from sputum and 1 from alveolar lavage fluid) were collected from the affiliated people's hospital of Xinxiang Medical University, Henan Province, China. The antibiotic susceptibility results showed that the antibiotic resistance of sputum isolates of *K. pneumoniae* was severe, with the highest resistance rate of 80.0% for PIP. While lower resistance rates for IPN and PIT 20.0% and 15.0%, respectively. Moreover, ESBLs-producing positive strains were found up to 55.0%. So, these results are consistent with the existing literature.^{6,19,20} The aforementioned literature also reported that resistance rates of cephalosporins and quinolone antibiotics rates were below 40.0% a while carbapenems were below 10.0%. In the current research, *K. pneumoniae* strains exhibited a higher resistance prevalence than the literature cited above. Current study results suggest that this difference may be related to the spread of the region's antimicrobial resistance genes due to

frequent antibiotic use.²¹ Therefore, further experimental work is still to be needed to explore the antimicrobial resistance genes and the local antibiotic use.

Many antibiotic-resistant genes are involved in the drug resistance of *K. pneumoniae*. The current study elaborates the mechanism of antibiotic resistance which is being used in clinical practice. β -lactam antibiotics are the most commonly used antibiotics in the clinic, among which ESBLs production is the primary mechanism of antibiotic resistance. In this study, all ESBLs-producing strains were MDR strains that are used in the current study. These findings support the conclusion that ESBLs-producing bacteria's resistance was enhanced, agreeing with the reported studies.^{22,23} In the current research, *bla_{SHV}* is more widely distributed among genes encoding resistance to ESBLs, which may be the main gene type in the prevalence of ESBLs in our region. The underlying mechanism of antibiotic resistance is the production of carbapenemases which can hydrolyze penicillins, cephalosporins, monocyclic β lactamides, aminoglycosides, quinolones, and other antibiotics.²⁴ The modified Hodge test and PCR were used to confirm the phenotype and genes of carbapenemase. The results showed that only 3 *K. pneumoniae* of producing carbapenemases were detected with KPC gene in this study, and these 3 strains were resistant to all antibiotics except Kanamycin, chloramphenicol and doxycycline. The details of antibiotics have been described in [Table S2](#). Fluoroquinolones are a class of powerful broad-spectrum antibiotics that have been used to treat severe or antibiotic-resistant infections since the end of the 20th century.²⁵ However, in recent years, with frequent use and abuse of antibiotics, the resistance rate of the pathogen to fluoroquinolones has been increasing and is currently up to 50%. Reported studies by Liam S. Redgrave et al showed that the prevalence of *PMQR* genes among the strains was not affected by quinolone antibiotics selection,²⁵ which may be the crucial factor in the rapid spread of resistance. In current experimental work, *qnr_A* was not detected, *qnr_B* and *aac* were scattered, while the detection rate of *oqx_{AB}* was higher, which is parallel with some reported literature.^{26,27}

The emergence of MDR strains would be hindered the clinical treatment of infection caused by *K. pneumoniae*. The MDR study mechanism has great importance in reducing the fatality rate, improving the spread of MDR strains, and delaying the occurrence of pan-resistant strains. The results show that the rate of *K. pneumoniae* in MDR is higher, about 70.0%. However, the antibiotic resistance genes *bla_{CTX-M}*, *bla_{TEM}*, and *aac* are closely associated with MDR strains. Also, all three genes are plasmid-mediated antibiotic resistance genes.²⁸ So, the strains that produced all three genes together accounted for about 30.0%. It has been reported that *PMQR* genes (*aac*, *qnr_A*, *qnr_B*, *oqx_A* and *oqx_B*) are usually carried on plasmids with other resistance genes, especially ESBLs-producing plasmids.²⁶ The results of the current study showed that ESBLs genes (*bla_{CTX-M}*, *bla_{TEM}*) and *PMQR* genes (*aac*) are more strongly correlated. Therefore, with combined literature²⁶ analysis, the above mentioned genes may be located in the same plasmid for transmission. Therefore, these genes may be critical for transmitting multi-drug resistant strains in this region.

The main virulence factors of *K. pneumoniae* included capsular (CPS), lipopolysaccharide (LPS), pili, and iron carriers, which are also the main factors leading to the characteristics of hypervirulent *K. pneumoniae* (hvKP).²⁹ Many genes encoding these virulence factors have been reported, and some representative genes were selected for amplification in the current study. The results show that HMV phenotype is strongly correlated with *rmp_A*, *map_A*, *iuc_A* and *iut_A*, which is aligned with previous studies. *rmp_A* and *mag_A* regulate the expression of CPS, closely related to the high HMV phenotype of *K. pneumoniae*.^{29,30} While *iuc_A* codes aerobactin and *iut_A* codes aerobactin transporter¹⁸ shows higher level virulence.³⁰ *wzi* and *wab_G* have been detected in 20 strains of *K. pneumoniae*, *wzi* encodes outer membrane protein *Wzi* and is involved in the attachment of the CPS to the outer membrane.¹⁶ *wab_G* gene encodes *Wab_G* protein and is involved in the synthesis of LPS.³¹ The detection rates of *fim_H* and *mrk_D* genes encoding type I and III pili are 90% and 85%, respectively. The detection rate of the above four genes is higher, which is consistent with the reported literature.^{6,26,32} All *K. pneumoniae* in this study can form the biofilms, which may be related to the source of samples. Type I and III pili are crucial factors for *K. pneumoniae* colonization, and *fim_H* encodes *Fim_H* adhesion molecule at the tip of type I pili,³² which is closely related to urinary tract infection.³³ *mrk_D* encodes adhesive subunit located at the tip of type III pili,³⁰ and mediates the binding of *K. pneumoniae* with organ cells and lung tissues,³⁴ which is related to lung infection. The current study results elaborate that SBF and *mrkD* showed a positive correlation, which supports our study.

To analyze *K. pneumoniae*, basic information about the isolated strains was taken from Xinxiang City, Henan Province, between July and November 2020. Overall, strains isolated from pulmonary infectious diseases tend to

exhibit greater drug resistance, which is relatively more virulent strains isolated from craniosynostosis (more common and critical). The high rate of resistance in pulmonary infectious diseases may be related to the use of β -lactamase inhibitor antibiotics prior to sampling.²¹ β -lactamase inhibitor antibiotics are more commonly available in the community in Henan Province. So, this phenomena may lead to the occurrence of drug-resistant bacteria in the community. Strains of current research collected from the People's Hospital of Xinxiang City, Henan Province (not the community) provide a possible history of antibiotic usage in the patients. Therefore, it is necessary to analyze the local dissemination of drug-resistant genes by incorporating antibiotic abuse in the local community. Patients with craniosynostosis are often associated with severe clinical symptoms, which may be related to the high virulence of the strains.

wzi, is a conserved open reading frame. So the first of four conserved gene blocks (*wzi-wza-wzb-wzc*) were found in all group 1 K-antigen serotypes.³⁵ Sequencing of the *wzi* genotype allows rapid prediction of K-serotypes, and this method can be used to determine K-types.^{36–38} In this study, we found *wzi* typing and K-antigen serotypes and some strains by *wzi* sequence analysis. Moreover, only exact matches were selected for analysis to ensure accurate experiments. *wzi* sequencing are not sufficient for accurate serotyping. Therefore, K serotypes of some strains could not be determined. Clear serotyping by immunological methods or sequencing the entire polysaccharide capsule synthesis gene cluster (35 kbp) is necessary for further genetic characterization. In the current study, *wzi* phylogenetic tree shows the genetic diversity of the experimental samples and the low probability of intra-sectional strain transmission. So, it indicates a representative sample.

Correlation analysis shows a strong relationship between the distribution of *iut_A* and *rmp_A*, which may be related to the fact that both the genes are located in the same *pLVPK* plasmid.³⁹ It has been reported that *mag_A* encodes CPS polymerase specific to K1 type,⁴⁰ K1 serotype and *mag_A* also shows an absolute correlation in this study. Serotype K19 is strongly correlated with *bla_{KPC}*, which might be the main epidemic serotype of carbapenemase in the local area. The antibiotic resistance of *K. pneumoniae* generally negatively correlates with the distribution of virulence characteristics. Thus, the multiresistant strains exhibited negative correlations with virulence phenotypes and genes. The HMV phenotype is negatively correlated with antibiotic resistance phenotypes and genes, which is consistent with previously reported literature.⁴¹ KP13 is a multiresistant strain of capsular serotype K1, carrying three types of ESBLs antibiotic-resistant genes in the current study, which shows serious concern with public health. The prevalence mechanism of antibiotic resistance and virulence factors has crucial implications for the patients.

Conclusions

In conclusion, our study describes antibiotic resistance and virulence characteristics of 20 Strains of *K. pneumoniae* isolated from the sputum samples in Xinxiang, China. The results show that local *K. pneumoniae* has severe drug resistance and contains antibiotic resistance genes (*bla_{CTX-M}*, *bla_{TEM}* and *aac*). Virulence genes *mag_A*, *rmp_A*, *iuc_A* and *iut_A* are crucial influencing factors of HMV. In general, virulence exhibited significant negative correlations with antibiotic resistance factors, and some strains exist simultaneously. Therefore, it is necessary to investigate their transmission mechanism to effectively slow down the emergence of such strains, ultimately reducing the medical treatment costs.

Data Sharing Statement

The datasets supporting the conclusions of this article are included within the article (and the [Supplementary Materials](#)).

Ethics Approval and Consent to Participate

This research was carried out in accordance with Declaration of Helsinki. The informed consent have been voluntarily obtained from the participants and participants have been informed of the study including any of the benefits and risks involved. The research was approved by the Ethics Committee of Xinxiang Medical University.

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Disclosure

The authors declare that they have no competing interests.

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