

Molecular Characterization of *Klebsiella Pneumoniae* Isolated From Sputum

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Abstract

Background: *K. pneumoniae* is a common opportunistic pathogen responsible in clinical practice and an important pathogen of nosocomial infection. This study aimed to analyze the trend of antimicrobial susceptibility and virulent characteristics of *K. pneumoniae* isolated from sputum in clinics and to provide data for clinical treatment of *K. pneumoniae* infection disease.

Results: The resistance rates of the 20 *K. pneumoniae* isolates against 13 antibiotics ranged from 15.0% to 80.0%. The detection rate of ESBLs was up to 55%, and *bla_{SHV}* were the most prevalent ESBLs genes. Four strains (25.0%) of *K. pneumoniae* presented HMV phenotype, and 18 strains (90.0%) had the stronger biofilm-forming ability. *wzi*, *wab_G*, *fim_H*, *mrk_D* were the most prevalent virulence genes. 10 strains were present capsule typing and the higher genetic diversity of colonizing *K. pneumoniae* in this region. K₁₉ exhibited a strong positive correlation with imipenem resistance, and K₁ showed **strong** correlations with *mag_A*. HMV phenotype show significantly negative correlations with multidrug-resistant.

Conclusions: The antibiotic resistance of *K. pneumoniae* isolated from sputum in this hospital was serious, and these strains show the higher genetic diversity.

Background

Klebsiella pneumoniae (*K. pneumoniae*) is a common opportunistic pathogen responsible in clinical practice and an important pathogen of nosocomial infection, leading to various types of infection, such as pneumonia, blood infection, urinary tract infection, and osteomyelitis.¹ The detection rate of Gram-negative pathogens was second only to *Escherichia coli*.² With the widespread use and even abuse of antibiotics in clinic, resistant strains were increasing year by year. In particular, the emergence of multidrug-resistant (MDR) strains led to the failure of clinical antibacterial treatment and the delay of the course of disease.³ This increased the medical costs of patients and the mortality of inpatients, which has emerged as an urgent threat to public health. A variety of 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 show different pathogenic characteristics and clinical characteristics.⁵ The number of studies of clinically isolated *K. pneumoniae* has been increased dramatically in recent years.^{6–8} However, there are limited studies about antibiotic resistance and molecular characteristics of *K. pneumoniae* isolated from sputum. Here we identify the antibiotic resistance and molecular characteristics of *K. pneumoniae* isolated from clinical sputum. This study aimed to obtain a better comprehension of the molecular epidemiological characteristics of *K. pneumoniae* in this region, which is of great clinical significance for the prevention and control of *K. pneumoniae* infection and transmission.

Results

Antimicrobial susceptibility testing

The resistance of 20 strains of *K. pneumoniae* to 13 commonly used antibiotics was shown in Table 1. Overall, the antibiotic test range of the 20 strains ranged from 15.0% to 80.0%, with multidrug-resistant strains up to 70.0%. The strains showed the highest resistance to PIP (80.0%), followed by CTX (65.0%) and CIP (65.0%), and the lowest resistance to PIT (15.0%), followed by IPN (20.0%) and CFX (25.0%). The range of other antibiotics tested was around 50.0%.

Table 1. Antibiotic resistance profiles of *K. pneumoniae* isolated from sputum

Antibiotic agents	Resistance against 13 antibiotics	
	Number of strains (n=20)	Resistant rates(%)
CAZ	10	50.0
CTX	13	65.0
CFX	5	25.0
PIP	16	80.0
AZT	9	45.0
PIT	3	15.0
CHL	9	45.0
IPN	4	20.0
DOX	8	40.0
K	8	40.0
GAT	10	50.0
LVF	9	45.00
CIP	13	65.0
MDR	14	70.0

ESBL phenotype and resistance genes of *K. pneumoniae*

Table 2 describes the distribution of antibiotic resistance genes in clinical *K. pneumoniae*. ESBLs-producing strains were prevalent in the region, with a detection rate of 55.0%, among which *bla_{SHV}* was the most widely distributed strain, while the other two strains were less detected. Only 3 *bla_{KPC}* positive strains of carbapenase genes were detected, but no other carbapenase genes were detected. Among plasmid-mediated quinolone resistance (*PMQR*) genes, *qnr_A* was not detected, while *oq_{XAB}* was distributed in almost all strains, *aac* and *qnr_B* were scattered, with detection rates of 40.0% and 25.0%, respectively. As shown in Table S1, *bla_{CTX-M}+bla_{TEM}+aac+oq_{XAB}* strains were widely distributed (30.0%),

all of which were multidrug-resistant. 2/3 showed co-production of *bla*_{KPC}+*bla*_{SHV}+*oqx*_{AB} among *bla*_{KPC} producing strains.

Table 2. 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</i> _{SHV}	12	60.0
<i>bla</i> _{CTX-M}	7	35.0
<i>bla</i> _{TEM}	8	40.0
<i>bla</i> _{KPC}	3	15.0
<i>bla</i> _{NDM}	0	0.0
<i>bla</i> _{OXA-48}	0	0.0
<i>bla</i> _{IMP}	0	0.0
<i>qnr</i> _A	0	0.
<i>qnr</i> _B	5	25.0
<i>oqx</i> _A	18	90.0
<i>oqx</i> _B	19	95.0
<i>aac</i>	8	40.0
<i>bla</i> _{KPC} + <i>bla</i> _{SHV} + <i>oqx</i> _{AB}	2	10.0
<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM} + <i>aac</i> + <i>oqx</i> _{AB}	6	30.0

Virulence phenotypes and genes of *K. pneumoniae*

The results of the crystal violet staining showed that all 20 *K. pneumoniae* could form biofilms, and 18 strains (90.0%) had strong biofilms. String test showed that 5 strains (25.0%) presented the HMV phenotype. Table 3 characterizes the distribution of the virulence genes of *K. pneumoniae* strains isolated from sputum. The LPS-encoding *wab*_G gene and CPS encoding *wzi* were present in all strains; The genes encoding type I *fim*_H, and type III *mrk*_D were 90% and 85%, respectively, and was pathogenic genes.

iucA (35.0%) and *iut_A* (30.0%) were the genes encoding and regulating the aerobactin. The positive rates of mucoviscosity-associated genes *rmp_A* and *mag_A* were 25.0% and 10.0%, respectively.

Table 3. Distribution of virulence genes of *K. pneumoniae* 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

Abbreviation: BF Biofilm, HMV hypermucoviscous phenotype.

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). Other 10 strains were unknown capsular serotyping, which belong *wzi* 168, *wzi*84, *wzi*679, *wzi*206, *wzi*187, *wzi*209, *wzi*150, *wzi*516, *wzi*275, and *wzi*401. Each sequence corresponds to a distinct *wzi* allele, as indicated. The allele number is followed by the corresponding capsular types. The phylogenetic tree showed more branches and higher genetic diversity (Fig.1).

Figure 1. Phylogenetic tree of *wzi* sequence of clinical isolates of *K. pneumoniae*

Analysis of the correlations

Pearson's correlation analysis was used to assess the existence the potential correlation between antibiotic-resistant phenotypes and molecular characteristics of *K. pneumoniae* strains. Figure 2 suggests that the MDR was significantly positively correlated with *bla_{TEM}* ($r = 0.535$, $p < 0.05$), *bla_{CTX-M}* ($r = 0.480$, $p < 0.05$) and *aac* ($r = 0.535$, $p < 0.05$), and negative correlations with HMV ($r = 0.630$, $p < 0.05$) and *rmp_A* ($r = 0.630$, $p < 0.05$). HMV exhibited positive correlations with *rmp_A* ($r = 1.000$, $p < 0.001$), *iuc_A* ($r = 0.545$, $p < 0.05$), *iut_A* ($r = 0.630$, $p < 0.05$) and *mag_A* ($r = 0.577$, $p < 0.05$). SBF was positively correlated with *mrk_D* ($r = 0.327$, $p < 0.05$). Some molecular types displayed positive or negative correlations with several resistance and virulence genes. K19 and CR ($r = 0.667$, $p < 0.001$), *bla_{KPC}* ($r = 0.793$, $p < 0.001$) displayed a strong positive correlation with BF ($r = -1.000$, $P < 0.001$), and a negative correlation with *mrk_D* ($r = 0.327$, $p < 0.05$). K1 was completely correlated with *mag_A* ($r = -1.000$, $p < 0.001$). According to Figure 2, virulence genes *rmp_A* and *iut_A* ($r = 0.630$, $p < 0.05$) showed significantly positive correlation, and all antibiotic resistance phenotypes had huge correlation with their genes.

Figure 2. Pearson's correlation analysis between phenotypes and genotypes of *K. pneumoniae*

Discussion

Reports of antibiotic resistance of *K. pneumoniae* isolated from sputum have increased year by year.⁶⁻⁸ But studies on molecular characteristics are still rare. In this 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 *K. pneumoniae* isolated from sputum showed high resistance to 15 antimicrobials. The resistance rate to PIP was the highest (80.0%), IPN and PIT were lower (20.0%,15.0% respectively), ESBLs-producing strains accounted for up to 55.0%. These results are consistent with literature reports.^{6,9-10} In previous studies, resistance rates of cephalosporins and quinolone antibiotics were less than 40.0%, and carbapenems were less than 10.0%. In this study, *K. pneumoniae* strains exhibited a higher prevalence of resistance than in the above study. We analyzed that the differences might be related to the molecular characteristics and prevalent antibiotic usage habits in the region. Therefore, combined with the antimicrobial resistance gene of this study and the investigation of antibiotics in patients, further analysis of the reasons.

Many antibiotic resistance genes are involved in the occurrence of drug resistance of *K. pneumoniae*, and we studied the mechanism of antibiotic resistance commonly used in clinical practice. β -lactam antibiotics are the most widely used antibiotics in clinic, among which ESBLs production is the main mechanism of antibiotic resistance. In this study, ESBLs-producing strains were absolutely multiresistant, which supports the conclusion that the resistance of ESBLs-producing bacteria was enhanced, consistent with the studies report.¹¹⁻¹² *bla_{SHV}* is more widely distributed among genes encoding ESBLs resistance, which may be the main mechanism of ESBLs epidemic distribution in this region. Carbapenems are the last line of defense for the treatment of ESBLs-producing Gram-negative pathogen infection. The main mechanism of antibiotic resistance is the production of carbapenemases that can hydrolyze penicillins, cephalosporins, monocyclic β lactamides, aminoglycosides, quinolones, and other antibiotics.¹³ Only 3 *K. pneumoniae* of KPC-producing carbapenemases were detected in this study, which was resistant to all antibiotics except chloramphenicol and doxycycline. Details of antibiotics refer to 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.¹⁴ However, with the widespread use and abuse of antibiotics in recent years, the resistance rate of the pathogen to fluoroquinolones has been increasing, and in this study has reached about 50%. Studies by Liam S. Redgrave et al showed that the prevalence of *PMQR* genes among strains was not affected by quinolone antibiotics selection,¹⁴ which may be the key to the rapid spread of resistance. In this study, *qnr_A* was not detected, *qnr_B* and *aac* were scattered, while the detection rate of *oqx_{AB}* was high, which was consistent with some reports.¹⁵⁻¹⁶

The emergence of MDR strains has caused great difficulties in the clinical treatment of infection caused by *K.pneumoniae*. The study of MDR mechanism is of great significance for reducing the fatality rate, improving the spread of MDR strains, and delaying the occurrence of pan-resistant strains. In this study, the rate of multidrug-resistant *K.pneumoniae* has reached 70.0%, and antibiotic resistance genes *bla_{CTX-}*

*bla*_{TEM}, and *aac* are closely related to multidrug-resistant strains. Moreover, these three genes are plasmid-mediated antibiotic resistance genes,¹⁷ and strains co-producing three genes account for 30.0%, which may be crucial genes for spreading multiresistant strains in this region. Studies have reported that *PMQR* gene is usually carried on the plasmids of other antibiotic resistance genes, especially ESBLs.¹⁵ Therefore, we speculated that the above-mentioned epidemic resistance genes in this region may be located in the same plasmid and spread, the specific mechanism needs further study.

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*).¹⁸ Many genes encoding these virulence factors have been reported, and some representative genes were selected for amplification in this study. The results showed that HMV phenotype was strongly correlated with *rmp*_A, *map*_A, *iuc*_A and *iut*_A, which was consistent with some studies: *rmp*_A and *mag*_A regulated the expression of CPS, which was closely related to the high HMV phenotype of *K. pneumoniae*.¹⁸⁻¹⁹ *iuc*_A codes *aerobactin*, and *iut*_A codes *aerobactin* transporter,²⁰ closely related to high virulence.¹⁹ *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 membrane²¹ and *wab*_G gene encodes *Wab*_G protein and is involved in the synthesis of LPS.²² The detection rates of *fim*_H and *mrk*_D genes encoding type I and III pili were 90% and 85%, respectively. The detection rate of the above four genes is high, which is consistent with the report.^{6,15,23} All *K. pneumoniae* in this study can form 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 1 pili,²³ which is closely related to urinary tract infection.²⁴ *mrk*_D encodes adhesive subunit located at the tip of type 3 hair,²⁵ and mediates the binding of *K. pneumoniae* with organ cells and lung tissues,²⁶ which is related to lung infection. Studies have shown that type 3 pili have been proved to play a major role in the formation of biofilms,²⁴ which is consistent with the results of this study.

Correlation analysis showed a strong correlation between the distribution of *iut*_A and *rmp*_A, which may be related to the fact that both are located in the same *pLVPK* plasmid.²⁷ Studies have shown that *mag*_A encodes CPS polymerase specific to K1 type,²⁸ K1 serotype and *mag*_A also showed an absolute correlation in this study. Serotype K19 was strongly correlated with *bla*_{KPC}, which might be the main epidemic serotype of carbapenase in the local area. The antibiotic resistance of *K. pneumoniae* was generally negatively correlated with the distribution of virulence characteristics, in which the multiresistant strains exhibited negative correlations with virulence phenotypes and genes, and the HMV phenotype was negatively correlated with antibiotic resistance phenotypes and genes, which was consistent with the literature reports.²⁹ KP13 is a multiresistant strain of capsular serotype K1, carrying three types of ESBLs antibiotic-resistant genes in this study, which seriously endangers public health. Studying the prevalence mechanism of antibiotic resistance and virulence factors has crucial implications.

Conclusions

In conclusion, our study describes antibiotic resistance and virulence characteristics of 20 Strains of *K. pneumoniae* isolated from sputum from Henan, China. The results showed that local *K. pneumoniae* had severe drug resistance, and antibiotic resistance genes *bla*_{CTX-M} *bla*_{TEM} and *aac* were the main mechanisms. Virulence genes *magA*, *rmpA*, *iucA* and *iutA* were crucial influencing factors of HMV. In general, virulence exhibited significant negative correlations with antibiotic resistance factors, and some strains exist at the same time. Therefore, investigate their transmission mechanism to effectively slow down the emergence of such strains and reduce medical costs is necessary.

Methods

Bacterial isolates

A total of 20 *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 isolates were identified according to the National Clinical Examination Procedures, and the isolates were numbered KP1-KP20 in sequence.

Antibiotics and Reagents

Following antibiotics were used in this study: Ceftazidime (CAZ,30µg), Ceftazidime-clavulanic acid (CAC,30µg/10µg), Cefotaxime (CTX,30µg), Ceftazidime-clavulanic acid (CTC,30µg/10µg), Cefoxitin (CFX,30µg), Aztreonam (AZT,30µg), Piperacillin-tazobactam (PIT,100µg/10µg), Piperacillin (PIP,100µg) Imipenem (IPN,10µg) Doxycycline (DOX,30µg) Chloramphenicol (CHL,30µg) Ciprofloxacin (CIP,5µg) Levofloxacin (LVF,5µg) Gatifloxacin (GAT,5µg) Kanamycin KAN, 30µg. LB agar plates and Columbia blood agar plates were purchased from Guangzhou Huankai Microbiology Technology Limited Company. The reagents of PCR were purchased from Kangwei Century Co., Ltd. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The primer sequences are shown in Table 4 and Table 5.

ESBLs Phenotype confirmation tests

ESBLs phenotypes of 20 Strains of *K. pneumoniae* were detected by the combined disc test (CDT) recommended by CLSI 2020. Briefly, the bacteria with turbidity equivalent to 0.5 McFarland standards were swabbed onto MH agar plates containing 30 µg of CTX and CAZ, with and without 10 µg of clavulanic acid, were placed independently, 25 mm apart (center to center) on a lawn culture. The plates were incubated at 37 °C for up to 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. Drug sensitivity results were determined according to CLSI 2020 criteria. In the antimicrobial sensitivity test results, the strain resistant to more than 3 antibiotics is considered MDR bacteria.

Amplify antimicrobial resistance genes

Bacterial DNA template was extracted by the water boiling method. Briefly, bacteria were inoculated and cultured overnight in LB liquid medium at 37 °C. 1 ml bacterial culture was added into the 1.5 mL EP tube, centrifuged at 12,000 RPM for 2 min, and the supernatant was discarded and resuspended in 500 µL water, then centrifuged again under the same conditions, and the supernatant was discarded. Add 200 µL water, boiling at 100 °C, 20-30 min to lyse 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. The following resistant genes of *K. pneumoniae* were amplified by PCR, including encode ESBLs (*bla_{CTX-M}*, *bla_{TEM}*, *bla_{SHV}*), carbapenemase genes (*bla_{KPC}*, *bla_{NDM}*, *bla_{OXA-48}*, *bla_{IMP}*), quinolones resistance genes (*qnr_A*, *qnr_B*, *oqxA*, *oqxB*), aminoglycoside resistance gene (*aac*). The primers information of these resistance genes is shown in Table 4.

Table 4. PCR primers 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: ACGCTCACCGGCTCCAGATTTAT	445	55.1	30
<i>bla_{SHV}</i>	P1: ATGCGTTATATTCGCCTGTG P2: TGCTTTGTTATTCGGGCCAA	753	58.4	30
<i>bla_{KPC}</i>	P1: ATGTCACTGTATCGCCGTC P2: TTA CTGCCCGTTGACGCC	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: CAAGAGGATTTCTCACGCCAGGAT 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: TCGGCGTGTTGGTGA ACTGC	648	60.4	
<i>aac</i>	P1: AGTACAGCATCGTGACCAACA P2: CTCGAATGCCTGGCGTGTTT	545	55.8	18

PS: Primer sequences without reference were obtained by primer 5.0 design (same below)

Determination of Mucinous phenotype

The mucinous phenotype of *K. pneumoniae* was analyzed by “String-forming test” according to previous. Briefly, *K. pneumoniae* was transferred to Columbia blood plate for overnight culture at 37 °C. Dip the

colony was with the inoculum ring, then lift the inoculum ring. If the adhesive wire formed was larger than 0.5 cm, it was judged as positive; otherwise, it was negative. The strain with high mucilage phenotype was positive in the “String-forming test”.

Analysis of biofilm formation ability

Biofilm-forming ability was measured by the determination of adhesion to flat-bottomed microtiter plates (96-well).³¹ Briefly, each well of the 96-well microtitration plates was filled with 200 µl sterile broth liquid medium. Bacterial cultures during biofilm formation (18 h incubation) was added to each well (1:100 in liquid medium, 200 µl liquid medium). Only liquid medium was used as a negative control. After incubation at 37 °C for 48 h, total cell mass was measured as absorbance at 570 nm (OD_1) while the blank was OD_{10} . Each well was washed three times with PBS, dried for 1 h at 60 °C and stained for 20 min with 200 µl of 1% crystal violet. After the crystal violet solution was removed, each well was washed with PBS 4 times to remove the remaining stain. Air dried in the aseptic processing table for 30 mins, each well was added with 200 µl of 95% ethanol. After vibrating for 30 min, the absorbance was measured at 570 nm (OD_2), while the blank was OD_{20} . The biofilm formation capacity was calculated by $B = (OD_2 - OD_{20}) / (OD_1 - OD_{10})$. 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 ($0.1 < B \leq 1.0$) and strong biofilm producers ($B > 1.0$).

Analysis capsular serotypes and virulence genes

Amplification of *wzi* genes for detection of 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 document,²¹ and the products were sent to Bioengineering (Shanghai) incorporated company for sequencing. Sequencing results submitted Institut Pasteur website³² 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* and *markD*), lipopolysaccharide (*wabG*), mucous phenotypic related genes (*rmpA*, *magA*), and ferritin genes (*iucA*, *iutA*). PCR primer information of virulence genes is shown in Table 5.

Table 5. PCR primer sequence information of virulence genes

Gene	Primer sequence	Product size (bp)	Ta opt [°C]	References
<i>wzi</i>	P1:GTGCCGCGAGCGCTTTCTATC TTG GTATTCC P2:GAGAGCCACTGGTTCCAGAA[CorT]TT[CorG]ACCGC	580	55	21
<i>mag_A</i>	P1:GGTGCTCTTTACATCATTGC P2:GCAATGGCCATTTGCGTTAG	1282	58	33
<i>rmp_A</i>	P1:ACTGGGCTACCTCTGCTTCA P2:CTTGCATGAGCCATCTTTCA	516	58	34
<i>iuc_A</i>	P1: CCGCTTCCCTACTTT P2: ATTCGCTTCGCTGTCC	575	54.8	
<i>iut_A</i>	P1:GGCTGGACATCATGGGAACTGG P2:CGTCGGGAACGGGTAGAATCG	300	55	34
<i>wab_G</i>	P1:ACCATCGGCCATTTGATAGA P2:CGGACTGGCAGATCCATATC	683	49	34
<i>fim_H</i>	P1:TGCTGCTGGGCTGGTCGATG P2:GGGAGGGTGACGGTGACATC	688	49	34
<i>mark_D</i>	P1:TTTCTGCACAGCGGTCCC P2:GATACCCGGCGTTTTTCGTTAC	480	49	34

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 correlation analysis was conducted by SPSS software, version 19.0, and data were plotted using Hiplot³⁵ for correlation Heatmap.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files).

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YH completed the acquisition, analysis, interpretation of data and the creation of new software used in the work, and was a major contributor in writing the manuscript.

YJ completed the methodology, the data curation, the formal analysis and the visualization.

HMI completed language editing and polishing.

Wenke Liu completed the data curation, the resources and the investigation.

Wei Liao completed the conceptualization, the supervision, the validation and the writing - review & editing.

CP completed the supervision, the validation and the writing - review & editing.

FY completed the conceptualization, the funding acquisition, the validation and the writing - review & editing.

All authors read and approved the final manuscript.

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Figures

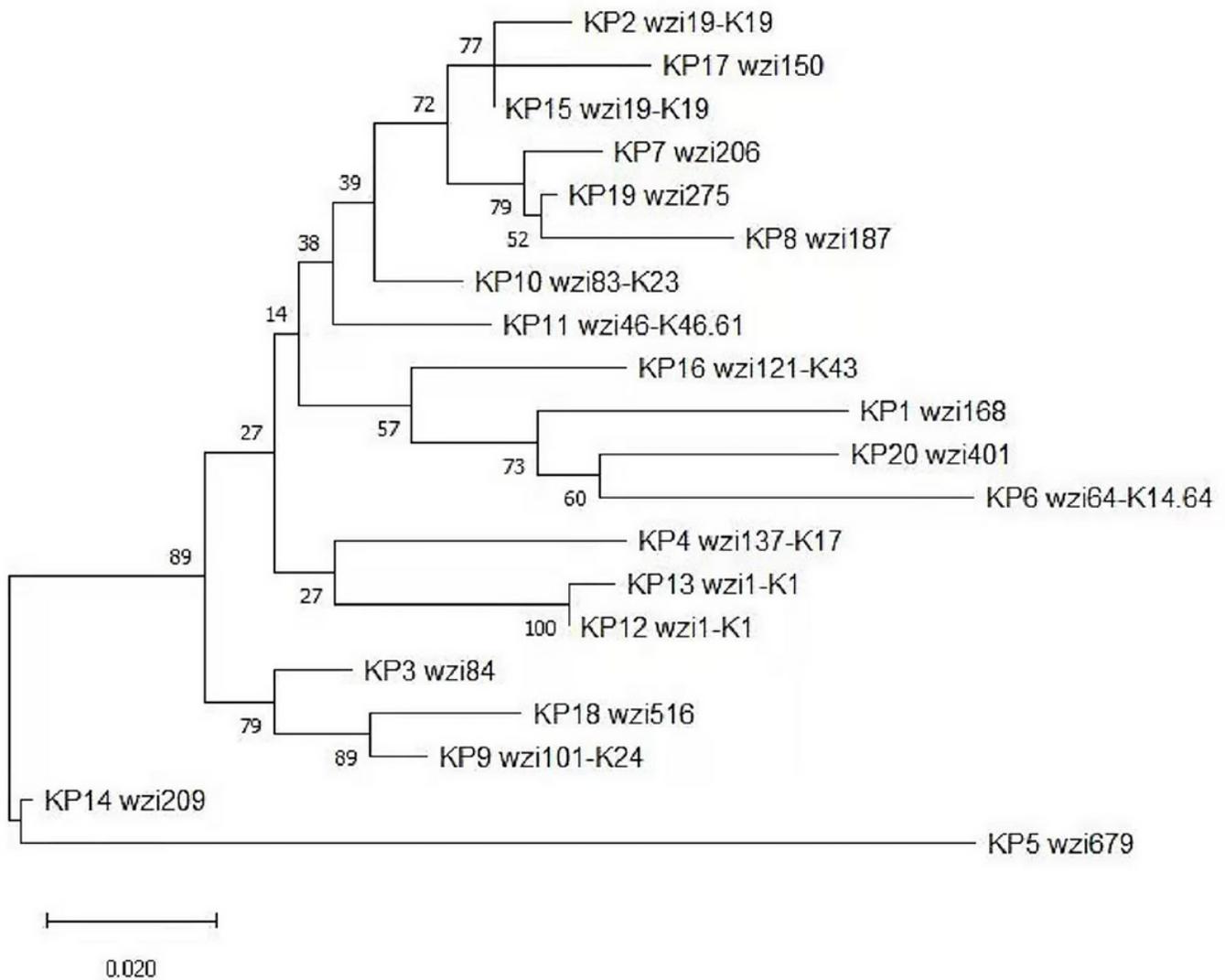


Figure 1

Phylogenetic tree of *wzi* sequence of clinical isolates of *K. pneumoniae*

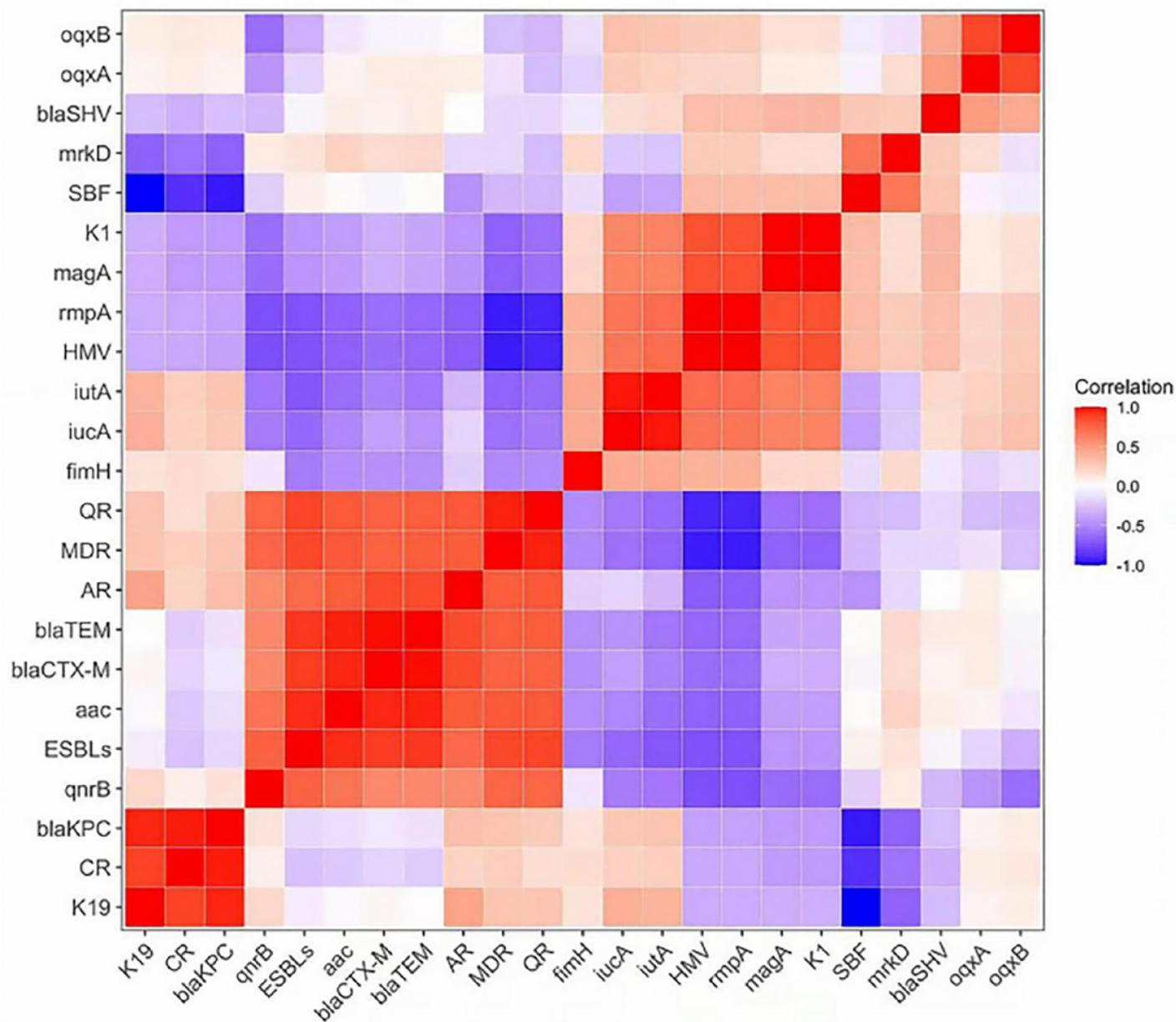


Figure 2

Pearson's correlation analysis between phenotypes and genotypes of *K. pneumoniae*

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