

# High rate of multiresistant *Klebsiella pneumoniae* from human and animal origin

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**Purpose:** The main objectives of the present study were to detect the antimicrobial susceptibility and molecular characteristics of *Klebsiella pneumoniae* isolated from different hosts and to investigate the possibility of *K. pneumoniae* transmission between animals and humans.

**Materials and methods:** A total of 189 nonduplicate *K. pneumoniae* isolates were collected from hospitals and four species of animals in Henan Province, China. The disk diffusion method was used for antimicrobial susceptibility testing, and resistance and virulence genes were screened by polymerase chain reaction (PCR). The molecular types were identified through multilocus sequence typing (MLST), and the hypermucoviscous (HMV) phenotype was identified using the "string-forming test". Pearson's parameters were used to determine the potential link among the molecular types and resistance and virulence genes of all *K. pneumoniae* strains.

**Results:** The resistance rates of the 189 *K. pneumoniae* isolates against 15 antibiotics ranged from 11.6% to 77.8%. The highest multidrug resistance rate was detected in the pig strains (93.6%), followed by the human strains (90.4%), chicken strains (88.9%), cow strains (52.0%) and sheep strains (50.0%). Forty-eight (25.4%) *K. pneumoniae* strains presented the HMV phenotype. *entB*, *fimH-1* and *mrkD* were the most prevalent of the detected virulence genes, and *magA* and *rmpA* were the least prevalent genes in all the isolates. The MLST analysis revealed 24 unique sequence types (STs) among from the 189 isolates. ST11, ST235 and ST258 were common STs among the five isolates of host origin. ST258 exhibited significantly positive correlations with *blaNDM*, *magA* and the HMV phenotype and a negative correlation with *qnrB*.

**Conclusion:** *K. pneumoniae* strains from different hosts, including humans and animals, have common molecular types and similar phenotypes, and these strains can potentially be transmitted between humans and animals.

**Keywords:** *K. pneumoniae*, antimicrobial resistance, virulence genes, sequence types

## Introduction

*Klebsiella pneumoniae* is an important facultative pathogen that causes hospital and community infections.<sup>1</sup> According to data provided by the Chinese antimicrobial resistance surveillance system (CARSS), *K. pneumoniae* is the second most prevalent (20.2%) among the isolated gram-negative pathogens.<sup>2</sup> An increasing number of studies have shown that *K. pneumoniae* also causes a variety of animal diseases, including pneumonia, bacteremia, and septicemia.<sup>3-5</sup> Due to the extensive use and abuse of antimicrobial agents for promoting growth and treating diseases in animals, *K. pneumoniae* has become severely resistant to most antibiotic agents. In

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particular, the emergence of pan-resistant strains, hyper-virulent strains and multidrug resistant (MDR) strains has caused great challenges for the prevention and treatment of infections caused by *K. pneumoniae*.<sup>6–10</sup> The aims of this study were to obtain a better understanding of the molecular epidemiological characteristics of *K. pneumoniae* and to assess the likelihood of *K. pneumoniae* transmission between humans and animals. Here, we report the drug-resistance profile and molecular characteristics of *K. pneumoniae* isolated from different host origins in Henan Province, China.

## Materials and methods

### Bacterial isolates and identification

A total of 137 strains of *K. pneumoniae* were isolated from cows (n=25), pigs (n=47), sheep (n=20) and chickens (n=45) at large-scale farms and animal hospitals in Henan Province, China, from March to December 2017. Moreover, 52 strains of nosocomial *K. pneumoniae* were isolated from the teaching hospital of Xinxiang Medical University, and they were part of the routine hospital laboratory procedure of the hospital. All isolated strains were identified through staining, biochemical tests and the VITEK-2 compact system (bioMérieux, Craponne, France). *K. pneumoniae* ATCC 700603 was used as the quality control strain for species identification.

### Detection of the mucous phenotype and extended-spectrum beta-lactamase (ESBL) bacterial phenotype

The hypermucoviscous (HMV) phenotype was tested using the “string-forming test” as previously described.<sup>11</sup> Briefly, *K. pneumoniae* was inoculated onto Columbia blood agar plates, which were incubated overnight at 37 °C, and a loop bacterial colony was used for stretching. If the length of the viscous string was greater than 5 mm, the sample was considered positive for the HMV phenotype. The ESBL phenotype was detected by a double disk synergy test according to the description provided by the Clinical and Laboratory Standards Institute (CLSI 2018).

### Antimicrobial susceptibility testing

The antibiotic susceptibility of *K. pneumoniae* was tested using the diffusion method on Mueller-Hinton agar according to the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI 2018). The following 15 commonly used antibiotic agents were

tested: amikacin (AK), ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ceftazidime (CAZ), cefotaxime (CTX), kanamycin (KAN), clindamycin (CLI), azithromycin (AZM), erythromycin (ERY), ciprofloxacin (CIP), gatifloxacin (GAT), tetracycline (TCY), imipenem (IMP), meropenem (MEM), and vancomycin (VAN). The results of the antibiotic susceptibility tests were interpreted according to the guidelines provided by the CLSI (2018). *Escherichia coli* ATCC 25922 was used as the quality control strain for antimicrobial susceptibility testing.

### Detection of resistance- and virulence-associated genes

Genomic DNA from all the isolates was extracted using a bacterial genomic DNA mini prep kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed to amplify the resistance genes *blaKPC*, *blaNDM*, *blaCTX-M*, *blaTEM*, *blaSHV*, *qnrA* and *qnrB* and five virulence genes, namely, *magA*, *rmpA*, *MrkD*, *fimH-1* and *entB*. The primers for the resistance and virulence genes were designed as previously described,<sup>12–14</sup> and the positive PCR products were sequenced by Majorbio Bio-Pharm Technology Co. (Shanghai, China). The sequencing results were analyzed and compared using NCBI BLAST (<http://www.ncbi.nlm.nih.gov/Blast>).

### Multilocus sequence typing (MLST)

Seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB*) from 189 *K. pneumoniae* isolates were amplified by PCR, and MLST was performed according to the protocol on the MLST website (<https://bigsdbs.pasteur.fr/klebsiella/klebsiella.html>).

## Results

### Antimicrobial susceptibility testing

In general, the resistance rates of the 189 *K. pneumoniae* isolates against the 15 tested antibiotics ranged from 11.6% to 77.8%. The nosocomial strains exhibited higher resistance to the 15 antibiotics than the strains from animal sources. The nosocomial strains showed the highest resistance to AMP (80.8%), followed by CIP (75.0%), TCY and KAN (69.2%), and the lowest resistance to AZM and MEM (38.5%). Among the strains of animal origin, the resistance rates of the isolates from pigs and chickens were higher than those of the isolates from cows and sheep. The isolates from cows and sheep showed resistance rates

greater than 50.0% for CIP and TCY, and the strains isolated from pigs showed resistance rates greater than 50.0% for all antibiotics with the exception of AZM, GAT, IMP, MEM and VAN. Among the chicken isolates, the highest resistance rate was found for CIP (82.2%), followed by TCY (73.3%), AMP (71.1%), ERY (64.4%) and KAN (62.2%). Most of the strains isolated from animals were sensitive to carbapenems (IMP and MEM), whereas only three isolates from pigs and two isolates from chickens showed resistance to these two antibiotics. However, the resistance rates of the nosocomial isolates against IMP and MEM reached 42.3% and 38.5%, respectively. In the present study, we considered strains resistant to at least three classes of antibiotics as MDR strains.<sup>15</sup> The highest multidrug resistance rates were found among the *K. pneumoniae* strains from pigs, reaching values of 93.6%, followed by the human strains (90.4%), chicken strains (88.9%), cow strains (52.0%) and sheep strains (50.0%) (Table 1).

## Mucous phenotype and virulence genes of *K. pneumoniae*

The results of the string test showed that 48 (25.4%) *K. pneumoniae* strains presented the HMV phenotype. Of the 48 string-test-positive isolates, 20 (38.5%) were isolated from humans, 15 (31.9%) were isolated from pigs, nine (20.0%) were isolated from chickens, three (12.0%) were

isolated from cows, and only one (5.0%) was isolated from sheep (Table 2). Table 2 characterizes the distribution of the virulence genes among *K. pneumoniae* strains isolated from different hosts. The siderophore gene *entB* (enterobactin) and the fimbrial adhesin genes *fimH-1* and *mrkD* were present in 96.8%, 91.5% and 81.5% of the isolates, respectively, and were the most prevalent virulence genes. The mucoviscosity-associated genes *magA* and *rmpA* were less prevalent in all the isolates, with prevalence rates of 6.9% and 10.6%, respectively. However, none of the strains isolated from cows and sheep contained the *magA* and *rmpA* genes.

## ESBL phenotype and resistance genes of *K. pneumoniae*

A total of 67 *K. pneumoniae* strains produced ESBLs. Of the 67 ESBL-positive isolates, 25 (48.1%) were isolated from humans, 21 (44.7%) were isolated from pigs, 14 (31.1%) were isolated from chickens, five (20.0%) were isolated from cows, and two (10.0%) were isolated from sheep (Table 3). Table 3 characterizes the distribution of the antibiotic resistance genes among *K. pneumoniae* strains isolated from different hosts. In general, *tolC* (64.0%) and *qnrA* (63.0%) were the most commonly detected antibiotic resistance genes. Among the genes encoding beta-lactamases, *blaKPC* (30.2%), *blaTEM* (29.1%) and *blaSHV* (26.5%) exhibited the highest

**Table 1** Antibiotic resistance profiles of *K. pneumoniae* isolates from different host origins

Antibiotic agents	Resistant strains of <i>K. pneumoniae</i> isolated from different host sources (%)					
	Humans (52)	Cows (25)	Sheep (20)	Pigs (47)	Chicken (45)	Total (189)
AK	59.6 (31)	32.0 (8)	20.0 (4)	76.6 (36)	46.7 (21)	52.9 (100)
AMP	80.8 (42)	36.0 (9)	15.0 (3)	85.1 (40)	71.1 (32)	66.7 (126)
AMC	53.9 (28)	28.0 (7)	15.0 (3)	63.8 (30)	48.9 (22)	47.6 (90)
AZM	38.5 (20)	12.0 (3)	10.0 (2)	44.7 (21)	42.2 (19)	34.4 (65)
CAZ	67.3 (35)	40.0 (10)	15.0 (3)	53.2 (25)	37.8 (17)	47.6 (90)
CTX	53.9 (28)	24.0 (6)	10.0 (2)	55.3 (26)	42.2 (19)	42.9 (81)
CLI	44.2 (23)	44.0 (11)	40.0 (8)	74.5 (35)	53.3 (24)	53.4 (101)
CIP	75.0 (39)	64.0 (16)	70.0 (14)	87.2 (41)	82.2 (37)	77.8 (147)
ERY	48.1 (25)	28.0 (7)	30.0 (6)	66.0 (31)	64.4 (29)	51.9 (98)
GAT	36.5 (19)	12.0 (3)	10.0 (2)	23.4 (11)	15.6 (7)	22.2 (42)
IMP	42.3 (22)	0.0 (0)	0.0 (0)	4.3 (2)	2.2 (1)	13.2 (25)
KAN	69.2 (36)	48.0 (12)	45.0 (9)	78.7 (37)	62.2 (28)	64.6 (122)
MEM	38.5 (20)	0.0 (0)	0.0 (0)	2.1 (1)	2.2 (1)	11.6 (22)
TCY	69.2 (36)	52.0 (13)	60.0 (12)	74.5 (35)	73.3 (33)	68.3 (129)
VAN	46.2 (24)	12.0 (3)	10.0 (2)	10.6 (5)	6.7 (3)	19.6 (37)
MDR	90.4 (47)	52.0 (13)	50.0 (10)	93.6 (44)	88.9 (40)	81.5 (154)

**Abbreviations:** AK, amikacin; AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AZM, azithromycin; CAZ, ceftazidime; CTX, cefotaxime; CLI, clindamycin; CIP, ciprofloxacin; ERY, erythromycin; GAT, gatifloxacin; IMP, imipenem; KAN, kanamycin; MEM, meropenem; TCY, tetracycline; VAN, vancomycin; MDR, multidrug resistant.

**Table 2** Distribution of virulence genes among *K. pneumoniae* isolates from different hosts

Strain origin	HMV	Distribution of virulence genes (number of strains, %)				
		<i>magA</i>	<i>rmpA</i>	<i>mrkD</i>	<i>fimH-I</i>	<i>entB</i>
Humans (52)	20 (38.5)	6 (11.5)	9 (17.3)	48 (92.3)	51 (98.1)	52 (100)
Cows (25)	3 (12.0)	0 (0)	0 (0)	18 (72.0)	22 (88.0)	23 (92.0)
Sheep (20)	1 (5.0)	0 (0)	0 (0)	11 (55.0)	12 (60.0)	16 (80.0)
Pigs (47)	15 (31.9)	3 (6.4)	6 (12.8)	42 (89.4)	45 (95.7)	47 (100)
Chicken (45)	9 (20.0)	4 (8.9)	5 (11.1)	35 (77.8)	43 (95.6)	45 (100)
Total (189)	48 (25.4)	13 (6.9)	20 (10.6)	154 (81.5)	173 (91.5)	183 (96.8)

**Abbreviation:** HMV, hypermucoviscous phenotype.

**Table 3** Distribution of antibiotic-resistance genes of *K. pneumoniae* isolates from different hosts

Strain origin	ESBL phenotype	Distribution of antibiotic resistance genes (number of strains, %)						
		<i>blaKPC</i>	<i>blaNDM</i>	<i>blaSHV</i>	<i>blaTEM</i>	<i>qnrA</i>	<i>qnrB</i>	<i>tolC</i>
Humans (52)	25 (48.1)	22 (42.3)	2 (3.8)	27 (51.9)	19 (36.5)	32 (61.5)	18 (34.6)	34 (65.4)
Cows (25)	5 (20.0)	1 (4.0)	–	2 (8.0)	3 (12.0)	14 (56.0)	10 (40.0)	8 (32.0)
Sheep (20)	2 (10.0)	1 (5.0)	–	1 (5.0)	2 (10.0)	11 (55.0)	7 (35.0)	12 (60.0)
Pigs (47)	21 (44.7)	24 (51.1)	1 (2.1)	7 (14.9)	14 (29.8)	29 (61.7)	19 (40.4)	35 (74.5)
Chicken (45)	14 (31.1)	9 (20.0)	2 (4.4)	13 (28.9)	17 (37.8)	33 (73.3)	16 (35.6)	32 (71.1)
Total (189)	67 (35.4)	57 (30.2)	5 (2.6)	50 (26.0)	55 (29.1)	119 (63.0)	70 (37.0)	121 (64.0)

prevalence, and *blaNDM* (2.7%) showed the lowest prevalence among all detected antibiotic resistance genes in *K. pneumoniae* strains isolated from different hosts. However, different distributions of resistance genes were detected in *K. pneumoniae* strains from different host sources. *blaKPC* (51.1%) showed the highest prevalence in pig isolates, followed by human isolates (42.3%), chicken isolates (20.0%), sheep isolates (5.0%) and cow isolates (4.0%). *blaSHV* was exhibited the highest prevalence in human isolates (51.9%), followed by chicken strains (28.9%), pig strains (14.9%), cow strains (8.0%) and sheep strains (5.0%). No *blaNDM* genes were detected in *K. pneumoniae* strains isolated from cows and sheep.

## MLST

The MLST analysis revealed that 24 unique sequence types (STs) were identified from the 189 isolates. The unique STs were identified in the human (7), pig (8), chicken (8), cow (5) and sheep strains (5) (Table 4). As clearly shown in the table, seven STs were associated with human strains, eight STs were associated with pig and chicken strains, and five STs were associated with cow and sheep strains. The most frequent ST in *K. pneumoniae* was ST11, which was commonly detected among the isolates originating from five hosts and was present in human

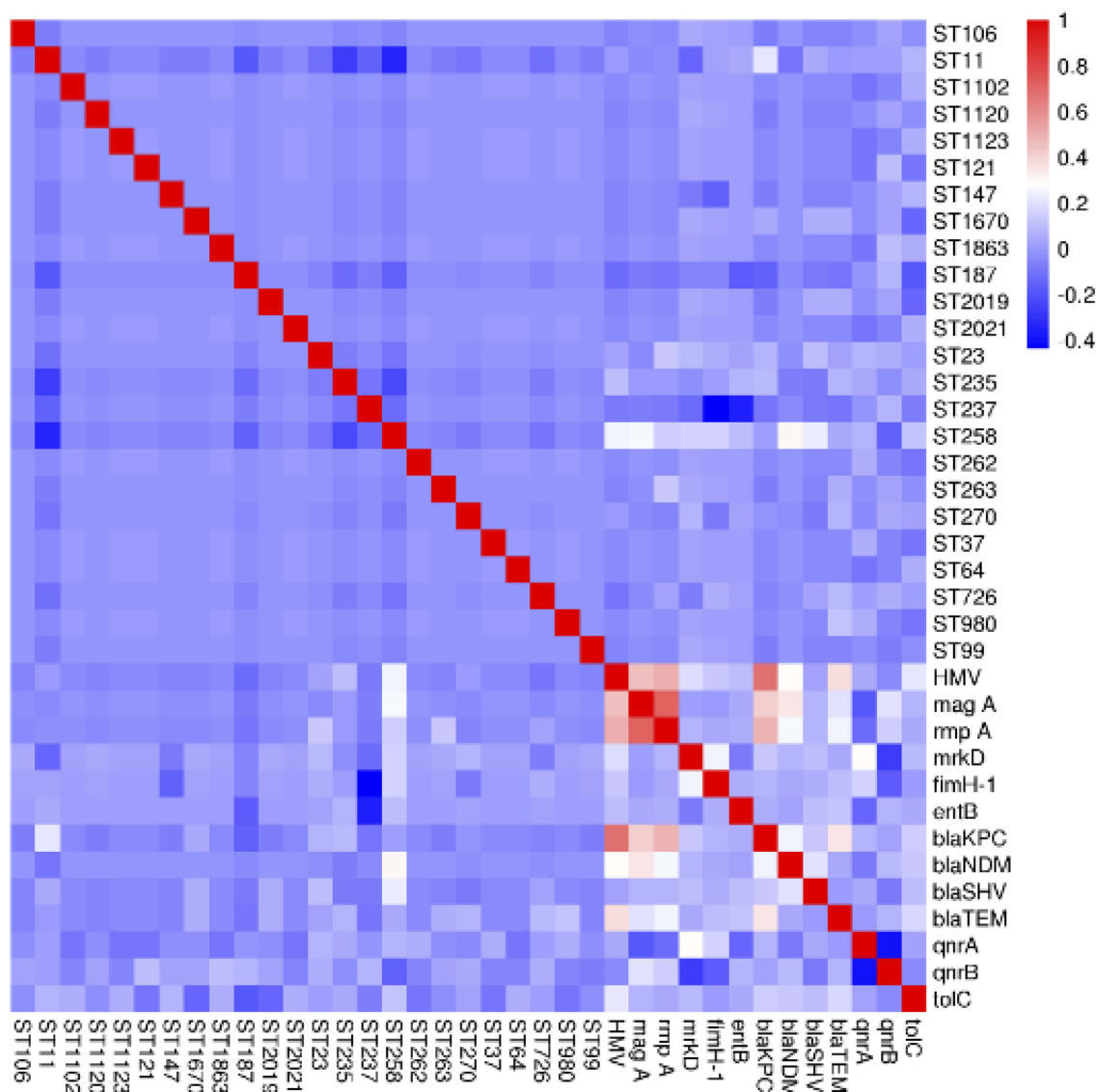
(34.6%), pig (36.2%), chicken (15.6%), cow (28.0%) and sheep (30.0%). ST235 and ST258 were also common STs of *K. pneumoniae* isolated from human (46.2%), pig (42.6%) and chicken strains (57.8%). ST187 was the most frequent ST among *K. pneumoniae* strains from cows, with a prevalence of at 52.0%, and ST237 was the most frequent ST among *K. pneumoniae* isolated from sheep, with a prevalence of 50.0%.

## Analysis of the correlations among STs and resistance and virulence genes

We performed Pearson's correlation analysis to assess the existence of potential correlations among the molecular types and resistance and virulence genes of all *K. pneumoniae* strains. Figure 1 suggests that the HMV phenotype of *K. pneumoniae* was significantly positively correlated with *rmpA* ( $R=0.51$ ,  $p<0.001$ ), *magA* ( $R=0.46$ ,  $p<0.001$ ), *blaKPC* ( $R=0.68$ ,  $p<0.001$ ), *blaTEM* ( $R=0.37$ ,  $p<0.001$ ) and *blaNDM* ( $R=0.28$ ,  $p<0.001$ ). The STs displayed positive or negative correlations with several resistance and virulence genes. For instance, ST258 exhibited significant positive correlations with *blaNDM* ( $R=0.31$ ,  $p<0.001$ ), *magA* ( $R=0.25$ ,  $p<0.001$ ), and HMV ( $R=0.24$ ,  $p<0.001$ ) and a negative correlation with *qnrB* ( $R=-0.15$ ,  $p<0.05$ ). ST237 was obviously negatively correlated with *fimH-I*

**Table 4** Multilocus sequence types of *K. pneumoniae* isolates from different host origins

Strain origin	Sequence type (number of strains, %)
Humans (52)	ST11 (18, 34.6%), ST23 (6, 11.5%), ST235 (8, 15.4%), ST258 (16, 30.8%), ST980 (1, 1.9%), ST1123 (1, 1.9%), ST1670 (2, 3.8%)
Cows (25)	ST11 (7, 28.0%), ST65 (1, 4.0%), ST99 (2, 8.0%), ST187 (13, 52.0%), ST1120 (2, 8.0%)
Sheep (20)	ST11 (6, 30.0%), ST64 (1, 5.0%), ST147 (2, 10.0%), ST237 (10, 50.0%), ST262 (1, 1.0%)
Pigs (47)	ST11 (17, 36.2%), ST106 (2, 4.3%), ST235 (8, 17.0%), ST258 (12, 25.5%), ST263 (2, 4.3%), ST270 (4, 8.5%), ST1102 (1, 2.1%), ST1863 (1, 2.1%)
Chickens (45)	ST11 (7, 15.6%), ST37 (1, 2.2%), ST187 (2, 4.4%), ST235 (12, 26.7%), ST258 (14, 31.1%), ST726 (6, 13.3%), ST2019 (2, 4.4%), ST2021 (1, 2.2%)

**Figure 1** Pearson's correlation analysis of the ST types, drug-resistance genes and virulence genes of the 189 *K. pneumoniae* isolates.

( $R=-0.43$ ,  $p<0.001$ ) and *entB* ( $R=-0.36$ ,  $p<0.001$ ). However, no potential links were found among the other ST types and any resistance and virulence genes of the *K.*

*pneumoniae* strains. As shown in Figure 1, the virulence gene *rmpA* displayed a strong positive correlation with *magA* ( $R=0.72$ ,  $p<0.001$ ) and a positive association with



the resistance genes *blaKPC* ( $R=0.049$ ,  $p<0.001$ ), *blaNDM* ( $R=0.26$ ,  $p<0.001$ ) and *blaTEM* ( $R=0.25$ ,  $p<0.001$ ).

## Discussion

*K. pneumoniae* is an important opportunistic pathogen responsible for both human and animal infections, and the emergence of MDR *K. pneumoniae* has made it difficult to control this pathogen worldwide.<sup>1</sup> An increasing number of studies have isolated that MDR *K. pneumoniae* strains from a variety of animals as well as humans,<sup>16,17</sup> but few studies have assessed the molecular relationships among *K. pneumoniae* isolates from livestock and human infections.<sup>18</sup> The present study was performed to assess the prevalence distribution of antibiotic-resistant genotypes, phenotypes, and virulence genes among *K. pneumoniae* isolated from different hosts, including cows, sheep, pigs, chickens and humans. The antibiotic susceptibility results showed that the resistance profiles of the strains isolated from different hosts were obviously different. Nosocomial *K. pneumoniae* strains exhibited a higher prevalence of resistance than animal strains. Fifty-two nosocomial *K. pneumoniae* strains showed high resistance to 15 antimicrobials, with resistance rates ranging from 36.5% to 80.8%, and resistance rates of 42.3% and 38.5% were obtained after the inclusion of imipenem and meropenem among the carbapenem antibiotics. These results are consistent with previous findings.<sup>19</sup> Among the animal isolates, the prevalence of resistant isolates from pigs and chickens was higher than that of resistant isolates from cows and sheep. The highest resistance rates among the isolates from chickens and pigs (82.2% and 87.2%, respectively) were found against ciprofloxacin. Quinolones are broad-spectrum antimicrobial agents that have been widely used to treat food-producing animals, including chickens and pigs, in China.<sup>20</sup> Some data have shown that 80.0% of isolates from chickens are resistant to ciprofloxacin,<sup>21</sup> and similar findings have reported that the incidence of ciprofloxacin resistance among *K. pneumoniae* from Chinese pigs is 68.9%.<sup>22</sup> However, another study found that more than 90% of 61 *K. pneumoniae* isolates from retail foods in China are susceptible to quinolone antibiotics, including ciprofloxacin,<sup>20</sup> which disagrees with our results. *K. pneumoniae* isolates from different hosts have different antibiotic resistance profiles, and we speculate that these differences might be due to the environmental selective pressures exerted on bacteria. Many antibiotics are often excessively and unreasonably

used in animal clinics for the treatment of multiple infections,<sup>23</sup> which increases the selective pressure for antibiotic and multidrug resistance. In modern livestock production systems, antimicrobials are heavily used for treating diseases and promoting animal growth, which has resulted in an environment conducive to the amplification of antibiotic resistance. The extensive use and abuse of antimicrobials are common in large-scale pig farms and chicken farms in China, but antibiotics are less commonly used in cows and sheep farms, which might explain the lower prevalence of antibiotic resistance among the *K. pneumoniae* strains isolated from cows and sheep compared that found among the strains isolated from pigs and chickens. Some expensive and newly synthesized antibiotics are rarely used in animal agriculture,<sup>24</sup> and thus, bacteria exhibit less drug resistance against these antibiotics than against traditional antibiotics. For example, GAT, IMP and MEM are rarely used for the treatment of animal infections. In the present study, we found that most *K. pneumoniae* isolates from animals were susceptible to GAT, IMP and MEM, and similar findings revealed that all *K. pneumoniae* isolates from food animals are susceptible to IMP and MEM.<sup>16</sup> In addition, multiresistant strains increase the risk of infections caused by treatment failure in humans and animals. Multiresistant *K. pneumoniae* isolates have emerged in many countries, including Northwest Iran, Turkey, Australia and China.<sup>25–28</sup> Our study also showed a high occurrence of multiresistant *K. pneumoniae* among humans and different animals, which suggest that these multiresistant strains can potentially be transmitted among humans and animals.

The pathogenicity of *K. pneumoniae* is due to various virulence factors, including capsule production, hypermucoviscosity, the presence of lipopolysaccharides and iron acquisition systems, all of which contribute to overcoming the innate immunity of mammalian hosts and to maintaining infections in these hosts.<sup>29,30</sup> *K. pneumoniae* strains with a hypermucoviscous phenotype are considered hypervirulent strains.<sup>31</sup> The molecular identification of these strains is related to the presence of the *rmpA* and *magA* genes. *rmpA* is the plasmid gene regulator of the mucoid phenotype A gene, which is a regulator of extracapsular polysaccharide synthesis.<sup>31</sup> *magA*, the chromosomally encoded hypermucoviscosity gene A, encodes the K1 mucoviscous serotype. Although most *K. pneumoniae* strains with the hypermucoviscous phenotype harbored *magA*, some *magA*-negative strains harboring the *rmpA* gene also exhibited this phenotype.<sup>32</sup> In the present

study, the overall frequency of the hypermucoviscous phenotype was 25.2%, which was similar to those found in other studies<sup>33</sup> but higher than those found in other investigations conducted in European countries.<sup>34</sup> Interestingly, the identification of virulence genes performed in our study showed that *rmpA* (10.6%) and *mgaA* (6.9%) were present in the hypermucoviscous isolates. Cubero et al also reported that half of the hypermucoviscous isolates included in their study were *mgaA*- and *rmpA*-negative.<sup>35</sup> This finding suggests that the string test (hypermucoviscosity) is not a reliable indicator of the presence of virulent clones with *mgaA* and *rmpA*. We speculate that other factors are involved in the development of the hypermucoviscosity phenotype. In fact, some researchers have suggested that variations in the composition of lipopolysaccharides plays an important role in the development of a hypermucoviscous phenotype that might resemble that found in virulent strains.<sup>36</sup> Our results also showed that more *K. pneumoniae* isolates with the hypermucoviscous phenotype were isolated from humans than from animals, which suggests that the hypermucoviscous characteristics of *K. pneumoniae* might be associated with the host.<sup>18</sup> In addition, the *entB* gene is a siderophore-associated gene of *K. pneumoniae*. Iron is essential for bacterial survival but is absent in the host plasma. *K. pneumoniae* mainly acquires iron via the secretion of siderophores, which have a higher affinity for iron than host transport proteins.<sup>37</sup> In the present study, the detection rate of the *entB* gene was 96.8%, which agrees with other reports.<sup>37</sup> *FimH* and *mrkD* are also important virulent factors that are essential in pili formation and are important for attachment to acellular surfaces and biofilms. In the present study, the detection rates of the *fimH* and *mrkD* genes were 91.5% and 81.5%, respectively, which is consistent with the results obtained in a previous study.<sup>37,38</sup>

ST11 is a common MDR ST that is mainly found in Asia and South America<sup>39–41</sup> and is the main ST in hospitals and animals in China.<sup>38,42,43</sup> The present results were consistent with these reports. Our data also showed that ST11 was an ST of *K. pneumoniae* isolates from both humans and animals (including pigs, cows, sheep and chickens), which suggests that ST11 *K. pneumoniae* can spread between humans and animals. Moreover, ST235 and ST258 were prevalent in the isolates obtained from humans, pigs and chickens, which indicated that these STs might be associated with transmission between humans and animals. The analysis of the correlations among STs, drug-resistance genes, virulence genes and phenotypic characteristics

showed that some molecular types exhibited significant correlations with some resistance and virulence genes. As we previously described, the *K. pneumoniae* strains with the HMV phenotype harbored *mgaA* and *rmpA* genes. Our correlation analysis also showed that the HMV phenotype was significantly positively correlated with *mgaA* ( $R=0.46$ ) and *rmpA* ( $R=0.51$ ), which was consistent with other reports (17). The major mechanism underlying the resistance of *K. pneumoniae* to carbapenems involves the expression of carbapenemases. Most of the carbapenemases present in the *K. pneumoniae* strains with the HMV phenotype are KPC.<sup>37</sup> A similar result was obtained in the present study: the HMV phenotype was strongly positively correlated with *blaKPC* ( $R=0.68$ ,  $p<0.001$ ). Although some researchers hypothesize that ST types of *K. pneumoniae* strains are associated with hypervirulent characteristics,<sup>44</sup> our data identified no significant correlations with the exception of correlations among ST258 and virulence and antibiotic-resistance genes. Because few strains with other STs were detected, no other potential correlations between STs and any resistance and virulence genes in *K. pneumoniae* strains were found in this study. Various factors associated with virulence and antibiotic resistance are important for bacterial pathogenesis. El Fertat-Aissani et al found that virulence factors are associated with antibiotic resistance in pathogenic bacteria;<sup>32</sup> however, this study found no significant correlation among virulence factors and resistance genes. Thus, more strains should be collected to determine the correlations among STs and resistance and virulence genes in the future.

Although the sources of *K. pneumoniae* strains were different, the phenotypic characteristics of some strains were similar, which suggested a risk of transmission between humans and animals. Therefore, to prevent further transmission of *K. pneumoniae* among humans and animals, strict infection control measures, such as the rational application of antibiotics in clinical settings and animal farms, the regular disinfection of the farm environment, decreased human contact with animals, and the screening of more effective drugs, should be implemented.

## Conclusion

In conclusion, our study described the antimicrobial resistance and molecular characterization of *K. pneumoniae* strains isolated from humans and animals in Henan, China. The results showed that some *K. pneumoniae* strains of different origins, including humans and animals, have common molecular types and similar phenotypes,

which indicates that these strains can potentially be transmitted between humans and animals. Therefore, the prudent use of antimicrobials in human clinical therapy and animal production as well as control measures for the transmission of *K. pneumoniae* between humans and animals are needed.

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## Disclosure

The authors report no conflicts of interest associated with this work.

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