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ORIGINAL RESEARCH

# Molecular Mechanism of Polymyxin Resistance in Multidrug-Resistant *Klebsiella pneumoniae* and *Escherichia coli* Isolates from Henan Province, China: A Multicenter Study



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**Purpose:** To evaluate polymyxin-resistant *Klebsiella pneumoniae* and *Escherichia coli* prevalence and characteristics in the Henan province, China.

**Materials and Methods:** A total of 2301 bacterial isolates collected at six hospitals were assessed. Their response to polymyxin was evaluated by minimum inhibitory concentration (MIC) analysis, and the mobilized colistin resistance (mcr) and carbapenemase gene were explored. Mutations on *mgrB, phoPQ, pmrAB*, and *crrAB* in polymyxin-resistant *K. pneumoniae* were detected by PCR. *phoP, phoQ, pmrK, pmrA, pmrB*, and *pmrC* transcriptional levels were quantified by RT-qPCR. Pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing were performed to determine the phylogenetic relationship between the polymyxin-resistant isolates.

**Results:** Of the *E. coli* and *K. pneumoniae* isolates identified, 0.3% and 1.4% were polymyxin-resistant, respectively, with MICs of 4–64 μg/mL. All polymyxin-resistant isolates were susceptible to tigecycline. Four *E. coli* isolates were *mcr-1*-positive and one was carbapenem-resistant, carrying $bla_{\text{NDM-5}}$ and *mcr-1*. One *K. pneumoniae* isolate was *mcr-1*-positive and nine were carbapenem-resistant (PRCRKP), carrying $bla_{\text{KPC-2}}$ but not *mcr-1*. The five *E. coli* isolates belonged to four sequence types (ST2, ST132, ST632, and ST983). All PRCRKP isolates belonged to ST11. However, all 16 isolates belonged to different PFGE types with <95% genetic similarity. Insertion sequences in *mgrB* were detected in nine (81.8%) polymyxin-resistant *K. pneumoniae* samples. Colistin resistance was linked with *pmrHFIJKLM* operon upregulation, with *phoP, phoQ*, and *pmrK* being overexpressed in all but one of the polymyxin-resistant *K. pneumoniae* samples. Furthermore, 33.3% of patients carrying polymyxin-resistant isolates had previously used polymyxin, and 66.7% patients displayed good clinical outcomes.

**Conclusion:** The *K. pneumoniae* polymyxin resistance rate was slightly higher than that of *E. coli* and *mcr-1* was more common in *E. coli* than in *K. pneumoniae*. Moreover, the insertion of ISkpn14 into *mgrB* may be the main contributor to polymyxin-resistance in *K. pneumoniae* in Henan.

**Keywords:** polymyxin, *Escherichia coli*, *Klebsiella pneumoniae*, *mcr*, Henan province

## Introduction

In recent years, antibiotic resistance has become a global public health priority. Colistin, also known as polymyxin, is one of the few therapeutic options available for the treatment of infectious diseases caused by multidrug-resistant gram-negative

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bacteria.[1] In China, polymyxin was approved in January 2017 as an injectable drug for the treatment of bacterial infections. However, because of the increased usage of polymyxin in the clinical setting, polymyxin-resistant strains, especially those carrying the plasmid-borne mobilized colistin resistance (*mcr*) gene, have appeared in China and various countries worldwide.[2] Moreover, intraspecies transmission of resistant isolates has already been reported.[1,3]

Since its discovery in southern China in late 2015,[4] *mcr-1* has spread to over 40 countries and regions, implying that it plays a prevalent role in the transferability of polymyxin resistance. *Mcr-1*-positive strains have also appeared in the Henan province, and it has been reported that *mcr* exists in pig-derived *Escherichia coli* isolates.[5] Clinical *E. coli* isolates coproducing $bla_{NDM}$ and *mcr-1* were previously reported by our laboratory.[6] A novel conjugative *mcr-8.2*-bearing plasmid was identified in an almost pan-resistant hypermucoviscous *Klebsiella pneumoniae* ST11 isolate in Henan.[7] However, overall, the report about *mcr* in human-derived *E. coli* and *K. pneumoniae* isolates was primarily focused outside Henan.

Except for *mcr*, colistin resistance in *K. pneumoniae* can be mediated by chromosomal mutations in genes involved in lipopolysaccharide synthesis, namely *phoPQ, pmrAB*, and *crrA*/*crrB*, as well as the regulatory *mgrB*.[8–10]

To better understand the epidemiological trends and characteristics of polymyxin-resistant clinical strains, these strains were screened using isolates collected at six hospitals located in the Henan province between 2018 and 2019. A total of 16 polymyxin-resistant strains were collected and their molecular resistance characteristics were analyzed. To the best of our knowledge, this is the first multicenter study to screen and investigate the molecular mechanisms of polymyxin resistance among *E. coli* and *K. pneumoniae* strains in the Henan province.

# Materials and Methods

## Sample Collection

Non-duplicated *E. coli* and *K. pneumoniae* strains were obtained from routine microbiological cultures of clinical samples from patients, including blood, urine, sputum, bronchoalveolar lavage fluid (BAL), bile, hydrothorax, ascites, and various other specimens. A total of 2301 strains were collected from six hospitals in the Henan province. Identification at the species level was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany).

## Susceptibility Testing and Minimum Inhibitory Concentrations (MICs) Determination

Susceptibility to polymyxin was screened using polymyxin B susceptibility test Strip (Antu, Zhengzhou, China). MICs higher than 2 μg/mL were confirmed by the microbroth dilution method based on the clinical break points defined by the European Committee on Antimicrobial Susceptibility Testing.[11]

Susceptibility of polymyxin-resistant strains to ampicillin (AMP), meropenem (MEM), imipenem (IPM), ceftazidime (CAZ), cefotaxime (CTX), cefazolin (KZ), ampicillin/sulbactam (SAM), aztreonam (ATM), cefepime (FEP), piperacillin/tazobactam (TZP), levofloxacin (LEV), amikacin (AK), gentamicin (GN), trimethoprim/sulfamethoxazole (SXT), ceftazidime/avibactam (CZA), and tigecycline (TGC) were determined using the microbroth dilution method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[12] *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as quality controls.

## Multi-Locus Sequence Typing (MLST)

Polymyxin-resistant *K. pneumonia* and *E. coli* isolates were typed using MLST following the scheme established by the Pasteur Institute (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html and https://bigsdb.pasteur.fr/ecoli/ecoli.html, respectively).

## Characterization of Mcr, mgrB, phoPQ, pmrAB, crrAB, and Carbapenem Resistance Genes

The modified carbapenem inactivation method (mCIM) and ethylenediaminetetraacetic acid-modified carbapenem inactivation method (eCIM), which are recommended by the CLSI, were used for phenotypic detection to confirm carbapenemase production. The presence of carbapenem resistance genes ($bla_{VIM}$, $bla_{IMP}$, $bla_{KPC}$, $bla_{NDM}$, and $bla_{oxa\text{-}48\text{-}like}$) and the polymyxin resistance gene *mcr1–8* in polymyxin-resistant isolates were screened by polymerase chain reaction (PCR) using the methods described previously.[13–15] The full-length *mgrB, pmrA, pmrB, phoP, phoQ, crrA*, and *crrB* genes in polymyxin-resistant

*K. pneumoniae* were amplified and sequenced. Next, the translated amino acid sequences were analyzed and compared with those of polymyxin-susceptible *K. pneumoniae*.

## Transcriptional Analysis Real-Time Quantitative PCR

RNA extraction and transcription were carried out as previously described.[16] Real-time quantitative PCR (RT-qPCR) was used to measure the expression of the *phoQ, phoP, pmrK, pmrA, pmrB*, and *pmrC* genes using the primers as previously described.[16–18] Normalization was performed against the *rpsL* gene using the ΔΔCT method (relative) with the *rpsL* gene as internal control. The obtained values were then normalized against those obtained with polymyxin-susceptible strains.

## Pulsed-Field Gel Electrophoresis (PFGE)

The molecular epidemiology of all polymyxin-resistant strains was determined by PFGE after total chromosomal DNA digestion with *Xba*I, in accordance with a previous report.[19] The PFGE patterns were analyzed with the BIONUMERICS software (Applied Maths NV, Sint-Martens-Latem, Belgium) using the dice similarity coefficient. Isolates were considered as the same strain (PFGE type) if they possessed a genetic similarity of ≥ 95%.

# Results

## Overall Prevalence of Polymyxin-Resistant Strains

Over the course of the study, 16 (0.7%) isolates were found to be polymyxin-resistant among the 2301 identified *E. coli* and *K. pneumoniae* isolates, of which 5 and 11 were *E. coli* and *K. pneumoniae* isolates, respectively. The prevalence of polymyxin resistance in *E. coli* and *K. pneumoniae* was of 0.3% and 1.4%, respectively (Table 1).

## Antimicrobial Susceptibility Testing for Polymyxin-Resistant Isolates

Antimicrobial susceptibility testing for the 16 polymyxin-resistant isolates showed that all of the isolates were resistant to AMP, KZ, and CTX; 93.3% were resistant to LEV; 86.6% were resistant to CAZ, FEP, and ATM; 80% were resistant to SAM and TZP; 66% were resistant to GN and AK; 62.5% were resistant to IPM and MEM; and 60% were resistant to SXT. Only one isolate was resistant to CZA (6.3%), and all of them were susceptible to TGC (Figure 1).

**Table 1** Prevalence of Polymyxin-Resistant Isolates in the Six Participating Hospitals

| Isolates | Hospitals | No. of Isolates | No. of Polymyxin-Resistant Isolates (%) |
|---|---|---|---|
| *Escherichia coli* | | | |
| | Hospital 1 | 326 | 3 (0.9) |
| | Hospital 2 | 231 | 1 (0.4) |
| | Hospital 6 | 942 | 1 (0.1) |
| Total | | 1499 | 5 (0.3) |
| *Klebsiella pneumoniae* | | | |
| | Hospital 3 | 141 | 1 (0.7) |
| | Hospital 4 | 133 | 1 (0.8) |
| | Hospital 5 | 78 | 2 (2.6) |
| | Hospital 6 | 450 | 7 (1.6) |
| Total | | 802 | 11 (1.4) |
| Overall total | | 2,301 | 16 (0.7) |

The MICs of these 16 strains to polymyxin ranged from 4–64 μg/mL. *K. pneumoniae* isolates had MICs in the range of 4–64 μg/mL (median: 64 μg/mL), whereas the MICs for polymyxin-resistant *E. coli* isolates were all of 4 μg/mL (Table 2).

## Detection of Antimicrobial Resistance Genes

Among the 16 polymyxin-resistant isolates, five carried *mcr-1*, including 1 *K. pneumoniae* and four *E. coli* isolates. No other mcr genes were detected in the 16 polymyxin-resistant isolates. In addition, nine *K. pneumoniae* and one *E. coli* isolates were carbapenemase-positive. The mCIM and eCIM results showed that nine *K. pneumoniae* isolates were serine carbapenemase-positive and one *E. coli* isolate was metallo-carbapenemase-positive. The PCR results showed that nine *K. pneumoniae* isolates were $bla_{\text{KPC-2}}$-positive, but none of them carried *mcr-1*. Furthermore, one *E. coli* isolate was both $bla_{\text{NDM-5}}$- and *mcr-1*-positive. No other carbapenemase genes, such as $bla_{\text{IMP}}$, $bla_{\text{VIM}}$, or $bla_{\text{OXA48-like}}$, were detected (Table 2).

Among the 11 polymyxin-resistant *K. pneumoniae* samples, *crrA* was detected in 72.7% (8/11) isolates, and *crrB* was detected in 54.5% (6/11) isolates. Nine (81.8%) strains carried sequence insertions at the coding region for the *mgrB* gene. Due to the generation of amplicons larger than the normal size of the *mgrB* gene, one strain carried the normal *mgrB* gene and one strain had a W47R mutation in *mgrB*. ISKpn14 was the most common insertion

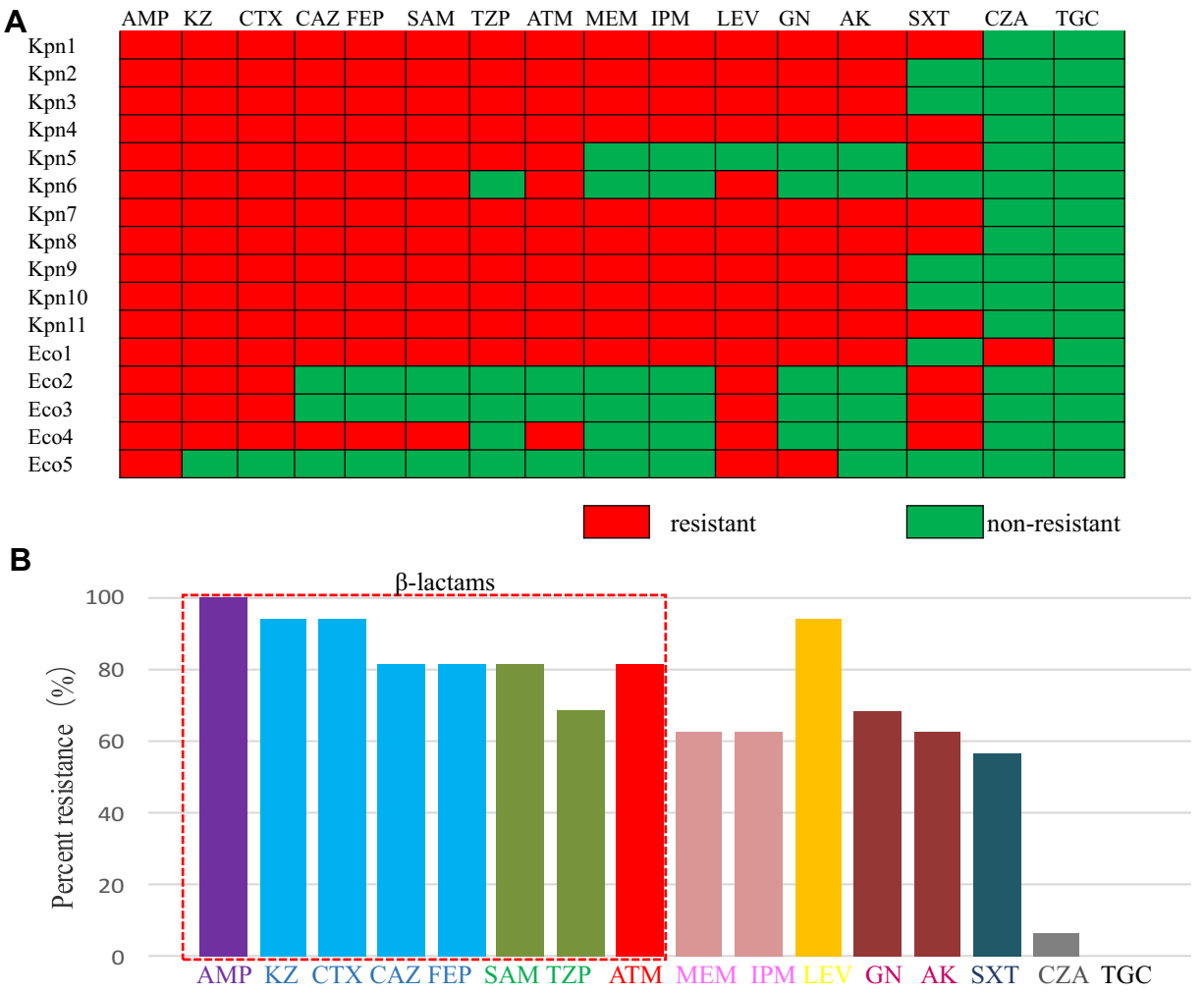


**Figure 1** Antimicrobial susceptibility profiles of the 16 polymyxin-resistant isolates. (**A**) Heatmap showing the resistance phenotypes of each of the isolates. (**B**) Percentage of strains resistant to the tested antibiotics.

**Abbreviations**: AK, amikacin; AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; CZA, ceftazidime Avibactam; FEP, cefepime; GN, gentamicin; IPM, imipenem; KZ, cefazolin; LEV, levofloxacin; MEM, meropenem; SAM, ampicillin/sulbactam; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline; TZP, piperacillin/tazobactam.

sequence found in *mgrB* and was identified in seven (63.6%) isolates, followed by IS5 (two, 18.2%). No mutations were identified in *phoP, phoQ, crrA*, or *pmrB*. Only one strain carried M66I in the *pmrA* gene and another carried a frameshift in the *crrB* gene (Table 3).

## Overexpression of phoPQ and pmrK Contribute to Polymyxin-Resistance in *K. pneumoniae*

In general, transcriptional upregulation of the *phoQ, phoP*, and *pmrK* genes was observed in all strains with *mgrB* alterations, except for KPN5 with a normal *mgrB* gene. Compared to the levels obtained for our laboratory polymyxin-susceptible wild-type strains, the transcription level of *phoP* was elevated from 8.4- to 24.9-fold, that of *phoQ* was elevated from 6.9- to 28.9-fold, and that of *pmrK* was elevated from 3.4- to 21.3-fold. Moreover, three polymyxin-resistant *K. pneumoniae* isolates were found to have increased expression of the *pmrA* gene from 4.2- to 10.1-fold, of the *pmrB* gene from 5.0- to 10.7-fold, and of the *pmrC* gene from 13.3- to 21.6-fold (Figure 2).

## Epidemiological Characterization

MLST analysis showed that nine *K. pneumoniae* isolates belonged to the sequence type (ST) 11, and the other two isolates belonged to ST37 and ST364, respectively. Among the five *E. coli* isolates, two belonged to ST132, and the other three isolates belonged to ST2, ST983, and ST632 (Figure 3).

**Table 2** Phenotypic and Genotypic Characteristics of the Polymyxin-Resistant Strains

| Site | Isolate | Polymyxin MIC (μg/mL) | mCIM | eCIM | *KPC* | *NDM* | *mcr–1* |
|---|---|---|---|---|---|---|---|
| Hospital 5 | Kpn1 | 4 | + | – | *KPC-2* | – | – |
| Hospital 4 | Kpn2 | 32 | + | – | *KPC-2* | – | – |
| Hospital 6 | Kpn3 | 64 | + | – | *KPC-2* | – | – |
| Hospital 5 | Kpn4 | 8 | + | – | *KPC-2* | – | – |
| Hospital 6 | Kpn5 | 64 | ND | ND | – | – | + |
| Hospital 3 | Kpn6 | 64 | ND | ND | – | – | – |
| Hospital 6 | Kpn7 | 16 | + | – | *KPC-2* | – | – |
| Hospital 6 | Kpn8 | 32 | + | – | *KPC-2* | – | – |
| Hospital 6 | Kpn9 | 64 | + | – | *KPC-2* | – | – |
| Hospital 6 | Kpn10 | 64 | + | – | *KPC-2* | – | – |
| Hospital 6 | Kpn11 | 64 | + | – | *KPC-2* | – | – |
| Hospital 1 | Eco1 | 4 | + | + | – | *NDM–5* | + |
| Hospital 1 | Eco2 | 4 | ND | ND | – | – | + |
| Hospital 1 | Eco3 | 4 | ND | ND | – | – | + |
| Hospital 2 | Eco4 | 4 | ND | ND | – | – | + |
| Hospital 6 | Eco5 | 4 | ND | ND | – | – | – |

**Abbreviations**: eCIM, ethylenediaminetetraacetic acid-modified carbapenem inactivation method; Eco, *Escherichia coli; KPC-2, K. pneumoniae* carbapenemase-2; Kpn, *Klebsiella pneumoniae*; mCIM, modified carbapenem inactivation method; *mcr-1*, mobilized colistin resistance-1; MIC, minimal inhibitory concentration; ND, data were not collected; *NDM-5*, New Delhi metallo-enzyme-5.

**Table 3** Chromosomal Mutations and Insertion Sequences (IS) in Polymyxin-Resistant *K. pneumoniae* Strains

| Site | Isolate | *mgrB* | *phoP* | *PhoQ* | *pmrA* | *pmrB* | *crrA* | *crrB* |
|---|---|---|---|---|---|---|---|---|
| Hospital 5 | Kpn1 | ISkpn14 | + | + | + | + | + | Fr at16L |
| Hospital 4 | Kpn2 | ISkpn14 | + | + | + | + | + | – |
| Hospital 6 | Kpn3 | ISkpn14 | + | + | + | + | – | – |
| Hospital 5 | Kpn4 | ISkpn14 | + | + | + | + | + | + |
| Hospital 6 | Kpn5 | + | + | + | M66I | + | + | + |
| Hospital 3 | Kpn6 | W47R | + | + | + | + | + | + |
| Hospital 6 | Kpn7 | ISkpn14 | + | + | + | + | + | + |
| Hospital 6 | Kpn8 | ISkpn5-like | + | + | + | + | - | - |
| Hospital 6 | Kpn9 | ISkpn5-like | + | + | + | + | + | - |
| Hospital 6 | Kpn10 | ISkpn14 | + | + | + | + | - | - |
| Hospital 6 | Kpn11 | ISkpn14 | + | + | + | + | + | + |

**Abbreviations**: Fr, frameshift, +, presence of PCR product and no change in the nucleotide/amino acid sequences; –, absence of PCR product.

Dendrogram analysis of PFGE at ≥ 95% similarity revealed that homology among the five *E. coli* and 11 *K. pneumoniae* isolates was low and sporadic, suggesting a very low likelihood of clonal spread (Figure 3).

## Clinical Characteristics of Patients Harboring Polymyxin-Resistant Isolates

The 16 polymyxin-resistant isolates were collected within 1 year from 15 patients aged 2 months to 93 years. One patient had two isolates collected separately from blood and urine, whereas from all other 14 patients each isolate had been collected from urine (n = 4), BAL (n = 3), blood (n = 3), secretion (n = 2), peritoneal puncture fluid (n = 1), and sputum (n = 1) samples. The diagnosed diseases for these patients included cerebrovascular disease (n = 3), urinary tract disease (n = 3), pneumonia (n = 2), sepsis (n = 1), fever (n = 1), acute coronary syndrome (n=1), pregnancy-induced hypertension (n = 1), premature baby (n = 1), infection around the prosthesis (n = 1), and Guillain-Barre syndrome (n = 1). Five patients received polymyxin treatment before the isolation of polymyxin-resistant strains. Ten patients displayed positive clinical outcomes (Table 4).

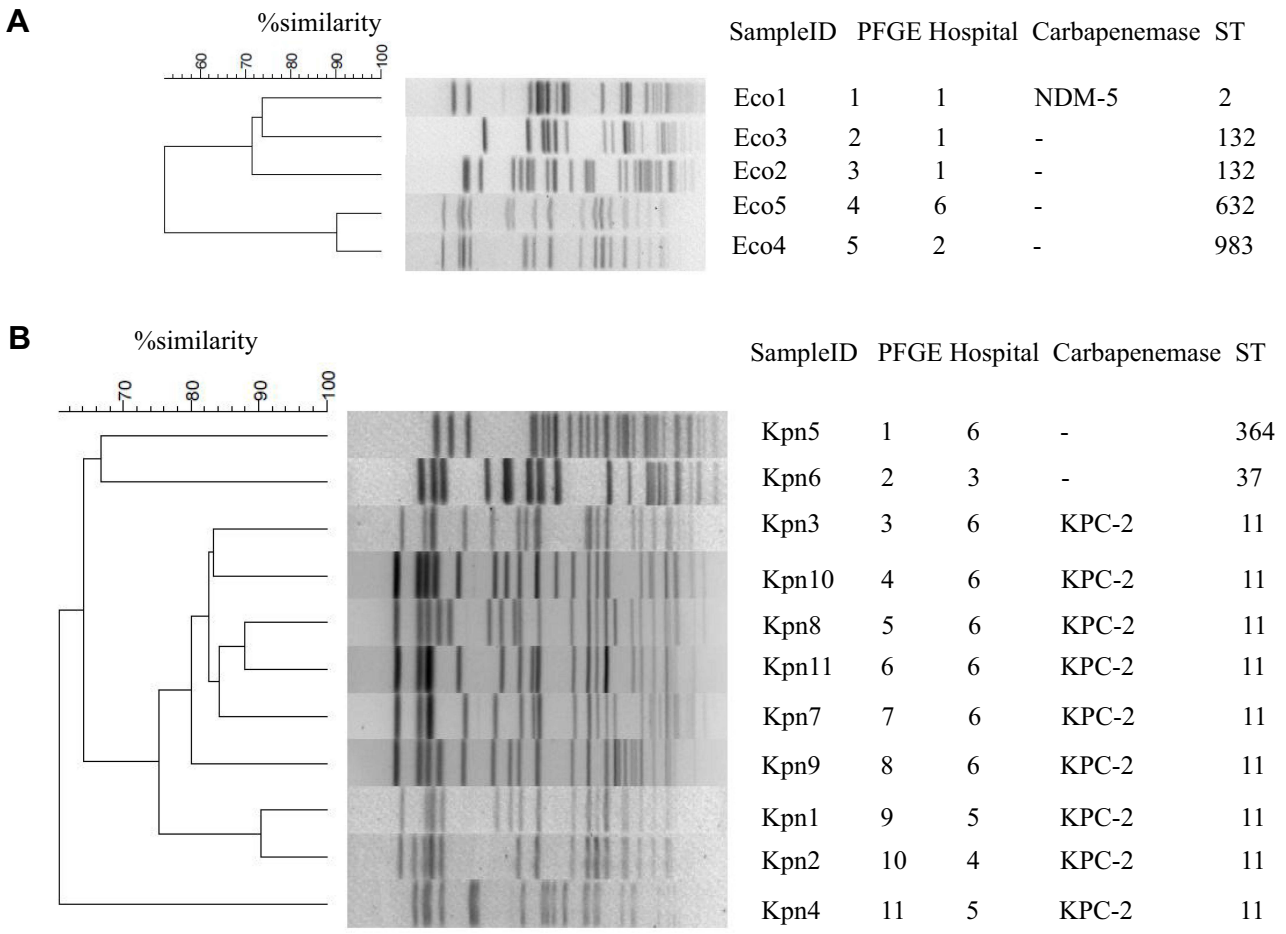


**Figure 2** Transcriptional levels of *phoP, phoQ, pmrK, pmrA, pmrB*, and *pmrC* in polymyxin-resistant *K. pneumoniae.*

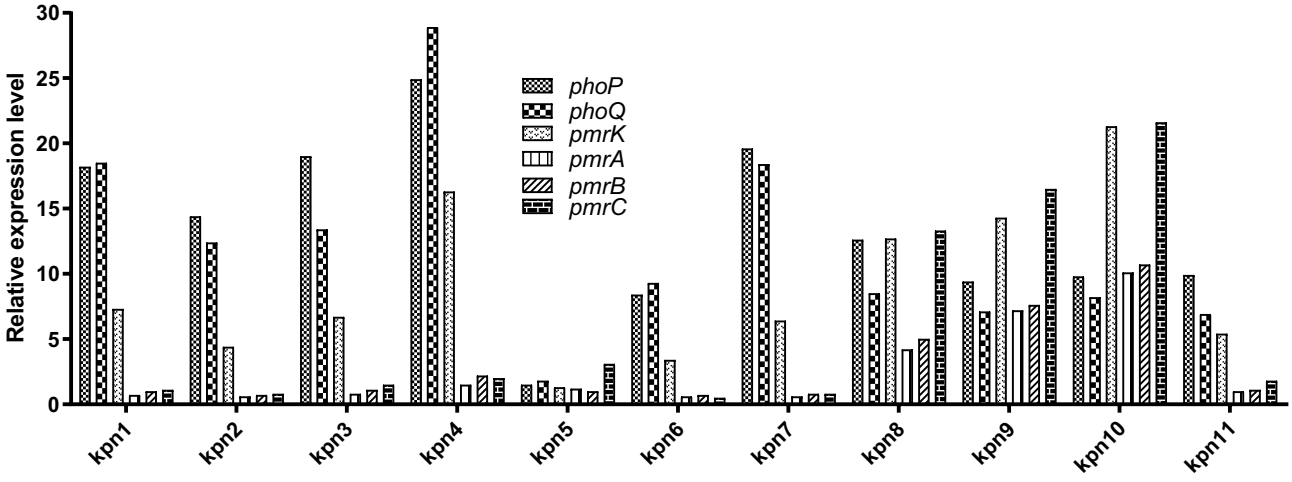


**Figure 3** Pulsed-field gel electrophoresis (PFGE)-based dendrogram of polymyxin-resistant *Escherichia coli* (**A**) and *Klebsiella pneumoniae* (**B**) strains.
**Abbreviations**: Eco, *E coli; KPC, K, pneumoniae* carbapenemase; Kpn, *K, pneumoniae; NDM-5*, New Delhi metallo-enzyme-5; ST, sequence type.

## Discussion

Polymyxin has been used against aggressive infections caused by multidrug-resistant bacteria, but its use has been severely compromised by the emergence of plasmid-mediated polymyxin resistance in *Enterobacteriaceae*. Hence, we surveyed polymyxin resistance rates and

**Table 4** Clinical Characteristics of Patients Carrying Polymyxin-Resistant Bacterial Strains

| Pt | Sex/Age (Years) | Isolate | Source | Clinical Diagnosis | Underlying Disease | Indwelling Devices | Antimicrobial Use Prior to Culture Within 30 Days | Outcome |
|---|---|---|---|---|---|---|---|---|
| 1 | M/87 | Kpn | Blood | Cerebral infarction | Diabetes | Tracheal cannula | TGC, Carbapenems | Discharge |
| 2 | F/33 | Kpn | Secretion | Pneumonia, AFE | No | No | Clindamycin, Quinolones | Discharge |
| 3 | M/38 | Kpn | Sputum | Septic shock | No | CVC, Tracheal cannula | β-lactam, Quinolones | Death |
| 4 | F/69 | Eco/ Eco | Blood/urine | Acute coronary syndrome | Diabetes, CHD | No | β-lactam, Quinolones | Discharge |
| 5 | M/81 | Eco | Blood | Fever | Hemodialysis | No | β-lactam, Quinolones, | Discharge |
| 6 | M/93 | Eco | BAL | Cerebral hemorrhage | Diabetes | CVC, Tracheal cannula | Carbapenems | Discharge |
| 7 | F/69 | Kpn | Urine | Urinary retention | No | CVC | β-lactam | Worsening |
| 8 | M/62 | Kpn | BAL | Cerebral hemorrhage | No | Tracheotomy | TGC, Polymyxin | Worsening |
| 9 | F/89 | Eco | Urethral secretions | cUTI | Hypertension, CHD | Urethral catheter | β-lactam, Quinolones | Death |
| 10 | M/56 | Kpn | Urine | Guillain-Barre syndrome | Hypertension | Tracheal cannula | TGC, Polymyxin, Carbapenems | Discharge |
| 11 | M/67 | Kpn | Urine | Urethral injury | Hypertension | Urethral catheter | β-lactam | Discharge |
| 12 | F/2 months | Kpn | BAL | Premature baby | No | Tracheal cannula | Polymyxin, Fosfomycin, fluconazole | Discharge |
| 13 | F/28 | Kpn | Peritoneal puncture fluid | Pregnancy-induced hypertension | SLE | Peritoneal drainage tube | TGC, Polymyxin, Carbapenems | Discharge |
| 14 | M/89 | Kpn | Blood | Severe pneumonia | No | Tracheal cannula | Polymyxin, Carbapenems, fluconazole | Death |
| 15 | F/54 | Kpn | Secretion | Infection around the prosthesis | No | No | Carbapenems, Quinolones | Discharge |

**Abbreviations**: AFE, amniotic fluid embolism; BAL, bronchoalveolar lavage fluid; Kpn, *Klebsiella pneumoniae*; CVC, central venous catheter; CHD, coronary heart disease; cUTI, complicated urinary tract infection; Eco, *Escherichia coli*; SLE, systemic lupus erythematosus; TGC, tigecycline.


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mechanisms in *E. coli* and *K. pneumoniae* isolates from hospitalized patients at six local hospitals in the Henan province.

Among the total 2301 *E. coli* and *K. pneumoniae* isolates, 16 (0.7%) were polymyxin-resistant, five of which carried *mcr-1*. In addition, 0.88% (34/3854) of the *E. coli* isolates and 0.21% (5/2410) of the *K. pneumoniae* isolates carrying *mcr-1* were previously reported in the China Antimicrobial Resistance Surveillance Trial.[20] Of the 1499 *E. coli* isolates in this study, 5 (0.3%) were polymyxin-resistant and 4 were *mcr-1*-positive. Previous research found that 1% (20/1495) of the *E. coli* isolates and 0.18% (1/571) of the *K. pneumoniae* isolates recovered from bloodstream infections in China were *mcr-1*--positive.[21] Herein, of the 802 *K. pneumoniae* isolates, 11 (1.4%) were polymyxin-resistant, one of which carried *mcr-1*. Although *mcr-1* was more common in *E. coli* than in *K. pneumoniae* isolates, the polymyxin resistance rate of *K. pneumoniae* was slightly higher than that of *E. coli* in our study, which is possibly due to antibiotic selection driven by the high detection rate (32.8%) of carbapenem-resistant *K. pneumoniae* (PRCRKP) in Henan among all Chinese provinces in 2019.[22]

Compared to polymyxin-resistant *E. coli*, polymyxin-resistant *K. pneumoniae* had 8–64 times higher MICs, and insertion sequences in the *mgrB* were detected in 81.8% of polymyxin-resistant *K. pneumoniae* isolates, which suggested that chromosomal mutations by insertional inactivation mediated by mobile insertion sequences may play important roles in polymyxin resistance in *K. pneumoniae* in the Henan province. Many studies found that *mgrB* alterations, including insertion elements, non-silent point mutations, and small intragenic deletions, mediate colistin resistance.[16,18,23] ISkpn14 was the most common insertion observed in our study. However, an ISKpn26-like element was predominant in Taiwan[23] and Greece.[10] One strain carried a W47R mutation in the *mgrB* gene, which has been previously reported.[24] M66I mutation in the *pmrA* gene and a frameshift at 16L in the *crrB* gene were newly found in this study, but further research is needed to clarify the mechanism.

The *mgrB* gene was a negative feedback regulator of the PhoQ-PhoP signaling system; therefore, *phoP, phoQ*, and *pmrK* were all upregulated in polymyxin-resistant *K. pneumoniae* with *mgrB* alterations in this study, as previously reported.[16,25] *pmrA, pmrB*, and *pmrC* overexpression was found only in three polymyxin-resistant *K. pneumoniae* isolates, which implies that *mgrB* alterations might be the main reason for polymyxin resistance in *K. pneumoniae*.

Two carbapenemase genes, $bla_{KPC}$ and $bla_{NDM}$, are responsible for the phenotypic resistance of 90% of carbapenem-resistant *Enterobacteriaceae* strains in China.[26] The coexistence of *mcr* and carbapenemase genes, such as $bla_{NDM-5}$,[27] $bla_{NDM-4}$,[28] $bla_{KPC}$,[29] and $bla_{OXA}$,[30] has been sporadically reported in different countries. In the national monitoring data from China, one report showed that the *mcr-1* was detected in 4.6% (13/282) of carbapenem-resistant *E. coli* isolates and coexisted with New Delhi metallo-enzyme (NDM)-5 in one strain.[31] In another study, *mcr-1* prevalence among carbapenem-resistant *E. coli* and PRCRKP isolates was 3.7% (14/376) and 0% (0/1134), respectively, and 14 carbapenem-resistant *E. coli* isolates coproduced $bla_{NDM4/5/9}$ with *mcr-1*.[32] In this study, only one *E. coli* isolate coproduced *mcr-1* and $bla_{NDM-5}$.

An *E. coli* isolate belonging to ST167 that coproduced $bla_{NDM}$ and *mcr-1* was previously reported in Henan,[6,33] but in our study, the aforementioned coproducing *E. coli* isolate belonged to ST2. The other *E. coli* strains in this study belonged to ST132, ST983, and ST632. Nine PRCRKP isolates belonged to ST11, but PFGE showed that they belonged to different types and were not clonal, even when isolated from the same hospital. These results demonstrated that polymyxin-resistant isolates were non-clonal and had different resistance potentials. It was reported that ST11 KPC-Kp was clonally heterogeneous and could be further classified into eleven mobile genetic element (MGE) types and fourteen PFGE subtypes.[34]

The patients carrying polymyxin-resistant isolates had varying severities of illness, and 33.3% of them had a history of polymyxin use. Moreover, 66.7% of them were cured, which could be explained by the retained susceptibility to other antimicrobials, such as CZA, SXT, and TGC, that most polymyxin-resistant isolates showed.

The limitation of this study is the use of the polymyxin B susceptibility test strip to screen polymyxin-resistant isolates, which is not the gold standard for identifying resistance to polymyxin. Indeed, it has been reported that results obtained by this method has high very major errors (VMEs), leading to an underestimation of the resistance.[35–37]

## Conclusions

In conclusion, the polymyxin resistance rate of *K. pneumoniae* was slightly higher than that of *E. coli*, and *mcr-1* occurrence was lower in polymyxin-resistant

*K. pneumoniae* than in polymyxin-resistant *E. coli* in the Henan province of China. Further molecular investigations showed that insertion sequences in the *mgrB* gene might be the main mechanism contributing to polymyxin resistance in *K. pneumoniae* in Henan. In addition, continuous and close monitoring is required to prevent the dissemination of polymyxin-resistant *K. pneumoniae* and *E. coli* strains.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of Henan Provincial People's Hospital, Henan, China (20210055). No personally identifiable information was collected in this study. The requirement for informed consent from patients was also waived.

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## Author Contributions

All authors made a significant contribution to the work reported, including the conception, study design, execution, acquisition of data, analysis and interpretation. All authors took part in drafting, revising and critically reviewing the article, and approved the final manuscript. In addition, the authors have agreed on the journal to which this manuscript has been submitted and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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