Emergence of mobilized colistin resistance-1 in multidrug-resistant Klebsiella pneumoniae and Escherichia coli isolates from the Henan province in China: a multicentre study

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Research

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Abstract

Background

The increased clinical use of polymyxin led to the emergence of polymyxin-resistant strains, especially those carrying plasmid-borne mobilized colistin resistance (mcr) gene variants. In this study, we aimed to evaluate the prevalence and characteristics of polymyxin-resistant Klebsiella pneumoniae and Escherichia coli isolates from the Henan province, China.

Methods

A total of 16 polymyxin-resistant isolates among 2301 E. coli and K. pneumoniae isolates collected in 6 local hospitals in the Henan province were studied. The isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and the minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique. Polymyxin-resistant isolates were further analysed for mcr-1 and carbapenemase-mediated resistance using the modified carbapenem inactivation method, the ethylenediaminetetraacetic acid-modified carbapenem inactivation method, and polymerase chain reaction. Pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) were performed to disclose the phylogenetic relationships of the polymyxin-resistant isolates. The clinical characteristics of patients infected with the polymyxin-resistant isolates were also retrospectively analysed.

Results

5/1499 (0.3%) and 11/802 (1.4%) E. coli and K. pneumoniae isolates, respectively, were polymyxin-resistant. The MICs of polymyxin were in the range of 4–64 µg/mL and all of the 16 polymyxin-resistant isolates were susceptible to tigecycline. Additionally, four of the five E. coli polymyxin-resistant isolates were mcr-1 positive; one of them was also carbapenem-resistant, carrying blaNDM-5. Conversely, only 1/11 K. pneumoniae isolates was mcr-1 positive, while 9 polymyxin-resistant isolates were also carbapenem-resistant (PRCRKP), carrying blaKPC-2 but not mcr-1. MLST results showed that the five E. coli isolates belonged to four sequence types (STs), including ST2, ST132, ST632, and ST983, while all PRCRKP isolates belonged to ST11. However, all 16 isolates showed different PFGE types using a genetic similarity of ≥ 95%. Furthermore, 33.3% (5/15) of the patients carrying polymyxin-resistant K. pneumoniae isolates showed a history of polymyxin use, and 10/15 (66.7%) patients displayed good clinical outcomes.

Conclusion

The polymyxin resistance rate of K. pneumoniae was slightly higher than that of E. coli in the Henan province; however, mcr-1 was only detected in one K. pneumoniae isolate. Thus, close monitoring is needed to prevent and control the spread of PRCRKP.

Background

Antibiotic resistance has become a global public health priority in recent years. 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 bacteria [1]. In China, injectable polymyxin was approved for the treatment of bacterial infections in January 2017. However, because of the increased usage of polymyxin in clinical settings, polymyxin-resistant strains, especially those carrying plasmid-borne mobilized colistin resistance (mcr) gene variants have emerged in China and various countries worldwide [2]. Moreover, the 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. Of note, mcr-1-positive strains have also emerged in the Henan province, including in pig-derived Escherichia coli isolates [5]. In fact, clinical E. coli isolates co-producing blaNDM and mcr-1 were previously reported by our laboratory [6], and a novel conjugative mcr-8.2-bearing plasmid was identified in an almost pan-resistant hypermucoviscous Klebsiella pneumoniae ST11 isolate [7]. However, overall, the reports of mcr in human-derived E. coli and K. pneumoniae isolates are mainly centred outside of Henan.

Additionally, 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 mgrB regulatory gene [8-10].
To better understand the epidemiological trends and characteristics of polymyxin-resistant clinical strains, here, we looked for polymyxin resistance among isolates collected at 6 hospitals in Henan from 2018 to 2019. A total of 16 polymyxin-resistant strains were collected, and their molecular resistance characteristics were analysed. To the best of our knowledge, this is the first multi-centre study screening for polymyxin-resistant isolates among *E. coli* and *K. pneumoniae* in the Henan province, China.

**Methods**

**Sample collection**

Non-duplicated *E. coli* and *K. pneumoniae* strains were obtained from routine microbiological cultures of clinical samples including blood, urine, sputum, bronchoalveolar lavage fluid (BAL), bile, hydrothorax, ascites, and various other specimens. A total of 2301 strains were isolated from 6 hospitals in Henan. Identification at the species level was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany).

**Susceptibility testing and determination of the minimum inhibitory concentrations (MICs)**

Susceptibility to polymyxin was screened using Etest strips (Antu, Zhengzhou, China); only isolates with MICs higher than 2 µg/mL were subjected to further susceptibility testing for validation using the microbroth dilution method based on the clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018) [11].

Additionally, susceptibility 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) was determined only in the context of polymyxin-resistant strains using the microbroth dilution method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

**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; https://bigsdb.pasteur.fr/ecoli/ ecoli.html).

**Characterization of mcr-1 and carbapenemase mediated resistance**

The modified carbapenem inactivation method (mCIM) and ethylenediaminetetraacetic acid-modified carbapenem inactivation method (eCIM), which are recommended by the CLSI, were used for the phenotypic detection of carbapenemase production. The presence of carbapenem resistance genes (*bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, and *bla*<sub>oxa-48-like</sub>) and of the polymyxin resistance gene *mcr-1* in polymyxin-resistant isolates were screened by polymerase chain reaction using the methods described previously [13, 14].

**Pulsed-field gel electrophoresis (PFGE)**

Molecular epidemiology of all polymyxin-resistant strains was determined by PFGE after total chromosomal DNA digestion with XbaI in accordance with a previous report [15]. The PFGE patterns were analysed using 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 out of the 2301 *E. coli* and *K. pneumoniae* isolates (0.7%) were found to be polymyxin-resistant: 5 *E. coli* and 11 *K. pneumoniae* isolates, collected from 6 different hospitals. The prevalence of polymyxin resistance in *E. coli* and *K. pneumoniae* was 0.3% and 1.4%, respectively (Table 1).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing showed that all of the isolates (100%) 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 (100%) (Figure 1).
The MICs of polymyxin in the context of these 16 strains ranged from $4-64 \mu g/mL$: the full range in the context *K. pneumoniae* isolates (median: $64 \mu g/mL$) and $4 \mu g/mL$ in the context of *E. coli* isolates (Table 2).

**Detection of antimicrobial resistance genes**

Among the 16 polymyxin-resistant isolates, 5 carried the *mcr-1* gene, including 1 *K. pneumoniae* and 4 *E. coli* isolates. In addition, 9 *K. pneumoniae* and 1 *E. coli* isolates were carbapenemase-positive. The mCIM and eCIM results showed that the 9 *K. pneumoniae* isolates were serine carbapenemase-positive, and the *E. coli* isolate was metallo-carbapenemase-positive. Furthermore, the PCR results showed that the 9 *K. pneumoniae* isolates were *bla*KPC-2-positive, but none of them carried *mcr-1*. On the other hand, the *E. coli* isolate was both *bla*NDM-5- and *mcr-1*-positive. Of note, no other carbapenemase genes, such as *bla*IMP, *bla*VIM, and *bla*OXA-like, were detected (Table 2).

**Epidemiological characterization**

MLST analysis showed that the nine *K. pneumoniae* carbapenem-resistant isolates all belonged to sequence type (ST) 11. On the other hand, among the five *E. coli* isolates, two belonged to ST132 and the other three belonged to ST2, ST983, and ST632 (Table 2).

Of note, dendrogram analysis of PFGE at 95% similarity revealed that homology among the 5 *E. coli* and 11 *K. pneumoniae* isolates was low and sporadic, suggesting a very low likelihood of clonal spread (Figure 2).

**Clinical characteristics of the patients infected with polymyxin-resistant isolates**

The 16 polymyxin-resistant isolates were collected within 1 year from 15 patients aged 2 months to 93 years old, in 6 hospitals. Two strains were isolate from a single patient, from blood and urine samples, while each of the other 14 patients showed only 1 strain, isolated from urine (n = 4), BAL (n = 3), blood (n = 3), secretion (n = 2), peritoneal puncture fluid (n = 1) and sputum (n = 1) samples. The underlying diseases in 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). Of note, five patients received polymyxin treatment before the isolation of polymyxin-resistant strains. Importantly, ten patients displayed positive clinical outcomes (Table 3).

**Discussion**

Polymyxin has been used against aggressive infections caused by multidrug-resistant bacteria; however, its use has been severely compromised by the emergence of plasmid-mediated polymyxin resistance in Enterobacteriaceae. Hence, in this study, we surveyed the polymyxin resistance rates in *E. coli* and *K. pneumoniae* isolates from hospitalized patients at six local hospitals in the Henan province, China.

Among the total 2301 *E. coli* and *K. pneumoniae* isolates, 16 (0.7%) strains were polymyxin-resistant, 5 of which carried the *mcr-1* gene. Of note, of the 1499 *E. coli* isolates, 5 (0.3%) were polymyxin-resistant and 4 were *mcr-1*-positive; on the other hand, of the 802 *K. pneumoniae* isolates, 11 (1.4%) were polymyxin-resistant, 1 of which carried *mcr-1*. Previously, 0.88% (34/3854) of the *E. coli* isolates and 0.21% (5/2410) of the *K. pneumoniae* isolates carrying *mcr-1* were reported in the China Antimicrobial Resistance Surveillance Trial [16]. Additionally, another study found that 1% (20/1945) 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 [17]. Our results are, therefore, in line with the previous ones, with rates not exceeding 1.5%. Although *mcr-1* was more common in *E. coli* isolates 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 presumably due to antibiotic selection because the detection rate (32.8%) of carbapenem-resistant *K. pneumoniae* (PRCRKP) in Henan ranked first among all Chinese provinces in 2019 [18]. Of note, compared to polymyxin-resistant *E. coli*, polymyxin-resistant *K. pneumoniae* were associated with 8–64 times higher MICs, suggesting chromosomal mutations in related genes, such as *phoP/phoQ, pmrA/pmrB*, and *mrgB*. Additionally, other intrinsic mechanisms might also play important roles in increasing polymyxin resistance in *K. pneumoniae* [17].

Two carbapenemase genes, *bla*KPC and *bla*NDM are responsible for the phenotypic resistance of 90% of carbapenem-resistant Enterobacteriaceae strains in China [18]. The co-existence of *mcr* and carbapenemase genes, such as *bla*NDM-5 [19], *bla*NDM-4 [20], *bla*KPC [21], and *bla*OXA [22], has been sporadically reported in different countries. In the national monitoring data from China, one report showed that the *mcr-1* gene was detected in 4.6% (13/282) of carbapenem-resistant *E. coli* isolates and coexisted with the New Delhi metallo-enzyme (NDM)-5 in one strain [23]. In another study, the *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* [24]. In this study, only one *E. coli* isolate coproduced *mcr-1* and *bla*NDM-5.
An *E. coli* isolate belonging to ST167 that co-expressed *bla*NDM and *mcr-1* was previously reported in Henan [6, 25], 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. Additionally, nine PRCRKP isolates belonged to ST11, but PFGE showed different types, suggesting these strains, all isolated from the same hospital, were unrelated. Altogether, our results demonstrate that polymyxin-resistant isolates are non-clonal and have different virulence and resistance potentials.

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, and these positive outcomes could be explained by the finding that most polymyxin-resistant isolates remained susceptible to other antimicrobials, such as CZA, SXT, and TGC.

In conclusion, here, we show that polymyxin resistance rate of *K. pneumoniae* is slightly higher than that of *E. coli*, while the presence of *mcr-1* is lower in polymyxin-resistant *K. pneumoniae* versus *E. coli* in the Henan province, China. Further molecular investigations and studies are warranted to elucidate the polymyxin resistance mechanism of PRCRKP. In addition, continuous and close monitoring is required to prevent the dissemination of polymyxin-resistant *K. pneumoniae* and *E. coli* strains.

**Abbreviations**

polymyxin and carbapenem resistant *K. pneumoniae* (PRCRKP), Minimum inhibitory concentrations (MICs), pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), sequence types (STs), mobilized colistin resistance (*mcr*), bronchoalveolar lavage fluid (BAL), 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), tigecycline (TGC), carbapenem inactivation method (mCIM), ethylenediaminetetraacetic acid-modified carbapenem inactivation method (eCIM)

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of the Henan Provincial People's Hospital, Henan, China (20190050). The requirement for informed consent from patients was waived.

**Consent for publication**

No personally identifiable information was collected in this study.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

WJY and YL contributed to conception and design of the article. YZ, SYR, DMH, WMZ, CQS and XJZ were responsible for microbiological tests. NJ and YHY carried out the molecular genetic studies. WJY and QZ wrote the first version of the manuscript. MYW and YL conceived and supervised the project. All authors critically revised the manuscript, and read and approved the final manuscript.

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Tables

**Table 1. Prevalence of polymyxin-resistant isolates in the six participating hospitals**

| Hospital      | 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 | 2301            | 16 (0.7)                               |

**Table 2 Phenotypic and genotypic characteristics of the polymyxin-resistant strains**

| Isolate | Polymyxin MIC (μg/mL) | mCIM | eCIM | KPC | NDM | mcr-1 |
|---------|----------------------|------|------|-----|-----|-------|
| spital5 Kpn1 | 4 | + | - | KPC-2 | - | - |
| spital4 Kpn2 | 32 | + | - | KPC-2 | - | - |
| spital6 Kpn3 | 64 | + | - | KPC-2 | - | - |
| spital5 Kpn4 | 8 | + | - | KPC-2 | - | - |
| spital6 Kpn5 | 64 | ND | ND | - | - | mcr-1 |
| spital3 Kpn6 | 64 | ND | ND | - | - | - |
| spital6 Kpn7 | 16 | + | - | KPC-2 | - | - |
| spital6 Kpn8 | 32 | + | - | KPC-2 | - | - |
| spital6 Kpn9 | 64 | + | - | KPC-2 | - | - |
| spital6 Kpn10 | 64 | + | - | KPC-2 | - | - |
| spital6 Kpn11 | 64 | + | - | KPC-2 | - | - |
| spital1 Eco1 | 4 | + | + | - | NDM-5 | mcr-1 |
| spital1 Eco2 | 4 | ND | ND | - | - | mcr-1 |
| spital1 Eco3 | 4 | ND | ND | - | - | mcr-1 |
| spital2 Eco4 | 4 | ND | ND | - | - | mcr-1 |
| spital6 Eco5 | 4 | ND | ND | - | - | - |

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

**Table 3 Clinical characteristics of the patients carrying polymyxin-resistant isolates**
| Patient | Gender/age (years) | Isolate | Source | Clinical diagnosis | Underlying disease | Indwelling devices | Antimicrobial use within 30 days prior to culture | Outcome |
|---------|-------------------|---------|--------|-------------------|-------------------|-------------------|-----------------------------------------------|---------|
| 1       | Male/87           | Kpn     | Blood  | Cerebral infarction | Diabetes          | Tracheal cannula  | TGC, Carbapenems                             | Discharge |
| 2       | Female/33         | Kpn     | Secretion | Pneumonia, AFE    | No                | No                 | Clindamycin, Quinolones, β-lactam, Quinolones | Discharge |
| 3       | Male/38           | Kpn     | Sputum | Septic shock       | No                | CVC, Tracheal cannula | No       | β-lactam, Quinolones, Carbapenems             | Discharge |
| 4       | Female/69         | Eco/Eco | Blood/urine | Acute coronary syndrome | Diabetes, CHD    | No                 | Discharge                                    | Die      |
| 5       | Male/81           | Eco     | Blood  | Haemodialysis      | No                | CVC, Tracheal cannula | CVC       | β-lactam, Quinolones, Carbapenems             | Discharge |
| 6       | Male/93           | Eco     | BAL    | Cerebral haemorrhage | Diabetes          | Tracheal cannula  | TGC, Polymyxin                               | Get worse |
| 7       | Female/69         | Kpn     | Urine  | Urinary retention | No                | No                 | β-lactam                                     | Get worse |
| 8       | Male/62           | Kpn     | BAL    | Cerebral haemorrhage | CVC              | Tracheotomy       | TGC, Polymyxin                               | Get worse |
| 9       | Female/89         | Eco     | Urethral secretions | cUTI | Hypertension, CHD | Urethral catheter | β-lactam, Quinolones                          | Die      |
| 10      | Male/56           | Kpn     | Urine  | Guillain-Barre syndrome | Hypertension      | Tracheal cannula  | TGC, Polymyxin, Carbapenems                  | Discharge |
| 11      | Male/67           | Kpn     | Urine  | Urethral injury    | Hypertension      | Urethral catheter | β-lactam                                     | Discharge |
| 12      | Female/2 months   | Kpn     | BAL    | Premature baby     | No                | Tracheal cannula  | Polymyxin, Fosfomycin, Fluconazole           | Discharge |
| 13      | Female/28         | Kpn     | Peritoneal puncture fluid | Pregnancy-induced hypertension | SLE | Peritoneal drainage tube | TGC, Polymyxin, Carbapenems | Discharge |
| 14      | Male/89           | Kpn     | Blood  | Severe pneumonia   | No                | Tracheal cannula  | Polymyxin, Carbapenems, Fluconazole          | Die      |
| 15      | Female/54         | Kpn     | Secretion | Infection around the prosthesis | No | No | Carbapenems, Quinolones                  | Discharge |

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