Comparative genomic analyses of Polymyxin-resistant Enterobacteriaceae strains from China

Zhien He1, Yongqiang Yang2,3,4, Wei Li1, Xiaoling Ma1, Changfeng Zhang2, Jingxiang Zhang3,6, Baolin Sun1*, Tao Ding3,6* and Guo-bao Tian2,3,7*

Abstract
Background: Mobile colistin resistance like gene (mcr-like gene) is a new type of polymyxin resistance gene that can be horizontally transferred in the Enterobacteriaceae. This has brought great challenges to the treatment of multidrug-resistant Escherichia coli and K. pneumoniae.

Results: K. pneumoniae 16BU137 and E. coli 17MR471 were isolated from the bus and subway handrails in Guangzhou, China. K. pneumoniae 19PDR22 and KP20191015 were isolated from patients with urinary tract infection and severe pneumonia in Anhui, China. Sequence analysis indicated that the mcr-1.1 gene was present on the chromosome of E. coli 17MR471, and the gene was in the gene cassette containing pap2 and two copies of ISApl1. The mcr-1.1 was found in the putative IncX4 type plasmid p16BU137_mcr-1.1 of K. pneumoniae 16BU137, but ISApl1 was not found in its flanking sequence. Mcr-8 variants were found in the putative IncFIB/IncFII plasmid pKP20191015_mcr-8 of K. pneumoniae KP20191015 and flanked by ISec1 and ISkp26.

Conclusion: This study provides timely information on Enterobacteriaceae bacteria carrying mcr-like genes, and provides a reference for studying the spread of mcr-1 in China and globally.

Keywords: Mcr-1, Klebsiella pneumoniae, Antibiotic resistance, Comparative genomic

Introduction
Polymyxin is a cyclic lipopeptide antibiotic discovered by Ainsworth et al. [1] in the 1940s. In 1959 [2], polymyxin B and colistin (polymyxin E) were introduced into clinical practice and used to treat infections caused by gram-negative bacteria. Due to the strong nephrotoxicity and neurotoxicity, and the popularity of more “safe” antibiotics such as beta-lactam antibiotics, polymyxins had not been used in clinical treatments in the following decades. In the past two decades, the outbreak of multidrug resistant (MDR) gram-negative bacteria and the lack of new antibiotics have caused polymyxins to return to clinical application as the last line of defense against gram-negative bacteria [3].

The resistance mechanisms of bacteria to polymyxins are mainly divided into two categories, two-component system [4, 5] and hyperproduction of CPS capsular polysaccharide (CPS) [6]. The two-component system mainly regulates polymyxin resistance by PhoPQ and PmrAB in Enterobacteriaceae, such as Pseudomonas aeruginosa and Salmonella enterica server Typhimurium. PhoQ can phosphorylate and activate PhoP in the presence of

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
polymyxin. PhoP can increase the positive charge of the outer membrane of the bacteria and the resistance to polymyxins by activating the pmrHFIJKLM operon, causing lipid A to be modified by 4-amino-4-arabinose. Hyperproduction of CPS generally occurs in *K. pneumoniae*. Some *K. pneumoniae* strains can reduce the interaction between polymyxin and bacterial surface by synthesizing large amounts of CPS, which lead to the development of polymyxin resistance. Efflux pumps of some Gram-negative bacteria (such as AcrAB [7] and KpEF [8] of *K. pneumoniae*) can participate in the resistance of bacteria to polymyxins, but the molecular mechanism is not yet clear. Although bacteria have evolved multiple polymyxin-resistance mechanisms, these mechanisms often require sacrificing their own development and are difficult to disseminate horizontally between strains. These factors limit the spread of these resistant genes among strains. However, in 2015, China reported a new colistin resistance gene, *mcr-1*, carried by *E. coli* in the intestine of edible pigs, can be transferred horizontally in *Enterobacteriaceae* [9]. According to statistics before 2016, *mcr-1* positive strains have been reported in more than 40 countries [10], spreading across 7 continents, and may be further expanded. Several reports have shown that many drug-resistant genes, such as New Delhi β-lactamase (NDM) and other extended spectrum β-lactamase genes (ESBLs), were frequently found in the strains carrying *mcr-1* [11, 12]. The emergence of *mcr-1* not only subverted our understanding of polymyxin resistance genes, but also greatly increased the difficulty of treating MDR pathogenic microorganisms.

MCR-1 is a phosphoethanolamine (PEA) transferase with a 5-fold hydrophobic transmembrane helix located in the periplasmic domain and can reduce the net negative charge of the outer membrane of the bacteria by modifying PEA on the negatively charged lipid A on the lipopolysaccharide (LPS) of the bacteria [13]. The modification reduces the interaction of the outer membrane of bacteria, which in turn produces resistance to polymyxin [14]. Generally, *mcr-1* forms a complex transposon *Tn* 6330 with the surrounding transposon sequence ISApl1 [15]. The complex transposon consists of a sequence of about 2600 bp containing *mcr-1* (1626 bp), a PAP2 superfamily protein encoding gene (765 bp), and ISApl1 transposon insertions on both sides [16]. ISApl1 belongs to the IS30 family and therefore has similar functions and activities to IS30 members [17]. It is flanked by 27 bp inverted repeats (referred to as *IRL* and *IRR*) and contains a 927 bp open reading frame (*orf*). The ISApl1 transposon will self-cleave to form a circular sequence intermediate (ISApl1)_2-mcr-1-pap2 [18, 19] if the ISApl1 transposon exists around the *mcr-1* gene. The circular intermediate contains 2 bp of host flanking DNA between adjacent ISApl1 transposon ends and generates 2 bp of target site duplications (TSDs) after integration [20]. When the *mcr-1* circular intermediate is integrated into the plasmid or genome of another strain, there is a probability that the ISApl1 transposon sequence will be lost. Loss of ISApl1 stabilizes *mcr-1* in the plasmid or genome, which is conducive to the widespread spread of *mcr-1*.

Epidemiological studies have found that *mcr-1* can be horizontally transferred in more than a dozen *Enterobacteriaceae*, mainly including *E. coli*, *K. pneumoniae*, *Salmonella* spp. [21], *Enterobacter aerogenes* [22], *P. aeruginosa* [23], *Proteus putida* [24], *Enterobacter cloacae* [22], *Cronobacter sakazakii* [25], *Shigella sonnei* [26], *Kluyvera ascorbata* [27], *Raoultella ornithinolytica* [28], *Achromobacter spp* [23] and *Citrobacter spp* [29]. These bacteria are mainly transmitted in nature through soil, water, food chains and animal migration [30, 31], and further lead to the global spread of *mcr-1*. The whole genome sequencing results of *mcr-1* positive strains showed that the *mcr-1*-bearing plasmids were mainly Incl2, IncX4, IncHI2 [32], IncP [33], IncHI1 [34], IncFI, IncFII [35], IncFIB [36], IncK [37], IncX [38], IncN [31], F18:*A--B+* [39]. Among them, IncI2, IncX4 and IncHI2 are the main replicons, and are all conjugative transfer plasmids. These carried plasmids can be stably present in the recipient bacteria even in the absence of polymyxin.

Since *mcr-1* was discovered, not only twenty-five genetic variants of the *mcr-1* gene (such as *mcr-1.1*, *mcr-1.2*, etc.) were reported all over the world [40, 41], but also a variety of *mcr*-like genes were discovered, which were named *mcr-1*, *mcr-2* [42], *mcr-3* [43], *mcr-4* [44], *mcr-5* [45], *mcr-6* [46], *mcr-7* [47], *mcr-8* [48], *mcr-9* [49] and *mcr-10* [50]. Among them, *mcr-2* and *mcr-3* were found in *E. coli*. *Mcr-4*, *mcr-5* and *mcr-9* were found in *S. enterica subsp*. The *mcr-7* and *mcr-8* were found in *K. pneumoniae*. The *mcr-6* was found in *Moraxella* spp. These proteins encoded by these *mcr*-like genes have different amino acid sequence identity with MCR-1. MCR-6 has the highest amino acid sequence similarity to MCR-1 (82.7%), while *mcr-4* has the lowest amino acid sequence identity with MCR-1.

In the current study, we performed a third-generation genome sequencing analysis of three strains of polymyxin...
B resistant *K. pneumoniae* (16BU137, KP20191015 and 19PDR22) and one strain of polymyxin B resistant *E. coli* (17MR471) from patients and environment. Then we combined with the phenotypes of related experiments to explain the resistance mechanism of *mcr*-like genes.

**Results**

Four multidrug-resistant strains all showed colistin resistance

We obtained four MDR strains resistant to polymyxin B, including three strains of *K. pneumoniae* (16BU137, KP20191015 and 19PDR22) and one strain of *E. coli* (17MR471). According to the whole-genome three-generation sequencing results, *E. coli* 17MR471 and *K. pneumoniae* 16BU137 carried the *mcr-1.1* genes, and *K. pneumoniae* KP20191015 carried the *mcr-8.2* gene (Fig. 1A). To determine the phenotypes of these four strains, we performed the determination of MIC value (Table 1). Based on polymyxin B resistance criteria (USCAST, MICs, ≥4 μg/ml) [53], these strains were identified as polymyxin B resistant strains. The MIC values of the four strains were 4 μg/ml (*E. coli* 17MR471), 8 μg/ml (*K. pneumoniae* 16BU137), 32 μg/ml (*K. pneumoniae* KP20191015) and 64 μg/ml (*K. pneumoniae* 19PDR22). Among these strains, *K. pneumoniae* 19PDR22 has the highest MIC value and *E. coli* 17MR471 has the lowest MIC value.

**Transferability of *mcr-1* - and *mcr-8* -carring plasmids**

Transconjugants conjugate of J53 and 16BU137 is called J53-16BU137, and transconjugants of J53 and KP20191015 is called J53-KP20191015. Through the drug susceptibility test, we found that neither 16BU137 nor KP20191015 can grow on MH plates containing 100 mg/L of sodium azide. PCR detection of *mcr*-like gene and *K. pneumoniae*-specific gene was performed.

---

**Table 1** Strains used in this study

| Strain     | Sourcea | MIC of polymyxin B (μg/ml) | MLST  | Datab | Regionc |
|------------|---------|---------------------------|-------|-------|---------|
| 16BU137    | Bus handrail | 8             | 37    | 2016.12.17 | Guangdong, China |
| 17MR471    | Subway handrail | 4            | 1437  | 2017.10.28 | Guangdong, China |
| KP20191015 | Sputum   | 32           | 340   | 2019.08.02 | Anhui, China |
| 19PDR22    | Urine    | 64           | 11    | 2019.09.16 | Anhui, China |
| J53 [52]   | Laboratory | 0.5          | 10    | 2018.05.24 | Kyoto, Japan |
| J53-16BU137| Laboratory | 4            | 10    | 2021.01.02 | Anhui, China |

*a* Source, source of isolates  
*b* Data, date of isolate collection  
*c* Region, geographic location of isolate collection
on all transconjugants. Using wzi gene as a K. pneumoniae-specific gene (Table 2), PCR product of J53 showed no bands in agarose gel electrophoresis, showing negative, and other K. pneumoniae showed positive. The PCR result of mcr-1 of J53-16BU137 was positive, and the PCR result of wzi was negative, indicating that the mcr-1 plasmid carried by 16BU137 was successfully transferred to the J53, and the conjugation efficiency was $1 \times 10^{-4}$ per donor cell. The MIC of J53 for polymyxin B is 0.5 μg/ml, and the MIC of J53-16BU137 for polymyxin B is 4μg/ml. It is proved that J53 is transformed from a polymyxin-sensitive strain into a polymyxin-resistant strain after receiving p16BU137_mcr-1.1. Although we also performed the same conjugation experiment on KP20191015, we did not observe the transfer of the plasmid carrying mcr-8 from KP20191015 to J53, which may indicate that the mcr-8 plasmid carried by KP20191015 is difficult to transfer between different strains.

### Genomic profiles of four colistin-resistant isolates

According to third-generation whole genome sequencing, the complete genome sequence of *E. coli* 17MR471 is 4,765,524 bp, containing 4433 CDS and 87 tRNA; the genome of *K. pneumoniae* 16BU137 is 5,269,011 bp, containing 4863 CDS and 86 tRNA; the genome of *K. pneumoniae* KP20191015 is 5,409,809 bp, containing 5110 CDS and 88 tRNA; the genome of *K. pneumoniae* 19PDR22 is 5,396,045 bp, containing 5077 CDS and 87 tRNA (Fig. 2). *E. coli* 17MR471 belongs to ST1437, harboring colistin resistance gene mcr-1.1 and other seven ARGs. *K. pneumoniae* 16BU137 belongs to ST37, harboring mcr-1.1 and other 25 ARGs. *K. pneumoniae* KP20191015 belongs to ST340, harboring a mcr-8 variant (1698 bp, 99.71% nucleotide identity to mcr-8) and other 28 ARGs. *K. pneumoniae* 19PDR22 belongs to ST11, while lacked known plasmid-mediated colistin resistance gene.

### Molecular epidemiological features of mcr-positive *E. coli* and *K. pneumoniae* isolates

To better understand the genetic background of these colistin-resistant strains, we collected all *E. coli* isolates from Guangdong, China and *K. pneumoniae* isolates from Anhui and Guangdong, China in the NCBI database, and conducted a phylogenetic analysis on them (Fig. 3). The MLST type of *E. coli* in Guangdong shows diversified characteristics. The MLST type of *K. pneumoniae* in Anhui and Guangdong is more concentrated, most of which are ST11. Among the isolates we obtained, except for KP20191015, none of the other isolates formed an independent branch. 17MR471 formed a branch with a ST6335 *E. coli* isolate GDA49. 16BU137 formed a branch with *K. pneumoniae* P10 and P12 isolates of MLST type ST4298 from Guangdong. 19PDR22 was clustered with ST11 type *K. pneumoniae* isolates. It is worth noting that KP20191015 formed a branch on its own. The *K. pneumoniae* isolates distributed in Anhui and Guangdong were intertwined in the phylogenetic tree, which seems to indicate that the *K. pneumoniae* in China has spread and needs to be controlled immediately.

### Virulence factors and colistin-related resistance genes of four isolates

A total of 48 virulence factors were predicted in *E. coli* 17MR471, 16 virulence factors were predicted in *K. pneumoniae* 16BU137, 10 virulence factors were predicted in *K. pneumoniae* KP20191015, 25 virulence factors were predicted in *K. pneumoniae* 19PDR22 (Table 3 and Table S1). In *E. coli* 17MR471, *K. pneumoniae* KP20191015 and *K. pneumoniae* 19PDR22, the identified virulence factors were all located on the chromosomes. In *K. pneumoniae* 16BU137, a total of 6 virulence factors located on the plasmid were identified. iucA, iucB, iucC, iutA and cseA are located on IncFIB(K)/IncFII type plasmids, while astA is located on IncQ1/IncFII type plasmid.

Colistin resistance related genes and other resistance genes in four isolates are shown in Tables 4 and 5 and Table S2. Colistin resistance related genes in *K. pneumoniae* KP20191015 are similar to *K. pneumoniae* 19PDR22. Compared with *K. pneumoniae* 16BU137, *K. pneumoniae* KP20191015 and *K. pneumoniae* 19PDR22 may contain more colistin resistance related genes. Among the three strains, the types of arnD, eptB, mgrB, oppE, pmrA, pmrB, pmrC, and pmrD are the same. Both *K. pneumoniae* KP20191015 and *K. pneumoniae* 19PDR22 contain two types of emrA, two types of emrB, and three types of phoP. *K. pneumoniae* 16BU137 contains one type of emrA, one type of emrB, and two types of phoP. In addition, IS903 inserted on the upstream sequence of mgrB in *K. pneumoniae* 19PDR22 (Fig. 1B), which may affect the normal expression of mgrB [56].

### Location of mcr-1.1 on chromosome and plasmid

The mcr-1.1 gene was found to locate on the chromosome of *E. coli* 17MR471. Specifically, the mcr-1-pap2

---

**Table 2** Sequences of primers used in this study

| Primer | Oligonucleotide (5’-3’) | Application |
|--------|--------------------------|-------------|
| mcr-1-F | GTCAATTCGTGGTGTCTTG | Detection |
| mcr-1-R | GTGACATCAACAGCCTT | Detection |
| mcr-8-F | CAACATAGCATCTTGGCA | Detection |
| mcr-8-R | GGAAGACATGGTGTTGG | Detection |
| wzi-F | ATGATAAAATTCGCCGCGCAT | Detection |
| wzi-R | GCGTGTACGGTTGCTGATCC | Detection |
gene cassette which encodes both MCR-1 and a hypothetical protein was flanked by two copies of ISApI1 (1070 bp, IS30 family) upstream and downstream in the same orientation. In *K. pneumoniae* 16BU137, *mcr-1.1* located in an IncX4-type plasmid which named p16BU137_mcr-1.1 (Table S3). This plasmid is 33,309 bp in size and is predicted to encode 41 ORFs for which *mcr-1.1* is the only resistance gene. No ISApI1 was found in the flanking sequences of *mcr-1.1* in p16BU137_mcr-1.1(Fig. 4). IncX4 is the dominant plasmid type to harbor *mcr-1.1* [57]. The *mcr-1*-bearing IncX4 plasmid was firstly identified in Germany in 2009. Since 2009, the majority of *mcr-1* genes have been found on IncX4 plasmids. BLASTn revealed that the genetic context of *mcr-1.1* in IncX4 plasmids are diverse. The examples included that *mcr-1.1* without flanking ISApI1 (pAF48, KX032520). Also, *mcr-1-pap2* could be flanked by ISApI1 upstream (pMCR-11EC-P293, KX555451), downstream (pPY1, KX711708) or both (pC214, KY120363). Plasmids like PN42 (MG557854) and pCDF8 (MF175191) have truncated IS elements in flanking regions of *mcr-1*. It has been hypothesized that after the loss of ISApI1, *mcr-1* is immobilized in the plasmids [18].
A mcr-8 variant was found in an IncFIB/IncFII plasmid

In *K. pneumoniae* KP20191015, a mcr-8 variant was found in an 107,661 bp IncFIB/IncFII plasmid which named pKP20191015_mcr-8. mcr-8 was flanked by a reverse IS*Ecl1*-like element (1336 bp, 99% identity to IS*Ecl1*) upstream. Also, it was flanked by an IS*Kpn26*-like element (1196 bp, 99% similarity to IS*Kpn26*) at the same direction downstream. Consistent with the sequences in the mcr-8-carrying pKP91 (95,983 bp, MG736312) [48], both of which carried *dgkA*, *baeS*, and *copR* close to mcr-8 (Fig. 5). While mcr-8 in pKP91 was flanked by two intact IS903B sequences up- and downstream [48], and significant differences were observed in the remaining plasmid backbone (Fig. 5). BLASTn indicated that pKP20191015_mcr-8 carried novel components that showed limited identity to those known plasmid sequences (coverage <75%). pKP20191015_mcr-8 is organized similar to that of plasmid pKP1814–2 (187,349bp, KX839208) (69% coverage, 99.84% identity) identified in *K. pneumoniae* in China; p002SK2_A (159,714bp, CP025516) (53% coverage, 99.80% identity) identified in *K. pneumoniae* in Switzerland; pKP121–2 (134,208bp, CP031851) (53% coverage, 99.75% identity) identified in *K. pneumoniae* in China. They all carried plasmid transfer associated *tra* locus with different combination and the replicon encoding gene *repB*.

**Discussion**

Polymyxins are cyclic, positively charged peptides, which were first discovered to possess antibiotic properties in the 1940s [58]. Polymyxins can bind to lipid A of lipopolysaccharide (LPS) on the outer membrane of Gram-negative bacteria, and then displace Mg$^{2+}$ and Ca$^{2+}$ from cationic binding sites leading to disruption of bacterial membrane integrity [58, 59]. Polymyxins (polymyxin B and colistin) are a last resort treatment against human infections caused by multidrug-resistant (MDR) Gram-negative bacteria [60]. Colistin resistance is often associated with chromosomal point mutations that affect the expression of regulators, which modify lipid A and lead to alterations of LPS [61]. Bacteria can add phosphoethanolamine (PEtN) and 4-amino-4-deoxy-L-arabinose (L-Ara4N) to lipid A via biosynthesis, thereby decreasing the net negative charge of lipid A to reduce its binding affinity to polymyxins [62, 63]. The synthesis and transfer of PETN and L-Ara4N are mediated by the expression of *pmrCAB* and *arnBCADTEF* (also called *pmrHFIJKLM*) [64] which were regulated by a two-component
system (TCS) PmrA/PmrB [65, 66]. Mutations in the genes encoding PmrA/PmrB were shown to contribute to colistin resistance [67, 68]. Moreover, another TCS PhoP/PhoQ is known to develop polymyxin resistance via activation of its posttranscriptional activator PmrD to induce expression of the PmrA/PmrB system [69]. Mutations in the genes encoding the PhoP/PhoQ were also associated with colistin resistance [70]. Here, we report four polymyxin-resistant Enterobacteriaceae strains. Among them, K. pneumoniae 16BU137 and E. coli 17MR471 carries mcr-1, and K. pneumoniae KP20191015 carry mcr-8. The mcr-1 in 17MR471 is located on the chromosome, and the surrounding sequence is a typical Tn 6330 structure, (ISApl1)2-mcr-1-pap2. The mcr-1 in 16BU137 lacks upstream ISApl1 and downstream ISApl1, but still retains pap2. ISApl1 may be lost due to its involvement in mcr-1 transposition [18, 19]. However, pap2 always exists downstream of mcr-1, which seems to suggest that pap2 may play an indispensable function for mcr-1. Through further experimental verification, we identified K. pneumoniae 19PDR22 which conferred high MIC of colistin, while no known plasmid-mediated colistin genes was found. We found an IS903B-like element (97% similarity to IS903B) inserted into the upstream sequence of mgrB. This insertion appeared at position −18 bp of the mgrB, which may lead to the inactivation of mgrB by interrupting its promoter region. The inactivation of mgrB conferred colistin resistance has been reported previously [71]. IS integration has also been reported to induce colistin resistance via transposition into the upstream putative promoter region of mgrB [72].

Table 3  Virulence factors predicted with VFDB database

| Gene     | 16BU137 | 17MR471 | 19PDR22 | KP20191015 |
|----------|---------|---------|---------|------------|
| axsA     | a       | b       | -       | -          |
| astA     | +       | +       | -       | -          |
| cseA     | +       | +       | -       | -          |
| csgB/F/G | -       | -       | -       | -          |
| ecpA/B/C/D/E/R | + | + | + | + |
| entA/B   | +       | +       | +       | +          |
| entC/D/E/F/S | -         | - | - | - |
| espL1    | -       | +       | -       | -          |
| espR1    | -       | +       | -       | -          |
| espX1    | -       | +       | -       | -          |
| espX4    | -       | +       | -       | -          |
| fepA     | -       | +       | -       | -          |
| fepB     | -       | +       | -       | -          |
| fepC     | +       | +       | +       | +          |
| fepD     | -       | -       | -       | -          |
| fpgG     | -       | +       | -       | -          |
| fes      | -       | -       | -       | -          |
| fimA/B/C/D/E/F/G/H/I | - | + | - | - |
| fyuA     | -       | -       | -       | -          |
| gspD/E/F/G/H/I | - | + | - | - |
| gspK/I/M | -       | +       | -       | -          |
| iir1/2   | -       | +       | -       | -          |
| iucA/B/C | +       | -       | +       | -          |
| iutA     | +       | -       | +       | -          |
| ompA     | +       | +       | +       | +          |
| mmpA2    | -       | -       | +       | +          |
| ybaE/F/P/Q/S/T/U/X | - | + | - | - |

<sup>a</sup> −, indicates virulence factor negative

<sup>b</sup> +, indicates virulence factor positive

Conclusion

We present the complete genome of four polymyxin-resistant strains (including two clinically isolated strains and two environmentally isolated strains, both clinically isolated strains are K. pneumoniae). The high-quality complete genome sequence generated in this study will help to further study the mechanism of polymyxin resistance of K. pneumoniae and the horizontal transfer pathway of mcr-like genes. Although two strains are isolated from the environment, they still have high polymyxin resistance. And the types of virulence factors are basically the same as clinical strains, and still have the risk of infecting humans. These also warns us that the multidrug resistant K. pneumoniae has spread seriously in China and needs to be controlled as soon as possible.

Methods

Bacterial isolation

The MIC of polymyxin B was tested on the MDR clinical isolates isolated from the inpatients in Affiliated Hospital of Anhui University of Traditional Chinese Medicine.
and the Anhui Provincial Hospital in 2019, and two polymyxin B resistant isolates were obtained (K. pneumoniae 19PDR22 and K. pneumoniae KP20191015). The environmental isolates of K. pneumoniae 16BU137 and E. coli 17MR471 were obtained from our previous studies [32]. They all carried mcr-1 and were resistant to polymyxin B. Briefly, the environmental samples were collected using sterilized swab with saline, and cultured by broth medium. Then, the cultured samples were plated on the MacConkey agar with colistin (2 μg/mL) and cultured under 37 °C overnight. Subsequently, we randomly selected 5 colonies for each plate which were subject to screen mcr-1 gene by PCR. Only one colony for each sample was included for the subsequent study. K. pneumoniae 19PDR22 was isolated from the urine of patient with urinary tract infection, and K. pneumoniae KP20191015 was isolated from the sputum of patient with severe pneumonia. Sputum and urine were plated on blood agar plates and cultured at 37 °C to isolate bacterial clones. VITEK 2 Compact System (bioMérieux, France) was used to identify positive culture strains.

Determination of minimum inhibitory concentration
K. pneumoniae and E. coli were cultured overnight in LB liquid medium at 37°C for 220 rpm according to 1:100, and a small amount of liquid medium was streaked on LB plate and incubated overnight in 37°C constant temperature incubator. Several monoclonal strains were selected to adjust the concentration of bacteria in MH (Mueller-Hinton Broth) medium so that the concentration of bacteria reached OD$_{600}$ = 0.4 and then diluted 200 times in MH medium [74]. Mix 75 ml of MH medium with different concentrations of polymyxin B and 75 ml of MH medium with diluted bacterial solution and add them to each well of a 96-well plate according to the polymyxin concentration gradient. The final CFU of the well is 5 × 10$^5$. Each concentration gradient was divided into three parallel groups and grown at 37°C and 220 rpm with shaking for 24 and 48h. The experiment was repeated three times independently.

Plasmid conjugation experiments
E. coli J53 (LacZ–, AzrR, RifR) was used as the recipient, and the mcr-like gene-positive strain (16BU137, KP20191015) was used as the donor. Overnight culture (2 mL) of each donor and recipient bacteria was mixed together at a ratio of donor to recipient of 1:3. The mixture was added to a final volume of 5 mL LB liquid medium, and incubate at 37°C for 12–18 h. Then spotted the mixture on Muller-Hinton agar plates containing 100 mg/L sodium azide and 2 mg/L polymyxin B as a selective medium for E. coli J53 transconjugants. Detection of mcr-like gene by PCR confirmed the putative transconjugants. Use wzl gene primers, mcr-1 gene primers and mcr-8 gene primers to distinguish the recipient strain (16BU137 and KP20191015) from the donor strain (J53).

Whole-genome sequencing and genotyping
K. pneumoniae and E. coli were cultured overnight in LB medium. Bacterial samples (5000 g 10 min at 4°C) were collected and frozen at −80°C. The genomes of

Table 4  Colistin resistance related gene in four isolates

| Gene      | 16BU137 Uniprot ID | 17MR471 Uniprot ID | 19PDR22 Uniprot ID | KP20191015 Uniprot ID |
|-----------|--------------------|--------------------|--------------------|-----------------------|
| amrD      | Uniprot ID: P76472 | Uniprot ID: P76472 | Uniprot ID: P76472 | Uniprot ID: P76472   |
| emrA      | Uniprot ID: P27303 | Uniprot ID: P27303 | Uniprot ID: P27303 | Uniprot ID: P76472   |
| emrB      | Uniprot ID: P0AEJ0 | Uniprot ID: P0AEJ0 | Uniprot ID: P0AEJ0 | Uniprot ID: P0AEJ0   |
| eptB      | Uniprot ID: P37661 | Uniprot ID: P37661 | Uniprot ID: P37661 | Uniprot ID: P37661   |
| mcr-like genes | mcr-1.1 | mcr-1.1 | None               | mcr-8               |
| mglB      | Uniprot ID: BSXQ45 | Uniprot ID: P64512 | Uniprot ID: BSXQ45 | Uniprot ID: BSXQ45   |
| opgE      | Uniprot ID: P75785 | Uniprot ID: P75785*2 | Uniprot ID: P75785 | Uniprot ID: P75785   |
| phoP      | Uniprot ID: P13792; Uniprot ID: D0ZV90 | Uniprot ID: P23836 | Uniprot ID: P0DM78; Uniprot ID: P13792; Uniprot ID: D0ZV90 | Uniprot ID: P23836; Uniprot ID: D0ZV90 |
| phoQ      | Uniprot ID: P23837 | Uniprot ID: P23837 | Uniprot ID: P23837 | Uniprot ID: P23837   |
| pmrA (basR) | Uniprot ID: P30843 | Uniprot ID: P30843 | Uniprot ID: P30843 | Uniprot ID: P30843   |
| pmrB (basS) | Uniprot ID: P30844 | Uniprot ID: P30844 | Uniprot ID: P30844 | Uniprot ID: P30844   |
| pmrC (eptA) | Uniprot ID: P36555 | Uniprot ID: P36555 | Uniprot ID: P36555 | Uniprot ID: P36555   |
| pmrD      | Uniprot ID: P37589 | Uniprot ID: P37590 | Uniprot ID: P37589 | Uniprot ID: P37589   |

...
four isolates were performed using a PacBio RS II platform and Illumina HiSeq 4000 platform at the Beijing Genomics Institute (BGI, Shenzhen, China). Four SMRT cells Zero-Mode Waveguide arrays of sequencing, were used by the PacBio platform to generate the subreads set. PacBio subreads (length < 1 kb) were removed. The program Pbdagcon (https://github.com/PacificBiosciences/pbdagcon) was used for self-correction. Draft genomic unitigs, which are uncontested groups of fragments, were assembled using the Celera Assembler against a high quality corrected circular consensus sequence subreads set. To improve the accuracy of the genome sequences, GATK (https://www.broadinstitute.org/gatk/) and SOAP

### Table 5 Antimicrobial resistance genes predicted with ResFinder-3.2

| Gene       | Identity(%) | Antibiotic Resistance | Position          |
|------------|-------------|-----------------------|-------------------|
| KP20191015 |             |                       |                   |
| aac(3)-IV  | 100         | Aminoglycoside        | Plasmid           |
| aadA1      | 100         | Aminoglycoside        | Plasmid           |
| aadA2b     | 99.87       | Aminoglycoside        | Plasmid           |
| aph(3’)-Ib | 100         | Aminoglycoside        | Plasmid           |
| aph(6)-Ib  | 100         | Aminoglycoside        | Plasmid           |
| armA       | 100         | Aminoglycoside        | Plasmid           |
| blaCTX-M-15| 100         | Beta-lactam           | Plasmid           |
| blaDHA-1   | 100         | Beta-lactam           | Plasmid           |
| blaSHV-182 | 99.88       | Beta-lactam           | Chromosome        |
| blaTEM-1B  | 100         | Beta-lactam           | Plasmid           |
| mcr-8      | 99.71       | Colistin              | Plasmid           |
| fosA       | 99.27       | Fosfomycin            | Plasmid           |
| mph(A)     | 100         | Macrolide             | Plasmid           |
| mph(E)     | 100         | Macrolide             | Plasmid           |
| msr(E)     | 100         | Macrolide             | Plasmid           |
| catA2      | 96.11       | Phenicol              | Plasmid           |
| cmlA1      | 99.92       | Phenicol              | Plasmid           |
| oxaA       | 100         | Quinolone             | Chromosome        |
| oxaB       | 100         | Quinolone             | Chromosome        |
| qnrB4      | 100         | Quinolone             | Plasmid           |
| sul1       | 100         | Sulphonamide          | Plasmid           |
| sul2       | 99.88       | Sulphonamide          | Plasmid           |
| tet(D)     | 100         | Tetracycline          | Plasmid           |
| tet(B)     | 100         | Tetracycline          | Plasmid           |
| tet(M)     | 96.15       | Tetracycline          | Plasmid           |

### Table 5 (continued)

| Gene       | Identity(%) | Antibiotic Resistance | Position          |
|------------|-------------|-----------------------|-------------------|
| dfrA17     | 100         | Trimethoprim          | Plasmid           |
| 16BU137    |             |                       |                   |
| aac(3)-IId | 99.88       | Aminoglycoside        | Plasmid           |
| aac(6’)-Ib-cr | 100     | Aminoglycoside        | Plasmid           |
| aadA16     | 99.65       | Aminoglycoside        | Plasmid           |
| aph(3’)-Ib | 100         | Aminoglycoside        | Plasmid           |
| aph(6)-Ib  | 99.88       | Aminoglycoside        | Plasmid           |
| blaCTX-M-3  | 100        | Beta-lactam           | Plasmid           |
| blaSHV-110 | 99.77       | Beta-lactam           | Chromosome        |
| blaTEM-18  | 100         | Beta-lactam           | Plasmid           |
| mcr-1.1    | 100         | Colistin              | Plasmid           |
| fosA       | 99.29       | Fosfomycin            | Chromosome        |
| mph(A)     | 100         | Macrolide             | Plasmid           |
| floR       | 98.27       | Phenicol              | Plasmid           |
| aac(6’)-Ib-cr | 100  | Quinolone             | Plasmid           |
| qoxA       | 100         | Quinolone             | Chromosome        |
| qoxB       | 100         | Quinolone             | Chromosome        |
| qnrB2      | 99.84       | Quinolone             | Plasmid           |
| qnrS1      | 100         | Quinolone             | Plasmid           |
| arr-3      | 100         | Quinolone             | Plasmid           |
| sul1       | 100         | Rifampicin            | Plasmid           |
| sul2       | 99.88       | Sulphonamide          | Plasmid           |
| tet(A)     | 100         | Tetracycline          | Plasmid           |
| dfrA27     | 100         | Trimethoprim          | Plasmid           |
| 19PDR22    |             |                       |                   |
| aac(3)-IId | 99.88       | Aminoglycoside        | Chromosome        |
| aadA2b     | 99.87       | Aminoglycoside        | Plasmid           |
| aadA5      | 100         | Aminoglycoside        | Plasmid           |
| aph(3’)-Ib | 100         | Aminoglycoside        | Plasmid           |
| aph(6)-Ib  | 100         | Aminoglycoside        | Plasmid           |
| armA       | 100         | Aminoglycoside        | Plasmid           |
| mdr(A)     | 99.92       | Macrolide             | Chromosome        |
| floR       | 98.19       | Phenicol              | Plasmid           |
| qoxA       | 100         | Quinolone             | Plasmid           |
| qoxB       | 99.97       | Quinolone             | Plasmid           |
| tet(B)     | 100         | Tetracycline          | Plasmid           |
| tet(M)     | 96.15       | Tetracycline          | Plasmid           |
| mcr-1.1    | 100         | Colistin              | Chromosome        |
| fosA       | 99.27       | Fosfomycin            | Chromosome        |
| mph(A)     | 100         | Macrolide             | Chromosome        |
| mph(E)     | 100         | Macrolide             | Plasmid           |
| mdr(A)     | 99.92       | Macrolide             | Chromosome        |
| floR       | 98.19       | Phenicol              | Plasmid           |
| qoxA       | 100         | Quinolone             | Plasmid           |
| qoxB       | 99.97       | Quinolone             | Plasmid           |
| tet(B)     | 100         | Tetracycline          | Chromosome        |
| tet(M)     | 96.15       | Tetracycline          | Plasmid           |
De novo hybrid assembly both of short Illumina reads and long PacBio reads was performed using Unicycler v0.4.3 [75]. Complete circular contigs were then corrected using Pilon v1.22 with Illumina reads. For each de novo assembled genome, coding sequences were predicted using Prodigal (v. 2.6) [76] and annotated using the rapid prokaryotic genome annotation tool Prokka [77]. Acquired antimicrobial resistance genes (ARGs) were identified using ABRicate version 0.5 (https://github.com/tseemann/abricate) by aligning genome sequences to the ResFinder database [78]. The virulence factors of the isolates were identified using VFDB database [79]. Insertion sequence (IS) elements were determined with ISFinder (https://www-isfinder.bioline.fr). In silico multilocus sequence typing (MLST) was performed by MLST 1.8 (https://cge.cbs.dtu.dk/services/MLST/). Plasmid replicon types were detected using PlasmidFinder v1.3 [80].

Phylogenetic analysis
We collected all 87 *E. coli* strains from Guangdong, China and all 182 *K. pneumoniae* strains from Guangdong and Anhui, China (182 strains from Guangdong and 70 from Anhui) in the NCBI database (https://www.ncbi.nlm.nih.gov/pathogens/) as of December 2020. HarvestTools kit (Parsnp, Gingr and HarvestTools) was used to perform
comparative genomics analysis and phylogenetic analysis of different isolates, Interactive tree of life (iTOL) v5 ([http://itol.embl.de/](http://itol.embl.de/)) was used to construct a maximum likelihood phylogenetic tree [54, 55].

**Abbreviations**

MDR: Multidrug resistant; CPS: Capsular polysaccharide; NDM: New Delhi β-lactamase; ESBL: Extended spectrum β-lactamase genes; PEA: Phosphoethanolamine; LPS: Lipopolysaccharide; TSDs: Target site duplications; WGS: Whole-genome sequencing; PEtN: Phosphoethanolamine.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08301-5.

Additional file 1.
Additional file 2.
Additional file 3.

**Acknowledgments**

We thank Zeyu Jin and Dehua Liu for technical assistance. We thank Professor Yingzhen Zhou from Southwest Medical University for providing us with *E. coli* J53.
Authors' contributions

BS, GBT and TD initiated the project, designed the research framework, review and edited the manuscript; ZH, YY and WL analyzed the data and drafted the manuscript. XM, CZ and JZ processed the whole-genome sequencing. JZ collected samples and provided the experimental materials. All authors read and approved the final manuscript.

Funding

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB29020000), the National Natural Science Foundation of China (grant numbers 81722030, 81830103, 81902123), National Key Research and Development Program (grant number 2017ZX100302031), Guangdong Natural Science Foundation (grant no. 2017A030306012), project of high-level health teams of Zhuhai at 2018 (The Innovation Team for Antimicrobial Resistance and Clinical Infection), and 111 Project (grant no. B1203). The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials

Nucleotide sequence accession number whole-genome sequencing data have been deposited in the NCBI database and are publicly available under BioProject: PRJNA622869. The complete genome sequence of E.coli 17MR471, K. pneumoniae 168BU137, K. pneumoniae KP20191015, and K. pneumoniae 19PDR22 reported in this study has been submitted to the NCBI database and assigned accession number CP051158, CP051161, CP051160, and CP051159, respectively. Sequences of p168BU137_mcr-1 and pKP20191015_mcr-8 were assigned accession number MT316509 and MT316510, respectively. The detailed prediction information of virulence factors, resistance genes and plasmids are located in Table S1, Table S2 and Table S3, respectively.

Declarations

Ethics approval and consent to participate

This study was approved by ethical committees of Sun Yat-Sen University Zhongshan School of Medicine (November 1st, 2014). Individual consent forms were obtained face to face to use the samples in research and being consented for publication.

Not applicable.

Competing interests

We declare no competing interests.

Author details

1 Department of Oncology, The First Affiliated Hospital, University of Science and Technology of China, Hefei, China. 2 Department of Microbiology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China. 3 Key Laboratory of Tropical Diseases Control (Sun Yat-sen University), Ministry of Education, Guangzhou 510080, China. 4 School of Pharmaceutical Sciences, Anhui University of Chinese Medicine, Hefei 230031, Anhui, China. 5 Department of Immunology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China. 6 Department of Pathology, the First Affiliated Hospital, Anhui University of Chinese Medicine, Hefei 230031, Anhui, China. 7 Clinical Laboratory of the First Affiliated Hospital, Anhui University of Chinese Medicine, Hefei 230031, Anhui, China. 8 Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China. 9 Xuzhou Minzu University School of Medicine, Xiangyang, China.

Received: 8 July 2020 Accepted: 11 January 2022

Published online: 31 January 2022

References

1. Ainsworth GC, Brown AM, Brownlee G, Aeroplatin, an antibiotic produced by Bacillus aerousporus Greer. Nature. 1947;159(4060):263.

2. Nang SC, Li J, Velkov T. The rise and spread of mcr plasmid-mediated polymyxin resistance. Crit Rev Microbiol. 2019;45(2):131–61.

3. Li J, Nation RL, Turnidge JD, Milne RW, Coughard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. Lancet Infect Dis. 2006;6(9):589–601.

4. Grosisman EA. The pleiotropic two-component regulatory system PhoP-PhoQ. J Bacteriol. 2001;183(6):1835–42.

5. Park SY, Grosisman EA. Signal-specific temporal response by the Salmonella PhoP/PhoQ regulatory system. Mol Microbiol. 2014;91(1):135–44.

6. Campos MA, Vargas MA, Queiroz V, Llopart CM, Alberti S, Bengoechea JA. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun. 2004;72(12):7107–14.

7. Padilla E, Llobet E, Domenech-Sanchez A, Martinez-Martinez L, Bengoechea JA, Alberti S. Klebsiella pneumoniae AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother. 2010;54(1):177–83.

8. Srivivasan VR, Rajamohan G. KpnEF, a new member of the Klebsiella pneumoniae cell envelope stress response regulon, is an SMR-type efflux pump involved in broad-spectrum antimicrobial resistance. Antimicrob Agents Chemother. 2013;57(9):4449–62.

9. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism mcr-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161–8.

10. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 2016;71(8):2066–70.

11. Zheng B, Dong H, Xu H, Lv J, Zhang J, Jiang X, et al. Coexistence of mcr-1 and NDM-1 in clinical Escherichia coli isolates. Clin Infect Dis. 2016;63(10):1393–5.

12. Yao X, Doi Y, Zeng L, Lv L, Liu JH. Carbapenem-resistant and colistin-resistant Escherichia coli co-producing NDM-9 and MCR-1. Lancet Infect Dis. 2016;16(3):288–9.

13. Xu Y, Lin J, Cui T, Srivinas S, Feng Y. Mechanistic insights into transferable polymyxin resistance among gut bacteria. J Biol Chem. 2018;293(12):4350–65.

14. Gao R, Hu Y, Li Z, Sun J, Wang Q, Lin J, et al. Dissemination and mechanism for the MCR-1 Colistin resistance. PLoS Pathog. 2016;12(11):e1005957.

15. Li R, Xie M, Zhang J, Yang Z, Liu L, Liu X, et al. Genetic characterization of mcr-1-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. J Antimicrob Chemother. 2017;72(2):393–401.

16. Snedsrud E, He S, Chandler M, Dekker JP, Hickman AB, McGann P, et al. A model for transposition of the Colistin resistance gene mcr-1 by ISApl1. Antimicrob Agents Chemother. 2016;60(11):6973–6.

17. Tegetmeyer HE, Jones SC, Langford PB, Baltes N. mcr-1, a novel inser- tion element of Actinobacillus pleuropneumoniae, prevents ApoV-based serological detection of serotype 7 strain AP76. Vet Microbiol. 2008;128(3–4):342–53.

18. Snedsrud E, McGann P, Chandler M. The birth and demise of the ISApl1-mcr-1-ISApl1 composite transposon: the vehicle for transferable Colistin resistance. mBio. 2018;9(1):e02381–17.

19. He YZ, Li XP, Miao YY, Lin J, Sun RY, Wang XY, et al. The ISApl1 2 dimer circular intermediate participates in mcr-1 transposition. Front Microbiol. 2018;9:1015.

20. Szabo M, Kiss J, Nagy Z, Chandler M, Olasz F. In vitro detection of ISAP2 forming an mcr-1-encoding plasmid mediated colistin resistant strain of Salmonella enterica from retail meat in Portugal. J Antimicrob Chemother. 2016;71(8):2338–40.

21. Figueiredo R, Card RM, Nunez J, Pomba C, Mendonca N, Anjum MF, et al. Detection of an mcr-1 encoding plasmid mediating colistin resistance in Salmonella enterica from retail meat in Portugal. J Antimicrob Chemother. 2016;71(8):2338–40.

22. Zeng JD, Alarcon RD, Malina M, Mazzacane S. Spread of mcr-1-driven Colistin resistance on hospital surfaces, Italy. Emerg Infect Dis. 2018;24(9):1752–3.

23. Liu BT, Song FJ, Zou M, Hao ZH, Shan H. Emergence of Colistin resistance gene mcr-1 in Cronobacter sakazakii producing NDM-9 and in Escherichia coli from the same animal. Antimicrob Agents Chemother. 2017;61(2):e00444–16.
26. Pham Thanh D, Thanh Tuyen H, Nguyen Thi Nguyen T, Chung The H, Wick RR, Thwaites GE, et al. Inducible colistin resistance via a disrupted plasmid-borne mcr-1 gene in a 2008 Vietnamese Shigella sonnei isolate. J Antimicrob Chemother. 2016;71(8):2314–7.
27. Zhao F, Zong Z. Kluyvera ascorbata strain from hospital sewage carry-resistant mcr-1. Antimicrob Agents Chemother. 2016;60(12):7127–9.
28. Luo Y, Yao X, Lv L, Dui Y, Huang X, Huang S, et al. Emergence of mcr-1 in Raoultella ornithinolytica and Escherichia coli isolates from retail vegetables in China. Antimicrob Agents Chemother. 2017;61(10):e01398–17.
29. Sennati S, Di Pilato V, Riccobono E, Di Maggio T, Villagran AL, Pallecchi L, et al. Citrobacter braakii carrying plasmid-borne mcr-1 colistin resistance gene from ready-to-eat food from a market in the Chaco region of Bolivia. J Antimicrob Chemother. 2017;72(7):2127–9.
30. Singer AC, Shaw H, Rhodes V, Hart A. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. Front Microbiol. 2016;7:1728.
31. Radhouani H, Silva N, Poeta P, Torres C, Corexia S, Igrejas G. Potential impact of antimicrobial resistance in wildlife, environment and human health. Front Microbiol. 2014;5:23.
32. Shen C, Feng S, Chen H, Dai M, Paterson DL, Zheng X, et al. Transmission of mcr-1-producing multidrug-resistant Enterobacteriaceae in public transportation in Guangzhou, China. Clin Infect Dis. 2018;67(suppl_2):S217–S24.
33. Zhao F, Feng Y, Lu X, McNally A, Zong Z. IncP plasmid carrying Colistin resistance gene mcr-1 in Klebsiella pneumoniae from hospital sewage. Antimicrob Agents Chemother. 2017;61(2):e02229–16.
34. Zurfluh K, Klumpp J, Nuesch-Inderbinen M, Stephan R. Full-length nucleotide sequences of mcr-1-producing multidrug-resistant Enterobacteriaceae. Antimicrob Agents Chemother. 2016;60(9):5389–91.
35. Xavier BB, Lammens C, Butaye P, Goossens H, Malhotra-Kumar S. Complete sequence of an IncFII plasmid harbouring the colistin resistance gene mcr-1 in Klebsiella pneumoniae from hospital sewage. Antimicrob Agents Chemother. 2017;61(2):e02229–16.
36. Zurlu H, Kummer N, Luesch-Indira E, Mestphan R. Full-length nucleotide sequences of mcr-1-borne plasmids isolated from extended-spectrum-beta-lactamase-producing Escherichia coli isolates of different origins. Antimicrob Agents Chemother. 2016;60(9):5389–91.
37. Dona V, Bernasconi OJ, Pires J, Collaud A, Overesch G, Ramette A, et al. Identification of a novel transposon-associated phosphoethanolamine transerase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting Salmonella enterica subsp. enterica serovar Paratyphi B. J Antimicrob Chemother. 2017;72(12):3317–24.
38. AbuOun M, Staberfeldt EJ, Jugdutt NA, Kirchner M, Dormer L, Nunez-Garca I, et al. Mcr-1 and mcr-2 (mcr-6.1) variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother. 2018;73(10):2904.
39. Yang YQ, Li YX, Lei CW, Zhang AY, Wang NH. Novel plasmid-mediated colistin resistance gene mcr-7.1 in Klebsiella pneumoniae. J Antimicrob Chemother. 2018;73(7):1791–5.
40. Zhu B, Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, et al. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg Microbes Infect. 2018;7(11):122.
41. Carroll LM, Gabbalia A, Guldbrannson C, Sullivan G, Henderson LO, Wiedmann M. Identification of novel mobilized Colistin resistance gene mcr-9 in a multidrug-resistant, Colistin-susceptible Salmonella enterica serotype typhimurium isolate. mBio. 2019;10(3):e00853–19.
42. Wang C, Feng Y, Liu L, Wei L, Kang M, Zong Z. Identification of novel mobile colistin resistance gene mcr-10. Emerg Microbes Infect. 2020;9(1):508–16.
43. Teo JWP, Kalisivar M, Venkatachalam I, Ng OT, Lin RTP, Octavia S. And variants in Carbenapenemase-producing clinical Enterobacteriaceae do not confer phenotypic Polymyxin resistance. J Clin Microbiol. 2018;56(3).
44. Mazur A, Urzua Y, Peirano G, Pitout JD. Complete genome sequence of Escherichia coli J53, an Azide-resistant laboratory strain used for conjugation experiments. Genome Announc. 2016;4(2):e00433–18.
45. Pogue JM, Jones RN, Bradley JS, Andes DR, Bhavnani SM, Drusano GL, et al. Polymyxin susceptibility testing and interpretive breakpoints: recommendations from the United States committee on antimicrobial susceptibility testing (USCAST). Antimicrob Agents Chemother. 2020;64(2):e01495–19.
46. Treangen TJ, Ondov BD, Koren S, Phillippy AM. The harvest suite for rapid core-genome alignment and visualization of thousands of intraspecies microbial genomes. Genome Biol. 2014;15(1):524.
47. Letunic I, Bork P. Interactive tree of life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. 2019;47(W1):W265–W9.
48. Yang TY, Wang SF, Lin JE, Griffith BTS, Lian SH, Hong ZD, et al. Contributions of insertion sequences conferring colistin resistance in Klebsiella pneumoniae. Int J Antimicrob Agents. 2020;55(3):105894.
49. Shen Y, Zhou H, Xu J, Wang Y, Zhang Q, Walsh TR, et al. Anthropogenic and environmental factors associated with high incidence of mcr-1 carriage in humans across China. Nat Microbiol. 2018;3(9):1054–62.
50. Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. Clin Microbiol Rev. 2008;21(3):449–65.
51. Groisman EA, Kayser J, Soncini FC. Regulation of polymyxin resistance and adaptation to low-Mg2+-enriched environments. J Bacteriol. 1997;179(23):7040–5.
52. Olatan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014;5(643):1–18.
53. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13(1):42–51.
54. Kline T, Trent MS, Stead CM, Lee MS, Sousa MC, Felise HB, et al. Synthesis and evaluation of lipid A modification by 4-substituted 3-deoxyarabinose analogs as potential inhibitors of bacterial polymyxin resistance. Bioorg Med Chem Lett. 2008;18(4):1507–10.
55. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67(4):593–656.
56. Reeves PR, Hobbs M, Valvano MA, Skurnik M, Whitlefield C, Coplin D, et al. Bacterial polysaccharide synthesis and gene nomenclature. Trends Microbiol. 1996;4(12):495–503.
57. Yan A, Guan Z, Raetz CR. An undecaprenyl phosphate-aminoarabinose mechanism required for polymyxin resistance in Escherichia coli. J Biol Chem. 2007;282(49):36077–89.
58. Gatzeva-Topalova PZ, May AP, Sousa MC. Structure and mechanism of ArnA: conformational change implies ordered dehydrogenase mechanism in key enzyme for polymyxin resistance. Structure. 2005;13(6):929–42.
59. Adams MD, Nickel GC, Bajaksouzian S, Lavender H, Murthy AR, Jacobs MR, et al. Resistance to colistin in Acinetobacter baumannii associated with mutations in the PrmR2 two-component system. Antimicrob Agents Chemother. 2009;53(9):3628–34.
68. Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother. 2012;67(7):1607–15.
69. Kato A, Latifi T, Grossman EA. Closing the loop: the PmrA/PmrB two-component system negatively controls expression of its posttranscriptional activator PmrD. Proc Natl Acad Sci U S A. 2003;100(8):4706–11.
70. Miller AK, Brannon MK, Stevens L, Johansen HK, Selgrade SE, Miller SJ, et al. PhoQ mutations promote lipid modification and polymyxin resistance of Pseudomonas aeruginosa found in colistin-treated cystic fibrosis patients. Antimicrob Agents Chemother. 2011;55(12):5761–9.
71. Cheng YH, Lin TL, Pan YJ, Wang YF, Lin YT, Wang JT. Colistin resistance mechanisms in Klebsiella pneumoniae strains from Taiwan. Antimicrob Agents Chemother. 2015;59(5):2909–13.
72. Poirel L, Jayol A, Bontron S, Villegas MV, Ozdamar M, Turkoglu S, et al. The mgrB gene as a key target for acquired resistance to colistin in Klebsiella pneumoniae. J Antimicrob Chemother. 2015;70(1):75–80.
73. Olaitan AO, Rolain JM. Interruption of mgrB in the mediation of colistin resistance in Klebsiella oxytoca. Int J Antimicrob Agents. 2015;46(3):354–5.
74. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163–75.
75. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS computational biology. 2017;13(6):e1005595.
76. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.
77. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9.
78. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67(11):2640–4.
79. Chen L, Zheng D, Liu B, Yang J, Jin Q. VFDB 2016: hierarchical and refined dataset for big data analysis—a 10 years on. Nucleic Acids Res. 2016;44(D1):D694–7.
80. Carattoli A, Zankari E, Garcia-Fernandez A, Larsen MV, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58(7):3895–903.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.