Infection and Drug Resistance

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SHORT REPORT

Characterization of a Novel *mcr-8.2*-Bearing Plasmid in ST395 *Klebsiella pneumoniae* of Chicken Origin

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Abstract: The emergence of mobile colistin resistance *mcr* genes undermines the efficacy of colistin as the last-resort drug for multi-drug resistance infections and constitutes a great public health concern. Plasmids play a critical role in the transmission of *mcr* genes among bacteria. One colistin-resistant *Klebsiella pneumoniae* strain of chicken origin was collected and analyzed by antimicrobial susceptibility testing, PCR, conjugation assay and S1-PFGE. Whole-genome sequencing (WGS) approach combining Illumina and MinION platforms was utilized to decipher the underlying colistin resistance mechanism and genetic context. A novel *mcr-8.2*-bearing plasmid p2019036D-mcr8-345kb with 345 655 bp in size encoding various resistance genes including *floR, sul1, aadA16, aadA2,* $bla_{\text{CTX-M-27}}$, $bla_{\text{DHA-1}}$, *tet*(D), *dfrA12* and *qnrB4* was identified responsible for the colistin resistance phenotype. Plasmid comparison has shown that the *mcr-8.2*-bearing plasmid differed from other reported plasmids positive for *mcr-8.2* but shared the same core *mcr-8.2*-bearing conserved region. This study demonstrates the emergence of *mcr-8.2*-bearing *K. pneumoniae* of animal origin is a potential risk to humans.

Keywords: *mcr-8.2*, *Klebsiella pneumoniae*, plasmids, animal origin

Antimicrobial resistance is posing a great public health concern worldwide. Since the first report of plasmid-mediated colistin resistance gene *mcr*-1 in 2015,[1] a variety of *mcr* genes up to *mcr-10* have been detected.[2,3] These different *mcr* genes and the *mcr*-bearing plasmids are wildly distributed in Enterobacterales from humans, animals and environments.[1–4] *Klebsiella pneumoniae* is ubiquitous in environments and is a major cause of nosocomial infections worldwide.[5] The emergence of *mcr* genes in *K. pneumoniae* is a challenge to clinical treatments. To date, several *mcr* genes and their variants have been detected in *K. pneumoniae* of both human and animal origins in different countries.[6–9] The first identified *mcr-8* was found in a transferrable IncFII plasmid pKP91 in *K. pneumoniae* of swine origin.[6] Then, another novel *mcr-8.2* variant was reported in *K. quasipneumoniae*, phylogenetically similar to *K. pneumoniae*, isolated from a pig farm during our surveillance study in 2018.[10] Recently, a cluster of *Klebsiella pneumoniae* carrying both $bla_{\text{NDM-1}}$ and *mcr-8.2* was also reported.[11] In this study, we characterized a novel *mcr-8.2*-bearing plasmid harbored by a multi-drug resistance (MDR) *K. pneumoniae* strain of chicken origin, which extended the understanding of large plasmids co-harboring *mcr-8.2* and other important resistance genes.

A colistin-resistant strain 2019036D, isolated through MacConkey agar plates supplemented with colistin (4 μg/mL), was recovered from a caecal microbiota

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http://doi.org/10.2147/IDR.S256544

sample at a broiler chicken slaughterhouse in Sichuan, China in June 2019. The genomic DNA of the purified bacteria was extracted using the TIANamp Bacteria DNA Kit (Tiangen, China) according to the manufacturer's instruction, and PCR targeting at *mcr* genes from *mcr*-1 to *mcr*-8 were performed using primers as previously described.[6] The PCR product was sequenced and confirmed positive for *mcr-8.2* after BLASTn analysis. 16S rRNA gene sequencing identified 2019036D isolate as *K. pneumoniae*. Minimum inhibitory concentrations (MICs) of different antimicrobials were measured with the broth microdilution method according to the CLSI standards with *E. coli* ATCC 25,922 as the control. The strain 2019036D was resistant to colistin, cefazolin, cefoxitin, cefotaxime, ceftazidime, tetracycline, nalidixic acid, erythromycin, trimethoprim/sulfamethoxazole, azithromycin, ciprofloxacin and chloramphenicol, but still susceptible to imipenem (Table S1). Conjugation assay was performed to verify the transferability of the colistin resistance gene with the *E. coli* C600 as the recipient strain but failed after three repeats. Subsequently, S1-PFGE showed that 2019036D harbored three plasmids of ca. 350kb, 50kb and 35kb in length. To investigate the genetic structure of *mcr-8.2*, the complete genome sequence of 2019036D was sequenced by short-read Illumina (150bp×2) Hiseq 2500 and long-read MinION with the rapid sequencing kit simultaneously, and de novo assembled with a hybrid strategy utilizing Unicycler.[12,13] The complete genome sequences were annotated using the online RAST tool (http://rast.nmpdr.org/) and were modified manually. Plasmid replicon typing and resistance genes identification were performed using the online tools (https://cge.cbs.dtu.dk/services/). Insertion sequences were identified based on the ISfinder database.[14] Circular plasmids comparison was performed by BRIG.[15] Phylogenetic analysis was based on the core genome analysis of Roary and FastTree,[16,17] and visualized by iTOL.[18]

One 5369 757 bp chromosome (CP047336) and three plasmids including p2019036D-mcr8-345kb (CP047337), p2019036D-50kb (CP047338) and p2019036D-35kb (CP047339) were obtained, which was consistent with the plasmid profile observed by S1-PFGE. MLST analysis indicated that 2019036D belonged to ST395, a clinical KPC-producing and NDM-producing *K. pneumoniae* ST lineage.[19,20] Kleborate analysis (https://github.com/katholt/Kleborate) identified no virulence genes indicating this strain was not a Hypervirulent *Klebsiella pneumoniae* (HvKP). Whole-genome analysis showed that a *mcr*-8 variant showing 100% identity to *mcr-8.2* was detected in the plasmid p2019036D-mcr8-345kb belonging to IncFIB(K) replicon type. Other *mcr-8.2*-bearing plasmids in NCBI databases were found to harbor the backbone of IncF-type plasmid. But they showed limited homologous region to p2019036D-mcr8-345kb (Figure 1A). Among them, pD120-1_83kb belonging to IncFIB(K) showed most identity (84%) to p2019036D-mcr8-345kb but differed in most plasmid backbone, highlighting this plasmid was a novel *mcr-8.2*-bearing plasmid. Meanwhile, all plasmids co-harboring *mcr* genes and IncFIB(K) replicon in NCBI databases were retrieved and they shared few common regions to p2019036D-mcr8-345kb (Figure S1), which implied that the structure of the *mcr*-8.2-bearing plasmid was novel among all *mcr*-bearing plasmids. In addition to the *mcr-8.2* gene, two multi-drug resistance regions (MRRs) were detected in p2019036D-mcr8-345kb but lacked in pD120-1_83kb, these MRRs contained *floR, sul1, aac(6')-Ib-cr, aadA16, aadA2, aph(3')-Ia,* $bla_{\text{CTX-M-27}}$, $bla_{\text{DHA-1}}$, *mph*(A), *tet*(D), *dfrA12, dfrA27, aac(6')-Ib-cr* and *qnrB4* (Figure 1A). They were also absent in other *mcr*-negative IncFIB(K) plasmids (pIncFIBK and p1_020143) sharing similar backbone to p2019036D-mcr8-345kb in NCBI databases (Figure S2). The core genetic structure of *mcr-8.2* with IS*903B*-ORF1-4-*dgkA-baeS-copR*-IS*Ecl1*-ORF5-*mcr-8.2*-ORF6-IS*Kpn26*-ORF7-8 in p2019036D-mcr8-345kb was identified nondistinctive to other five available *mcr-8.2*-bearing plasmids in nr databases (Figure 1B), demonstrating that the *mcr-8.2* containing region might have a common ancestor and translocate among different plasmids. IS*Ecl1* was inserted in the intergenic region of *mcr-8.2* and *copR*, reconfirming the assumption that IS*Ecl1* insertion occurred before *mcr-8.2* mobilization and has no association with the translocation of *mcr-8.2*.[10] Comparatively, IS*903B* and IS*Kpn26* located in the boundary regions and may play roles in the dissemination of *mcr-8.2*, but no circular intermediate harboring *mcr-8.2* was detected. Until now, all strains positive for *mcr-8.2*-bearing plasmids are *K. pneumoniae* besides strain D120-1 identified as *K. quasipneumoniae*, both of which were derived from the same clade.[10] Although all *mcr-8.2*-positive strains from different sources were *Klebsiella* spp., they belonged to different sequence types (STs) (Figure S3), implying that the dissemination of *mcr-8.2* and its corresponding plasmids were likely limited by genus and widely spread in different clones. Thus, the prevalence of *mcr-8.2* gene among *Klebsiella* spp. should be monitored consistently.

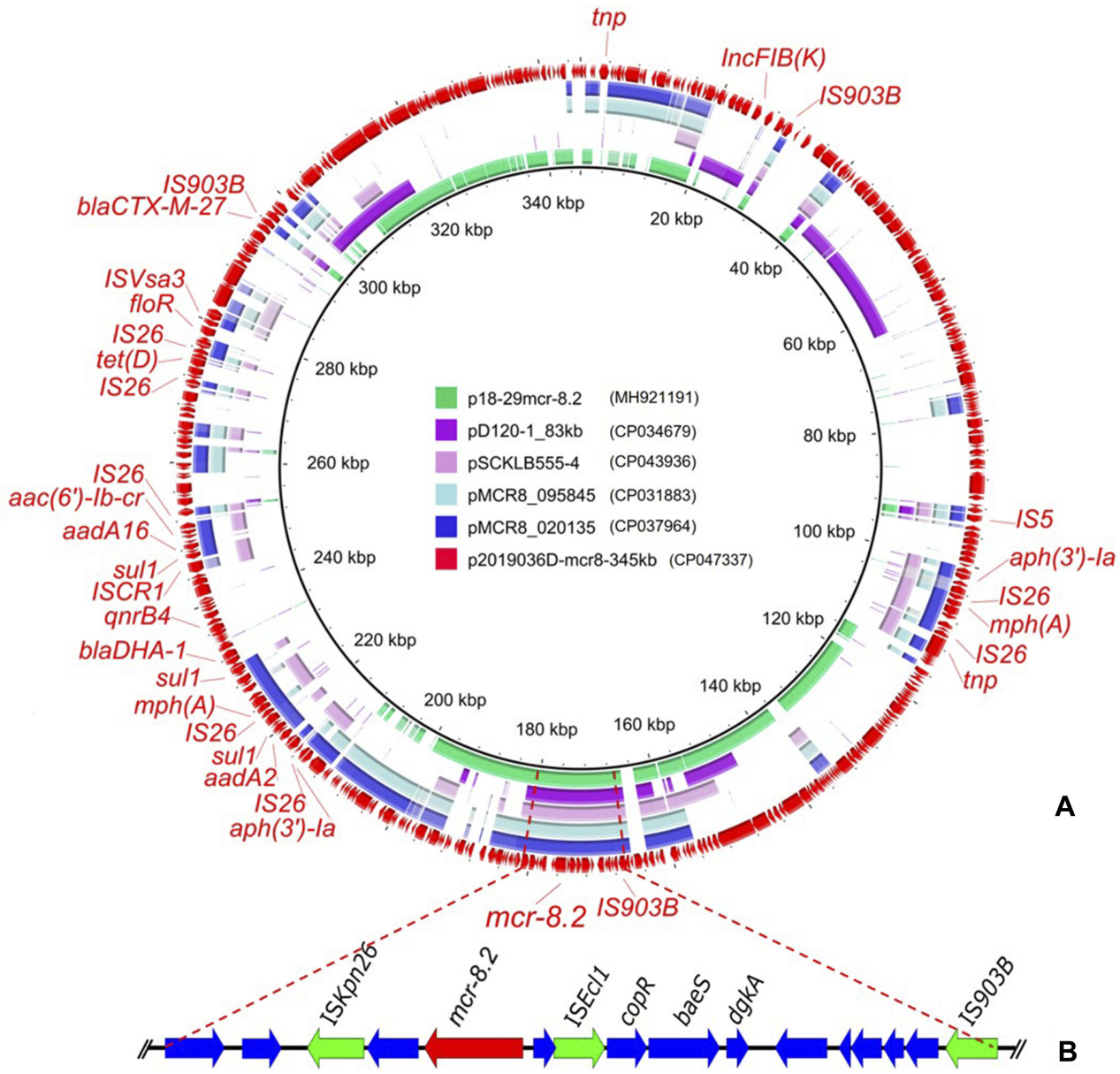

Figure 1 (**A**) Circular comparative analysis of *mcr-8.2*-bearing plasmids in this study and nr database. (**B**) The core genetic structure in the *mcr-8.2*-bearing plasmids. Circular comparison diagram of *mcr-8.2*-bearing plasmids were generated using BRIG v0.95. The outmost ring denotes the reference plasmid p2019036D-mcr8-345kb with labels for resistance genes, insertion sequences and other highlighted genes.

In addition to p2019036D-mcr8-345kb, another two resistance plasmids were identified. A multireplicon (IncR/IncN) plasmid p2019036D-50kb with 50,845 bp in length showed 100% identity at 84% coverage to plasmid sequence tig00000003 (CP021547) (Figure S4a). Another multireplicon (IncX1/IncN) plasmid p2019036D-35kb with 35,955 bp in length shared 99.67% identity at 71% coverage with p16EC-IncN (MN086778) (Figure S4b). The resistome analysis of the two plasmids indicated the presence of genes encoding resistance for beta-lactams ($bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}141}$), aminoglycosides (*aac(3)-IV, aadA1, aadA2b, aph(3')-IIa, aph(3')-Ia, aph(4)-Ia*) and sulphonamides (*sul3*). Together, the three MDR plasmids rendered the strain resistant to multiple antimicrobials.

In conclusion, a ST395 *K. pneumoniae* strain of chicken origin was found positive for a novel *mcr-8.2*-bearing MDR plasmid. Plasmids and core *mcr-8.2*-bearing structure are the genetic basis underlying the transmission of *mcr-8.2* in *K. pneumoniae*. Continuous surveillance of *mcr-8.2* in *Klebsiella* spp. and other bacterial pathogens of different origins is necessary to understand its potential dissemination and risk.

Acknowledgments

This work was supported by the Key Research and Development of Food Safety of the Ministry of Science and Technology of China (no. SQ2017YFC1601501), the National Key Research and Development Program of China (no. 2019YFC1605205), the Natural Science Foundation of Jiangsu Province (no. BK20180900) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Disclosure

The authors report no conflicts of interest in this work.

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