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

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, blaCTX-M-27, blaDHA-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 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

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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, cefotaxin, cefotaxime, ceftazidine, 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(6ʹ)-Ib-cr, aadA16, aadA2, aph(3’)Ia, blaCTX-M-27, bladHIA-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 (plmFIBK and p1_020143) sharing similar backbone to p2019036D-mcr8-345kb in NCBI databases (Figure S2). The core genetic structure of mcr-8.2 with IS903B-ORF1-4-dgkA-bacS-copR-ISEcl1-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. ISEcl1 was inserted in the intergenic region of mcr-8.2 and copR, reconfirming the assumption that ISEcl1 insertion occurred before mcr-8.2 mobilization and has no association with the translocation of mcr-8.2.\(^10\) Comparatively, IS903B and ISKpn26 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.
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<sub>CTX-M-55</sub>, bla<sub>TEM-141</sub>), 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 <i>K. pneumoniae</i> strain of chicken origin was found positive for a novel <i>mcr-8.2</i>-bearing MDR plasmid. Plasmids and core <i>mcr-8.2</i>-bearing structure are the genetic basis underlying the transmission of <i>mcr-8.2</i> in <i>K. pneumoniae</i>. Continuous surveillance of <i>mcr-8.2</i> in <i>Klebsiella</i> spp. and other bacterial pathogens of different origins is necessary to understand its potential dissemination and risk.
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Disclosure
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References
7. Page AJ, Cummins CA, Hunt M, et al. Unicycler: resolving antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.