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A Phage-Like IncY Plasmid Carrying the *mcr-1* Gene in *Escherichia coli* from a Pig Farm in China

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ABSTRACT We report here a new type of plasmid that carries the *mcr-1* gene, the pMCR-1-P3 plasmid, harbored in an *Escherichia coli* strain isolated from a pig farm in China. pMCR-1-P3 belongs to the IncY incompatibility group and is a phage-like plasmid that contains a large portion of phage-related sequences. The backbone of this plasmid is different from that of other *mcr-1*-carrying plasmids reported previously.

KEYWORDS DNA sequencing, antibiotics, plasmid

The discovery of the plasmid-mediated colistin resistance gene *mcr-1* has captured global attention. Unlike colistin resistance raised by chromosomal mutations, the plasmid carrying the *mcr-1* resistance gene can be rapidly disseminated through horizontal gene transfer. Since the first report of plasmid pHNSHP45 carrying the *mcr-1* gene (1), a number of plasmids with different backbones from human and animal sources carrying the same *mcr-1* gene have been discovered worldwide (2–10). This suggests that the use of colistin in farm animals, particularly in pigs and chickens, is associated with the emergence and spread of colistin resistance (2). Of particular note is evidence of *mcr-1* gene transfer from farm animals to humans through the food chain (3, 4). Therefore, analyzing the diversity of *mcr-1*-carrying plasmids is important to gain an understanding of the pattern of colistin resistance dissemination.

In this study, a total of 262 *Escherichia coli* strains were isolated from chicken cloacal and pig anal swabs in Shandong Province, China, between July 2015 and June 2016. We screened for the *mcr-1* gene by performing PCR, confirmed by Sanger sequencing. Among these *E. coli* isolates, six *mcr-1*-positive strains were derived from chicken farms and seven were derived from pig farms. Sequences of the *mcr-1* gene from these isolates showed 100% nucleotide identity with the published *mcr-1* sequence (1). The results of multilocus sequencing typing (MLST) (11) showed that the 13 *mcr-1*-carrying isolates belong to 12 different sequence types, indicating wide distribution of the *mcr-1* gene (see Table S1 in the supplemental material). To further investigate the genetic background of *mcr-1*-carrying plasmids, we sequenced all plasmids from each isolate using the Illumina MiSeq platform. The results revealed that one *mcr-1*-carrying plasmid from *E. coli* isolate P3 (sequence type 877) displayed a different gene sequence, while the others were mapped to plasmids pHNSHP45 (1), pHNSHP45-2 (GenBank accession number KU341381), and pMCR-1-IncI2 (12). Strain P3 was resistant to ampicillin, colistin, doxycycline, florfenicol, and tetracycline but susceptible to ceftazidime, enrofloxacin, gentamicin, and spectinomycin, among others (see Table S2). Further analysis of plasmid

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contigs using PlasmidFinder tools (https://cge.cbs.dtu.dk/services/PlasmidFinder/) suggested that isolate P3 contains more than one plasmid. Aiming to isolate the *mcr-1*-carrying plasmid from this isolate and to determine the host range of the plasmid, conjugation transfer experiments were performed using *E. coli* J53 Azi^r (azide resistance) (2 ng/μl colistin and 100 ng/μl NaN_3), *Pseudomonas aeruginosa* ATCC 9027 (2 ng/μl colistin and *Pseudomonas* isolation agar), and *Acinetobacter baumannii* ATCC 19606 (2 ng/μl colistin and 8 ng/μl cefepime) as recipient strains. The conjugation frequencies of the *mcr-1*-positive plasmid were determined to be approximately 10^{-4}, 10^{-1}, and 0 per donor cell, respectively. The conjugation did not succeed with the *A. baumannii* strain, demonstrating that the *mcr-1*-positive plasmid is unlikely to be a broad host plasmid. The plasmid DNA was then extracted from positive *E. coli* J53 Azi^r conjugants and used for single-molecule real-time (SMRT) sequencing (Pacific Biosciences). The complete sequence of the plasmid, named pMCR-1-P3, was obtained by assembling the PacBio long reads and corrected by assembling Illumina short reads (see Table S3 for sequencing metrics) (10, 11).

The plasmid sequence was 97,386 bp in length with a GC content of 47.8% (Fig. 1A). It was assigned to the IncY group and encodes a total of 108 open reading frames (ORFs). It contained a fragment of ~23 kb with 97% nucleotide identity to *E. coli* genome assembly FHI87 (GenBank accession number LM996988) and a fragment of ~17 kb with 98% identity to a plasmid sequence from *E. coli* strain O177:H21 (GenBank accession number CP016549) downstream of the *mcr-1* gene (Fig. 1A). It has been found that the *mcr-1* gene in some plasmids is surrounded by two IS*Apl1* elements, one on each end, which may form a composite transposon structure that can potentially move as one complete unit (6, 13–16). In pMCR-1-P3, the sequence of 668 bp on the 5′ end of the second IS*Apl1* was inverted, and thus the entire 924-bp of the IS*Apl1* transposase gene was split into two ORFs transcribed in opposite directions (Fig. 1B). This structure was further verified by PCR and sequencing. The fact that the second IS*Apl1* element is often missing (e.g., in the first reported plasmid pHNSHP45) (Fig. 1B) may indicate that the composite transposon Tn*Apl* (16) experiences dynamic changes during the transposition process.

Interestingly, a total of 72 genes encode phage-related proteins in pMCR-1-P3, such as portal protein, tail fiber protein, outer membrane lytic protein, terminase, and others (see Table S4 in the supplemental material). Among them, 47 genes are carried only by phages, while the other 25 genes are carried by both known plasmids and phages. Therefore, this suggests that pMCR-1-P3 is a phage-like plasmid that usually carries many phage-like elements (17–19). No other antibiotic resistance genes were found in pMCR-1-P3.

The IncY plasmids are normally identified from Enterobacteriaceae, such as *E. coli*, *Salmonella*, and *Klebsiella pneumoniae* (1–3). The common antibiotic resistance gene carried by IncY plasmids is $bla_{CTX\text{-}M\text{-}15}$, an extended-spectrum beta-lactamase gene. To the best of our knowledge, this is the first report of an IncY plasmid carrying the *mcr-1* gene. To date, the *mcr-1* gene has been found in seven different plasmid incompatibility groups (IncI2 [20], IncFII [21], IncX4 [22], IncHI1 [15], IncHI2 [23], IncP [22], and IncF [10]), suggesting the wide distribution and high dissemination capacity of *mcr-1*-carrying plasmids. Given the fact that many phage-related genes are encoded by pMCR-1-P3 and most are homologous to the complete *E. coli* genome (GenBank accession number CP007392), this plasmid was likely formed via homologous recombination from a prophage that was originally located in the *E. coli* genome.

Under such heavy selection pressure, the high frequency of antibiotic resistance gene exchange and recombination in animal farms is expected. The identification of new *mcr-1*-containing phage-like plasmids with an IncY replicon from farm animals suggests that the diversity of *mcr-1*-carrying plasmids in livestock environments may be higher than expected. Given that phage-like plasmids carrying the *mcr-1* gene are not common according to the National Center for Biotechnology Information (57 plasmids carrying the *mcr-1* gene currently published), we suggest that pMCR-1-P3 may not have a dissemination advantage. However, results of previous research indicate that expo-

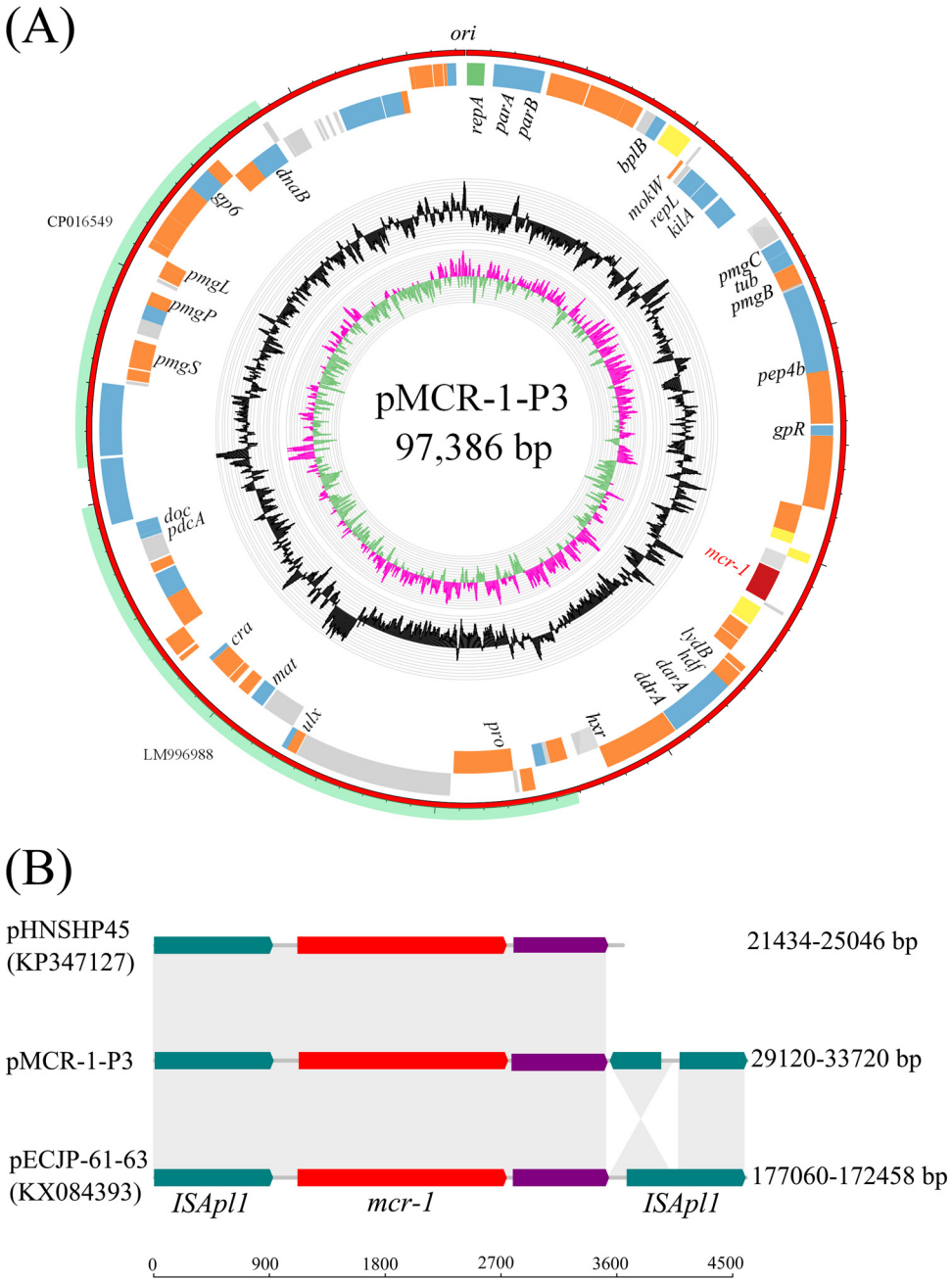

FIG 1 (A) Structure of plasmid pMCR-1-P3 carrying *mcr-1* from *Escherichia coli* isolate P3. Colored rectangles represent open reading frames, with green, blue, orange, yellow, red, and gray rectangles representing replication genes, ORFs with known function, phage-related genes, mobile elements, the *mcr-1* gene, and ORFs with unknown function, respectively. Individual rings range from 1 (outer ring) to 4 (inner ring): ring 1, genes encoded clockwise; ring 2, genes encoded anticlockwise; ring 3, GC content; ring 4, GC skew of the plasmid ([G−C]/[G+C]): magenta, >0; green, <0. Outer ring represents the high similarity of pMCR-1-P3 to LM996988 and CP016549, respectively. (B) Comparison of the immediate genetic environment of representative *mcr-1*-containing plasmids. Plasmid sequences were retrieved from GenBank; accession numbers are shown in brackets. Gray shading indicates >99% nucleotide identity.

sure to antibiotics induces prophages that can transfer antibiotic resistance genes to susceptible bacterial hosts (24). Therefore, pMCR-1-P3 may have emerged by transducing the phage into the plasmid, transferring the *mcr-1* gene into the plasmid at the same time.

Accession number(s). The nucleotide sequences reported in this work have been deposited in GenBank under accession no. KX880944.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02035-16.

TEXT S1, PDF file, 0.3 MB.

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