Supplementary materials

Complete genetic analysis of plasmids carried by two non-clonal \textit{bla}_{NDM-5} \text{ and } \textit{mcr-1}-bearing \textit{Escherichia coli} strains isolated from a chicken meat sample: insights into plasmid transmission among bacteria on food samples

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Running title: Complete genetic analysis of MDR plasmids

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\textbf{Keywords:} Foodborne E. coli, \textit{bla}_{NDM-5}, \textit{mcr-1}, Copy repeats, Genetic analysis
Supplementary materials and methods

Bacterial isolation

*E. coli* isolates 1106 and 1107 were recovered from a chicken meat sample purchased from a supermarket in Shenzhen, Guangdong Province, China in March 27th, 2017. However, they were obtained in two different ways for the reason described as follows. In our previous studies on isolation of *Salmonella* from meat samples, we found that some of the colonies picked up from XLT4 plates were *E. coli*. These *E. coli* isolates were phenotypically very different from *E. coli* isolates that we isolated from the same meat products using MacConkey agar plates. Although we did not understand the underlying reason for discrepancy of colony morphology in *E. coli* isolated by the two methods, we intended to simultaneously use these two methods for isolation of *E. coli* during our surveillance. In brief, 25 g of chicken meat sample were placed in a sterile homogeneous bag containing 50 ml of sterilized saline. Strain 1106 was obtained after a loopful of food suspension was spread onto MacConkey agar plates supplemented with 0.5μg/ml meropenem, followed by incubation at 37°C overnight. Strain 1107 was obtained by the following procedure. Briefly, 1ml of homogenate was transferred to Lactose broth which was then incubated at 42°C for 12-16 h. One milliliter of this pre-enriched broth was transferred to Rappaport-Vassiliadis broth (RV broth) and incubated overnight at 37°C. A loopful of the culture was inoculated onto the XLT4 agar plate supplemented with 0.5μg/ml meropenem. After incubation at 37°C overnight, a colony with typical *E. coli* morphology was selected. Both 1106 and 1107 were identified to be *E. coli* by MALDI-TOF MS using a Bruker MicroFlex LT mass spectrometer (Bruker Daltonics) and API20E test strip (BioMerieux, Inc).
**Antimicrobial susceptibility tests**

Antimicrobial susceptibility tests for strain 1106 and 1107 were performed using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI)(1). Antibiotics used were listed in Table 1. Resistance breakpoints were interpreted according to CLSI recommendations. E. coli strain ATCC 25922 was used as the quality control strain.

**Conjugation, S1-PFGE and Southern hybridization**

The transferability of resistance phenotypes was confirmed by filter mating assays performed as previously described, with slight modification(2). Briefly, cultures of donor strains (E. coli 1106 and 1107) and the recipient strain (sodium-azide-resistant E. coli strain J53) were mixed at a ratio of 4:1, inoculated onto a filter membrane which was then placed on LB agar medium without antibiotics, and incubated at 37°C for 16 hours. Transconjugants were selected on MacConkey agar plates supplemented with meropenem (1μg/mL) and sodium azide (200μg/mL) or on Eosin Methylene Blue Agar containing sodium azide (100μg/mL) and colistin (2μg/mL). The genetic relatedness of strain 1106 and 1107 were determined by PFGE using the Chef-Mapper pulsed-field electrophoresis system (Bio-Rad, USA) upon XbaI digestion. The BioNumerics (Applied Maths) system was used to perform cluster analysis of PFGE patterns. S1-nuclease PFGE was performed to characterize the plasmids that conferred resistance to meropenem and colistin. The genetic location of blaNDM-5 and mcr-1 in strain 1106 and 1107 and their corresponding transconjugants were identified by Southern hybridization, using blaNDM-1 and mcr-1 digoxigenin-labeled probes in accordance with the instructions of the
DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics). The genomic DNA of strain 1106 and 1107 were sequenced and their multilocus sequence types (MLST) were assigned using online databases (http://bigsdb.pasteur.fr/) for *E. coli*.

**Plasmid sequencing and bioinformatics analyses**

To determine the complete nucleotide sequences of the plasmids harbored by *E. coli* 1106 and 1107, plasmids were extracted from *E. coli* strain 1106 and 1107 by using the QIAGEN Plasmid Midi Kit (Qiagen, Valencia, CA). The quality of plasmid DNA was measured by Nanodrop and subjected to short-read and long-read sequencing of Illumina Nextseq 500 and ONT MinION sequencers to obtain accurate complete plasmid sequences. Library preparation and data analysis were performed according to published methods with minor modification(3). The sequence accuracy of raw Nanopore read is below 97%, and consensus accuracy of assembly with high sequencing coverage could reach up to 99%, but still did not meet the high-quality genome sequences requirements. We therefore used hybrid assembly strategy combining short-read Illumina data and long-read Nanopore data with Unicycler (v0.4.4) to generate high-quality sequences. In Unicycler, the Pilon tool was used to correct assemblies with accurate short-read Illumina data. This quality control steps could be retrieved from the software manual (4). Alignment of MDR plasmids and nanopore long reads were visualized by the Easyfig and BRIG tools(5, 6). All complete sequences were submitted to NCBI GenBank database with accession numbers as listed in Table 2.
**Supplementary results**

Apart from the plasmids described above in these two *E. coli* strains, strain 1106 also harbored another plasmid and 1107 harbored three more plasmids. One plasmid from strain 1106, designated as p1106-IncFIB, and three plasmids from strain 1107 designated as p1107-99K, p1107-111K and p1107-118K respectively, were all bacteriophage like plasmids. Bacteriophage, one of mobile genetic elements involved in horizontal gene transfer in microorganisms, could be integrated into the chromosome or transmitted to the new host through self-replication(7). Plasmid p1106-IncFIB was 190,401bp in size, with a GC content of 49.8%. It exhibits 99% nucleotide similarity with two ColV plasmids including the plasmid p1ColV5155(CP005931)(8) carried by *E. coli* strain IMT5155 at 90% coverage and pAPEC-O1-ColBM (DQ381420)(9) carried by an avian pathogenic *E. coli* (APEC) strain O1 at 63% coverage (**Fig. S6**). The plasmid comprises a virulence region and a large transfer region; the virulence region contains several virulence genes associated with APEC strains, including the *iroBCDEN* genes encoding the siderophore receptor, the *iucABCD* and *iutA* genes of the aerobactin iron transport system, the *sitABCD* genes of the manganese ABC transport system, and the *hlyF* and *iss* genes of the salmochelin operon. Such structure was similar to those of p1ColV5155 and pAPEC-O1-ColBM(8, 9) (**Fig. S6**). However, p1106-IncFIB lacked the *cvaA* and *cvaB* genes of ColV operon, indicating that p1106-IncFIB might not belong to ColV plasmids. On the other hand, the 32kb transfer region of p1106-IncFIB was also structurally similar to a phage-harboring MDR plasmid, pKP12226 (KP453775), which was carried by a *K. pneumoniae* strain isolated in South Korea(10)(**Fig. S6**).

BLASTN analysis revealed that p1107-99K exhibited the highest similarity with the plasmid
RCS47 harbored by an *E. coli* strain isolated in Paris, and that both plasmids belonged to an unknown incompatibility group. RCS47 was found to harbor a *bla*SHV-2 gene(11), however, no resistance genes were detected in p1107-99K, of which 81% of the sequence was identical to P1 bacteriophage (AF234172)(12) (**Fig. S7**). The sequence of plasmid p1107-111K was similar to that of the *E. coli* plasmid LF82 (CU638872)(13), which was associated with Crohn’s disease and at a high query coverage of 89% and a bacteriophage-like plasmid pECOH89(HG530657)(14) from an *E. coli* strain containing the ISEcp1-*bla*CTX-M-15 transposition unit at 84% coverage (**Fig. S8**). Nevertheless, p1107-118K exhibited high sequence similarity to an IncHI2 plasmid pP2-3T (MG014722)(15) and an IncFII plasmid pSMS35_130 (CP000971)(16). A large region of ~35kb that contained representative virulence genes including *sitABCD*, *iroBCDEN*, *ompT*, *hlyF*, *colicinM* and *iss* genes was observed, in which the corresponding gene clusters are also located in pP2-3T. Worryingly, some resistance genes such as *aph*(3′)-*Ia*, *sul*3 and *aac*(3′)-*IId* were also found in p1107-118K (**Fig. S9**). It was noteworthy that phage-like sequences are more commonly reported in *E. coli* and *Acinetobacter baumannii* strains(10, 17), thus dissemination of resistance genes-bearing phage-like plasmids among various bacterial pathogens is a new concern.
### Supplementary Table S1. MICs of strain 1106, 1107 and their corresponding transconjugants.

| Strains      | TIG | CLS | MRP | CAZ/AVB | FOS | KAN | CHL | NAL | AMK | CIP | CTX | AMP | SXT |
|--------------|-----|-----|-----|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1106         | 0.25| 4   | ≥16 | 32      | ≥512| ≥256| ≥128| ≥128| 2   | 16  | ≥32 | ≥128| ≥64 |
| CTC1106      | 0.12| 4   | 0.12| 0.12    | 4   | 1   | 4   | 4   | 0.5 | 1   | 0.25| ≥128| 8   |
| MTC1106      | 0.25| 0.5 | ≥16 | 64      | 4   | 1   | 4   | 2   | 0.5 | 0.015| ≥32 | ≥128| 32  |
| 1107         | 0.25| 8   | 8   | 32      | ≥512| ≥256| ≥128| ≥128| 2   | 16  | ≥32 | ≥128| 32  |
| MTC1107      | 0.12| 0.5 | 4   | ≥128    | 4   | 1   | 4   | 4   | 0.5 | 1   | ≥32 | ≥128| 32  |

TIG, tigecycline; CLS, colistin; MRP, meropenem; CAZ/AVB, ceftazidime/Avibactam; FOS, fosfomycin; KAN, kanamycin; CHL, chloramphenicol; NAL, nalidixic acid; AMK, amikacin; CIP, ciprofloxacin; CTX, cefotaxime; AMP, ampicillin; SXT, trimethoprim-sulfamethoxazole.
### Supplementary Table S2. Genetic features of 10 plasmids identified in *E. coli* strain 1106 and 1107.

| Plasmid       | Size  | G+C  | Inc type | Antimicrobial resistance genes                                      | IS elements or transposons                  | Accession    |
|---------------|-------|------|----------|---------------------------------------------------------------------|---------------------------------------------|--------------|
| p1106-Incl2MCR | 60,960| 42.3 | IncI2    | *mcr-1*                                                             | NT                                          | MG825374     |
| p1106-IncFII  | 92,438| 51.9 | IncFII   | NT                                                                  | ISEc27, ISCfr3, IS2                         | MG825371     |
| p1106-NDM-5   | 113,687| 54.7 | IncFII   | *dfra12, aadA2, sull, ble_{MBl}, ble_{NDM}, aph(3')-Ia*              | Tn2, IS26, ISCR1, IS6100, Tn2, Tn5393      | MG825375     |
| p1106-IncFIB  | 190,401 | 49.8 | IncFIB   | NT                                                                  | IS5, ISCro3, IS1595, IS2, ISEc27, ISKpn28, IS3, ISEc8, IS30, IS91, IS110, IS1, Tn3, ISEc38, IS21 | MG825372     |
| p1106-IncHI2MCR | 265,799 | 46.6 | IncHI2   | *mph(A), mcr-1, tet(M), bla_{CTX-M-14}, fosA3, aph(4)-Ia, sul2, floR, aadA1, aadA2, cmlA1, sul3, aph(3')-Ia* | ISAp11, IS26, IS6100, IS1R, ISEcpl1, Tn3, ISEc59, ISAbal, ISVsa3, IS1006, Tn5393, IS4321R, ISCro1, IS10L, ISEc23, IS186B, IS150, IS2 | MG825373     |
| p1107-99K     | 99,453 | 47.7 | NT       | NT                                                                  | ISPa38, IS1294                              | MH580301     |
| p1107-111K    | 111,455| 46.3 | IncFIB   | NT                                                                  | IS1294                                     | MG825385     |
| p1107-NDM-5   | 116,042| 54.6 | IncFII   | *dfra12, aadA2, sull, ble_{MBl}, ble_{NDM-5}, aph(3')-Ia, sul3, aac(3')-IIC* | Tn3, ISCR1, IS26, Tn2, IS6100, Tn5393      | MG601057     |
| p1107-118K    | 118,160| 49.2 | IncFIB   | NT                                                                  | ISEc32, IS1203, IS2, ISSso4, IS26, ISVsa5, Tn2, ISVsa5, IS1006, Tn5393, ISAbal, ISVsa3, IS150, ISCro1 | MH580302     |
| p1107-IncHI2MCR | 226,105 | 45.7 | IncHI2   | *mcr-1, fosA3, bla_{CTX-M-14}, aac(3)-IVa, aph(4)-Ia, sul2*          | ISVsa5, ISSso4, ISAp11, Tn3, IS26, ISEcpl1, ISEc59, Tn5393, ISAbal, ISVsa3, IS150, ISCro1 | MG662415     |

*aNT, not detected.*
Supplementary Figure S1. XbaI-PFGE pattern of *E. coli* 1106 and 1107.
Supplementary Figure S2. S1-PFGE and Southern hybridization analysis of *E. coli* strains 1106 and 1107, and their transconjugants. CTC, transconjugants selected by colistin and sodium azide; MTC, transconjugants selected by meropenem and sodium azide; SH, Southern hybridization.
Supplementary Figure S3. Sequence alignment of plasmids p1106-NDM(MG825375), p1107-NDM(MG601057), p92944(MG838206), pHNSD138-1(MG271839), pHUSEC41-1 (HE603110) and p3521(GU256641). The outer circle with red arrows signifies annotation of the reference sequence. Gaps in the circle refer to plasmid regions which are missing compared to the reference.
Supplementary Figure S4. Sequence alignment of plasmids p1106-IncFII (MG825371), pMb1488 (KY565558) and pMb1536 (KY689635). The outer circle with red arrows signifies annotation of the reference sequence. Gaps in the circle refer to plasmid regions which are missing compared to the reference.
Supplementary Figure S5. Sequence alignment of plasmids p1106-MCR (MG825373), p1107-MCR (MG662415), pHNSHP45-2 (KU341381), pECJS-59-244 (KX084394), pHSHLJ1-MCR1 (KX856066) and pXGE1mcr (KY990887). The outer circle with red arrows signifies annotation of the reference sequence. Gaps in the circle refer to plasmid regions which are missing compared to the reference.
Supplementary Figure S6. Sequence alignment of plasmids p1106-Incl2(MG825374), pColR644SK1(MF175188) and pP111(KY120365). The outer circle with red arrows signifies annotation of the reference sequence. Gaps in the circle refer to plasmid regions which are missing compared to the reference.
Supplementary Figure S7. Sequence alignment of plasmids p1106-IncFIB (MG825372), pKP12226 (KP453775), pAPEC-O1-ColBM (DQ381420) and p1ColV5155 (CP005931). The latter one was used as a reference. The outer circle with red arrows denotes annotation of the reference sequence, the gaps represent sequences that were found to be missing when compared to the reference plasmid.
Supplementary Figure S8. Sequence alignment of plasmids p1107-99K(MH580301), P1 phage (AF234172) and RCS47(FO818745). The outer circle with red arrows signifies annotation of the reference sequence. Gaps in the circle refer to plasmid regions which are missing compared to the reference.
Supplementary Figure S9. Sequence alignment of plasmids p1107-111K(MG825385), pECOH89 (HG530657) and LF82(CU638872). The outer circle with red arrows signifies annotation of the reference sequence. Gaps in the circle refer to plasmid regions which are missing compared to the reference.
Supplementary Figure S10. Sequence alignment of p1107-118K (MH580302), pSMS35_130 (CP000971) and pP2-3T(MG014722). Light gray shading denotes shared regions of homology. Gray shading indicates homologies between the corresponding genetic loci in each plasmid. Arrows indicate CDSs, with arrowheads indicating the direction of transcription. purple, virulence genes; red, resistance genes; blue, mobile elements; yellow, replication genes; cyan, genes coding for plasmid transfer; gray, hypothetical proteins or other plasmid scaffold regions.
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