Emergence of Colistin Resistance Gene mcr-10 in Enterobacterales Isolates Recovered from Fecal Samples of Chickens, Slaughterhouse Workers, and a Nearby Resident

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ABSTRACT The widespread spread of plasmid-borne mobilized colistin resistance (mcr) genes from animals to humans broadly challenges the clinical use of polymyxins. Here, we evaluated the incidence of a recently reported mcr variant, mcr-10, in animals and humans in the same area. Our results revealed the presence of novel mcr-10-carrying plasmids in two Klebsiella pneumoniae isolates from chickens, one Escherichia coli isolate from slaughterhouse workers, and a chromosome-borne mcr-10 gene in Enterobacter kobei from a healthy resident in the same region. It is worth mentioning that the multidrug-resistant ST11 K. pneumoniae isolates coharboring mcr-10 and mcr-8 genes in two separate plasmids not only were resistant to polymyxins (MIC = 8 mg/L) but also showed reduced susceptibility to tigecycline (MIC ≥ 2 mg/L) due to the tet(A) mutation or the tmexCD1-toprJ1 gene cluster. The structure xerC-mcr10-insCinsD-like was found in genetic environments of both the plasmid and chromosome carrying mcr-10. We compared genomic epidemiological characteristics of mcr-10-harboring bacteria available in 941,449 genomes in the NCBI database (including strains of K. pneumoniae, E. coli, and E. kobei) with isolates in this study. The results indicated a sporadic distribution of mcr-10 all around the world and in a variety of sources, including humans, environments, and animals, which confirms that mcr-10 has spread among various hosts and warrants close monitoring and further future studies.

IMPORTANCE We discovered mcr-10-harboring isolates in the “one health” approach and reported for the first time multidrug-resistant clinically threatening ST11 K. pneumoniae isolates coharboring mcr-10 and mcr-8 genes that are resistant to polymyxins and show reduced susceptibility to tigecycline. The exhaustive screening of 941,449 bacterial genomes in the GenBank database discovered a sporadic distribution of mcr-10 harboring isolates all around the world in a variety of sources, especially humans, which warrants close monitoring and a particular concern in clinical settings.

KEYWORDS polymyxins, antibiotic resistance, mcr-10, Enterobacterales, one health
pathogens (5, 6). Enterobacterales strains with acquired colistin resistance have emerged worldwide, significantly jeopardizing the efficacy of colistin (7–9). Mobile colistin resistance genes (mcr) have become a major mechanism mediating decreased colistin susceptibility in Enterobacterales and pose a serious challenge to public health, the livestock industry, and the environment.

Liu et al. (10) first reported the emergence of mcr-1 in Enterobacterales isolates of human and animal origin in China, and in the past 5 years, 10 major mcr variants (mcr-1 to -10) have been reported worldwide (11–19). However, higher prevalence rates of mcr-1-positive isolates are observed in Asian countries, especially in food animals and farm environments, which can be caused by long-term exposure to high colistin concentrations (5, 20, 21). In addition, multiple mcr variants, mcr-2 to -10, have been occasionally reported and are not as widely disseminated as mcr-1. Recently, Wang et al. (19) identified mcr-10 on an IncFIA plasmid of an Enterobacter roggenkampii clinical strain, which has the highest nucleotide identity with mcr-9 and confers a 4-fold increase in colistin MIC. Following this report, studies reported mcr-10-harboring Enterobacter spp. isolated from animal and hospital sewage water and Cronobacter sakazakii isolated from a healthy person (22–24). Since it was a newly discovered mcr variant, case reports about mcr-10 were still rare.

This study discovered plasmid-borne mcr-10-harboring Klebsiella pneumoniae from chickens, Escherichia coli from slaughterhouse workers, and Enterobacter kobei from a nearby healthy resident. It is worth mentioning that the K. pneumoniae isolates were multidrug-resistant ST11 isolates that cohabored mcr-10 and mcr-8 on two separate plasmids, had a reduced susceptibility to tigecycline, and had several virulence genes. We extensively screened the mcr-10-carrying isolates in the NCBI database and compared their genomes and plasmids with the isolates in our study. To our knowledge, this is the first detailed report of mcr-10-harboring plasmids from K. pneumoniae and E. coli and various mcr-10-harboring Enterobacterales in the “one health” approach. Our results suggest that mcr-10 has spread among humans and animals, which requires increasing efforts to closely monitor the emergence of more resistant isolates and further studies to investigate the current situation.

RESULTS

Identification of mcr-10 in Enterobacterales isolates and transferability of the mcr-10-harboring plasmids. To study the spread and transmission of mcr-10 between animals and humans, we chose the rural area near Hangzhou, China, where there are a chicken slaughterhouse and villages nearby. Fecal samples of chickens, slaughterhouse workers, and healthy people in villages were collected. A total of 200 Enterobacterales strains were isolated from fecal samples in this study, including 102 from chickens, 58 from slaughterhouse workers, and 40 from nearby residents. PCR was conducted to screen mcr-10 using primers as described in Materials and Methods. Four mcr-10-positive isolates were obtained, including two K. pneumoniae isolates (KP46 and KP57) from chickens, one E. coli isolate (EC81) from a slaughterhouse worker, and one E. kobei isolate (EK6) from a nearby resident. PCR and Sanger sequencing demonstrated that these four mcr-10 genes are all mcr-10.1 (GenBank accession number MN179494.1). KP46 and KP57 had low-level resistance to polymyxins (MIC = 8 mg/L), and EK6 had high-level resistance to polymyxins (MIC = 128 mg/L), while EC81 was susceptible to polymyxins (MIC = 2 mg/L) but close to the breakpoint defined by EUCAST (Table 1).

Antimicrobial susceptibility analysis of these four mcr-10-harboring isolates indicated that K. pneumoniae isolates from chickens were resistant to most antimicrobial agents, including most cephalosporins and one of the clinical last-resort antibiotics, tigecycline (MIC = 8 mg/L and 2 mg/L, respectively), but remained susceptible to the carbapenems fosfomycin and moxalactam (Table 1). However, EK6 and EC81, the isolates from humans, were susceptible to almost all the tested antibiotics (Table 1).

Conjugation experiments showed that isolates KP46 and KP57 could partially transfer their colistin resistance phenotypes to E. coli J53, while EK6 and EC81 could not. The conjugants of KP46 and KP57 did not contain mcr-10, which meant that mcr-10 was not transferable in both isolates. However, the colistin-resistant conjugants were both harboring mcr-8 genes that were transferred from plasmids in both KP46 and KP57. S1-PFGE (pulsed-field gel
electrophoresis) and Southern hybridization showed that KP46 and KP57 carried five and four plasmids, respectively, on which the mcr-10 gene was located at both ~173.4 kb and ~216.9 kb while the mcr-8 gene was located at both ~78.2 kb and ~104.5 kb on plasmids (see Fig. S1 in the supplemental material). EC81 carried two plasmids, and the mcr-10 gene was located between ~54.7 kb and ~78.2 kb (Fig. S1). EK6 did not have the mcr-10-carrying plasmids, which indicates that mcr-10 might be found on the chromosome.

**Genome analysis of the four mcr-10-harboring isolates in this study.** Complete genomes of these four isolates were analyzed. In silico multilocus sequence typing (MLST) analysis revealed that KP46 and KP57 both belonged to ST11, the dominant pan-drug-resistant sequence type widely disseminated in China, which causes an infection that is challenging to cure. EK6 belongs to ST1605, and EC81 belongs to ST216.

KP46 consists of a 5,253,530-bp chromosome and five plasmids (pKP46-mcr10, pKP46-mcr8, pKP46-3, pKP46-4, and pKP46-5) (Table 2). KP57 consists of a 5,253,236-bp chromosome and four plasmids (pKP57-mcr10, pKP57-mcr8, pKP57-3, and pKP57-4) (Table 2). Resistance determinants fosA, qoxA, qoxB, and bla<sub>SHV-182</sub> were detected on the chromosomes of both KP46 and KP57, pKP57-mcr10, pKP57-mcr8, pKP57-3, and pKP57-4 had 99.9% identity (100%
coverage) to pKP46-mcr10, pKP46-mcr8, pKP46-4, and pKP46-5, respectively. Six resistance genes were carried on pKP57-mcr10 including mcr-10, qnrB52, sul1, tet(A), blaTEM-1B, and floR, which confer multidrug resistance. Only one antimicrobial resistance gene, mcr-8, was identified on both pKP57-mcr8 and pKP46-mcr8. Other resistance genes such as blaCTX-M-15 and qnrS1 were found on the other two plasmids (Table 2). Considering there were only 56 core-genome single nucleotide polymorphisms (SNPs) of KP46 compared with KP57, and four plasmids were almost the same, KP46 might have evolved from a KP57-like strain that gained the big plasmid pKP46-3 or KP46 lost pKP46-3 to produce KP57. EC81 consists of a 4,785,944-bp chromosome and two plasmids (pEC81-mcr10 and pEC81-2); no other resistance genes were identified in pEC81-mcr10, except mcr-10 (Table 2). EK6 consists of a 4,860,688-bp chromosome that contains resistance genes mcr-10 and blaATC-9, and no plasmids were identified (Table 2).

The mrk gene cluster (mrkABCDF), encoding type 3 fimbrial adhesins, which mediate adhesion to the surface of endothelial cells and are usually found in K. pneumoniae clinical isolates, was present on the pKP46-mcr10 and pKP57-mcr10 plasmids. The mrk cluster remained truncated in pEC-mcr10 lacking mrkD. Serum complement killing assay and Galleria mellonella infection experiments indicated an increased virulence of EC81 compared with E. coli ATCC 25922 but no significantly increased virulence of KP46, KP57, and EK6 (Fig. S2).

We found two amino acid mutations in EK6 PmrB (Q168P and N233T) compared with the reference strain Enterobacter kobei UCI-24 but did not find mutations in PmrA and PhoPQ. Q168P is in the His kinase A (phosphoacceptor) domain, which is very important for the function of PmrB.

Features of mcr-10-harboring isolates and plasmids. A total of 151 mcr-10-harboring isolates were screened out from 941,449 genomes of the GenBank database (Table S1), including 15 species of 7 genera (Citrobacter, Cronobacter, Enterobacter, Escherichia, Klebsiella, Kluyvera, and Raoultella) of the family Enterobacterales, distributed in 17 countries on five continents. Of the strains with a clear origin, most of the strains were from humans (101/132), and the next most common were from water (24/132); only a few were from animals or animal food (6/132). Of the strains with clear locations, the top six were from the United States, the United Kingdom, Singapore, China, Canada, and Japan (Fig. 1; see also Table S1).
mcr-10-Harboring Isolates in the One Health Approach

Phylogenetic comparison of the mcr-10-positive isolates in this study with their corresponding mcr-10-positive species from the GenBank database was performed with categories of phylogroups, location, multilocus sequencing types, antimicrobial resistance genes, virulence-associated genes, and sources (Fig. 2A to C). The results indicated a sporadic distribution of mcr-10 worldwide and a broad spectrum of sequence types. There was no animal source of mcr-10-harboring K. pneumoniae from the database except for two isolates from this study (Fig. 2A). Most of the mcr-10-harboring E. coli isolates were isolated from the environment (water) located in the United Kingdom, but that might have been because of the sample bias (Fig. 2B). ST125 accounted for 47.4% (9/19) of all mcr-10-positive E. kobei isolates (Fig. 2C).

BLAST searching in GenBank suggested that the three mcr-10-bearing plasmids were novel, and replicon typing showed that they belong to IncFIB-FII (pKP46-mcr10 and pKP57-mcr10) and IncFIA (pEC81-mcr10). No genes encoding conjugation-related proteins were found in pKP46-mcr10, pKP57-mcr10, or pEC81-mcr10, suggesting that the plasmid is non-self-transmissible, which was consistent with the results of the conjugation experiment. pKP57-mcr10 and pEC81-mcr10 shared only 18% coverage, showing that they were from different plasmids with no direct transmission relationship. Comparing the three plasmids with the only 10 mcr-10-harboring completely sequenced plasmids in GenBank, we found a diversity of genes in the plasmids (Fig. 3A) but a relatively undiversified plasmid replicon type which most likely belongs to IncF (Table S2).
This study did not find any complete site-specific recombination sites in mcr-10-harbor- ing plasmids.

In all mcr-10-positive plasmids (Fig. 3 and Table S2), the tyrosine site-specific recombinase gene xerC, which mediates the mobilization of genetic elements, was found upstream of all the plasmids, as well as the insCinsD-like region (Fig. 3B). The ~6-kb xerC-mcr10-insCinsD-like gene arrangement is quite well conserved through the spreading process of mcr-10-mediated colistin resistance among Enterobacter species, whereas ISEc36 transposase InsC was identified downstream in all three isolates in this study but in only 3/10 plasmids from the GenBank database, as insC and insD were not complete genes in some of the plasmids (Fig. 3B). Moreover, we also performed synteny analysis with the mcr-10 gene detected on the chromosome genome of EK6. The result was the same as that of xerC located upstream of mcr-10 (Fig. 3B). The structure of the three mcr-10-positive plasmids is detailed in Fig. 4.
The virulence gene mrkABCDFJ cluster was closely arranged in KP46 and KP57, but for EC81, only mrkA, mrkB, mrkF, and mrkJ were present (Fig. 4).

**Genetic features of mcr-8-carrying and other plasmids in KP46 and KP57.** The transconjugation assay showed that pKP46-mcr8 and pKP57-mcr8 were self-transferable, and the frequency of plasmid transfer was $10^{-6}$ per recipient cell. The plasmid replicon type of pKP57-mcr8 was identified as multireplicon plasmids with IncFIA-FII replicons (Table 2). Sequences of pKP46-mcr8 and pKP57-mcr8 displayed high identity (99.79%) by BLASTN. The genetic environment of the mcr-8-flanking region of pKP57-mcr8 was 90% identical to pHNAH81-1 (accession number MK347425), a representative plasmid-encoded resistance-nodulation-division (RND) efflux pump conferring resistance to multiple drugs in *K. pneumoniae* (Fig. 5). Phylogenetic analysis and gene context comparison revealed that mcr-8 was flanked by complete insertion sequence IS903B (Fig. 5). This increased the risk for the transposition and dissemination of pKP46-mcr8 and pKP57-mcr8.

We also noticed that KP46 showed the highest resistance level to tigecycline, up to 8 mg/L. After screening for the tigecycline resistance genes, we identified the newly reported resistance-nodulation-division (RND) efflux pump gene cluster *tnfxB1-tmexCD1-toprJ1* located on plasmid pKP46-3, an IncFIB-HI1B plasmid containing multiple resistance genes (Table 2). The *tnfxB1-tmexCD1-toprJ1* cluster was flanked by two mobile element proteins. A transposase, TnpA, is located upstream of this cluster, indicating that *tnfxB1-tmexCD1-toprJ1* might transfer from other antibiotic resistance plasmids by genetic recombination.

**DISCUSSION**

Monitoring the distribution of *mcr* genes in the “one health” vision is important for the development of effective control measures. Since the novel *mcr* gene was identified on a nonconjugative IncFIA plasmid of a clinical *Enterobacter roggenkampii* isolate in 2020 in China, *mcr-10* was mainly reported on plasmids or chromosomes of *Enterobacter* spp. isolated from different sources, including clinical fecal samples, hospital sewage water, and animals. Both transferable and nontransferable *mcr-10*-carrying plasmids were reported previously (19, 23, 25). There is also a case of an *mcr-10*-carrying plasmid reported in *Cronobacter sakazakii* (24). However, *mcr-10*-harboring plasmids in *K. pneumoniae* and *E. coli* isolates were not reported in previous studies (26). This study discovered four *mcr-10*-harboring isolates distributed among three different bacterial species among the 200 *Enterobacteriales* isolates recovered from chickens and humans in the same region (4/200, 2%). Kim et al. (27) reported a relatively high rate of *mcr-10* prevalence (17/3,675, 0.46%, *K. pneumoniae* [n = 1] and *Enterobacter cloacae* complex [n = 16]) in clinical carbapenem-resistant *Enterobacteriales* (CRE) isolates in Seoul, Republic of Korea, which was the second-highest rate in all *mcr* variants, just behind *mcr-9*. The high isolation rate of *mcr-10* indicated a high transmission capacity of *mcr-10*, either by self-transmission or by site-specific recombination. A study has reported that the number of *mcr-10*-carrying fecal samples was more elevated in Asia than in Europe or the United States (28). However, after the exhaustive screening of *mcr-10* in the
GenBank database, we reported significantly more mcr-10-positive strains in the United States, isolated from humans, water, and food. Though this might be due to sample bias, we demonstrated a worldwide distribution of mcr-10 within different hosts. All these findings revealed a silent distribution of mcr-10 in different sources. Thus, there is an urgent need for further surveillance to understand the prevalence and dissemination of mcr-10-positive isolates.

No genes encoding conjugation-related proteins were found in the Cronobacter sakazakii mcr-10-carrying plasmid pMCR10_145005. At the same time, two recombination sites were identified flanking the genetic element containing mcr-10 and an integrase-encoding gene, suggesting that site-specific recombination mediated by an integrase of an integrative mobile element is a potential mechanism for mobilizing mcr-10 (24). The three mcr-10-carrying plasmids in this study did not contain conjugation-related genes, and the plasmids were non-self-transmissible. We did not find any complete site-specific recombination sites but transposases in the mcr-10-harboring plasmids of this study. It is worth noting that xerC-mcr-10 is quite well conserved, no matter whether it is located on a plasmid or in the genome with diverse insertion sequences (ISs) found upstream and downstream of this site, which implies that the area surrounding xerC-mcr-10 is the high-frequency region for insertion of mobile genetic elements. Considering the non-self-transmissibility of most reported mcr-10 plasmids (19, 24), these reports indicated diversified paths for mcr-10 transfer. In the three novel mcr-10-carrying plasmids we discovered, pEC81-mcr10 showed few similarities with pKP46-mcr10 and pKP57-mcr10, so we speculate that the spreading process of mcr-10 from chicken to human would go through several rounds of insertion or integration events among different types of plasmids.

The MIC assay in this study revealed that the colistin resistance mediated by the mcr-10-carrying plasmid caused a moderate increase in colistin MIC, which was consistent with previous reports (19, 24, 25), while EK6, the chromosomal mcr-10-harboring isolate, displayed high resistance to colistin with a MIC of 128 mg/mL, because of mutations in the two-component systems. mcr-10 in KP46 and KP57 functions synergistically with mcr-8, resulting in relatively higher resistance to colistin than that of the single mcr-10 carrier EC81.

We would like to emphasize that mcr-8 was also found in the mcr-10-harboring MDR ST11 K. pneumoniae isolates. This is the first report of MDR ST11 K. pneumoniae harboring both non-self-transferable mcr-10 and self-transferable mcr-8. MDR ST11 K. pneumoniae is the dominant clinical prevalent clone of KPC-producing K. pneumoniae in China and Asia and brings great difficulty to clinical treatment (26, 29). Both KP46 and KP57 are isolates with not only multiple resistance genes but also multiple virulence genes. An efficient enterobactin system and other known virulence factors indicate a potential threat to public health. Such strains may spread and evolve into MDR hypervirulent strains that cause severe and untreatable invasive infections in the clinic. Therefore, close surveillance is urgently needed to monitor the prevalence of mcr genes, especially in ST11 carbapenem-resistant hypermucoviscous K. pneumoniae clones in the clinical setting.

In conclusion, to our knowledge, this is the first detailed report of mcr-10-harboring plasmids from K. pneumoniae and E. coli and various mcr-10-harboring Enterobacterales in a “one health” approach. Extensive screening of mcr-10-carrying isolates in the NCBI database in this study indicated a sporadic distribution of mcr-10 all around the world and from a variety of sources, including humans, environments, and animals, which confirms that mcr-10 has spread among various hosts and warrants close monitoring and further studies.

MATERIALS AND METHODS

**Bacterial isolation, detection of mcr-10, and antimicrobial susceptibility testing.** A surveillance study of mcr-harboring Enterobacterales recovered from feces of chickens, slaughterhouse workers, and healthy people nearby was conducted in 2019. This study was approved by the Ethical Committee of the First Affiliated Hospital of Zhejiang University with waiver of informed consent. Fecal samples (10 g) were diluted in 5 mL of sterile Luria-Bertani broth and cultured overnight at 37°C. The cultures grown overnight were plated on MacConkey agar for 18 to 24 h at 37°C. Colonies with different morphologies were repeatedly streaked on MacConkey agar to obtain pure isolates. Bacterial species were identified by using the Vitek MS and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF). All isolated
**Enterobacteriales** were subjected to PCR and Sanger sequencing on the *mcr-10* gene, using primers *mcr-10*-F, GGACCGTATTACACGCG, and *mcr-10*-R, GGCTATTGTCGACAGCG (25).

Agar or broth microdilution methods evaluated antimicrobial susceptibility according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The MICs of polymyxins (colistin and polymyxin B) and tigecycline were determined by the broth dilution method, and MICs of the other antibiotics were determined by the agar dilution method. Polymyxin and tigecycline resistances were defined according to clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (version 10.0) ([https://ecucast.org/clinical_breakpoints/](https://ecucast.org/clinical_breakpoints/)), and the others were interpreted according to CLSI guidelines. *Escherichia coli* ATCC 25922 was used as a quality control standard for antimicrobial susceptibility testing.

**WGS and bioinformatic analysis.** Genomic DNA of *mcr-10*-positive isolates was extracted using the Gentra Puregene Yeast/Bact kit (Qiagen, CA, USA), subjected to whole-genome sequencing (WGS) on a Nanopore PromethION platform (Nanopore, Oxford, UK) following a 10-kbp library protocol, and checked with the Illumina HiSeq X Ten 6000 systems (Illumina, San Diego, CA, USA), using paired-end libraries. The hybrid assembly of short Illumina reads and long PromethION reads was performed using Unicycler v0.4.8. PCR, and sequencing confirmed plasmid circularity. The annotation of the WGS data was performed by Prokka v1.17.

Multilocus sequence typing (MLST) was performed and antimicrobial resistance genes were identified using both Bacillus WGSTdb 2.0 (30) and the Center for Genomic Epidemiology (CGE) platform. Plasmid incompatibility type was determined by PlasmidFinder 2.0. Circular comparisons between multiple genomes and plasmids were prepared using BLAST Ring Image Generator (BRIG). Linear comparisons of multiple plasmids were generated using EasyFig.2. The phylogenetic tree was generated using Prokka and Roary. The presence of strains harboring *mcr-10* was screened by performing a BLAST search on *mcr-10.1* (GenBank accession number MN179494.1) in the NCBI nr database. We screened for the presence of *mcr-10* in sequences, including complete, draft, or raw-read genome sequences deposited in GenBank, by BLAST search ([https://blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi), accessed 26 September 2021). Matches with >90% identity and >90% coverage were retrieved from GenBank.

**Conjugation and electroporation experiments.** Conjugation assays were mainly performed according to a method described previously (31). *mcr-10*-harboring isolates were used as the donor, while *E. coli* J53 (sodium azide resistant) served as the recipient strain. Transconjugants were selected on Mueller-Hinton agar supplemented with sodium azide (100 mg/L) and colistin (1 mg/L). PCR and DNA sequencing were used to detect the presence of the *mcr-10* gene in transconjugants.

**S1-PFGE and Southern hybridization.** To estimate the sizes of *mcr*-positive plasmids, S1-PFGE and Southern hybridization were performed. Briefly, bacterial whole-cell DNA of *mcr*-positive isolates and their transconjugants was prepared in agarose plugs and digested with S1 nuclease (TaKaRa, Dalian, China). The DNA was separated using the CHEF-Mapper PFGE system (Bio-Rad) under the following conditions: 14°C, 6 V/cm, and a 120° pulse angle for 16 h, with the initial and final pulses conducted for 2.16 and 63.8 s, respectively. The separated DNA fragments were transferred to nylon membranes, hybridized with digoxigenin-labeled *mcr*-10 or *mcr*-8-specific probes, and detected using the nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl-phosphate (NBT-BCIP) color detection kit (Roche, catalog no. 11745832910).

**Measurement of virulence.** The virulence of these isolates was estimated by the anticomplement killing test and *Galleria mellonella* infection experiment. For the anticomplement killing test, serum was collected from healthy volunteers, centrifuged to obtain normal serum, and placed in a water bath at 56°C for 30 min to inactivate complement, generating inactive serum. One hundred eighty microliters of serum was separately mixed with 20 μL diluted bacterial suspension (2 × 10^8 CFU/mL) and incubated at 37°C for 1 h. Samples were diluted 100-fold, spread onto plates, and incubated overnight, and colonies were counted. The bacterial survival rate was calculated using the following formula: bacterial survival rate = (number of colonies with normal serum/number of colonies with inactivated serum) × 100%. For the *G. mellonella* infection experiment, the overnight bacterial culture was diluted to a cell density of 1 × 10^8 CFU/mL, and *G. mellonella* individuals weighing ~250 mg were randomly divided into groups with 25 individuals in each group. Each individual was injected with 20 μL of bacterial suspension, incubated at 37°C, and assessed once every 8 h for 7 days. The Kaplan-Meier estimator method was used to plot a survival curve for *G. mellonella*. Phosphate-buffered saline (PBS) served as the negative control. All experiments were done in triplicate.

**Data availability.** WGS data for KP46, KP57, EC81, and EK6 have been deposited in GenBank under accession no. CP088120 to CP088125, CP088126 to CP088130, CP088131 to CP088133, and CP088119, respectively.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.**

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We declare that we have no known competing financial interests.
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