Identification of Tn6835 and a Novel Genomic Island, MMGI-1, in a Pan-Resistant *Morganella morganii* Strain

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Morganella morganii is a member of the *Morganellaceae* that shares closest features with the *Proteus* and *Providencia* genera (1) and is widespread in the environment and the intestinal tracts of animals and humans. It is also recognized as an opportunistic pathogen causing clinical infections (2). Acquisition of the *bla*_{NDM-1} gene in *M. morganii* strains that are intrinsically resistant to multiple antibiotics will make clinical treatment extremely difficult (2). Here, we report the isolation and identification of a highly drug-resistant *M. morganii* strain harboring a novel Tn7-like transposon and a peculiar genomic island, *M. morganii* genomic island 1 (MMGI-1).

M. morganii GDMM86 was recovered from a fecal swab of a healthy chicken in the routine surveillance of animal-origin bacterial antimicrobial resistance in Guangdong Province, China. Antimicrobial susceptibility testing was performed and interpreted using CLSI reference broth microdilution (3). Strain GDMM86 was resistant to cefotaxime (MIC, >256 µg/ml), ceftazidime (>256 µg/ml), cefoxitin (>256 µg/ml), cefepime (>256 µg/ml), piperacillin (>256 µg/ml), imipenem (>32 µg/ml), meropenem (>64 µg/ml), ertapenem (>64 µg/ml), ciprofloxacin (>32 µg/ml), levofloxacin (>64 µg/ml), gentamicin (>128 µg/ml), amikacin (>64 µg/ml), streptomycin (>128 µg/ml), trimethoprim-sulfamethoxazole (>128 µg/ml), chloramphenicol (>128 µg/ml), and aztreonam (>128 µg/ml). Whole-genome sequencing data were generated using short-read technology (Illumina, San Diego, CA) and MinION (Oxford Nanopore Technologies, Oxford, UK). Short and long reads were subjected to *de novo* hybrid assembly. A single contig, assembled with the Canu (version 1.8) protocol and corrected using Pilon (version 1.22), contained a full chromosome of 4,111,737 bp, with 3,890 putative coding sequences (Fig. 1A) (GenBank accession no. CP061513). The resistome was investigated using ResFinder 3.1 (<https://cge.cbs.dtu.dk/services/ResFinder/>). The chromosome-borne *bla*_{DHA-10} and *catA2* genes were detected in the isolate, resulting in its intrinsic resistance to ampicillin and phenicols.

Sequence analysis showed that 11 resistance genes were carried by a novel multidrug-resistant (MDR) transposon that was designated Tn6835 (Fig. 1B), according to the nomenclature of transposons (4; <http://transposon.lstmed.ac.uk/>). Tn6835 contains a hybrid class 2/class 1 integron with a large IS26 flanked multiresistance region inserted. It is located at the usual Tn7 insertion site near *glmS* (encodes glucosamine 6-phosphate synthase), with a 5-bp direct repeat (AGATA) on both sides. Tn6835 is 41,915 bp (corresponding to bases 35338 to 77252 of GenBank accession no. CP061513) and harbors 11 resistance genes, including *aadA1*, *aac(3)-IV*, *aadA2*, *aph(4)-Ia*, *aphA1*, *floR*, *lnu(F)*, *dfrA1*, *dfrA12*, *sul1*, and *sul2*. Tn7 contains a transposition module formed by the *tnsA*, *tnsB*, *tnsC*, *tnsD*, and *tnsE* genes, in which *tnsD* and *tnsE* encode transposition target site selection proteins (5, 6). In this study, *tnsD* in Tn6835 was truncated by insertion of a fragment of Tn1721, and *tnsE* is missing. The transposition module (*tnsA*, *tnsB*, *tnsC*, and Δ *tnsD*) and the class 2 integrase gene of Tn6835 shows 99% nucleotide sequence identity to the Tn7 of *M. morganii* strain FDAARGOS_172 (CP014026). The truncation of *tnsD* and the loss of *tnsE* may enhance the

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FIG 1 Genomic analysis of *M. morganii* strain GDMM86. (A) The genome of *M. morganii* strain GDMM86 was used as the reference to compare with *M. morganii* strains GN28 (GenBank accession no. CP026651), AR_0133 (CP028956), and FDAARGOS_63 (CP026046). (B) Genetic structure of Tn6835. Linear comparison of transposon Tn6835 with the homologous regions of the genome of *M. morganii* strain FDAARGOS_172 (CP014026), *Klebsiella pneumoniae* plasmid pK1HV (HF545434), and *Salmonella* plasmid pST45-1 (CP050754) and the genome of *Proteus mirabilis* strain SNYG17 (MF805806). IRI, inverted repeat at the integrase-encoding end of the class 1 integron. (C) Genetic structure of MMGI-1 compared with that of PAGI-16 of *P. aeruginosa* and the chromosome of *Escherichia coli* strain Y5. L, left; R, right; T4SS, type 4 secretion system.

stability of Tn6835 in the chromosome of *M. morganii*. On the right side of the inverted long repeat (IRL), the *mcp* gene is interrupted by Tn4352 containing kanamycin and neomycin resistance determinant *aphA1*. Tn6835 harbors a class 1 integron, including the *dfrA12* *gcuF* *aadA2* cassette array, and the right-hand inverted repeat at the *tni* transposition module outer end of the class 1 integron (IRt) was lost. BLAST analysis showed that the 8,738-bp region (from bp 42391 to 51128 in CP061513) containing Tn4352 and a class 1 integron shows almost 100% identity to the corresponding regions of the five DNA sequences, including plasmid pK1HV (HF545434), *Providencia alcalifaciens* strain 1701003's chromosome (CP059346), *Proteus mirabilis* strain JPM24's chromosome (CP053894), *Providencia* sp. strain 1701091's chromosome (CP042860), and *Providencia* sp. strain 1701011's chromosome (CP042859), with only 0 to 4 single-base changes. An 18,147-bp region (from bp 51149 to 69295 in CP061513) containing the resistance genes *floR*, *sul2*, *aph* (4)-*la*, and *aac*(3)-*IV*, which had a 2-bp nucleotide substitution and a 3-bp deletion compared with plasmid pST45-1 (CP050754), was recovered from a clinical *Salmonella* strain in China. A hybrid integron consisting of *intI2* and the 3' coding sequence (3'-CS) of a class 1 integron was found in Tn6835, carrying a single gene cassette containing three open reading frames (ORFs), *lnu*(F), *dfrA1*, and *aadA1* (7). In this gene cassette, *lnu*(F) and *dfrA1* have lost *attC* sites, which appears to have been replaced by part of *attI2*, causing them to be "fused" to the next cassette (8) (Fig. 1B). An identical hybrid integron was identified in the Tn6450 region in *P. mirabilis* strain SNYG17 (MF805806), which may have been created by recombination in gene cassettes shared by a class 2 integron and a class 1 integron (9, 10).

Interestingly, in addition to Tn6835, a *Pseudomonas aeruginosa* genetic island 16 (PAGI-16)-like *M. morganii* genomic island (MMGI-1) was identified in the *M. morganii* GDMM86 genome by using IslandViewer 4 (11) and ICEfinder 2.0 (12). MMGI-1 is 167,062 bp (corresponding to bases 2776872 to 2943933 in GenBank accession no. CP061513) in length and harbors 7 types of resistance genes, including *sul1*, *arr-3*,

*bla*_{NDM-1}, *catB3*, *mph(A)*, *aphA6*, and *erm(42)*. Sequence analysis revealed that MMGI-1 was integrated into the chromosome at the 3' end of tRNA^{Lys}, flanked by the recombinant sites *attL* and *attR*. The integrase gene in MMGI-1 was identical to *int* in PAGI-15 (*int*_{PAGI-15}) and *int*_{PAGI-16}. BLAST analysis showed that the backbone of MMGI-1, which consists of 203 ORFs, is almost identical in nucleotides (99%) to PAGI-16 in *P. aeruginosa* (13), indicating that MMGI-1 and PAGI-16 might have a common origin (Fig. 1C). The 105-kb MDR region of MMGI-1 (corresponding to bases 64494 to 116774 in GenBank accession no. [MG773277](#)), bracketed by *IS6100* and *IS1017*, harbors 52 antimicrobial resistance genes, including *erm(42)*, *aphA6*, and 12 copies of *bla*_{NDM-1}. The region bracketed by *ISpa38* and Δ TnAs3 in MMGI-1 exhibited 99% nucleotide identity to the corresponding region of plasmid pZD02 ([CP013143](#)). Additionally, MinION genome sequencing verified the presence of 12 tandem repeats of an *ISCR1*-*bla*_{NDM-1} element (6,400 bp) in MMGI-1, each consisting of the cluster *sul1 qacEΔ1 arr-3 ΔcatB3 ΔISAbA125 bla*_{NDM-1} *ble*_{MBL} *ΔtnpF ISCR1*. All 12 copies of *ISCR1 bla*_{NDM-1} elements were completely intact, and no additional DNA sequences were identified at the join sites (Fig. 1C). The *ISCR1* element is an unusual insertion sequence that demonstrates IS91-like characteristics and may mobilize adjacent DNA sequences via a process called rolling-circle transposition (14). *ISCR1* is usually adjacent to various antibiotic resistance genes and shows the potential to mobilize adjacent antibiotic resistance genes (15). The coexistence of 12 *bla*_{NDM-1} genes has not been reported before, but two identical copies of *ISCR1 bla*_{NDM-1} elements on the chromosome of *Escherichia coli* strain Y5 ([CP013483](#)) have previously been described (16). Inside the *ISCR1 bla*_{NDM-1} element, *bla*_{NDM-1} together with the bleomycin resistance gene *ble*_{MBL}, *tat*, *ΔISAbA125* (151-bp remnant), and *ΔtnpF* (440-bp remnant) form part of the Tn125 transposon (Δ Tn125). After sequence alignment with *Citrobacter freundii* plasmid pCfr-36808cz ([MG557997](#)), we found that the subfragment *sul1 qacEΔ1 arr-3 ΔcatB3* is a truncated class 1 integron that shares 100% nucleotide sequence identity with the corresponding region of plasmid pCfr-36808cz. As previously reported, an *ISCR1*-mediated circular intermediate can be formed (17), and circular intermediates containing resistance genes can integrate either into the chromosomal DNA or into plasmids by recombination events (18, 19). Based on the observed structural features and the sequence analyses, we predicted that an *ISCR1 bla*_{NDM-1} circular intermediate was first integrated into the chromosome of GDMM86 through homologous recombination. Then, repetition of the incorporation process with the same circular intermediate led to the formation of 12 *ISCR1 bla*_{NDM-1} tandem repeats.

Previous studies have reported that several genomic islands in *P. aeruginosa* can be precisely excised from the integration site on the chromosome and form an extrachromosomal circular intermediate, such as the element pKLC102 (20) and *P. aeruginosa* pathogenicity island 1 (PAPI-1) (21). The nucleotide sequences of the backbone of MMGI-1 have 99% nucleotide identical to PAGI-16 in *P. aeruginosa*, indicating that the entire MMGI-1 element in this study may be transferred horizontally by conjugation of circular intermediates excised from the *P. aeruginosa* chromosome. Because the spontaneous excision rate of MMGI-1 may occur at a very low frequency, we used two steps of nested PCR to detect the circular intermediate of MMGI-1 and the chromosomal locus after MMGI-1 excision (see Table S1 in the supplemental material). The PCR product of the circular intermediate of MMGI-1 and the chromosomal locus after the MMGI-1 excision was detected in the second step of the nested PCR; no PCR product was detected in the first step of the nested PCR (Fig. S1). This observation suggests that MMGI-1 can be excised from the chromosome and form an extrachromosomal circular intermediate with low frequency. Sanger sequencing and sequence analysis showed that the circular intermediate of MMGI-1 consisted of partial sequences at the 5' and 3' ends of MMGI-1 and the downstream 20-bp direct repeat sequence (*attR*). The chromosomal locus after the MMGI excision contains the upstream 20-bp direct repeat sequence (*attL*). Mobilization assays showed that MMGI-1 in GDMM86 could not be conjugally mobilized to *E. coli* J53 despite three independent attempts.

In conclusion, we characterized two novel mobile genetic elements, Tn6835 and MMGI-1, in a highly drug-resistant *M. morganii* isolate in China. Twelve copies of *bla*_{NDM-1} genes were tandemly located on MMGI-1, with the ISCR1 *bla*_{NDM-1} structure (*sul1 qacEΔ1 arr-3 ΔcatB3 ΔISAb125 bla*_{NDM-1} *ble*_{MBL} *ΔtnpF ISCR1*), which was presumably created as a result of insertion of multiple ISCR1 *bla*_{NDM-1} circles through homologous recombination. Further studies are required to assess the prevalence of MMGI-1 among *Enterobacteriales* of medical interest, especially in *M. morganii* and *Proteus* spp., and to determine whether MMGI-1 is a mobilizable element.

Data availability. The complete nucleotide sequences of *M. morganii* strain GDM86 characterized in this study were submitted to the GenBank database and assigned GenBank accession no. [CP061513](#) and BioProject accession no. [PRJNA663257](#).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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We have no conflicts of interest to declare.

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