



Complete Nucleotide Sequence of pGA45, a 140,698-bp IncFII γ Plasmid Encoding *bla*_{IMI-3}-Mediated Carbapenem Resistance, from River Sediment

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Plasmid pGA45 was isolated from the sediments of Haihe River using *Escherichia coli* CV601 (*gfp*-tagged) as recipients and indigenous bacteria from sediment as donors. This plasmid confers reduced susceptibility to imipenem which belongs to carbapenem group. Plasmid pGA45 was fully sequenced on an Illumina HiSeq 2000 sequencing system. The complete sequence of plasmid pGA45 was 140,698 bp in length with an average G + C content of 52.03%. Sequence analysis shows that pGA45 belongs to IncFII γ group and harbors a backbone region which shares high homology and gene synteny to several other IncF plasmids including pNDM1_EC14653, pYDC644, pNDM-Ec1GN574, pRJF866, pKOX_NDM1, and pP10164-NDM. In addition to the backbone region, plasmid pGA45 harbors two notable features including one *bla*_{IMI-3}-containing region and one type VI secretion system region. The *bla*_{IMI-3}-containing region is responsible for bacteria carbapenem resistance and the type VI secretion system region is probably involved in bacteria virulence, respectively. Plasmid pGA45 represents the first complete nucleotide sequence of the *bla*_{IMI}-harboring plasmid from environment sample and the sequencing of this plasmid provided insight into the architecture used for the dissemination of *bla*_{IMI} carbapenemase genes.

Keywords: carbapenem resistance, plasmid, pGA45, T6SS, antibiotic resistance

INTRODUCTION

The overuse and misuse of antibiotics have contributed to the emergence and spread of antibiotic resistance genes (ARGs) and multidrug resistance pathogens (Zhang and Zhang, 2011; He et al., 2014). Now ARGs have been recognized as a new type of pollutants (Pruden et al., 2006). Among various ARGs, carbapenem resistance genes, especially plasmid mediated carbapenem resistance genes, have raised worldwide concern, leading to the extensive research on some of these genes and related plasmid architecture (McGann et al., 2012; Tiwari et al., 2012; Villa et al., 2012, 2013; Lo et al., 2013; Tiwari and Moganty, 2014). Acquired carbapenem resistance can be resulted from carbapenemases of Amber class A (IMI, GES and KPC), Amber class B (metallo β -lactamases including IMP, VIM and NDM) or Amber class D (OXA-48 and OXA-181) (Nordmann et al.,

2012). The bla_{KPC} gene of Amber class A and the metallo-β-lactamase genes have been the research focus but there is rare reports about the other carbapenem resistance genes especially little is known about the bla_{IMI} genes and the plasmid architecture involved in the dissemination of this type of genes (Aubron et al., 2005; Yu et al., 2006; Rojo-Bezares et al., 2012; Teo et al., 2013; Chen et al., 2015).

The bla_{IMI-1} gene was first identified in 1996 and found to be located in the chromosome of *Enterobacter cloacae* isolates whereas bla_{IMI-2} was first identified in 2005 in *Enterobacter asburiae* isolates and found to be related to plasmids (Rasmussen et al., 1996; Aubron et al., 2005). In 2009, a new variant of bla_{IMI-1}, bla_{IMI-3}, was identified in Hong Kong in *Enterobacter cloacae* isolates (Chu et al., 2011). The bla_{IMI-3} was also located in the conjugative plasmids. The bla_{IMI}-mediated carbapenem resistance is an infrequent mechanism but it has been reported both in clinical strains and environmental bacteria from rivers. Horizontal transfer may occur between environmental bacteria and clinical strains. With the horizontal transfer, the bla_{IMI} genes would broaden their hosts and inevitably pose serious risks to the public health. For further research on the dissemination of bla_{IMI} genes, the full sequence of bla_{IMI}-related plasmid is needed. Here we report the first complete nucleotide sequence of bla_{IMI}-carrying conjugative plasmid from the environment sample.

MATERIALS AND METHODS

Studying Sites and Sample Collection

Sediment sample was collected under JinGang Bridge of Haihe River. JinGang Bridge was located in densely populated urban areas with frequent human activities. Sediment sample was collected with a grab sampler and then put into sterile containers. The sample was immediately taken to the laboratory and stored in -20°C for subsequent experiments after sampling was completed.

Isolation of the Conjugative Plasmids Conferring Resistance to Imipenem

In order to obtain conjugative plasmids which confer resistance to imipenem, ampicillin resistant plasmids were first isolated by filter mating assays and these ampicillin resistant plasmids were then subjected to antibiotic susceptibility testing against imipenem and other antibiotics. The filter mating assays were applied using *Escherichia coli* CV601 (*gfp*-tagged, kanamycin and rifampicin resistant) as recipients and sediment samples as donors (Heuer et al., 2002). Transconjugants were selected by Mueller-Hinton agar plates supplemented with ampicillin (100 mg L⁻¹), kanamycin (50 mg L⁻¹), rifampicin (50 mg L⁻¹) and cycloheximide (100 mg L⁻¹). *E. coli* CV601 recipient culture was plated on the same selective plates as controls. The procedure used for filter mating assays was described by Heuer et al. (2002) with slight modification. Briefly, the sediment samples from which the indigenous bacteria were used as donors were doubled to 2 g and the Luria-Bertani (LB) broth used for resuspending the sediment samples was accordingly doubled to 18 ml. After

incubation for 2 days, transconjugants were determined by green fluorescence which is resulted from green fluorescence protein (GFP) gene. All the ampicillin resistant transconjugants were then streaked on the ampicillin selective plates. Overnight culture of these transconjugants were stored in -80°C for further study.

Antibiotic Susceptibility Testing of the Ampicillin Resistant Transconjugants

Kirby-Bauer disk diffusion method was applied to determine which ampicillin resistant transconjugants confer resistance to imipenem. According to the criteria of the Clinical and Laboratory Standards Institute (CLSI), the disks used in this study are as follows: imipenem (10 μg), ampicillin (10 μg), gentamicin (10 μg), streptomycin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), sulfamethoxazole (300 μg) and erythromycin (15 μg). *E. coli* ATCC25922 was used as quality control strain. In this study, one transconjugant designated GA45 was found to confer resistance to imipenem and ampicillin. The conjugative plasmid harbored by GA45 was named pGA45 and stored for further analysis.

Conjugative Transfer Experiments and the Role Determination of pGA45 in Recipient Strains

To assess the conjugative frequency of plasmid pGA45, liquid mating assays were employed using *E. coli* CV601 (pGA45) as donor strains and *E. coli* J53 (azide and nalidixic acid resistance) as recipient strains. For liquid mating assay, overnight cultures of donor and recipient strains were centrifuged, washed and adjusted to the optical density of 0.6 at the wavelength of 600 nm (OD₆₀₀) with LB broth. Then 0.5 ml cultures of each donor and recipient strains were mixed and make up to the volume of 5 ml with LB broth. After incubation of 16 h in 37°C, transconjugants were selected on LB plates containing azide (200 mg L⁻¹), nalidixic acid (20 mg L⁻¹) and ampicillin (100 mg L⁻¹). Conjugative frequency was determined by the following formula: conjugative frequency = transconjugants (CFU/ml)/recipients (CFU/ml). The *E. coli* J53 transconjugants were then tested against imipenem to confirm the role of pGA45 in recipient strains. The results showed that pGA45 also conferred resistance to imipenem in recipient strains.

Plasmid Sequencing and Bioinformatics

Plasmid DNA from the *E. coli* J53 transconjugants was extracted using a Qiagen plasmid midikit (Qiagen, Inc). The plasmid DNA was sequenced on an Illumina HiSeq 2000 sequencing system. Sequencing reads were *de novo* assembled into contigs using the SOAPdenovo 2.04 software (Li et al., 2008, 2010). Gaps between contigs were closed by PCR with standard Sanger sequencing. Glimmer 3.02 was used to predict putative open reading frames (ORFs) (Salzberg et al., 1998; Delcher et al., 1999, 2007). All ORFs were translated and aligned with different protein databases including NR (version: 20121005), KEGG (version: 59), COG (version: 20090331), SwissProt (version: 201206) and GO (version: 1.419).

Nucleotide Sequence Accession Number

The complete nucleotide sequence of pGA45 was deposited in GenBank under accession no. KT780723.

RESULTS

Sequencing of plasmid pGA45 generated 237,937,000 reads in total. Reads either of low quality or representing the host chromosome contamination through comparison with the sequence of reference strain *E. coli* MG1655 were filtered. In the end, 34 contigs were obtained and then assembled into 10 scaffolds. Through PCR and Sanger sequencing, gaps between contigs and scaffolds were closed. The complete sequence of plasmid pGA45 was 140,698 bp in length with an average G + C content of 52.03% (Figure 1). Analysis of the sequence identified 157 ORFs, 64 of which were transcribed in the opposite direction. The backbone of this plasmid included the replication region, stability region 1, and transfer region (51524 bp), making up 36.6% of the total sequence. This backbone region shared high homology and gene synteny to several other IncF plasmids including pNDM1_EC14653 (Wu et al., 2015), pYDC644 (GenBank accession no.: KR351290), pNDM-Ec1GN574 (GenBank accession no.: KJ812998), pRJF866 (Qu et al., 2015), pKOX_NDM1 (Huang et al., 2013) (86% query coverage and 97% nucleotide identity, NCBI database) and pP10164-NDM (Sun et al., 2015) (86% query coverage and 94% nucleotide identity, NCBI database). Notably, all these plasmids were recently sequenced and four of them were found in China. Thus, to our best of knowledge, pGA45 and other recently sequenced plasmids represent a new IncF subtype and this type of plasmids exhibit a high prevalence in China. By contrast, the rest parts of pGA45 [including the variable region, the stability region 2 and the type VI secretion system (T6SS) region] showed no significant similarities with other sequenced plasmids in GenBank.

The replication region (1,431 bp) of pGA45 (positions 75496–76926), including the replication initiation protein gene *repA* and replication regulatory protein gene *repA2*, shared 93% nucleotide similarity with the six IncF plasmids mentioned above with 100% query coverage. Plasmid pGA45 was further assigned to the IncFIIy incompatibility group through sequence queries against the plasmid MLST databases¹.

Plasmid pGA45 contained one transfer region (position 42643–73867 bp) that comprised 21 *tra* genes, 3 *trb* genes (ordered as follows: *traM*, *traY*, *traA*, *traL*, *traE*, *traK*, *traB*, *traV*, *traP*, *traC*, *trbI*, *traW*, *traU*, *trbC*, *traN*, *traF*, *traQ*, *trbB*, *traH*, *traG*, *traT*, *traD*, *traI*, *traX*) and *finO*. Mating out experiments demonstrated that this region made pGA45 self-transmissible at a relatively high frequency of $(7.81 \pm 7.15) \times 10^{-3}$ transconjugants per recipient between *E. coli* CV601 and *E. coli* J53.

The genes on plasmid pGA45 that are responsible for plasmid stability and maintenance included *umuC-umuD* genes

which confer resistance to UV light, *relE-relB* genes encoding a toxin-antitoxin system, *ardA* gene with antirestriction function, *parA-parB* genes for partition, *psiA-psiB* genes involved in the bacterial SOS inhibition and *ssb* gene involved in recombination and repair.

The variable region of plasmid pGA45 contained two resistance genes including one ARG *bla*_{IMI-3} and one copper resistance gene *copC* (with 54% coverage and 94% amino acid identity to *Klebsiella pneumoniae* subsp. *pneumoniae* DSM 30104, NCBI database). Plasmid pGA45 also harbored a T6SS region (position 82987–114739bp) which may be related to bacterial pathogenesis.

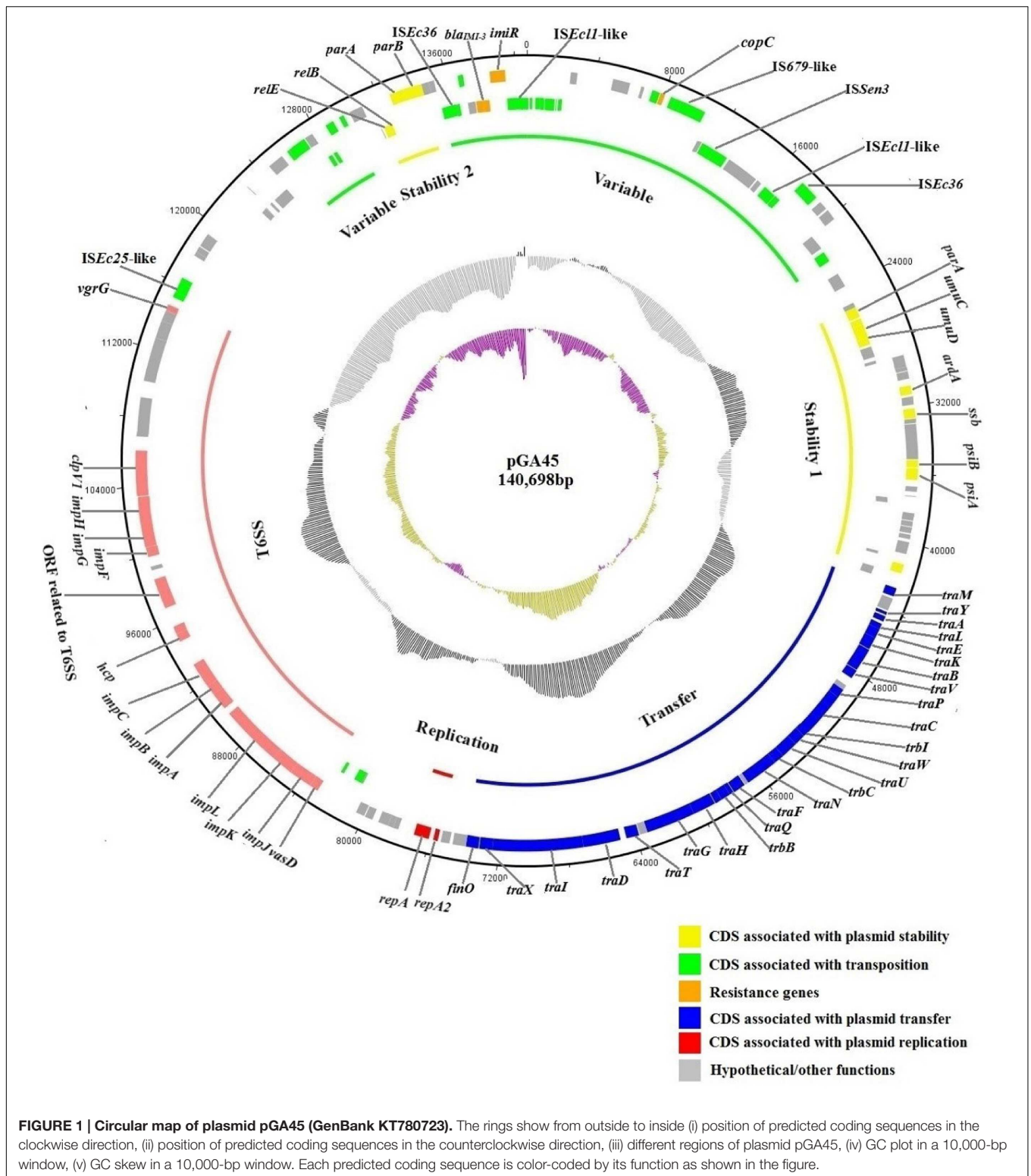
DISCUSSION

The *bla*_{IMI-3} gene from the variable region was the only ARG harbored by pGA45. The *bla*_{IMI-3}-containing region (position 58145–74139 bp) was bracketed by one copy of insertion sequence *ISEc36*² and one copy of insertion sequence highly similar to *ISEc11*² (with 100% coverage and 94% nucleotide identity to *ISEc11*) in the same orientation (Figure 2). *ISEc36* was first identified in a *bla*_{IMI-2}-bearing *E. coli* W635 strain (Rojo-Bezares et al., 2012). In this strain, *bla*_{IMI-2} was detected upstream the IMI-2R gene. However, in plasmid pGA45, *bla*_{IMI-3} and IMI-3R changed positions with each other and IMI-3R was located upstream the *bla*_{IMI-3} gene. Another well characterized *bla*_{IMI}-containing structure was from *Enterobacter cloacae* plasmid pT103 (GenBank accession no.: NG_036022.1) (Yu et al., 2006). In this partially sequenced plasmid, the *bla*_{IMI}-containing region comprises two *ISEc13*² (one is partial) elements flanking the *bla*_{IMI-3} and *bla*_{IMI-3R} genes in the opposite directions and one partial *ISEc36* located downstream of this region. In all these characterized *bla*_{IMI}-containing regions, *bla*_{IMI} genes have close relationships with insertion sequence *ISEc36*. Therefore, *ISEc36* may play an essential role in the dissemination of *bla*_{IMI} genes between different plasmids. It is also noteworthy to point out that the six plasmids mentioned above similar to pGA45 in backbones mainly have two different bacterial hosts, *Klebsiella pneumoniae* and *Enterobacter cloacae*. In addition, the identified *bla*_{IMI} genes were mostly from *Enterobacter* species. In view of this, the most probable hosts for plasmid pGA45 were *Enterobacter* species.

Another notable feature harbored by pGA45 was the T6SS region. Compared to other similar IncFII plasmids, the T6SS region was unique to pGA45. This region was most closely related to the T6SS system of plant pathogen *Erwinia amylovora* not only in nucleotide identity (71% coverage and 82% nucleotide identity) but also in gene organization. Previous studies showed that *bla*_{IMI-2} and *bla*_{IMI-3} genes were located on plasmids with sizes ranging from 48.5 to 80 kb (one of these plasmids had been identified to belong to IncF group). In this study, pGA45 was much bigger than these plasmids. This perhaps resulted from the

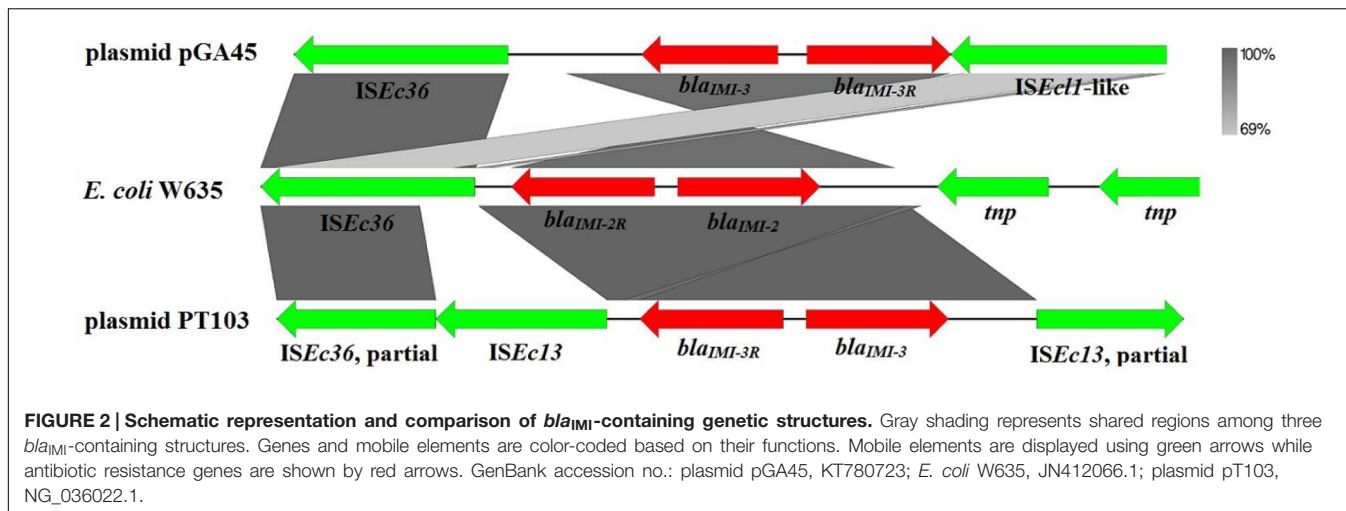
¹<http://pubmlst.org/plasmid>

²<https://www-is.biotoul.fr/>



integration of this T6SS region (31753 bp). The T6SS region of plasmid pGA45 was flanked by a copy of ISEc25-like element (with 100% coverage and 85% nucleotide identity to ISEc25) downstream and two transposase genes (with weak

amino acid identity to known transposase) upstream. The T6SS region of plasmid pGA45 comprised 14 T6SS-related genes including *vasD* (with 93% coverage and 64.46% amino acid identity to *Erwinia amylovora* ATCC 49946, KEGG database),



impJ (with 100% coverage and 82.55% amino acid identity to *Erwinia pyrifoliae* Ep1/96, KEGG database), *impK* (with 99% coverage and 84.26% amino acid identity to *Erwinia pyrifoliae* Ep1/96, KEGG database), *impL* (with 100% coverage and 84.38% amino acid identity to *Erwinia tasmaniensis*, KEGG database), *impA* (with 99% coverage and 77.45% amino acid identity to *Erwinia amylovora* ATCC 49946, KEGG database), *impB* (with 97% coverage and 86.52% amino acid identity to *Erwinia amylovora* ATCC 49946, KEGG database), *impC* (with 100% coverage and 94.99% amino acid identity to *Erwinia billingiae*, KEGG database), *hcp* (with 99% coverage and 86.79% amino acid identity to *Enterobacter cloacae subsp. cloacae* ATCC 13047, KEGG database), an ORF encoding a FHA-domain containing protein (with 97% coverage and 70.38% amino acid identity to *Erwinia pyrifoliae* Ep1/96, KEGG database), *impF* (with 99% coverage and 85.03% amino acid identity to *Erwinia amylovora* ATCC 49946, KEGG database), *impG* (with 100% coverage and 87.5% amino acid identity to *Erwinia pyrifoliae* Ep1/96, KEGG database), *impH* (with 99% coverage and 76.95% amino acid identity to *Erwinia tasmaniensis*, KEGG database), *clpV* (with 99% coverage and 89.64% amino acid identity to *Erwinia amylovora* ATCC 49946, KEGG database) and *vgrG* (with 100% coverage and 96.92% amino acid identity to *Kosakonia radicincitans* DSM 16656, NR database).

The T6SS was a recently discovered phage-like secretion apparatus. First reported in *Vibrio cholerae* and *Pseudomonas aeruginosa*, T6SS was likely to be involved in bacterial pathogenesis through acting like a potential nano-syringe for the translocation of effector proteins into the host cell (Pukatzki et al., 2006; Sarris et al., 2011). The Hcp (haemolysin co-regulated protein) and VgrG (valine-glycine repeat G protein) proteins are putative effectors for T6SS (Russell et al., 2014) and the genes encoding these two proteins are also found to be located in the T6SS region of plasmid pGA45. T6SS-related genes are harbored by many kinds of Gram-negative bacterial pathogens which can result in human or animal diseases. In this study, plasmid pGA45 was isolated from

river sediment which was collected from urban section of Haihe River. This area was densely populated and strongly affected by human activities. In previous published literatures, most of the bla_{IMI} isolates were from in clinical settings. Therefore, the occurrence of T6SS and bla_{IMI-3}-containing plasmid pGA45 in the river environment are a potential risk for human health and the horizontal transfer of this plasmid between *Enterobacteriaceae* bacteria may aggravate this situation.

CONCLUSION

This report demonstrated the complete nucleotide sequence of the bla_{IMI}-harboring plasmid. The sequencing of this plasmid provided insight into the architecture used for the dissemination of bla_{IMI} carbapenemase genes. In addition to the bla_{IMI} gene, plasmid pGA45 also harbored a T6SS cluster probably involved in bacteria virulence. Notably, this plasmid was isolated from environment sample, which will increase the risks of obtaining infections resulted from various types of pathogens carrying this plasmid.

AUTHOR CONTRIBUTIONS

DM and YL designed experiments; BD carried out experiments and analyzed experimental results; BD wrote the manuscript.

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REFERENCES

- Aubron, C., Poirel, L., Ash, R. J., and Nordmann, P. (2005). Carbapenemase-producing Enterobacteriaceae, U.S. rivers. *Emerg. Infect. Dis.* 11, 260–264. doi: 10.3201/eid1102.030684
- Chen, Z., Li, H., Feng, J., Li, Y., Chen, X., Guo, X., et al. (2015). NDM-1 encoded by a pNDM-BJ01-like plasmid p3SP-NDM in clinical *Enterobacter aerogenes*. *Front. Microbiol.* 6:294. doi: 10.3389/fmicb.2015.00294
- Chu, Y. W., Tung, V. W., Cheung, T. K., Chu, M. Y., Cheng, N., Lai, C., et al. (2011). Carbapenemases in enterobacteria, Hong Kong, China, 2009. *Emerg. Infect. Dis.* 17, 130–132. doi: 10.3201/eid1701.101443
- Delcher, A. L., Bratke, K. A., Powers, E. C., and Salzberg, S. L. (2007). Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23, 673–679. doi: 10.1093/bioinformatics/btm009
- Delcher, A. L., Harmon, D., Kasif, S., White, O., and Salzberg, S. L. (1999). Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* 27, 4636–4641. doi: 10.1093/nar/27.23.4636
- He, L.-Y., Liu, Y.-S., Su, H.-C., Zhao, J.-L., Liu, S.-S., Chen, J., et al. (2014). Dissemination of antibiotic resistance genes in representative broiler feedlots environments: identification of indicator ARGs and correlations with environmental variables. *Environ. Sci. Technol.* 48, 13120–13129. doi: 10.1021/es5041267
- Heuer, H., Krögerrecklenfort, E., Wellington, E., Egan, S., Van Elsland, J., Van Overbeek, L., et al. (2002). Gentamicin resistance genes in environmental bacteria: prevalence and transfer. *FEMS Microbiol. Ecol.* 42, 289–302. doi: 10.1111/j.1574-6941.2002.tb01019.x
- Huang, T. W., Wang, J. T., Lauderdale, T. L., Liao, T. L., Lai, J. F., Tan, M. C., et al. (2013). Complete sequences of two plasmids in a blaNDM-1-positive *Klebsiella oxytoca* isolate from Taiwan. *Antimicrob. Agents Chemother.* 57, 4072–4076. doi: 10.1128/AAC.02266-12
- Li, R., Li, Y., Kristiansen, K., and Wang, J. (2008). SOAP: short oligonucleotide alignment program. *Bioinformatics* 24, 713–714. doi: 10.1093/bioinformatics/btn025
- Li, R., Zhu, H., Ruan, J., Qian, W., Fang, X., Shi, Z., et al. (2010). De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.* 20, 265–272. doi: 10.1101/gr.097261.109
- Lo, W. U., Cheung, Y. Y., Lai, E., Lung, D., Que, T. L., and Ho, P. L. (2013). Complete sequence of an IncN plasmid, pIMP-HZ1, carrying blaIMP-4 in a *Klebsiella pneumoniae* strain associated with medical travel to China. *Antimicrob. Agents Chemother.* 57, 1561–1562. doi: 10.1128/AAC.02298-12
- McGann, P., Hang, J., Clifford, R. J., Yang, Y., Kwak, Y. I., Kuschner, R. A., et al. (2012). Complete sequence of a novel 178-kilobase plasmid carrying bla(NDM-1) in a *Providencia stuartii* strain isolated in Afghanistan. *Antimicrob. Agents Chemother.* 56, 1673–1679. doi: 10.1128/AAC.05604-11
- Nordmann, P., Dortet, L., and Poirel, L. (2012). Carbapenem resistance in Enterobacteriaceae: here is the storm! *Trends Mol. Med.* 18, 263–272. doi: 10.1016/j.molmed.2012.03.003
- Pruden, A., Pei, R., Storteboom, H., and Carlson, K. H. (2006). Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environ. Sci. Technol.* 40, 7445–7450. doi: 10.1021/es060413l
- Pukatzki, S., Ma, A. T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W. C., et al. (2006). Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1528–1533. doi: 10.1073/pnas.0510322103
- Qu, H., Wang, X., Ni, Y., Liu, J., Tan, R., Huang, J., et al. (2015). NDM-1-producing Enterobacteriaceae in a teaching hospital in Shanghai, China: IncX3-type plasmids may contribute to the dissemination of blaNDM-1. *Int. J. Infect. Dis.* 34, 8–13. doi: 10.1016/j.ijid.2015.02.020
- Rasmussen, B. A., Bush, K., Keeney, D., Yang, Y. J., Hare, R., O'gara, C., et al. (1996). Characterization of IMI-1 β -lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. *Antimicrob. Agents Chemother.* 40, 2080–2086.
- Rojo-Bezares, B., Martin, C., Lopez, M., Torres, C., and Saenz, Y. (2012). First detection of blaIMI-2 gene in a clinical *Escherichia coli* strain. *Antimicrob. Agents Chemother.* 56, 1146–1147. doi: 10.1128/AAC.05478-11
- Russell, A. B., Peterson, S. B., and Mougous, J. D. (2014). Type VI secretion system effectors: poisons with a purpose. *Nat. Rev. Microbiol.* 12, 137–148. doi: 10.1038/nrmicro3185
- Salzberg, S. L., Delcher, A. L., Kasif, S., and White, O. (1998). Microbial gene identification using interpolated Markov models. *Nucleic Acids Res.* 26, 544–548. doi: 10.1093/nar/26.2.544
- Sarris, P. F., Zoumadakis, C., Panopoulos, N. J., and Scoulica, E. V. (2011). Distribution of the putative type VI secretion system core genes in *Klebsiella* spp. *Infect. Genet. Evol.* 11, 157–166. doi: 10.1016/j.meegid.2010.09.006
- Sun, F., Yin, Z., Feng, J., Qiu, Y., Zhang, D., Luo, W., et al. (2015). Production of plasmid-encoding NDM-1 in clinical *Raoultella ornithinolytica* and *Leclercia adecarboxylata* from China. *Front. Microbiol.* 6:458. doi: 10.3389/fmicb.2015.00458
- Teo, J. W., La, M. V., Krishnan, P., Ang, B., Jureen, R., and Lin, R. T. (2013). *Enterobacter cloacae* producing an uncommon class A carbapenemase, IMI-1, from Singapore. *J. Med. Microbiol.* 62, 1086–1088. doi: 10.1099/jmm.0.053363-0
- Tiwari, V., Kapil, A., and Moganty, R. R. (2012). Carbapenem-hydrolyzing oxacillinase in high resistant strains of *Acinetobacter baumannii* isolated from India. *Microb. Pathog.* 53, 81–86. doi: 10.1016/j.micpath.2012.05.004
- Tiwari, V., and Moganty, R. R. (2014). Conformational stability of OXA-51 β -lactamase explains its role in carbapenem resistance of *Acinetobacter baumannii*. *J. Biomol. Struct. Dyn.* 32, 1406–1420. doi: 10.1080/07391102.2013.819789
- Villa, L., Carattoli, A., Nordmann, P., Carta, C., and Poirel, L. (2013). Complete sequence of the IncT-type plasmid pT-OXA-181 carrying the blaOXA-181 carbapenemase gene from *Citrobacter freundii*. *Antimicrob. Agents Chemother.* 57, 1965–1967. doi: 10.1128/AAC.01297-12
- Villa, L., Poirel, L., Nordmann, P., Carta, C., and Carattoli, A. (2012). Complete sequencing of an IncH plasmid carrying the blaNDM-1, blaCTX-M-15 and qnrB1 genes. *J. Antimicrob. Chemother.* 67, 1645–1650. doi: 10.1093/jac/dks114
- Wu, W., Feng, Y., Carattoli, A., and Zong, Z. (2015). The emergence of *Enterobacter cloacae* producing both KPC and NDM carbapenemases: characterization by whole genome sequencing. *Antimicrob. Agents Chemother.* AAC.1275-15. doi: 10.1128/AAC.01275-15
- Yu, Y. S., Du, X. X., Zhou, Z. H., Chen, Y. G., and Li, L. J. (2006). First isolation of blaIMI-2 in an *Enterobacter cloacae* clinical isolate from China. *Antimicrob. Agents Chemother.* 50, 1610–1611. doi: 10.1128/AAC.50.4.1610-1611.2006
- Zhang, X. X., and Zhang, T. (2011). Occurrence, abundance, and diversity of tetracycline resistance genes in 15 sewage treatment plants across China and other global locations. *Environ. Sci. Technol.* 45, 2598–2604. doi: 10.1021/es103672x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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