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Genome Note

Genomic characterisation of a *Proteus mirabilis* clinical isolate from China carrying bla_{NDM-5} on an IncX3 plasmid

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ABSTRACT

Objectives: Acquisition of carbapenemases is of particular concern in *Proteus mirabilis*, which is intrinsically resistant to tigecycline and colistin, as it makes clinical therapy extremely difficult. Here we report the whole genome sequence of a *P. mirabilis* clinical isolate from China (CRPM10) harbouring bla_{NDM-5} on an IncX3-type plasmid.
Methods: Whole-genome sequencing of the isolate was performed using an Illumina HiSeq™ 4000 platform and MinION sequencer. Hybrid assembly of short Illumina reads and long MinION reads was performed using Unicycler v.0.4.7. Functional annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) server, and further genomic analyses were performed.
Results: The complete genome sequence of *P. mirabilis* CRPM10 consisted of a chromosome of 4 158 695 bp and one IncX3-type plasmid of 46 161 bp (pNDM-5). Fourteen antimicrobial resistance genes (ARGs) were identified in CRPM10. The ARGs were all located on the chromosome except for bla_{NDM-5}, which was located on the IncX3-type plasmid pNDM-5. Plasmid sequence alignment of pNDM-5 with the NCBI GenBank database revealed several highly identical plasmids from different Enterobacteriaceae strains.
Conclusion: Here we report the complete genome sequence of a *P. mirabilis* clinical isolate from China carrying bla_{NDM-5} on an IncX3-type plasmid. The 46 161-bp IncX3-type plasmids may play an important role in the distribution of NDM mutants among Enterobacteriaceae strains. Considering the global emergence of the NDM-5 carbapenemase, an epidemiological survey and analysis of bla_{NDM-5}-harbouring Enterobacteriaceae strains are urgently required to prevent its future prevalence.
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Proteus mirabilis, part of the Enterobacteriaceae family, is an important pathogen causing nosocomial infections. The flagella *of P. mirabilis* allows its motility. Colonisation of the skin and mucosa of patients in long-term care facilities by *P. mirabilis* has been shown to cause infections. *Proteus mirabilis* is a common aetiological agent of urinary tract infections (UTIs) in humans, particularly catheter-associated UTIs [1]. *Proteus mirabilis* is able to harbour several antimicrobial resistance genes (ARGs), including carbapenemases. Acquisition of carbapenemases is of particular concern in *P. mirabilis*, which is intrinsically resistant to tigecycline and colistin, as it makes clinical therapy extremely difficult.

New Delhi metallo-β-lactamase (NDM), an Ambler class B β-lactamase, is the main type of carbapenemase that confers resistance to almost all β-lactams [2]. Isolates producing NDM enzymes have been detected worldwide, most frequently in *Klebsiella pneumoniae* and *Escherichia coli*. NDM-5-producing *P. mirabilis* is seldom reported. NDM-5, which differs from NDM-1 by mutation of two amino acids, was first identified in *E. coli* in the UK in 2011 and exhibits high carbapenemase activity. Here were report an NDM-5-producing *P. mirabilis* strain (CRPM10) isolated from a urine sample of a patient hospitalised long-term in a tertiary hospital in Zhejiang Province, China, in 2018.

The strain was preliminarily identified using a VITEK® MS system (bioMérieux, Marcy-l'Étoile, France) and was further confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing was performed using a VITEK®2 system (bioMérieux) with a Gram-negative antimicrobial susceptibility testing card (AST-GN16) or by Etest following Clinical and Laboratory Standards

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Institute (CLSI) guidelines. The minimum inhibitory concentrations (MICs) are presented in Supplementary Table S1. Strain CRPM10 was an extensively drug-resistant (XDR) bacterium exhibiting a typical NDM-producing phenotype, i.e. resistant to cephalosporins and carbapenems but susceptible to aztreonam.

Genomic DNA of the isolate was extracted using a QIAamp DNA Mini Kit (QIAGEN, Germantown, MD, USA) and was subjected to whole-genome sequencing (WGS) using an Illumina HiSeq™ 4000 platform (Illumina Inc., San Diego, CA, USA). To obtain the complete genome sequence, CRPM10 was also subjected to WGS using the long-read MinION sequencer (Oxford Nanopore Technologies, Oxford, UK). Hybrid assembly of short Illumina reads and long MinION reads was performed using Unicycler v.0.4.7 under conservative mode for increased accuracy. Complete circular contigs generated were then corrected using Pilon v.1.23 with Illumina reads for several rounds until no change was detected. The whole genome sequence was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) server. ARGs and plasmids were analysed using the BacWGSTdb server [3]. Circular comparison between the bla_{NDM-5}-carrying plasmid and similar plasmids was conducted by BLAST Ring Image Generator (BRIG) v.0.95 as concentric rings [4].

The complete genome sequence of *P. mirabilis* CRPM10 consisted of a chromosome of 4 158 695 bp and one plasmid of 46 161 bp (pNDM-5). A total of 82 tRNA genes, 3 rRNA operons and 3717 protein-coding sequences were identified in the chromosome by PGAP server. An IncX3 plasmid replicon was identified in plasmid pNDM-5.

ARGs present in the genome of the isolate are presented in Supplementary Table S2. Fourteen ARGs were identified in CRPM10, including aminoglycoside resistance genes [*aadA2* and *aph(3')-Ia*], β-lactam resistance genes (bla_{TEM-1B}, bla_{OXA-1}, $bla_{CTX-M-3}$ and bla_{NDM-5}), macrolide resistance genes [*mph(E)* and *msr(E)*], tetracycline resistance genes [*tet(A)* and *tet(J)*], the phenicol resistance gene *floR* and three copies of the sulfonamide resistance gene *sul1*. The ARGs were all located on the chromosome except for bla_{NDM-5}.

The carbapenem resistance gene bla_{NDM-5} was located on the IncX3-type plasmid pNDM-5, preceded by Tn*3*–IS*3000*–IS*30*–IS*5* in the upstream region and followed by ble_{MBL}–*trpF*–*dsbC*–*cutA*–IS*26* in the downstream region. According to the result of similarity analysis by Basic Local Alignment Search Tool (BLAST), several previously reported plasmids were found to be 99% identical to pNDM-5 (Fig. 1), including p1079-NDM (from *E. coli*, accession no.

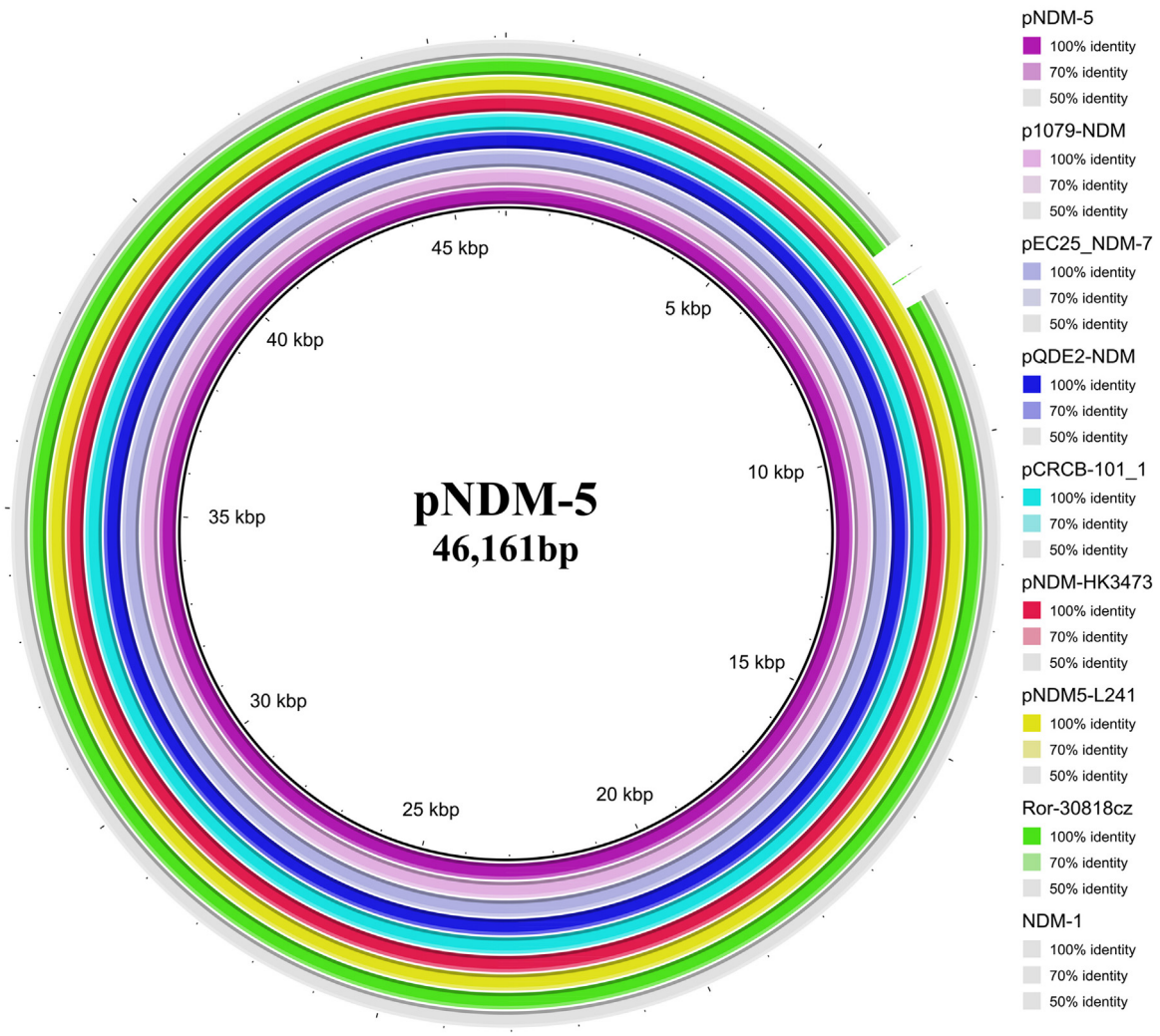

Fig. 1. Sequence alignment of plasmid pNDM-5 from *Proteus mirabilis* strain CRPM10 with the NCBI GenBank database revealed several highly identical plasmids from different Enterobacteriaceae strains, including p1079-NDM (*Escherichia coli* strain 1079, accession no. MG825384), pEC25_NDM-7 (*E. coli* strain EC25, accession no. CP035125), pQDE2-NDM (*Klebsiella pneumoniae* strain QDE2, accession no. MH917280), pCRCB-101_1 (*Citrobacter freundii* strain CRCB-101, accession no. CP024820), pNDM-HK3473 (*Enterobacter cloacae* strain CRE3473, accession no. MH234506), pNDM5-L241 (*Morganella morganii* strain L241, accession no. CP033057), pRor-30818cz (*Raoultella ornithinolytica* strain pRor-30818cz, accession no. MG252893) and NDM-1 (*Raoultella planticola* strain RJA274, accession no. KF877335).

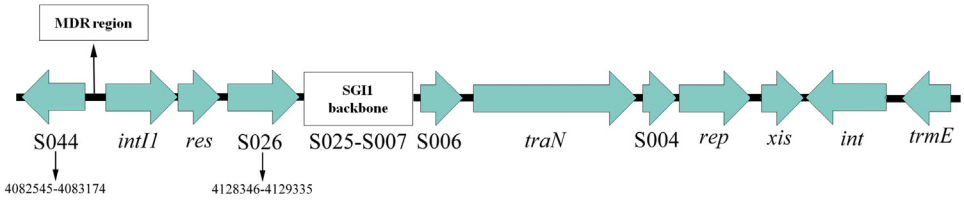

Fig. 2. Backbone structure of the *Salmonella* genomic island 1 (SGI1) element in *Proteus mirabilis* CRPM10. MDR, multidrug resistance.

MG825384), pEC25_NDM-7 (from *E. coli*, accession no. **CP035125**), pQDE2-NDM (from *K. pneumoniae*, accession no. **MH917280**), pCRCB-101_1 (from *Citrobacter freundii*, accession no. **CP024820**), pNDM-HK3473 (from *Enterobacter cloacae*, accession no. **MH234506**) and pNDM5-L241 (from *Morganella morganii*, accession no. **CP033057**). Most of these plasmids originated from China. It is interesting that pNDM-5 is similar to plasmid pEC25_NDM-7 that we reported previously [5]. pEC25_NDM-7 was identified in an *E. coli* strain isolated in the same hospital in 2017 carrying bla_{NDM-7} in its backbone. The 46 161-bp IncX3-type plasmids may play an important role in the distribution of NDM mutants in Enterobacteriaceae strains.

A new *Salmonella* genomic island 1 (SGI1) variant was identified in the chromosome of *P. mirabilis* CRPM10 (Fig. 2). The first report of a *P. mirabilis* containing an SGI1 variant was by Ahmed et al. in 2007 [6]. SGI1 variants are widely distributed in the chromosome of *P. mirabilis* isolates and contribute to multiple drug resistance [7–9]. In the current study, the SGI1 variant was ~76 kb in size. A multidrug resistance (MDR) region including the genes *aadA2*, *aph (3')-Ia*, bla_{TEM-1B}, bla_{OXA-1}, $bla_{CTX-M-3}$, *mph(E)*, *msr(E)*, *floR*, *tet(A)* and three copies of *sul1* was inserted between ORF_S044 (position 4 082 545–4 083 174) and ORF_S026 (position 4 128 346–4 129 335).

In summary, here we report the complete genome sequence of a *P. mirabilis* clinical isolate from China carrying bla_{NDM-5} on an IncX3-type plasmid. Considering the global emergence of NDM-5, an epidemiological survey and analysis of bla_{NDM-5}-harbouring Enterobacteriaceae strains are urgently needed to prevent its future prevalence.

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession nos. **CP043332**–**CP043333**. The version described in this paper is the first version.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.10.025.

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