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The coexistence of two *bla*$_{NDM-5}$ genes on an IncF plasmid as revealed by nanopore sequencing

Yu Feng[1, 2], Lu Liu[1, 2], Alan McNally[5], Zhiyong Zong[1, 2, 3,4]*

[1]Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China. [2]Division of Infectious Diseases, State Key Laboratory of Biotherapy, Chengdu, China. [3]Department of Infection Control, West China Hospital, Sichuan University, Chengdu, China.[4]Center for Pathogen Research, West China Hospital, Sichuan University, Chengdu, China. [5]Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK.

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*Corresponding author. Mailing address: Center of Infectious Diseases, West China Hospital (Huaxi), Guoxuexiang 37, Chengdu 610041, China. Phone: 86-28-8542-2637. Fax: 86-28-8542-3212. E-mail: zongzhiy@scu.edu.cn

Abstract

In a carbapenem-resistant *Escherichia coli* clinical isolate of sequence type 167, two copies of *bla*$_{NDM-5}$ were found on a 144,225-bp IncF self-transmissible plasmid of the F36:A4:B- type. Both *bla*$_{NDM-5}$ genes were located in 11,065-bp regions flanked by two copies of IS*26*. The two regions were identical in sequence but were present at different locations on the plasmid, suggesting a duplication of the same region. This study highlights the complex genetic contexts of *bla*$_{NDM-5}$.

New Delhi metallo-β-lactamase (NDM) is a type of carbapenem-hydrolysing enzyme~~s~~ (carbapenemase~~s~~) with the ability to hydrolyze all β-lactams except monobactams (1), representing a serious challenge for treatment of bacterial infections, infection control and public health. ~~Up to now~~To date, there are 21 variants of NDM, ~~among which~~with NDM-5 ~~is~~ one of the most common variants encountered in the Enterobacteriaceae (2-5). The NDM-5-encoding gene, *bla*$_{NDM-5}$, usually exists in a single copy on plasmids. However, we have found the peculiar presence of two copies of *bla*$_{NDM-5}$ on a single plasmid within an *Escherichia coli* clinical isolate, which is reported here.

E. coli strain SCEC020007 was recovered from urine of a female outpatient with urinary tract infection in October 2016 in China. The strain was resistant to amikacin (MIC, >512 μg/ml), ceftazidime (>512 μg/ml), ceftazidime-avibactam (>512/4 μg/ml), ciprofloxacin (256 μg/ml), imipenem (64 μg/ml), meropenem (256 μg/ml), piperacillin-tazobactam (>512/4 μg/ml) and trimethoprim-sulfamethoxazole (128/2,432 μg/ml), but was susceptible to aztreonam (8 μg/ml), colistin (2 μg/ml) and tigecycline (0.25 μg/ml) as determined using the broth dilution method of the Clinical Laboratory Standards Institute (6). As there are no breakpoints of colistin and tigecycline from CLSI, those defined by EUCAST (http://www.eucast.org/) were applied.

A draft genome sequence of the strain was generated on the Illumina HiSeq X10 platform, which generated 5,557,833 clean reads and 1.67 Gb clean bases. A total of 113 contigs (102 >1,000 bp; *N50* 126,680 bp) with a 50.76% GC content were *de novo* assembled using SPAdes (7). Strain SCEC020007 belonged to phylogenetic group A as determined using PCR as described previously (8) and sequence type

167 (ST167) as determined using the genomic sequence to query the *E. coli* multi-locus sequence typing database (http://enterobase.warwick.ac.uk/species/index/ecoli). Antimicrobial resistance genes were identified from genome sequences using the ABRicate program (https://github.com/tseemann/abricate) to query the ResFinder database (http://genomicepidemiology.org/). Strain SCEC020007 had 9 antimicrobial resistance genes mediating resistance to aminoglycosides (*aadA2*, *aadA5*, *rmtB*), β-lactams (bla_{NDM-5} and bla_{TEM-1}), tetracycline (*tet(A)*), sulphonamides (*sul1*) and trimethoprim (*dfrA12* and *dfrA17*). Plasmid replicon types within strain SCEC020007 were determined using ~~by~~ the PlasmidFinder tool at http://genomicepidemiology.org/. Surprisingly, strain SCEC020007 had an IncFIA, an IncFII and an IncB/O/K/Z replicon but no IncX3, which is the common replicon type of plasmids associated with bla_{NDM-5}.

To untangle the genetic context of bla_{NDM-5}, strain SCEC020007 was subjected to sequencing using the long-read ~~real-time~~ MinION Sequencer (Nanopore, Oxford, UK). ~~The~~ A *de novo* hybrid assembly of both short Illumina reads and long MinION reads was ~~performed~~ constructed using Unicycler v0.4.3 (9) under conservative mode for ~~an~~ increased accuracy. The ~~C~~complete circular contigs generated were then corrected using Plion v1.22 (10) with Illumina reads for several rounds until no change was detected. The hybrid assembly of Illumina and MinION reads revealed that strain SCEC020007 had a 4.8-Mb circular chromosome, a 144,225-bp plasmid containing ~~an~~ IncFIA and ~~a~~ FII replicons (designated pNDM5_020007) and an 84,952-bp plasmid with an IncB/O/K/Z replicon (designated pBOKZ_020007). Surprisingly, there were two copies of bla_{NDM-5} in strain SCEC020007, both of which were present on pNDM5_020007. Both bla_{NDM-5} genes were located in 11,065-bp

regions flanked by two copies of IS*26* and the two regions were identical in sequence but were present at different locations on pNDM5_020007 (Figure 1), suggesting that the 11,065-bp region is duplicated. The presence of the two bla_{NDM-5} genes and their locations on pNDM5_020007 were confirmed by PCR. The 11,065-bp region contained a complex class 1 integron with a *dfrA17-aadA5* cassette array and IS*CR1* (insertion sequence common region 1), which is truncated by IS*26* at its 5' conserved segment, a 69-bp remnant of *ctuA1* (encoding an ion tolerant protein), *dsbC* (encoding an oxidoreductase), *trpF* (encoding a phosphoribosylanthranilate isomerase), *ble* (mediating bleomycin resistance), bla_{NDM-5}, a truncated IS*Aba125* and a truncated IS*Ecp1*/IS*Ec9* element (Figure 1). The co-existence of two bla_{NDM-5} genes has~~ve~~ not been reported before but the co-existence of two bla_{NDM-1} genes has been described previously (11, 12). Two tandem copies of bla_{NDM-1} genes have been found in the chromosome of an ST167 *E. coli* in China (11) and a *Pseudomonas aeruginosa* strain in Serbia (12). In both cases, the tandem copies of bla_{NDM-1} are associated with IS*CR1* but not IS*26*. It is known that IS*CR1* uses the rolling circle mechanism for transposition and may generate tandem duplication of its mobilized sequence via homologous recombination (13). However, the duplication of the 11,065-bp region carrying bla_{NDM-5} on pNDM5_020007 is not tandem, suggesting that the duplication might not result from the action of IS*CR1* but could be mediated by IS*26*. The exact mechanism for the duplication of such a large region warrants further studies.

Assembly based on Illumina reads alone generated only a single contig containing bla_{NDM-5} and was unable to reveal that there were actually ~~were~~ two identical copies of the same contig. This imposes difficulties for completing the bla_{NDM-5}-carrying plasmid

sequence by conventional methods including PCR and Sanger sequencing to close gaps between contigs. By contrast, MinION sequencing ~~is~~ was able to resolve the copy numbers of genes and contigs and their exact position on the plasmid relative to each other.

Plasmid multi-locus sequence typing (pMLST) was performed using the pMLST tool (https://cge.cbs.dtu.dk/services/pMLST/). pNDM5_020007 belongs to the F36:A4:B- type. pNDM5_020007 ~~was~~ has closest similarity (97% coverage and 99% identity) to a 149.5-kb unnamed plasmid (GenBank accession no. CP023871) from *E. coli* strain FDAARGOS_434, which was recovered from a human rectal swab in British Colombia, Canada, in 2014. This unnamed plasmid also carries bla_{NDM-5} (a single copy) and belongs to the F36:A4:B- type. Backbones of pNDM5_020007 and the unnamed plasmid of strain FDAARGOS_434 are almost identical, suggesting that they might have originated from a common plasmid. Conjugation experiments were carried out in broth and on filters with the azide-resistant *E. coli* strain J53 as the recipient. pNDM5_020007 was able to be transferred by conjugation, suggesting that it is self-transmissible.

In conclusion, we identified the presence of two bla_{NDM-5} genes on an F36:A4:B- self-transmissible plasmid. The co-existence of two bla_{NDM-5} genes was due to the duplication of an IS*26*-bracketed region containing IS*CR1*.

Nucleotide sequence accession numbers. The complete sequence of pBOKZ_020007, pNDM5_020007 and the chromosome of strain SCEC020007 has

been deposited into GenBank under the accession no. CP025625, CP025626 and CP025627, respectively.

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Figure legend

Figure 1. pNDM5_020007 and the genetic context of *bla*$_{NDM-5}$. The two 11,065-bp *bla*$_{NDM-5}$-containing regions bracketed by IS*26* are indicated by orange circles in the map of pNDM5_020007 and are shown in detail at the bottom. Δ represents truncated genes or mobile genetic elements.