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Short Communication

Emergence of ST654 *Pseudomonas aeruginosa* co-harbouring bla_{NDM-1} and bla_{GES-5} in novel class I integron In1884 from Bulgaria

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Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is a common cause of hospital-acquired infections. Carbapenem resistance, in particular, represents a substantial problem in terms of treatment of infections due to this pathogen and leads to increased mortality, longer duration of hospital stay and increased healthcare costs [1]. The class B New Delhi metallo-β-lactamase 1 (NDM-1) carbapenemase has spread to and has been described in many bacterial species, including *P. aeruginosa* [2] Another carbapenem-hydrolysing enzyme, the Guiana extended-spectrum β-lactamase-5 (GES-5), initially found in *Escherichia coli* in Greece and belonging to class A β-lactamase family, has also been detected in *P. aeruginosa* in Brazil, China, Spain and South Africa [3].

Here, we report the first detection of NDM-1-producing *P. aeruginosa* isolates in Bulgaria and the chromosomal co-harbouring of bla_{NDM-1} and bla_{GES-5} genes in these MDR *P. aeruginosa* in novel class I integron In1884.

Five carbapenem-resistant (CR) *P. aeruginosa* strains were isolated from clinical samples of patients in two Bulgarian hospitals. The first two isolates, named Pae1250 and Pae1251, were recovered in September 2017 from urine samples (>10^5 CFU/mL) of two different patients hospitalised in Alexandrovska University Hospital in Sofia. In August 2018, we detected three other CR *P. aeruginosa* isolates (Pae1252, Pae1255 and Pae1257) from tracheobronchial aspirates of three patients hospitalised in 'St. Ivan Rilski' University Hospital in Sofia. A CR *Klebsiella pneumoniae* (Kpn1256) was also isolated from the patient from whom CR *P. aeruginosa* Pae1255 was collected.

Antibiotic susceptibility testing was performed using Etest (bioMérieux, la Balme-les-Grottes, France). Colistin susceptibility was tested by broth microdilution method using MICRONAUT plate (MERLIN Diagnostika GmbH, Bornheim, Germany) and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines v9.0 (http://www.eucast.org/clinical_breakpoints/). All six strains were resistant to carbapenems and had minimum inhibitory concentration (MIC) values of imipenem and meropenem >32 mg/L. They were also resistant to ceftazidime, cefepime, piperacillin/tazobactam, ceftolozane/tazobactam, ceftazidime/avibactam, ciprofloxacin, levofloxacin, amikacin, tobramycin and gentamicin, but were susceptible to colistin (MIC range 1–2 mg/L). Genomic DNA from all strains (five *P. aeruginosa* and one *K. pneumoniae*) was extracted using the MasterPure™ DNA Purification Kit (Epicentre Technologies Inc.) and sequenced (via 2 × 250 bp, MiSeq, Illumina, San Diego, USA), and long-read sequencing (MinION, Oxford Nanopore Technologies, Oxford, UK) of Pae1250 and Pae1255 was done according to the manufacturer's instructions. Data analysis was performed using an in-house tool (BacPipe) [4], and single-nucleotide polymorphism (SNP) calling and genetic context analysis was performed using CLC Genomics Workbench v.9.5.1 (Qiagen, Hilden, Germany).

Utilising short-read sequencing and read mapping, the bla_{NDM-1} gene in Kpn1256 was carried on an IncFII plasmid. bla_{NDM-1} in Kpn1256 was also flanked upstream by the truncated IS*Aba125* (IS30 family) and bleomycin resistance gene downstream. In addition, the isolate also carried $bla_{CTX-M-15}$, bla_{SHV-11}, bla_{CMY-4} and bla_{TEM-1B} genes and belonged to ST11, a high-risk clone repeatedly reported in the Balkan region [5]. The close relatedness between the two Bulgarian NDM-1-producing isolates in 2016 and Kpn1256, recovered in 2018, potentially indicates a persistent circulation of this NDM-1-carrying ST11 clone.

The five *P. aeruginosa* were clonally related, all belonging to ST654, carrying similar accessory genomes with no plasmids and the same profile of resistance genes. The core genomes of these *P. aeruginosa* encompassed 11–19 SNP differences, indicating the close relatedness between these strains (GenBank accession no. BioProject ID: PRJNA628735). In addition to the bla_{NDM-1} gene, isolates Pae1250 and 1251 also carried bla_{GES-1}, while Pae1252, 1255 and 1257 harboured bla_{GES-5}. GES-5 is a variant of GES-1 with only one amino acid difference (Gly165Ser, previously reported as Gly170Ser) and, unlike GES-1, possesses carbapenem-hydrolysing activity.

Genes bla_{GES-5} and bla_{NDM-1} were integrated into the chromosome of PA1255 in a ca. 42-kb region of divergence (125 054–167 819 bp) situated between universal stress protein (*Usp*) and nucleotidyltransferase. *Bla*GES-5 harboured the novel class 1 integron In1884 with the 5′CS-*bla*GES-5/*aad*B-3′CS gene cassette array. However, the 3′CS was interrupted by a second integron 5′CS with sul3 as first (not a gene cassette but a structure called In0 found in other class 1 integrons). It seems that a truncated attI1 follows the last gene cassette of the In1884 integron. The composite genetic element was organised as follows: In1884 with a regular 3′CS on one side, then an In0 element with *attI1-sul3* fusion and *ISCR1* and bla_{NDM-1} on the other side (Fig. 1).

This divergence region consisted of other genes conferring resistance towards aminoglycosides [*strA*, *strB*, *aph(3′)-Via*, *aadB*], sulfonamide (*sul1*) and tetracycline (*tetA* and *tetR*). Although *P. aeruginosa* isolates are intrinsically resistant to sulfonamides and

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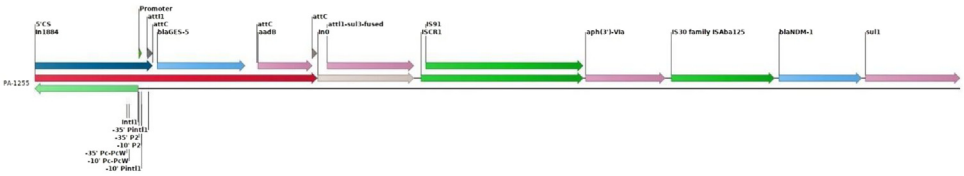

Fig. 1. Composite genetic structure of novel integron In1884 harbouring 5′CS-*bla*GES-5/*aad*B-3′CS gene cassette array. The new integron numbering is assigned based on the INTEGRALL database (http://integrall.bio.ua.pt/).

tetracyclines, the presence of this composite and its potential mobilisation are of particular concern as its transfer to other microorganisms, such as Enterobacterales, could incur resistance to multiple antimicrobial agents simultaneously, including the new β-lactam β-lactamase inhibitor combinations. To the best of our knowledge, this is the first report of the co-existence of bla_{NDM-1} and bla_{GES-5} in the same genetic element harboured in the chromosome and the first report of NDM-1-producing *P. aeruginosa* from hospitals in Bulgaria and a novel class 1 integron In1884. Moreover, the simultaneous presence of NDM-1 *K. pneumoniae* and *P. aeruginosa* isolates in one of the patient's samples implied an initial hypothesis of a potential horizontal transfer of bla_{NDM-1} between these strains. Based on the analysis of the adjacent genetic structures by long-read sequencing, this hypothesis was rejected with the conclusion that the co-existence of two CR bla_{NDM-1}-harbouring microorganisms in this patient was coincidental. However, this might have happened in other unresearched MDR isolates.

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Competing interests

No conflict of interest to declare.

Ethical approval

Not required.

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