International Journal of Antimicrobial Agents 53 (2019) 703–704

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Letter to the Editor

Emergence of Extremely Drug-Resistant and Uropathogenic New Delhi Metallo-β-Lactamse-6 (bla_{NDM-6})-producing *Citrobacter werkmanii*

Sir,

Antibiotic resistance has become a major public health problem. In India, the infectious disease mortality rate is 416.75 per 100 000 persons, which is twice the rate in the United States when antibiotics were introduced (approximately 200 per 100 000 persons) [1]. In 2009, a novel carbapenemase, "New Delhi Metallo-β-Lactamases-1" (NDM-1), was first reported in *Klebsiella pneumoniae* isolated from a Swedish patient of Indian origin [2]. The NDM producers were resistant to all β-lactams, including carbapenems. Carbapenems are a backup drug to treat patients who are gravely ill or who are suspected of harboring multidrug-resistant bacteria. The rapid worldwide dissemination and emergence of NDM in Enterobacteriaceae members resembles a move towards the pre-antibiotic era. With this scenario in mind, we initiated the current study to understand the emergence, spread, and genetic features of carbapenem-resistant Enterobacteriaceae in the intensive care unit (ICU) of a tertiary care hospital in north India.

Sixty patient samples (urine, pus or stool) were screened during August 2013 to September 2013 from patients admitted to the tertiary care hospital ICU. Of these samples, only one, isolated from a 57-year-old male with chronic kidney disease (CKD), was found to be a carbapenemase producer. Despite aggressive treatment and hemodialysis, the patient died on the sixth day of admission. The isolate was identified as *Citrobacter werkmanii* (AK-8) using the BD Phoenix™ automated microbiology system.

Antibiogram using standard disc diffusion of AK-8 strain showed the isolate was resistant to all β-lactams (including carbapenems), aztreonam, aminoglycosides, fluoroquinolones, oxacephem (moxalactam) and antibiotic-inhibitor combinations (Supplementary Table S1). However, the isolate was susceptible to colistin, tigecycline, and tetracycline (Supplementary Table S1). Double-disc synergy test using imipenem and imipenem with ethylenediaminetetraacetic acid (EDTA) disc confirmed the presence of metallo-β-lactamase (MBL). Minimum inhibitory concentrations (MICs) of antibiotics (Sigma-Aldrich, Co., St. Louis, MO, USA) in Supplementary Table S1 were determined by the microbroth dilution method. The results of these tests were interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines, 2016 [3]. The strain showed very high MIC values against all tested antibiotics. Polymerase chain reaction (PCR) amplification, sequencing, and conjugation confirmed the presence and co-existence of bla_{NDM-6} with $bla_{CTX-M-15}$, bla_{TEM-1} and *armA* genes on a self-transmissible plasmid (Supplementary Fig. S1-S3, Table S2). Replication machinery of the plasmid harboring bla_{NDM-6} belonged to IncFIC replicon type and carried class 1 integron, as confirmed by amplification of *intI* 1 and *sul* 1 gene.

In AK-8, PCR-based genetic environment analysis was performed as per Poirel et al. [4], but we did not find IS*Aba125* upstream or bleomycin resistance gene (ble_{MBL}) downstream of bla_{NDM-6}.

Citrobacter werkmanii was sequenced on Illumina NextSeq 500 platform for paired-end 150-bp sequencing. De Novo assembly of clean high-quality reads was performed by CLC Genomics Workbench version 9.0 (Qiagen, Germany). The location of bla_{NDM-6} in the AK-8 genome was identified by stand-alone Basic Local Alignment Search Tool (BLAST) against the assembled genome of AK-8 using NDM-6 nucleotide sequence as a query. The potential open reading frames (ORFs) were predicted by the NCBI ORFfinder program (https://www.ncbi.nlm.nih.gov/orffinder/). The NDM-6 gene was mapped and showed complete matching on scaffold 33, which was 10 208 bases. NCBI ORFfinder program predicted a total of 18 ORFs on this scaffold. Of these, 10 were collinear and 8 were nested ORFs (Supplementary Table S3, Supplementary Fig. S4). However, we found IS*30* family transposase upstream of NDM-6 instead of IS*Aba125* (Fig. 1). We also found transposase, IS*110* family transposase, TnpA transposase and Transposon Tn21 resolvase upstream of NDM-6 (Supplementary Table S3, Fig. 1). The presence of multiple mobile elements indicates these might help dissemination of bla_{NDM-6} among Enterobacteriaceae members. Moreover, for the first time, we found the bleomycin gene to be mapped as nested ORF within a hypothetical ORF downstream of NDM-6 in AK-8, in contrast to earlier reports [4].

The promoter of NDM was recently documented to be in part of the inverted repeat upstream of IS*Aba125* [5]; however, we did not find IS*Aba125* upstream of bla_{NDM-6}. Therefore, we performed qRT-PCR to investigate how the new genetic element affects the expression of bla_{NDM-6}. In the presence of imipenem, we found significantly high expression of bla_{NDM-6}, i.e. 4.75-fold compared with bla_{NDM-1}, associated with IS*Aba125*. However, when strains were grown in the absence of imipenem, a low level of bla_{NDM} expression was observed, indicating its constitutive expression (Supplementary Fig. S5, Table S4). This constitutive expression indicates that bacteria might be an opting strategy for NDM as an integral part of its existence. We urge policymakers to implement judicious use of antibiotics across all healthcare settings to impede the emergence of resistance.

The bla_{NDM-6} sequence was deposited at the NCBI database under accession number KJ872581. The whole genome next generation sequencing project was deposited at DDBJ/ENA/GenBank under accession number MRVL00000000.

https://doi.org/10.1016/j.ijantimicag.2019.02.006

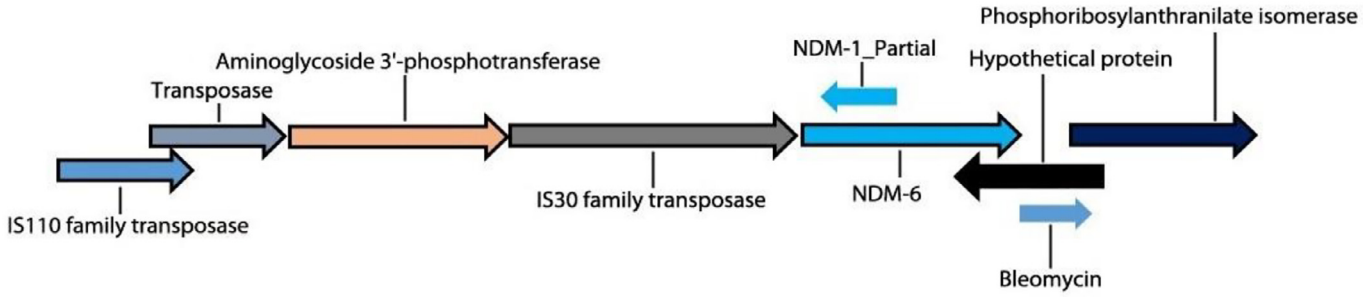

Fig. 1. Genetic map representing IS30 transposase upstream and bleomycin, as nested ORF, downstream of bla_{NDM-6}.

Acknowledgments

We thank Dr. Rafiq, Medicine Department, J N Medical College and Hospital, Aligarh, India, for providing samples and history of the patient.

Declarations

Funding

This work was supported by DBT (India) grants; BT/PR11453/BID/07/296/2009, BT/HRD/NBA/34/01/2012 and ICMR (India) grant AMR/5/2011-ECD-I to AUK. SP's Ph.D. fellowship was awarded by UGC-BSR, Government of India.

Competing Interests

None.

Ethical Approval

This work was approved by the ethical committee, "Institutional Ethical Committee of Interdisciplinary Biotechnology Unit [Biot/307/01.06.13]" at Aligarh Muslim University, Aligarh, India.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.02.006.

References

[1] Laxminarayan R, Chaudhury RR. Antibiotic resistance in India: drivers and opportunities for action. PLOS Med 2016;13:e1001974.
[2] Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 2009;53:5046–54.
[3] Clinical Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100S. 26th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
[4] Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother 2011;55:5403–7.
[5] Poirel L, Bonnin RA, Boulanger A, Schrenzel J, Kaase M, Nordmann P. Tn125-related acquisition of blaNDM-like genes in Acinetobacter baumannii. Antimicrob Agents Chemother 2012;56:1087–9.

Shadab Parvez
Lama Misba
Quazi T. Ahmad
Asad U. Khan*
Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, 202 002, India
*Corresponding author: Prof. Asad U. Khan, Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh-202 002, UP, India. Ph.: 0091-9837021912, Fax: 0091-571-2721776.
E-mail address: asad.k@rediffmail.com (A.U. Khan)

Received 21 November 2018
Accepted 6 February 2019