Identification of a Novel NDM Variant, *blaNDM-3*, From a Multidrug-Resistant *Acinetobacter baumannii*

To the Editor—Acinetobacter baumannii is a rapidly emerging pathogen in the healthcare setting. *Acinetobacter* infections usually occur in severely ill patients in the intensive care unit, and the associated mortality rate is high, ranging from 26% to 68%.[1] *Acinetobacter* species have a tendency to rapidly develop antimicrobial resistance to many classes of antibiotics. Increasing antimicrobial resistance leaves few therapeutic options to treat *Acinetobacter* infections. In the present study, *A. baumanii* was isolated from a patient who suffered serious burn injuries. The susceptibility of *A. baumanii* was tested against antimicrobial agents according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution procedure and interpretation criteria. *A. baumanii* showed resistance to the following antibiotics: aztreonem (≥32 mg/L), ceftazidime/clavulanic acid (≥128 mg/L), cefepime (≥64 mg/L), tobramycin (≥128 mg/L), ceftriaxone (≥32 mg/L), gentamicin (≥16 mg/L), amikacin (≥64 mg/L), imipenem (≥32 mg/L), meropenem (≥32 mg/L), colistin (≥16 mg/L) and tigecycline (≥16 mg/L).

Multiplex PCR approaches were used to search for Ambler class B (*blaIMP*, *blaVIM*, *blaSIM*, *blaSPM*, and *blaNDM-1*) and carbapenem-hydrolyzing class D β-lactamase genes (*blaOXA-51*, *blaOXA-23*, *blaOXA-24*, and *blaOXA-58*) as previously described.[2,3] Multiplex PCRs for class D β-lactamases and MBL genes were positive only for *blaOXA-51* and *blaNDM-1* genes in *A. baumanii*. The sequence analysis of the PCR product showed 99% identity with the *blaNDM-2* previously reported in *A. baumannii* from Egypt.[4] The sequence of the *blaNDM* gene detected in this study showed 4 substitutions at the protein level, compared with *blaNDM-1*. The first change resulted in an amino acid substitution from P (proline) to A (alanine) at position 28, as was previously described and named *blaNDM-2*.[4] The other 3 amino acid substitutions revealed in this study have not been reported previously: (1) from A to T (threonine) at position 99, (2) from P to L (leucine) at position 150, and (3) from L to P at position 221. This new variant was designated as bla*NDM-3* (GenBank accession no. KU220611).

Plasmid identification experiments were unsuccessful.[5] Genetic transformation of electrocompetent *Escherichia coli* DH5α with cell-free extract of *A. baumannii* also failed, which suggests that the *blaNDM-3* gene was chromosomally encoded in *A. baumannii*. Detection of *blaNDM-1* gene on the chromosome has also been reported previously.[4] To determine the genetic structure surrounding the *blaNDM-3* gene, inverse PCR was performed. An analysis of the genetic surroundings showed that the *blaNDM-2* gene was similar to that described for plasmid pNDM-HK[6] with *ble* (bleomycin resistance) and *trpF* (N-[5′-phosphoribosyl] anthranilate isomerase) genes downstream and an insertion sequence (ISAba125) upstream close to the promoter region (Figure 1). Thus, *blaNDM-3* flanked by an insertion sequence can be shuttled between plasmids and the chromosome.

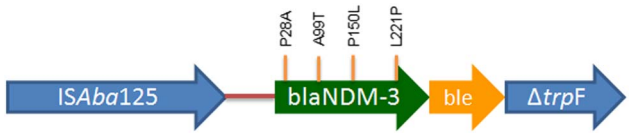

FIGURE 1. Genetic surroundings of the bla_{NDM-3} in *A. baumannii*. IS, insertion sequence; *ble*, bleomycin resistance gene; *trpF*, *N*-(5′-phosphoribosyl) anthranilate isomerase.

In conclusion, this study reports for the first time a new variant of *blaNDM-1* producing a multidrug-resistant *A. baumannii* isolate from India. The presence of an insertion sequence around the *blaNDM-3* gene may contribute to the dissemination of the *blaNDM*-like genes with a likelihood of the emergence of more variants. Epidemiological control and identification of new mechanisms of resistance will contribute to better applications of therapeutic measures and to the prevention of an increase in resistance.

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Mohit Kumar, PhD[1]

Affiliations: Biotechnology and Bioinformatics, NIIT University, Neemrana, Rajasthan, India

Address correspondence to Mohit Kumar, PhD, Biotechnology and Bioinformatics, NIIT University, Neemrana, Rajasthan-301705, India (kumarmohit@yahoo.com).

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Menacing Emergence of Fosfomycin Resistance Among *Klebsiella pneumoniae* Carbapenemase–2-Producing *K. pneumoniae* Driven by Prior Use in Critically Ill Patients

To the Editor—Owing to the widespread prevalence of carbapenemase-producing Enterobacteriaceae resistant to last-resource therapeutic options, including extended-spectrum β-lactams, fluoroquinolones, and aminoglycosides, an interest in old antimicrobial agents, such as polymyxins and fosfomycin, has reignited.[1] The latter is an agent that acts inhibiting the formation of a precursor of peptidoglycan (ie, a cell wall–acting agent); it was first used in the treatment of uncomplicated urinary tract infections but, nowadays, is being used (still on a small scale in our institution) as an adjunct to other active agents for the treatment of *Klebsiella pneumoniae* carbapenemase (KPC)–2-producing *K. pneumoniae* (KPC-2-Kp) infections.[2]

Although high in vitro frequency of fosfomycin resistance mutations has been reported,[3] resistance rates to this agent have remained relatively low since its introduction in clinical practice. On the other hand, a substantially higher resistance rate has been noted when carbapenemase producers are considered.[3,4] Furthermore, a report by Karageorgopoulos et al[5] identified patients who were treated with fosfomycin for an initially fosfomycin-susceptible KPC-2-Kp bacteremia but from whom a fosfomycin-resistant isolate was subsequently collected. However, the potential of in vivo emergence of fosfomycin-resistance among KPC-2-Kp isolates has not been systematically investigated so far.

Thus, this study aimed to perform a survey on the subsequent emergence of fosfomycin resistance among intensive care unit patients from whom a fosfomycin-susceptible KPC-2-Kp isolate was previously collected at a tertiary hospital in southern Brazil from April 1, 2013, through May 31, 2015. KPC-2-Kp was defined according to carbapenem resistance patterns, with phenotypic testing results determined as proposed by Clinical and Laboratory Standards Institute guidelines[6] and through $bla_{\text{KPC-2}}$ gene detection by polymerase chain reaction as previously reported.[7] Cases were defined as patients from whom a fosfomycin-resistant isolate was recovered from urine and/or blood cultures more than 48 hours but less than 90 days after the day a urinary fosfomycin-susceptible isolate was collected. Data on antibiotic exposures between the first KPC-2-Kp isolation and the day on which a positive culture for a fosfomycin-resistant isolate was obtained were recorded.

Eighty-five patients had a urinary KPC-2-Kp isolate collected during the period of this study and 35 of them (41.2%; 95% CI, 31.3%–51.8%) had a subsequent isolation of this same pathogen: 20 patients with a recurrent bacteriuria, 10 patients presenting a bloodstream infection, and 5 patients with both. Each patient was considered only once as a case and therefore for those patients in whom a KPC-2-Kp was recovered from a recurrent bacteriuria and blood, only the latter was considered. Among these 35 patients, in 32 (91.4%) a fosfomycin-susceptible KPC-2-Kp isolate had been previously recovered. For the 3 patients presenting a fosfomycin-resistant KPC-2-Kp isolate, the subsequent fosfomycin susceptibility remained unaltered. On the other hand, for those 32 patients with a prior fosfomycin-susceptible isolate, in 8 patients (25%) the subsequent KPC-2-Kp isolate was resistant to fosfomycin. When evaluating the previous use of antibiotics, 5 of these 8 patients (62.5%; odds ratio, 9.6 [95% CI, 1.6–56.9], $P = .013$) had already received fosfomycin to treat the first urinary KPC-2-Kp isolate (Table 1).

TABLE 1. Microbiologic Features and Patients' Data in Study of Emergence of Fosfomycin Resistance

	Fosfomycin Etest MIC, mg/L[a]			
Patients	First[b]	Subsequent[c]	Antibiotic used to treat the first isolate	Clinical site
1	12.0	128	None	Urine
2	6.0	128	Polymyxin B / meropenem	Urine
3	32.0	>1024	**Fosfomycin**	Urine and blood
4	32.0	>1024	**Fosfomycin**	Urine
5	16.0	1024	**Fosfomycin**	Urine
6	32.0	>1024	Polymyxin B /meropenem / ertapenem	Urine and blood
7	32.0	>1024	**Fosfomycin**	Urine
8	32.0	>1024	**Fosfomycin**	Urine

NOTE. All isolates were *Klebsiella pneumoniae* with carbapenem resistance via $bla_{\text{KPC-2.}}$ MIC, minimum inhibitory concentration.
[a]Considering ≤64 mg/L and >64 mg/L as susceptible and resistant, respectively.
[b]First urinary *K. pneumoniae* carbapenemase (KPC)–2-producing *K. pneumoniae* (KPC-2-Kp) isolate.
[c]Subsequent KPC-2-Kp isolate, considering a hospitalization period of >48 hours and <90 days following first isolation.

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