Emergence of $\text{bla}_{\text{NDM-1}}$ associated with the $\text{aac}(6')$-$\text{lb}$-$\text{cr}$, $\text{acrB}$, $\text{cps}$, and $\text{mrkD}$ genes in a clinical isolate of multi-drug resistant *Klebsiella pneumoniae* from Recife-PE, Brazil

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**Abstract**

**Introduction**: The emergence of New Delhi metallo-$\beta$-lactamase (NDM) is concerning because it reduces the antibiotic therapy options for bacterial infections. **Methods**: Resistant and virulent genes from an isolate of *Klebsiella pneumoniae* derived from a patient with sepsis in a hospital in Recife-PE, Brazil, were investigated using PCR and DNA sequencing. **Results**: $\text{bla}_{\text{NDM-1}}$, $\text{aac}(6')$-$\text{lb}$-$\text{cr}$ and $\text{acrB}$ resistance genes, and $\text{cps}$ and $\text{mrkD}$ virulence genes were detected. **Conclusions**: To our knowledge, this is the first report on $\text{bla}_{\text{NDM-1}}$ in Recife-PE. This detection alerts researchers to the need to control the spread of $\text{bla}_{\text{NDM-1}}$ resistance gene by this bacterium in Brazil.

**Keywords**: $\text{bla}_{\text{NDM-1}}$, *Klebsiella pneumoniae*. Resistance. Virulence.

New Delhi metallo-$\beta$-lactamase (NDM) is a $\beta$-lactamase classified as Ambler class B, and it differs from other carbapenemases because it uses zinc in its active site, which facilitates antimicrobial hydrolysis and confers resistance against all $\beta$-lactam antibiotics except aztreonam. The $\text{bla}_{\text{NDM-1}}$ gene was first detected in 2009 in isolates of *Klebsiella pneumoniae* and *Escherichia coli* from the feces of a Swedish patient in India[1]. Since this first description, $\text{bla}_{\text{NDM-1}}$ has been reported worldwide[2]. In South America, $\text{bla}_{\text{NDM-1}}$ was reported in Uruguay in a *Providencia rettgeri* isolate and in Brazil in the state of Rio Grande do Sul. In both countries, $\text{bla}_{\text{NDM-1}}$ was reported for the first time in the same species[3].

In addition to its resistance mechanisms, these *K. pneumoniae* isolates may present several virulence factors, those that stand out are the production of polysaccharide capsules, fimbrial adhesin type 3, and yersiniabactin. Fimbrial adhesins type 3 can mediate the binding of *K. pneumoniae* isolates to various human cells, such as the endothelial and epithelial cells of the respiratory tract and urinary tract[4]. The accumulation of virulence genes along with resistance genes may facilitate infection and limit therapeutic options.

This paper analyzes a *K. pneumoniae* isolate (K2-R2) from a female patient with sepsis who was admitted to the clinical medicine department of a public hospital in Recife, Brazil, on 12/04/2016. The K2-R2 isolate was pre-selected because it is involved in sepsis and is multi-drug resistant (MDR), including to carbapenems. The isolate was biochemically identified using the automated (Bactec 9120/Phoenix-BD system). The culture was preserved in 20% glycerol at -70 °C and grown in the medium of Brain Heart Infusion (BHI) at 37 °C for 18 hours prior to analysis. Susceptibility to several classes of antimicrobials was detected using the automated (Bactec 9120/Phoenix-BD system). The culture was preserved in 20% glycerol at -70 °C and grown in the medium of Brain Heart Infusion (BHI) at 37 °C for 18 hours prior to analysis. Susceptibility to several classes of antimicrobials was tested: amikacin, ampicillin, ampicillin/sulbactam, ceftazidime, cefepime, cefotaxim, ceftriaxone, cefuroxime, colistin, gentamycin, ertapenem, imipenem, meropenem, and tigecycline. Interpretation was performed according to the criteria of the Clinical and Laboratory Standards Institute (CLSI)[5]. The genomic DNA of the K2-R2 isolate was extracted using the Wizard Genomic DNA purification kit (Promega) in accordance with the manufacturer’s instructions. The genes encoding resistance to carbapenems ($\text{bla}_{\text{KPC}}$, $\text{bla}_{\text{VIM}}$, $\text{bla}_{\text{GES}}$).
*bla*<sub>NDM</sub>-1 and *bla*<sub>NDM</sub>-1), those encoding resistance to aminoglycoside (*aac(3')-Ia; aac(3')IIa, and *aac(6')-Ib*), the efflux pump gene (*acrB*), and the virulence genes (*cps, mrkD and irp2*) were investigated using the (polymerase chain reaction (PCR) technique. A description of the primers and amplification conditions utilized are presented in Table 1<sup>6-12</sup>. Negative and positive controls were included in each PCR. The amplified products were electrophoresed in 1% agarose gel under a constant voltage of 100V in 0.5X (Tris-base boric acid (TBE) buffer and (Ethylene di amine tetra-acetic acid (EDTA).

The amplicons were purified using the Wizard®SV Gel and PCR Clean-Up System (Promega). After purification, they were quantified in nano-drops and sequenced (3500 Genetic Analyzer - Applied Biosystems). Sequences were analyzed using Chromas software (http://www.mybiosoftware.com/sequence-analysis) and compared to sequences deposited in the GenBank databases (http://www.ncbi.nlm.nih.gov/blast/) using the (Basic Local Alignment Search (BLAST) tool. After the BLAST comparison, the nucleotide sequences were translated into proteins with the (Sequence Manipulation Suite (http://www.bioinformatics.org/sms2/trans_map.html) using the Translation Map tool.

The *K. pneumoniae* isolate exhibited resistance to multiple drugs, such as penicillin, β-lactamase inhibitors, cephalosporins, aminoglycoside, and carbapenems (Table 2), and only exhibited sensitivity to amikacin, ciprofloxacin, colistin, and tigecycline. The PCR and sequencing analyses demonstrated the presence of the resistance genes *bla*<sub>NDM</sub>-1 and *aac(6')-Ib-cr*, the virulence genes *cps* and *mrkD* and the gene for the efflux pump *acrB*. The sequence of the gene *bla*<sub>NDM</sub>-1 was deposited into GenBank under the following accession number: MH818328. The genes

### Table 1: Primers used in PCR and sequencing to detect resistance genes, efflux pump and virulence genes in *Klebsiella pneumoniae* clinical isolate.

| Primer       | Sequence (5’-3’)                                                        | Temp.<sup>a</sup> | Reference                | Gene       |
|--------------|------------------------------------------------------------------------|-------------------|--------------------------|------------|
| KPC1a        | TGTCACTGTATCGCCGTC                                                      | 63°C              | Cabral et al. (2017)<sup>6</sup> | *bla*<sub>KPC</sub> |
| KPC1b        | CTCACTGTATCGCCGTC                                                      |                   |                          |            |
| VIM-F        | CAG ATT GCC GAT GGT GTT TGG                                            | 64°C              | Cabral et al. (2017)<sup>6</sup> | *bla*<sub>VIM</sub> |
| VIM-R        | AGG TGG GCC ATT CAG CCA GA                                              |                   |                          |            |
| GES-F        | ATGGCGGTTCATTTACGAGTC                                                  | 60°C              | Bagheri-Nesami et al. (2016)<sup>7</sup> | *bla*<sub>GES</sub> |
| GES-R        | CTGGCGGTTCATTTACGAGTC                                                  |                   |                          |            |
| IMP-F        | GGA ATA GAG TGG CTT AAT TCT C                                         | 60°C              | Cabral et al. (2017)<sup>6</sup> | *bla*<sub>IMP</sub> |
| IMP-R        | GTG ATG CTT CYCCAA AYTT CAC T                                         |                   |                          |            |
| NDM-F        | GGTGGGCGATCTGGTTC                                                     | 52°C              | Poirel et al. (2011)<sup>8</sup> | *bla*<sub>NDM</sub> |
| NDM-R        | GGAAATTGCTCATCAGGAT                                                    |                   |                          |            |
| AAC(3')-Ia-F | GACATAAGCTCTGGGTT                                                     | 55°C              | Noppe-Leclercq et al. (1999)<sup>9</sup> | aac(3')-Ia |
| AAC(3')-Ia-R | CTCCGAATCTACGAGGA                                                     |                   |                          |            |
| AAC(3')-IIa-F| GCGAAACCGGCTTC                                                       | 55°C              | Noppe-Leclercq et al. (1999)<sup>9</sup> | aac(3')-IIa; |
| AAC(3')-IIa-R| TCCGAGGCTTC                                                          |                   |                          |            |
| AAC(6')-Ib-F | TATGATGCTACGGTGGTT                                                     | 55°C              | Noppe-Leclercq et al. (1999)<sup>9</sup> | aac(6')-Ib-cr |
| AAC(6')-Ib-R | CCGCGGTGCAGGTGTA                                                      |                   |                          |            |
| ACRB-F       | TCAACCGGTGCTGGGCT                                                        | 61°C              | Scavuzzi et al. (2017)<sup>10</sup> | acrB       |
| ACRB-R       | TTAATCCACGAGGGGAGGTGC                                                   |                   |                          |            |
| CPS-F        | TCCAAATTGCTGGGGA                                                       | 63°C              | Hennequin e Forestier (2007)<sup>11</sup> | cps        |
| CPS-R        | GGCTGGCGGACGGATGGA                                                     |                   |                          |            |
| MRKD-2 F     | CCA CCA ACT CCC TGG AA                                                 | 58°C              | Melo et al. (2014)<sup>12</sup> | mrkD       |
| MRKD-2 R     | ATGGCGGCTTC                                                          |                   |                          |            |
| IRP2 F       | ATT TCT GCC GCA CCA TCT                                                | 65°C              | Melo et al. (2014)<sup>12</sup> | irp2       |
| IRP2 R       | GCCTGGCGGTATT AGC GAC TGC TGGA                                        |                   |                          |            |

(a) Temp: annealing temperature of the primers.
The co-production of bla\textsubscript{NDM-1}, with other \(\beta\)-lactamases or with genetic determinants related to resistance to quinolones, such as \(aac(6')\)-Ib-cr, are also frequently detected in enterobacteria; this corroborates the findings presented in this paper\textsuperscript{6}. Besides the association of bla\textsubscript{NDM-1} and \(aac(6')\)-Ib-cr, the presence of an efflux pump and virulence genes was also verified, which demonstrates the presence of different associated genetic mechanisms. The virulence factors detected in the K2-R2 isolate suggest that, in addition to multi-antimicrobial resistance, this bacterium exhibits important mechanisms that lead to infection, such as the potential to resist phagocytosis due to the presence of the \(cps\) gene and the ability to adhere and form biofilm on the surface of catheters due to the gene encoding type 3 fimbria (\(mrkD\))\textsuperscript{12}.

This accumulation of resistance genes in association with the efflux pump and virulence genes in \(K.\) \textit{pneumoniae} limit the therapeutic options, which explains many failures in the attempts to control healthcare-associated infections (HAIs) caused by this species. The detection of bla\textsubscript{NDM-1} in \(K.\) \textit{pneumoniae} in Recife, Brazil, highlights the need to adopt urgent and rigorous effective measures to control the spread of this carbapenemase in all regions of the country. If a set of control measures is not adopted, the proliferation of bla\textsubscript{NDM-1} will likely occur in Brazil, in the same manner as the proliferation of bla\textsubscript{KPC-2}.

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### Conflict of Interest

The authors declare that there is no conflict of interest.

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