

Short Communication

First report of a bla_{NDM} -resistant gene in a *Klebsiella aerogenes* clinical isolate from Brazil

Cynthia Regina Pedrosa Soares^[1], Jorge Belém Oliveira-Júnior^[2]
and Elza Ferreira Firmo^[1]

[1]. Universidade Federal de Pernambuco, Departamento de Medicina Tropical, Recife, PE, Brasil.

[2]. Instituto Aggeu Magalhães, Laboratório de Biologia Molecular e Celular, Departamento de Parasitologia, Recife, PE, Brasil.

Abstract

Introduction: Carbapenemase-resistant enterobacteria that produce the bla_{NDM} gene are found worldwide. However, this is the first report of bla_{NDM} in *Klebsiella aerogenes* in Brazil. **Methods:** The identification of bacterial species was performed using an automated system and confirmed by biochemical tests, 16S rRNA gene sequencing, and detection of resistance genes. **Results:** The clinical isolate showed minimum inhibitory concentration resistance to meropenem and polymyxin B at 8mg/L and 4mg/L, respectively. Only the bla_{NDM} gene was detected. **Conclusions:** The current report of the bla_{NDM} gene in isolated MDR enterobacteria indicates that this gene can spread silently in a hospital setting.

Keywords: *Klebsiella aerogenes*. bla_{NDM} gene. metallo-beta-lactamase. Carbapenemase. Multidrug-resistant.


New Delhi metallo-beta-lactamase (NDM), which codes for the bla_{NDM} gene, is found in carbapenemase-producing Enterobacteriaceae, and the highest distributions of bla_{NDM} variants have been detected in *Klebsiella pneumoniae* and *Escherichia coli*^{1,2}. The occurrence of the bla_{NDM} gene in *Klebsiella aerogenes* has also been documented in countries such as China³, South Korea⁴, Tunisia⁵, Japan⁶, and India^{7,8}. In South America, the bla_{NDM} gene was first reported in 2012 in Uruguay⁹. In Brazil, this gene has been detected in only 11 isolates (0.97%) from Porto Alegre, with nine from *Enterobacter cloacae* and two from *Morganella morganii* complexes. Moreover, other isolates showed high resistance to carbapenems¹⁰. The spread of the bla_{NDM} gene in various species of gram-negative bacilli has occurred in different Brazilian regions¹¹. In 2019, the first report of *Proteus mirabilis* and *Serratia marcescens* carrying bla_{KPC-2} and bla_{NDM-1} genes in Brazil was published¹². The bla_{NDM-1} gene product is NDM-1, which hydrolyzes a wide range of β -lactam antimicrobials, including carbapenems, which are the last-resort antimicrobials in the treatment of infections caused by antimicrobial-resistant bacteria. The emergence of bacteria carrying

these genes represents a challenge in the treatment of infections¹. The objective of this study was to describe the occurrence of the bla_{NDM} gene in a clinical isolate of *K. aerogenes* resistant to aminopenicillin, beta-lactam + beta-lactamase inhibitors, and 1st, 2nd, 3rd, and 4th generation beta-lactams, as well as carbapenems.

Identification of bacterial species was performed using a Vitek-2 automated system (bioMérieux, Marcy l'Etoile, France), and confirmed by biochemical tests and 16S rRNA gene sequencing. PCR and DNA sequencing were performed to track the presence of the bla_{NDM} gene in two duplicates, collected at a referral hospital in Recife (Pernambuco, Brazil) in 2019. A universal primer pair, NDM-F (5-CGGAATGGCTCATCACGATC-3) and NDM-R (5-GGTTTGGCGATCTGGTTTTC-3)¹³ were used for screening, as previously described. Furthermore, the entire bla_{NDM} region was amplified and sequenced based on consensus sequences flanking the gene. The analysis showed that the bla_{NDM} gene was present in a *K. aerogenes* clinical isolate from a patient with bacteremia hospitalized in an intensive care unit (ICU). The minimum inhibitory concentrations (MIC) of meropenem and polymyxin B for this isolate were 8mg/L and 4mg/L, respectively. In addition, this isolate presented a susceptibility of 8mg/L to amikacin and 1mg/L to colistin, according to the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST)¹⁴. These data were confirmed by colorimetric assays with resazurin¹⁵.

Corresponding author: Msc. Cynthia Regina Pedrosa Soares.

e-mail: cynthiaregina@msn.com

 <https://orcid.org/0000-0001-8915-859X>

Received 11 May 2020

Accepted 2 September 2020

The *K. aerogenes* isolate showed an antimicrobial resistance profile (R) to cefepime, ceftazidime, ceftoxitin, ceftriaxone, ciprofloxacin, ampicillin, ampicillin/sulbactam, cefotaxime, and carbapenems (imepenem, meropenem, ertapenem), which differs from its resistance profile in China³. High resistance to carbapenems has also been observed in other Enterobacteriaceae isolates¹⁰. However, it was susceptible (S) to amikacin, gentamicin, tobramycin, tigecycline, colistin, and polymyxin B, and sensitive, with increasing exposure (I), to ciprofloxacin, according to BrCAST¹⁴. The present study detected the presence of the broad-spectrum beta-lactamase and beta-lactamase class C beta-lactamase phenotypes in *K. aerogenes* isolates. The presence of *bla*_{NDM} was confirmed by polymerase chain reaction (PCR). To our knowledge, this is the first report of the *bla*_{NDM} gene in clinical isolates of *K. aerogenes* from Brazil. In addition, other carbapenemase genes, such as *bla*_{IMP}, *bla*_{VIM}, *bla*_{GES}, *bla*_{OXA-48}, and *bla*_{KPC} were investigated, as well as the *mcr-1* gene. Despite being susceptible to polymyxin B by automated testing, the MIC showed resistance, but the *mcr-1* gene was not detected. However, none of the other genes were detected in the clinical isolates used. NDM-producing strains, including carbapenems, cephamycin, extended-spectrum cephalosporins, aminoglycosides, monobactams, tetracyclines, fluoroquinolones, and polymyxin and colistin⁸, were detected in neonates in the ICU with high antibiotic resistance.

NDM-1-producing Enterobacteriaceae has spread rapidly in hospitalized patients in several countries. Thus, the spread of carbapenemase-resistant genes has been a major concern worldwide, as it poses a threat to the antimicrobial treatment of infections caused by multidrug-resistant isolates. Although strains that produce *bla*_{NDM} appear less frequently in Brazil, it is nonetheless necessary to control epidemiological surveillance to control infections.

Nucleotide sequence accession number: The nucleotide sequence of the *bla*_{NDM} gene in the *K. aerogenes* isolate was GenBank/EMBL/DDBJ, accession N^o. MN735678.1.

This study was approved by the ethics committee of our institution (CAAE: 20195119.3.0000.5586), opinion number 3,787,364, and (CAAE: 20195119.3.3001.5198) opinion number 3,838,892, following Resolution 466/12 of the National Health Council.

ACKNOWLEDGMENTS

We thank all the staff and patients of the referral public hospital for their collaboration.

FINANCIAL SUPPORT

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHORS' CONTRIBUTION

CRPS: Conception and design of the study, acquisition, analysis and interpretation of data; JBOJ: Conception, implementation of the MIC and translation of the article into English. EFF: Conception and design of the study, analysis and interpretation of data and final approval of the version to be submitted.

CONFLICTS OF INTEREST

All authors report no conflicts of interest.

REFERENCES

- Khan AU, Maryam L, Zarrilli R. Structure, Genetics and Worldwide Spread of New Delhi Metallo-β-lactamase (NDM): a threat to public health. *BMC Microbiol.* 2017; 17(1):101.
- Pérez-Vázquez M, Sola Campoy PJ, Ortega A, Bautista V, Monzón S, Ruiz-Carrasco G, et al. Emergence of NDM-producing *Klebsiella pneumoniae* and *Escherichia coli* in Spain: phylogeny, resistome, virulence and plasmids encoding bla_{NDM}-like genes as determined by WGS. *J Antimicrob Chemother.* 2019;74(12):3489-96.
- Shen Y, Xiao WQ, Gong JM, Pan J, Xu QX. Detection of New Delhi Metallo-Beta-Lactamase (Encoded by bla_{NDM-1}) in *Klebsiella aerogenes* in China. *J Clin Lab Anal.* 2017;31(2):1-5.
- Yoon EJ, Kang DY, Yang JW, Kim D, Lee H, Lee KJ, et al. New Delhi Metallo-Beta-Lactamase-Producing Enterobacteriaceae in South Korea Between 2010 and 2015. *Front Microbiol.* 2018; 29:9:571.
- Ben Helal R, Dziri R, Chedly M, Klibi N, Barguelli F, El MS Asli, et al. Occurrence and Characterization of Carbapenemase-Producing Enterobacteriaceae in a Tunisian Hospital. *Microb Drug Resist.* 2018;24(9):1361-7.
- An J, Guo L, Zhou L, Ma Y, Luo Y, Tao C, et al. NDM-producing Enterobacteriaceae in a Chinese hospital, 2014-2015: identification of NDM-producing *Citrobacter werkmanii* and acquisition of bla_{NDM-1}-carrying plasmid in vivo in a clinical *Escherichia coli* isolate. *J Med Microbiol.* 2016;65(11):1253-9.
- Khalid S, Ahmad N, Ali SM, Khan AU. Outbreak of Efficiently Transferred Carbapenem-Resistant bla_{NDM}-Producing Gram-Negative Bacilli Isolated from Neonatal Intensive Care Unit of an Indian Hospital. *Microb Drug Resist.* 2020;26(3):284-9.
- Ahmad N, Khalid S, Ali SM, Khan AU. Occurrence of bla_{NDM} Variants Among Enterobacteriaceae From a Neonatal Intensive Care Unit in a Northern India Hospital. *Front Microbiol.* 2018;9:407.
- Carvalho-Assef AP, Pereira PS, Albano RM, Berião GC, Chagas TP, Timm LN, et al. Isolation of NDM -producing *Providencia rettgeri* in Brazil. *J Antimicrob Chemother.* 2013;68(12):2956-7.
- Rozales FP, Ribeiro VB, Magagnin CM, Pagano M, Lutz L, Falci DR, et al. Emergence of NDM-1-producing Enterobacteriaceae in Porto Alegre, Brazil. *Int J Infect Dis.* 2014;(25):79–81.
- da Silva IR, Aires CAM, Conceição-Neto OC, de Oliveira Santos IC, Ferreira Pereira N, Moreno Senna JP, et al. Distribution of Clinical NDM-1-Producing Gram-Negative Bacteria in Brazil. *Microbial Drug Resistance.* 2019;25(3):394-9.
- Firmo EF, Beltrão EMB, Silva FRF, Alves LC, Brayner FA, Veras DL, et al. Association of bla_{NDM-1} with bla_{KPC-2} and aminoglycoside-modifying enzymes genes among *Klebsiella pneumoniae*, *Proteus mirabilis* and *Serratia marcescens* clinical isolates in Brazil. *J Glob Antimicrob Resist.* 2019;21:255-61.
- Nithia N, Remitha R, Jayasree PR, Faisal M, Manish Kumar PR. Analysis of beta-lactamases, bla_{NDM-1} phylogeny & plasmid replicons in multidrug-resistant *Klebsiella* spp. from a tertiary care centre in south India. *Indian J Med Res.* 2017;146(1):38–45.
- BrCAST - Brazilian Committee on Antimicrobial Susceptibility Testing. Tabelas de pontos de corte clínicos 2020-01-05-2020 [updated 2020 January 05; cited 2020 Feb 14]. Available in: <http://brcast.org.br/documentos/>.
- Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.* 2002;46(8):2720-2.