Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:52:e20180352: 2019
doi: 10.1590/0037-8682-0352-2018

Short Communication

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

***Alexsandra Maria Lima Scavuzzi*[1], *Elza Ferreira Firmo*[1], *Érica Maria de Oliveira*[1], *and Ana Catarina de Souza Lopes*[1].**

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

Abstract

Introduction: The emergence of New Delhi metallo-β-lactamase (NDM) is concernig 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:** bla_{NDM-1}, *aac(6')-Ib-cr* and *acrB* resistance genes, and *cps* and *mrkD* virulence genes were detected**. Conclusions**: To our knowledge, this is the first report on bla_{NDM-1} in Recife-PE. This detection alerts researchers to the need to control the spread of bla_{NDM-1} resistance gene by this bacterium in Brazil.

Keywords: bla_{NDM-1}. *Klebsiella pneumoniae*. Resistance. Virulence.

New Delhi metallo-β-lactamase (NDM) is a β-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 β-lactam antibiotics except aztreonam. The bla_{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, bla_{NDM-1} has been reported worldwide[2]. In South America, bla_{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, bla_{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 ºC and grown in the medium of Brain Heart Infusion (BHI) at 37 ºC for 18 hours prior to analysis. Susceptibility to several classes of antimicrobials was detected using the automated Bactec 9120 (Phoenix BD) system, and susceptibility to the following antimicrobials was tested: amikacin, ampicillin, ampicillin/sulbactam, ceftazidime, cefepime, cefoxitin, ciprofloxacin, 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 (bla_{KPC}, bla_{VIM}, bla_{GES},

Corresponding author: Profª Drª Ana Catarina de Souza Lopes.
e-mail: ana.lopes.ufpe@gmail.com
Orcid: 0000-0003-0277-108X
Received 30 August 2018
Accepted 1 March 2019

bla$_{IMP}$ and *bla*$_{NDM}$), 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**[6-12]. 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 (Ethylenediamine 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*$_{NDM-1}$ 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*$_{NDM-1}$ 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.[(a)]	Reference	Gene
KPC1a	TGTCACTGTATCGCCGTC	63°C	Cabral et al. (2017)[6]	*bla*$_{KPC}$
KPC1b	CTCAGTGCTCTACAGAAAACC			
VIM-F	CAG ATT GCC GAT GGT GTT TGG	64°C	Cabral et al. (2017)[6]	*bla*$_{VIM}$
VIM-R	AGG TGG GCC ATT CAG CCA GA			
GES-F	ATGCGCTTCATTCACGCAC	60°C	Bagheri-Nesami et al. (2016)[7]	*bla*$_{GES}$
GES-R	CTATTTGTCCGTGCTCAGG			
IMP-F	GGA ATA GAG TGG CTT AAT TCT C	60°C	Cabral et al. (2017)[6]	*bla*$_{IMP}$
IMP-R	GTG ATG CGT CYC CAA YTT CAC T			
NDM-F	GGTTTGGCGATCTGGTTTTC	52°C	Poirel et al. (2011)[8]	*bla*$_{NDM}$
NDM-R	CGGAATGGCTCATCACGATC			
AAC(3')-Ia-F	GACATAAGCCTGTTCGGTT	55°C	Noppe-Leclercq et al. (1999)[9]	*aac(3')-Ia*
AAC(3')-Ia-R	CTCCGAACTCACGACCGA			
AAC(3')-IIa-F	GGCAATAACGGAGGCGCTTCAAAA	55°C	Noppe-Leclercq et al. (1999)[9]	*aac(3')-IIa;*
AAC(3')-IIa-F	TTCCAGGCATCGGCATCTCATACG			
AAC(6')-Ib-F	TATGAGTGGCTAAATCGAT	55°C	Noppe-Leclercq et al. (1999)[9]	*aac(6')-Ib-cr*
AAC(6')-Ib-R	CCCGCTTTCTCGTAGCA			
ACRB-F	TCAAACCAGGTGTGCAGGTA	61°C	Scavuzzi et al. (2017)[10]	*acrB*
ACRB-R	TTAATACCCAGACCGGATGC			
CPS-F	TCCCAATTGTGACCGAAATC	63°C	Hennequin e Forestier (2007)[11]	*cps*
CPS-R	GCTCGCGGCACCAGCTGA			
MRKD-2 F	CCA CCA ACT ATT CCC TCG AA	58°C	Melo et al. (2014)[12]	*mrkD*
MRKD-2 R	ATG GAA CCC ACA TCG ACA TT			
IRP2 F	ATT TCT GGC GCA CCA TCT	65°C	Melo et al. (2014)[12]	*irp2*
IRP2 R	GCG CCG GGT ATT ACG GAC TTC			

(a) Temp: annealing temperature of the primers.

TABLE 2: Minimum inhibitory concentration values (MICs) for *Klebsiella pneumoniae* K2-R2 clinical isolate, a carrier of the resistance genes bla_{NDM-1}, *aac(6´)-Ib-cr,* and the *acrB* efflux pump from Recife-PE, Brazil.

Antimicrobial	MIC µg/mL	Interpretation
Amikacin	≤ 2	S
Ampicillin	≥ 32	R
Ampicillin/Sulbactam	≥ 32	R
Cefepime	8	I
Cefoxitin	≥ 64	R
Ceftazidime	≥ 64	R
Ceftriaxone	≥ 64	R
Cefuroxime	≥ 64	R
Ciprofloxacin	≤ 0,25	S
Colistin	≤ 0,5	S
Ertapenem	≥ 8	R
Gentamycin	≥ 16	R
Imipenem	≥ 16	R
Meropenem	≥ 16	R
Tigecycline	≤ 0,5	S

S: sensitive; **R:** resistant; **I:** intermediary.

bla_{KPC}, $bla_{VIM,}$ $bla_{GES,}$ $bla_{IMP,}$ *aac(3')-Ia; aac(3')IIa,* and *irp2* were not found.

To the best of our knowledge, this is the first report on the bla_{NDM-1} gene in *K. pneumoniae* isolate in Recife-PE, Brazil. In South America, the bla_{NDM-1} gene was first recorded in 2012 in Uruguay, a country bordering the Brazilian state of Rio Grande do Sul, where NDM was first described in Brazil[3]. The following year, Carvalho Assef et al. (2014)[13] performed a retrospective study and detected six strains of *Enterobacter hormaechei subsp. oharae,* which are NDM producers related to the isolates recovered in 2012, prior to the first report on bla_{NDM-1} in Brazil. Since then, other NDM-producing Enterobacteriaceae have been isolated in the South and Southeast Regions of Brazil[14].

In Northeast Brazil, Baberino et al.[15] first detected bla_{NDM-1} in *K. pneumoniae* and *Citrobacter freundii* in 2015. The present report on a *K. pneumoniae* isolate harboring the bla_{NDM-1} gene in Recife-PE demonstrates the dispersion of this gene in the Brazilian Northeast.

The resistance profile of the *K. pneumoniae* isolate suggests that the high resistance to carbapenems is related to the association of more than one resistance mechanism, which was confirmed with the concomitant presence of bla_{NDM-1} and efflux pump AcrB. In Brazil, Scavuzzi et al.[10] reported a bla_{KPC-2} resistant to various drugs that presented alongside the co-production of the *acrB* gene and an efflux pump that reduces susceptibility to several drugs in *K. pneumoniae* isolates. This may explain the MDR resistance profile of the isolate of this study.

The co-production of bla_{NDM-1} with other β-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[2]. Besides the association of bla_{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)*[12].

This accumulation of resistance genes in association with the efflux pump and virulence genes in *K. 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_{NDM-1} in *K. 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_{NDM-1} will likely occur in Brazil, in the same manner as the proliferation of bla_{KPC-2}.

Acknowledgments

We are grateful to the Central Laboratory of the CCB/UFPE-LABCEN, Brazil, for assisting us with sequencing, especially Prof. Dr. Marcos Antonio de Morais and Dr. Heidi Lacerda.

Financial Support

Fundação de Amparo a Ciência e Tecnologia de Pernambuco (FACEPE) (PPSUS)**.**

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

1. 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(12):5046-54.
2. Al-Agamy MH, Aljallal A, Radwan HH, Shibl AM. Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive *Escherichia coli* and *Klebsiella pneumoniae* in Riyadh hospitals. J Infect Public Health. 2018;11(1):64-8.
3. 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.
4. Podschun R, Fischer A, Ullman U. Expression of putative virulence factors by clinical isolates of *Klebsiella planticola*. J Med Microbiol. 2000;49(2):115-9.
5. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 16th

Informational Supplement. CLSI/NCCLS Publication M100-S17. Wayne, PA: CLSI, 2007.

6. Cabral AB, Maciel MA, Barros JF, Antunes MM, Barbosa de Castro CM, Lopes AC. Clonal spread and accumulation of β-lactam resistance determinants in *Enterobacter aerogenes* and *Enterobacter cloacae* complex isolates from infection and colonization in patients at a public hospital in Recife, Pernambuco, Brazil. J Med Microbiol. 2017;66(1):70-7.
7. Bagheri-Nesami M, Rafiei A, Eslami G, Ahangarkani F, Rezai MS, Nikkhah A, et al. Assessment of extended-spectrum β-lactamases and integrons among *Enterobacteriaceae* in device-associated infections: multicenter study in North of Iran. Antimicrob Resist Infect Control. 2016;5:52.
8. Poirel L, Revathi G, Bernabeu S, Nordmann P. Detection of NDM-1-producing *Klebsiella pneumoniae* in Kenya. Antimicrob Agents Chemother. 2011;55(2):934-6.
9. Noppe-Leclercq I, Wallet F, Haentjens S, Courcol R, Simonet M. PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for *Acinetobacter baumannii*. Res Microbiol. 1999;150(5):317-22.
10. Scavuzzi AML, Maciel MAV, de Melo HRL, Alves LC, Brayner FA, Lopes ACS. Occurrence of qnrB1 and qnrB12 genes, mutation in gyrA and ramR, and expression of efflux pumps in isolates of *Klebsiella pneumoniae* carriers of blaKPC-2. J Med Microbiol. 2017;66(4):477-84.
11. Hennequin C, Forestier C. Influence of capsule and extended-spectrum beta-lactamases encoding plasmids upon *Klebsiella pneumoniae* adhesion. Res Microbiol. 2007;158(4):339-47.
12. de Cássia Andrade Melo R, de Barros EM, Loureiro NG, de Melo HR, Maciel MA, Lopes ACS. Presence of *fimH*, *mrkD*, and *irp2* virulence genes in KPC-2 producing *Klebsiella pneumoniae* isolates in Recife-PE, Brazil. Curr Microbiol. 2014;69(6):824-31.
13. Carvalho-Assef AP, Pereira OS, Albano RM, Berião GC, Tavares CP, Chafgas TP, et al. Detection of NDM-1, CTX-M-15-, and qnrB4 producing *Enterobacter hormaechei* isolates in Brazil. Antimicrob Agents Chemother. 2014;58(4):2475-6.
14. Pereira PS, Albano RM, Asensi MD, Carvalho-Assef AP. Draft genome sequences of three NDM - 1 -producing Enterobacteriaceae species isolated from Brazil. Mem Inst Oswaldo Cruz. 2015;110(4):580-2.
15. Barberino MG, Cruvinel SA, Faria C, Salvino MA, Silva MO. Isolation of bla_{NDM}-producing Enterobacteriaceae in a public in Salvador, Bahia, Brazil. Braz J Infect Dis. 2018;22(1):47-50.