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Short Communication

# New sequence type in multidrug-resistant *Klebsiella pneumoniae* harboring the $bla_{NDM-1}$-encoding gene in Brazil

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ABSTRACT

*Objectives:* The aim of this study was to investigate the $bla_{NDM}$ gene, pathogenic potential, and antimicrobial resistance of clinical isolates of carbapenem-resistant *Klebsiella pneumoniae* isolated from patients admitted to the University Hospital of Londrina between January 2014 and March 2017.
*Methods:* $bla_{NDM-1}$ and virulence genes were investigated using conventional PCR methods Antimicrobial susceptibility testing was performed by disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines of 2017. Clonal relationships of the New Delhi metallo-β-lactamase (NDM)-positive isolates were determined by enterobacterial repetitive intergenic consensus (ERIC)-PCR and multilocus sequence typing (MLST).
*Results:* A total of 825 *K. pneumoniae* were identified, with four isolates (Kp6408, Kp6410, Kp6411, and Kp6715) presenting the $bla_{NDM-1}$ gene. All NDM-1-producing isolates showed co-production of $bla_{KPC-2}$ and $bla_{TEM}$ genes and also the virulence genes *kfu*, *entB*, *mrkD*, and *fimH*. Three isolates (Kp6408, Kp6410, and Kp6715) were classified as multidrug-resistant (MDR) and one (Kp6411) as extensively drug-resistant (XDR). ERIC-PCR analyses demonstrated that the isolates shared about 60% genetic similarity. MLST revealed four different sequence types (STs), described for the first time in Brazil, with two novel STs described in this study: ST3371 and ST3372.
*Conclusion:* This study reports the identification of NDM-1 associated with KPC and virulence genes in four MDR *K. pneumoniae* with STs first described in Brazil.


## Introduction

The first description of New Delhi metallo-β-lactamase (NDM) in Brazil was in 2013 in an isolate of *Providencia rettgeri* in the city of Porto Alegre (southern region). In 2014, the first case of NDM in *Acinetobacter baumannii* was reported in Londrina, in the state of Paraná (Pillonetto et al., 2014); however, no further NDM-producing bacteria were reported in this region after this case. Taking this into consideration, a surveillance study was conducted on clinical isolates of carbapenem-resistant *Klebsiella pneumoniae* (CRKpn) isolated from patients admitted to the University Hospital of Londrina between January 2014 and March 2017.

## Methods

From January 2014 to March 2017, 825 CRKpn isolates were isolated from different patients admitted to the University Hospital of Londrina. These isolates were identified using a Vitek 2 instrument (Vitek AMS; bioMérieux Vitek Systems, Hazelwood, MO, USA).

Antimicrobial susceptibility testing was performed by disk diffusion method as described in the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017) and by minimum inhibitory concentration (MIC) for colistin using Vitek systems. Thirty-eight different antibiotic disks (Oxoid, Basingstoke, UK) were tested; these are listed in the footnotes to Table 1. Each bacterial isolate was classified as multidrug-resistant (MDR), extensively drug-resistant (XDR), or pandrug-resistant (PDR) according to the definitions of Magiorakos et al. (2012). The strains *Escherichia coli* ATCC 25922 and ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls for these experiments.

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**Table 1**
General data for the four NDM-1-producing *Klebsiella pneumoniae* isolates from this study.

| Isolate | Year | Source | Clinical ward | Resistance profile (non-susceptible)[a] | Resistance classification[b] | β-lactamase genes | Virulence genes | MLST |
|---|---|---|---|---|---|---|---|---|
| Kp6408 | 2016 | Tissue | Burn plastic surgery | APS, AMC, CFC, CFL, CTL, CPM, CTX, CAZ, CRO, CRX, DOX, IPM, ERT, LMX, MPM, NAL, NIT, PIT, SUL, TRI, TOB, SUT | MDR | *bla*NDM-1, *bla*KPC, $bla_{TEM}$ | *kfu*, *ent*B, *mrk*D, *ycf*M, *fim*H | ST1773 |
| Kp6410 | 2016 | Urine | Orthopedic | AMI, AMC, APS, ATM, CPM, CTL, CRO, CAZ, CTX, CRX, CFL, CIP, ERT, GEN, IPM, LMX, MPM, NAL, NIT, NOR, OFX, PIT, SUL, TRI, TOB, SUT | MDR | *bla*NDM-1, *bla*KPC, *bla*CTX-M-Gp1, *bla*TEM, *bla*OXA-1-like | *kfu*, *ent*B, *mrk*D, *ybt*S, *ycf*M, *fim*H | ST3372 |
| Kp6411 | 2016 | Urine | Emergency room | AMI, AMC, APS, ATM, CFC, CFZ, CPM, CFM, CFO, CTL, CRO, CAZ, CTX, CRX, CFL, CIP, CLO, DOR, DOX, ERT, GEN, IPM, LEV, LMX, MPM, MIN, NAL, NIT, NOR, OFX, PIT, EST, SUL, TET, TAC, TRI, TOB, SUT | XDR | *bla*NDM-1, *bla*KPC, *bla*CTX-M-Gp1, *bla*TEM, blaOXA-1-like | *kfu*, *ent*B, *mrk*D, *ybt*S, *fim*H | ST1067 |
| Kp6715 | 2017 | Blood | Orthopedic | AMC, ATM, APS, ATM, CFC, CFZ, CPM, CFM, CFO, CTL, CRO, CAZ, CTX, CRX, CFL, CIP, CLO, DOX, ERT, IPM, LEV, LMX, MPM, MIN, NAL, NIT, NOR, OFX, PIT, EST, SUL, TET, TAC, TRI, TOB, SUT | MDR | *bla*NDM-1, *bla*KPC, *bla*CTX-M-Gp9, *bla*TEM, *bla*OXA-1-like | *kfu*, *ent*B, *mrk*D, *ycf*M, *fim*H | ST3371 |

NDM-1, New Delhi metallo-β-lactamase type 1; MLST, multilocus sequence type.
[a] AMI, amikacin; AMC, amoxicillin–clavulanate; APS, ampicillin–sulbactam; ATM, aztreonam; CAZ, ceftazidime; CFC, cefaclor; CFL, cephalothin; CFM, cefixime; CFO, cefoxitin; CFZ, cefazolin; CIP, ciprofloxacin; CLO, chloramphenicol; CPM, cefepime, CRO, ceftriaxone; CRX, cefuroxime; CTL, ceftaroline; CTX, cefotaxime; DOR, doripenem; DOX, doxycycline; ERT, ertapenem; EST, streptomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; LMX, lomefloxacin; MIN, minocycline; MPM, meropenem; NAL, nalidixic acid; NIT, nitrofurantoin; NOR, norfloxacin; OFX, ofloxacin; PIT, piperacillin–tazobactam; SUL, sulfonamide; SUT, trimethoprim–sulfamethoxazole; TAC, ticarcillin–clavulanate; TET, tetracycline; TOB, tobramycin; TRI, trimethoprim.
[b] MDR, multidrug-resistant; XDR, extensively drug-resistant.

Genomic DNA from the isolates was extracted using the QIAamp DNA Mini Kit (Qiagen, Redwood City, CA, USA). PCR reactions were performed using specific primers described previously for the detection of the following β-lactamase-encoding genes: *bla*CTX-M-Gp1-2-8-9 group, *bla*VEB, *bla*GES, *bla*TEM, *bla*KPC, *bla*VIM, *bla*OXA-48-like, *bla*OXA-58-like, $bla_{IMP}$, $bla_{SPM}$, $bla_{SIM}$, $bla_{GIM}$, $bla_{NDM-1}$, $bla_{OXA-1-like}$, $bla_{CMY}$, $bla_{BEL}$ (Clímaco et al., 2013; Peirano et al., 2011; Dallenne et al., 2010; Ellington et al., 2007). The following virulence genes were also screened for in all *K. pneumoniae* isolates: *all*S, *ent*B, *fim*H, *iut*A, *kfu*, *mag*A, *mrk*D, *rmp*A, *ybt*S, and *ycf*M (Gonçalves et al., 2017; El Fertas-aissani et al., 2013).

For confirmation of the amplified genes, one of each amplicon was randomly selected and assessed by sequencing. The nucleotide sequences obtained were compared with those available in GenBank (http://www.ncbi.nlm.nih.gov/Genbank) using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences obtained were deposited in GenBank under accession numbers **MH818545**–**MH818548** and **MH818571**–**MH818576**.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR was performed as described previously by Versalovic et al. (1994). A similarity dendrogram was constructed using BioNumerics version 5.1 software. Multilocus sequence typing (MLST) analyses were performed according to the protocol available in the *K. pneumoniae* MLST database (http://bigsdb.pasteur.fr/klebsiella/primers_used.html).

## Results and discussion

Among the 825 CRKpn isolates obtained, the $bla_{NDM}$ gene was found in 0.38% (4/825), $bla_{VIM}$ in 0.72% (6/825), and $bla_{OXA}$ in 0.72% (6/825). The four isolates that presented the $bla_{NDM}$ gene were Kp6408, Kp6410, Kp6411, and Kp6715 (Table 1). Subsequent sequence analysis of the $bla_{NDM}$ gene identified the NDM-1 variant in all of the aforementioned isolates. All NDM-1-producing *K. pneumoniae* isolates also demonstrated co-production of the $bla_{KPC-2}$ and $bla_{TEM}$ genes. CTX-M and OXA-1 production was also observed in three isolates (Kp6410, Kp6411, and Kp6715). The virulence genes *kfu* and *ent*B (siderophores), *mrk*D gene (type 3 fimbrial adhesin), and *fim*H gene (type 1 fimbrial adhesin) were found in all isolates. The genes *ybt*S and *ycf*M (also siderophores) were also found among the isolates (Table 1). *K. pneumoniae* that produces both NDM-1 and *K. pneumoniae* carbapenemase type 2 (KPC-2) has been reported previously in a middle-aged patient with a urinary tract infection admitted to an intensive care unit of a tertiary care hospital in Chennai, India (Kumarasamy and Kalyanasundaram, 2012) (Figure 1).

Three isolates (Kp6408, Kp6410, and Kp6715) were classified as MDR and one (Kp6411) as XDR. Although Kp6411 did not demonstrate susceptibility to any of the antimicrobials tested, it was not classified as PDR because not all antibiotics recommended by Magiorakos et al. (2012) were tested (Table 1). All isolates were susceptible to colistin. A dendrogram based on ERIC-PCR fingerprint analysis grouped the isolates sharing a similarity of 59.8%. Isolates Kp6408 and Kp6410 were genetically closest, sharing a similarity of 95.7% (Figure 1). MLST revealed four different sequence types (STs), which to the authors' knowledge are described in Brazil for the first time, with two novel STs identified in this study: ST3371 (Kp6715) and ST3372 (Kp6410) (Table 1; Figure 1). ST1076 belongs to the clonal group (CG) CG258/11, which contains the STs 258 and 11, detected worldwide, and considered the main international high-risk clones. The new STs were not associated with CG258/11; ST3371 belongs to the CG107, a small clonal group not connected to CG258/11, and ST3737 was not associated with any CG.

This study reports the identification of NDM-1 associated with carbapenemase-producing *K. pneumoniae* and virulence genes in four MDR *K. pneumoniae* that have STs described for the first time in Brazil, including two novel STs. The fact that these isolates were encountered in a hospital is cause for concern for the public health

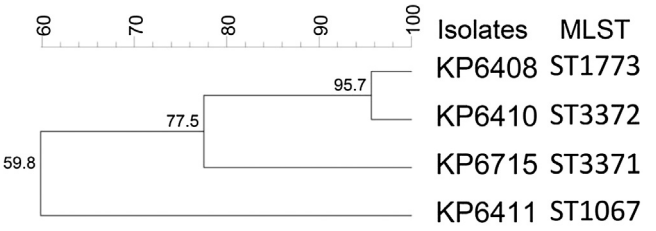


**Figure 1.** Dendrogram representing the genetic relationships among the four NDM-1-producing *Klebsiella pneumoniae* studied. Clusters were determined using the unweighted pair group method with arithmetic mean (UPGMA) and the Dice similarity coefficient. Similarity (%) among patterns is represented by the numbers next to the nodes. For each isolate, their respective sequence type (ST) is represented.

sector. Immediately after the identification of these *K. pneumoniae* isolates, the local infection control committee was notified so that infection control measures could be implemented, which included the isolation of the infected patients and surveillance cultures to avoid dissemination of the strain in the hospital.

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## Ethical approval

The study was approved by the Research Ethics Committee of the State University of Londrina (CAAE0015.0.268.000-11).

## Conflict of interest

All authors report no conflicts of interest relevant to this article.

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