Dissemination of blaNDM-1 Gene Among Several Klebsiella pneumoniae Sequence Types in Mexico Associated With Horizontal Transfer Mediated by IncF-Like Plasmids

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Nosocomial infections caused by multidrug-resistant (MDR) Klebsiella pneumoniae are a major health problem worldwide. The aim of this study was to describe NDM-1-producing K. pneumoniae strains causing bacteremia in a tertiary referral hospital in Mexico. MDR K. pneumoniae isolates were screened by polymerase chain reaction for the presence of resistance genes. In resistant isolates, plasmids were identified and conjugation assays were performed. Clonal diversity and the sequence types were determined by pulsed-field gel electrophoresis and multilocus sequence typing. A total of 80 K. pneumoniae isolates were collected from patients with bacteremia over a 1-year period. These isolates showed a level of resistance of 59% (47/80) to aztreonam, 56–60% (45–48/80) to cephalosporins, 54% (43/80) to colistin and 12.5% (10/80) to carbapenems. The carbapenem resistant isolates were blaNDM-1 carriers and negative for blaKPC, blaNDM, blaIMP, blaVIM and blaOXA-48-like carbapenemases genes. Conjugative plasmids IncFIIA and IncF group with sizes of 82–195 kbp were carriers of blaNDM-1, blaCTX-M-15, blaTEM-1, aac(6’)-Ib and/or aac(3’)-IIa. Clonal variability and nine different multilocus sequence types were detected (ST661, ST683, ST1395, ST2706, ST252, ST1198, ST690, ST1535, and ST3368) for the first time in the isolates carrying blaNDM-1 in Mexico. This study demonstrates that blaNDM-1 has remained within this hospital in recent years and suggests that it is currently the most prevalent carbapenemase among K. pneumoniae MDR strains causing bacteremia in Mexico. The horizontal transfer of blaNDM-1 gene through IncF-like plasmids among different clones demonstrates the dissemination pathway of antimicrobial resistance
INTRODUCTION

*Klebsiella pneumoniae* (K. pneumoniae) is the causative agent of community and hospital acquired infections (Ramírez et al., 2019). In recent years, this bacterium has acquired high resistance to broad-spectrum antibiotics such as β-lactams, aminoglycosides, and quinolones (Ferreira et al., 2019). At the present time, the spread of carbapenemase-producing *K. pneumoniae* is a global public health concern (Villa et al., 2017). *Klebsiella pneumoniae* carbapenemase (KPC) was first reported in North Carolina in 2001. In the last decade it has disseminated globally due to the clonal spread of KPC-producing *K. pneumoniae* and in some countries its nosocomial dissemination has caused outbreaks (Martin et al., 2017). In Mexico, the first report of KPC-3-producing *K. pneumoniae* causing an outbreak was in 2013 (Rodríguez-Zulueta et al., 2013). Two subsequent works have reported the presence of a small number of strains of *K. pneumoniae* producing this carbapenemase (Bocanegra-Ibarias et al., 2017; Aquino-Andrade et al., 2018). One of the carbapenemases initially described in *K. pneumoniae* is New Delhi metallo-β-lactamase 1 (NDM-1), the dissemination of which is mostly hospital associated (Politi et al., 2019). The prevalence of carbapenemases in *K. pneumoniae* has been little studied in Mexico, however, recent research has demonstrated that NDM-1 carbapenemase is more frequent than that of KPC (Rodríguez-Zulueta et al., 2013; Bocanegra-Ibarias et al., 2017; Aquino-Andrade et al., 2018; Alcántar-Curiel et al., 2019b), which has been reported as endemic in the United States, Brazil, Argentina, Colombia and sporadically in Canada (Lee et al., 2016; Hammoudi Halat and Ayoub Moubareck, 2020).

Although *bla*<sub>NDM-1</sub> has been found on the bacterial chromosome, the vast majority is carried on plasmids (Wu et al., 2019). Currently there are 20 different incompatibility groups (Inc) of *bla*<sub>NDM-1</sub> carrying plasmids in Enterobacteriaceae, including IncA/C, IncFIA, IncFIB, IncFII and IncX3 (Wu et al., 2019), indicating the different possibilities of acquisition of *bla*<sub>NDM-1</sub> and the horizontal spread between bacteria of the same or different species.

In addition to this phenomenon, some *K. pneumoniae* carbapenemase producers are defined as high-risk clones because of their ability to colonize, spread and persist (Pitout et al., 2015). The multilocus sequence types (ST) ST258, and ST11, both belonging to the clonal complex (CC) 258, are prototypes of an epidemic clone which was identified as early 2000s and are currently spread around the world (Pitout et al., 2015; Lee et al., 2016).

The aims of this study were to investigate antimicrobial resistant genes, the plasmids associated with horizontal gene transfer and to determine the expansion of multilocus sequence types in *K. pneumoniae* causing bacteremia in a tertiary referral hospital in Mexico.

and underscore the need for strong and urgent joint measures to control the spread of NDM-1 carbapenemase in the hospital.

**Keywords:** *Klebsiella pneumoniae*, *bla*<sub>NDM-1</sub>, IncF-like plasmids, carbapenems, MLST, Mexico

MATERIALS AND METHODS

**Bacterial Isolation**

Non-duplicate isolates of *K. pneumoniae* were consecutively collected from all blood cultures of patients with nosocomial bacteremia identified from January to December 2017 at Hospital Civil de Guadalajara, an 899-bed tertiary-care teaching hospital in Guadalajara, Jalisco, Mexico. The hospital infrastructure is made up of two buildings, the old, the architecture of the building is mixed, horizontal in its old area and a vertical tower of specialties with ten levels, also there is a building for the Care of Neonates and Women, an outpatient tower, the ophthalmology Unit and a Geriatric Care Unit.

Nosocomial infections were defined according to criteria published by the Centers for Disease Control and by Infectious Diseases Unit physicians (Horan et al., 2008). The isolates were stored in Luria Bertani (LB) broth (Difco, BD Biosciences, Franklin Lakes, NJ, United States) with 20% glycerol (Sigma-Aldrich, St. Louis, MO, United States) at −70°C.

**Antimicrobial Susceptibility Testing**

Identification and antimicrobial susceptibility against piperacillin–tazobactam, aztreonam, cefazolin, cefepime, ceftriaxone, cefotaxime, cefotaxolone–tazobactam, imipenem, meropenem, ciprofloxacin, amikacin, gentamycin, tobramycin, nitrofurantoin, tigecycline and trimethoprim-sulfamethoxazole were performed using the Vitek® 2 system (BioMérieux Durham, NC, United States). Minimal inhibitory concentrations (MIC) of colistin were determined by a microdilution method following the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2018). The production of extended spectrum β-lactamases (ESBLs) was confirmed phenotypically in all isolates resistant to penicillin/tazobactam and cephalosporins using the agar diffusion method (CLSI, 2018). Metallo-β-lactamases (MBLs) production in carbapenem-resistant isolates was determined by the diffusion test on agar using meropenem and imipenem sensidsiks with or without 0.5 M EDTA and in combination with 400 μg/mL of phenylboronic acid for the presumptive identification of carbapenemase KPC (Alcántar-Curiel et al., 2019a).

**Detection of Resistance Genes**

Genes that encode antimicrobial resistance were detected by polymerase chain reaction (PCR) assay described previously (Alcántar-Curiel et al., 2019b). The presence of carbapenemase genes *bla*KPC, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub> and *bla*<sub>OXA-48</sub>–like was determined by multiplex PCR (Poirel et al., 2011). Endpoint PCR was performed to detect *bla*<sub>TEM</sub>, *bla*<sub>CTX–M</sub>. Additional genes of aminoglycoside modifying enzymes (AMEs) genes aac(3′)-Ia, aac(6′)-Iib, the methyltransferases genes rmtB and armA and the colistin resistance *mer*–I gene (Liu et al., 2016)
were included in order to further characterize the strains. The specific oligonucleotides used are described in Supplementary Table 1. The amplified fragments were purified using the Zymogen Purification Kit (Promega) and sequenced (Instituto de Biotecnología, Universidad Nacional Autónoma de México). The sequence analysis was performed with the BioEdit and Kaling bioinformatics tools to subsequently undergo a Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information database.

Plasmid Analysis and Conjugation Assays
Plasmids profile was obtained from isolates carrying ESBLs or MBLs according to Eckhardt technique (Eckhardt, 1978). Horizontal transfer of antibiotic resistant was confirmed by bacterial conjugation with Escherichia coli J53-2 as the recipient strain using Miller method (Miller, 1992). Transconjugants were selected on MacConkey agar supplemented with rifampicin (200 µg/mL) plus ceftazidime (16 µg/mL) and plus meropenem (16 µg/mL) for isolates carrying carbapenemases genes and tested for antimicrobial susceptibility. Successful conjugation was confirmed by specific PCR amplification and the electrophoretic pattern of the conjugated plasmids was obtained. The bacterial artificial chromosomes (BACs) of 67, 86, 101, 122, 145, and 195 kb were used as a molecular weight markers (González et al., 2006). Plasmids of transconjugant strains were purified using the QIAGEN Plasmid Midi Kit (Qiagen, Hilden, Germany), following the manufacturer's specifications. Plasmids diversity was determined by restriction fragment length polymorphism (RFLP) (Ho et al., 2012) with EcoRI and HindIII (Invitrogen) restriction enzymes following the manufacturer's specifications. Finally, the groups Inc of conjugative plasmids were determined by PCR-based replicon typing (Carattoli et al., 2005).

Genotyping by Pulsed-Field Gel Electrophoresis
Clonality among all of the isolates was confirmed by PFGE (Alcántar-Curiel et al., 2019a) and the electrophoretic pattern of the conjugated plasmids was obtained. The bacterial artificial chromosomes (BACs) of 67, 86, 101, 122, 145, and 195 kb were used as a molecular weight markers (González et al., 2006). Plasmids of transconjugant strains were purified using the QIAGEN Plasmid Midi Kit (Qiagen, Hilden, Germany), following the manufacturer's specifications. Plasmids diversity was determined by restriction fragment length polymorphism (RFLP) (Ho et al., 2012) with EcoRI and HindIII (Invitrogen) restriction enzymes following the manufacturer's specifications. Finally, the groups Inc of conjugative plasmids were determined by PCR-based replicon typing (Carattoli et al., 2005).

Multilocus Sequence Typing
To determine the sequence type (ST) of K. pneumoniae isolates harboring blaNDM−1, MLST was performed according to the Pasteur scheme (Diancourt et al., 2005). The housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, tonB) were amplified, sequenced, and analyzed with the MLST database of the Pasteur Institute to identify allelic profile. In order to identify the clonal complex (CC) and visualize evolutionary relationships among isolates carrying blaNDM−1, we used Phyloviz 2.0 program that generates eBURST and neighbor-joining diagram (Sepp et al., 2019).

RESULTS
Clinical Isolates and Antibiotic Susceptibility Pattern
A total of 80 isolates of K. pneumoniae causing bacteremia were collected over the course of 1 year at the Hospital Civil de Guadalajara. These isolates representing 8% of the total documented bacteremias. K. pneumoniae were more frequently derived in patients from surgical ward (32.5%) and medicine ward (31.2%) (Table 1 and Supplementary Data Sheet 1). The isolates were resistant to penicillin/tazobactam 26% (21/80), aztreonam 59% (47/80), cepafirin 56% (45/80), ceftriaxone 60% (48/80), imipenem and meropenem 12.5% (10/80), ciprofloxacin 18% (14/80), tobramycin 50% (40/80), gentamicin 55% (44/80), nitrofurantoin 16% (13/80), ticagrelor 5% (4/80), trimethoprim-sulfamethoxazole 56% (45/80) and colistin 53% (42/80) (Table 2).

Antibiotic Resistance Enzymes
A total of 10 strains resistant to all β-lactams including the two carbapenems were detected. These strains were MBL producers and carried the blaNDM−1 carbapenemase gene (Supplementary Data Sheet 1). Furthermore, three of these strains carried the blaTEM−1 penicillinase gene and four strains blaTEM−1 and blaSHV−1.

| Hospital wards | Area | No. isolates (%) |
|----------------|------|------------------|
| Surgical       | Old  | 26 (32.5)        |
| Neurology (NEU)| Old  | 13 (16)          |
| General surgery (GES)| Old | 10 (12.5)        |
| Plastic surgery (PSU)| Old | 1 (3.8)          |
| Oral and maxillofacial surgery (OMS)| Old | 1 (3.8)          |
| Otorhinolaryngology (OTO)| Old | 1 (3.8)          |
| Medicine       | Old  | 25 (31.2)        |
| Internal medicine (IME)| Old | 7 (8.4)          |
| Cardiology (CAR)| Old | 5 (6.3)          |
| Nephrology (NEP)| Tower 5 floor | 5 (6.3) |
| Haematology (HEM)| Tower 9 floor | 3 (3.8) |
| Infectious diseases unit (IDU)| Tower 7 floor | 3 (3.8) |
| Gastroenterology (GAS)| Tower 6 floor | 1 (1.3) |
| HIV/AIDS Unit (HIV)| Tower 5 floor | 1 (1.3) |
| Pediatric intensive care unit | Newborn unit | 13 (16.2) |
| Neonatal intensive care unit (NICU)| Tower 1 floor | 7 (8.7) |
| Intensive Care Unit (ICU)| Tower 1 floor | 9 (11.2) |
| Total          |      | 80 (100)         |

1www.ncbi.nlm.nih.gov/blast/
**TABLE 2** | Minimum inhibitory concentration data and antimicrobial susceptibility of 80 *Klebsiella pneumoniae* isolates from January to December 2017 at Hospital Civil de Guadalajara.

| Drug class | Antimicrobial agent | MIC* (µg/mL) | Antimicrobial susceptibility (%) |
|------------|---------------------|--------------|---------------------------------|
|            |                     | MIC 90 | MIC 50 | Susceptible | Intermediate | Resistant |
| β-lactam combination agents | Piperacillin/Tazobactam | 128 | 16 | 65 | 9 | 26 |
| Monobactam | Aztreonam | 64 | 2 | 36 | 5 | 59 |
| Cepham | Cefazolin | 64 | 8 | 41 | 0 | 59 |
| | Ceftape | 64 | 2 | 44 | 0 | 56 |
| | ceftiraxone | 64 | 32 | 40 | 0 | 60 |
| β-lactam combination agents | Cefotiozyn/Tazobactam** | N/A | N/A | 87 | 0 | 13 |
| | Carbapenems | Imipenem | 8 | 0.06 | 87 | 0 | 13 |
| | | Meropenem | 4 | 0.03 | 87 | 0 | 13 |
| Fluoroquinolones | Ciprofloxacin | 4 | 1 | 68 | 14 | 18 |
| Aminoglycosides | Amikacin | 128 | 8 | 56 | 0 | 24 |
| | Gentamicin | 128 | 32 | 45 | 0 | 55 |
| | Tobramycin | 32 | 1 | 50 | 0 | 50 |
| Nitrofurans | Nitrofurantoin | 128 | 64 | 26 | 58 | 16 |
| Glycycline** | Tigecycline** | 2 | 1 | 91 | 4 | 5 |
| Folate pathway antagonists | Trimethoprim/Sulfamethoxazole | 16/304 | 16/304 | 43 | 1 | 56 |
| Lipopeptides | Colistin*** | 32 | 4 | 47 | 0 | 54 |

*Susceptibility breakpoint categories were derived from CLSI (2018).
**Susceptibility was determined by agar disk diffusion method.
***Susceptibility categorization was determined according to the EUCAST criteria.
N/A, Not applicable.

With respect to thirty-three *bla* _TEM_–1 and *bla* _CTX–M_–15 carriers, 26 transconjugants were obtained (Supplementary Table 3), of which 13 harbored a >195 kbp plasmid, 12 carried a ~195 kbp plasmid and only one acquired a ~67 kbp plasmid. From the total 26 conjugative plasmids, 25 of them harbored the *bla* _CTX–M_–15, *bla* _TEM_–1, *aac(3′)-Ila* and *aac(6′)-Ib*, and only one of them harbored the *bla* _CTX–M_–15, *bla* _TEM_–1 and *aac(6′)-Iia*. Twenty-five of these plasmids belonged to the IncF group and one only plasmid to the IncFIIA group.

Fragment length polymorphism (RFLP) analysis of the conjugative plasmids carrying both *bla* _NDM–1_ and *bla* _CTX–M_–15 showed an average similarity of 88% (Figure 1). The five plasmids carriers *bla* _NDM–1_ revealed two different restriction profiles (P), four of them belonged to P8. The 26 plasmids carriers of *bla* _TEM_–1 and *bla* _CTX–M_–15 belonged to eight different restriction profiles.

**Plasmid Analysis**

Conjugation experiments in ten isolates *bla* _NDM–1_ carriers showed that five transconjugants acquired the *bla* _NDM–1_ gene (Table 3). Plasmid analysis indicated that one transconjugant harbored the *bla* _NDM–1_ gene on a ~82 kbp plasmid. The other four transconjugants harbored the *bla* _NDM–1_ gene on a ~195 kbp plasmid, only two of these plasmids were carriers of a single *bla* _NDM–1_ resistance gene, the other plasmid was a carrier of both *bla* _NDM–1_ and *bla* _TEM–1_ genes and the fourth plasmid was a carrier of *bla* _NDM–1_, *bla* _TEM–1_, *bla* _CTX–M–15_ and *aac(3′)-Ila* and *aac(6′)-Ib*. All the five transconjugants were resistant to all β-lactams and aminoglycosides with the exception of one. Plasmid replicon typing showed that four of the conjugative plasmids belonged to the IncFIIA and one to the IncF group.

**Clonality Analysis**

Pulsed-field gel electrophoresis (PFGE) analysis was conducted with an average similarity of 66%. Sixty-nine different clones were detected among the 80 isolates, which showed clonal heterogeneity (data not shown). Clone 26 was the most prevalent with three isolates collected in May and June, all carriers of *bla* _CTX–M–15_, *bla* _TEM–1_, *aac(3′)-Ia* and *aac(6′)-Ib*. Clones 6, 15, 34, 35, 37, 39, 42, and 47 had two isolates each, while the rest of the isolates belonged to different clones (Supplementary Data Sheet 1). The 43 strains of *K. pneumoniae* carrying resistance genes belonged to 38 different clones, the 10 isolates carriers of *bla* _NDM–1_ belonged to different clones (Supplementary Figure 1).
IncF-Like Plasmids From Klebsiella pneumoniae

Table 3 shows the plasmid patterns and resistant genes of K. pneumoniae carriers of NDM-1 gene. The plasmids were found in various sizes ranging from 82 kbp to 195 kbp. The table also shows the transconjugants and the acquired multi-resistance profile.

MLST Analysis

The analysis demonstrated nine different STs among ten isolates carriers of blaNDM-1 gene. ST661, ST683 belonged to CC258, ST1395, ST2706, ST252, ST1198, ST690, ST1535, and ST3368 (Supplementary Figure 2). Isolates 07-KP-17, and 11-KP-17 belonged to ST661 which corresponds to the founder member of CC661, these isolates were recovered in February and March 2017 respectively.

Phylogenetic analysis using the neighbor-joining method detected the genetic distance between the 10 isolates carriers of blaNDM-1 gene (Figure 2). Isolates recovered in February and March as well as the isolates recovered between April and June were significantly associated, while the October isolates including the 69-KP isolate were not.

DISCUSSION

Klebsiella pneumoniae is included in the global priority list of antibiotic resistant bacteria, and there is a need for enhanced K. pneumoniae surveillance to rapidly identify and monitor convergent strains and/or plasmids (Wyres et al., 2020). The infections caused by K. pneumoniae during the present study period were 8%, lower than those reported for developing countries ranging from 16 to 28% (Khaeretynov et al., 2018).

The rate of antimicrobial resistance observed in the isolates from our study was lower than the rate from the isolates that caused nosocomial outbreaks previously reported in the same hospital (Bocanegra-Ibarias et al., 2017). While this was a short study period, our findings are consistent with the high rate of resistance reported in Asia where they detected 60–75.8% to cephalosporins, 47–65.6% to imipenem and 40.8–76% to amikacin (Effah et al., 2020). Detection of 54% colistin resistance in this study is undoubtedly our biggest concern, a significant increase compared to the 4.7% reported 2 years earlier in this hospital (Bocanegra-Ibarias et al., 2017). This may be due to the increased use of colistin for the treatment of bacteremia caused by carbapenemase-producing isolates in hospital and because the isolates from the previous study were mostly derived from a nosocomial outbreak (Bocanegra-Ibarias et al., 2017).

The spread of NDM-producing bacteria and their association with nosocomial outbreaks is of concern worldwide (Dortet et al., 2014). The first report of NDM in K. pneumoniae was described in Mexico in 2014 (Barrios et al., 2014). The detection of blaNDM-1 in ten isolates included in this study, demonstrates the spread and persistence of this carbapenemase among K. pneumoniae isolates for at least two consecutive years at Hospital Civil de Guadalajara (Bocanegra-Ibarias et al., 2017). Additionally, the co-transfer of blaNDM-1 and AMEs genes together with fluoroquinolone resistance in 19-Kp isolate (Table 3) demonstrates the concurrence of these genes, which represents a major challenge in the treatment of patients (Mitra et al., 2019).

It has been documented that in K. pneumoniae these resistance genes are encoded in small 25 kbp conjugative plasmids or smaller (Ramirez et al., 2019), which contrasts with the >195 kbp conjugative plasmids identified in this
study (Figure 1). RFLP analysis showed that isolates in our study harbor different types of plasmids carrying blaNDM−1, ESBLs and AMEs, suggesting that genetic rearrangements occurred at the plasmid level during this period of study. Furthermore, similar plasmids were detected among different clones, which indicates that the transfer of genes is common among bacteria allowing the spread of resistance genes in the hospital environment.

The blaNDM−1 gene has been identified in plasmids from different replicon types, in this study the plasmid carrying blaNDM−1 gene belongs to the IncF and IncFIA subgroups; which are different from the IncFIIk and IncFIIy subgroups previously reported in this hospital (Bocanegra-Ibarias et al., 2017) and the IncFIA reported in isolates in Mexico City (Alcántar-Curiel et al., 2019a).

The prevalence of plasmid replicons is led by the IncX group, while the IncF group ranks third, worldwide (Partridge et al., 2018; Wu et al., 2019). However, in Latin America, particularly in Mexico, Brazil, and Colombia, the IncF group seems be the most prevalent (Torres-González et al., 2015; Bocanegra-Ibarias et al., 2017; Alcántar-Curiel et al., 2019b; Wu et al., 2019).

In this study, gentamicin and tobramycin resistance was associated with the production of AAC(3′)-Ila and AAC(6′)-Ib, but not for amikacin resistance since the resistant isolates did not carry these genes studied (Supplementary Table 2). The five transconjugants of the isolates that produced ESBLs and were resistant to the 3 aminoglycosides acquired both AMEs but only had resistance to gentamicin and tobramycin (Table 2), suggests that amikacin resistance may be due to another AME or another resistance mechanism not encoded in plasmids. Because none of the studied AMEs were detected in transconjugants carriers blaNDM−1 that acquired resistance to aminoglycosides, we consider that these isolates may carry others AMEs that were not investigated.

One of the most prevalent genes in Enterobacteriaceae, Pseudomonadales and Vibrionaceae worldwide is aac(6′)-Ib (Ramirez and Tolmasky, 2017; Fernández-Martínez et al., 2018; Galani et al., 2019), which is mostly associated with amikacin and gentamicin resistance (Ramirez and Tolmasky, 2017; Ramirez et al., 2019) and frequently encoded in plasmids and coexisting with ESBLs such as CTX-M. Our results are in agreement with these data with the exception that aac(6′)-Ib seems to be associated with tobramycin resistance since it was detected in isolates resistant only to this antibiotic. The observation that aac(3′)-Ila is more frequent in Enterobacteriaceae and is associated with gentamicin and tobramycin resistance (Fernández-Martínez et al., 2018) is in agreement with our results.

Colistin resistance was not transferred by conjugation and this was supported by the fact that none of the isolates carried the mcr-1 gene, suggesting that resistance may be due to be associated with chromosomal mutations that are directly involved in LPS.
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**CONCLUSION**

This study shows the prevalence of *K. pneumoniae* MDR isolates causing bacteremia in a tertiary referral hospital in Mexico. The carbapenem-resistant isolates were carriers of the *blaNDM−1* gene harbored in similar IncF-like plasmids among clones with different STs, which supports their nosocomial dissemination and persistence in different plasmids which can be associated with genetic rearrangements that might be in favor the microevolution of this nosocomial pathogen. These results underscore the importance of maintaining microbiological and epidemiological surveillance actions to detect and prevent the spread of MDR bacteria.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**ETHICS STATEMENT**

This study was evaluated and approved by the Institutional Research and Ethics Committee of the Hospital Civil de Guadalajara project number HCG/CEI-009316; 27 January 2016. The study does not involve humans it is an in vitro study. Written informed consent was not required for this study according to the institutional ethical, biosecurity and investigation committees because the Hospital Clinical Laboratory provided every bacterial isolates included in this study.

**AUTHOR CONTRIBUTIONS**

JET-T conceived and designed the study and performed the experiments, analyzed the data, and wrote and edited the manuscript. CG-V, MDJ-Q, JLF-V, and JDC performed the experiments, analyzed the data, and revised the manuscript. RM-O, ER-N, SG-C, GG, and JS-P analyzed the data and revised the manuscript. MA-C conceived, designed, and supervised the study, analyzed the data, and wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.611274/full#supplementary-material

Supplementary Figure 1 | Genetic relationship and molecular characteristics of the 43 strains of K. pneumoniae carrying blαNDM-1 and other resistance genes.

Supplementary Figure 2 | Diagram of the different clonal complexes (CC) and the multilocus sequence types (STs) identified by eBURST in blαNDM-1-producing K. pneumoniae isolates. The ST (red circles) and the CC (green circles) obtained for each strain 2B.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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