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Antimicrobial Susceptibility Studies

Genetic characterization of multiple NDM-1–producing clinical isolates in Mexico

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ABSTRACT

This work describes the molecular characteristics and the genetic structure of plasmid-borne NDM-1 gene of 5 different bacterial species associated with nosocomial infections in Mexico. In *Acinetobacter* spp. the NDM-1 gene structure is conserved, suggesting an intraspecies spread. In Enterobacteriaceae, a dissemination of IncFII-related plasmid encoding the NDM-1 suggests horizontal transfer, but *E. coli* showed a different molecular mechanism, related to class 1 integron and ISCR1 element.

Multidrug-resistant Gram-negative bacteria have emerged as a worldwide serious infectious disease challenge (Hudson et al., 2014). The emergence of carbapenem-resistant bacteria has become a clinical problem associated to the production of carbapenemases (Sidjabat et al., 2011). One of the principal families of carbapenemases described is the NDM (New Delhi metallo-β-lactamase), which has been described worldwide and in the American continent (Barrios et al., 2013, 2014; Carvalho-Assef et al., 2013; Escobar Perez et al., 2013; Kumarasamy et al., 2010). Particularly in Mexico, the NDM-1–producing *Providencia rettgeri*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli* have been described. *K. pneumoniae* has been the most prevalent and reported in several Mexican hospitals (Aquino-Andrade et al., 2018; Barrios et al., 2013, 2014; Bocanegra-Ibarias et al., 2017; Torres-Gonzalez et al., 2015). Nevertheless, the genetic context of plasmid-borne NDM-1 in Mexican isolates has not been studied.

The NDM-1 encoding carbapenemase gene is commonly found on large conjugative plasmids along with additional antibiotic resistance determinants (Chen et al., 2011). **In *Acinetobacter* spp., NDM-1 was originally identified within the genetic element *Tn*125, a 10,099-bp transposon (Poirel et al., 2012). The NDM-1 gene has been suggested to be originally mobilized from *Acinetobacter* species into plasmids, facilitating the spread among other species such as those found in the Enterobacteriaceae family (Hu et al., 2012; Marquez-Ortiz et al., 2017; Pfeifer et al., 2011).** In this work, we report the molecular characteristics and the genetic structure of plasmid-borne NDM-1 gene of 5 different bacterial species associated with nosocomial infections in Mexico.

The first NDM-1 carbapenemase-producing isolates from this study were detected in 2012 and later in 2017 from a routine analysis in a tertiary care hospital; after this detection, no more *A. baumannii* and *A. haemolyticus* strains harboring the NDM-1 gene were detected (data not published).

In a previous work, from 3044 clinical isolates of Enterobacteriaceae, 52 (1.71%) carbapenem-resistant isolates were identified by VITEK 2 compact system (BioMérieux, Durham, NC) (Bocanegra-Ibarias et al., 2017). Five species were positive for production of carbapenemases according to the Carba NP test (CLSI, 2012; Dortet et al., 2012). *K. pneumoniae* was identified in 88% of the isolates, and PCR screening for carbapenemases showed higher prevalence of NDM- (92.3%) than VIM- (5.6%), KPC- (1.9%), and IMP-type genes (1.9%) (Bocanegra-Ibarias et al., 2017). The antibiotic resistance determined by the minimal

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Table 1
Clinical and molecular characteristics and antimicrobial susceptibility of **NDM-producing** clinical isolates.

Isolate	Species	Collection date	Clinical specimen	Hospital ward	City of origin	Plasmid profile[a]	Mating assay	Incompatibility plasmid group (Inc)	MLST (ST)	Susceptibility MIC (μg/mL)										
										AMP	CRO	ETP	IMP	MEM	ATM	SXT	GEN	AMK	CIP	FOF	CHL	TGC	CST
12465	*A. baumannii*	04/06/2012	Blood	ICU	Nuevo León	**55**, 180	ND	ND[b]	ND	N/A	>64	N/A	128	>128	N/A	16/304	>32	64	>16	512	N/A	<0.5	<0.5
9250	*K. pneumoniae*	21/10/2014	BAL	ICU	Jalisco	**120**, 165, 180	+	FII(Yp)[c]	392	>128	>64	16	4	8	16	16/304	>32	>128	>16	512	16	2	<0.5
9263	*E. cloacae*	13/11/2014	Peritoneal fluid	Nephrology	Jalisco	55, **110**, 140, 150	+	A/C2, FII(Yp)[c]	182	>128	>64	32	8	16	16	16/304	>32	>128	>16	512	>128	1	<0.5
9267	*E. coli*	26/02/2015	Catheter	Neonatology	Jalisco	**140**	−	A/C2, FIC(FII), FIB (AP001918)[d]	10	>128	>64	8	4	4	8	8/152	>32	8	2	32	>128	<0.5	<0.5
10256	*A. haemolyticus*	22/06/2011	Blood	IMS	México	**55**, 110	−	ND[b]	NA	N/A	>64	N/A	>128	>128	N/A	8/152	2	128	16	128	N/A	<0.5	<0.5

Abbreviations: BAL = bronchoalveolar lavage; ICU = intensive care unit; IM = Internal Medicine Service; AMP = ampicillin; CRO = ceftriaxone; ETP = ertapenem; IMP = imipenem; MEM = meropenem; ATM = aztreonam; SXT = trimethoprim/sulfamethoxazole; GEN = gentamicin; AMK = amikacin; CIP = ciprofloxacin; FOF = fosfomycin; CHL = chloramphenicol; TGC = tigecycline; CST = colistin; ND = not determined; N/A = not applicable.

[a] The boldface plasmids contain the NDM-1 gene identified by Southern hybridization (Supplementary file).

[b] Incompatibility group could not be determined *in silico* in the clinical isolates by whole-genome *sequencing*.

[c] Incompatibility group determined *in silico* in the transconjugants by whole-genome *sequencing*.

[d] Incompatibility group determined *in silico* in the clinical isolate by whole-genome *sequencing*.

inhibitory concentration (MIC) (CLSI, 2012) showed an extensively drug-resistance profile (Table 1) (Magiorakos et al., 2012). From this previous study, the 3 species that harbored the NDM-1 gene were selected.

In the present study, 5 different NDM-1 carbapenemase-producing species were selected and identified as *A. baumannii*, *Acinetobacter haemolyticus*, *K. pneumoniae*, *E. cloacae*, and *E. coli*. In the 5 isolates, the carbapenemase gene was identified, and the presence of NDM-1 gene was confirmed by PCR sequencing. The isolates were obtained from blood ($n = 2$), bronchoalveolar lavage ($n = 1$), peritoneal fluid ($n = 1$), and catheter ($n = 1$). Demographic and additional clinical characteristics of the isolates are shown in Table 1.

The plasmid profile and mating experiments were determined according to Kieser and Miller methods (Kieser, 1984; Miller, 1972). The plasmid-borne NDM-1 gene identification was carried out by Southern hybridization with a nonradioactive probe of the NDM-1 gene. *A. baumannii*, *A. haemolyticus*, and *E. coli* isolates and transconjugants of *K. pneumoniae* and *E. cloacae* were subjected to whole-genome sequencing by Illumina MiSeq platform. The MultiLocus Sequencing Typing (MLST) for *K. pneumoniae* and *E. cloacae* was determined by Sanger sequencing (Diancourt et al., 2005; Miyoshi-Akiyama et al., 2013). The MLST for *E. coli* (http://www.genomicepidemiology.org) and incompatibility groups (Carattoli et al., 2014) was determined *in silico* analysis.

The plasmid profile was heterogeneous (Table 1, Supplementary file), and the Southern hybridization showed that *Acinetobacter* spp. carried the NDM-1 gene on the 55-kb plasmid; however, the incompatibility group could not be identified. **In the Enterobacteriaceae isolates, the NDM-1 gene was identified on plasmids of 120 kb in *K. pneumoniae*, 110 kb in *E. cloacae*, and 140 kb in the unique plasmid of *E. coli*** (Supplementary file). The mating was successful in the 120-kb and 110-kb plasmids, and these shared the incompatibility group FII(Yp); however, in the *E. cloacae* transconjugant, the IncA/C2 was also identified. The mating experiment in the *E. coli* isolate was unsuccessful, and the IncA/C2, IncFIC (FII), and IncFIB (AP001918) incompatibility groups were identified in the same NDM-1 harboring 140-kb plasmid (Table 1).

Whole-genome sequencing revealed the genetic contexts of the plasmid-borne NDM-1 genes (Fig. 1). A contig sequence of 55-kb long containing NDM-1 was obtained from *Acinetobacter* spp., which matched in size with the plasmid-born NDM-1 gene observed in the hybridization experiments. This plasmid has more than 99% identity with the genetic context of the pNDM-BJ02 plasmid sequence of the *A. lwoffii* strain WJ10659 isolated from a urinary tract infection in China (GenBank: JQ060896.1) (Hu et al., 2012). The NDM-1 genetic context detected in *K. pneumoniae* (120-kb plasmid) and *E. cloacae* (110-kb plasmid) transconjugants was similar and shared the incompatibility group IncFII (Yp). A plasmid belonging to the same incompatibility group IncFII (Yp) was observed in *Yersinia* sp., and this group was closely related to IncFII (K) of *K. pneumoniae* (Carattoli et al., 2014). The plasmid-borne NDM-1 gene in *E. coli* 9267 showed a 99% of identity with class 1 integron structure: *tnpA*, *tnpR*, *IntI1*, *arr-3*, *dfrA27*, *aadA16*, *sul*, and *ISCR* from pYNKP001 plasmid of *Raoultella ornithinolytica* strain YNKP001 (GenBank: KY270853.1). A similar structure was identified in Non-O139 *Vibrio cholerae* strain plasmid pRJ354C (GenBank: KP076293.1), except that the PER-1 gene was substituted by NDM-1 gene in the plasmid of the *E. coli* 9267 isolate.

This work describes for the first time the NDM-1–producing *Acinetobacter* spp. isolates in Mexico**. The blaNDM-1 has been commonly detected among Enterobacteriaceae isolates in Mexico, and diverse sequence types were previously reported in *K. pneumoniae* (ST22, ST76, ST307, ST309, ST392, ST846, and ST2004), *E. cloacae* (ST182), and *E. coli* (ST10, ST131, ST617)** (Aquino-Andrade et al., 2018; Barrios et al., 2013, 2014; Bocanegra-Ibarias et al., 2017; Torres-Gonzalez et al., 2015). Likewise, in these bacterial species, the plasmids-borne NDM-1 gene were associated with FII, FII_y, FII_k, $FIII_k$, and K incompatibility groups, with FII_y being the most prevalent (Aquino-Andrade et al., 2018; Barrios et al., 2013, 2014; Bocanegra-

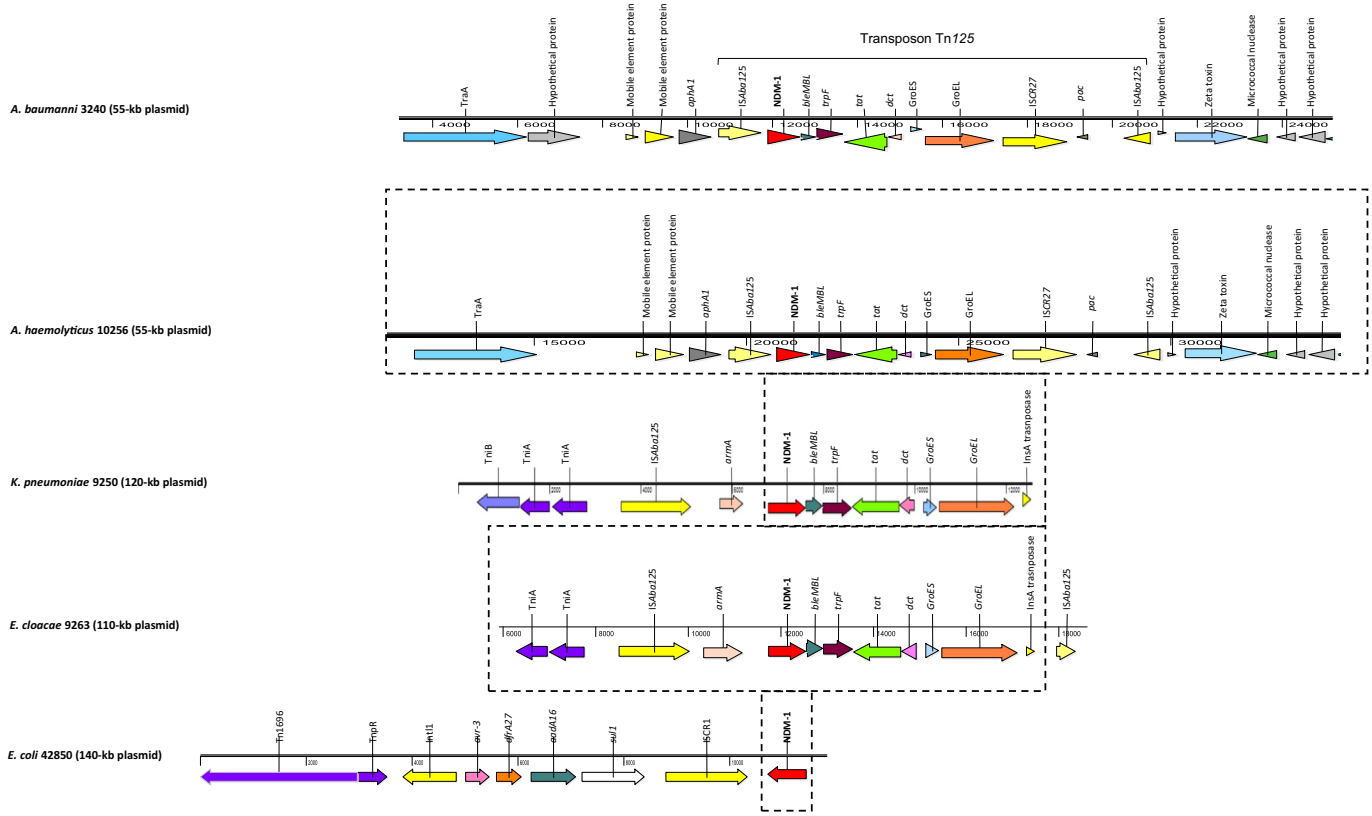

Fig. 1.

Ibarias et al., 2017; Torres-Gonzalez et al., 2015). **The results obtained here indicated that the NDM-1 genetic context in *Acinetobacter* spp. appeared more conserved, suggesting intraspecies plasmid dissemination.** For its part, *K. pneumoniae* and *E. cloacae* were isolated from the same hospital and period of the year. Both isolates contain an autoconjugative NDM-1 plasmid-borne of different size. *In silico* analysis showed that both plasmids share a common structure and FII(Yp) incompatibility group, suggesting horizontal transfer among these enteric bacterial species. This is not the case for *E. coli* since it showed a different molecular mechanism, related to class 1 integron and ISCR1 element on nonconjugative plasmid.

In conclusion, the genetic contexts of β-lactamase NDM-1 gene are described for the first time in the isolates that caused infections in Mexico. The main genetic context identified is preserved as previously reported. However, in this work, we describe the existence of NDM in a class 1 integron that was previously reported with PER-1 in *V. cholerae*.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2018.12.002.

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Transparency declarations

None to be declared.

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Nucleotide sequence accession number. The annotated genomes sequences are available at the European Nucleotide Archive under the following accession numbers: in process (*A. baumannii 12465*), in process (*A. haemolyticus* 10256), in process (*E. cloacae* 9263), in process (*E. coli* 9267), in process (*K. pneumoniae* 9250).

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