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Initial Assessment of the Molecular Epidemiology of bla_{NDM-1} in Colombia

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We report complete genome sequences of four bla_{NDM-1}-harboring Gram-negative multidrug-resistant (MDR) isolates from Colombia. The bla_{NDM-1} genes were located on 193-kb Inc FIA, 178-kb Inc A/C2, and 47-kb (unknown Inc type) plasmids. Multilocus sequence typing (MLST) revealed that these isolates belong to sequence type 10 (ST10) (*Escherichia coli*), ST392 (*Klebsiella pneumoniae*), and ST322 and ST464 (*Acinetobacter baumannii* and *Acinetobacter nosocomialis*, respectively). Our analysis identified that the Inc A/C2 plasmid in *E. coli* contained a novel complex transposon (Tn*125* and Tn*5393* with three copies of bla_{NDM-1}) and a recombination "hot spot" for the acquisition of new resistance determinants.

At this time, bla_{NDM} is recognized as a major global health threat. Guatemala and Colombia reported the first cases of bla_{NDM-1}-harboring isolates in Latin America (1, 2). In both instances, bla_{NDM-1} was discovered in hospital-acquired, clonally related *Klebsiella pneumoniae* isolates that were recovered from patients who had not travelled recently. The molecular epidemiology of bla_{NDM} carbapenemases in South America has been investigated only in a limited fashion, and data in Colombia are very scarce. In order to understand the dissemination of bla_{NDM-1} in Colombia, we performed a genomic analysis of four sentinel isolates, *Acinetobacter baumannii*, *Acinetobacter nosocomialis*, *Escherichia coli*, and *K. pneumoniae* collected in 2012, shortly after the first reported outbreak (1).

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Phenotypic characterization revealed that two of the isolates (*E. coli* and *A. nosocomialis*) were resistant to all antibiotics tested, including polymyxin B and tigecycline (see Table S1 in the supplemental material). On the other hand, the *A. baumannii* isolate was also multidrug resistant but susceptible to polymyxin B, tigecycline, and ciprofloxacin. The *K. pneumoniae* isolate was susceptible to polymyxin B and exhibited relatively low susceptibility to carbapenems, highlighting once again the difficulty clinical microbiology laboratories have detecting carbapenemase genes that are expressed at low levels (3, 4). None of the isolates tested positive for carbapenemases using the modified Hodge test, but they tested positive (with the exception of the *E. coli* isolate) in the three-dimensional (3D) bioassay using an imipenem disk (5). Double-disk synergy testing using EDTA (DDST+EDTA) confirmed the presence of a metallo-β-lactamase for the members of the family *Enterobacteriaceae*, but not in the *Acinetobacter* species isolates.

In order to understand the genetic background of these early bla_{NDM-1}-containing strains, the complete chromosome and plasmid sequences were obtained by assembly of Pacific Biosciences single-molecule real-time (SMRT) sequence data, with the exception of *K. pneumoniae*, where the chromosome was assembled into three ordered contigs (Table 1). Genome sequencing results showed that all isolates possessed multiple plasmids (Table 1) and revealed that bla_{NDM-1} was localized in one plasmid per strain, as confirmed by S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) (6).

Multilocus sequence type (MLST) analysis revealed that *Acinetobacter* species isolates belonged to sequence type 322 (ST322) (*A. baumannii*) and ST464 (*A. nosocomialis*), none regarded as "high-risk" clones (7). Both *A. baumannii* and *A. nosocomialis* harbored three plasmids and carried the bla_{NDM-1} gene on a Tn*125* backbone (Fig. 1) located on a 47,274-bp plasmid that was 99% similar to plasmid pNDM-BJ01 (GenBank accession no. JQ001791.1) reported in an *Acinetobacter lwoffi* isolate from China (8, 9). This plasmid also carried the aminoglycoside phosphotransferase *aph*(3′)*VIIa* gene, and a type IV secretion system (T4SS) gene cluster encoding a P-type T4SS that has been reported to encode a short, rigid pilus characteristic of broad-host-range conjugative plasmids (10).

We next discovered that a chromosomally encoded class C β-lactamase bla_{ADC-80} was found in both *Acinetobacter* species isolates. This surprising finding is particularly interesting, as it highlights the possibility that bla_{ADC} evolved similarly in two different species of *Acinetobacter*. Consistent with the species identification, *A. baumannii* also harbored bla_{OXA-94} (a $bla_{OXA-51-like}$ derivative). None of the other plasmids contained additional resistance genes, and interestingly, *A. baumannii* resistance islands (AbaRI) were

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not found in the chromosome or on plasmids harbored by either of the *Acinetobacter* species isolates.

In contrast with *Acinetobacter* species isolates, both members of the *Enterobacteriaceae* contained multiple resistance genes in two large plasmids (151 to 198 kb) (Table 1), including $bla_{CTXM-15}$, consistent with the previously documented predominance of that extended-spectrum β-lactamase (ESBL) in Colombia (11, 12). The *K. pneumoniae* isolate belonged to ST392, previously associated with the dissemination of bla_{KPC}, bla_{OXA-48}, and other ESBLs (13, 14). In addition, this isolate also harbored a chromosomal bla_{SHV-11} and $bla_{CTX-M-15}$, the latter located downstream of IS*Ecp1*, as previously reported in other *Enterobacteriaceae* isolates from Spain, Japan, Germany, the Netherlands, and the United Kingdom (15–18). The largest plasmid of *K. pneumoniae* (198 kb) was a multireplicon Inc FII/FIB type plasmid, and it carried antibiotic determinants conferring resistance to aminoglycosides [*aph*(3′)-*Ib*, *aph*(6′)-*Id*, *aac*(3)-*IIa*, and *aac*(6′)-*Ib-cr*], quinolones (*qnrB66*), sulfonamides (*sul2*), tetracycline [*tet*(*A*)], trimethoprim (*dfrA14*), chloramphenicol (*catB3*), and β-lactams (bla_{TEM-1}, $bla_{CTX-M-15}$, and bla_{OXA-1}). In this case, the second copy of $bla_{CTX-M-15}$ was found to be part of a previously reported structure: a Tn*3*-like transposon also carrying bla_{TEM-1} which has its *tnpA* gene disrupted by IS*Ecp1*-$bla_{CTX-M-15}$ (19). In this plasmid, this entire structure is also followed by an IS*26* element, previously shown to have a critical role in the mobilization and reorganization of antibiotic resistance genes in Gram-negative bacteria (20, 21).

The bla_{NDM-1}-bearing plasmid (178 kb) contained an Inc A/C2 replicon, extensively associated with antibiotic resistance in Gram-negative bacteria (22). The plasmid backbone shares similarity with other plasmids carrying bla_{NDM-1} and other β-lactamases in a variety of Gram-negative species (see Table S2 in the supplemental material). Additionally, this plasmid carried determinants conferring resistance to most antimicrobial classes, including β-lactams (bla_{CARB-2}), aminoglycosides [*aph*(3′)-*VIa*, *aacA29*, and *aadA2*], chloramphenicol (*catB3* and *cmlA1*), sulfonamides (*sul2* and *sul1*), macrolides [*mph*(*E*)], streptogramin B (*strB*), and lincosamide [*msr*(*E*)].

The *E. coli* isolate belonged to ST10 (phylogroup A), which has been associated with ESBLs and hyperexpressed AmpC enzymes (7). *E. coli* harbored most of the resistance determinants in plasmids; only the sulfonamide resistance gene *sul2* was present in the chromosome. The Inc FIA/FIB 151-kb plasmid carried bla_{TEM-1}, *catA1*, *sul1*, *tetB*, and *dfrA7*, while the 193-kb Inc A/C2 plasmid harbored not only bla_{NDM-1} but also *strA*. *strB*, *catA1*, *sul2*, *sul1*, *tetB*, and *dfrA1*. It is noteworthy that there were three tandem repeats of bla_{NDM-1} in the 193-kb plasmid, two of them within a Tn*125* structure and the last one lacking the right side copy of IS*Aba125* (*Aba* stands for *A. baumannii*) (Fig. 1). We interpret this to be a consequence of insertion of Tn*125* within a Tn*5393*-like structure, as evidenced by the presence of *tnpA*, *tnpR*, *strA*, and *strB*, characteristic of this Tn*3* transposon, originally reported for *Erwinia amylovora* (23), but now found in several Gram-negative species in clinical, ecological, and agricultural niches (24–27). This complex array of transposons is followed by an aminoglycoside resistance gene (*aadA16*) flanked by transposable genetic elements, indicating that this whole region could be serving as a "hot spot" for the incorporation of genetic determinants either by homologous recombination via IS elements, site-specific recombination, or transposition. A similar 20-kb resistance region is

TABLE 1 Accession number and resistome of bla_{NDM-1}-harboring isolates

Species	Parameter	Chromosome	Plasmids		
A. baumannii	Accession no.	NZ_CP010397.1	CP010398.1	CP010399.1	CP010400.1
	Size (bp)	3,902,527	114,848	47,274	9,327
	Resistance determinant(s)	bla_{ADC-80} and bla_{OXA-94}	None	*aph*(3′)*VIIa* and bla_{NDM-1}	None
A. nosocomialis	Accession no.	CP010368.1	NZ_CP010369.1	CP010903.1	CP010370.2
	Size (bp)	3,858,956	89,111	66,409	47,274
	Resistance determinant(s)	bla_{ADC-80}	None	None	*aph*(3′)*VIIa* and bla_{NDM-1}
K. pneumoniae	Accession no.	NZ_JWRK01000001.1	CP010390.1	CP010391.1	
	Size (bp)	5,329,244[a]	198,371	178,193	
	Resistance determinant(s)	$bla_{CTXM-15}$, bla_{SHV-11}, *oqxA*, *oqxB*, and *fosA*	*strA*, *strB*, *aac*(3′)*IIa*, *aac*(6′)*Ib-a*, *qnrB66*, *sul2*, *tetA*, *dfrA14*, *catB3*, bla_{TEM-1}, $bla_{CTXM-15}$, and bla_{OXA-1}	*aph*3′*VIa*, *aacA29*, *aadA2*, *mph*(*E*), *msr*(*E*), *catB3*, *cmlA1*, *sul2*, *sul1*, bla_{NDM-1}, and bla_{CARB-2}	
E. coli	Accession no.	NZ_CP010371.1	NZ_CP010373.2	NZ_CP010372.1	
	Size (bp)	4,761,012	193,908	151,583	
	Resistance determinant(s)	*sul1*	*strA*, *strB*, *catA1*, *sul2*, *sul1*, *tetB*, *dfrA1*, *aadA16*, and bla_{NDM-1} (3 times)	*catA1*, *sul1*, *tetB*, *dfrA7*, and bla_{TEM-1}	

[a] The genome was not closed. The size is estimated based on the size of the three contigs.

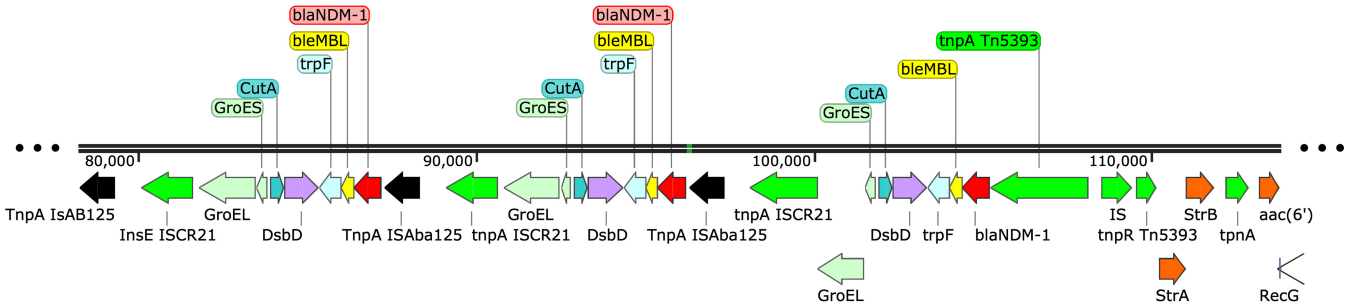

FIG 1 Organization of the bla_{NDM-1}-containing element in *E. coli*. The first two copies of bla_{NDM-1} are contained within the Tn*125* transposon (flanked by IS*Aba125* [shown in black]). The third copy lacks the right-flanking IS*Aba125* and is followed instead by a Tn*5393*-like element. Multiple transposable elements are found within this region, indicating that it may serve as a "hot spot" for the incorporation of new resistance determinants through homologous recombination via IS elements, site-specific recombination, or transposition.

found on environmental plasmid pRSB101 which was originally isolated from bacterial populations residing in the activated sludge compartment of a wastewater treatment plant (28). Furthermore, one of those transposable elements was identified as IS*26* and found not only flanking the above-mentioned region, but also next to the first copy of bla_{NDM-1} containing Tn*125*. This would reinforce the hypothesis of a "hot spot region," given the replicative transposition mechanism of IS*26* and its previously shown critical role in the mobilization and reorganization of antibiotic resistance genes in Gram-negative bacteria (20, 21). Most importantly, although bla_{NDM} in tandem repeats has been observed before, this is to our knowledge the first report of such a structure in *E. coli*. In both previously reported cases, it occurred in *K. pneumoniae* isolates: the first from a Taiwanese patient with a hospitalization history in New Delhi, India (250-kb Inc FIB/FII type plasmid) (29) and the second from an outbreak in a neonatal unit in Nepal (304-kb Inc HIB/FIB type plasmid) (30).

All strains were nosocomially acquired and isolated from elderly patients with severe systemic infections, three patients, who presented several comorbidities, died (see Table S3 in the supplemental material). First, since evidence of international travel or travel to Bogota, Colombia (where the first Colombian bla_{NDM-1} was reported) could not be established for any of the patients or their families and second, given that they originally lived in rural areas or small cities, this emergence in a variety of species in two different geographic locations, is extremely worrisome. Colombia has often been among the first countries in the region to report the circulation of important resistance determinants, including CTX-M-15, KPC, and NMC-A, all of which have become widely disseminated, even becoming endemic, as is the case for KPC (31–34). Even though information regarding molecular epidemiology of bla_{NDM-1} in Colombia is still very limited, the National Institute of Health of Colombia is reporting increased numbers of patients infected with NDM-producing bacteria. Interestingly, *K. pneumoniae* and *Providencia rettgeri* are the most prevalent bla_{NDM-1}-expressing Gram-negative bacterial species (35, 36). We hypothesize that the rapid spread of this resistance gene (bla_{NDM-1}) is aided by the circulation of broad-host-range, transferable plasmids such as Inc A/C found in this study.

The widespread dissemination of bla_{NDM} in Colombia portends a significant antibiotic resistance problem in Latin America (1). Colombia's situation may be only the "tip of the iceberg"; therefore, studies assessing the real prevalence of bla_{NDM}, especially in countries where few reports are available, are warranted. It is of great importance that the findings of surveillance and genomic studies like the present one help inform new and more-effective infection control and stewardship programs that can be translated into appropriate national policies to prevent a situation where it becomes endemic.

Sequence accession numbers. Sequences have been deposited in GenBank under the accession numbers given in Table 1.

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