Distinct mechanisms of dissemination of NDM-1 metallo- β-lactamase in *Acinetobacter* spp. in Argentina

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Abstract

A four-year surveillance of carbapenem-resistant *Acinetobacter* spp. in Argentina identified 40 strains carrying bla_{NDM-1}. Genome sequencing revealed that most were *A. baumannii*, while seven represented other *Acinetobacter* spp. The *A. baumannii* genomes were closely related, suggesting recent spread. bla_{NDM-1} was located in the chromosome of *A. baumannii* strains and on a plasmid in non-*baumannii* strains. A resistance gene island carrying bla_{PER-7} and other resistance determinants was found on a plasmid in some *A. baumannii* strains.

The predominant species expressing the NDM-1 carbapenemase are *Klebsiella pneumoniae* and *Escherichia coli*. However, *Acinetobacter spp.* are recognized as an intermediate reservoir for the bla_{NDM-1} resistance determinant (1, 2). bla_{NDM} is a metallo-beta-lactamase (MBL) generally found on a plasmid or other mobile element that carries resistance determinants for other antibiotic classes, rendering many NDM-positive isolates extensively drug resistant (XDR). Infections caused by carbapenem-resistant (CR) *A. baumannii* are associated with mortality rates as high as 60% (3, 4).

The Argentina National Reference Laboratory (NRL) identified an increase in the prevalence of NDM-containing *Acinetobacter* spp. beginning in 2015. Of the 20,028 clinical isolates screened since 2010, 15,621 were carbapenem resistant and 144 had a MBL phenotype (20-22). PCR for common MBLs confirmed that 68/144 (47%) were producers with 40 bla_{NDM} and 28 bla_{IMP}, while in the remaining strains the MBL-like

phenotype observed in the institution of origin was due to the presence of *bla*$_{OXA-23}$ or *bla*$_{OXA-58}$.

NDM-producing strains were recovered from 19 hospitals in nine cities and seven provinces of Argentina (Figure S1). *A. baumannii* isolates had more extensive antimicrobial resistance profiles than the non-*baumannii* isolates (Supplemental Table S1). Genome sequences were obtained on an Illumina NextSeq 500 and assembled using velvet (8). BLASTN search at NCBI classified seven genomes as representing five different non-*baumannii* species. (Supplemental Table S1). MLST analysis (9) showed that all 33 *A. baumannii* isolates belonged to ST25. The ST25 genomes were most closely related to isolates found throughout the world including HEU3 (Honduras), HWBA8 (Korea), NM3 (United Arab Emirates), and two genomes with no geographic origin provided: AR_0088 and AB5256. The AR_0088 genome has been completely sequenced (CP027530.1) and was used as the reference genome for SNP and IS element annotation.

The *A. baumannii* genomes differed by only 14-36 sequence variants, suggesting recent divergence. Patterns of shared SNPs (10), insertion sequence locations (11), and epidemiological data were highly concordant (Figure 1). Genomes were predominantly clustered by isolation location, with a few exceptions. For example, AMA19 from Hospital General de Agudos Vélez Sarsfield (VELE) is essentially identical to AMA9 from Hospital Dr. Cosme Argerich (COS), and on the same branch as other COS isolates. These hospitals belong to the public care sector in the Buenos Aires capital district, where patient exchange is frequent suggesting a possible transmission event.

The AR_0088 reference genome contained two plasmids: pAR_0088_1 (CP027531.1) and pAR_0088_2 (CP027532.1). pAR_0088_2 carries the *bla*$_{NDM-1}$ gene in Tn*125* and this plasmid is the likely location of the *bla*$_{NDM-1}$ gene in the non-*baumannii* *Acinetobacter* genomes (1, 12, 13). pAR_0088_2 sequences were not present in the *A. baumannii* ST25 genomes. To ascertain the location of Tn*125* in the *A. baumannii* genomes, we identified the locations of IS*Aba*125 insertions, which flank the transposon, in the draft genome assembly of a representative strain (AMA16). Six IS*Aba*125 insertion sites were inferred, all in the chromosome based on alignment of flanking sequences to the AR_0088 reference sequence. PCR and Sanger sequencing was used to demonstrate that Tn*125* is inserted at base 3,921,386 of the AR_0088 genome in AMA16 and all other *A. baumannii* isolates, interrupting a gene encoding a hypothetical protein, AM467_RS18915 (Supplemental Table S2).

A large resistance gene island (RI) was identified in fourteen of the ST25 strains (RI-PER-7). This ~23.8 kb sequence is bounded by a pair of IS*26* elements in direct repeat orientation (Figure 2). The island carries genes encoding resistance to aminoglycosides (*armA*), rifampicin (*arr*), cephalosporins (*bla*$_{PER-7}$), and fosfomycin (GST) (Figure 2). This RI also carries two copies of IS*CR1* (*IS*91-like) and three other IS elements that are found predominantly in *Enterobacteriaceae*, but are rare in *Acinetobacter*: IS*10A*, IS*Ec28*, and IS*Ec29*. The 7.5 kb at the 3' end of the island is found in several non-*Acinetobacter* genomes. The complete structure was identified in seven accessions in GenBank, most of which are plasmid sequences. Five of the ST25 genomes harbored a shorter version of the RI, lacking the *bla*$_{PER-7}$ gene. Genetic structures similar to RI-PER-7 were identified in other species, including *K.*

pneumoniae, *Proteus mirabilis*, and *E. coli*. In these structures *bla*$_{PER-7}$ was not present, but other β-lactamases were found (Figure 2).

RI-PER-7 is not present in AR_0088, but was found on one of the plasmids in HWBA8 (14). pHWBA8_1 (CP020596.1) is 195,838 bases and a large portion of this sequence is present in the ST25 *A. baumannii* genomes, including the complete RI-PER-7. Interestingly, pHWBA8_1 is also similar to pAR_0088_1, but the latter plasmid lacks the RI, another ~17.5kb segment, and has extensive rearrangements covering another ~20kb of sequence. The location of RI-PER-7 in the plasmid was confirmed by PCR amplification and Sanger sequencing of the junction regions (Supplemental Table S2). This large plasmid also carries five additional AR genes located outside RI-PER-7.

The *comM* gene, a common location for insertion of RIs in *A. baumannii*, is intact in the ST25 strains. IS elements were not found upstream of the chromosomal *bla*$_{ADC}$ or *bla*$_{OXA}$ genes. The ST25 strains carry S84L and S81L substitutions in the *parC* and *gyrA* genes, consistent with their non-susceptibility to ciprofloxacin.

The NRL also confirmed the presence of *bla*$_{NDM}$-producing Enterobacteriaceae in 12 of 19 hospitals (data not shown). In 8 of the 12 hospitals, this emergence occurred 1-4 months after the first detection of NDM-producing *Acinetobacter*. Additional work is needed to determine whether *Acinetobacter* could have played a role in the inter-species dissemination of NDM in these institutions.

Although reports of *bla*$_{NDM}$ in Latin America are limited, sentinel investigations have described the presence of Tn*125* in *Acinetobacter* spp. (6, 15-17). An ST25 *A. baumannii* strain from a patient with an abdominal infection in Honduras was determined to have Tn*125* on a plasmid (18), but this plasmid is not present in the ST25

strains analyzed here. Tn*125* has been reported in the chromosome of *Acinetobacter* spp as well (19, 20).

ST25 strains in Argentina typically carry the bla_{OXA-23} β-lactamase (17). According to the available data (12, 21-23), the co-occurrence of bla_{NDM-1} and $bla_{OXA-23/58}$ seems to be an uncommon event, a scenario that could change due to the increasing emergence of NDM-1 in *A. baumannii* isolates.

In summary, the genetic context of the bla_{NDM-1} differs between *A. baumannii* and non-*baumannii* isolates in Argentina, with non-*baumannii* strains mostly retaining susceptibility to some antibiotics. We also describe an RI in a subset of *A. baumannii* genomes that likely contributes substantially to the MDR phenotype of these strains. The escalating number of reports of NDM-1 among *A. baumannii* isolates is suggestive of a switch regarding the genetic basis of carbapenem resistance in this species and intensive tracking of patient contacts is warranted for ST25.

Data availability.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the BioProject accession PRJNA562922. Contig sequences for each genome are available as GenBank accession numbers VYSH00000000-VYTU00000000.

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Figure legends

Figure 1. Phylogenetic tree of *A. baumannii* genomes. A neighbor-joining tree was constructed using shared SNP and IS insertion sites. The inner circle is colored by the year of isolation. The outer circle is colored by the hospital in which the isolate was recovered. Refer to Table S1 for further information on the location of each hospital. Isolates that are positive for components of RI-PER-7 are denoted by colored circles at the outside of the tree. The scale bar represents the combined number of SNPs and IS insertion events. The AR_0088 genome was used as the outgroup, but the branch length is not shown so as to highlight the relationships of the AMA strains. The figure was created using iTOL (24).

Figure 2. Comparison of genetic structure of RI-PER-7 genomic island. Grey bars denote regions that are shared between isolates. Red arrows denote insertion sequence elements, green arrows denote antibiotic resistances genes. The figure was created using EasyFig version 2.2.2**.**