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EPIDEMIOLOGY AND SURVEILLANCE

Distinct Mechanisms of Dissemination of NDM-1 Metallo-β-Lactamase in *Acinetobacter* Species in Argentina

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ABSTRACT A 4-year surveillance of carbapenem-resistant *Acinetobacter* spp. isolates in Argentina identified 40 strains carrying bla_{NDM-1}. Genome sequencing revealed that most were *Acinetobacter baumannii*, whereas 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-*A. baumannii* strains. A resistance gene island carrying bla_{PER-7} and other resistance determinants was found on a plasmid in some *A. baumannii* strains.

KEYWORDS *Acinetobacter baumannii*, NDM-1, carbapenem resistance, *Acinetobacter*, carbapenems

The predominant species expressing the NDM-1 carbapenemase are *Klebsiella pneumoniae* and *Escherichia coli*. However, *Acinetobacter* spp. isolates are recognized as intermediate reservoirs for the bla_{NDM-1} resistance determinant (1, 2). bla_{NDM} is a metallo-β-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. Infections caused by carbapenem-resistant *Acinetobacter baumannii* isolates 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. isolates beginning in 2015. Of the 20,028 clinical isolates screened since 2010, 15,621 were carbapenem resistant and 144 had an MBL phenotype (5–7). PCR assays for common MBLs confirmed that 68 (47%), i.e., 40 bla_{NDM} and 28 bla_{IMP}, of 144 isolates were producers; whereas 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

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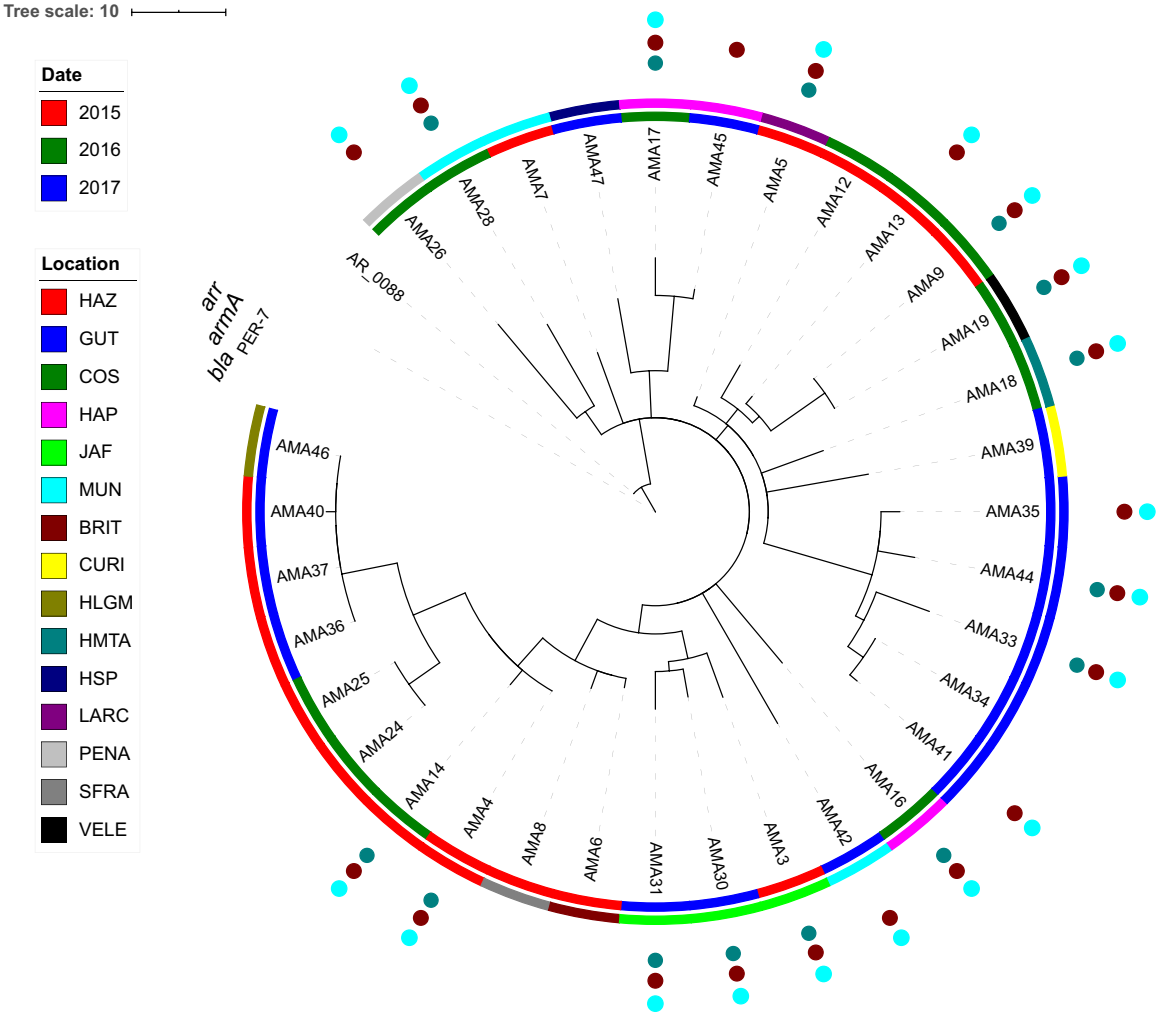

FIG 1 Phylogenetic tree of *A. baumannii* genomes. A neighbor-joining tree was constructed using shared SNP and IS insertion sites. Inner circle shows year of isolation. Outer circle shows hospital in which the isolate was recovered. See Table S1 for further information on the location of each hospital. Isolates that are positive for components of RI-PER-7 are denoted by colored circles 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 out group, but the branch length is not shown to highlight the relationships of the AMA strains. The figure was created using iTOL (24).

provinces in Argentina (see Fig. S1 in the supplemental material). *A. baumannii* isolates had more extensive antimicrobial resistance profiles than the non-*baumannii* isolates (see Table S1 in the supplemental material). Genome sequences were obtained on an Illumina NextSeq 500 and assembled using Velvet (8). A BLASTN search at NCBI classified seven genomes as representing five different non-*baumannii Acinetobacter* species. (see Table S1). MLST analysis showed that all 33 *A. baumannii* isolates belonged to sequence type ST25 (9). The ST25 genomes were most closely related to isolates found throughout the world, including HEU3 (Honduras), HWBA8 (South Korea), NM3 (United Arab Emirates), and two genomes with no geographic origin provided, i.e., AR_0088 and AB5256. The AR_0088 genome has been completely sequenced (GenBank accession no. CP027530.1) and was used as the reference genome for single nucleotide polymorphisms (SNPs) and insertion element (IS) annotation.

The *A. baumannii* genomes differed by only 14 to 36 sequence variants, suggesting recent divergence. Patterns of shared SNPs (10), IS locations (11), and epidemiological data were highly concordant (Fig. 1). Genomes were predominantly clustered by isolation location, with a few exceptions. For example, AMA19 from Hospital General de Agudos Vélez Sarsfield (VELE) was 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, i.e., pAR_0088_1 (GenBank accession no. CP027531.1) and pAR_0088_2 (GenBank accession no. 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 were used to demonstrate that Tn*125* was 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 (see Table S2 in the supplemental material).

A large resistance gene island (RI) was identified in 14 of the ST25 strains (RI-PER-7). This ~23.8-kb sequence is bound by a pair of IS*26* elements in direct-repeat orientation (Fig. 2). The island carries genes encoding resistance to aminoglycosides (*armA*), rifampin (*arr*), cephalosporins (bla_{PER-7}), and fosfomycin (GST) (Fig. 2). This RI also carries two copies of IS*CR1* (*IS*91-like) and three other IS elements that are found predominantly in *Enterobacteriaceae* isolates but are rare in *Acinetobacter* isolates, i.e., 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, mostly 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 (Fig. 2).

RI-PER-7 is not present in AR_0088, but it was found on one of the plasmids in HWBA8 (14). pHWBA8_1 (GenBank accession no. 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 and another ~17.5-kb segment and has extensive rearrangements covering another ~20 kb of the sequence. The location of RI-PER-7 in the plasmid was confirmed by PCR amplification and Sanger sequencing of the junction regions (see Table S2). This large plasmid carries five additional AR genes located outside RI-PER-7.

The *comM* gene, a common location for insertion of RIs in *A. baumannii* isolates, is intact in ST25 strains. IS elements were not found upstream of the chromosomal bla_{ADC} or bla_{OXA} genes in this study. The ST25 strains carry S84L and S81L substitutions in the *parC* and *gyrA* genes, consistent with their nonsusceptibility 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 to 4 months after the first detection of NDM-producing *Acinetobacter* isolates. Additional work is needed to determine whether *Acinetobacter* spp. could have played a role in the interspecies 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 was 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 bla_{OXA-23} β-lactamase (17). According to available data, the cooccurrence of bla_{NDM-1} and $bla_{OXA-23/58}$ seems to be an uncommon event (12, 21–23), a scenario that may change due to the increasing emergence of NDM-1 in *A. baumannii* isolates.

In summary, the genetic context of bla_{NDM-1} differs between *A. baumannii* and non-*baumannii* isolates in Argentina, with non-*baumannii* strains mostly retaining

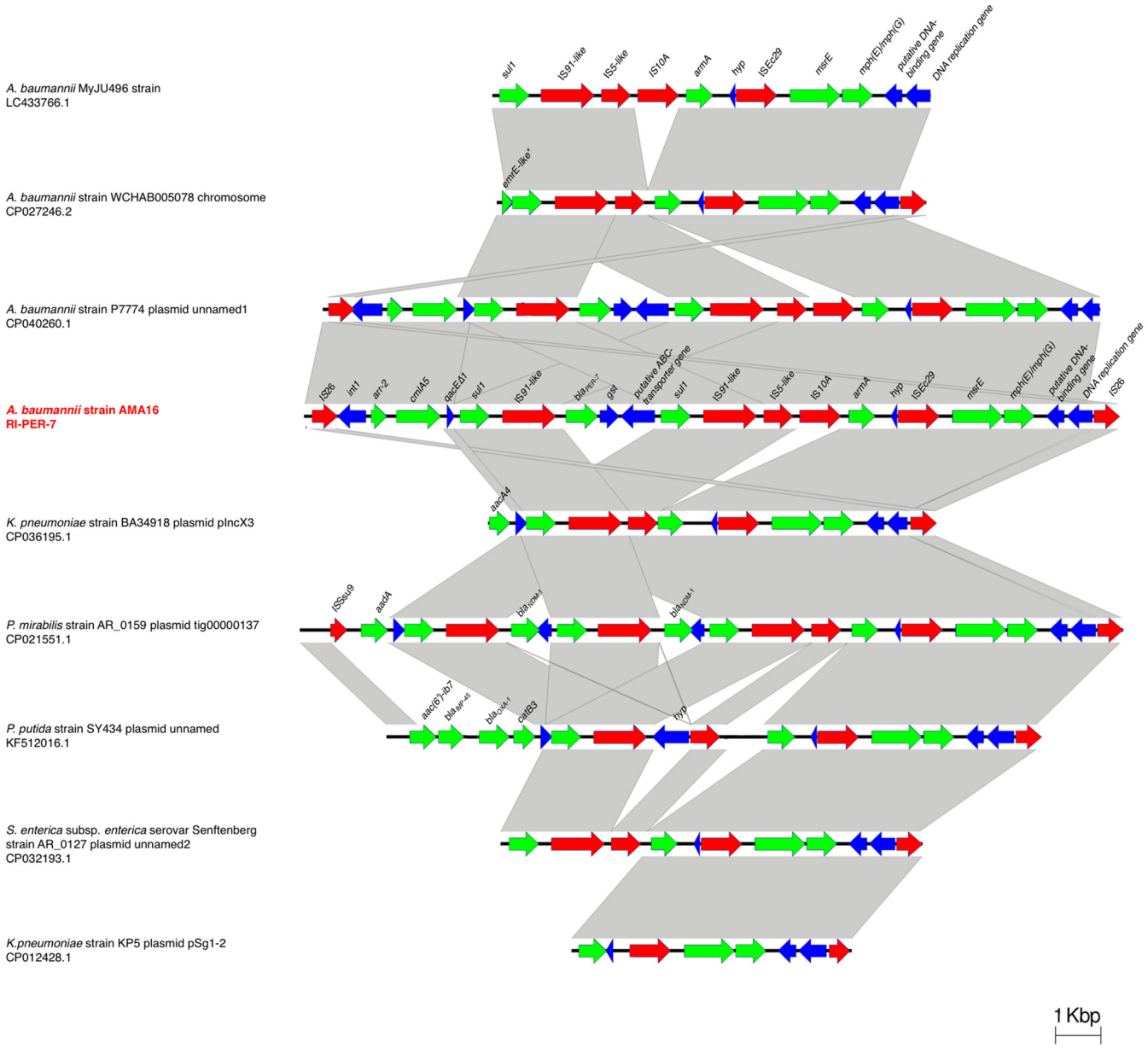

FIG 2 Comparison of genetic structure of RI-PER-7 genomic island. Gray bars, regions shared between isolates; red arrows, IS elements; green arrows, antibiotic resistances genes. The figure was created using EasyFig, version 2.2.2.

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 suggests 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 BioProject accession no. PRJNA562922. Contig sequences for each genome are available under GenBank accession nos. VYSH00000000 to VYTU00000000.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.01 MB.

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We have no conflicts of interest to declare.

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