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# Characterization of ST25 *bla*$_{NDM-1}$ producing *Acinetobacter* spp. strains leading the increase in NDM-1 emergence in Argentina.

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Sir,

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) are recognized by the Centers for Disease Control and Prevention (CDC) as an urgent threat (1). The most prevalent mechanism of carbapenem resistance in *Acinetobacter* spp. is due to the presence of carbapenem-hydrolyzing class D β-lactamases (CHDLs) with OXA-23 the most common. The observed dispersion of *bla*$_{OXA-23}$ is attributed in part to the spread of successful global clones such as GC1 and GC2 (2). However, in the most recent years an increased emergence of *bla*$_{NDM-1}$ harboring *Acinetobacter* spp. isolates were reported (3).

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A surveillance program in Argentina reported the increased occurrence of *Acinetobacter* NDM-1 isolates (3). A total of 15,621 carbapenem resistant isolates were included in the surveillance 40 of them were $bla_{NDM}$ positive (33 *A. baumannii* and 7 non-*A. baumannii* strains). All the *A. baumannii* isolates belonged to the ST25 and were closely related, suggesting a recent spread of NDM-1. In these strains, $bla_{NDM-1}$ was located in the chromosome rather than on a plasmid as was found in non-*A. baumannii* strains. In addition, a resistance island carrying $bla_{PER-7}$ was found on a plasmid in some *A. baumannii* strains (3). Reports of NDM-1 positive ST25 isolates have been restricted to some regions, including Latin America, Central and Western Europe, East Africa and Western Asia (3, 8–12).

In the present work, we further characterized the ST25 NDM-1 *A. baumannii* isolates and compared them to representative strains of the most prevalent clonal complexes (GC1 and GC2) (4, 5). Twenty-two representative strains recovered from different sources, years, and location were selected (Table S1).Among the selected strains, we included eight NDM-1 positive *A. baumannii* strains, seven OXA-23 positive *A. baumannii* strains, two strain with upregulation of the endogenous OXA-51-like (OXA-66) carbapenemase, and five NDM-1 positive non-*A. baumannii* strains (Table S1). Phenotypic assays including motility, macrocolony formation (6), hydrogen peroxide susceptibility, osmotolerance, and desiccation tolerance (7), were performed.

Surface-motility assays were performed on lysogeny broth, LB, agar plates with 0.3% of agarose. None of the tested *A. baumannii* strains exhibited surface-associated motility (Figure 1). Most of the non-*A. baumannii* strains also did not exhibit surface-associated motility with the exception of AMA 43. To study biofilm formation, macrocolonies were used as a biofilm model system. The *A. baumannii* strains were grown on LB agar supplemented with Congo red (6). Macrocolony formation varied among the ST25, GC1, GC2, and non-*A. baumannii* strains. A few of the ST25 strains produced more extracellular matrix (ECM) than others, i.e. AMA 40 and AMA 46 produced more ECM in comparison to AMA 16 (Figure S1). Among the non-*A. baumannii* strains, AMA 23 was the one that produced more ECM. In general, we observed that GC1, GC2 and the non-*A. baumannii* strains produced less ECM compare with the ST25 strains. However, substantial variability of ECM production was observed among the studied strains (Figure S1).

Desiccation assays were performed for seven days (7). Data collected on days zero, one, four and seven were recorded. Dried cells were incubated at room temperature and at the indicated times dried samples were suspended, and survival was assessed by CFU/mL counts. ST25, GC1, and GC2 *A. baumannii* strains demonstrated a decrease in viability as the days progressed. After day four, there were no GC2 strains that survived for the day seven collection (Figure 1). Select ST25 strains had an increase in viability from day one to day four. Non-*A. baumannii* strains were not viable after one day of desiccation (Figure 1).

To assess osmotolerance, bacterial strains were treated and incubated with 0.6 M NaCl (7). Data were collected at hours four, eight and twenty-four. We observed that all of the NDM-1 positive *A. baumannii* strains and the GC1 and GC2 strains, were able to resist the NaCl

treatment and were viable after treatment. Only the AMA23 (non-*baumannii*) strain was not viable after treatment (Figure 1).

Finally, hydrogen peroxide susceptibility was performed only in *A. baumannii* strains (7). We observed that hydrogen peroxide treated cells were less viable than non-treated cells. Some strains were not able to resist the peroxide treatment such as AMA3, AMA16, AB0057 (GC2), ABUH796, ABUH628, ABUH747, AB5075 (Figure 1). Collectively, even among the closely related NDM-1 positive ST25 *A. baumannii* isolates, strain-specific behavior was observed in phenotypic traits. To further explore the characteristics of these isolates, genomic analysis of known *Acinetobacter* virulence genes, such as iron uptake systems, biofilm-associated proteins, motility, etc., were analyzed using a combination of blastn and sequences of virulence determinants from *A. baumannii*. The genes that code for the Baumanoferrin siderophore and the *feoABC* genes related to iron uptake were found in all of *A. baumannii* and *A. nosocomialis* genomes studied. Also, *feoABC* genes were found in *A. pittii, A. junii*, and *A. haemolyticus* (Figure S2). The Heme cluster 1 was found only in six genomes of *A. baumannii*. However, the Heme cluster genes 2 and Acinetobactin genes were identified in *A. baumannii* ST25 clones, *A. baumannii* AB0057, and *A. baumannii* AB5075-UW (Figure S2). However, the genes coding for the Fimsbactin siderophore and the *entAB* genes were not found in the included *Acinetobacter* genomes (Figure S2). Genes linked to biofilm formation, motility, and poly-N-acetyl glucosamine (PNAG) production were also analyzed. The presence of *pgaABC* and *bfmSR* genes related to biofilm formation were found in *A. baumannii, A. nosocomialis*, and *A. pittii* (Figure S2). Strikingly, we identified an orphan *bfmR* gene in *A. junii.* The *CsuAB/ABCDE* genes cluster linked to biofilm formation was also found in fifteen of seventeen genomes of *A. baumannii* (Figure S2). In *A. baumannii* and *A. nosocomialis* the presence of *prpABCD* genes involved in fimbriae biogenesis and motility were also observed (Figure S2). The K locus (KL) and OC locus (OCL) responsible for the production of capsular polysaccharides and O-antigen, respectively, were identified and characterized using to Kaptive tool (13) and the results were confirmed by BLAST using GenBank database. Seven ST25 genomes contain KL14/OCL6, one has KL14/OCL5 (AMA50), and AMA42 has KL22-like/OCL6 (Figure S2). Among the GC2 genomes included in the study five contain KL22/OCL3-like. However, *A. baumannii* AMA51 (GC2) recovered from Argentina contained KL2/OCL2. *A. nosocomialis* and *A. junii* genomes contained KL48-like/OCL7 and KL38-like/OCL6-like (Figure S2). In the rest of non-*baumannii* genomes, the KL and OCL genetic structures were not found. Furthermore, we found orphan genes from OCL and KL in *A. lwoffii, A. junii* and *A. haemolyticus* with an 80-85 % amino acid identity against to reference sequence of KL and OCL (Kaptive tool, reference sequences) (Figure S2). Overall, no definitive correlations could be made between the observed phenotypes and the presence or absence of specific genes, highlighting the complex nature of most virulence characteristics.

In summary, we found that ST25 isolates had similar characteristics to GC1 and GC2 strains. The majority of strains did not exhibit surface-associated motility. Macrocolony biofilms assays showed variation in structural characteristic among the different strains. All selected strains were able to resist desiccation for up to seven days and some strains were able to resist hydrogen peroxide treatment. In addition, ST25 strains were able to resist NaCl treatment. We did not observe a clear difference between NDM-1 ST25 strains and the

isolates from the two major GC. However, in addition to carbapenem-resistance, we identified different traits, such as desiccation, osmotolerance, etc, that can allow the strains to persist in the hospital environment and can in part explain the potential of ST25 to spread as GC1 and GC2. Further *in vivo* studies will shed more light on the success and pathogenicity of ST25 *A. baumannii*.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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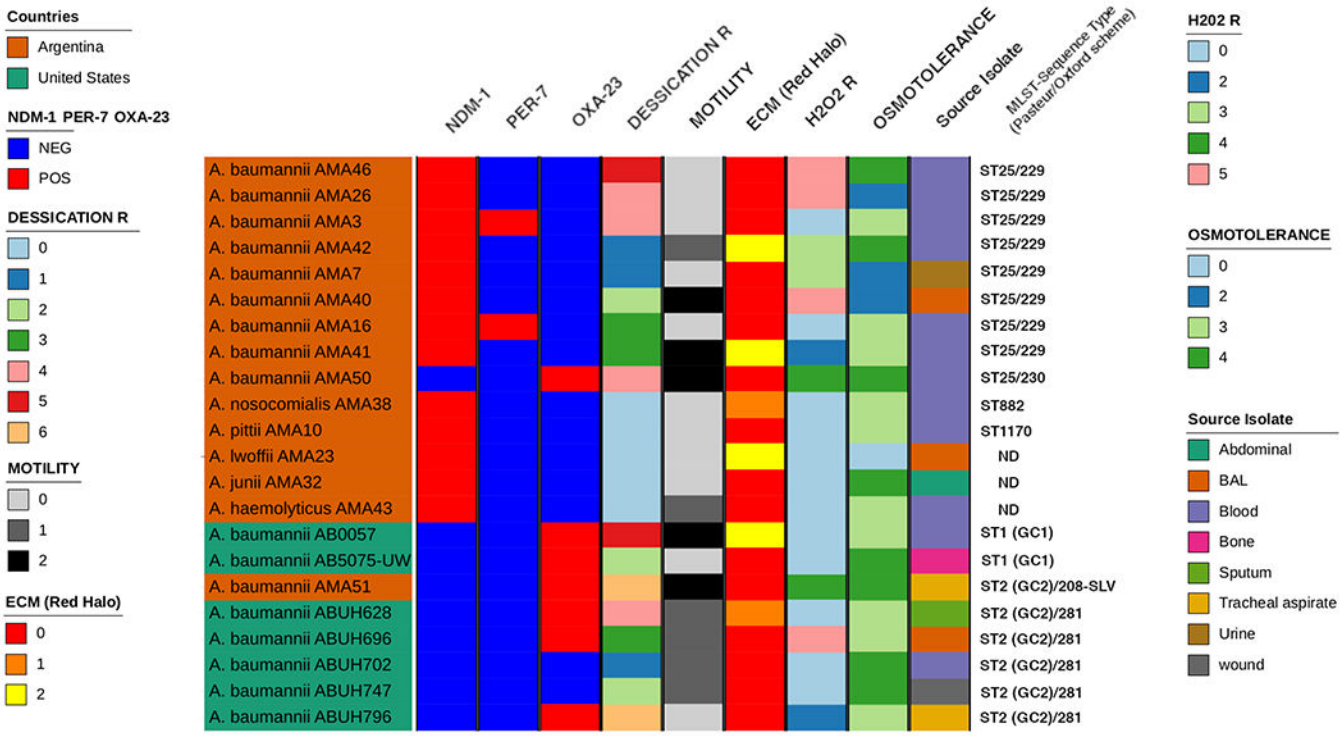


**Figure 1.**

Graphic representation of the different phenotypes of *Acinetobacter* sp. used in this study. The graphic representation was performed by ComplexHeatmap package (cita_1). The sequence type data was determined by MLST script (https://github.com/tseemann/mlst).