Letter to the Editor

Characterisation of ST25 NDM-1-producing Acinetobacter spp. strains leading the increase in NDM-1 emergence in Argentina

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Sir,

Carbapenem-resistant *Acinetobacter baumannii* are recognised by the US 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-hydrolysing class D β-lactamases, with OXA-23 being the most common. The observed dispersion of bla_{OXA-23} is attributed in part to the spread of successful *A. baumannii* global clones such as GC1 and GC2 [2]. However, in the most recent years an increased emergence of bla_{NDM-1}-harbouring *Acinetobacter* spp. isolates has been reported [3].

A surveillance programme in Argentina reported the increased occurrence of NDM-1-producing *Acinetobacter* isolates [3]. A total of 15 621 carbapenem-resistant isolates were included in the surveillance, of which 40 were bla_{NDM}-positive (33 *A. baumannii* and 7 non-*baumannii Acinetobacter* spp. strains). All of the *A. baumannii* isolates belonged to sequence type 25 (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 the non-*baumannii Acinetobacter* spp. 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 certain regions, including Latin America, Central and Western Europe, East Africa and Western Asia [3–8].

In the present work, we further characterised the ST25 NDM-1-producing *A. baumannii* isolates and compared them with representative strains of the most prevalent clonal complexes (GC1 and GC2) [9,10]. A total of 22 representative strains recovered from different sources, years and location were selected (Supplementary Table S1). Among the selected strains, we included eight NDM-1-positive *A. baumannii*, seven OXA-23-positive *A. baumannii*, two strains with upregulation of the endogenous OXA-51-like (OXA-66) carbapenemase, and five NDM-1-positive non-*baumannii Acinetobacter* spp. strains (Supplementary Table S1). Phenotypic assays including motility, macrocolony formation [11], hydrogen peroxide susceptibility, osmotolerance and desiccation tolerance [12] were performed.

Surface motility assays were performed on lysogeny broth (LB) agar plates with 0.3% agarose. Most of the tested *A. baumannii* strains did not exhibit surface-associated motility (Fig. 1). Most of the non-*baumannii* strains also did not exhibit surface-associated motility with the exception of strain AMA43. To study biofilm formation, macrocolonies were used as a model biofilm system.

Acinetobacter baumannii strains were grown on LB agar supplemented with Congo red [11]. Macrocolony formation varied among the ST25, GC1, GC2 and non-*baumannii Acinetobacter* strains. A few of the ST25 strains produced more extracellular matrix (ECM) than others, i.e. AMA40 and AMA46 produced more ECM comparison with AMA16 (Supplementary Fig. S1). Among the non-*baumannii* strains, AMA23 was the one that produced more ECM. In general, we observed that GC1, GC2 and the non-*baumannii Acinetobacter* strains produced less ECM compared with the ST25 strains. However, substantial variability of ECM production was observed among the studied strains (Supplementary Fig. S1).

Desiccation assays were performed for 7 days [12]. Data collected on Days 0, 1, 4 and 7 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 4, there were no GC2 strains that survived for the Day 7 collection (Fig. 1). Select ST25 strains had an increase in viability from Day 1 to Day 4. Non-*baumannii Acinetobacter* strains were not viable after 1 day of desiccation (Fig. 1).

To assess osmotolerance, bacterial strains were treated and incubated with 0.6 M NaCl [12]. Data were collected after 4, 8 and 24 h. 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 strain AMA23 (non-*baumannii*) was not viable after treatment (Fig. 1).

Finally, hydrogen peroxide susceptibility was performed only in *A. baumannii* strains [12]. 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 (GC1), ABUH796, ABUH628, ABUH747 and AB5075 (Fig. 1).

Collectively, even among the closely related NDM-1-positive ST25 *A. baumannii* isolates, strain-specific behaviour 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 analysed using a combination of BLASTN and sequences of virulence determinants from *A. baumannii*. The genes encoding the baumanoferrin siderophore and the *feoABC* genes related to iron uptake were found in all of the *A. baumannii* and *Acinetobacter nosocomialis* genomes studied. Also, *feoABC* genes were found in *Acinetobacter pittii*, *Acinetobacter junii* and *Acinetobacter haemolyticus* (Supplementary Fig. S2). Heme cluster 1 was found in only 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 (Supplementary Fig. S2). However, genes encoding the fimsbactin siderophore and
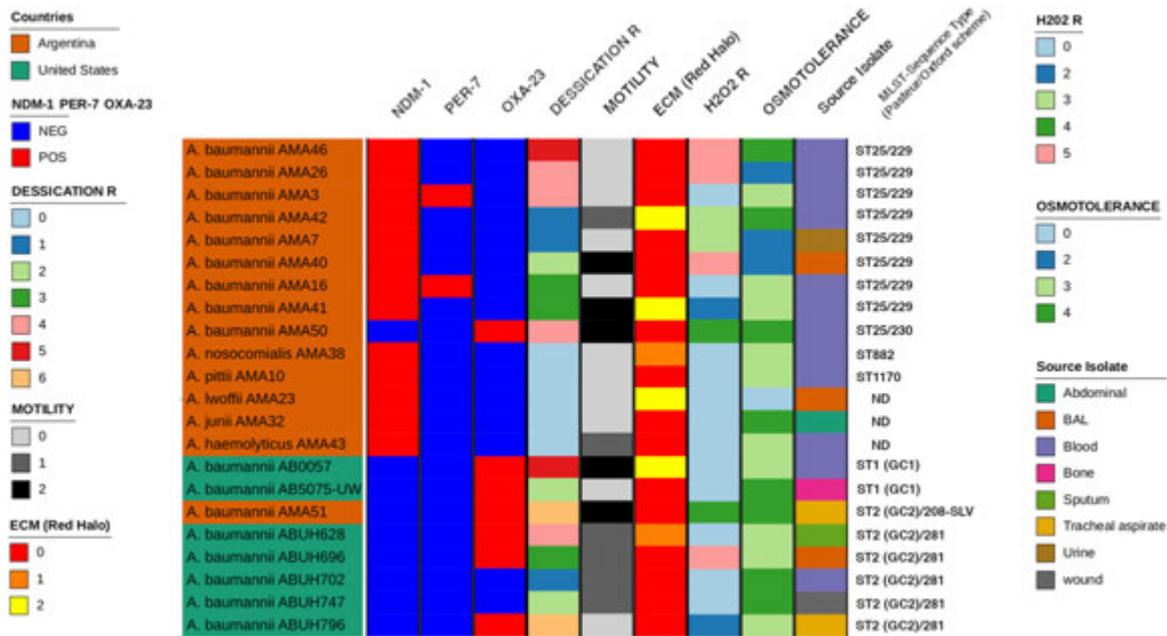
Fig. 1. Graphical representation of the different phenotypes of *Acinetobacter* spp. used in this study. The graphical representation was performed by ComplexHeatmap package [14]. The sequence type data were determined by MLST script (https://github.com/tseemann/mLst).

the *entAB* genes were not found in the included *Acinetobacter* genomes (Supplementary Fig. S2). Genes linked to biofilm formation, motility and poly-*N*-acetyl glucosamine (PNAG) production were also analysed. The presence of *pgaABC* and *bfmSR* genes related to biofilm formation were found in *A. baumannii*, *A. nosocomialis* and *A. pittii* (Supplementary Fig. S2). Strikingly, we identified an orphan *bfmR* gene in *A. junii*. The *csuAB/ABCDE* gene cluster linked to biofilm formation was also found in 15 of 17 genomes of *A. baumannii* (Supplementary Fig. S2). In *A. baumannii* and *A. nosocomialis*, the presence of *prpABCD* genes involved in fimbriae biogenesis and motility was also observed (Supplementary Fig. S2). The K locus (KL) and OC locus (OCL) responsible for production of capsular polysaccharides and O-antigen, respectively, were identified and characterised using Kaptive tool [13] and the results were confirmed by BLAST using the GenBank database. Seven ST25 genomes contained KL14/OCL6, one had KL14/OCL5 (AMA50), and AMA42 had KL22-like/OCL6 (Supplementary Fig. S2). Among the GC2 genomes included in the study, five contained KL22/OCL3-like. However, *A. baumannii* AMA51 (GC2) recovered from Argentina contained KL2/OCL2. *Acinetobacter nosocomialis* and *A. junii* genomes contained KL48-like/OCL7 and KL38-like/OCL6-like (Supplementary Fig. S2). In the rest of the non-*baumannii* genomes, the KL and OCL genetic structures were not found. Furthermore, we found orphan genes from OCL and KL in *Acinetobacter lwoffii*, *A. junii* and *A. haemolyticus* with an 80–85% amino acid identity with the reference sequence of KL and OCL (Kaptive tool, reference sequences) (Supplementary Fig. 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 biofilm assays showed variation in structural characteristic among the different strains. All selected *A. baumannii* strains were able to resist desiccation for up to 7 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 GCs. 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*.

Funding

Conflict of interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2020.08.015.

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