## Supplementary Data

### Materials and methods

#### Bacterial strains and serial passage

The *A. baumannii* strains, XH386 and XH731, were isolated from two tertiary hospitals in Hangzhou, China.[1](#_ENREF_1), [2](#_ENREF_2) The Mueller-Hinton (MH) Broth (OXOID, England) supplemented 16 mg/L imipenem (TIENAM®, Merck Sharp & Dohme Corp), and 0 mg/L imipenem (as the control), was used for culturing the two strains. The serial passage lasted for 14 days. Each day, 2 µL from a densely grown culture was inoculated into a fresh 2 mL MH broth supplemented with the same concentration of imipenem; the remainder was frozen for subsequent experiments at -80 oC. The entire serial passage experiment was repeated three times independently, for the biological replicates.

#### Antimicrobial susceptibility testing

The MIC of imipenem was determined using a modified agar dilution method. Briefly, the frozen bacterial culture was plated on an MH agar (imipenem free) medium and incubated overnight at 37 oC. The bacteria were then harvested and diluted with a 0.9% normal saline solution to 0.5 MCF. Next, the bacterial suspension was diluted a further 10 times with 0.9% normal saline, and 2 µl of the diluted bacteria suspension was loaded onto an MH agar medium containing imipenem at the serial concentrations of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, 104, 112, 120 and 128 mg/L.

For the MIC determination, only the bacteria spread onto the entire plate were interpreted as effective growth, with the exception of a minority of colonies, which were ignored. The *Escherichia coli* strain ATCC 25922 was used as the reference control. The MIC was measured three times for each culture as technical replicates.

#### Measurement of the *bla*OXA-23 copy number

The quantification of the *bla*OXA-23 copy number was performed as previously described.[2](#_ENREF_2) Briefly, frozen bacterial culture was plated onto an MH agar (imipenem free) medium and incubated overnight at 37 oC. The bacteria were harvested, and their genomic DNA extracted from the culture using a QIAamp DNA Mini Kit (Qiagen, Germany). The concentration of the genomic DNA was measured by a Nanodrop 2000c spectrometer (Thermo Scientific, USA), with the quality being evaluated at the value of A260/A280. 20 ng genomic DNA was used as the template for the qRT-PCR. Each of the qPCR reaction samples were created according to the kit’s instructions, as follows: 5 µl SYBR® 2x Premix Ex Taq (Takara, Japan), 1 µl diluted genomic DNA, 1 µl mixed primer (forward and reverse primer, 5 µM each), and 3 µl distilled water, with the entire reaction volume being 10 µl. The qRT-PCR was performed using a Roche LightCycler 480II instrument (Roche Molecular Diagnostics, Switzerland) and SYBR Premix Ex TaqTM PCR kit (Takara Bio, Japan). The gene *rpoB* served as the internal control. The relative copy number of *bla*OXA-23 was calculated according to the ΔΔCt method. Three technical replicates were made for each biological replicate strain.

#### Genome sequencing and analyses

XH386 and XH731 Replicate Two, collected on Day 14, were chosen for whole genome sequencing. The frozen bacterial culture was plated onto an MH agar (imipenem free) medium and incubated overnight at 37 oC. The bacteria were harvested, and their genomic DNA extracted from the culture using the QIAamp DNA Mini Kit (Qiagen, Germany). The genomic DNA was sequenced using the PacBio RS II sequencer (Pacific Biosciences, USA). A *de novo* assembly was performed following the Hierarchical Genome Assembly Process workflow available in SMRT Analysis v2.3.0. Complete genomic sequences were deposited in the NCBI database. The accession numbers for strain XH386 on Day 0 and 14 were CP010779 and CP021326; the accession numbers for XH731 deposited on Day 0 and 14 were CP019217 and CP021321.

### References

1. Fang Y, Quan J, Hua X et al. Complete genome sequence of Acinetobacter baumannii XH386 (ST208), a multi-drug resistant bacteria isolated from pediatric hospital in China. *Genom Data* 2016; **7**: 269-74.

2. Hua X, Shu J, Ruan Z et al. Multiplication of blaOXA-23 is common in clinical Acinetobacter baumannii, but does not enhance carbapenem resistance. *J Antimicrob Chemother* 2016; **71**: 3381-5.

### Table S1. Mutations occurring in XH386, identified by comparing the genomes before and after serial passage

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Position (bp) | Mutation | Reference | Allele | Coding region |
| 3840067 | Insertion | - | T | Intergenic |

Note: CP010779.1 is used as the reference genome.

### Table S2. Mutations occurring in XH731, identified by comparing the genomes before and after serial passage

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Position (bp) | Mutation | Reference | Allele | Coding region |
| 617438 | Insertion | - | T | Intergenic |
| 1974425 | Deletion | G | - | BWI80\_09325 (encoding chromate transporter) |
| 2984934 | Insertion | - | A | BWI80\_14355 (encoding tyrosyl-tRNA synthetase) |

Note: CP019217.1 is used as the reference genome.