

Two carbapenem-resistant ST1:ST231:KL1:OCL1 *Acinetobacter baumannii* strains recovered in Tehran, Iran, carry AbaR31 in the chromosome and AbaR4 and TnaphA6 in a RepAci6 plasmid

Masoumeh Douraghi¹, Parisa Aris¹, Joyce To², Garry S. A. Myers² and Mohammad Hamidian ^{2*}

¹Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran;

²The iThree institute, University of Technology Sydney, Ultimo, NSW, Australia

*Corresponding author. E-mail: mohammad.hamidian@uts.edu.au or mohamidian@gmail.com

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Objectives: To analyse the context of genes conferring antibiotic resistance in two carbapenem-resistant *Acinetobacter baumannii* isolates recovered in Tehran, Iran.

Methods: The antibiotic resistance phenotype for 28 antibiotics was determined using disc diffusion. The whole genome sequences of ABH008 and ABS200 were determined using the Illumina HiSeq X Ten platform. Resistance genes were identified using ResFinder and multilocus sequence types were determined using the Oxford and Institut Pasteur schemes.

Results: Isolates ABH008 and ABS200, recovered in 2012 and 2013, respectively, in two different Tehran hospitals, belong to the common global clone 1 lineage, ST1_{IP} and ST231_{OX}. They are resistant to sulfamethoxazole, tetracycline, gentamicin, amikacin, third-generation cephalosporins and carbapenems. Despite being isolated in different hospitals, phylogenetic analysis indicated they are closely related. Consistent with this, both isolates carry *catA1*, *sul1*, *aacC1* and *aadA1* in a novel variant of the AbaR3-type resistance island, named AbaR31. Both isolates are resistant to amikacin and carbapenems owing to *aphA6* and *oxa23*, respectively. The *oxa23* gene is located in the AbaR4 resistance island, and *aphA6* in TnaphA6, and both mobile elements are in an ~90 kbp plasmid encoding the putative RepAci6 replication initiation protein. Resistance to third-generation cephalosporins is due to the acquisition by homologous recombination of a 5 kb DNA segment that contains ISAbal1-*ampC* from a ST623 strain.

Conclusions: The resistance gene complements of ABH008 and ABS200 were found in AbaR31 and a plasmid that encodes RepAci6. The close genetic relationship of ABH008 and ABS200, despite each being recovered from different hospitals, indicates transmission between the two hospitals.

Introduction

Resistance to carbapenems in *Acinetobacter baumannii*, the number one WHO priority pathogen, is widespread, posing a global threat as carbapenems are the frontline option for treating infections caused by multi-antibiotic-resistant strains of this opportunistic pathogen.^{1,2} In *A. baumannii* carbapenem resistance is most often caused by acquired class D carbapenem oxacillinases, typically OXA-23, OXA-24 (OXA-40) and OXA-58.^{2,3}

Two clonal complexes of *A. baumannii* are distributed globally: global clone 1 (GC1) and global clone 2 (GC2). Within each is a myriad of lineages and sub-lineages. The genetic variation among these lineages informs understanding of how resistance evolves and enables epidemiological tracking of antibiotic-resistant strains. For *A. baumannii*, the majority (70%) of the whole genome

sequence data feeding variant analysis is geographically skewed.² It is sourced from isolates obtained from only four countries, all outside the Middle East region² while the source of many resistant lineages of *A. baumannii*, however, is believed to be the Middle East region.⁴⁻⁷ This discrepancy makes it difficult to draw an accurate global picture of *A. baumannii*. We recently reported analysis of whole genome sequence data from an outbreak in an Iranian hospital that supports the view that lineage 2 GC1 strains may have an origin in the region.⁵

Here, we report analysis of whole genome sequence data for two antibiotic-resistant *A. baumannii* strains isolated from two different hospitals in Iran. Both belong to lineage 1 of GC1 indicating that strains belonging to lineage 1 are also present in the country.

Methods

Antibiotic resistance phenotype and PCR mapping

Antibiotic resistance profiles of ABS200 and ABH008 were determined using the standard Kirby-Bauer disc diffusion assay to 28 antibiotics as previously described.⁵ Resistance to colistin was determined by measuring the MICs using the standard micro-broth dilution method.⁸ Antibiotic discs tested were ampicillin, streptomycin, spectinomycin, sulfamethoxazole, tetracycline, trimethoprim, kanamycin, neomycin, cefotaxime, ceftazidime, gentamicin, ciprofloxacin, amikacin, nalidixic acid, tobramycin, netilmicin, imipenem, meropenem, ticarcillin/clavulanic acid, rifampicin, ampicillin/sulbactam, cefepime, doripenem, piperacillin/tazobactam, ceftriaxone, minocycline, doxycycline and levofloxacin. Strains were classified as resistant or susceptible according to the CLSI guidelines for *Acinetobacter* spp.⁹ and Calibrated Dichotomous Sensitivity (CDS) disc diffusion assay (<http://web.med.unsw.edu.au/cdstest>) where an *Acinetobacter* spp. CLSI breakpoint was not available (e.g. for netilmicin, streptomycin, spectinomycin, sulfamethoxazole, nalidixic acid and rifampicin).

AbaR resistance islands were mapped using the PCR mapping strategy previously developed including the novel AbaR31 junction, which was mapped using the RH513: 5'-GTACTGTTGTAATTCATTAAGCAT-3' and intI1-RV: 5'-GGGCATGGTGGCTGAAGGACC-3' primers generating a 3 kb product.^{10,11}

AbaR4 was also mapped using previously designed primers¹² and located in a RepAci6 plasmid using two mapping PCRs (i) MH5 (5'-CTAAAAGGCGTTTGGGCATA-3') and RH909 (5'-GCGATTCAAAATATCG GTCAA-3')¹³ (generating a 3105 bp product) and (ii) MH7 (5'-TGCTG AACCGTACAACCAGA-3') and MH6 (5'-CAGGATGAGCTGGATCAACA-3') (generating a 3645 bp product) developed here. PCR conditions were the same as those described elsewhere.

Whole genome sequencing, assembly and phylogenetic analysis

Genomic DNA isolated from ABH008 and ABS200 was sequenced using Illumina HiSeq X Ten. Paired-end reads of 150 bp were assembled using SPAdes (v. 3.14.1) yielding 75 and 77 contigs for ABH008 and ABS200, respectively, and with an average depth of ~100-fold for each genome. Multi-locus Sequence Types in the Institut Pasteur (ST_{IP}) and Oxford (ST_{OX}) schemes as well as the *ampC* alleles were determined from the genome sequence data using the PubMLST database (<http://pubmlst.org/abaumannii/>).

Antibiotic resistance genes and contigs carrying them were identified using ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>), and recovered using standalone BLAST (<ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/>).

Genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (v.4.11).¹⁴ Pfam (<http://pfam.xfam.org/>) and ISFinder (<https://www-is.biotoul.fr/>) search tools were used to identify proteins and insertion sequences, respectively. Here, we used the ST_{IP}#:ST_{OX}#:KL#:OCL# formula that we recently developed¹⁵ to group/distinguish *A. baumannii* isolates.

Phylogenetic analysis was done by constructing a recombination-free tree by generating a whole genome alignment using snippy (v.4.6.0) (publicly available at <https://github.com/tseemann/snippy>) followed by removing regions potentially introduced by recombination using Gubbins¹⁶ (v2.1.025) as previously described in detail.^{5,6} A set of nine GC1 strains known to belong to lineage 1 and 2, including the A1 GC1 reference genome (GenBank accession number CP010781), were also used as controls (Figure 1). Figures were drawn to scale using SnapGene[®] (v. 5.2.4.) and Inkscape (v. 1.0).

Nucleotide accession numbers

The genome sequence of ABH008 and ABS200 have been deposited in GenBank GenBank/EMBL/DBJ database and are publicly available under the accession numbers JADPVA000000000 and JADPVB000000000.

Results and discussion

Resistance profiles, MLST and phylogenetic relationship

ABH008 and ABS200 are carbapenem-resistant isolates from two different hospitals in Tehran, Iran, isolated in 2012 and 2013, respectively. We determined their resistance phenotype and their relationship to global *A. baumannii* clones. ABH008 and ABS200 are resistant to carbapenems, third-generation cephalosporins, ticarcillin/clavulanic acid, tetracycline, streptomycin, spectinomycin, gentamicin, kanamycin, neomycin, amikacin sulphonamides, trimethoprim, nalidixic acid and ciprofloxacin, (Table S1, available as [Supplementary data](#) at JAC-AMR Online) while remaining susceptible to colistin (MIC <0.5 mg/L). Hence, they are considered extensively drug resistant (XDR).¹⁷ ABH008 and ABS200 genomes both contain the *catA1*, *tetA(A)*, *sul1*, *aacC1*, *aadA1*, *aphA6* and *oxa23* genes, accounting for the resistance phenotype observed. ABH008 and ABS200 were found to be GC1 based on analysis of the allelic-specific PCR¹⁸ which determines clones and their draft genomes. Consistent with other GC1s, both strains encode the OXA-69 variant of the intrinsic *oxaAb* gene and belong to ST_{1P}(1-1-1-1-1-5-1) and ST_{231OX}(10-12-4-11-4-98-5). Phylogenetic analysis showed ABH008 and ABS200 were tightly clustered together, both falling within lineage 1 (Figure 1). It is possible that the ABH008 and ABS200 branch might represent the lineage 1 strains circulating in Tehran's hospitals; however, more genome sequences would be needed to confirm this.

ABH008 and ABS200 carry AbaR31; a novel antibiotic resistance island derived from AbaR3

To determine the genetic context of the resistance genes of ABH008 and ABS200, we used a combination of draft genomes and PCR mapping strategies.¹⁰⁻¹³ The *catA1*, *tetA(A)*, *sul1*, *aacC1*, *aadA1* resistance genes were localized in a novel variant of AbaR0/3-type islands with a Tn6019 transposon backbone located in the *comM* gene of ABH008 and ABS200. Our draft genome analysis indicated that this island derives from AbaR3 rather than AbaR0 as it contains a 108 bp sequence in the 5'-CS of the class 1 integron, which is characteristic of AbaR3 (e.g. in AB0057; GenBank accession number CP001182) and its variants.¹⁹ We named this new AbaR3 variant AbaR31. It is predicted to be 51 113 kbp (Figure 1). AbaR31 has been generated via an IS26-mediated adjacent deletion of an 11 877 bp internal segment of the AbaR3 island that includes the *bla_{TEM}* and *aphA1b* antibiotic resistance genes. IS26-mediated deletions are known to be important in generating variants of AbaR0/3 islands.¹⁹ We confirmed the novel junction in AbaR31 generated by the IS26-mediated deletion by manual sequencing of the ~3 kbp product of a PCR that links *intI1* with *catA1*. The presence of AbaR31 in both ABH008 and ABS200 indicates their close relationship as well as transmission between hospitals.

AbaR4 and TnaphA6 carried on a RepAci6 plasmid

The two antibiotic resistance genes of ABH008 and ABS200, *aphA6* and *oxa23*, that are not carried on the AbaR31 island were found in different transposons on the same plasmid. Using published primers¹³ we previously showed that the *aphA6* amikacin resistance gene is located in TnaphA6 in ABH008 and ABS200 in a context

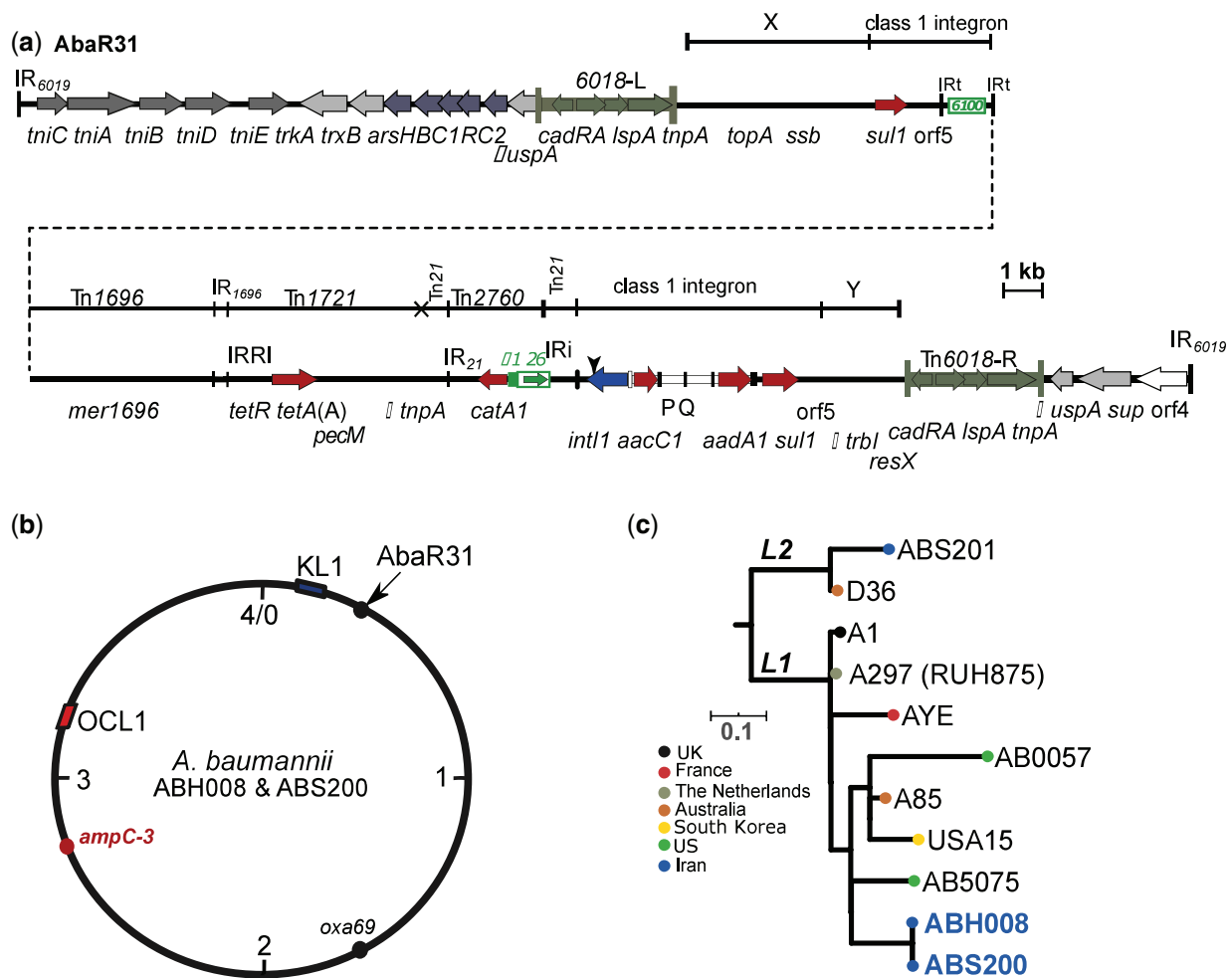


Figure 1. Structure of AbaR31 (a), circular map representing the chromosomes of ABH008 and ABS200 (b) and phylogenetic tree of representative GC1 strains (c). In (a), The thick central line shows the AbaR31 resistance island with horizontal arrows indicating the extent and orientation of genes. Antibiotic resistance genes are shown using red arrows. Two terminal vertical lines indicate the inverted repeats (IRs) of AbaR31. Tn6018 copies are shown using dark green. The origin of each segment of the multiple antibiotic resistance region (MARR), flanked by Tn6018 copies, is shown using a thin line above the central line. The vertical arrowhead above the *intI1* gene indicates the 108 bp deletion found in AbaR31. (b) Represents a schematic of the ABH008 and ABS200 chromosome with the position of AbaR31, *ampC*, K and OC loci marked. (c) Shows the phylogenetic relationship of ABH008 and ABS200 compared with known GC1 strains including GC1 reference strain A297 (RUH875).

similar to that seen in RepAcI6 plasmids.²⁰ Here, analysis of draft genomes confirmed that the *aphA6* gene is in TnaphA6, in precisely the location that this transposon is in pAb-G7-2 (GenBank accession number KF669606).¹³ The plasmid pAb-G7-2 is a RepAcI6 plasmid found in an Australian GC1.¹³ All remaining segments of pAb-G7-2 were recovered in multiple contigs, indicating that the RepAcI6 plasmid in ABH008 and ABS200 is closely related to pAb-G7-2 (with 99.9% DNA identities in plasmid backbone). The *oxa23* gene is in Tn2006 in AbaR4; this was confirmed by retrieving all of the contigs making up the complete AbaR4 from draft genomes. Using the genome sequence data, AbaR4 was predicted to be located on the same RepAcI6 plasmid that contains TnaphA6. This prediction was confirmed by developing a PCR mapping strategy, followed by manual sequencing of products, to link unique sequences of either end of AbaR4 (*tniB* on the left and *oxa23* on the right) to the backbone of RepAcI6 plasmid (see Methods). This led to the localization of AbaR4 in a spot equivalent to the position 9601 of pAb-G7-2 (GenBank accession number

KF669606) interrupting an open reading frame that encodes a hypothetical protein (protein id AGY56207.1). Sequence analysis also confirmed that AbaR4 is flanked by 5 bp target site duplication (TSD) 5'-AAAAG-3', a typical property of transposons that belong to this family.¹² AbaR4 has already been detected in RepAcI6 plasmids, e.g. pA85-3 (GenBank accession number CPO21787); however, in the genomes of ABH008 and ABS200 it is present in a unique location in the plasmid backbone, which indicates local acquisition is likely. RepAcI6 plasmids are potentially conjugative,^{13,21-23} and have been associated with the *aphA6* (amikacin resistance) and *oxa23* (carbapenem resistance) genes singly,^{6,24} or together,²³ which combined makes RepAcI6 plasmids important vehicles to spread amikacin and carbapenem resistance genes. To date, RepAcI6 plasmids have not been seen in other Gram-negative bacteria e.g. enterobacteria or *Klebsiella pneumoniae*.

Analysis of the draft genomes of ABH008 and ABS200 also showed both strains contain a copy of a cryptic plasmid encoding

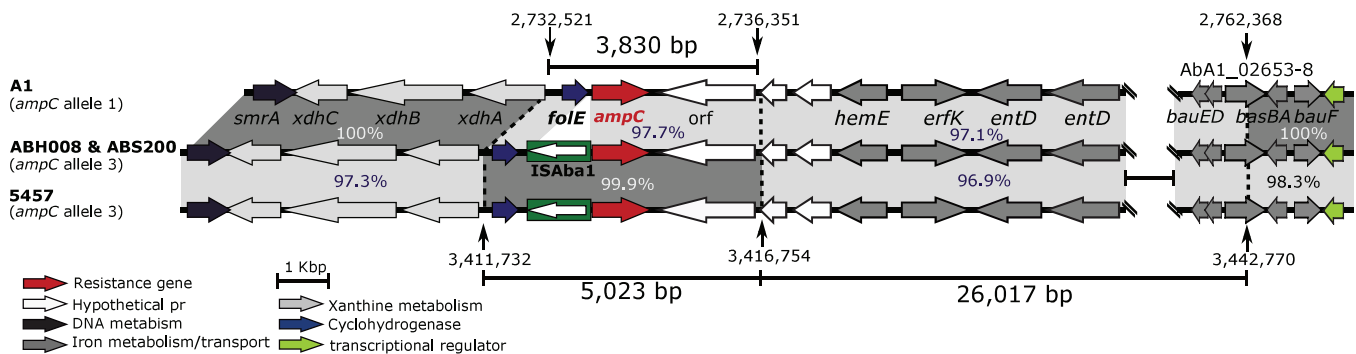


Figure 2. Schematic representation of the *ampC* region in ABH008 and ABS200 compared with A1 (GenBank accession number CP010781) and 5457 (GenBank accession number CP045541). The thick central line shows the chromosomes and horizontal arrows indicate the extent and orientation of genes. Shades of grey indicate regions with significant identity and numbers show their percentage DNA identity. Vertical arrows with numbers (above or below them) indicate chromosomal positions of A1 and 5457 as in CP010781 and CP045541, respectively.

RepAci1 that was identical to pA1-1 (GenBank accession number CP010782) found in *A. baumannii* A1 recovered in the UK in 1982.²⁵ The plasmid pA1-1 and its variants encode the AbkA (pfam14384) and AbkB (pfam04365) toxin-antitoxin system, which helps plasmids to be stably maintained in the cells. This plasmid type is widespread in *A. baumannii* strains regardless of clonal type and geographical distribution.^{6,24}

Resistance to third-generation cephalosporins due to acquisition of a DNA segment containing ISAbal-ampC

The presence of ISAbal upstream of the intrinsic *ampC* gene is known to be responsible for increasing the expression level of this gene, leading to resistance to third-generation cephalosporins.²⁶ Both ABH008 and ABS200 include a copy of ISAbal 9 bp away from the start of *ampC*, explaining their resistance to ceftazidime and cefotaxime. However, analysis of the *ampC* sequences of ABH008 and ABS200 revealed that they do not include the standard ST1 *ampC* sequence (allele 1). Instead, they carry the allele 3 *ampC* sequence, typical in ST623 strains (e.g. strain 5457; GenBank accession number CP045541). We have previously reported several cases of the acquisition of DNA segments containing an ISAbal-activated *ampC* gene by homologous recombination leading to third-generation cephalosporin resistance.^{27,28} Similarly, comparative analysis of the *ampC* region in ABH008 and ABS200 with the corresponding regions in A1 (GenBank accession number CP010781 containing allele 1) and 5457 (GenBank accession number CP045541 containing allele 3) also indicated that the ISAbal-*ampC* structure has been acquired from 5457 and incorporated into their chromosome as part of an ~5 kbp DNA segment (Figure 2). A ~26 kbp DNA patch was also found immediately adjacent to the 5 kbp fragment that contains ISAbal-*ampC* (Figure 2). This segment differed from the corresponding regions in A1 and 5457 by 97.1% and 96.9%, respectively (Figure 2). The source for this 26 kbp chromosomal segment could not be found. However, interestingly, an identical 5022 bp *ampC*-3 patch, along with its adjacent ~26 kbp segment, was also found in the *A. baumannii* strain DA33382 (GenBank accession number CP030106), indicating it is closely related to ABH008 and ABS200. DA33382 is an ST1:ST1567:KL40:OCL2 GC1 strain recovered from a tracheal secretion sample in Germany with an unknown isolation date. The fact that DA33382 has a different KL and OCL, compared with

ABH008 and ABS200, indicates that it has diverged by switching its outer core and capsular surface polysaccharides. We previously proposed that the Middle East region might be the source for many of the globally distributed resistance lineages of GC1s.^{5,6} The relationship of DA33382, ABH008 and ABS200 provides further evidence of this, but more genomes from the region will be needed to confirm the proposition.

Conclusions

The ST1:ST231:KL1:OCL1 strains ABH008 and ABS200 belong to lineage 1 within global clone 1. Both strains are extensively antibiotic resistant due to the presence of AbaR31 and a RepAci6 plasmid that carries AbaR4 and TnaphA6. ABH008 and ABS200 show that strains belonging to lineage 1 are circulating in Tehran hospitals and that they are closely related to a strain from Germany.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as [Supplementary data](#) at JAC-AMR Online.

References

1 WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 2017: 1–7.

<https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/>.

- 2** Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. *Microb Genom* 2019; **5**: e000306.
- 3** Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β -lactamases. *Antimicrob Agents Chemother* 2010; **54**: 24–38.
- 4** Adams MD, Goglin K, Molyneaux N et al. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J Bacteriol* 2008; **190**: 8053–64.
- 5** Douraghi M, Kenyon JJ, Aris P et al. Accumulation of antibiotic resistance genes in carbapenem-resistant *Acinetobacter baumannii* isolates belonging to lineage 2. Global Clone 1, from Outbreaks in 2012–2013 at a Tehran Burns Hospital. *mSphere* 2020; **5**: e00164–20.
- 6** Holt K, Kenyon JJ, Hamidian M et al. Five decades of genome evolution in the globally distributed, extensively antibiotic-resistant *Acinetobacter baumannii* global clone 1. *Microb Genom* 2016; **2**: e000052.
- 7** Hujer KM, Hujer AM, Hulten EA et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. *Antimicrob Agents Chemother* 2006; **50**: 4114–23.
- 8** Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* 2001; **48**: 5–16.
- 9** CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Twenty-Ninth Edition: M100*. 2019.
- 10** Post V, Hall RM. AbaR5, a large multiple-antibiotic resistance region found in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; **53**: 2667–71.
- 11** Post V, White PA, Hall RM. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010; **65**: 1162–70.
- 12** Hamidian M, Hall RM. AbaR4 replaces AbaR3 in a carbapenem-resistant *Acinetobacter baumannii* isolate belonging to global clone 1 from an Australian hospital. *J Antimicrob Chemother* 2011; **66**: 2484–91.
- 13** Hamidian M, Holt KE, Pickard D et al. A GC1 *Acinetobacter baumannii* isolate carrying AbaR3 and the aminoglycoside resistance transposon TnaphA6 in a conjugative plasmid. *J Antimicrob Chemother* 2014; **69**: 955–8.
- 14** Tatusova T, DiCuccio M, Badretdin A et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016; **44**: 6614–24.
- 15** Hamidian M, Ambrose SJ, Blackwell GA et al. An outbreak of multiply antibiotic-resistant ST49:ST128:KL11:OCL8 *Acinetobacter baumannii* isolates at a Sydney hospital. *J Antimicrob Chemother* 2021; **76**: 893–900.
- 16** Croucher NJ, Page AJ, Connor TR et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res* 2015; **43**: e15.
- 17** Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268–81.
- 18** Turton JF, Gabriel SN, Valderrey C et al. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. *Clin Microbiol Infect* 2007; **13**: 807–15.
- 19** Hamidian M, Hall RM. The AbaR antibiotic resistance islands found in *Acinetobacter baumannii* global clone 1 – structure, origin and evolution. *Drug Resist Updat* 2018; **41**: 26–39.
- 20** Aris P, Boroumand MA, Douraghi M. Amikacin resistance due to the *aphA6* gene in multi-antibiotic resistant *Acinetobacter baumannii* isolates belonging to global clone 1 from Iran. *BMC Microbiol* 2019; **19**: 221.
- 21** Hamidian M, Hall RM. pACICU2 is a conjugative plasmid of *Acinetobacter* carrying the aminoglycoside resistance transposon TnaphA6. *J Antimicrob Chemother* 2014; **69**: 1146–8.
- 22** Hamidian M, Kenyon JJ, Holt KE et al. A conjugative plasmid carrying the carbapenem resistance gene *bla_{OXA-23}* in AbaR4 in an extensively resistant GC1 *Acinetobacter baumannii* isolate. *J Antimicrob Chemother* 2014; **69**: 2625–8.
- 23** Nigro SJ, Holt KE, Pickard D et al. Carbapenem and amikacin resistance on a large conjugative *Acinetobacter baumannii* plasmid. *J Antimicrob Chemother* 2015; **70**: 1259–61.
- 24** Hamidian M, Hawkey J, Wick R et al. Evolution of a clade of *Acinetobacter baumannii* global clone 1, lineage 1 via acquisition of carbapenem- and aminoglycoside-resistance genes and dispersion of ISAbA1. *Microb Genom* 2019; **5**: e000242.
- 25** Holt KE, Hamidian M, Kenyon JJ et al. Genome sequence of *Acinetobacter baumannii* strain A1, an early example of antibiotic-resistant global clone 1. *Genome Announc* 2015; **3**: e00032-15.
- 26** Hamidian M, Hall RM. ISAbA1 targets a specific position upstream of the intrinsic *ampC* gene of *Acinetobacter baumannii* leading to cephalosporin resistance. *J Antimicrob Chemother* 2013; **68**: 2682–3.
- 27** Hamidian M, Hall RM. Resistance to third-generation cephalosporins in *Acinetobacter baumannii* due to horizontal transfer of a chromosomal segment containing ISAbA1-*ampC*. *J Antimicrob Chemother* 2014; **69**: 2865–6.
- 28** Hamidian M, Hancock DP, Hall RM. Horizontal transfer of an ISAbA125-activated *ampC* gene between *Acinetobacter baumannii* strains leading to cephalosporin resistance. *J Antimicrob Chemother* 2013; **68**: 244–5.