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

Masoumeh Douraghi,a Johanna J. Kenyon,b Parisa Aris,a Mahla Asadian,a Sedighe Ghourchian,a Mohammad Hamidianc

aDivision of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
bInstitute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia
cThe ithree institute, University of Technology Sydney, Ultimo, New South Wales, Australia

ABSTRACT The worldwide distribution of carbapenem-resistant Acinetobacter baumannii (CRAB) has become a global concern, particularly in countries where antibiotic prescription is not tightly regulated. However, knowledge of the genomic aspects of CRAB from many parts of the world is still limited. Here, 50 carbapenem-resistant A. baumannii isolates recovered at a single hospital in Tehran, Iran, during several outbreaks in 2012 and 2013 were found to be resistant to multiple antibiotics. They were examined using PCR mapping and multilocus sequence typing (MLST). All Iranian strains belonged to sequence type 328 in the Institut Pasteur MLST scheme (ST328IP), a single-locus variant of ST81IP, and all Iranian strains contained two carbapenem resistance genes, oxa23 and oxa24. The oxa23 gene is in the transposon Tn2006 in AbaR4, which interrupts the chromosomal comM gene. Phylogenetic analysis using whole-genome sequence (WGS) data for 9 isolates showed that they belonged to the same clade, designated the ST81/ST328 clade, within lineage 2 of global clone 1 (GC1). However, there were two groups that included either KL13 or KL18 at the K locus (KL) for capsular polysaccharide synthesis and either a tet39 or an aadB resistance gene, respectively. The genetic context of the resistance genes was determined, and the oxa24 (OXA-72 variant) and tet39 (tetracycline resistance) genes were each in a plasmid module in different plasmids. The aadB gene cassette (which encodes gentamicin, kanamycin, and tobramycin resistance) was harbored by prAY*, and the aphA6 gene (which encodes amikacin resistance) and sul2 gene (which encodes sulfamethoxazole resistance) were each harbored by a different plasmid. The sequences obtained here will underpin future studies of GC1 CRAB strains from the Middle East region.

IMPORTANCE Carbapenem-resistant Acinetobacter baumannii strains are among the most critical antibiotic-resistant bacteria causing hospital-acquired infections and treatment failures. The global spread of two clones has been responsible for the bulk of the resistance, in particular, carbapenem resistance. However, there is a substantial gap in our knowledge of which clones and which specific lineages within each clone are circulating in many parts of the world, including Africa and the Middle East region. This is the first genomic analysis of carbapenem-resistant A. baumannii isolates from Iran. All the isolates, from a single hospital, belonged to lineage 2 of global clone 1 (GC1) but fell into two groups distinguished by genes in the locus for capsule biosynthesis. The analysis suggests a potential origin of multiply antibiotic-resistant lineage 2 in the Middle East region and highlights the ongoing evolution of carbapenem-resistant GC1 A. baumannii strains. It will enhance future studies on the local and global GC1 population structure.

Citation Douraghi M, Kenyon JJ, Aris P, Asadian M, Ghourchian S, Hamidian M. 2020. 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 5:e00164-20. https://doi.org/10.1128/mSphere.00164-20.

Editor Ana Cristina Gales, Escola Paulista de Medicina/Universidade Federal de São Paulo
Copyright © 2020 Douraghi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.
Address correspondence to Mohammad Hamidian, mohammad.hamidian@uts.edu.au.
Received 19 February 2020
Accepted 25 March 2020
Published 8 April 2020
Acinetobacter baumannii is a Gram-negative opportunistic pathogen that causes a range of nosocomial infections. It has become a major global threat because of its high level of resistance to a wide range of antibiotics, which often complicates treatment and leads to treatment failure (1–4). Most A. baumannii isolates resistant to all or most of the antibiotics currently used for treatment belong to one of two major globally distributed clones, known as global clone 1 (GC1) and global clone 2 (GC2) (5, 6).

Carbapenem antibiotics are active against most β-lactamase-producing organisms, including those with extended-spectrum β-lactamase enzymes (7). They are the antibiotics of choice and are considered frontline treatment for infections caused by multidrug-resistant bacteria (8), but alarmingly, the rate of carbapenem resistance is increasing among A. baumannii isolates, imposing huge financial and health care burdens (1, 9–11). Indeed, carbapenem-resistant A. baumannii (CRAB) has emerged as one of the biggest challenges in the treatment of infections caused by this organism, especially when it involves GC1 or GC2 isolates that are already resistant to a wide range of alternative antibiotics (12, 13). Among several carbapenem resistance genes identified so far, oxa23 appears to be the most widespread in A. baumannii, regardless of clonal type (12).

In the last decade, there have been a number of studies reporting the emergence and rise of resistance to multiple antibiotics, including high levels of carbapenem resistance in different geographic regions of Iran (14–16). In 2012 and 2014, two separate studies reported high incidences of carbapenem resistance caused by oxa23 in isolates that belong to GC1 and GC2 (17, 18). Recently, a systematic review examining the rate of CRAB estimated an overall ~80% rate of resistance to carbapenems across the country, with 73%, 21%, and 6.2% of carbapenem-resistant isolates containing the oxa23, oxa24, and oxa58 oxacillin carbapenemase genes, respectively (14). However, to date, none of the studies investigated the genetic context and/or genomic location of the oxa23 gene, which is the most widely encountered carbapenem resistance gene in the country (14) and globally (19).

In 2015, we reported the resistance profiles of 401 clinical A. baumannii isolates recovered from 5 hospitals in Iran between 2011 and 2013, with 90% of the isolates being found to be extensively drug resistant (XDR) (20). Later, all the isolates were further examined using allele-specific PCRs (21), and 57 (14%) and 86 (22%) isolates were found to belong to GC1 and GC2, respectively (22). It was also shown that the majority of multiply drug-resistant isolates, including all GC1 and GC2 strains, contained an interrupted comM gene (22). This location is where AbaR-type (23) and AbGRI1-type (24) resistance islands are often present in members of the GC1 and GC2 clonal complexes, respectively. Interruption of comM in the Iranian isolates therefore provided evidence that they might carry a resistance island in the comM gene (22). Interestingly, 50 out of 57 GC1 isolates were isolated during several outbreaks in 2012 and 2013 at a single hospital (hospital H5) (22).

Here, we sought to further examine this set of 50 GC1 outbreak isolates to investigate the distribution of sequence types (STs) and class D carbapenemase genes and the structure(s) of the resistance island (RI) occupying the comM gene. The whole-genome sequences (WGS) of 9 representative strains were also determined to examine the phylogenetic relationship of representative isolates by comparing them to known GC1 isolates from different countries that belong to the defined GC1 lineages, lineage 1 and lineage 2.

RESULTS

PCR screening, resistance island mapping, and resistance profiles. (i) Identification of GC1 isolates and antibiotic resistance profiles. All 50 GC1 isolates (Table 1)
were previously examined (22) and identified to be GC1 using the allelic-specific PCR described previously (21). Here, representatives from each ward were also tested using PCR and sequencing and found to carry an allele that encodes an OXA-69 variant of the intrinsic A. baumannii oxa (oxa-Ab) gene, consistent with their assignment to GC1, as previously described (5, 6). They were all found to be resistant to multiple antibiotics, including ampicillin, streptomycin, spectinomycin, sulfonamides, trimethoprim, ceftazidime, cefotaxime, ticarcillin-clavulanate (Timentin), ceftriaxone, imipenem, meropenem, zidime, cefotaxime, ticarcillin-clavulanate (Timentin), ceftriaxone, imipenem, meropenem, and ticarcillin.
penem, doripenem, ciprofloxacin, nalidixic acid, and kanamycin (see Table S1 in the supplemental material). Forty-nine strains were resistant to amikacin (only strain ABS230 exhibited complete susceptibility), and 33 strains were resistant to tetracycline (Table S1). All were defined to be extensively drug resistant (XDR), based on the criteria defined previously (nonsusceptibility to one agent in all but two antibiotic classes or less) (25). The complete antibiotic resistance profiles (28 antibiotics) are included in Table S1.

(ii) Antibiotic resistance genes. In all isolates, a copy of ISAba1 was detected upstream of the chromosomal ampC gene (Table 1), accounting for their resistance to 3rd-generation cephalosporins, consistent with the role of ISAba1 in increasing the expression of the ampC gene (26, 27). All 50 strains carried the oxa23 and oxa24 carbapenem resistance genes (Table 1), accounting for their resistance to carbapenems, as well as ticarcillin-clavulanate. All isolates but ABS230 were resistant to amikacin, and we recently showed that these isolates carry the aphA6 gene in TnaphA6 (28), consistent with this phenotype. All isolates were also resistant to sulfonamide compounds, and consistent with this profile, a copy of the sul2 gene was found in them.

Thirty-four strains were resistant to tobramycin, in addition to gentamicin and kanamycin (Table S1), and consistent with this phenotype, the aadB gene (which encodes gentamicin, kanamycin, and tobramycin resistance) was detected in these strains (Table 1). The aadB cassette was not in a class 1 integron but was instead found by PCR to be in the location that it occupies in the small plasmid pRAY (29). Though 33 strains were found to be tetracycline resistant, here, a copy of the tetA(A) and tetA(B) tetracycline resistance genes was not detected in any strain. However, the genome sequencing data for 5 tetracycline-resistant strains determined (see below) here revealed a copy of the tet39 resistance gene. Hence, we screened by PCR the entire set and found a 100% correlation between the presence of tet39 and the tetracycline resistance phenotype in all 33 tetracycline-resistant strains (PCR data not shown).

(iii) All outbreak isolates carry the oxa23 gene in AbaR4, located in comM. We previously showed that the comM gene was interrupted in all 50 GC1 isolates recovered in hospital H5 (22). Here, PCR was used to screen these strains for general features of the AbaR0/3-type resistance islands that are often found in the chromosomal comM gene in GC1, including the class 1 integron, AbaR junctions with comM (junctions J1 and J2) (5, 6), and the AbaR internal junctions (junctions J3 to J6) (5, 6), as well as resistance genes frequently found in AbaRs [arsB, aphA1b, cata1, tetA(A), and bla TEM] (23). Unexpectedly, none of the features associated with AbaR-type RIs, including a class 1 integron with the sul1 gene and the associated aacC1-orfP-orfP-orfQ-aadA1 gene cassettes, the cadmium/zinc transposon Tn6018, and the ars operon (encoding arsenic resistance), were detected in any isolates. However, among the AbaR0/3-type junctions (J1 to J6), only J1 and J2 produced the expected amplicons, suggesting that a genomic island with a backbone related to that of AbaR0/3-type islands (such as Tn6021, Tn6022, or AbaR4, which has a Tn6022 transposon backbone) might be present in the comM gene of these Iranian outbreak isolates.

The boundaries between comM and the genomic island (the J1 and J2 junctions) were amplified and sequenced for strain ABS201, which was randomly chosen as a representative. Sequence analysis revealed that these amplicons were identical to the corresponding junctions of the AbaR4 island previously described in D36, an Australian GC1 lineage 2 strain that carries the oxa23 gene in Tn2006 located in AbaR4, which interrupts the chromosomal comM gene (30). Using a PCR mapping strategy developed previously (30, 31), we found a complete copy of AbaR4 located in comM in all 50 isolates by linking oxa23 to comM (Fig. 1) and detecting the central portion of the Tn6022/AbaR4 transposons (Table 1). This finding raised the possibility that all 50 outbreak strains might be related to D36, a strain that contains AbaR4 in comM and that was recovered from a member of the military who had returned to Australia (30, 31). The fact that D36 belongs to lineage 2 within GC1 (10) suggested that all Iranian strains may also belong to the same lineage.
The sequence type (ST) in the Institut Pasteur multilocus sequence typing (MLST) scheme (STIP) was determined using PCR and sequencing for all 50 Iranian isolates. All strains were found to belong to ST328 (cpn60-1, fusA1, gltA1, pyrG25, recA5, rplB1, and rpoB2), which is a single-locus variant (SLV) of ST81 (cpn60-1, fusA1, gltA1, pyrG1, recA5, rplB1, and rpoB1), which includes isolate D36. ST81 is a double-locus variant of ST1 (cpn60-1, fusA1, gltA1, pyrG1, recA5, rplB1, and rpoB1), which represents the majority of strains that belong to GC1 lineage 1. ST328 and ST81 differ by only 2 nucleotides in the pyrG gene (a pyrG1 allele type in ST81 versus a pyrG25 allele in ST328). ST328 is a rare sequence type, with only one representative in the Institut Pasteur MLST database (http://pubmlst.org/abaumannii/), isolate 67 from Tehran, Iran, suggesting that ST328 might be commonly found in the country.

Whole-genome analysis and comparison of genomes. (i) Placing the Iranian isolates in the global GC1 phylogeny. To examine if the Iranian outbreak strains fall within lineage 2 of GC1, the whole-genome sequences of 9 representative strains (the strains shown in bold in Table 1) were determined using the Illumina MiSeq technology (Table 2) and used to construct a recombination-free phylogenetic tree in combination with other GC1 isolates previously determined to belong to lineage 1 and lineage 2 (Fig. 2) (10), including the A1 (lineage 1) (33) and D36 (lineage 2) (31) genomes. Two further ST81 strains (strains PR332 and MRSN 3527; Table 3), found by screening the entire NCBI GenBank and whole-genome shotgun databases (as of July 2019), were also included in the phylogenetic analysis. The resulting phylogeny (Fig. 2) demonstrated that all 9 Iranian strains clustered together, branching from the D36 subclade, indicating that they all belong to lineage 2 of GC1. The two ST81 strains (PR332 and MRSN

TABLE 2 Genome sequence data statistics for Iranian GC1 isolates

| Isolate | Assembly length (bp) | No. of contigs | No. of read pairs | Read depth (mean fold)* | N50 (kbp) | GenBank accession no. |
|---------|----------------------|----------------|------------------|-------------------------|------------|----------------------|
| ABS029  | 4,102,995            | 106            | 1,370,402        | 197                     | 101        | WIOH000000000        |
| ABS042  | 4,172,082            | 106            | 1,370,826        | 149                     | 140        | WIOG000000000        |
| ABS062  | 4,104,184            | 116            | 1,371,953        | 107                     | 102        | WIOF000000000        |
| ABS063  | 4,102,168            | 109            | 1,367,413        | 195                     | 102        | WIOE000000000        |
| ABS078  | 4,183,937            | 97             | 1,369,455        | 125                     | 145        | WIOD000000000        |
| ABS103  | 4,244,771            | 107            | 1,368,430        | 170                     | 150        | WIOC000000000        |
| ABS104  | 4,136,827            | 102            | 1,370,892        | 144                     | 150        | WIOB000000000        |
| ABS122  | 4,102,555            | 108            | 1,367,643        | 113                     | 102        | WIOA000000000        |
| ABS201  | 4,198,099            | 125            | 1,365,667        | 115                     | 90         | VJZY000000000        |

*Estimated by dividing the total number of reads generated (in base pairs) by the genome size of about 4.1 Mb (4,100,000 bp).

N50, the minimum contig length needed to cover 50% of the genome, indicating that half of the genome sequence is in contigs larger than or equal to the N50 contig size.
3527) were also found to belong to lineage 2. Analysis of the single nucleotide differences (SNDs) across the genomes indicated that, on average, the Iranian strains differed from D36 by 50 SNDs, indicating a close relationship. The complete SNDs between all strains analyzed here can be found in Table S2.

Further, analysis of the recombination patches across the entire chromosomes showed that the Iranian strains share several recombinant regions with other lineage 2 strains, while they also include few recombination blocks specific to their branch (Fig. 2). The Iranian strains were placed on three branches, and interestingly, each branch contained novel (branch-specific) recombination patches. These analyses confirmed the assignment of the Iranian strain to lineage 2 of GC1 and indicated their continued evolution and genetic exchange via homologous recombination.

(ii) Surface polysaccharide loci. Capsular polysaccharide (CPS) is a major virulence factor for \textit{A. baumannii}, and the genes at the K locus (KL), which directs its synthesis, have previously been shown to vary between isolates of the GC1 clone (10). In that study, we demonstrated that strain D36 and the related strains 6013113 and 6013150 carry the KL12 CPS biosynthesis gene cluster (10). Here, KL12 was found in one more isolate, PR332, belonging to ST81 (Table 3). However, the Iranian strains were found to carry either KL13 or KL18, with the KL type separating the two distinct phylogenetic subclades in which the Iranian strains are positioned (Fig. 2 and Table 3). The genomes of other lineage 2 isolates that do not belong to the ST81/ST328 subclade were shown to carry either KL1 or KL15 (10).

The arrangements of the KL13 and KL18 gene clusters (Fig. 3) have been described previously (34, 35), though neither has been reported in a GC1 isolate to date. Interestingly, the KL13 gene cluster is closely related to KL12, and both include genes for the synthesis of the rare non-2-ulosonic acid sugar 5,7-di-N-acetylaceaminic acid, also known as Aci5Ac7Ac (34). The two genetic arrangements differ only in a small...
segment that includes a single gene (Fig. 3). However, this small replacement leads to a structural change in the K12 and K13 CPS that are produced (34). Similarly, the KL18 gene cluster is closely related to KL17, which has so far been reported in only a single ST81 clade were previously shown to carry OCL2 (31, 32). Isolates in the ST81 clade were previously shown to carry OCL2 (31, 32).

In addition to the K locus, a second chromosomal region involved in the synthesis of the outer core (OC) component of the lipooligosaccharide (known as the OC locus [OCL]) (36) was also found to vary in our previous analysis of GC1 (10). Isolates in the ST18 clade were previously shown to carry OCL2 (10), and the OCL2 arrangement was identified in the additional isolates belonging to the ST81/ST328 subclade, though

### TABLE 3 Properties of strains belonging to GC1, lineage 2

| Isolate | Date | Country | Source | IP | OX | KL | OCL | RI in comM<sup>a</sup> | ISAbα<sup>b</sup> | ampC<sup>b</sup> | oxa<sup>c</sup> | cmlA1 | ahpA<sup>d</sup> | mer | sul2 | aadB | tet<sup>e</sup> | GyrA81 | ParC84 | GenBank accession no. |
|---------|------|---------|--------|----|----|----|-----|----------------------|------------------|----------------|------------|-------|------------------|-----|-------|------|-------------------|--------|----------|----------------------|
| TG19582 | nk* | nk | nk | 1 | 231 | 1 | 1 | Intact | – (1) | – | – | – | + | + + | – | – | – | L | S | AMIV000000000 |
| PR332 | nk USA | nk | 81 | 498 | 12 | 2 | Intact | + (17) | – | – | 1a | + + | + | – | – | L | L | NGDV010000000 |
| OFC074 | 2003 USA | nk | 19 | 231 | 1 | 5 | Tn6022 | + (3) | – | + | 1b | – | – | + | – | A(B) | L | S | AMDE010000000 |
| Naval-21 | 2006 USA | Wound | 19 | 946 | 15 | 1 | Tn6022 | + (3) | – | + | 1b | – | – | + | – | A(B) | L | L | AMSY010000000 |
| 6013150 | 2007 UK | Skin | 81 | 498 | 12 | 2 | Intact | + (17) | – | – | 1a | + + | + | – | L | L | ACQ000000000 |
| 6013131 | 2007 UK | Skin | 81 | 372 | 12 | 2 | Intact | + (17) | – | – | 1a | + + | + | – | L | L | ACYR000000000 |
| D36 | 2008 Australia | Wound | 81 | 498 | 12 | 2 | AbaR4 | + (17) | 23 | – | 1a | + + | + | – | L | L | CP012952/ |
| MRSN 3527 | 2011 USA | Wound | 81 | 498 | 12 | 2 | AbaR4 | + (17) | 23 | – | 6 | + + | + | – | L | L | JPHZ000000000 |
| ABS029 | 2012 Iran | Wound | 328 | 1972 | 18 | 2 | AbaR4 | + (80) | 23 | 24 | – | 6 | + + | – | 39 | L | L | WIOG000000000 |
| ABS042 | 2012 Iran | Wound | 328 | 498 | 13 | 2a | AbaR4 | + (81) | 23 | 24 | – | 6 | + + | – | + | – | L | L | WIOG000000000 |
| ABS062 | 2012 Iran | Wound | 328 | 1972 | 18 | 2 | AbaR4 | + (80) | 23 | 24 | – | 6 | + + | – | 39 | L | L | WIOF000000000 |
| ABS063 | 2012 Iran | Wound | 328 | 1972 | 18 | 2 | AbaR4 | + (80) | 23 | 24 | – | 6 | + + | – | 39 | L | L | WIOE000000000 |
| ABS078 | 2012 Iran | Wound | 328 | 498 | 13 | 2a | AbaR4 | + (81) | 23 | 24 | – | 6 | – | + + | – | L | L | WIOD000000000 |
| ABS103 | 2013 Iran | Wound | 328 | 498 | 13 | 2a | AbaR4 | + (81) | 23 | 24 | – | 6 | + | + | + | L | L | WIOC000000000 |
| ABS104 | 2013 Iran | Blood | 328 | 498 | 13 | 2a | AbaR4 | + (81) | 23 | 24 | – | 6 | + | + | + | L | L | WIOB000000000 |
| ABS122 | 2013 Iran | Wound | 328 | 1972 | 18 | 2 | AbaR4 | + (80) | 23 | 24 | – | 6 | + + | + | – | 39 | L | L | WIOA000000000 |
| ABS201 | 2013 Iran | Wound | 328 | 1972 | 18 | 2 | AbaR4 | + (80) | 23 | 24 | – | 6 | + + | + | – | 39 | L | L | V2Y000000000 |

---

**a** Genomes sequenced or analyzed in this study are shown in bold. Analysis of the remaining genomes from reference 10 is shown for ease of comparison.

**b** IP, Institut Pasteur scheme, which uses the cprn60, fusA, gltA, pyrG, recA, rplB, and rpoD genes; OX, Institut Oxford scheme, which uses the cprn60, gltA, gyrB, gdhB, recA, cpn60, and rpoD genes.

**c** RI, the resistance island found in the chromosomal comM gene. In lineage 1, this gene is often occupied by the AbaR0/3-type RI, while it is either intact or interrupted by AbaR4 and Tn6022.

**d** Leucine (L) and serine (S) at positions of 81 and 84 of the GyrA and ParC proteins. Fluoroquinolone-resistant strains often include an L at these positions, and sensitive strains tend to include S.

**e** Numbers in parentheses indicate the ampC allele numbers. All allele numbers are those used in the ampC database, publicly available at [https://pubmlst.org/abaumannii/](https://pubmlst.org/abaumannii/).

**f** A complete genome is available for D36 (31). D36 carries 4 plasmids (GenBank accession numbers CP012953 to CP012956).

---

**FIG 3** Arrangement of the CPS biosynthesis gene clusters at the K locus in isolates belonging to the ST81/ST328 subclade of lineage 2. Genes are colored according to the functions of their predicted products, and the scheme is shown at the bottom. The figure is drawn to scale from representative KL sequences available as GenBank accession numbers MF522881 (KL18), MF522810.1 (KL13), and JN107991.2 (KL12). Shading between gene clusters indicates regions of nucleotide sequence similarity.
OCL2 in the Iranian isolates with KL13 had an IS\textsubscript{Aba12} interrupting the gtr\textsubscript{OC9} gene, and this variant was designated OCL2a.

(iii) Sequence types. We previously showed that, despite belonging to the same lineage within GC1, lineage 2 strains belong to several sequence types of STIP and the Oxford MLST scheme (STOX)\(^{10}\). Using PCR and sequencing, we showed that all Iranian strains belong to ST328IP, while D36 and the 4 other strains in D36 clade belong to ST81 (a pyrG SLV of ST328; see above), indicating an ST81/ST328-specific clade within lineage 2. Analysis of the Oxford sequence types indicated that the strains also exhibited a higher degree of diversity. These differences were mainly due to the presence of a different gpi allele, as previously shown\(^{10}\). This gene is located within the K locus for capsular polysaccharide (CPS) biosynthesis\(^{36}\), and the differences showed structural diversity on the cell surface (Table 3). This corresponds to the difference in STOX, as KL13 isolates belonged to ST498 and KL18 isolates belonged to ST1972\(^{10}\) (Table 3). The K locus represents the tallest peak in the SND density plot shown in Fig. 2, indicating extensive recombination events in this region.

(iv) Resistance to 3rd-generation cephalosporins and acquisition of the \textit{ampC} gene. Resistance to 3rd-generation cephalosporins may be conferred by the insertion of IS\textit{Aba1} or IS\textit{Aba125} upstream of the \textit{ampC} gene, which enhances expression\(^{37}\). We previously showed that IS\textit{Aba1} is present in the same location in all other lineage 2 strains studied before, except strain TG19582\(^ {10}\) (Table 3). Here, consistent with the ceftazidime and cefotaxime resistance profiles of the Iranian strains and the results of PCR analysis (see above), IS\textit{Aba1} was found to be 9 bp away from the start of the chromosomal \textit{ampC} gene in all 9 sequenced Iranian strains.

We also previously showed that GC1 strains can gain IS\textit{Aba125}- and IS\textit{Aba1}-activated \textit{ampC} genes, along with a surrounding segment of the chromosome, by horizontal transfer from an exogenous source\(^{37, 38}\). Hence, we explored the surrounding regions of the \textit{ampC} gene in all lineage 2 strains for evidence of whether different \textit{ampC} alleles were the result of horizontal transfer events. Analysis of the 1,152-bp \textit{ampC} gene in the 9 Iranian strains revealed two potential internal recombination patches of about 550 bp with ~94% DNA identity compared to the sequences of their corresponding regions in strains D36 and TG19582 (Fig. 4). However, we could not identify the source of these patches in GenBank or WGS database searches.

---

**FIG 4** Alignment of the chromosomal \textit{ampC} gene and its surrounding regions (10 kbp on either side). Horizontal arrows indicate the directions and orientations of the genes, and the green filled box indicates IS\textit{Aba1}, with the arrow inside indicating the direction of the transposase gene. Genes are color coded based on their function, and the key is shown at the bottom. Shades of gray indicate regions with significant identity, and red numbers indicate their percent DNA sequence identities to the D36 genome.
new alleles found here in Iranian strains, *ampC80* and *ampC81*, have been deposited in the *ampC* database, publicly available at [http://pubmlst.org/abaumannii/](http://pubmlst.org/abaumannii/). All other *ampC* allele numbers in Table 3 are also those used in the *ampC* database.

We previously showed that strains OIFC074 and Naval-21 contain a different *ampC* allele (10). Here, analysis of *ampC* and its surrounding sequence indicated a 4.6-kb recombination patch in OIFC074 and Naval-21, which shared 97.6% DNA identity with the corresponding region in D36 and TG19582 (Fig. 4), indicating that a short segment, including an IS*Aba1*-activated *ampC* gene, has been horizontally transferred and incorporated into these two genomes. However, searches of the GenBank and draft genomes in WGS databases did not result in identification of the source for this *ampC* recombinant region.

(v) Resistance to fluoroquinolones. All Iranian strains contained the same *gyrA* and *parC* alleles as strain D36, encoding a leucine (L) at positions 81 and 84 of the GyrA and ParC proteins, respectively, which is consistent with their nalidixic acid and ciprofloxacin resistance phenotype (Table S1 and Table 3). The remaining lineage 2 strains also had the same *gyrA* and *parC* gene alleles as D36 (GenBank accession no. CP012952) (31).

(vi) Prophage genomes. Three intact prophage genomes of 36.3 kb, 36.4 kb, and 95.9 kb were previously identified in strain D36 (31). Here, screening of the Iranian strains indicated the presence of a variant of prophage region 1 in all strains, while prophage region 2 was missing. Interestingly, all KL13 strains also contained prophage region 3, while KL18 strains lacked this region (Fig. 5). All ST81 strains
included all three prophage regions. Analysis of the other lineage 2 genomes indicated that the majority had either complete copies of all three prophage genomes or a remnant of them. In the first subclade, which included strains TG19582, Naval-21, and OIFC074, large portions of all 3 prophage regions appeared to be missing, with only very small portions remaining (Fig. 5), indicating a complex history. This was not investigated further.

(vii) Antibiotic resistance genes in plasmids. Analysis of resistance genes in Iranian strains and other lineage 2 strains (Table 3) confirmed that, as previously shown (10), strains in lineage 2 included a set of antibiotic resistance genes completely different from those in the bulk of GC1 strains belonging to lineage 1 (10). Moreover, the 9 sequenced Iranian strains differed from other lineage 2 strains and other members of the ST81/ST328 clade by the presence of an oxa24 carbapenem resistance gene, in addition to oxa23 (Table 3). The oxa24 gene, encoding the OXA-72 variant, was found within a pdf module and was identical to the oxa24 modules previously described (39, 40). The oxa24 pdf module was found in an ~15-kb contig, which appears to represent a plasmid, as it encodes a putative replication initiation protein (Rep) that is 98.9% identical to that encoded by the A. baumannii plasmid pMAC (GenBank accession no. AY541809.1). Among the strains sequenced, the five tetracycline-resistant isolates that contained tet39 belonged to the same KL18 subclade in the recombination-free phylogeny (Fig. 2). The tet39 gene was found in an approximately 4-kb contig within a pdf module identical to that described previously (41). This contig also encodes a putative plasmid replication initiation protein (Rep) that is 98% identical to the one in A. baumannii RCH52 (GenBank accession no. KT346360) (42), suggesting the presence of a similar plasmid in the Iranian strains. However, the complete plasmid sequence could not be assembled due to the presence of several repeated sequences. Here, the tet39 tetracycline resistance gene was shown to be present in all 33 tetracycline-resistant Iranian strains (Table 3) and to account for their tetracycline resistance phenotype (see the PCR screening results above). To the best of our knowledge, this is the first time that tet39 has been seen in lineage 2 of GC1. Interestingly, the sul2 sulfonamide resistance gene was carried by all GC1 lineage 2 isolates, while this gene has so far rarely been seen in strains belonging to lineage 1, which carry sul1 embedded within the AbaR0/3-type resistance islands as part of the 3’ conserved sequence segment of the class 1 integron (23). Sequence analysis showed that in the Iranian isolates the sul2 gene is located in a plasmid (Table 4), similar to pD36-4 previously reported in D36 (43).

Using PCR, a 100% correlation between the presence of the aadB gene in the pRAY context and the tobramycin, gentamicin, and kanamycin resistance phenotype was shown (Table 1). Here, the presence of pRAY* was confirmed in 4 genomes (Tables 3 and 4). Interestingly, all these 4 genomes contained KL13 (Table 3). We recently showed that amikacin resistance is due to the presence of the aphA6 amikacin resistance gene, located in TnaphA6, in a large set of GC1 Iranian strains, including the ones analyzed here (28). TnaphA6 is made up of a central segment containing aphA6 flanked by two copies of ISAba125. To locate TnaphA6, here, the 9 sequenced genomes were searched for contigs with ISAba125 at their ends. In each genome, 9 contigs were found; 7 contigs had the ISAba125 sequence at one end, 1 contig which also had the aphA6 sequence contained the ISAba125 sequence at both ends, and an additional contig contained an internal sequence of ISAba125. All of these contigs had a coverage of >15-fold relative to contigs containing chromosomal genes, indicating that all ISAba125 copies and, hence, TnaphA6 must be located on a plasmid estimated to be 15 to 20 kb. A more detailed context of the plasmids was not pursued further. However, the analysis performed here indicates that, except for oxa23, which is located in AbaR4 in the chromosome, all other resistance genes appear to have been acquired via 5 different plasmids (Table 4; Fig. 6).
(viii) Cryptic plasmids. In addition to two plasmids that carry antibiotic resistance genes (plasmids pD36-2 and pD36-4), we previously showed that the lineage 2 strain D36 contains two cryptic plasmids (plasmids pD36-1 and pD36-3) (43). We also previously screened other members of lineage 2 to find them (43). Here, the cryptic pD36-3 plasmid was found in all strains belonging to the ST81/ST328 subclade (Table 4), while the pD36-1 plasmid was present only in D36 and the Iranian strains (Table 4).

**DISCUSSION**

CRAB was placed as the number 1 critical priority pathogen on the World Health Organization’s list of 12 multiresistant bacteria for which immediate research on new therapeutics and antibiotic development is required (44). However, despite the global distribution of CRAB and the need to have data from all countries, there is a substantial gap in WGS data from most geographical regions across the globe, as the bulk of the current publicly available genome sequence data is from only 4 countries, namely, the United States, China, Thailand, and Australia (19). The lack of sequence data has made it difficult to track the spread of resistance at the global level and determine the population structure of this microorganism (19).

Over the last decade, a large number of studies have reported the alarming rate of carbapenem resistance among *A. baumannii* isolates in Iran (14–16). However, virtually all these studies have included only the phenotypic determination of carbapenem resistance and PCR screening for carbapenem resistance genes, combined with very limited phylogenetic analysis using traditional methods (14–16), making it impossible to place strains recovered from this region in the global context. Here, for the first time, we investigated the phylogenetic relationships among a set of 9 strains, representing 50 CRAB Iranian isolates that caused several outbreaks and that were recovered in 2012 and 2013 from a single hospital in Tehran, Iran, in the context of isolates belonging to the same lineage recovered in regions around the world. This study showed that all Iranian outbreak strains belong to the same sequence type, ST328 in the Institut Pasteur scheme, and share many properties, such as resistance to the frontline carbapenem antibiotics, conferred by the carbapenem resistance *oxa23* gene in Tn2006 within AbaR4, which is located in the chromosomal *comM* gene, and a plasmid that carries *oxa24*. This study further represents the first analysis of the genetic context of the most widespread carbapenem resistance gene, *oxa23* (19), in *A. baumannii* isolates recovered from Iran. In *A. baumannii* strains that belong to the two major global clones, resistance genes often reside in the chromosome within large genomic islands (6, 23, 24).

**TABLE 4** Plasmid content of strains belonging to GC1, lineage 2, ST81/ST328 clade

| Isolate | pD36-1 | pD36-2 (pRAY*) | pD36-3 | pD36-4 | poxa24 | pter39 | pTnaphA6 |
|---------|--------|----------------|--------|--------|--------|--------|----------|
| D36     | +      | +              | +      | +      | +      | +      | +        |
| 601350  | –      | +              | +      | +      | +      | –      | –        |
| 6013113 | –      | +              | +      | +      | +      | –      | –        |
| MRSN 3527 | +          | +              | +      | +      | +      | –      | –        |
| PR332   | –      | +              | +      | +      | +      | –      | –        |
| ABS029  | +      | +              | +      | +      | +      | +      | +        |
| ABS042  | +      | +              | +      | +      | +      | –      | –        |
| ABS062  | +      | –              | +      | +      | +      | +      | +        |
| ABS063  | +      | –              | +      | +      | +      | +      | +        |
| ABS078  | +      | +              | +      | +      | +      | +      | +        |
| ABS103  | +      | +              | +      | +      | +      | –      | +        |
| ABS104  | +      | +              | +      | +      | +      | –      | –        |
| ABS122  | +      | –              | +      | +      | +      | +      | +        |
| ABS201  | +      | +              | +      | +      | +      | –      | –        |

*a* Includes indels of ~0.3 kbp and 1.3 kbp.

*b* A total of 438 bp is missing.

*c* Containing only 2 fragments (0.7 and 2.2 kb) of pD36-3.

*d* The Tn4352::IS4352 structure is missing, likely due to an IS26-mediated deletion event.

*e* The mer module is missing.
However, the only chromosomal resistance gene found in Iranian strains is oxa23, and all other resistance genes, aadB, aphA6, oxa24, tet39, and sul2, have been acquired by 5 different plasmids (Fig. 6). This highlights the role of plasmids in the acquisition of these important resistance determinants in this subset of lineage 2 strains.

Though we showed that the Iranian isolates studied here fall into the second defined lineage of GC1, it is noteworthy that the Iranian strains differed from each other in many ways. Differences included the presence of different recombination patches across their genomes, different plasmid and prophage contents, and different ampC alleles, as well as the carriage of different OC and K loci. The different K loci explain their assignment to different sequence types in the Oxford MLST scheme. Interestingly, each of KL13 and KL18 was correlated with certain properties, as all strains with KL13 contained OCL2a, ampC81, pRAY*, and phage region 3, while strains with KL18 contained OCL2, tet39, ampC80, and prophage regions 1 and 2. These differences could suggest a single entry into the hospital environment, followed by continued evolution, leading to the separation of the KL13 and KL18 subclades over 2012 and 2013. This raises concerns about the hospital’s colonization with CRAB and the need to review infection control measures regularly.

Previously, it was shown that the most recent common ancestor of GC1 arose in about 1960, and subsequently, in about 1967, members of GC1 diverged into two phylogenetically distinct lineages (10). All strains belonging to lineage 1 included either an AbaR-type resistance island or a remnant of it in the comM gene, whereas lineage 2 strains, including D36, either contained an intact comM or carried Tn6022 or AbaR4 in the comM gene (10). This study further demonstrates that the multidrug-resistant ST81/ST328 subclade of lineage 2 within global clone 1 has clearly diverged from a common precursor and from the bulk of GC1 isolates and is defined by the acquisition of the AbaR4 resistance island and ISAba1 upstream of the ampC gene. These genetic features confer the ability to resist broad-spectrum β-lactam antibiotics, including 3rd-generation cephalosporins and the frontline antibiotics, the carbapenems.

This study provides clear evidence that strains belonging to lineage 2 have undergone different evolutionary changes to achieve their resistance phenotypes, evidenced by the presence of a resistance gene complement completely different from that of lineage 1 strains, and that all but oxa23 have been acquired by a different plasmid. This, in many ways, indicates the versatility of A. baumannii genomes and the wide range of antibiotic resistance genes and mobile genetic elements that could be involved with antibiotic resistance in this microorganism. It also indicates that all these plasmids must be compatible.

Given that D36 is a military isolate, it was previously hypothesized that GC1 isolates and, in particular, strains belonging to lineage 2 might have originated from the Middle
East (10), and evidence was provided to support the suggestion that D36 might be a carbapenem-resistant strain that was introduced into an Australian hospital by a member of the military (30). This study demonstrates a close relationship of the Iranian outbreak isolates recovered from hospital H5 in Iran with the Australian military isolate D36 and showed that the Iranian outbreak isolates belong to the same lineage as D36. These findings, combined with finding a single ST328 strain in the MLST database, provide further evidence that the Middle East region might be a reservoir for GC1 strains that belong to lineage 2. However, determining a more accurate distribution rate of lineage 2 strains in the Middle East and also globally warrants further investigation and more genome sequence data.

**MATERIALS AND METHODS**

**Bacterial strains.** A total of 50 isolates resistant to carbapenems were recovered from several outbreaks in a single Tehran hospital (hospital H5) between 2012 and 2013 and identified as GC1 (22) and were further examined in this study. Their features are listed in Table 1.

**Antimicrobial susceptibility testing.** In addition to the profiles of resistance to 20 antibiotics previously reported (22), the profiles of resistance to an additional 8 antibiotics, including ampicillin (25 μg), kanamycin (30 μg), neomycin (30 μg), nalidixic acid (30 μg), netilmicin (30 μg), streptomycin (25 μg), spectinomycin (25 μg), and sulfonamide (100 μg), were determined using the standard Kirby-Bauer disk diffusion method as described elsewhere (30). Strains were classified as resistant and susceptible according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for *Acinetobacter* spp. (45) and calibrated dichotomous sensitivity disk diffusion assay (CDS) (http://cdtest.net/) when a CLSI breakpoint for *Acinetobacter* spp. was not available (for netilmicin, streptomycin, spectinomycin, sulfamethoxazole, nalidixic acid, and rifampin).

**PCR amplification, DNA sequencing, and sequence analysis.** PCR amplification was carried out using published primers for various antibiotic resistance genes, including *aphA1*, *tetA1*, *blaIM*, and *catA1* (5, 6, 29); the *comH* gene; features of the AβA0/3-type islands (AβA0-type J1 to J6 junctions, *intI1*, *top*, and Tn6018-L and Tn6018-R) (6); and *aadB* in pRAY (29). Published primers and conditions were also used to identify the carbapenemase resistance genes *oxa23*, *oxa24*, and *oxa58* (46) and to detect IS*AbaI upstream of the chromosomal *ampC* gene (47). The intrinsic *oxa-Ab* gene (also referred to as *bla*<sub>oxa-A1</sub> elsewhere) was also amplified and sequenced for representative strains using primers previously published (21). For primers and amplicons up to 3 kb, reaction and cycling conditions were as described elsewhere (30). For larger amplicons, Phusion DNA polymerase (New England Biolabs) and Phusion HF buffer replaced Taq polymerase and PCR buffer. Cycling conditions included an initial denaturation cycle at 98°C for 30 s, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s per 1 kbp of expected PCR product. The final extension was at 72°C for 10 min. The PCR amplicons were separated using standard 1% agarose gels, stained with ethidium bromide, and visualized as described elsewhere. Sequencing was performed as described previously (30).

**Whole-genome sequencing, genome assembly, and in silico screening of genomes.** Genomic DNA isolated from 9 representative strains (identified in boldface in Table 1), including 1 strain from each hospital ward isolated in 2012 and 1 representative strain from each hospital ward isolated in 2013 (in total, 2 strains from each ward), were sequenced in-house at the University of Technology Sydney, using Illumina MiSeq technology. Whole-genome sequence data were obtained, and paired-end reads of 250 bp were assembled *de novo* using the SPAdes algorithm (48). Antibiotic resistance genes and the contigs carrying them were identified in all genomes, as well as in all lineage 2 sequences found in GenBank, using the ResFinder program (https://cge.cbs.dtu.dk/services/ResFinder/). A local database was created and used to screen for specific genomic regions using the NCBI standalone software BLAST (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/). Prophage genomes were found using searches of the PHASTER database (https://phaster.ca/) (49). Plasmid content was examined using the full sequence of plasmids previously found in strain D36, which was used as a representative of lineage 2 strains. BLAST results with >90% coverage and >95% DNA identity were considered positive and, hence, indicated that a given plasmid was present.

**Phylogenetic analysis.** To determine the locations of the 9 Iranian outbreak isolates in a GC1 phylogenetic tree, a maximum likelihood tree was constructed from the core genome alignment as previously described (10). The core genome alignment also included the sequences of a number of GC1 isolates known to belong to either lineage 1 or lineage 2, which were used as controls (10). In addition to strains previously shown to be members of lineage 2, two isolates (PR322 and MRSN 3527) that were identified as ST81 (a known ST in lineage 2) in the GenBank Whole-Genome Shotgun database were also included in the phylogenetic analysis. To draw a whole-genome phylogenetic tree, Illumina sequence reads for all isolates were mapped to the A1 GC1 reference genome (GenBank accession no. CP010781) (33), using the snplord pipeline, available at https://github.com/CJREID/snplord. The snplord pipeline uses the Snippy tool (available at https://github.com/leemann/snippy) to generate a whole-genome alignment. Briefly, Snippy mapped all reads to the reference genome using the bwa (v0.7.12) and minimap2 (v2.0) programs and default parameters. High-quality variant sites were called using SAMTools (v1.3.12) with standard quality filtering, as described previously (10). Single nucleotide differences (SNDs) in recombinant regions were identified and removed using the Gubbins (v2.1.025) program (50) with default parameters, including a default taxa filtering percentage of 25%. A maximum likelihood phylogenetic tree was inferred from the resulting recombination-filtered alignment using the RAxML (v8.2.7)
program with the GAMMA model. The tree was visualized and annotated using the R package ggtree (v.1.12.027). Recombination blocks were plotted against the phylogeny tree in R (v.3.5.2) using the ggtree (v.1.16.6) and ggplot2 (v.3.2.1) packages and the PlotTree program, available at https://github.com/katholt/plotTree. Bootstrap values were calculated using 10 independent runs of RAxML with 1,000 bootstraps, which each gave nearly identical results.

MLST. The Institut Pasteur (IP) multilocus sequence typing (MLST) scheme, which uses 7 housekeeping genes (cpn60, ftsA, gilA, pyrG, recA, rplB, and rpoB), was used to determine the sequence type of all 50 strains using the primers and conditions specified previously (32). An ST number was assigned by comparing the allele sequences to the ones on the MLST site (http://pubmlst.org/abaumannii/). The Oxford and Institut Pasteur multilocus sequence types were also determined in silico for the strains sequenced here.

Capsule and lipooligosaccharide biosynthesis genes. Genetic arrangements located at the K locus (KL) for capsular polysaccharide (CPS) biosynthesis and the OC locus (OCL) for synthesis of the outer core component of the lipooligosaccharide (LOS) were determined using the curated A. baumannii KL and OCL sequence databases available through Kaptive (51).

Data availability. Draft genome sequences of strains AB0529, AB0542, AB0562, AB0663, AB0768, AB0103, AB014, AB0122, and AB0201 have been deposited in the GenBank/EMBL/DDBJ database and are publicly available under accession numbers WIOH00000000, WIOG00000000, WIOF00000000, WIE00000000, WIOD00000000, WIOC00000000, WIOB00000000, WIOA00000000, and VJZ00000000, respectively. The new ampC alleles found here in Iranian strains, ampC80 and ampC81, have been deposited in the ampC database, publicly available under http://pubmlst.org/abaumannii/.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TABLE S1, DOCX file, 0.04 MB.
TABLE S2, CSV file, 0.1 MB.

ACKNOWLEDGMENTS

We express our sincere gratitude to Ruth M. Hall of the University of Sydney, Sydney, NSW, Australia, for her support during the initial stage of this study and guidance on revising the manuscript.

M.H. and the bioinformatics analysis were supported by a Chancellor’s Research Fellowship (fellowship DE180101563). J.J.K. was supported by an Australian Research Council (ARC) DECRA fellowship (fellowship CPDRF PRO17-4005) received from the University of Technology Sydney, Sydney, NSW, Australia. The experimental part of this study was supported by a Tehran University of Medical Sciences grant (grant no. 37548) and an Academy of Science for Developing World (TWAS) grant (grant no. 11-119 RG/PHA/AS_C-UNESCO FR:3240262646). J.J.K. was supported by an Australian Research Council (ARC) DECRA fellowship (fellowship DE180101563).

REFERENCES

1. Adams MD, Chan ER, Molyneaux ND, Bonomo RA. 2010. Genomewide analysis of divergence of antibiotic resistance determinants in closely related isolates of Acinetobacter baumannii. Antimicrob Agents Chemother 54:3569–3577. https://doi.org/10.1128/AAC.00057-10.
2. Adams MD, Goglin K, Molyneaux N, Hujer KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. 2008. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Bacteriol 190:8053–8064. https://doi.org/10.1128/JB.00834-08.
3. Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, Richet H, Robert C, Mangenot S, Abergel C, Nordmann P, Weissenbach J, Raoult D. 2008. Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS Genet 2:e17. https://doi.org/10.1371/journal.pgen.0020007.
4. Zarrilli R, Pouranis S, Giannouli M, Tsakris A. 2013. Global evolution of multidrug-resistant Acinetobacter baumannii clonal lineages. Int J Antimicrob Agents 41:11–19. https://doi.org/10.1016/j.ijantimicag.2012.09.008.
5. Post V, Hall RM. 2009. AblaRS, a large multiple-antibiotic resistance region found in Acinetobacter baumannii. Antimicrob Agents Chemother 53:2667–2671. https://doi.org/10.1128/AAC.01407-08.
6. Post V, White PA, Hall RM. 2010. Evolution of Abla-type genomic resistance islands in multiply antibiotic-resistant Acinetobacter baumannii. J Antimicrob Chemother 65:1162–1170. https://doi.org/10.1093/jac/dkq099.
7. Bush K, Jacoby GA. 2010. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 54:969–976. https://doi.org/10.1128/AAC.01009-09.
8. Codjoe FS, Donkor ES. 2017. Carbapenem resistance: a review. Med Sci 6:1. https://doi.org/10.3390/medsci6010001.
9. Perez F, Ponce-Terashima R, Adams MD, Bonomo RA. 2011. Are we closing in on an "elusive enemy"? The current status of our battle with Acinetobacter baumannii. Virulence 2:86–90. https://doi.org/10.4161/viru.2.2.15748.
10. Holt K, Kenyon JJ, Hamidian M, Schultz MB, Pickard DJ, Dougan G, Hall R. 2016. Five decades of genome evolution in the globally distributed, extensively antibiotic-resistant Acinetobacter baumannii global clone 1. Microb Genom 2:e000052. https://doi.org/10.1099/mgen.0.000052.
11. Hamidian M, Hawkey J, Wick R, Holt KE, Hall RM. 2019. 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 5:e000242. https://doi.org/10.1099/mgen.0.000242.
12. Nigro SJ, Hall RM. 2016. Structure and context of Acinetobacter transposons carrying the aoxA23 carbapenemase gene. J Antimicrob Chemother 71:1135–1147. https://doi.org/10.1093/jac/dkv440.
13. Nigro SJ, Holt KE, Pickard D, Hall RM. 2015. Carbapenem and aminoglycoside resistance on a large conjugative Acinetobacter baumannii plasmid. J Antimicrob Chemother 70:1259–1261. https://doi.org/10.1093/jac/dku486.
14. Beigverdi R, Sattari-Maraji A, Emaneini M, Jabalameli F. 2019. Status of carbapenem-resistant Acinetobacter baumannii harboring carbapenemase: first systematic review and meta-analysis from Iran. Infect Genet Evol 73:433–443. https://doi.org/10.1016/j.meegid.2019.06.008.
15. Nasiri MJ, Zamani S, Farsadani F, Arshadi M, Bigverdi R, Hajikhani B, Goudarzi H, Tabarsi P, Dabiri H, Feizabadi MM. 2020. Prevalence and mechanisms of carbapenem resistance in Acinetobacter baumannii: a comprehensive systematic review of cross-sectional studies from Iran. Microb Drug Resist 26:270–283. https://doi.org/10.1016/j.mdr.2018.04.035.
16. Razavi Nikoo H, Ardebili A, Mardaneh J. 2017. Systematic review of

March/April 2020 Volume 5 Issue 2 e00164-20
mSphere.asm.org
antimicrobial resistance of clinical Acinetobacter baumannii isolates in Iran: an update. Microb Drug Resist 23:744–756. https://doi.org/10.1089/mdr.2016.0118.

17. Hojjati Z, Pajand O, Bonura C, Aleo A, Gimmanco A, Mammma C. 2014. Molecular epidemiology of Acinetobacter baumannii in Iran: endemic and epidemic spread of multiresistant isolates. J Antimicrob Chemother 69:2383–2387. https://doi.org/10.1093/jac/dku045.

18. Peymani A, Higgin PG, Naheiri MR, Farajnia S, Seifert H. 2012. Characterization and clonal dissemination of OXA-23-producing Acinetobacter baumannii in Tabriz, northwest Iran. Int J Antimicrob Agents 39:526–528. https://doi.org/10.1016/j.ijantimicag.2012.02.014.

19. Hamidian M, Ngrio SJ. 2019. Emergence, molecular mechanisms and global spread of carbapenem-resistant Acinetobacter baumannii. Microbiom Genom 5:x000306. https://doi.org/10.1093/mgen/000306.

20. Jasemi S, Douraghi M, Adibhesami H, Zeraati H, Rahbar M, Boroumand MA. 2016. Prevalence and trend of extensive drug-resistant Acinetobacter baumannii and the remaining therapeutic options: a multicenter study in Tehran, Iran over a 3-year period. Lett Appl Microbiol 63:466–472. https://doi.org/10.1111/lam.12669.

21. Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Ptl TL. 2007. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of Acinetobacter baumannii. Clin Microbiol Infect 13:807–815. https://doi.org/10.1111/j.1469-0691.2007.01759.x.

22. Douraghi M, Jasemi S, Kodari M, Rahbar M, Boroumand MA. 2016. Evidence of interruption of the comG gene in a large series of clinical isolates of multidrug-resistant Acinetobacter baumannii. J Mol Microbiol Biotechnol 26:410–413. https://doi.org/10.1159/000448785.

23. Hamidian M, Hall RM. 2018. The Abar antibiotic resistance islands found in Acinetobacter baumannii global clone 1 structure, origin and evolution. Drug Resist Updat 41:26–39. https://doi.org/10.1016/j.drup.2018.10.003.

24. Ngio SJ, Hall MJ. 2012. Tn6167, an antibiotic resistance island in an Australian carbapenem-resistant Acinetobacter baumannii strain. J Antimicrob Chemother 67:1342–1346. https://doi.org/10.1093/jac/dkt037.

25. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Marttinen P, Meseguer J, Moret J, Moreillon P, Nordmann P, Partington M, Polk M, Polyer W, de Roscoff M-C, Woodford N, Erratt J, Hiramatsu K, Suski K. 2009. Multiresistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:286–288. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

26. Heritier C, Poirel L, Nordmann P. 2005. Cephalosporinase overproduction and characterization in pathogenic isolates of Acinetobacter baumannii from Australian hospitals. J Antimicrob Chemother 66:1504–1509. https://doi.org/10.1093/jac/dki122.

27. Hamidian M, Hall RM. 2018. Genetic structure of four plasmids found in Acinetobacter baumannii isolates belonging to global clone 1. J Mol Microbiol Biotechnol 61:e00780-17. https://doi.org/10.1128/AAC.01478-09.

28. Nigro SJ, Post V, Hall RM. 2011. Aminoglycoside resistance in multiply antibiotic-resistant Acinetobacter baumannii belonging to global clone 2 from Australian hospitals. J Antimicrob Chemother 66:1504–1509. https://doi.org/10.1093/jac/dkr163.

29. Hamidian M, Hall RM. 2013. ISAba1 targets a specific position upstream of the intrinsic ampC gene of Acinetobacter baumannii leading to cephalosporin resistance. J Antimicrob Chemother 68:2682–2683. https://doi.org/10.1093/jac/dkt233.

30. Antu P, Boroumand MA, Douraghi M. 2019. Amikacin resistance due to the ampC gene in multi-antibiotic-resistant Acinetobacter baumannii isolates belonging to global clone 1 from Iran. BMC Microbiol 19:221. https://doi.org/10.1186/s12866-019-1592-6.

31. Hamidian M, Hall RM. 2014. Resistance to third-generation cephalosporins in Acinetobacter baumannii due to horizontal transfer of a chromosomal segment containing ISAba1-ampC. J Antimicrob Chemother 69:2865–2866. https://doi.org/10.1093/jac/dku202.

32. D’Andrea MM, Giani T, D’Arezzo S, Capone A, Perssolin S, Visca P, Luzzaro F, Rossolini GM. 2009. Characterization of pABVA01, a plasmid encoding the OXA-24 carbapenemase from Italian isolates of Acinetobacter baumannii. Antimicrob Agents Chemother 53:3528–3533. https://doi.org/10.1128/AAC.00178-09.

33. Morino M, Acosta J, Pozza F, Sanz F, Becerre A, Chaves F, Bou G. 2010. OXA-24 carbapenemase gene flanked by XerC/XerD-like recombination sites in different plasmids from different Acinetobacter species isolated during a nosocomial outbreak. Antimicrob Agents Chemother 54:2384–2385. https://doi.org/10.1128/AAC.01277-09.

34. Blackwell GA, Hall RM. 2017. The tetT determinant and the mepE-mepH genes in Acinetobacter plasmids are each part of discrete modules flanked by inversely oriented pdf (XerC-XerD) sites. Antimicrob Agents Chemother 61:e00780-17. https://doi.org/10.1128/AAC.00780-17.

35. Hamidian M, Holt KE, Pickard D, Hall RM. 2016. A small Acinetobacter baumannii plasmid carrying the tetT99 tetracycline resistance determinant. J Antimicrob Chemother 71:269–271. https://doi.org/10.1093/jac/dkv293.

36. Hamidian M, Hall RM. 2018. Genetic structure of four plasmids found in Acinetobacter baumannii isolate D36 belonging to lineage 2 of global clone 1. PLoS One 13:e0204357. https://doi.org/10.1371/journal.pone.0204357.

37. World Health Organization. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organization, Geneva, Switzerland.

38. Chaisson ML. 2019. Performance standards for antimicrobial susceptibility testing, 29th ed. CLSI, Wayne, PA.

39. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Ames SY, Livermore DM. 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 27:351–353. https://doi.org/10.1016/j.ijantimicag.2006.01.004.

40. Mak JK, Kim MJ, Pham J, Tapsall J, White PA. 2009. Antibiotic resistance determinants in nosocomial strains of multidrug-resistant Acinetobacter baumannii. J Antimicrob Chemother 63:47–54. https://doi.org/10.1093/jac/dkr163.

41. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pyrbyskij AD, Pyshkin AV, Sotvkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2013. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/jcb.2012.0021.

42. Arumugam G, Grant JR, Maru A, Sajad T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.

43. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res 43:e13. https://doi.org/10.1093/nar/gku1996.

44. Myres KL, Catalin VH, Holt KE, Hall RM, Kenyon JJ. 2 March 2020. Identification of Acinetobacter baumannii loci for capsular polysaccharide (KL) and lipooligosaccharide outer core (OCL) synthesis in genome assemblies using curated reference databases compatible with Kaptive. Microbiom Genom https://doi.org/10.1093/mgen/0000339.