Complete genome of the extensively antibiotic-resistant GC1 Acinetobacter baumannii isolate MRSN 56 reveals a novel route to fluoroquinolone resistance

Christopher J. Harmer 1, Francois Lebreton 2, Jason Stam 2, Patrick T. McGann 2 and Ruth M. Hall 1*

1School of Life and Environmental Sciences, The University of Sydney, Sydney, Australia; 2Multidrug Resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, MD, USA

*Corresponding author. E-mail: ruth.hall@sydney.edu.au

Received 16 January 2022; accepted 14 March 2022

Objectives: To examine the causes of antibiotic resistance in the extensively resistant global clone 1 (GC1) Acinetobacter baumannii isolate MRSN 56 recovered at a US military treatment facility.

Methods: MRSN 56 was sequenced using MinION (Oxford Nanopore) and the reads combined with available Illumina MiSeq data using Unicycler. Acquired resistance genes were identified using ABRicate and their environment examined. ISAba1 and ISAba125 copies were located.

Results: MRSN 56 is ST1IP:ST231Ox:KL1:OCL1 and the complete genome includes four small plasmids, none of which carry resistance genes. The acquired resistance genes were found at four locations in the chromosome in addition to AbaR28 (aphA1, aacC1, aadA1, sul1) in comM. Tn2006 (oxa23, carbapenem resistance) was both in AbaR4 and alone elsewhere. Two copies of Tn7 (dfrA1, sat, aadA1) were identified. One was associated with a 22852 bp adjacent segment [tetA(B), sul2] derived from the AbGRI1 island, and this novel configuration was designated Tn7+. Tn7+ was incorporated in the position preferred by Tn7, downstream of glmS, by transposition using a sequence in AbGRI1 resembling the Tn7 terminal inverted repeats. Tn7 was found at a secondary site. Fluoroquinolone resistance appears to involve a mutation in gyrA combined with inactivation by ISAba1 of the marR gene in the mar operon and constitutive expression of marA from the promoter internal to ISAba1.

Conclusions: MRSN 56 represents a new sublineage of GC1 lineage 1 with novel features that had not been detected previously. The involvement of the mar operon in fluoroquinolone resistance has not been noted previously.
and OCL loci were annotated manually. The ampC1852 carbapenems imipenem and meropenem, and the third- and fourth-generation cephalosporins ceftazidime, ceftriaxone and cefepime. It was also resistant to the fluoroquinolones ciprofloxacin, levofloxacin and moxifloxacin, and the aminoglycoside gentamicin, as well as to trimethoprim/sulphamethoxazole, tetracycline and nitrofurantoin. MRSN 56 was susceptible only to tigecycline, amikacin and tobramycin and to colistin (MIC < 0.25 mg/L).

Methods

Genome sequencing and annotation

The complete genome of MRSN 56 was determined by combining newly determined MinION long-read data (Oxford Nanopore) and available short-read Illumina MiSeq data (SRR14998418). A hybrid assembly was generated using Unicycler as described elsewhere.6,7 Briefly, the MinION reads were filtered using Filtlong version 0.2.1 (https://github.com/rrwick/Filtlong) to remove reads <1000 bp and 500 Mb of long reads were combined with the short-read data using Unicycler version 0.4.0 with default settings.5 The copy number of plasmids was determined by comparing the coverage of the plasmid to that of the chromosome (copy number = 1) using an average of the coverage of Institut Pasteur MLST alleles.

Protein coding, rRNA and tRNA genes were initially annotated using a combination of Prokka (version 1.12)9 and RAST.10 Antibiotic resistance genes were identified using ABRicate version 0.8.13 (https://github.com/tseemann/abricate) and plasmids and resistance regions were annotated manually using an in-house database of standard sequences. The PubMLST database (www.pubmlst.org) was used to determine STs. Kaptive11 was used to identify surface polysaccharide types and the KL and OCL loci were annotated manually. The ampC database12 in PubMLST was used to identify ampC alleles.

Resistance and analysis of resistance regions

Resistance to antibiotics was determined as described previously.13 Antibiotic resistance regions were identified and analysed using an in-house database of known transposons and resistance islands with stand-alone BLAST. Regions that did not match a known structure in our database were used as queries in BLASTn searches of the GenBank nucleotide database.

GenBank accession numbers

The sequences of the MRSN 56 chromosome and four plasmids, pMRSN56-1, pMRSN56-2, pMRSN56-3 and pMRSN56-4, have been deposited in GenBank (BioProject PRJNA742487) under accession numbers CP080452, CP080453, CP080454, CP080455 and CP080456, respectively. Reads are available under accession numbers SRR14998418 (Illumina MiSeq) and SRR14008417 (Oxford Nanopore).

Results and discussion

Resistance profile of MRSN 56

Among the antibiotics tested, MRSN 56 was recorded as resistant to ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, the carbapenems imipenem and meropenem, and the third- and fourth-generation cephalosporins ceftazidime, ceftriaxone and cefepime.5 It was also resistant to the fluoroquinolones ciprofloxacin, levofloxacin and moxifloxacin, and the aminoglycoside gentamicin, as well as to trimethoprim/sulphamethoxazole, tetracycline and nitrofurantoin. MRSN 56 was susceptible only to tigecycline, amikacin and tobramycin and to colistin (MIC < 0.25 mg/L).

Complete genome of MRSN 56

The complete genome of MRSN 56 assembled from newly determined long-read data and available short-read data includes the chromosome and four small plasmids (Table 1). MRSN 56 belongs to ST1 in the Institut Pasteur scheme6 and to ST231 in the Oxford scheme, making it a member of GC1. The chromosome carries the GC1-associated variant of the intrinsic oxaAb gene encoding the OXA-69 variant, and includes the KL1 gene cluster at the capsule locus and OCL1 at the variable outer core locus, a configuration seen in several other GC1/CC1 isolates.1,13 Twenty copies of ISAba1 were detected in the MRSN 56 chromosome. Five are part of the resistance regions described below, four at the boundaries of Tn2006 and one in Tn7+. The remaining 15, including one upstream of ampC, are located throughout the chromosome as shown in Figure 1a. Two copies of ISAba125 were also present.

Acquired resistance involving intrinsic genes

The copy of ISAba1 found upstream of the intrinsic ampC gene in MRSN 56 would lead to cephalosporin resistance. The ampC allele was compared with that found in early GC1 isolates such as A116 (GenBank accession number CP010781) representing the standard GC1 sequence, and a recombination patch internal to ampC with 28 single-base substitutions between bases 77 and 1044 was detected. This allele is classified as ampC-78.12 No IS was present upstream of the intrinsic chromosomal oxaAb gene encoding OXA-69 and this allele is not known to be associated with reduced susceptibility to carbapenems.15

Fluoroquinolone resistance could not be explained by the presence of codon alterations at known positions in the resistance-determining regions of gyrA and parC, as only the S81L substitution in gyrA, which confers resistance to nalidixic acid, was detected. Inspection of the gyrB, parC and parA genes revealed no substitution in GyrB, ParC or ParA relative to the standard GC1 alleles seen in the chromosome of the early fluoroquinolone-susceptible isolate A1. As we were unable to

| Table 1. MRSN 56 genome content |
|--------------------------------|
| Replicon | Size (bp) | Copy number | Replicon type | Accession number | Comments |
|-----------------|----------|-------------|--------------|-----------------|----------|
| Chromosome      | 4033258  | 1           | —            | CP080452        | Novel Rep |
| pMRSN56-1       | 2178     | 4           | Rep_1        | CP080453        | Identical to pA85-1a |
| pMRSN56-2       | 2725     | 3           | Rep_1        | CP080454        | Identical to pMRSN56-1b, RepAcI1 |
| pMRSN56-3       | 6772     | 3-4         | Rep_3        | CP080455        | Novel Rep |
| pMRSN56-4       | 8731     | 3           | Rep_3        | CP080456        | Identical to pA1-1b, RepAcI1 |
| aGenBank accession number CP021783. |
| bGenBank accession number CP010782. |
Figure 1. Features of MRSN 56. (a) Circular map of the chromosome of A. baumannii strain MRSN 56. Locations of important features, e.g. gyrA, parC, oxaAb, mar and ampC genes, KL and OCL, and the location of insertions (e.g. AbaR, Tn2006, Tn7, Tn7+) are indicated. Thin black numbered lines indicate the locations of solo ISAba1 copies. Further copies surround Tn2006 (the solo copy shown and the copy in AbaR4) and Tn7+ includes a copy. Drawn to scale from GenBank accession number CP080452 (MRSN 56). (b) Structure of the mar operon showing the location and orientation of ISAba1 copy 8. (c) Structure of Tn7+. The chromosome on each side of the island is represented by a thin dashed line. ISs are shown as boxes, and inverted repeats (IRs) are shown as vertical bars. The nucleotide sequence of target site duplications is indicated by letters above. The orientation and extent of genes are indicated by horizontal arrows. Structures of known origin are labelled. Drawn to scale from GenBank accession numbers CP080452 and JN968483. (d) Comparison of the TIRs of Tn7 and Tn6167 with the novel TIR found at the right boundary of Tn7+.
find the source of the fluoroquinolone resistance at these locations, we searched among the genes that would be expressed from the strong outward-facing promoter in an ISAba1 located near them. The ISAba1 numbered 8 in Figure 1a was found to be located in a gene encoding a protein that is 37% identical to the MarR repressor of the marAB genes of *Escherichia coli*. In MRSN 56, the marR gene is followed by genes encoding MarA and MarB proteins that are 32% and 29% identical, respectively, to the well-studied Mar proteins of *E. coli* and the ISAba1 is oriented such that the marA and marB genes would be constitutively expressed from its internal promoter (Figure 1b). In *E. coli*, it has been shown that expression of MarA, a transcriptional activator, increases expression of a set of genes that lead to multiple antibiotic resistance. In addition, expression of MarA has been shown to reduce susceptibility to norfloxacin and it is likely that the inactivation of the marR gene plus provision of a replacement promoter internal to the ISAba1 combined with the S81L substitution in GyrA accounts for the observed fluoroquinolone resistance.

**Context of acquired antibiotic resistance genes in MRSN 56**

The acquired antibiotic resistance genes in the MRSN 56 genome were all included in the chromosome but were distributed among five different transposon insertions (or islands). As reported previously, MRSN 56 retains only a remnant of the AbaR island typically found in *comM* in GC1 isolates and the IS26 at one end of Tn6020 in Abar28 has caused a deletion (see Figure 1b in) that extends for 55.7 kb into the adjacent chromosomal sequence. Abar28 retains the aphA1b gene in Tn6020 and the aacC1, aadA1, and sul1 genes of the class 1 integron. The small (108 bp) deletion in intI1 that distinguishes the two main groups in GC1 lineage 1 that carry an AbaR derived from Abar0 (intI1 intact) or from Abar3 (intI1 deletion) was present. The Abar3 branch of GC1 lineage 1 is estimated to have arisen around 1985. The oxa23 carbapenem resistance gene is in Tn2006, which is present twice, once located in Abar4 and once alone (target site duplication acaTAgAT) in Tn2006. The second copy of Tn2006 may be derived from Abar4.

Two copies of Tn7, carrying the *dfrA1* trimethoprim resistance gene, the *sat2* streptomycin resistance gene and another copy of *aadA1*, were identified. One copy, part of Tn7+, was found in the preferred location for Tn7, namely downstream of *glmS*, and the second was in a secondary site (see Figure 1a for locations of Tn7+ and Tn7). However, the copy near glmS was surrounded by an adjacent segment and together they were surrounded by the expected 5 bp target site duplication, suggesting that they had moved to this location together. The additional segment included the sul2 sulphonamide resistance gene and the tet(B) tetracycline resistance determinant (Figure 1c) and the presence of tet(B) explains the observed tetracycline resistance. The combined structure was designated Tn7+.

**Origin of Tn7+**

The segment adjacent to Tn7 is identical to part of the backbone of the full-length AbGR1 and the configuration found on plasmid pAb3 from ATCC 17978 from which AbGR1 originated. To explain how this segment could have moved together with Tn7, the sequence at the outer boundary with the chromosome was examined and a sequence related to the inner transposase-binding region of the Tn7 terminal inverted repeat (TIR) was detected (Figure 1d). This sequence is more closely related to the TIR of Tn6171, a transposon related to Tn7 that includes the fimbriabactin synthesis and uptake genes and also targets attTn7. However, the presence of this sequence in the Tn6022 portion of AbGR1 appears to be fortuitous.

The proposed route to generation of Tn7+ is as follows. First, Tn7 jumped into pAB3 or AbGRI1 at the position in strB marked by the vertical arrow in Figure 1c. Then it was cut out from this position by the Tn7 transposition machinery using the fortuitous TIR-like sequence at one end (IR in Figure 1c) and this combination was pasted into the standard attTn7 location downstream of *glmS*.

**Conclusions**

MRSN 56 represents a new sublineage of GC1 that was not detected in a previous detailed analysis of sequenced GC1 isolates. Unusual chromosomal modifications and the locations of acquired antibiotic resistance regions (e.g. AbaR, Tn7) and ISAba1 copies should simplify identification of close relatives among complete and draft genomes. The fluoroquinolone resistance appears to have arisen via an unusual route but further work will be needed to confirm the role of MarR inactivation in fluoroquinolone resistance.

**Acknowledgements**

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation. The opinions and assertions contained herein are private views of the authors and are not to be construed as official, or reflecting the views of the U.S. Department of the Army or the Department of Defense.

**Funding**

This work was supported by National Health and Medical Research Council grant GNT1194178 to R.M.H. and the Defence Medical Research and Development Program to P.T.M.

**Transparency declarations**

None to declare.

**References**

1. 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.
2. Hamidian M, Wynn M, Holt KE et al. Identification of a marker for two lineages within the GC1 clone of *Acinetobacter baumannii*. *J Antimicrob Chemother* 2014; 69: 557–8.
3. 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.
4. 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.

5 McGann P, Courvalin P, Snesrud E et al. Amplification of aminoglycoside resistance gene aphA1 in *Acinetobacter baumannii* results in tobramycin therapy failure. *mBio* 2014; 5: e00915.

6 Harmer CJ. HI1 and I1 resistance plasmids from *Salmonella enterica* serovar Typhimurium strain SRC27 are epidemic. *Microb Drug Resist* 2021; 27: 1495-504.

7 Hamidian M, Wick RR, Judd LM et al. Complete genome sequence of A388, an antibiotic-resistant *Acinetobacter baumannii* global clone 1 isolate from Greece. *Microbiol Resour Announc* 2019; 8: e00971-19.

8 Wick RR, Judd LM, Gorrie CL et al. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; 13: e1005595.

9 Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014; 30: 2068–9.

10 Aziz RK, Bartels D, Best AA et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008; 9: 75.

11 Wyres KL, Cahill SM, Holt KE et al. Identification of *Acinetobacter baumannii* loci for capsular polysaccharide (KL) and lipoooligosaccharide outer core (OCL) synthesis in genome assemblies using curated reference databases compatible with Kaptive. *Microb Genom* 2020; 6: e000339.

12 Karah N, Jolley KA, Hall RM et al. Database for the ampC alleles in *Acinetobacter baumannii*. *PLoS One* 2017; 12: e0176695.

13 Galac MR, Snesrud E, Lebreton F et al. A diverse panel of clinical *Acinetobacter baumannii* for research and development. *Antimicrob Agents Chemother* 2020; 64: e00840-20.

14 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.

15 Takebayashi Y, Findlay J, Heesom KJ et al. Variability in carbapenemase activity of intrinsic OxaAb (OXA-51-like) β-lactamase enzymes in *Acinetobacter baumannii*. *J Antimicrob Chemother* 2021; 76: 587–95.

16 Alekshun MN, Levy SB. Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. *Antimicrob Agents Chemother* 1997; 41: 2067–75.

17 Alekshun MN, Levy SB. The mar regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends Microbiol* 1999; 7: 410–3.

18 Harmer CJ, Hall RM. IS26-mediated formation of transposons carrying antibiotic resistance genes. *mSphere* 2016; 1: e00038-16.

19 Post V, Hall RM. AbaR5, a large multiple-antibiotic resistance region found in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; 53: 2667–71.

20 Nigro SJ, Brown MH, Hall RM. AbGRI1-5, a novel AbGRI1 variant in an *Acinetobacter baumannii* GC2 isolate from Adelaide, Australia. *J Antimicrob Chemother* 2019; 74: 821–3.

21 Hamidian M, Hall RM. Origin of the AbGRI1 antibiotic resistance island found in the comM gene of *Acinetobacter baumannii* GC2 isolates. *J Antimicrob Chemother* 2017; 72: 2944–7.

22 Hamidian M, Hall RM. Dissemination of novel Tn7 family transposons carrying genes for synthesis and uptake of fimbriactin siderophores among *Acinetobacter baumannii* isolates. *Microb Genom* 2021; 7: e000548.