AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequences of Two Extensively Drug-Resistant Strains of *Acinetobacter baumannii* Isolated from Clinical Samples in Pakistan

Sara Lomonaco,[a] Matthew A. Crawford,[b] Christine Lascols,[c,d] Debra J. Fisher,[b] Kevin Anderson,[e] David R. Hodge,[e] Segaran P. Pillai,[f] Stephen A. Morse,[d] Erum Khan,[g] Molly A. Hughes,[b] Marc W. Allard,[a] Shashi K. Sharma[a]

[a]Division of Microbiology, Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland, USA
[b]Department of Medicine, Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, USA
[c]National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
[d]IHRC, Inc., Atlanta, Georgia, USA
[e]Science and Technology Directorate, U.S. Department of Homeland Security, Washington, DC, USA
[f]Office of the Commissioner, U.S. Food and Drug Administration, Silver Spring, Maryland, USA
[g]Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan

ABSTRACT Infections in immunocompromised patients that are caused by extensively drug-resistant (XDR) *Acinetobacter baumannii* strains have been increasingly reported worldwide. In particular, carbapenem-resistant *A. baumannii* strains are a prominent cause of health care-associated infections. Here, we report draft genome assemblies for two clinical XDR *A. baumannii* isolates obtained from hospitalized patients in Pakistan.

Acinetobacter baumannii, a Gram-negative opportunistic pathogen of the *Moraxellaceae* family, can carry multiple antimicrobial resistance (AMR) determinants. Infections and outbreaks caused by multidrug-resistant (MDR) or extensively drug-resistant (XDR) *A. baumannii* strains in immunocompromised patients have been increasingly reported worldwide (1), with isolates often belonging to international clone 2/sequence type 2 (ST2) (2). Carbapenem-resistant *A. baumannii* (CRAB) strains are a significant cause of health care-associated infections in Pakistan, with various prevalence rates (62% to 100%) (3). The most common β-lactamases in CRAB strains are acquired (e.g., $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-143}}$, and $bla_{\text{OXA-235}}$) and intrinsic (e.g., $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-69}}$) carbapenem-hydrolyzing oxacillinases (4).

Draft genomes are reported here for two clinical XDR *A. baumannii* isolates obtained from urine (CFSAN059604, isolated in 2004) and throat (CFSAN059618, isolated in 1998) specimens from hospitalized patients in Pakistan. Patient samples were inoculated onto nonselective (e.g., blood agar) and differential (e.g., MacConkey agar) plates and incubated for 24 to 48 h at 37°C. API 20E and Vitek 2 (bioMérieux) systems were used for species identification and confirmation, respectively. Susceptibility testing against clinically relevant antimicrobials was performed by conventional broth microdilution (5) following CLSI and EUCAST guidelines and breakpoints (5–7). The two isolates were resistant to 17 antibiotics with the same MICs, as follows: ≥128 μg/ml for piperacillin-tazobactam; ≥64 μg/ml for cefotaxime and aztreonam; ≥32 μg/ml for ampicillin, ceftriaxone, and tetracycline; ≥16 μg/ml for cefoxitin, ceftazidime-avibactam, gentamicin, and chloramphenicol; and ≥8 μg/ml for cefazolin, doripenem, meropenem, ertapenem, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole. CFSAN059604 and CFSAN059618 were also resistant, but with different MICs, to ampicillin-sulbactam (≥32 and ≥16 μg/ml, respectively), ceftazidime (≥128 and ≥16 μg/ml, respectively), cefepime (≥32 and ≥16 μg/ml, respectively), and imipenem (≥8 and ≥16 μg/ml, respectively). Both isolates were susceptible to colistin (≤2 μg/ml) and minocycline (≤4 μg/ml). Finally, CFSAN059604 and CFSAN059618 had markedly dif-

Citation Lomonaco S, Crawford MA, Lascols C, Fisher DJ, Anderson K, Hodge DR, Pillai SP, Morse SA, Khan E, Hughes MA, Allard MW, Sharma SK. 2020. Draft genome sequences of two extensively drug-resistant strains of *Acinetobacter baumannii* isolated from clinical samples in Pakistan. Microbiol Resour Announc 9:e00026-20. https://doi.org/10.1128/MRA.00026-20.

Editor Julia A. Maresca, University of Delaware

Address correspondence to Sara Lomonaco, sara.lomonaco@fda.hhs.gov.

Received 13 January 2020
Accepted 23 April 2020
Published 14 May 2020

TABLE 1 Assembly statistics, accession numbers, and AMR genes for the XDR *Acinetobacter baumannii* isolates examined in this work

Sample[a]	BioSample accession no.	Yr isolated	SRA accession no.	GenBank accession no.	No. of contigs	Total length (bp)	N_{50} (bp)	GC content (%)	Genome coverage (×)	Avg read quality score for:		No. of reads	No. of AMR genes
										Read 1	Read 2		
CFSAN059604	SAMN10086814	2004	SRR8837010	SSMO00000000	48	3,944,772	261,641	38.9	712	32[b]	30[b]	21,896,362	14[c]
CFSAN059618	SAMN10086821	1998	SRR8837150	SSMN00000000	79	3,907,254	116,856	38.8	93	35	33	1,792,448	8[d]

[a] The two isolates belong to NCBI BioProject PRJNA342326.

[b] Average of four lanes.

[c] Genes *aac(3)-I*, *aadA1*, *adeC*, *ant(3″)-IIa*, *aph(3″)-Ib*, *aph(3′)-Ia*, *aph(6)-Id*, bla_{ADC}, $bla_{OXA-254}$, bla_{TEM-1}, *qacEΔ1*, *sul1*, *sul2*, and *tet*(B).

[d] Genes *ant(2″)-Ia*, *ant(3″)-IIa*, *aph(3′)-VIa*, bla_{ADC-76}, $bla_{OXA-235}$, bla_{OXA-68}, *sul2*, and *tet*(39).

ferent resistance profiles for amikacin (≤2 and ≥64 μg/ml, respectively) and tobramycin (≤1 and ≥16 μg/ml, respectively).

Isolates were grown overnight in lysogeny broth (Lennox), and DNA was extracted using the DNeasy blood and tissue kit (Qiagen). Libraries were prepared using the Nextera XT DNA library preparation kit and sequenced on a MiSeq (CFSAN059618) or NextSeq (CFSAN059604) sequencer (Illumina), with paired-end sequencing technology (2 × 250-bp and 2 × 150-bp sequencing, respectively). Minimum sequence quality was represented by average coverage greater than 50× and Q scores for reads 1 and 2 greater than 30 (8). Absence of contamination was confirmed with Kraken (9). Default parameters were used unless otherwise noted. *De novo* assemblies were obtained with Shovill v0.9 (https://github.com/tseemann/shovill), available in the GalaxyTrakr pipeline (10). The "trim reads" option was selected, and 500 bp was set as the minimum contig length. Draft genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (11). Table 1 lists the number of reads, number of contigs per assembly, genome size, and GC content for each isolate. The isolates were assigned to ST2 (CFSAN059604) and ST23 (CFSAN059618), based on the multilocus sequence typing Pasteur scheme (https://pubmlst.org/abaumannii). NCBI Pathogen Detection (PD) (https://www.ncbi.nlm.nih.gov/pathogens) was used to identify 14 (CFSAN059604) and 8 (CFSAN059618) AMR genes. NCBI PD uses AMRFinderPlus to assign the most specific AMR protein by using a hierarchy of gene families/symbols/names (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder). Results are available in Table 1 and from the NCBI PD website (CFSAN059604 and CFSAN059618).

The described draft genomes will be useful in comparative genomic analyses of *A. baumannii* strains from different regions and clinical settings. These data can also provide phylogenetic insights into the emergence of XDR *A. baumannii* strains and support epidemiological investigations of outbreaks.

Data availability. The complete genome sequences of *A. baumannii* CFSAN059604 (SRA number SRR8837010) and CFSAN059618 (SRA number SRR8837150) are available in GenBank under accession numbers SSMO00000000 and SSMN00000000, respectively (first versions).

ACKNOWLEDGMENTS

S.L. received support by appointment to the Research Participation Program at the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration. M.A.C. and M.A.H. received support from the U.S. National Institutes of Health (grant R21 AI139947). This work was also supported by the U.S. Department of Homeland Security, Science and Technology Directorate (interagency agreement contract HSHQPM-17-X-00057).

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the U.S. Department of Homeland Security. The use of trade names and commercial sources is for identification purposes only and does not imply endorsement.

REFERENCES

1. Peleg AY, Seifert H, Paterson DL. 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 21:538–582. https://doi.org/10.1128/CMR.00058-07.
2. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. 2010. The population structure of *Acinetobacter baumannii:* expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 5:e10034. https://doi.org/10.1371/journal.pone.0010034.
3. Hsu L-Y, Apisarnthanarak A, Khan E, Suwantarat N, Ghafur A, Tambyah PA. 2017. Carbapenem-resistant *Acinetobacter baumannii* and *Enterobacteriaceae* in South and Southeast Asia. Clin Microbiol Rev 30:1–22. https://doi.org/10.1128/CMR.00042-16.
4. Higgins PG, Pérez-Llarena FJ, Zander E, Fernández A, Bou G, Seifert H. 2013. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 57:2121–2126. https://doi.org/10.1128/AAC.02413-12.
5. Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing; 27th informational supplement. CLSI document M100-S. Clinical and Laboratory Standards Institute, Wayne, PA.
6. Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—10th ed. CLSI document M07. Clinical and Laboratory Standards Institute, Wayne, PA.
7. EUCAST. 2016. Breakpoint tables for interpretation of MICs and zone

diameters, version 6.0. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf.
8. Timme RE, Sanchez Leon M, Allard MW. 2019. Utilizing the public GenomeTrakr database for foodborne pathogen traceback. Methods Mol Biol. 2019; 1918:201–212. https://doi.org/10.1007/978-1-4939-9000-9_17.
9. Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46. https://doi.org/10.1186/gb-2014-15-3-r46.
10. Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Čech M, Chilton J, Clements D, Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltemann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544. https://doi.org/10.1093/nar/gky379.
11. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.