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GENOME SEQUENCES





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

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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.

A cinetobacter 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_{OXA-23} , bla_{OXA-40} , bla_{OXA-58} , $bla_{OXA-143}$, and $bla_{OXA-235}$) and intrinsic (e.g., bla_{OXA-51} and bla_{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: \geq 128 μ g/ml for piperacillin-tazobactam; \geq 64 μ g/ml for cefotaxime and aztreonam; \geq 32 μ g/ml for ampicillin, ceftriaxone, and tetracycline; $\geq 16 \ \mu q/ml$ for cefoxitin, ceftazidime-avibactam, gentamicin, and chloramphenicol; and $\geq 8 \mu g/ml$ for cefazolin, doripenem, meropenem, ertapenem, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole. CFSAN059604 and CFSAN059618 were also resistant, but with different MICs, to ampicillin-sulbactam (\geq 32 and \geq 16 μ g/ml, respectively), ceftazidime (\geq 128 and \geq 16 μ g/ml, respectively), cefepime (\geq 32 and \geq 16 μ g/ml, respectively), and imipenem (\geq 8 and \geq 16 μ g/ml, respectively). Both isolates were susceptible to colistin ($\leq 2 \mu$ g/ml) and minocycline ($\leq 4 \mu g/ml$). Finally, CFSAN059604 and CFSAN059618 had markedly dif-

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	No. AM
	Avg read quality score for: No. of
s work	Genome
nii isolates examined in this woi	GC content Genome
ıcter baumanni	Total
DR Acinetoba	No. of
AMR genes for the XDR Acinetob	GenBank
umbers, and	SRA
accession n	۲r
TABLE 1 Assembly statistics,	BioSample

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										Avg read quality	quality		No. of
	BioSample	۲r	SRA	GenBank	No. of	Total		GC content	Genome	score for:		No. of	AMR
Sample ^a	accession no. isolated accession no. accession no.	isolated	accession no.	accession no.	contigs	length (bp) N ₅₀ (bp)	N ₅₀ (bp)	(%)	coverage (×) Read 1 Read 2	Read 1	Read 2	reads	genes
CFSAN059604	CFSAN059604 SAMN10086814 2004	2004	SRR8837010	SSMO00000000	48	3,944,772	261,641 38.9	38.9	712	32 ^b	30 ^b	21,896,362	14 ^c
CFSAN059618	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)-1, aadA1, adeC, ant(3")-1la, aph(3")-1b, aph(3')-1a, aph(6)-1d, bla_{ADC} bla_{OXA-254}, bla_{TEM-1}, qacEΔ1, sul1, sul2, and tet(B). ^d Genes ant(2")-1a, aph(3")-1la, aph(3")-1/a, bla_{ADC-76}, bla_{OXA-255}, bla_{OXA-68}, sul2, and tet(39).

ferent resistance profiles for amikacin (≤ 2 and $\geq 64 \ \mu g/ml$, respectively) and tobramycin (≤ 1 and $\geq 16 \ \mu g/ml$, 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 $(2 \times 250$ -bp and 2×150 -bp sequencing, respectively). Minimum sequence quality was represented by average coverage greater than $50 \times$ 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).

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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

- 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.
- 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.
- 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.

- 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.
- 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.

- Timme RE, Sanchez Leon M, Allard MW. 2019. Utilizing the public Genome-Trakr database for foodborne pathogen traceback. Methods Mol Biol. 2019; 1918:201–212. https://doi.org/10.1007/978-1-4939-9000-9_17.
- 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.

 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.