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# Genome Sequence of a Multidrug-Resistant Strain of *Klebsiella pneumoniae*, BAMC 07-18, Isolated from a Combat Injury Wound

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***Klebsiella pneumoniae* is an important infectious agent of surgical sites and combat wounds. Antibiotic resistance and tolerance are common impediments to the healing of chronic infections. Here, we report the genome sequence of a highly multidrug-resistant strain of *K. pneumoniae*, BAMC 07-18, isolated from a combat wound of a soldier.**

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*Klebsiella pneumoniae*, a Gram-negative bacterium, is commonly found in the soil and as a component of the normal human flora (1). However, *K. pneumoniae* has been increasingly implicated as a causative agent of nosocomial and/or chronic infections (2). Multidrug-resistant (MDR) strains of *K. pneumoniae* are also one of the most common pathogens isolated from infections in soldiers wounded in combat (3–5). The tendency of these strains to form biofilms on biotic and abiotic surfaces, including catheters and other medical devices, is a contributing factor to their antibiotic resistance (6). *K. pneumoniae* BAMC 07-18 (kindly provided by Clinton Murray of the San Antonio Military Medical Center, Fort Sam Houston, San Antonio, TX) is a biofilm-forming MDR strain isolated from a patient at the San Antonio Military Medical Center (SAMMC). This strain was highly resistant to many antimicrobials, including azithromycin, ceftazidime, chloramphenicol, and tetracycline; however, BAMC 07-18 is sensitive to imipenem, a carbapenem, both *in vivo* and *in vitro* (7). High doses of imipenem are still unable to completely clear biofilm infections, though it leads to significant reductions in viability and alterations in morphology (7), leading us to question the genetic mechanisms of the pleiotropic effects of imipenem against this carbapenem-sensitive strain of *K. pneumoniae*.

As a first step to understanding these effects, we sequenced the genome of *K. pneumoniae* BAMC 07-18. *De novo* genomic sequencing service was provided by BGI Tech Solutions Co., Ltd. (Cambridge, MA, USA) using the Illumina HiSeq 2000 platform. A total of 602 Mb of data was produced for BAMC 07-18 from the 500-bp library, 604 Mb of data from the 2,000-bp library, and 351 Mb of data from the 6,000-bp library. The raw sequence data were quality filtered and then assembled using the SOAPdenovo software (8).

The preliminary total assembled genome size was 5.5 Mb, consisting of a 5.0-Mb chromosome and 8 contigs ranging from 0.5 to 447.6 kb, with a G+C content of 57.20%, without low-coverage regions.

An analysis of the genome sequence revealed the presence of

many genes for antibiotic resistance, such as extended-spectrum  $\beta$ -lactamases (CTX-M, SHV, and TEM), polymyxin, tetracycline, and chloramphenicol, with a lack of any genes implicated in carbapenem resistance (9–12). We also found common virulence factors, such as genes necessary for biofilm and capsule formation, adhesion, and iron sequestration (13–19). The use of this genomic sequence as a reference for RNA sequencing analysis (RNA-seq) will allow us to explore the pleiotropic effects of carbapenems on *K. pneumoniae* biofilms and provide novel opportunities to exploit the overall fitness of *K. pneumoniae* under carbapenem stress.

**Nucleotide sequence accession number.** This genome sequence is deposited in GenBank under the accession no. [JRQE00000000](https://genbank.ncbi.nlm.nih.gov/GenBank/FASTA/JRQE00000000).

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## REFERENCES

- Childers BM, Van Laar TA, You T, Clegg S, Leung KP. 2013. MrkD1P from *Klebsiella pneumoniae* strain IA565 allows for coexistence with *Pseudomonas aeruginosa* and protection from protease-mediated biofilm detachment. *Infect. Immun.* 81:4112–4120. <http://dx.doi.org/10.1128/IAI.00521-13>.
- Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV. 2007. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. *J. Clin. Microbiol.* 45:3352–3359. <http://dx.doi.org/10.1128/JCM.01284-07>.
- Weintrob AC, Roediger MP, Barber M, Summers A, Fieberg AM, Dunn J, Seldon V, Leach F, Huang XZ, Nikolich MP, Wortmann GW. 2010. Natural history of colonization with gram-negative multidrug-resistant

- organisms among hospitalized patients. *Infect. Control Hosp. Epidemiol.* 31:330–337. <http://dx.doi.org/10.1086/651304>.
4. Tribble DR, Conger NG, Fraser S, Gleeson TD, Wilkins K, Antonille T, Weintrob A, Ganesan A, Gaskins LJ, Li P, Grandits G, Landrum ML, Hospenhal DR, Millar EV, Blackbourne LH, Dunne JR, Craft D, Mende K, Wortmann GW, Herlihy R, McDonald J, Murray CK. 2011. Infection-associated clinical outcomes in hospitalized medical evacuees after traumatic injury: trauma infectious disease outcome study. *J. Trauma* 71:S33–S42. <http://dx.doi.org/10.1097/TA.0b013e318221162e>.
  5. Petersen K, Riddle MS, Danko JR, Blazes DL, Hayden R, Tasker SA, Dunne JR. 2007. Trauma-related infections in battlefield casualties from Iraq. *Ann. Surg.* 245:803–811. <http://dx.doi.org/10.1097/01.sla.0000251707.32332.c1>.
  6. Custovic A, Smajlovic J, Hadzic S, Ahmetagic S, Tihic N, Hadzagic H. 2014. Epidemiological surveillance of bacterial nosocomial infections in the surgical intensive care unit. *Mater. Sociomed.* 26:7–11. <http://dx.doi.org/10.5455/msm.2014.26.7-11>.
  7. Chen P, Seth AK, Abercrombie JJ, Mustoe TA, Leung KP. 2014. Activity of imipenem against *Klebsiella pneumoniae* biofilms *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.* 58:1208–1213. <http://dx.doi.org/10.1128/AAC.01353-13>.
  8. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J. 2010. *De novo* assembly of human genomes with massively parallel short read sequencing. *Genome Res.* 20:265–272. <http://dx.doi.org/10.1101/gr.097261.109>.
  9. Livermore DM. 2012. Current epidemiology and growing resistance of Gram-negative pathogens. *Korean J. Intern. Med.* 27:128–142. <http://dx.doi.org/10.3904/kjim.2012.27.2.128>.
  10. Livermore DM. 2012. Fourteen years in resistance. *Int. J. Antimicrob. Agents* 39:283–294. <http://dx.doi.org/10.1016/j.ijantimicag.2011.12.012>.
  11. Schultzs C, Geerlings S. 2012. Plasmid-mediated resistance in *Enterobacteriaceae*: changing landscape and implications for therapy. *Drugs* 72:1–16. <http://dx.doi.org/10.2165/11597960-000000000-00000>.
  12. Tzouveleki LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL. 2012. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. *Clin. Microbiol. Rev.* 25:682–707. <http://dx.doi.org/10.1128/CMR.05035-11>.
  13. Baltes N, Hennig-Pauka I, Jacobsen I, Gruber AD, Gerlach GF. 2003. Identification of dimethyl sulfoxide reductase in *Actinobacillus pleuropneumoniae* and its role in infection. *Infect. Immun.* 71:6784–6792. <http://dx.doi.org/10.1128/IAI.71.12.6784-6792.2003>.
  14. Ge RG, Wang DX, Hao MC, Sun XS. 2013. Nickel trafficking system responsible for urease maturation in *Helicobacter pylori*. *World J. Gastroenterol.* 19:8211–8218. <http://dx.doi.org/10.3748/wjg.v19.i45.8211>.
  15. Naylor J, Cianciotto NP. 2004. Cytochrome *c* maturation proteins are critical for *in vivo* growth of *Legionella pneumophila*. *FEMS Microbiol. Lett.* 241:249–256. <http://dx.doi.org/10.1016/j.femsle.2004.10.028>.
  16. Nishiyama S, Murakami Y, Nagata H, Shizukuishi S, Kawagishi I, Yoshimura F. 2007. Involvement of minor components associated with the FimA fimbriae of *Porphyromonas gingivalis* in adhesive functions. *Microbiology* 153:1916–1925. <http://dx.doi.org/10.1099/mic.0.2006/005561-0>.
  17. Pullinger GD, van Diemen PM, Dziva F, Stevens MP. 2010. Role of two-component sensory systems of *Salmonella enterica* serovar Dublin in the pathogenesis of systemic salmonellosis in cattle. *Microbiology* 156:3108–3122. <http://dx.doi.org/10.1099/mic.0.041830-0>.
  18. Seyedmohammad S, Born D, Venter H. 2014. Expression, purification and functional reconstitution of FeoB, the ferrous iron transporter from *Pseudomonas aeruginosa*. *Protein Expr. Purif.* 101:138–145. <http://dx.doi.org/10.1016/j.pep.2014.06.012>.
  19. Wang N, Ozer EA, Mandel MJ, Hauser AR. 2014. Genome-wide identification of *Acinetobacter baumannii* genes necessary for persistence in the lung. *mBio* 5(3):e01163-14. <http://dx.doi.org/10.1128/mBio.01163-14>.