genomeA
Journals.ASM.org

Genome Sequences of Two Carbapenemase-Resistant *Klebsiella pneumoniae* ST258 Isolates

Maria Soledad Ramirez,[a,b] Gang Xie,[c] Shannon Johnson,[c] Karen Davenport,[c] David van Duin,[f] Federico Perez,[d] Robert A. Bonomo,[d,e] Patrick Chain,[c] Marcelo E. Tolmasky[a]

Center for Applied Biotechnology Studies, Department of Biological Science, California State University, Fullerton, Fullerton, California, USA[a]; IMPaM (UBA-CONICET), Buenos Aires, Argentina[b]; Los Alamos National Laboratory, Los Alamos, New Mexico, USA[c]; Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA[d]; Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA[e]; University of North Carolina, Chapel Hill, North Carolina, USA[f]

***Klebsiella pneumoniae*, an ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogen, has acquired multiple antibiotic resistance genes and is becoming a serious public health threat. Here, we report the genome sequences of two representative strains of *K. pneumoniae* from the emerging *K. pneumoniae* carbapenemase (KPC) outbreak in northeast Ohio belonging to sequence type 258 (ST258) (isolates Kb140 and Kb677, which were isolated from blood and urine, respectively). Both isolates harbor a bla_{KPC} gene, and strain Kb140 carries bla_{KPC-2}, while Kb677 carries bla_{KPC-3}.**

Received 4 June 2014 **Accepted** 6 June 2014 **Published** 19 June 2014

Citation Ramirez MS, Xie G, Johnson S, Davenport K, van Duin D, Perez F, Bonomo RA, Chain P, Tolmasky ME. 2014. Genome sequences of two carbapenemase-resistant *Klebsiella pneumoniae* ST258 isolates. Genome Announc. 2(3):e00558-14. doi:10.1128/genomeA.00558-14.

Address correspondence to Marcelo E. Tolmasky, mtolmasky@fullerton.edu.

Klebsiella pneumoniae, a bacterium belonging to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), is responsible for serious community- and hospital-acquired infections (1–5). The difficulty in treating infections caused by *K. pneumoniae* is increasing due to its ability to acquire antibiotic resistance genes (6–8), including carbapenemases (9).

Despite the clinical importance of this bacterium, the number of identified and characterized virulence factors has remained relatively low (10–13). Nucleotide and whole-genome mapping comparisons among several strains led to the identification of a high heterogeneity zone (HHZ) (14), which includes the capsular polysaccharide biosynthesis gene cluster (HHZ subregion 4) and a hot spot (HHZ subregion 3), which in strain *K. pneumoniae* NTUH-K2044 is the point of insertion of a fragment that includes a pathogenicity island related to that found in *Yersinia* species. This fragment includes the genes coding for the yersiniabactin siderophore system, a region previously identified in a *Klebsiella* plasmid, and genes coding for conjugation functions (10, 14, 15).

K. pneumoniae strains harboring carbapenem resistance genes, such as bla_{KPC} or bla_{NDM-1}, are becoming more frequent and extremely problematic. *K. pneumoniae* Kb140 and Kb677, two representative strains from an emerging KPC outbreak in northeast Ohio, were isolated in 2012. Kb140 was isolated from a patient with fatal bloodstream infection and pneumonia, while Kb677 was isolated from a patient with urinary tract colonization. Both strains were draft sequenced using Illumina (353× and 293× genome coverage for Kb140 and Kb677, respectively) and PacBio RS II (100× and 105× genome coverage, respectively). The submitted *de novo* assemblies utilized Velvet (version 1.2.08), Newbler (version 2.6), AllPaths (version 44837), HGAP (version 2.1.1), and parallel Phrap (SPS-4.24), along with manual review and curation. The draft genomes of Kb140 and Kb677 consist of 5,677,714-bp and 5,894,762-bp sequences, respectively.

There are 5,420 and 5,499 predicted protein-coding genes within the genomes of *K. pneumoniae* isolates Kb140 and Kb677, respectively. Of these, 20.7% and 21.9% of the protein-coding genes, respectively, are annotated as hypothetical or conserved hypothetical proteins. Of those with functional predictions, 31 (Kb140) and 68 (Kb677) are associated with phages/prophages, while 103 (Kb140) and 126 (Kb677) are associated with resistance to antibiotics or toxic compounds. The bla_{KPC} genes are likely located within the sequence with accession no. AQRD01000007 of the Kb140 assembly (positions 1431915 to 1432775) and the sequence with accession no. AQPG01000002 of the Kb677 assembly (positions 7281 to 8240), respectively. The strain Kb140 and Kb677 genomes have 86 and 85 tRNA genes, respectively, as well as 24 rRNA genes.

K. pneumoniae strains usually harbor several plasmids (4, 6, 16). Analysis of the nucleotide sequences of strains Kb140 and Kb677 showed homology to the $IncFII_k$-FIB-like plasmids pKPN- and pKpQIL-type (17, 18), pIncX-SHV (17), and pKP1780-kpc (accession no. KF874497), pNJST258C1, pNJST258C2 (19), pBK31551, pBK15692 (20), p1 (accession no. CP006657), pR55 (21), and the *K. pneumoniae* subsp. *rhinoscleromatis* pKRH (22).

Nucleotide sequence accession numbers. The GenBank accession no. for *K. pneumoniae* Kb140 and Kb677 are AQRD01000001 to AQRD01000008 and AQPG01000001 to AQPG01000012, respectively.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants 2R15AI047115 (to M.E.T.), the U.S. Department of Energy Joint Genome Institute through the Office of Science of the U.S. Department of Energy under contract DE-AC02-05CH11231, the Cleveland Department of Veterans Affairs, the Veterans Affairs Merit Review Program award 1I01BX001974, and the Geriatric Research Education and Clinical Center VISN 10. This work was also supported by funds from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, under award numbers R01AI063517 and R01AI10056 (to R.A.B.), and through the Antibiotic Resistance Leadership Group under National Institutes of Health award UM1AI104681 (to D.v.D.) and the Clinical and Translational Science Collaborative of Cleveland, UL1TR000439 from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health (to D.v.D. and F.P.).

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

REFERENCES

1. **Liu Y, Wang JY, Jiang W.** 2013. An increasing prominent disease of *Klebsiella pneumoniae* liver abscess: etiology, diagnosis, and treatment. Gastroenterol. Res. Pract. 2013:258514. http://dx.doi.org/10.1155/2013/258514.
2. **Nordmann P, Cuzon G, Naas T.** 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect. Dis. **9:**228–236. http://dx.doi.org/10.1016/S1473-3099(09)70054-4.
3. **Tiwana H, Natt RS, Benitez-Brito R, Shah S, Wilson C, Bridger S, Harbord M, Sarner M, Ebringer A.** 2001. Correlation between the immune responses to collagens type I, III, IV and V and *Klebsiella pneumoniae* in patients with Crohn's disease and ankylosing spondylitis. Rheumatology (Oxf.) **40:**15–23. http://dx.doi.org/10.1093/rheumatology/40.1.15.
4. **Woloj M, Tolmasky ME, Roberts MC, Crosa JH.** 1986. Plasmid-encoded amikacin resistance in multiresistant strains of *Klebsiella pneumoniae* isolated from neonates with meningitis. Antimicrob. Agents Chemother. **29:**315–319. http://dx.doi.org/10.1128/AAC.29.2.315.
5. **Rice LB.** 2010. Progress and challenges in implementing the research on ESKAPE pathogens. Infect. Control Hosp. Epidemiol. **31**(Suppl 1): S7–S10. http://dx.doi.org/10.1086/655995.
6. **Ramirez MS, Traglia G, Lin DL, Tran T, Tolmasky ME.** 2014. Plasmid-mediated antibiotic resistance and virulence in gram-negatives: the *Klebsiella pneumoniae* paradigm. Microbiol. Spectrum, in press.
7. **D'Andrea MM, Arena F, Pallecchi L, Rossolini GM.** 2013. CTX-M-type β-lactamases: a successful story of antibiotic resistance. Int. J. Med. Microbiol. **303:**305–317. http://dx.doi.org/10.1016/j.ijmm.2013.02.008.
8. **Ramirez MS, Tolmasky ME.** 2010. Aminoglycoside modifying enzymes. Drug Resist. Update. **13:**151–171. http://dx.doi.org/10.1016/j.drup.2010.08.003.
9. **Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP.** 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect. Dis. **13:**785–796. http://dx.doi.org/10.1016/S1473-3099(13)70190-7.
10. **Lin TL, Lee CZ, Hsieh PF, Tsai SF, Wang JT.** 2008. Characterization of integrative and conjugative element ICEKp1-associated genomic heterogeneity in a *Klebsiella pneumoniae* strain isolated from a primary liver abscess. J. Bacteriol. **190:**515–526. http://dx.doi.org/10.1128/JB.01219-07.
11. **Putze J, Hennequin C, Nougayrède JP, Zhang W, Homburg S, Karch H, Bringer MA, Fayolle C, Carniel E, Rabsch W, Oelschlaeger TA, Oswald E, Forestier C, Hacker J, Dobrindt U.** 2009. Genetic structure and distribution of the colibactin genomic island among members of the family *Enterobacteriaceae*. Infect. Immun. **77:**4696–4703. http://dx.doi.org/10.1128/IAI.00522-09.
12. **Russo TA, Olson R, Macdonald U, Metzger D, Maltese LM, Drake EJ, Gulick AM.** 2014. Aerobactin mediates virulence and accounts for the increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*. Infect. Immun. **82:**2356–2367. http://dx.doi.org/10.1128/IAI.01667-13.
13. **Struve C, Bojer M, Nielsen EM, Hansen DS, Krogfelt KA.** 2005. Investigation of the putative virulence gene *magA* in a worldwide collection of 495 *Klebsiella* isolates: *magA* is restricted to the gene cluster of *Klebsiella pneumoniae* capsule serotype K1. J. Med. Microbiol. **54:**1111–1113. http://dx.doi.org/10.1099/jmm.0.46165-0.
14. **Ramirez MS, Xie G, Marshall SH, Hujer KM, Chain PS, Bonomo RA, Tolmasky ME.** 2012. Multidrug-resistant (MDR) *Klebsiella pneumoniae* clinical isolates: a zone of high heterogeneity (HHZ) as a tool for epidemiological studies. Clin. Microbiol. Infect. **18:**E254–E258. http://dx.doi.org/10.1111/j.1469-0691.2012.03886.x.
15. **Wu KM, Li LH, Yan JJ, Tsao N, Liao TL, Tsai HC, Fung CP, Chen HJ, Liu YM, Wang JT, Fang CT, Chang SC, Shu HY, Liu TT, Chen YT, Shiau YR, Lauderdale TL, Su IJ, Kirby R, Tsai SF.** 2009. Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. J. Bacteriol. **191:**4492–4501. http://dx.doi.org/10.1128/JB.00315-09.
16. **Soler Bistué AJ, Birshan D, Tomaras AP, Dandekar M, Tran T, Newmark J, Bui D, Gupta N, Hernandez K, Sarno R, Zorreguieta A, Actis LA, Tolmasky ME.** 2008. *Klebsiella pneumoniae* multiresistance plasmid pMET1: similarity with the *Yersinia pestis* plasmid pCRY and integrative conjugative elements. PLoS One **3:**e1800. http://dx.doi.org/10.1371/journal.pone.0001800.
17. **García-Fernández A, Villa L, Carta C, Venditti C, Giordano A, Venditti M, Mancini C, Carattoli A.** 2012. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrob. Agents Chemother. **56:**2143–2145. http://dx.doi.org/10.1128/AAC.05308-11.
18. **Chen L, Chavda KD, Melano RG, Jacobs MR, Koll B, Hong T, Rojtman AD, Levi MH, Bonomo RA, Kreiswirth BN.** 2014. Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York hospitals. Antimicrob. Agents Chemother. **58:**2871–2877. http://dx.doi.org/10.1128/AAC.00120-14.
19. **Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN.** 2014. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. Proc. Natl. Acad. Sci. U. S. A. **111:**4988–4993. http://dx.doi.org/10.1073/pnas.1321364111.
20. **Chen L, Chavda KD, Fraimow HS, Mediavilla JR, Melano RG, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2013. Complete nucleotide sequences of *bla*KPC-4- and *bla*KPC-5-harboring IncN and IncX plasmids from *Klebsiella pneumoniae* strains isolated in New Jersey. Antimicrob. Agents Chemother. **57:**269–276. http://dx.doi.org/10.1128/AAC.01648-12.
21. **Doublet B, Boyd D, Douard G, Praud K, Cloeckaert A, Mulvey MR.** 2012. Complete nucleotide sequence of the multidrug resistance IncA/C plasmid pR55 from *Klebsiella pneumoniae* isolated in 1969. J. Antimicrob. Chemother. **67:**2354–2360. http://dx.doi.org/10.1093/jac/dks251.
22. **Fevre C, Passet V, Deletoile A, Barbe V, Frangeul L, Almeida AS, Sansonetti P, Tournebize R, Brisse S.** 2011. PCR-based identification of *Klebsiella pneumoniae* subsp. *rhinoscleromatis*, the agent of rhinoscleroma. PLoS Negl. Trop. Dis. **5:**e1052. http://dx.doi.org/10.1371/journal.pntd.0001052.