AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Genome Sequences of Multidrug-Resistant, Colistin-Susceptible and -Resistant *Klebsiella pneumoniae* Clinical Isolates from Pakistan

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The emergence and spread of colistin resistance among multidrug-resistant (MDR) *Klebsiella pneumoniae* represent a critical threat to global health. Here, we report the complete genome sequences of 10 MDR, colistin-susceptible and -resistant *K. pneumoniae* clinical isolates obtained in Pakistan between 2010 and 2013.

Received 21 October 2016 **Accepted** 25 October 2016 **Published** 15 December 2016

Citation Crawford MA, Timme R, Lomonaco S, Lascols C, Fisher DJ, Sharma SK, Strain E, Allard MW, Brown EW, McFarland MA, Croley T, Hammack TS, Weigel LM, Anderson K, Hodge DR, Pillai SP, Morse SA, Khan E, Hughes MA. 2016. Genome sequences of multidrug-resistant, colistin-susceptible and -resistant *Klebsiella pneumoniae* clinical isolates from Pakistan. Genome Announc 4(6):e01419-16. doi:10.1128/genomeA.01419-16.

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Klebsiella pneumoniae is a Gram-negative bacterial pathogen that causes a range of clinical diseases including pneumonia, bacteremia, and wound and urinary tract infections. The continuing increase of antibiotic resistance in *K. pneumoniae* presents a considerable challenge to global health. In particular, carbapenemase and/or extended spectrum β-lactamase production by this important human pathogen greatly limits therapeutic options and is associated with frequent treatment failures and increased mortality (1). The serious challenge posed by multidrug-resistant (MDR) *K. pneumoniae* has been countered by the clinical use of colistin (CST), a decades-old polymyxin considered to be the last line of defense against infections caused by MDR Gram-negative bacterial pathogens. Unfortunately, reports of CST-resistant *K. pneumoniae* are becoming increasingly common, and surveillance studies have demonstrated an increase in the prevalence of CST resistance (2). Infections caused by MDR, CST-resistant *K. pneumoniae* are of great concern as there are no suitable therapeutic agents available to treat them.

Here, we report the whole-genome sequences for 10 MDR *K. pneumoniae* strains isolated from patients in Pakistan between 2010 and 2013. Among these strains, seven are CST resistant and three are CST susceptible as determined using the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and by disk diffusion. These data provide a comparative genetic context for CST resistance in *K. pneumoniae* that will inform infectious diseases epidemiology and the identification of antimicrobial resistance determinants. Importantly, such information is likely to be broadly applicable to CST resistance among *Enterobacteriaceae*.

DNA libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA), and whole-genome sequencing was performed on an Illumina MiSeq system using MiSeq reagent kit v2 (2 × 250 bp paired-end reads). *De novo* genome assemblies were created using SPAdes Genome Assembler and evaluated in comparison to the genome of *K. pneumoniae* HS11286 (3) using the quality assessment tool QUAST (4). Assembled genomes were annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Multi-locus sequence typing (MLST) was performed according to the scheme described for the Institut Pasteur *K. pneumoniae* database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The presence of NDM-1 and/or OXA-48 β-lactamases was determined by PCR and confirmed using whole-genome sequencing data. The presence of NDM-1 and/or OXA-48, CST resistance, and sequence type (ST) are indicated for each strain: BA2880 (OXA-48, CST^R, ST101), BA3783 (NDM-1 and OXA-48, CST^R, ST14), BL13802 (NDM-1 and OXA-48, CST^S, ST11), BA2664 (OXA-48, CST^R, ST11), BL8800 (OXA-48, CST^R, ST101), BL12456 (NDM-1, CST^S, ST14), BL849 (NDM-1, CST^R, ST11), BU19801 (NDM-1, CST^R, ST307), MS84 (NDM-1, CST^R, ST15), BL12125 (NDM-1, CST^S, ST14).

Accession number(s). The genome assemblies described in this manuscript are available in DDBJ/ENBL/GenBank under the accession numbers MAGC00000000 (BA2880), MAGE00000000 (BA3783), MAGF00000000 (BL13802), MAGG00000000 (BA2664), MAGH00000000 (BL8800), MAGI00000000 (BL12456), MAGJ00000000 (BL849), MAGK00000000 (BU19801), MAGL00000000 (MS84), and MAGM00000000 (BL12125).

ACKNOWLEDGMENTS

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Food & Drug Administration, Centers for Disease Control & 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.

FUNDING INFORMATION

This work was supported by the U.S. Department of Homeland Security, Science and Technology Directorate (Interagency Agreement no. HSHQPM-16-X-00066).

REFERENCES

1. **Ben-David D, Kordevani R, Keller N, Tal I, Marzel A, Gal-Mor O, Maor Y, Rahav G.** 2012. Outcome of carbapenem resistant *Klebsiella pneumoniae* bloodstream infections. Clin Microbiol Infect **18:**54–60. http://dx.doi.org/10.1111/j.1469-0691.2011.03478.x.
2. **Ah YM, Kim AJ, Lee JY.** 2014. Colistin resistance in *Klebsiella pneumoniae*. Int J Antimicrob Agents **44:**8–15. http://dx.doi.org/10.1016/j.ijantimicag.2014.02.016.
3. **Liu P, Li P, Jiang X, Bi D, Xie Y, Tai C, Deng Z, Rajakumar K, Ou HY.** 2012. Complete genome sequence of *Klebsiella pneumoniae* subsp. *Pneumoniae* HS11286, a multidrug-resistant strain isolated from human sputum. J Bacteriol **194:**1841–1842. http://dx.doi.org/10.1128/JB.00043-12.
4. **Gurevich A, Saveliev V, Vyahhi N, Tesler G.** 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics **29:**1072–1075. http://dx.doi.org/10.1093/bioinformatics/btt086.