

# UC Berkeley

## UC Berkeley Previously Published Works

### Title

Draft Genome Sequence of a Community-Associated Methicillin-Resistant Panton-Valentine Leukocidin-Positive Staphylococcus aureus Sequence Type 30 Isolate from a Pediatric Patient with a Lung Infection in Brazil

### Permalink

<https://escholarship.org/uc/item/012375zh>

### Journal

Microbiology Resource Announcements, 3(4)

### ISSN

2576-098X

### Authors

Stephens, Craig  
Cho, Paul Jang-Yeon  
de Araujo, Veronica Afonso  
et al.

### Publication Date

2015-08-27

### DOI

10.1128/genomea.00907-15

Peer reviewed

# Draft Genome Sequence of a Community-Associated Methicillin-Resistant Panton-Valentine Leukocidin-Positive *Staphylococcus aureus* Sequence Type 30 Isolate from a Pediatric Patient with a Lung Infection in Brazil

Craig Stephens,<sup>a,b</sup> Paul Jang-Yeon Cho,<sup>b</sup> Veronica Afonso de Araujo,<sup>c</sup> Ivete Martins Gomes,<sup>d</sup> Selma Maria de Azevedo Sias,<sup>d</sup> Claudete A. Araújo Cardoso,<sup>c,d</sup> Lee W. Riley,<sup>b</sup> Fábio Aguiar-Alves<sup>e,f</sup>

Biology Department and Public Health Program, Santa Clara University, Santa Clara, California, USA<sup>a</sup>; School of Public Health, University of California, Berkeley, Berkeley, California, USA<sup>b</sup>; Faculdade de Medicina, Programa de Pós Graduação em Ciências Médicas, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil<sup>c</sup>; Departamento Materno-Infantil, Faculdade de Medicina, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil<sup>d</sup>; Faculdade de Medicina, Programa de Pós Graduação em Patologia, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil<sup>e</sup>; Laboratório de Epidemiologia Molecular e Biotecnologia (LEMB), Laboratório Universitário Rodolfo Albino, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil<sup>f</sup>

**The sequence of methicillin-resistant *Staphylococcus aureus* strain B6 (sequence type 30 [ST30], *spa* type t433, staphylococcal chromosomal cassette *mec* element [SCC*mec*] type IVc, Panton-Valentine leukocidin [PVL] positive), isolated from a pediatric patient with a lung infection in Niterói, Rio de Janeiro, Brazil, is described here. The draft genome sequence includes a 2.8-Mb chromosome, accompanied by a 20-kb plasmid containing *blaZ* and two small cryptic plasmids.**

Received 9 July 2015 Accepted 14 July 2015 Published 20 August 2015

**Citation** Stephens C, Cho PJ-Y, Afonso de Araujo V, Gomes IM, de Azevedo Sias SM, Araújo Cardoso CA, Riley LW, Aguiar-Alves F. 2015. Draft genome sequence of a community-associated methicillin-resistant Panton-Valentine leukocidin-positive *Staphylococcus aureus* sequence type 30 isolate from a pediatric patient with a lung infection in Brazil. *Genome Announc* 3(4):e00907-15. doi:10.1128/genomeA.00907-15.

**Copyright** © 2015 Stephens et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Craig Stephens, [cstephens@scu.edu](mailto:cstephens@scu.edu).

The emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has contributed significantly to an increase in the global staphylococcal disease burden (1, 2). We present here the genome sequence of an unusual CA-MRSA isolate from Niterói, Rio de Janeiro, Brazil. The source of the isolate was a 17-month-old boy diagnosed with pneumonia and pleural effusion of one lung. The *S. aureus* strain (designated B6) isolated from drainage fluid was resistant to methicillin and cefepime. The strain was characterized by multilocus sequence typing as sequence type 30 (ST30), by *spa* typing as t433, and as staphylococcal chromosomal cassette *mec* element (SCC*mec*) type IVc by PCR. The boy's HIV-positive aunt, living in the same household, was asymptotically colonized by a strain typed identically to B6 that may have been the source of the boy's infection. ST30 strains are a major source of high-risk staphylococcal infection globally (3) but are rare in Brazil, where the ST239 Brazilian epidemic clone (SCC*mec* type III) is epidemiologically dominant (4). ST30 strains carrying Panton-Valentine leukocidin (PVL), as B6 does, are often associated with necrotizing pneumonia, which causes high morbidity and mortality rates in children (5, 6). Strain B6 is also unusual as a *spa* type t433 strain; most Brazilian ST30 isolates are t318, suggesting that this strain had a distinct origin. The genome sequence was therefore determined for use as a reference in the future.

DNA preparation for whole-genome sequencing was done with the Qiagen DNeasy kit (Qiagen, USA). Preparation of Illumina-compatible libraries (with index tags) for 300-bp paired-end reads were conducted according to a standard protocol (Wa-

fergen Biosystems). Libraries were sequenced on a MiSeq instrument using V3 chemistry. Read trimming and contig assembly and analysis were performed using Geneious software (Biomatters Ltd.). Annotation of the draft genome employed RAST (7). Plasmids, resistance genes, and virulence factors were initially identified using Web-based tools from the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org>), and prophages were identified using PHAST (8).

The *S. aureus* B6 draft genome sequence was assembled into 71 contigs >1 kb in length, with a mean contig length of 39,889 bp, maximum contig length of 222,661 bp, and an  $N_{50}$  of 72,253 bp. The mean read coverage of the assembled contigs was approximately 25-fold. The contigs comprise a 2,808,089-bp chromosome, a 20,335-bp circular plasmid, and two small circular plasmids of 1,990 and 1,503 bp. The SCC*mec* type IVc element containing *mecA* was 21,398 bp in length. The genome contained two prophages associated with virulence factors  $\phi$ PVL (37 kb), containing the PVL subunit genes *lukF* and *lukS*, and a prophage highly similar to  $\phi$ NM-3 (44 kb), which contains the enterotoxin A (*sea*) and staphylokinase genes, as well as other known virulence factors (9). The 20-kb plasmid, which closely resembled pMW2 from CA-MRSA strain MW2 (10), contained *blaZ* ( $\beta$ -lactamase) and genes for heavy metal resistance. The B6 genome encodes numerous additional virulence factors potentially relevant to pulmonary infection, including the *ica* operon (for the synthesis of extracellular poly-*N*-acetyl-glucosamine for biofilm formation); adhesins binding elastin (*ebpS*), fibronectin (*fnbA*), and collagen

(*cna*); and multiple staphylococcal enterotoxins, exotoxins, and superantigens (11).

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [LDIT00000000](https://www.ncbi.nlm.nih.gov/nuclink/LDIT00000000). The version described in this paper is version LDIT01000000.

## ACKNOWLEDGMENTS

We thank the team of professionals from the pediatric unit of Antonio Pedro University Hospital at Fluminense Federal University for assistance with this study.

This research was supported by The Fulbright International Visiting Scholar Program, Fluminense Federal University, and FAPERJ (Rio de Janeiro, Brazil) grants FOPESQ-UFF and APQ1, Fogarty International Center/NIH Global Infectious Disease Research grant TW006563, and a training grant from the Fogarty International Center (TW009338). The Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley has been supported by NIH S10 Instrumentation grants S10RR029668 and S10RR027303.

## REFERENCES

- David MZ, Glikman D, Crawford SE, Peng J, King KJ, Hostetler MA, Boyle-Vavra S, Daum RS. 2008. What is community-associated methicillin-resistant *Staphylococcus aureus*? *J Infect Dis* 197:1235–1243. <http://dx.doi.org/10.1086/533502>.
- Guzmán-Blanco M, Mejía C, Isturiz R, Alvarez C, Bavestrello L, Gotuzzo E, Labarca J, Luna CM, Rodríguez-Noriega E, Salles MJ, Zurita J, Seas C. 2009. Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Latin America. *Int J Antimicrob Agents* 34:304–308. <http://dx.doi.org/10.1016/j.ijantimicag.2009.06.005>.
- Isobe H, Takano T, Nishiyama A, Hung WC, Kuniyuki S, Shibuya Y, Reva I, Yabe S, Iwao Y, Higuchi W, Khokhlova OE, Okubo T, Yamamoto T. 2012. Evolution and virulence of Pantón-Valentine leukocidin-positive ST30 methicillin-resistant *Staphylococcus aureus* in the past 30 years in Japan. *Biomed Res* 33:97–109. <http://dx.doi.org/10.2220/biomedres.33.97>.
- Rodríguez-Noriega E, Seas C, Guzmán-Blanco M, Mejía C, Alvarez C, Bavestrello L, Zurita J, Labarca J, Luna CM, Salles MJ, Gotuzzo E. 2010. Evolution of methicillin-resistant *Staphylococcus aureus* clones in Latin America. *Int J Infect Dis* 14:e560–e566. <http://dx.doi.org/10.1016/j.ijid.2009.08.018>.
- Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piémont Y, Brousse N, Floret D, Etienne J. 2002. Association between *Staphylococcus aureus* strains carrying gene for Pantón-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 359:753–759. [http://dx.doi.org/10.1016/S0140-6736\(02\)07877-7](http://dx.doi.org/10.1016/S0140-6736(02)07877-7).
- David MZ, Daum RS. 2010. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 23:616–687. <http://dx.doi.org/10.1128/CMR.00081-09>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsa K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
- Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res* 39(Suppl 2):W347–W352. <http://dx.doi.org/10.1093/nar/gkr485>.
- Bae T, Baba T, Hiramatsu K, Schneewind O. 2006. Prophages of *Staphylococcus aureus* Newman and their contribution to virulence. *Mol Microbiol* 62:1035–1047. <http://dx.doi.org/10.1111/j.1365-2958.2006.05441.x>.
- Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, Nagai Y, Iwama N, Asano K, Naimi T, Kuroda H, Cui L, Yamamoto K, Hiramatsu K. 2002. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* 359:1819–1827. [http://dx.doi.org/10.1016/S0140-6736\(02\)08713-5](http://dx.doi.org/10.1016/S0140-6736(02)08713-5).
- Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K. 2001. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 357:1225–1240. [http://dx.doi.org/10.1016/S0140-6736\(00\)04403-2](http://dx.doi.org/10.1016/S0140-6736(00)04403-2).