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Complete Genome Sequences of Bioluminescent *Staphylococcus aureus* Strains Xen31 and Xen36, Derived from Two Clinical Isolates

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ABSTRACT Here, we report complete genome sequences of two clinical isolates of *Staphylococcus aureus*, namely, Xen31 and Xen36, which have been genetically modified to express an optimized *Photobacterium luminescens* luciferase operon. Xen31 and Xen36 are bioluminescent strains used widely for investigation of bacterial pathogenesis, drug discovery, and development of novel therapies.

Staphylococcus aureus is a saprophytic bacterium that can infect multiple animal hosts, including humans (1–11). Methicillin-resistant *S. aureus* (MRSA) infections led to more than 100,000 deaths in 2019 worldwide (12). Thus, the development of innovative therapeutics is urgently needed. Bioluminescent microorganisms are powerful tools to use to investigate pathogenesis, screen antimicrobial drugs, and assess therapies (13–31). Here, we report genome sequences of bioluminescent *S. aureus* strains Xen31 and Xen36 (Perkin Elmer 119242 and 119243), derived from clinical isolates (16, 32). A modified *Photobacterium luminescens luxABCDE* operon is integrated in the Xen31 chromosome and Xen36 plasmid. Genomic characterization of these strains is of interest as they are commonly used in infection models and preclinical studies (22, 27, 28, 31, 33–42).

Genomic DNA was isolated from bacteria grown in brain heart infusion broth (BD) using a PureLink DNeasy kit (Invitrogen), sheared in Covaris g-tubes at 5,500 rpm for 60 s twice, and size selected using Pacific Biosciences (PacBio) magnetic beads diluted 3.5-fold. Sequencing was performed using a PacBio Sequel I instrument. Libraries were prepared according to the PacBio protocol for multiplex SMRT sequencing with v3 chemistry using a SMRTbell express template prep kit 2.0 and barcoded adapters kit 8A/8B. Circular consensus sequences (CCSs) were constructed using SMRT Link software v6.2.0 (PacBio) with default settings except that minimum accuracy was set to 0.7. Bioconvert v0.6.2 (<https://github.com/bioconvert/bioconvert>) converted CCS files to FastQ files resulting in 40,653 and 29,054 CCS reads for Xen31 and Xen36 genomes, respectively. N_{50} values were 9,122 and 9,890 bp, respectively. CCS data had high quality with a Phred score above 40 for 95% of reads. Taxonomic analysis using the Sequanamultitax pipeline (43) showed 99.9% *Staphylococcus aureus* reads. Assemblies were performed using the LORA pipeline from Sequana (43). Default parameters were used for all software. Using the Canu v1.6 assembler (44), four contigs were obtained for Xen31. Circularization was performed with Circlator v1.5.5 (45). The main circularized contig was 2,966,802 bp long with a G+C content of 32.95% (Table 1) and exhibited uniform coverage of 98 \times . The second circularized 32,188-bp contig had a uniform 105 \times coverage and was identified as plasmid CP041009.1 by BLAST. The 43,128-bp-long third contig had a uniform coverage

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TABLE 1 General features of the *Staphylococcus aureus* strains Xen31 and Xen36^a

Feature	Data by strain	
	Xen31	Xen36
Strain origin	Clinical isolate	Clinical isolate
Resistance type	MRSA	MSSA
Sequence type	ST247	ST8037
Genome size (bp)	2,966,802	2,749,617
G+C content (%)	32.95	32.91
No. of protein-coding genes	2,742	2,528
No. of rRNAs	19	19
No. of tRNAs	62	60
No. of tmRNAs	1	1
No. of CRISPR regions	5	10
Prophage (accession no.)	PT1028 (AY954948.1), ϕ NM3 (NC_008617), ϕ JB (NC_028669), AJ_2017 (NC_048644)	PT1028 (AY954948.1)
Plasmid accession no.	CP041009.1	CP082814 and CP029650.1
Antibiotic resistance genes	<i>aac(6')-aph(2'')</i> , <i>aadD</i> , <i>ant(9)-Ia</i> , <i>bla</i> TEM-1A, <i>bleO</i> , <i>cat</i> (pC194), <i>erm</i> (A), <i>erm</i> (B), <i>fosB</i> , <i>mecA</i> , <i>tet</i> (M)	<i>aph(3')-III</i> and <i>blaZ</i>
Virulence determinant genes ^b	<i>adsA</i> , <i>agr</i> , <i>ahpCF</i> , <i>arlRS</i> , <i>aur</i> , <i>cap</i> , <i>chp</i> , <i>clf</i> , <i>coa</i> , <i>codY</i> , <i>crt</i> , <i>dlt</i> , <i>eap</i> , <i>ebp</i> , <i>efb</i> , <i>esa</i> , <i>ess</i> , <i>esx</i> , <i>flr</i> , <i>fnb</i> , <i>fur</i> , <i>geh</i> , <i>graRSX</i> , <i>hla</i> , <i>hld</i> , <i>hlgABC</i> , <i>hysA</i> , <i>ica</i> , <i>isd</i> , <i>katA</i> , <i>lukED*</i> , <i>lukGH</i> , <i>lytRS</i> , <i>mgrA</i> , <i>mprF</i> , <i>oatA</i> , <i>psm</i> , <i>RNAIII</i> , <i>rot</i> , <i>rsr</i> , <i>saeRS</i> , <i>sak</i> , <i>sar</i> , <i>sarT*</i> , <i>sarU*</i> , <i>saxG</i> , <i>sbi</i> , <i>scn</i> , <i>sdr</i> , <i>sea</i> , <i>see</i> , <i>selP*</i> , <i>set1*</i> , <i>set3*</i> , <i>set5*</i> , <i>set6*</i> , <i>set9*</i> , <i>set12*</i> , <i>set21*</i> , <i>set30–34*</i> , <i>set36–40*</i> , <i>set2</i> , <i>set4</i> , <i>set7</i> , <i>set8</i> , <i>sigB</i> , <i>sodA</i> , <i>sodM</i> , <i>spa</i> , <i>spI*</i> , <i>spn</i> , <i>srrAB</i> , <i>ssl</i> , <i>ssp</i>	<i>adsA</i> , <i>agr</i> , <i>ahpCF</i> , <i>arlRS</i> , <i>aur</i> , <i>cap</i> , <i>chp</i> , <i>clf</i> , <i>cna*</i> , <i>coa</i> , <i>codY</i> , <i>crt</i> , <i>dlt</i> , <i>eap</i> , <i>ebp</i> , <i>efb</i> , <i>esa</i> , <i>ess</i> , <i>esx</i> , <i>flr</i> , <i>fnb</i> , <i>fur</i> , <i>geh</i> , <i>graRSX</i> , <i>hla</i> , <i>hld</i> , <i>hlgABC</i> , <i>hysA</i> , <i>ica</i> , <i>isd</i> , <i>katA</i> , <i>lukGH</i> , <i>lytRS</i> , <i>mgrA</i> , <i>mprF</i> , <i>oatA</i> , <i>psm</i> , <i>RNAIII</i> , <i>rot</i> , <i>rsr</i> , <i>saeRS</i> , <i>sak</i> , <i>sar</i> , <i>saxG</i> , <i>sbi</i> , <i>scn</i> , <i>sdr</i> , <i>sea</i> , <i>seb*</i> , <i>sec*</i> , <i>see</i> , <i>seg*</i> , <i>sei*</i> , <i>selm*</i> , <i>seln*</i> , <i>selo*</i> , <i>selu*</i> , <i>set2</i> , <i>set4</i> , <i>set7</i> , <i>set8</i> , <i>set11*</i> , <i>set13*</i> , <i>set15*</i> , <i>set16*</i> , <i>set22*</i> , <i>sigB</i> , <i>sodA</i> , <i>sodM</i> , <i>spa</i> , <i>spn</i> , <i>srrAB</i> , <i>ssl</i> , <i>ssp</i> , <i>yent1,2*</i>

^a tmRNAs, transfer-messenger RNAs; MSSA, methicillin-susceptible *Staphylococcus aureus*.

^b *, Strain-specific genes.

of 500 \times . It was identified as phage Ecel-2020n (CP062457.1) by BLAST and phage AJ_2017 (NC_048644) by the PHASTER Web server (46). The fourth contig of poor coverage was discarded. Analysis of the Xen36 sequence with Canu v1.6 and Flye v2.9 (47) resulted in a main contig of 2,749,617 bp with 80 \times coverage, 32.91% G+C content (Table 1), and 4 small contigs, identifying 28,816-bp plasmid CP082814 and 13,162-bp plasmid CP029650.1. Phages of the Xen31 and Xen36 main contigs were detected by PHASTER (Table 1).

A total of 2,742 and 2,528 protein-coding sequences were identified in Xen31 and Xen36, respectively (Table 1). Sequence types were defined using PubMLST. Putative clustered regularly interspaced short palindromic repeat sequences were identified by CRISPRCasFinder (48). Annotation was performed with Prokka v1.14.5 (49). Antimicrobial resistance genes were detected using ResFinder-4.1 (50) and GenBank. Virulence genes were identified using Virulence Factor of Pathogenic Bacteria Database (VFDB) and GenBank.

Data availability. Sequences were deposited in ENA under the accession number E-MTAB-12210. Raw data, assemblies, and annotations can be accessed online at <https://doi.org/10.5281/zenodo.7517362>.

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REFERENCES

- Davis MF, Iverson SA, Baron P, Vasse A, Silbergeld EK, Lautenbach E, Morris DO. 2012. Household transmission of methicillin-resistant *Staphylococcus aureus* and other staphylococci. *Lancet Infect Dis* 12:703–716. [https://doi.org/10.1016/S1473-3099\(12\)70156-1](https://doi.org/10.1016/S1473-3099(12)70156-1).
- Kozajda A, Ježak K, Kapsa A. 2019. Airborne *Staphylococcus aureus* in different environments—a review. *Environ Sci Pollut Res Int* 26:34741–34753. <https://doi.org/10.1007/s11356-019-06557-1>.
- Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 5:751–762. [https://doi.org/10.1016/S1473-3099\(05\)70295-4](https://doi.org/10.1016/S1473-3099(05)70295-4).
- Krismer B, Weidenmaier C, Zipperer A, Peschel A. 2017. The commensal life-style of *Staphylococcus aureus* and its interactions with the nasal microbiota. *Nat Rev Microbiol* 15:675–687. <https://doi.org/10.1038/nrmicro.2017.104>.
- Abdullahi IN, Fernández-Fernández R, Juárez-Fernández G, Martínez-Álvarez S, Eguizábal P, Zarazaga M, Lozano C, Torres C. 2021. Wild animals are reservoirs and sentinels of *Staphylococcus aureus* and MRSA clones: a problem with “One Health” concern. *Antibiotics* 10:1556. <https://doi.org/10.3390/antibiotics10121556>.
- Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 28:603–661. <https://doi.org/10.1128/CMR.00134-14>.
- Ahmad-Mansour N, Loubet P, Pouget C, Dunyach-Remy C, Sotto A, Lavigne J-P, Molle V. 2021. *Staphylococcus aureus* toxins: an update on their pathogenic properties and potential treatments. *Toxins* 13:677. <https://doi.org/10.3390/toxins13100677>.
- Thomer L, Schneewind O, Missiakas D. 2016. Pathogenesis of *Staphylococcus aureus* bloodstream infections. *Annu Rev Pathol Mech Dis* 11:343–364. <https://doi.org/10.1146/annurev-pathol-012615-044351>.
- Cheung GYC, Bae JS, Otto M. 2021. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence* 12:547–569. <https://doi.org/10.1080/21505594.2021.1878688>.
- Foster TJ. 2005. Immune evasion by staphylococci. *Nat Rev Microbiol* 3:948–958. <https://doi.org/10.1038/nrmicro1289>.
- Foster TJ, Geoghegan JA, Ganesh VK, Höök M. 2014. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat Rev Microbiol* 12:49–62. <https://doi.org/10.1038/nrmicro3161>.
- Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC, Browne AJ, Chipeta MG, Fell F, Hackett S, Haines-Woodhouse G, Kashaf Hamadani BH, Kumaran EAP, McManigal B, Agarwal R, Akech S, Albertson S, Amuasi J, Andrews J, Aravkin A, Ashley E, Bailey F, Baker S, Basnyat B, Bekker A, Bender R, Bethou A, Bielicki J, Boonkasidecha S, Bukosia J, Carvalho C, Castañeda-Orjuela C, Chansamouth V, Chaurasia S, Chiurchiù S, Chowdhury F, Cook AJ, Cooper B, Cressley TR, Criollo-Mora E, Cunningham M, Darboe S, Day NPJ, De Luca M, Dokova K, et al. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399:629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0).
- Avci P, Karimi M, Sadasivam M, Antunes-Melo WC, Carrasco E, Hamblin MR. 2018. *In-vivo* monitoring of infectious diseases in living animals using bioluminescence imaging. *Virulence* 9:28–63. <https://doi.org/10.1080/21505594.2017.1371897>.
- Syed AJ, Anderson JC. 2021. Applications of bioluminescence in biotechnology and beyond. *Chem Soc Rev* 50:5668–5705. <https://doi.org/10.1039/d0cs01492c>.
- Guo Y, Ramos RI, Cho JS, Donegan NP, Cheung AL, Miller LS. 2013. *In vivo* bioluminescence imaging to evaluate systemic and topical antibiotics against community-acquired methicillin-resistant *Staphylococcus aureus*-infected skin wounds in mice. *Antimicrob Agents Chemother* 57:855–863. <https://doi.org/10.1128/AAC.01003-12>.
- Francis KP, Joh D, Bellinger-Kawahara C, Hawkinson MJ, Purchio TF, Contag PR. 2000. Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel *luxABCDE* construct. *Infect Immun* 68:3594–3600. <https://doi.org/10.1128/IAI.68.6.3594-3600.2000>.
- Tenhami M, Hakkila K, Karp M. 2001. Measurement of effects of antibiotics in bioluminescent *Staphylococcus aureus* RN4220. *Antimicrob Agents Chemother* 45:3456–3461. <https://doi.org/10.1128/AAC.45.12.3456-3461.2001>.
- Xiong YQ, Willard J, Kadurugamuwa JL, Yu J, Francis KP, Bayer AS. 2005. Real-time *in vivo* bioluminescent imaging for evaluating the efficacy of antibiotics in a rat *Staphylococcus aureus* endocarditis model. *Antimicrob Agents Chemother* 49:380–387. <https://doi.org/10.1128/AAC.49.1.380-387.2005>.
- Lambrechts SAG, Demidova TN, Aalders MCG, Hasan T, Hamblin MR. 2005. Photodynamic therapy for *Staphylococcus aureus* infected burn wounds in mice. *Photochem Photobiol Sci* 4:503–509. <https://doi.org/10.1039/b502125a>.
- Martin LI, Li T, Van Praagh ADG, Zhang S, Zhang X-X, Alder JD. 2007. Rapid bactericidal activity of daptomycin against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* peritonitis in mice as measured with bioluminescent bacteria. *Antimicrob Agents Chemother* 51:1787–1794. <https://doi.org/10.1128/AAC.00738-06>.
- Zhang H, Kalker G, Mani N, Grossman TH. 2008. Development and validation of a multi-dose neutropenic rat thigh infection model using real-time monitoring of *Staphylococcus aureus* growth *in vivo*. *In Vivo* 22:667–672.
- Munro P, Benchetrit M, Nahori M-A, Stefani C, Clément R, Michiels J-F, Landraud L, Dussurget O, Lemichez E. 2010. The *Staphylococcus aureus* epidermal cell differentiation inhibitor toxin promotes formation of infection foci in a mouse model of bacteremia. *Infect Immun* 78:3404–3411. <https://doi.org/10.1128/IAI.00319-10>.
- Lorenz U, Schäfer T, Ohlsen K, Tiurbe GC, Bühler C, Germer C-T, Kellersmann R. 2011. *In vivo* detection of *Staphylococcus aureus* in biofilm on vascular prostheses using non-invasive biophotonic imaging. *Eur J Vasc Endovasc Surg* 41:68–75. <https://doi.org/10.1016/j.ejvs.2010.09.007>.
- Cho JS, Zussman J, Donegan NP, Ramos RI, Garcia NC, Uslan DZ, Iwakura Y, Simon SI, Cheung AL, Modlin RL, Kim J, Miller LS. 2011. Noninvasive *in vivo* imaging to evaluate immune responses and antimicrobial therapy against *Staphylococcus aureus* and USA300 MRSA skin infections. *J Invest Dermatol* 131:907–915. <https://doi.org/10.1038/jid.2010.417>.
- Plaut RD, Mocca CP, Prabhakara R, Merkel TJ, Stibitz S. 2013. Stably luminescent *Staphylococcus aureus* clinical strains for use in bioluminescent imaging. *PLoS One* 8:e59232. <https://doi.org/10.1371/journal.pone.0059232>.
- van Staden ADP, Heunis T, Smith C, Deane S, Dicks LMT. 2016. Efficacy of antibiotic treatment of *Staphylococcus aureus*-induced skin infections, monitored by *in vivo* bioluminescent imaging. *Antimicrob Agents Chemother* 60:3948–3955. <https://doi.org/10.1128/AAC.02938-15>.
- Bostian PA, Karnes JM, Cui S, Robinson LJ, Daffner SD, Witt MR, Emery SE. 2017. Novel rat tail discitis model using bioluminescent *Staphylococcus aureus*: rat tail discitis model. *J Orthop Res* 35:2075–2081. <https://doi.org/10.1002/jor.23497>.
- Rozenbaum RT, Su L, Umerska A, Eveillard M, Håkansson J, Mahlapuu M, Huang F, Liu J, Zhang Z, Shi L, van der Mei HC, Busscher HJ, Sharma PK. 2019. Antimicrobial synergy of monolaurin lipid nanocapsules with adsorbed antimicrobial peptides against *Staphylococcus aureus* biofilms *in vitro* is absent *in vivo*. *J Control Release* 293:73–83. <https://doi.org/10.1016/j.jconrel.2018.11.018>.
- Miller RJ, Crosby HA, Schilcher K, Wang Y, Ortines RV, Mazhar M, Dikeman DA, Pinsky BL, Brown ID, Joyce DP, Zhang J, Archer NK, Liu H, Alphonse MP, Czupryna J, Anderson WR, Bernthal NM, Fortunato-Miranda L, Bulte JWM, Francis KP, Horswill AR, Miller LS. 2019. Development of a *Staphylococcus aureus* reporter strain with click beetle red luciferase for enhanced *in vivo* imaging of experimental bacteremia and mixed infections. *Sci Rep* 9:16663. <https://doi.org/10.1038/s41598-019-52982-0>.
- Kobayashi H, Fujita R, Hiratsuka S, Shimizu T, Sato D, Hamano H, Iwasaki N, Takahata M. 2022. Differential effects of anti-RANKL monoclonal antibody and zoledronic acid on necrotic bone in a murine model of *Staphylococcus aureus*-induced osteomyelitis. *J Orthop Res* 40:614–623. <https://doi.org/10.1002/jor.25102>.
- Benjamin AB, Moule MG, Didwania MK, Hardy J, Saenkham-Huntsinger P, Sule P, Nielsen JE, Lin JS, Contag CH, Barron AE, Cirillo JD. 2022. Efficacy of cathelicidin-mimetic antimicrobial peptoids against *Staphylococcus aureus*. *Microbiol Spectr* 10:e00534-22. <https://doi.org/10.1128/spectrum.00534-22>.
- Schaefer S, Perry W, Jones D. 1979. Methicillin-resistant strains of *Staphylococcus aureus* phage type 92. *Antimicrob Agents Chemother* 15:74–80. <https://doi.org/10.1128/AAC.15.1.74>.
- Lebeau D, Chauhan A, Létoffé S, Fischer F, de Reuse H, Beloin C, Ghigo J-M. 2014. pH-mediated potentiation of aminoglycosides kills bacterial persisters and eradicates *in vivo* biofilms. *J Infect Dis* 210:1357–1366. <https://doi.org/10.1093/infdis/jiu286>.
- Chauhan A, Ghigo J-M, Beloin C. 2016. Study of *in vivo* catheter biofilm infections using pediatric central venous catheter implanted in rat. *Nat Protoc* 11:525–541. <https://doi.org/10.1038/nprot.2016.033>.

35. Gelin M, Paoletti J, Nahori M-A, Huteau V, Leseigneur C, Jouvion G, Dugué L, Clément D, Pons J-L, Assairi L, Pochet S, Labesse G, Dussurget O. 2020. From substrate to fragments to inhibitor active *in vivo* against *Staphylococcus aureus*. *ACS Infect Dis* 6:422–435. <https://doi.org/10.1021/acscinfecdis.9b00368>.
36. Vesperas A, Santana D, Ju M, Milbrandt NB, Tsai YH, Wickramasinghe S, Klika AK, Piuze NS, Samia ACS, Higuera-Rueda CA. 2022. Standardized quantification of biofilm in a novel rabbit model of periprosthetic joint infection. *J Bone Jt Infect* 7:91–99. <https://doi.org/10.5194/jbji-7-91-2022>.
37. Trikha R, Greig D, Sekimura T, Cevallos N, Kelley B, Mamouei Z, Hart C, Ralston M, Turkmani A, Sassoon A, Stavrakis A, Bernthal NM. 2021. Active rheumatoid arthritis in a mouse model is not an independent risk factor for periprosthetic joint infection. *PLoS One* 16:e0250910. <https://doi.org/10.1371/journal.pone.0250910>.
38. Quan K, Jiang G, Liu J, Zhang Z, Ren Y, Busscher HJ, van der Mei HC, Peterson BW. 2021. Influence of interaction between surface-modified magnetic nanoparticles with infectious biofilm components in artificial channel digging and biofilm eradication by antibiotics *in vitro* and *in vivo*. *Nanoscale* 13:4644–4653. <https://doi.org/10.1039/d0nr08537e>.
39. Trikha R, Greig D, Kelley BV, Mamouei Z, Sekimura T, Cevallos N, Olson T, Chaudry A, Magyar C, Leisman D, Stavrakis A, Yeaman MR, Bernthal NM. 2020. Inhibition of angiotensin converting enzyme impairs anti-staphylococcal immune function in a preclinical model of implant infection. *Front Immunol* 11:1919. <https://doi.org/10.3389/fimmu.2020.01919>.
40. Jørgensen NP, Meyer R, Dagnæs-Hansen F, Fuursted K, Petersen E. 2014. A modified chronic infection model for testing treatment of *Staphylococcus aureus* biofilms on implants. *PLoS One* 9:e103688. <https://doi.org/10.1371/journal.pone.0103688>.
41. Ovchinnikov KV, Kranjec C, Thorstensen T, Carlsen H, Diep DB. 2020. Successful development of bacteriocins into therapeutic formulation for treatment of MRSA skin infection in a murine model. *Antimicrob Agents Chemother* 64:e00829–20. <https://doi.org/10.1128/AAC.00829-20>.
42. Grønseth T, Ovchinnikov KV, Carlsen H, Benth JS, Diep DB, von Unge M, Silvola JT. 2022. Lugol's solution and Gentian violet eradicate methicillin-resistant *Staphylococcus aureus* biofilm in skin wound infections. *Int Wound J* 20:120–130. <https://doi.org/10.1111/iwj.13846>.
43. Cokelaer T, Desvilledhabrol D, Legendre R, Cardon M. 2017. "Sequana": a set of snakemake NGS pipelines. *J Open Source Softw* 2:352. <https://doi.org/10.21105/joss.00352>.
44. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
45. Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biol* 16:294. <https://doi.org/10.1186/s13059-015-0849-0>.
46. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 44:W16–W21. <https://doi.org/10.1093/nar/gkw387>.
47. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 37:540–546. <https://doi.org/10.1038/s41587-019-0072-8>.
48. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C. 2018. CRISPRCasFinder, an update of CRISPRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res* 46:W246–W251. <https://doi.org/10.1093/nar/gky425>.
49. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
50. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wiczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 75:3491–3500. <https://doi.org/10.1093/jac/dkaa345>.