



Sequence Variation in Multidrug-Resistant Plasmid pLUH01, Isolated from Human Nasopharyngeal Swabs

Kate V. Atkinson,^{a*} Lisa A. Bishop,^{a,b} Glenn Rhodes,^c Nicolas Salez,^d Neil R. McEwan,^{e*} Matthew J. Hegarty,^e Julie Robey,^f Nicola Harding,^f Simon Wetherell,^f Robert M. Lauder,^a Roger W. Pickup,^a Mark Wilkinson,^b  Derek Gatherer^a

^aDivision of Biomedical & Life Sciences, Faculty of Health & Medicine, Lancaster University, Lancaster, United Kingdom

^bRoyal Lancaster Infirmary, Lancaster, United Kingdom

^cCentre for Ecology & Hydrology, Lake Ecosystems Group, Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom

^dUMR_D 190, Emergence des Pathologies Virales, Aix-Marseille University, Marseille, France

^eInstitute of Biological, Environmental & Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom

^fQueen Square Medical Practice, Lancaster, United Kingdom

ABSTRACT Three variants of the multidrug-resistant plasmid pLUH01 were assembled by deep sequencing from nasopharyngeal swabs. All have a 21-bp deletion in the RS14515 hypothetical gene. Variants 1 through 3 have 2, 6, and 3 nucleotide substitutions, respectively, compared to the pLUH01 reference genome. We named the new plasmid variants pLUH01/Lancaster/2015/1 to pLUH01/Lancaster/2015/3.

Lindqvist et al. first sequenced pLUH01 (NCBI reference sequence number [NC_017346](#)) as part of a study on multidrug-resistant strains of *Staphylococcus aureus* (1). pLUH01 is 2,241 bp in length with two genes of known function, *rep*, encoding a replication protein, and *qacC*, encoding a small-molecule efflux transporter conferring resistance to quaternary ammonium compounds. A third open reading frame encodes a hypothetical protein. Plasmids pSK108 (reference sequence number [NC_013395](#)) and pKH8 (GenBank accession number [U50077](#)) (2) are 0.4% and 0.5% divergent from pLUH01, respectively. More distant relatives include pSA1308 (reference sequence number [NC_007928](#)) (2), pWBG754 (reference sequence number [NC_013350](#)), pKH4 (GenBank accession number [U81980](#)), and pNVH01 (reference sequence number [NC_004562](#)). The last two of these have *qacJ* in place of *qacC*, at 27% amino acid divergence (3).

Volunteers were recruited from a general practice surgery and a general hospital in Lancaster, United Kingdom (54.05°N, 2.80°W). Ethical approval was granted by the UK National Research Ethics Service (reference 14/LO/1634, NIHR Clinical Research Network [UKCRN] Portfolio, identification number 17799). All relevant guidelines and regulations were observed. Nasopharyngeal swabs were taken between 16 December 2014 and 25 February 2015. Nucleic acid was extracted from 51 swabs. Sequencing library preparation was performed in March 2015.

Deep sequencing was performed using an Illumina Nextera XT library and HiSeq 2500 system (SRA accession number [SRP092324](#)) (4, 5). The variant sequences were assembled from the following sequence pools: pLUH01/Lancaster/2015/1 from 2 pediatric patients with respiratory symptoms (BioSample accession number [SAMN05954284](#)) and from 10 asymptomatic adults ([SAMN05954287](#)); pLUH01/Lancaster/2015/2 from 6 chronic obstructive pulmonary disease (COPD) patients ([SAMN05954289](#)); and pLUH01/Lancaster/2015/3 from a single asymptomatic adult ([SAMN05954290](#)). Assembly was performed using Bowtie 1.1.1 (6), using reference sequence number [NC_017346](#) as template (parameters, bowtie -solexa-quals -S -p 8). Average and maximum coverage

Received 7 June 2018 **Accepted** 18 June 2018 **Published** 12 July 2018

Citation Atkinson KV, Bishop LA, Rhodes G, Salez N, McEwan NR, Hegarty MJ, Robey J, Harding N, Wetherell S, Lauder RM, Pickup RW, Wilkinson M, Gatherer D. 2018. Sequence variation in multidrug-resistant plasmid pLUH01, isolated from human nasopharyngeal swabs. *Microbiol Resour Announc* 7:e00835-18. <https://doi.org/10.1128/MRA.00835-18>.

Editor Catherine Putonti, Loyola University Chicago

Copyright © 2018 Atkinson et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Derek Gatherer, d.gatherer@lancaster.ac.uk.

* Present address: Kate V. Atkinson, Luton & Dunstable Hospital NHS Foundation Trust, Luton, United Kingdom; Neil R. McEwan, Robert Gordon University, Aberdeen, United Kingdom.

depths, in reads, for the 3 variants were 127 and 318, 7 and 24, and 24 and 66, respectively. All 3 sequence variants are 2,220 bp long, having a 21-bp deletion starting at position 1933 in comparison to reference sequence number [NC_017346](#). This removes a 21-bp/7-amino-acid tandem repeat in the hypothetical protein. Substitutions are observed at positions (numbers as in reference sequence number [NC_017346](#)) 780 (all variants), 1306 (all variants), 1615 (variant 2), 1642 (variant 2), 1663 (variants 2 and 3), and 1678 (variant 2). All of them are synonymous substitutions in the replication protein, except position 780, which is in a noncoding region.

Resistance of *Staphylococcus aureus* to the quaternary ammonium compound ethidium bromide was first detected in 1969 (7), and plasmids encoding resistance to similar compounds, present in disinfectants and antiseptics typically used in hospitals, have become important subjects of study in the field of antimicrobial resistance (reviewed in reference 8). We assembled full-length pLUH01 variants from 4 of our 9 nasopharyngeal swab sequencing pools (4) and detected fragments in the remaining 5, suggesting widespread prevalence of this plasmid in the population. Although none of our sequence variants has nonsynonymous point substitutions and the significance of the 7-residue deletion in the hypothetical protein remains a matter for speculation, the presence of such variation in samples collected from a single town within a time window of a few months highlights the evolutionary potential of pLUH01 as an antimicrobial resistance determinant.

BAM files are available from <https://doi.org/10.17635/lancaster/researchdata/220>.

Data availability. The complete sequences of pLUH01/Lancaster/2015/1 to pLUH01/Lancaster/2015/3 have been deposited in GenBank under the accession numbers [MH251945](#) to [MH251947](#).

ACKNOWLEDGMENTS

K.V.A. received a Service Increment from Teaching (SIFT) studentship from the University Hospitals of Morecambe Bay (UHMB) National Health Service (NHS) Foundation Trust, United Kingdom, and performed this work as part of the requirements for the degree of Master of Science (M.Sc.). Rosetrees Trust, United Kingdom, provided additional funding for deep sequencing (grant M395).

REFERENCES

1. Lindqvist M, Isaksson B, Grub C, Jonassen TØ, Hallgren A. 2012. Detection and characterisation of SCCmec remnants in multiresistant methicillin-susceptible *Staphylococcus aureus* causing a clonal outbreak in a Swedish county. *Eur J Clin Microbiol Infect Dis* 31:141–147. <https://doi.org/10.1007/s10096-011-1286-y>.
2. Im SH, Yoon SJ, Kim WK, Shin CK, Lee DW, Moon KH. 1996. Characterization of cryptic plasmid of multidrug-resistant *Staphylococcus aureus* SA2. *J Microbiol Biotechnol* 6:145–146.
3. Bjorland J, Steinum T, Sunde M, Waage S, Heir E. 2003. Novel plasmid-borne gene *qacJ* mediates resistance to quaternary ammonium compounds in equine *Staphylococcus aureus*, *Staphylococcus simulans*, and *Staphylococcus intermedius*. *Antimicrob Agents Chemother* 47:3046–3052. <https://doi.org/10.1128/AAC.47.10.3046-3052.2003>.
4. Atkinson KV, Bishop LA, Rhodes G, Salez N, McEwan NR, Hegarty MJ, Robey J, Harding N, Wetherell S, Lauder RM, Pickup RW, Wilkinson M, Gatherer D. 2017. Nasopharyngeal metagenomic deep sequencing data, Lancaster, UK, 2014–2015. *Sci Data* 4:170161. <https://doi.org/10.1038/sdata.2017.161>.
5. Atkinson KV, Bishop LA, Rhodes G, Salez N, McEwan NR, Hegarty MJ, Robey J, Harding N, Wetherell S, Lauder RM, Pickup RW, Wilkinson M, Gatherer D. 2017. Influenza C in Lancaster, UK, in the winter of 2014–2015. *Sci Rep* 7:46578. <https://doi.org/10.1038/srep46578>.
6. Langmead B, Trapnell C, Pop M, Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10:R25. <https://doi.org/10.1186/gb-2009-10-3-r25>.
7. Johnston LH, Dyke KG. 1969. Ethidium bromide resistance, a new marker on the staphylococcal penicillinase plasmid. *J Bacteriol* 100:1413–1414.
8. Wassenaar TM, Ussery D, Nielsen LN, Ingmer H. 2015. Review and phylogenetic analysis of *qac* genes that reduce susceptibility to quaternary ammonium compounds in *Staphylococcus* species. *Eur J Microbiol Immunol* 5:44–61. <https://doi.org/10.1556/EuJMI-D-14-00038>.