Whole-Genome Sequencing for Risk Assessment of Long-term Shiga Toxin–producing Escherichia coli

To the Editor: Long-term carriage of Shiga toxin–producing Escherichia coli (STEC) can greatly affect the social and work lives of infected patients. We describe the use of whole-genome sequencing to assess the risk from long-term STEC carriage in a patient who had been denied surgery because of the infection.

On August 18, 2013, a 64-year-old woman reporting to be a carrier of STEC since March 2013 contacted the University Medical Center Lübeck, Lübeck, Germany, seeking decolonization therapy. This therapy had been provided to long-term STEC carriers during the 2011 STEC O104:H4 outbreak (1). STEC had initially been identified in the patient during an epidemiologic investigation of long-term STEC carriage in a patient who had been denied surgery because of the infection.

Before responding to the request, we asked the patient to provide a fecal sample for STEC strain typing. A sample provided on August 22, 2013, was confirmed positive for STEC by culturing an STEC strain on MacConkey agar (bioMérieux, Marcy l’Etoile, France) that did not grow on selective agar (CHROMagar STEC, Mast Diagnostika, Reinford, Germany) optimized for the detection of classical enterohemorrhagic E. coli strains. Total DNA was extracted from the isolate, and a sequencing library was generated by using the Nextera XT Sample Preparation Kit (Illumina, San Diego, CA, USA). Sequencing was performed (MiSeq Benchtop Sequencer, Illumina) in 2 batches of paired 250-bp sequencing runs. Sequencing reads were further analyzed by using the CLC Genomics Workbench software package (CLC bio, Aarhus, Denmark). De novo assembly resulted in 120 contigs with an average length of 44,331 bp (N50 = 126,317 bp). A predefined dataset of 2,456 sequences was aligned with the generated contigs in a single step by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the alleles and subtypes of genes usually used for E. coli and STEC strain typing and for seropathotype detection.

Presence of a Shiga toxin subtype 1a with >99.9% and 100% identity to the stx1aA and stx1aB subunit genes, respectively (GenBank accession no M19473.1), was confirmed. The STEC strain carried genes with high homology to the O91 antigen–encoding operon (GenBank accession no. AY035396.1) and the H14 flagellin gene (GenBank accession no. AY249998.1). This observation was confirmed by a 100% sequence identity of a 643-bp fragment of the gnd gene of the sequenced strain with that of a gnd reference sequence of STEC O91:H14 (www.corefacility.ca/ecoli typer). These 2 sequences are different from the gnd sequence of reference strain STEC O91:H21. In vitro multilocus sequence typing (2) identified
sequence type (ST) 33. These data were used for risk assessment.

Only strains displaying serotype O91:H21 and a single O91:H10 isolate have been associated with HUS in humans (3, 4). ST33, identified in the patient in this study, has not been associated with HUS in humans despite being the most frequently identified ST of O91 STEC strains in humans (3). In addition, the identified strain carried only Shiga toxin 1a, whereas the HUS-associated strain HUSEC034 of serotype O91:H21 carried Shiga toxins 1a, 2a, and 2d (5). This data indicated the patient strain was a serotype D strain (6) with a relative low risk for HUS development in the patient.

The assumption that the patient strain had low pathogenicity was further corroborated by the analysis of additional marker genes (6–9) indicating the lack of pathogenicity islands associated with high virulence of STEC in humans. None of the 25 marker genes suggested for the LEE locus or pathogenicity islands OI-122, OI-44, OI-48, OI-50, OI-57, OI-71 or OI-122 were identified in the patient strain, whereas most of these markers could be detected in highly pathogenic STEC/enterohemorrhagic E. coli strains used to establish the method for identifying markers (online Technical Appendix, wwwnc.cdc.gov/EID/article/20/4/13-1728-Techapp1.pdf).

After completing the STEC risk assessment, we advised the patient’s general practitioner that antimicrobial drug prophylaxis could be administered for surgery with a low calculated risk for HUS development, as observed for other non-O157 strains (1, 10). In addition, we described our experience with 4 long-term carriers of STEC O91:H14 strains; the patients had been decolonized of STEC by the use of azithromycin decolonization therapy (data not shown).

The patient was added to a waiting list for surgery, and she elected to receive azithromycin as experimental decolonization therapy while awaiting surgery. Azithromycin was administered orally for 3 days (500 mg/day); fecal specimens on post-treatment days 7, 14, and 21 were negative by Shiga toxin ELISA. In addition, an sct-specific PCR using enrichment broth confirmed the sustainable eradication of the STEC infection. Our findings show that whole-genome sequencing can be used in the diagnostic process for long-term STEC carriers and might extend or replace other methods used for risk assessment (6–8, 10) and treatment decision guidance.

Johannes K.-M. Knobloch, Stefan Niemann, Thomas A. Kohl, Ulrich Lindner, Martin Nitschke, Friedhelm Sayk, and Werner Solbach

Author affiliations: University Hospital Schleswig-Holstein, Lübeck, Germany (J.K.-M. Knobloch, U. Lindner, M. Nitschke, F. Sayk, W. Solbach); Research Center Borstel, Borstel, Germany (S. Niemann, T.A. Kohl); and German Centre for Infection Research (DZIF), Partner Site Hamburg–Lübeck–Borstel, Germany (J.K.-M. Knobloch, S. Niemann, U. Lindner, W. Solbach)

DOI: http://dx.doi.org/10.3201/eid2004.131782

References
1. Nitschke M, Sayk F, Härtel C, Roseland RT, Hauswaldt S, Steinhoff J, et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin–producing enteroaggregative Escherichia coli O104:H4. JAMA. 2012;307:1046–52. http://dx.doi.org/10.1001/jama.2012.264
2. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol. 2006;60:1136–51. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x
3. Mellmann A, Fruth A, Friedrich AW, Wieler LH, Harmsen D, Werber D, et al. Phylogeny and disease association of Shiga toxin–producing Escherichia coli O91. Emerg Infect Dis. 2009;15:1474–7. http://dx.doi.org/10.3201/eid1509.090161
4. Pradel N, Boukhors K, Bertin Y, Forestier C, Martin C, Livrelli V. Heterogeneity of Shiga toxin–producing Escherichia coli strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. Appl Environ Microbiol. 2001;67:2460–8. http://dx.doi.org/10.1128/AEM.67.6.2460-2468.2001
5. Hauser E, Mehlmann A, Semmler T, Stoeber H, Wieler LH, Karch H, et al. Phylogenetic and molecular analysis of food-borne Shiga toxin–producing Escherichia coli. Appl Environ Microbiol. 2013;79:2731–40. http://dx.doi.org/10.1128/AEM.03552-12
6. Kamal MA, Mascarènas M, Shen S, Zieblik K, Johnson S, Reid-Smith R, et al. Association of genomic O island 122 of Escherichia coli EDL 933 with verocytotoxin-producing Escherichia coli serotypes that are linked to epidemic and/or serious disease. J Clin Microbiol. 2003;41:4930–40. http://dx.doi.org/10.1128/JCM.41.11.4930–4940.2003
7. Delannoy S, Beutin L, Fach P. Discrimination of enterohemorrhagic Escherichia coli (EHEC) from non-EHEC strains based on detection of various combinations of type III effector genes. J Clin Microbiol. 2013;51:3257–62. http://dx.doi.org/10.1128/JCM.01471-13
8. Delannoy S, Beutin L, Fach P. Towards a molecular definition of enterohemorrhagic Escherichia coli (EHEC): detection of genes located on O island 57 as markers to distinguish EHEC from closely related enteropathogenic E. coli strains. J Clin Microbiol. 2013;51:1083–8. http://dx.doi.org/10.1128/JCM.02864-12
9. Coombes BK, Wickham ME, Mascarènas M, Gruenheid S, Finlay BB, Kamal MA. Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin–producing Escherichia coli strains. Appl Environ Microbiol. 2008;74:2153–60. http://dx.doi.org/10.1128/AEM.02566-07
10. Jensen C, Schiellerup P, Olsen K, Schuetz F, Petersen E, Gerner-Smidt P, et al. Antimicrobial treatment of asymptomatic carriers of verocytotoxin-producing Escherichia coli: an empiric study. Scand J Infect Dis. 2005;37:61–3. http://dx.doi.org/10.1080/0036554050102427

Address for correspondence: Johannes K.-M. Knobloch, University Hospital Schleswig-Holstein, Campus Lübeck, Department of Medical Microbiology and Hygiene, Ratzeburger Allee 160, 23538 Lübeck, Germany; email: johannes.knobloch@uksh.de

Find emerging infectious disease information on Facebook

http://www.facebook.com
Whole-Genome Sequencing for Risk Assessment of Long-term Shiga Toxin–producing *Escherichia coli*

**Technical Appendix**

Technical Appendix Table. Presence of marker genes in 2 independent clinical strains and the patient strain in a whole-genome sequencing assessment of the risk of long-term Shiga toxin–producing *Escherichia coli* infection*

| Strain  | eae  | ent/egpL2 | nleB  | nleE  | nleG2–3  | nleG6 | nleG5–2 | nleB2  | nleC  | nleH1–1  | nleD  | nleE  | nleG1 | nleF  | nleH1–2 | nleH2–1 | nleG2–1 | nleG9 | nleM1  | ureD  | espV  | espK  | espN  | espM1  | espM2 | espM5 |
|---------|------|-----------|-------|-------|----------|-------|----------|-------|-------|----------|-------|-------|-------|-------|----------|----------|----------|-------|-------|-------|-------|-------|-------|-------|
| O26:H11 | +    | +         | +     | +     | +        | +     | +        | +     | +     | +        | +     | +     | +     | +     | +        | +        | +        | +     | +     | +     | +     | +     | +     | +     |
| O146:H21| –    | –         | –     | –     | –        | –     | –        | –     | –     | –        | –     | –     | –     | –     | –        | –        | –        | –     | –     | –     | –     | –     | –     | –     |
| O91:H14 | –    | –         | –     | –     | –        | –     | –        | –     | –     | –        | –     | –     | –     | –     | –        | –        | –        | –     | –     | –     | –     | –     | –     | –     |

*Marker designation and locus tags in the annotated genome of enterohemorrhagic *E. coli* O157:H7 strain EDL933 (GenBank accession no. AE005174.1) are shown. Gray shading indicates the patient strain. +, detection of the respective sequences in the contigs of de novo assembled whole-genome sequencing procedures.