

Use of Whole-Genome Sequencing at the Food Safety and Inspection Service to Detect and Investigate Foodborne Illness Outbreaks

Sheryl L. Shaw,^{1*} J. Emilio Esteban,² Bonnie W. Kissler,³ Jennifer L. Freiman¹ and Glenn E. Tillman⁴

¹Food Safety and Inspection Service, U.S. Dept. of Agriculture, 355 E. St., S.W., Patriot Plaza III, 9-195, Washington, D.C. 20024, USA

²Food Safety and Inspection Service, U.S. Dept. of Agriculture, 1400 Independence Ave., S.W., Room 2129, South Bldg., Washington, D.C. 20250, USA

³Food Safety and Inspection Service, U.S. Dept. of Agriculture, 100 Alabama St., 1924 Bldg., Suite 3R95, Atlanta, GA 30303, USA

⁴Food Safety and Inspection Service, U.S. Dept. of Agriculture, Eastern Laboratory, 950 College Station Road, Athens, GA 30605, USA

SUMMARY

Whole-genome sequencing (WGS) is a powerful tool used for surveillance and monitoring for foodborne illnesses. WGS can be used to generate hypotheses about food products that might be making people sick and to determine and prioritize actions taken by the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) during outbreaks. The use of WGS, in conjunction with epidemiologic evidence and food purchase histories, improves the likelihood of identifying associations between a food source and illness. Foodborne illness outbreaks due to *Listeria monocytogenes* and *Salmonella* are examples of the USDA-FSIS's use of WGS in solving foodborne illness outbreaks. Based on evidence from WGS, epidemiologic investigation, and food purchase histories, the USDA-FSIS was able to identify and facilitate removal of adulterated product from commerce and prevent further illnesses.

OVERVIEW

The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) collects and analyzes more than 100,000 food and environmental samples each year as part of pathogen reduction performance standards (e.g., *Salmonella* performance standards), the National Antimicrobial Resistance Monitoring System surveillance, and other pathogen detection programs. The USDA-FSIS performs molecular characterization for all isolates (>12,000/year) derived from those samples. In April 2019, USDA-FSIS laboratories discontinued the use of Pulsed-field Gel Electrophoresis (PFGE) and fully transitioned to whole genome sequencing (WGS). WGS is a laboratory tool used to identify unique characteristics of isolates and compare genetic similarities between isolates with greater precision than PFGE. WGS provides a detailed “genetic fingerprint” for bacteria isolated from food, the environment, and humans. The USDA-FSIS uses WGS information in several

ways to protect public health, one of which is to detect and investigate foodborne illness outbreaks. State and federal public health partners use WGS to generate hypotheses about a food product or other potential source that might be making people sick and to determine and prioritize actions during an outbreak. WGS information is also used to conduct surveillance, to identify emerging or novel strains, to follow strains that may reoccur as outbreaks year after year, and to track strains that seem to be persistent at a low level and are causing human illness. Central coordination of WGS activities is managed by Genomics for Food and Feed Safety (Gen-FS), a collaboration between federal and state regulatory and public health partners. Genomics for Food and Feed Safety coordinates analytical tools, laboratory approaches, data sharing, harmonized proficiency testing and training, and extramural communications (1).

DISCUSSION

The USDA-FSIS uses WGS to support foodborne illness surveillance in several ways. The USDA-FSIS participates in the Centers for Disease Control and Prevention (CDC) PulseNet, a nonpublic network of 83 local, state, and federal laboratories that follow strict microbiological quality control and data communication procedures. Data contributed to the CDC PulseNet are protected and available to network local, state, and federal public health partners that agree to secure certain metadata that are considered as personally identifiable information. State and local health officials share epidemiologic information related to foodborne illness and collaborate on WGS data sharing with the CDC and other federal partners. The USDA-FSIS also participates in foodborne illness surveillance by uploading WGS and limited metadata to the public National Institutes of Health (NIH), National Center for Biotechnology Information (NCBI) Pathogen Detection Browser (<https://www.ncbi.nlm.nih.gov/pathogen>). To enhance transparency and

collaboration, the USDA-FSIS uploads WGS data and limited metadata such as sample type (food, environmental), geographic location (state), and year to the NCBI. The NCBI Pathogen Detection Browser is the public-facing platform that the USDA-FSIS and other public health partners use to share isolate genetic information. The USDA-FSIS uploads sequences in real-time and is a significant contributor of food and food processing environment isolate sequences; however, other domestic and international partners contribute sequences as well, making this a highly diverse, robust database. As of 5 February 2020, there were ~255,000 *Salmonella* sequences, ~99,000 *Escherichia coli* sequences, ~48,500 *Campylobacter jejuni* sequences, and ~32,000 *Listeria monocytogenes* sequences in the NCBI Pathogen Detection Browser. With the transition to WGS, the USDA-FSIS continues to explore options for sharing WGS identifiers with stakeholders so they can use the NCBI Pathogen Detection Browser as well. The USDA-FSIS currently shares information with public health and regulatory partners to investigate outbreaks in accordance with an established information sharing processes. The USDA-FSIS also shares information with establishments from which isolates have been detected that match clinical isolates in an investigated outbreak.

Surveillance and investigations

The USDA-FSIS Office of Public Health Science uses WGS information uploaded to NCBI to conduct surveillance for clinical isolates that may be associated with isolates derived from USDA-FSIS-regulated products. Epidemiologists, microbiologists, and other food safety professionals use the NCBI Pathogen Detection Browser to determine whether USDA-FSIS isolates are related genetically (via single-nucleotide polymorphism analyses) to other isolates in the database. This information can help guide further investigation to determine whether USDA-FSIS-regulated products are associated with human illness. Genetic similarities between pathogens detected in food and humans increase the likelihood that they share a common source, but genetic similarity alone is not sufficient to determine whether a specific food is causing human illness. WGS may help to generate hypotheses and guide the investigation. It is essential however to consider all investigative information, such as epidemiologic (e.g., what product was consumed by ill patients) and traceback (where product was purchased and produced), as part of the decision-making process. Depending on the evidence collected during an investigation, the USDA-FSIS may take various actions to limit exposure and prevent additional illnesses. Actions taken by the USDA-FSIS at an establishment may include issuing a notice of intended enforcement, retaining product, withholding marks of inspection, or suspending a federal establishment. In addition, the USDA-FSIS may notify the public of potential food safety concerns through the issuance of a press release

announcing a public health alert or may request that an establishment recall product linked to human illness.

The USDA-FSIS has successfully used WGS to investigate and solve outbreaks in coordination with CDC and public health and regulatory partners. Two *Listeria monocytogenes* (*L. monocytogenes*) outbreaks investigated in 2018 highlight how WGS was used in conjunction with epidemiologic information to determine the source of illnesses and recall product from commerce. In addition, WGS has been used to detect outbreaks with a small number of cases and isolates spread out over a long period. In both situations, WGS, in conjunction with case interview and traceback data, increased the confidence that illnesses were linked to consumption of USDA-FSIS-regulated products.

The first outbreak involved clinical patients who became sick in 2017 and 2018. WGS data showed that the *L. monocytogenes* strains obtained from the patients were genetically similar to isolates collected from ready-to-eat ham samples in an establishment in 2016 and 2018. Patient interviews revealed that they had eaten deli ham or country ham biscuits. USDA-FSIS investigators visited and reviewed records from settings where patients purchased or consumed these ham products and determined that the ham products were received from the same establishment with the positive samples. The USDA-FSIS evaluated the WGS, case exposure, and traceback information to link illnesses to consumption of the product and the establishment voluntarily recalled the ready-to-eat ham products. In the second *L. monocytogenes* outbreak example, WGS data showed genetic similarity between clinical patient, food, and environmental isolates collected between 2014 and 2018 from an establishment that produced Asian-style pork products. Patients were not able to provide detailed food histories but did report eating Asian-style foods and shopping at Asian specialty grocery stores. The grocery stores sold ready-to-eat pork products manufactured at the establishment with the positive samples. This was the only establishment where the USDA-FSIS had isolated a strain that was closely related to the illness strain by WGS. Along with available epidemiologic information, this WGS evidence linked patients to the consumption of the ready-to-eat pork products and the establishment voluntarily recalled the products.

WGS has also been useful in investigations of illnesses due to *Salmonella*. Between 2018 and 2019, the USDA-FSIS, in coordination with the CDC and public health partners, investigated a *Salmonella* Reading outbreak. Exposure information from clinical patients suggested consumption of turkey as the common cause of the illnesses. The outbreak strain of *Salmonella* Reading was identified in samples of ground turkey collected from homes of patients and from raw turkey used for pet food. The outbreak strain was also identified in samples of raw turkey products from 24 slaughter and 14 processing establishments through routine USDA-FSIS testing and from an additional 120 retail turkey

samples collected as part of the National Antimicrobial Resistance Monitoring System retail meat sampling program. WGS showed that the bacteria isolated from the clinical samples from patients and turkey products were closely related genetically, providing further evidence that a common product was making people sick. Although no specific type, brand, or manufacturing source of turkey was identified as a common factor in all illnesses, the USDA-FSIS requested and two companies agreed to voluntarily recall turkey products when presented with epidemiologic, traceback, and laboratory evidence supporting a link between illnesses and their brand. A common supplier of raw turkey products connected to all illnesses was not identified, suggesting this strain may have been present at a low level in numerous facilities. During 2019, the USDA-FSIS and public health partners investigated a multistate outbreak of *Salmonella* Dublin illnesses linked to consumption of ground beef. Epidemiologic, traceback, and laboratory evidence indicated that ground beef was the source. The outbreak strain was identified in six samples of raw beef products from slaughter and processing establishments collected as part of the USDA-FSIS' routine testing and in repackaged leftover ground beef collected from a clinical patient's home. WGS showed that *Salmonella* bacteria from the USDA-FSIS samples and leftover ground beef sample were closely related genetically to the *Salmonella* bacteria from clinical patients. Using purchase history and receipts, the USDA-

FSIS determined that ground beef purchased by one patient was produced by a USDA-FSIS-regulated establishment. The WGS information, in conjunction with epidemiologic and trace information, supported a link between illnesses and the USDA-FSIS-regulated product. As a result, the company voluntarily recalled the lot of ground beef based on the USDA-FSIS's request.

CONCLUSIONS

WGS is a powerful tool. When combined with epidemiologic, production, and purchase trace evidence, WGS can be used to determine whether a USDA-FSIS-regulated product is causing human illness and to include or exclude potential exposure sources. Based upon this evidence, the USDA-FSIS can facilitate removal of adulterated product from commerce or prevent potentially contaminated product from reaching consumers. The USDA-FSIS continues to use the best available science to investigate outbreaks and protect public health.

REFERENCE

1. Brown, E., U. Dessai, S. McGarry, and P. Gener-Smidt. 2019. Use of whole-genome sequencing for food safety and public health in the United States. *Foodborne Pathog. Dis.* 16:441–450.

***Author for correspondence:**

Telephone: +1 202.690.6645

Email: sheryl.shaw@usda.gov