How WGS Is Changing the Landscape of Multistate Foodborne Outbreak Investigations

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Foodborne Diseases in the United States: A Changing Landscape

Disseminated Foodborne Outbreak Linked to a Commercially Distributed Product
Detecting Disseminated Outbreaks with PulseNet

- Subtyping enteric bacteria is essential to identifying highly disseminated outbreaks
- PulseNet laboratory network established in 1996
  - Over 80 participating laboratories in the US
  - 60,000+ isolates subtyped annually
- Bacteria collected from ill people undergo DNA “fingerprinting” using pulse-field gel electrophoresis (PFGE) and whole genome sequencing (WGS)

Bacteria with the same “DNA fingerprint” are more likely to come from a common source than bacteria with different fingerprints

The more specific the fingerprint, the higher the likelihood the bacteria come from a common source
Detecting Outbreaks with PulseNet

- PFGE/WGS data from illness-causing bacteria are transmitted to CDC
- Monitored for temporal clusters
- PulseNet notifies epidemiologists to investigate clusters
Strengths and Limitations of PFGE-Based Subtyping

- Successful over the last 20 years in detecting highly disseminated outbreaks
  - Would not have otherwise been detected
  - Would have been detected later

- Limitations to PFGE-based subtyping
  - Some PFGE patterns common, limiting utility
  - PFGE patterns are indirectly reflective of underlying genetic relatedness of bacteria
  - Genetically related bacteria can appear different by PFGE and vice versa
2010 Outbreak of *Salmonella* Enteritidis Infections Linked to Shell Eggs

Number of *Salmonella* Enteritidis cases matching PFGE pattern JEGX01.0004 reported to PulseNet, United States, 2010*

- Most common PFGE pattern in the PulseNet database
- 3,578 illnesses reported during the outbreak period
- 1,639 presumed to be unrelated “background” cases
- Complicated investigation into the source
WGS Provides a Higher Resolution View of the Bacterial Genome

PFGE gives us indirect information about the bacterial genome

WGS has the ability to give us information at nearly every position in the genome

Serotype, virulence, and resistance information can be directly identified from the sequence data
WGS Impacts Many Aspects of Multistate Outbreak Investigations

- **Identifying outbreaks**
  - Potential outbreaks linked to certain “common” PFGE patterns have traditionally been very hard to solve
  - These “common” PFGE patterns will likely be broken up by WGS into smaller, but more solvable outbreaks

- **Generating hypothesis about the cause of the outbreak through patient interviews**
  - Excludes unrelated illnesses from the analysis
  - Can groups together seemingly unrelated illnesses, increasing statistical power
WGS Impacts Many Aspects of Multistate Outbreak Investigations

- Identifying good candidates for tracing back the source of suspected foods
  - Helps ensure that traceback is performed on ill people most likely to be linked to the outbreak vehicle

- Identifying opportunities to test ill people’s leftover foods or sample at their purchase locations
  - Helps ensure that product testing is performed on ill people most likely to be linked to the outbreak vehicle
  - Increases the confidence in the link between bacteria from people and foods
Testing Hypotheses: How Do We Determine a Food is the Cause of an Outbreak?

- Three types of evidence used:
  - **Epidemiologic**: association between illness and exposure
  - **Traceback**: suspected food item converges on a common source of contamination
  - **Microbiologic**: pathogen found in the food, farm or facility
WGS Impact Extends Beyond Just Improving Outbreak Investigations

- WGS will clearly help identify more outbreaks and enhance the types of evidence used to link these outbreaks to specific food categories

- WGS may also help identify and characterize other food safety problems beyond traditional outbreaks
  - Spread of antimicrobial resistance genes/plasmids across multiple strains of bacteria
  - Persistent sources of food contamination that result in consistent numbers of illnesses without a rapid rise in cases
  - Repeated outbreaks that link back to pathogen reservoirs in animals or the environment
  - Better understanding the burden of illness attributable to certain food categories by combining genomic data with other data sources
Example
Some Background

- In late 2017, CDC and the Public Health Agency of Canada identified a bi-national outbreak of *E. coli* O157 infections.
- The investigation in Canada clearly linked the illnesses to romaine lettuce.
- In the US, all ill people also ate leafy greens, but only about half reported romaine.
- Were the outbreak vehicles different in the two countries? Did they come from the same source?
Outbreak Detection

- October 29, 2018: PulseNet identifies a cluster of 10 *E. coli* O157:H7 infections from 3 states
  - Three very similar PFGE pattern combinations
- WGS showed these isolates are highly related to the 2017 bi-national outbreak
- CDC and state colleagues initiated a multistate investigation to identify the outbreak source
November 2018: Hypothesis Generation

- 34 cases from 11 states
  - Case definition now includes 6 PFGE pattern combinations
  - WGS indicates isolates are closely related within 0-2 alleles
  - Closely related to isolates from Canada with romaine exposure

- Notable food exposures reported by ill people:
  - 24/25 (96%) report consuming any leafy greens
  - 13/16 (81%) report consuming romaine lettuce specifically
  - Two restaurant sub-clusters identified in MI and CA with some reporting romaine salad exposure

- FDA and state partners worked to traceback romaine lettuce exposures to the farm level
November 20, 2018: CDC Food Safety Alert

- FDA and states working to traceback romaine lettuce
- No common grower, supplier, distributor, or brand of romaine lettuce has been identified
- “CDC is advising that consumers not eat and restaurants and retailers not sell any romaine lettuce, until we learn more about this outbreak and the source of the contaminated lettuce.”

People infected with the outbreak strain of E. coli O157:H7, by state of residence
Romaine Traceback Investigation

- Traceback information from the FDA indicated ill people in this outbreak ate romaine lettuce harvested from the California Central Coastal
November 26: CDC/FDA Update Advice to Consumers and Retailers

- 43 ill people reported from 12 states
- 88% reported eating romaine lettuce in the week before their illness started
- Ill people reported eating different types of romaine lettuce in several restaurants and at home
- “CDC is advising that consumers not eat, restaurants not serve, and retailers not sell any romaine lettuce from the Central Coastal growing regions of northern and central California.”
November/December 2018: Environmental Investigation

- FDA, CDC, and state partners investigated farms and cooling facilities in California that were identified in romaine traceback.

- CDC analyzed water and sediment samples from farms identified during romaine traceback.

- *E. coli* O157:H7 found in an agricultural water reservoir was highly related by WGS to the outbreak strain.
**WGS Results**

- cgMLST tree of highly related STEC isolates within 0-4 alleles
- Includes 2016, 2017, and 2018 clinical isolates
- Environmental isolates from Adam Bros. Farm

**Legend:**
- **Historical isolates**
- **Environmental isolates**

0-4 alleles
WGS Strain of Concern: A Timeline

2016 outbreak
• 20 cases
• Vehicle: unknown

2017 outbreak
• 25 cases
• Vehicle: leafy greens

2018 outbreak
• 62 cases
• Vehicle: romaine lettuce
Outbreak Summary

People infected with the outbreak strain of *E. coli* O157:H7, by state of residence, as of January 9, 2019 (n=62)

- 62 cases from 16 states and DC
- Patient median age of 25 years with 66% of patients female
- 46% of patients were hospitalized and 2 developed HUS
Conclusions

- Role of WGS in the investigation
  - Linked current outbreak to a cluster in 2016 and an outbreak in 2017 associated with leafy greens in the U.S.
  - WGS link to 2017 leafy green outbreak informed the investigation, hypothesis generation, and traceback strategy with a focus on romaine lettuce
  - Confirmed genetic relatedness of 10 different PFGE pattern combinations

- FDA/CDC/CA farm investigation and isolation of the WGS outbreak strain
  - Key to identifying one of the farm level sources for reoccurring STEC outbreaks of the same WGS strain
  - Provides additional evidence that water contamination may be an important factor in STEC outbreaks associated with romaine lettuce
Thoughts on WGS Implementation
Full Implementation of WGS in PulseNet

- PulseNet is rapidly transitioning from PFGE to WGS
  - Already switched for *Listeria* and *Campylobacter*
  - Transition is happening during April/May 2019 for *Salmonella* and STEC

- This will require fundamental shifts in:
  - How outbreaks are defined and detected
  - The current processes for interagency, interdisciplinary, and private-public-academic collaboration

- Despite the advantages of WGS, it does not solve all problems
  - Multi-vehicle outbreaks with complex contamination routes
“Strains of Concern” Investigations Becoming More Common and Are Important

- **2016-2017 Salmonella Newport outbreak** linked to ground beef
  - Illnesses over a 10-month period
  - Outbreak strain found in 4 dairy cattle from a single state and ground beef collected from an ill person’s home
  - Traceback of ground beef did not converge on a single slaughter facility

- **Strain returned in 2018**, causing a large outbreak of over 400 illnesses, also linked to ground beef
No Change in Overall Incidence of Salmonellosis Since 1996
Outbreaks Are a Small Fraction of All Illness

- Only about 12% of all *Salmonella*, STEC, and *Listeria* illnesses reported to PulseNet are associated with a potential outbreak.
- The vast majority of illnesses do not have a known or suspected cause (the “baseline”).
- Focusing only on acute outbreaks misses potential opportunities for understanding illness sources.