KEY POINTS
for Successful Foodborne Outbreak Detection and Investigation

PRIORITIZATION OF WHOLE GENOME SEQUENCING CLUSTERS FOR INVESTIGATION

It can be challenging to determine which whole genome sequencing (WGS) clusters should be investigated, and once investigations are initiated, how aggressively they should be pursued. Epidemiologists have to balance preserving limited public health resources by minimizing investigations of clusters that are less likely to have a common source, with the goal of identifying as many outbreaks as possible as soon as possible. Here are some key points for determining how to prioritize *Salmonella* and STEC WGS clusters for investigation and how hard to pursue them, based on Minnesota Department of Health's (MDH) experience and the peer-reviewed literature.

Cluster definition

- For multi-state outbreak detection, CDC uses broad criteria - ≥7 *Salmonella* cases or ≥5 STEC cases with isolates ≤10 alleles and specimen collection dates ≤60 days. These cluster defiinitions are useful to initiate multi-state investigations and can also serve as useful starting-point thresholds for cluster detection at the state/local level; however, in practice, cluster definitions incorporating higher degrees of isolate relatedness and lower numbers of cases are more meaningful at the state/local level.
- For local *Salmonella* and STEC outbreak detection, a cluster definition of ≥2 cases in different households with isolates within 0-5 alleles by core genome multiple locus sequence typing (cgMLST) and specimen collection dates within 60 days is a practical starting point.

Certain characteristics of WGS clusters can inform which clusters are more likely to be solved (and by extension, which represent common source outbreaks vs. unrelated sporadic cases):

- Allele range of cluster case isolates
  - Once a cluster is detected using a broader screening threshold, a good strategy is to start out by focusing on the more closely related cluster cases to try to establish a common source among them, and then to evaluate more distantly related cases by determining whether they share that common source. Start tring to link cases that have 0 allele diffferences, then add in the cases with 1-2 allele differences, etc. This approach can be much more efficient than trying to fit more remotely related clusters cases into a single outbreak, as they can represent unrelated sporadic cases or even multiple outbreaks.
  - Generally most foodborne outbreak-associated case isolates are within 0-2 alleles. Zoonotic transmission outbreaks can have wider allele ranges (see additional resources).
  - Some *Salmonella* serotypes (such as Enteritidis, Javiana, or Newport, Hadar, I 4,5:12:i-, etc.) are more clonal, so starting with a tigher allele range (0-2 alleles) from the beginning can be helpful. It is not unusual for seemingly closely related cases to clearly have different sources.

- Requesting a SNP analysis from your laboratory will provide greater discrimination, which can be useful in identifying which cases are more likely to have a common exposure when investigating clusters of clonal *Salmonella* serotypes or difficult to solve clusters

- Cluster size (number of cases)
  - Generally, as the number of cases in a cluster increases, the cluster of highly related isolates is more likely to represent a common source outbreak, and the more epidemiologic information is available to identify the vehicle or outbreak source. However, this is not always true, as highly clonal serotypes (e.g., Enteritidis) can manifest as never-ending clusters when broader definitions are used.

- Temporality
  - Clusters with isolates bunched more closely in time are generally more likely to represent true common source outbreaks. However, always be aware that cases can be low in number and spread out in time in "slow burn" restaurant outbreaks associated with contaminated environments and/or infected food workers.

Additional questions to consider when deciding how to prioritize a WGS cluster

- Are cases geographically clustered?
- Do the cases have distinct demographics?
- Is the cluster clearly ongoing?
- Are there any closely related food, animal, or environmental isolates in NCBI?
  - Search using the PNUSA identifier for your cases at https://www.ncbi.nlm.nih.gov/pathogens/
- Do any of the cases have a "red flag" exposure like sprouts or unpasteurized milk?

If any of these are true, this argues for prioritizing clusters higher.

Often the decision isn't <u>if</u> you should investigate a cluster, but rather how aggressive you should be

- If there are only 2-3 cases, compare routine interviews to identify potential common exposures
- As additional cases occur, the next step is to identify specific exposures which you will use to re-interview older cases and incorporate into the initial interviews of newer cases.
  - Create a short supplemental questionnaire.
  - Ask specifically about all of the restaurants mentioned by all of the cases.
  - Ask about interesting exposures mentioned by other cases.
  - Get additional details (e.g., brand, variety) on suspect foods.
- Investigators can have more confidence that WGS clusters truly represent a common source outbreak and that investigation efforts will be worthwhile, or that food isolates that match a cluster are meaningful.

Additional Resources:

Genetic Diversity in Salmonella enterica in Outbreaks of Foodborne and Zoonotic Origin in the USA in 2006–2017

Prospective *Salmonella* Enteritidis surveillance and outbreak detection using whole genome sequencing, Minnesota 2015–2017

Transition to Whole Genome Sequencing Surveillance: The Impact on National Outbreak Detection and Response for *Listeria monocytogenes*, *Salmonella*, Shiga Toxin-Producing *Escherichia coli*, and *Shigella* Clusters in Canada, 2015-2021

Challenges Associated with Investigating *Salmonella* Enteritidis with Low Genomic Diversity in New York State

Enhancing genomics-based outbreak detection of endemic Salmonella enterica serovar Typhimurium using dynamic thresholds

MINNESOTA
Integrated Food Safety Center of Excellence
MINNESOTA DEPARTMENT OF HEALTH • UNIVERSITY OF MINNESOTA
http://mnfoodsafetycoe.umn.edu/