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Laboratory Testing Methods and Applications in Bacterial Enteric Case and Outbreak Investigations

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Public Health Ontario Rounds

Disclosures

- Ms. Lee does not have any conflicts of interest to disclose
- Dr. Corbeil does not have any conflicts of interest to disclose

Learning Objectives

1. Utilize terminology for different laboratory testing methods.
2. Describe the use of culture-independent diagnostic tests (CIDT) in Ontario laboratories for enteric bacterial detection.
3. Describe the use of whole genome sequencing (WGS) for enteric bacterial outbreak investigation and surveillance.
4. Assign case classification correctly based on laboratory results.
5. Explain the impact of CIDT on the incidence of bacterial enteric diseases of public health significance.

Overview

- Increase use of CIDT
- Applications and limitations of CIDT
- How to classify cases for public health based on lab results
- WGS clustering and visualization
- Application of WGS
- Who does what with WGS results?

Culture-Independent Diagnostic Tests

History of Enteric Bacterial Diagnostic Testing

- 1890s: Culture tests
- 1990s: Antigen tests, e.g. enzyme immunoassays
  - e.g. “EIA”, “ELISA”, “LFA”, “LFIA”, “ICT”, “RDT”
- 2000s: Single organism molecular tests
  - e.g. “NAT”, “NAAT”, “PCR”, “RT-PCR”, “LAMP”
- 2010s: **Multi-organisms (“multiplex”) molecular tests**

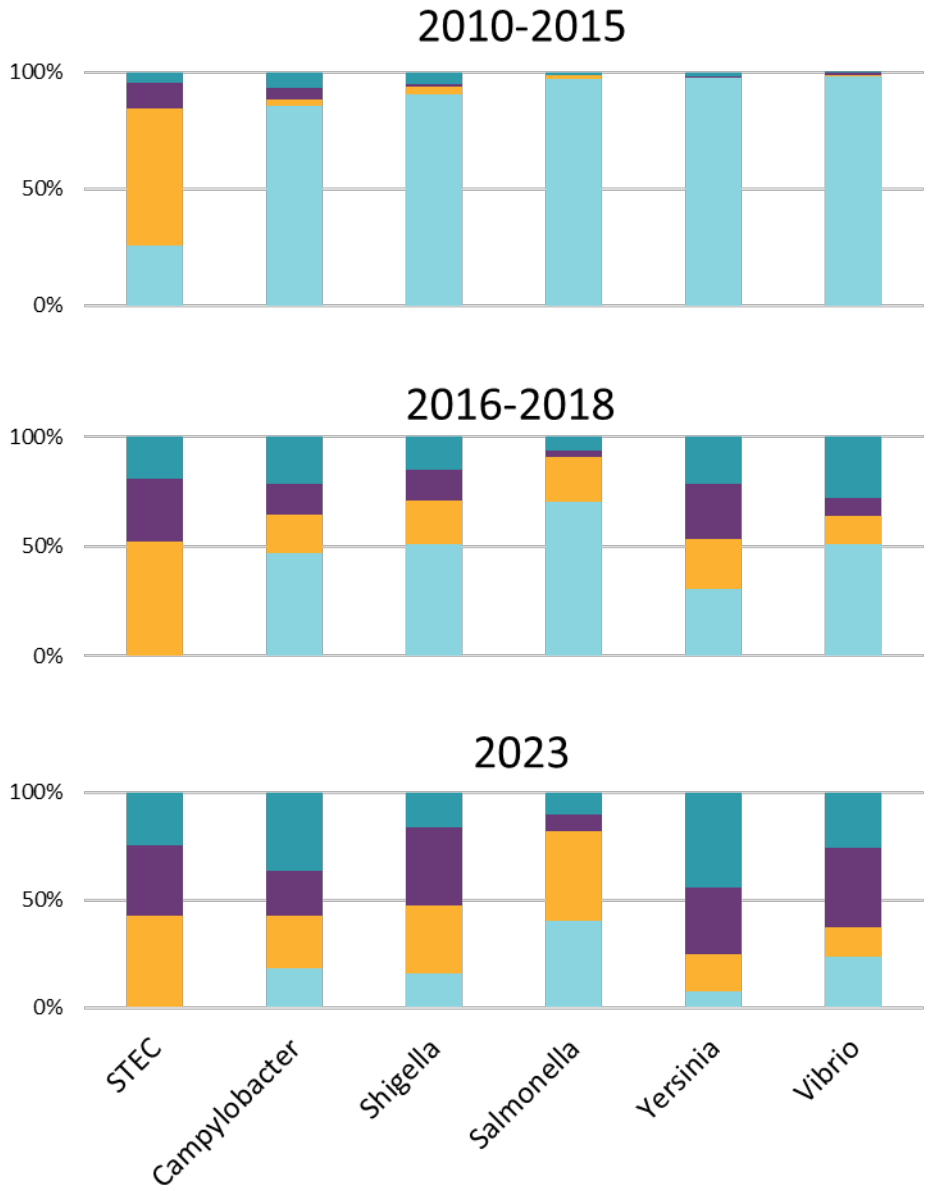
CIDTs

CIDT = Culture-independent diagnostic test
(i.e. any antigen or molecular test)

Increasing Use of CIDT Methods

- In the United States in 2023, bacterial enteric cases were diagnosed as follows:
  - 23% by **culture only**
  - 32% by **CIDT and positive culture**
  - 31% by **CIDT but negative culture**
  - 16% by **CIDT only** (culture not done)

47%

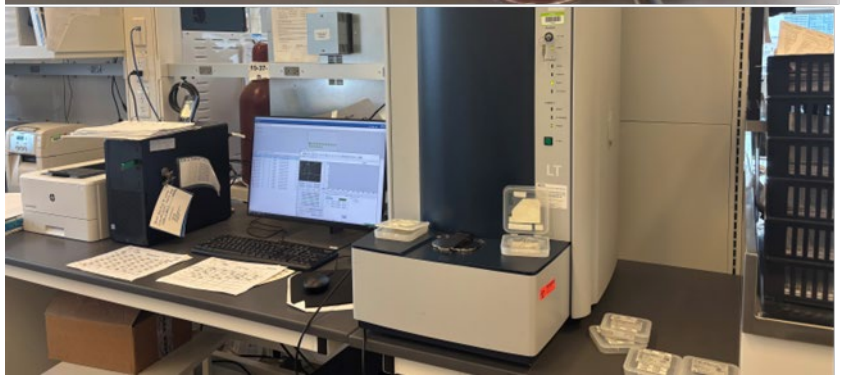


Shah HJ, Jervis RH, Wymore K, Rissman T, LaClair B et al. Reported incidence of infections caused by pathogens transmitted commonly through food: impact of increased use of culture-independent diagnostic tests — Foodborne Diseases Active Surveillance Network, 1996–2023. MMWR Morb Mortal Wkly Rep. 2024;73(26):584-93. Available from: https://doi.org/10.15585/mmwr.mm7326a1

Components of Conventional Culture Panels

Pathogens Routinely Isolated by Culture:

- *Salmonella*
- *Shigella*
- *Campylobacter*
- *E. coli* O157
- Other pathogens based on risk factors or upon request



Average Turnaround Time: 2 to 7 days

Isolate available for further analysis

"[Organism] **isolated**"

Components of Multiplex Molecular CIDT Panels

"[Organism] **detected**"

Reflex culture if CIDT positive (not always performed)

Pathogens Potentially Tested by CIDT Panels:

- *Salmonella*
- *Shigella*
- *Campylobacter*
- *E. coli* O157
- Shiga toxin-producing *E. coli*
- Enterotoxigenic *E. coli*
- Enteroinvasive *E. coli*
- Enteroaggregative *E. coli*
- Enteropathogenic *E. coli*
- *Yersinia* species
- *Yersinia enterocolitica*
- *Clostridioides difficile*
- *Clostridium perfringens*
- *Vibrio* species
- *Vibrio cholerae*
- *Aeromonas*
- *Plesiomonas*
- Viruses (e.g. norovirus) and/or
- Parasites (e.g. *Giardia*)

Average Turnaround Time: under 1 day

No isolate available for further analysis unless reflex cultures are done

Benefits and Limitations of Molecular CIDT (versus Culture)

Benefits
Simpler lab workflow
Faster clinical result
More permissive organism stability
Increased sensitivity (usually)
Increased number of organisms found

These highlight the importance of maintaining culture methods

Limitations
Higher cost per test
Variable positive predictive value
Unclear relevance for some organisms detected
Unclear relevance of co-detecting organisms
Unable to distinguish some organisms (for some assays)
No species identification (for some assays)
No typing
No susceptibility testing
Detects non-infectious residual material

CIDT Limitation 1: Unable to Distinguish Between Some Organisms

Example: Some commercial CIDTs cannot distinguish between *Shigella* and enteroinvasive *E. coli* (EIEC)

- *Shigella* is usually more severe and may need treatment, EIEC does not
- *Shigella* is a disease of public health significance (DoPHS), EIEC is not
- Lab would report as: "*Shigella*/EIEC detected"

To distinguish between *Shigella* and EIEC, labs may use a different molecular test or perform reflex culture locally.

CIDT Limitation 2: No Species Identification

Example: Some commercial CIDTs only identify *Vibrio* at the genus level

- *V. cholerae* and non-cholera *Vibrio* have different clinical and public health implications.
- Lab would report as "*Vibrio* species detected"

To distinguish between species, labs may use a different molecular test or perform reflex culture locally.

CIDT Limitation 3: No Typing

Example: Commercial CIDTs only identify *Salmonella* at the genus level

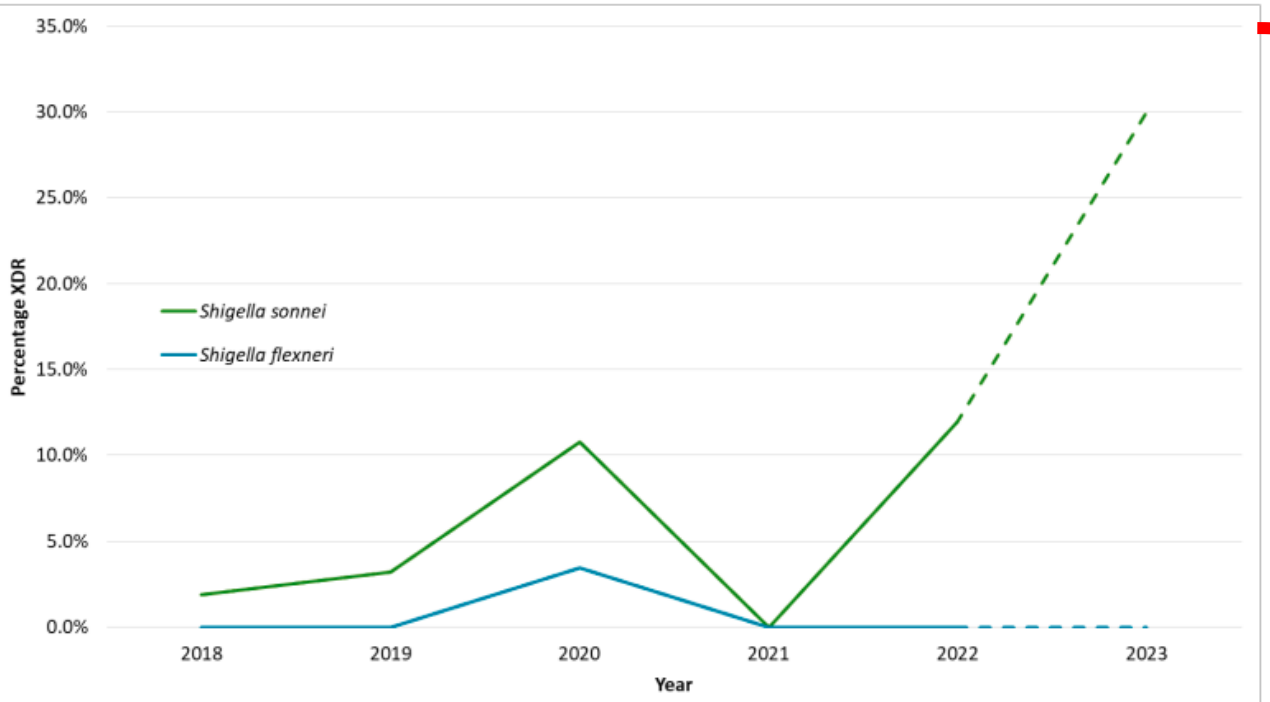
- Typhoidal and nontyphoidal *Salmonella* have different clinical and public health implications
- Lab would report as "*Salmonella* species detected"

To distinguish between species, labs may perform reflex culture locally and initial serogrouping locally.

CIDT Limitation 4: No Susceptibility Testing

Example: Rise in extremely drug-resistant *Shigella* infections in Ontario and worldwide

Figure 2. Proportion of shigellosis cases identified as XDR by species and year, January 1, 2018 to March 31, 2023



Note: Dashed line indicates a partial year including data up to March 31, 2023. *Shigella boydii* and *Shigella dysenteriae* were excluded due to low numbers. Excludes isolates where partial AST results were available (n=13).
Data source: Public Health Ontario Laboratory Information Management System

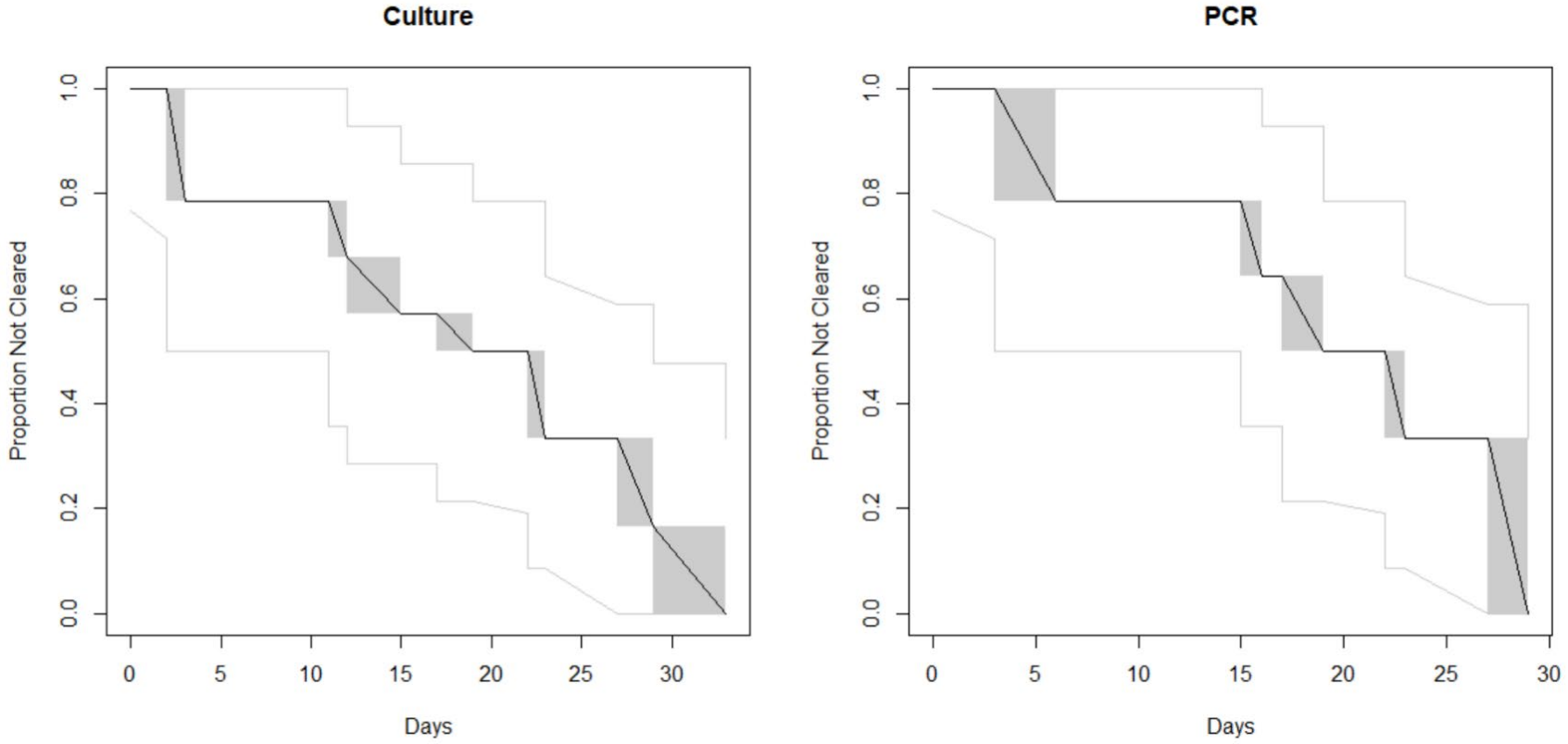
Ontario Agency for Health Protection and Promotion (Public Health Ontario). Shigella antimicrobial resistance. Toronto, ON: King's Printer for Ontario; 2023.

If culture not performed, lack of action on increasing resistance trends

CIDT Limitation 5: Detects Non-Infectious Residual Material

Example: For STEC, PCR is rapid with high sensitivity and negative predictive value

→ but post-infection clearance may take 2-7 more days vs. culture



Bording-Jorgensen M, Parsons BD, Tarr GAM, Shah-Gandhi B, Lloyd C, Chui L. Association of Ct values from real-time PCR with culture in microbiological clearance samples for shiga toxin-producing Escherichia coli (STEC). Microorganisms. 2020;8(11):1811.

Labs in Ontario are expected to perform reflex cultures, but culture yield can sometimes be low

Organism	Culture Yield Following Positive CIDT
Salmonella	70-80%
Shigella	50-60%
STEC	50-60%
Campylobacter	30-60%
Vibrio	30-40%
Yersinia	20-40%

Potential Causes of Negative Culture:

- Low burden of organisms shed
- Loss of viability during storage
- High background bacterial flora
- Culture not supporting growth for some strains
- Residual DNA or antigen detected
- False non-specific CIDT signal

Source: Shah HJ, Jervis RH, Wymore K, Rissman T, LaClair B, Boyle MM, et al. Reported incidence of infections caused by pathogens transmitted commonly through food: impact of increased use of culture-independent diagnostic tests - Foodborne Diseases Active Surveillance Network, 1996-2023. MMWR Morb Mortal Wkly Rep. 2024;73(26):584-93. Available from: https://doi.org/10.15585/mmwr.mm7326a1

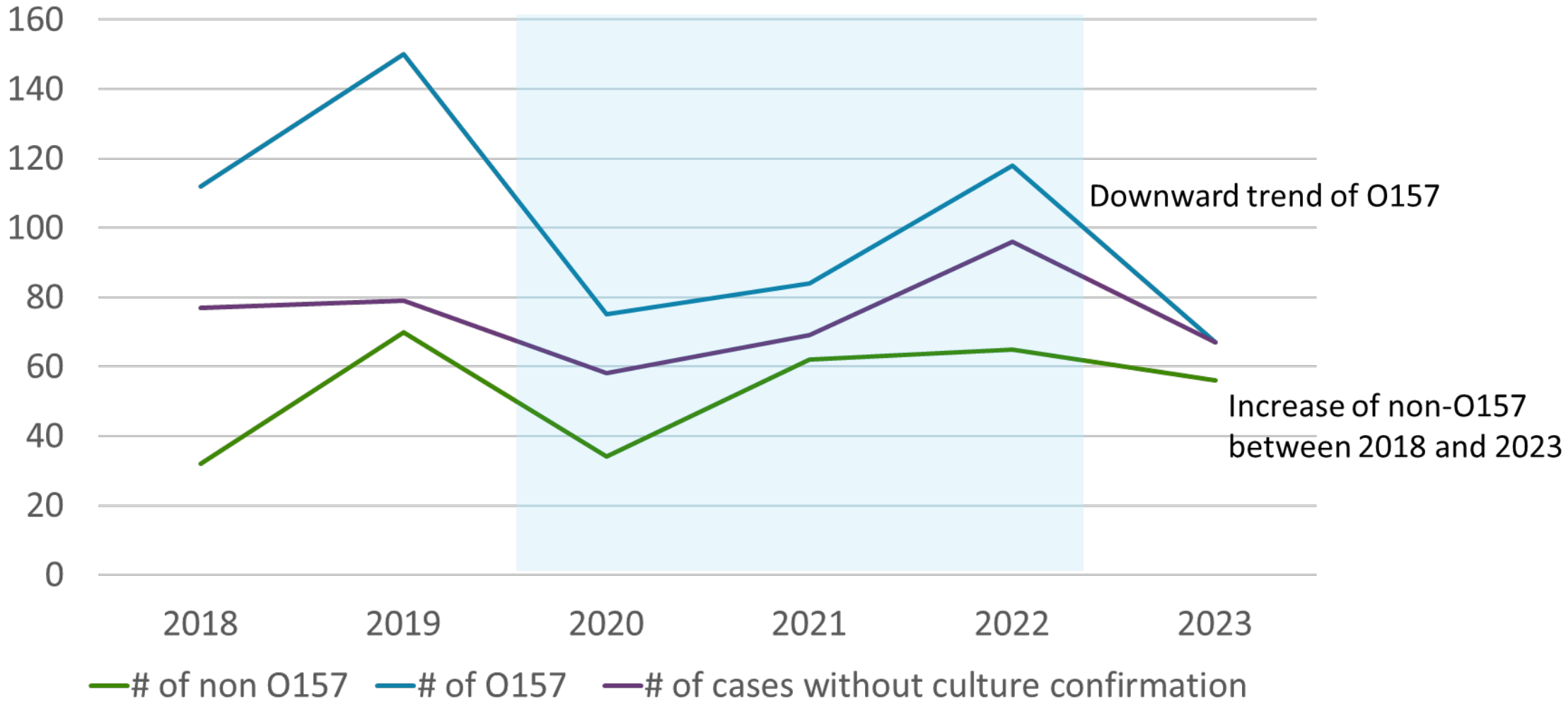
Rule of Thumb in Case Classification with CIDT/Culture Results for Bacterial Enteric Pathogens

CIDT Result (e.g.: RT-PCR, PCR, NAAT, EIA, etc.)	**Culture Result**	**Case Classification**
Detected (positive)	**Isolated (positive)**	**Confirmed**
	Not isolated (negative) or not performed	Probable
Not detected (negative) or not performed	**Isolated (positive)**	**Confirmed**
	Not isolated (negative) or not performed	DNM

Examples of How to Classify Cases and Creation of Cases in iPHIS

- For STEC
  - **Detected** by CIDT = probable STEC case
  - **Isolated** by culture = confirmed STEC case
- For *Salmonella*
  - **Detected** by CIDT = probable salmonellosis case
  - **Isolated** by culture as a non-typhoidal *S*almonella = confirmed salmonellosis case
  - **Isolated** by culture as *S*. Typhi/Paratyphi = confirmed typhoid or paratyphoid case
- For *Vibrio*
  - **Detected** by CIDT for *Vibrio* species only = consider classifying as a confirmed case of food poisoning if reported by laboratory
  - **Isolated** by culture as *V. cholerae* = confirmed cholera case

Incidence of O157, Non-O157 and Probable STEC Cases in Ontario



Data source: Ontario Agency for Health Protection and Promotion (Public Health Ontario). Shig VTEC 01JAN2018 to 20SEP2024 [dataset]. Toronto, ON: Integrated Public Health Information System (iPHIS) [producer]; DataMart [producer]; 2024 Sep 20 [data extracted 2024 Sep 20].

Whole Genome Sequencing

History of Enteric Bacterial Typing

- 1960s: Seroagglutination typing (“serotyping”)
- 1980s: Plasmid typing
- 2000s: Pulse-field gel electrophoresis (PFGE)
- 2010s: Whole genome sequencing

PulseNet

PulseNet = Network of public health labs conducting routine typing surveillance and outbreak investigations (including *Salmonella, Shigella,* Shiga toxin-producing *E. coli*, and *Listeria*)

Whole Genome Sequencing (WGS) for Enteric Bacterial Typing

- WGS: tally of the full DNA sequence of an organism
- Currently feasible only from pure bacterial colonies
- Can be read as individual DNA letters (“nucleotides”)

Example: CGTCAATATCA ATCTTACGACT ACTACTGACG

- Or as DNA segments (“genes” or “loci”)

Example:

WGS Analysis: Two Different Approaches

We can compare between two DNA sequences based on the number of…

A. Different DNA letters (called “single nucleotide variants” or SNVs)

5 single nucleotide variants (**SNVs**) between isolate A and B

B. Different DNA segments (called “alleles”)

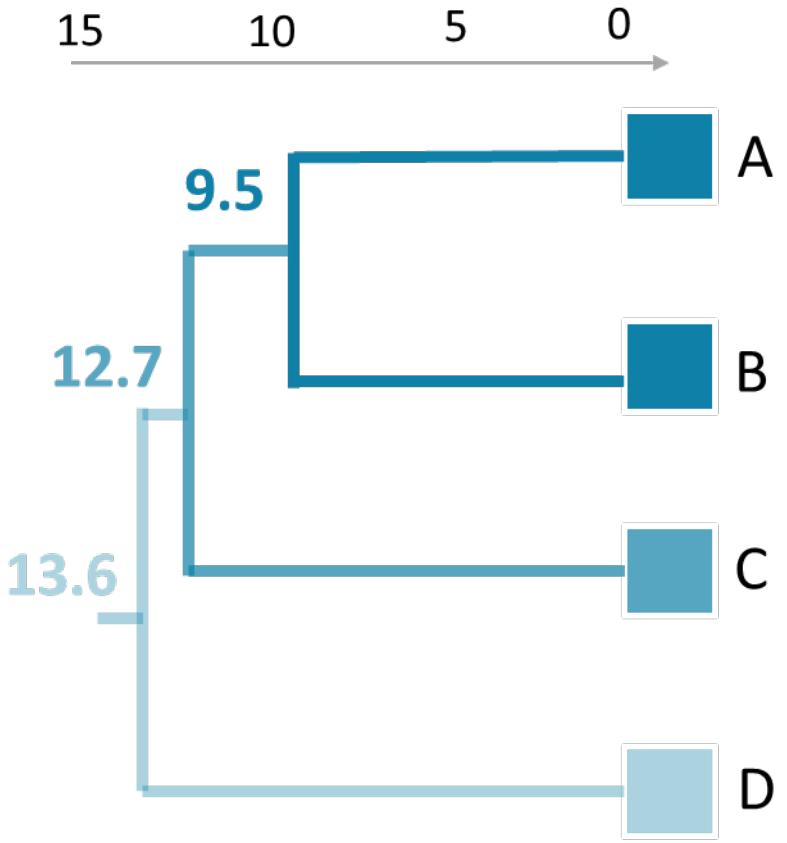
via technique called “multilocus sequence typing” or MLST, i.e. typing by sequencing multiple loci/genes

2 gene variants (i.e. **alleles**) between isolate A and B

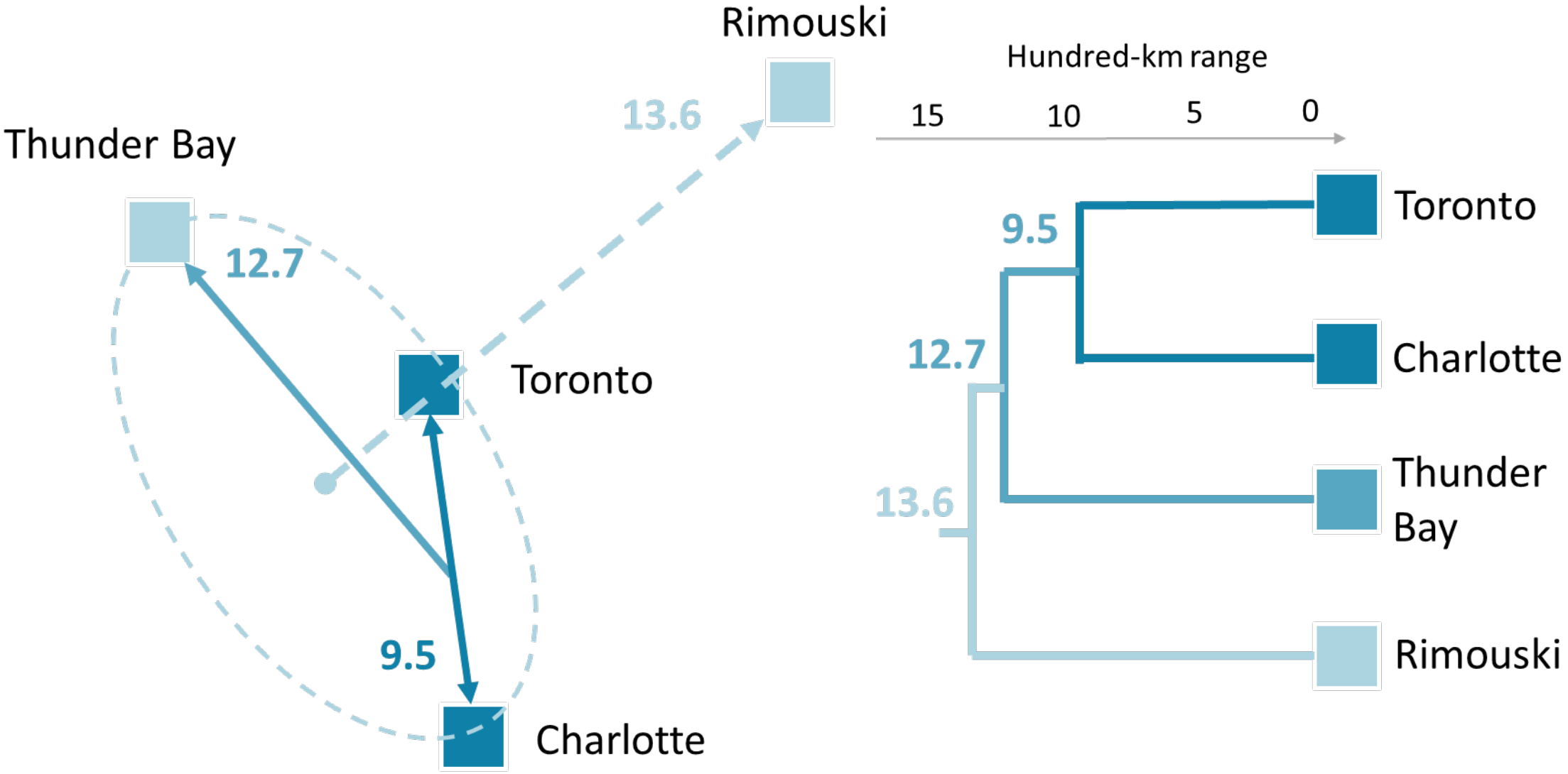
Visualizing DNA Sequence Differences Using Evolutionary Trees

Genetic relatedness can be viewed on an evolutionary tree (“dendrogram”)

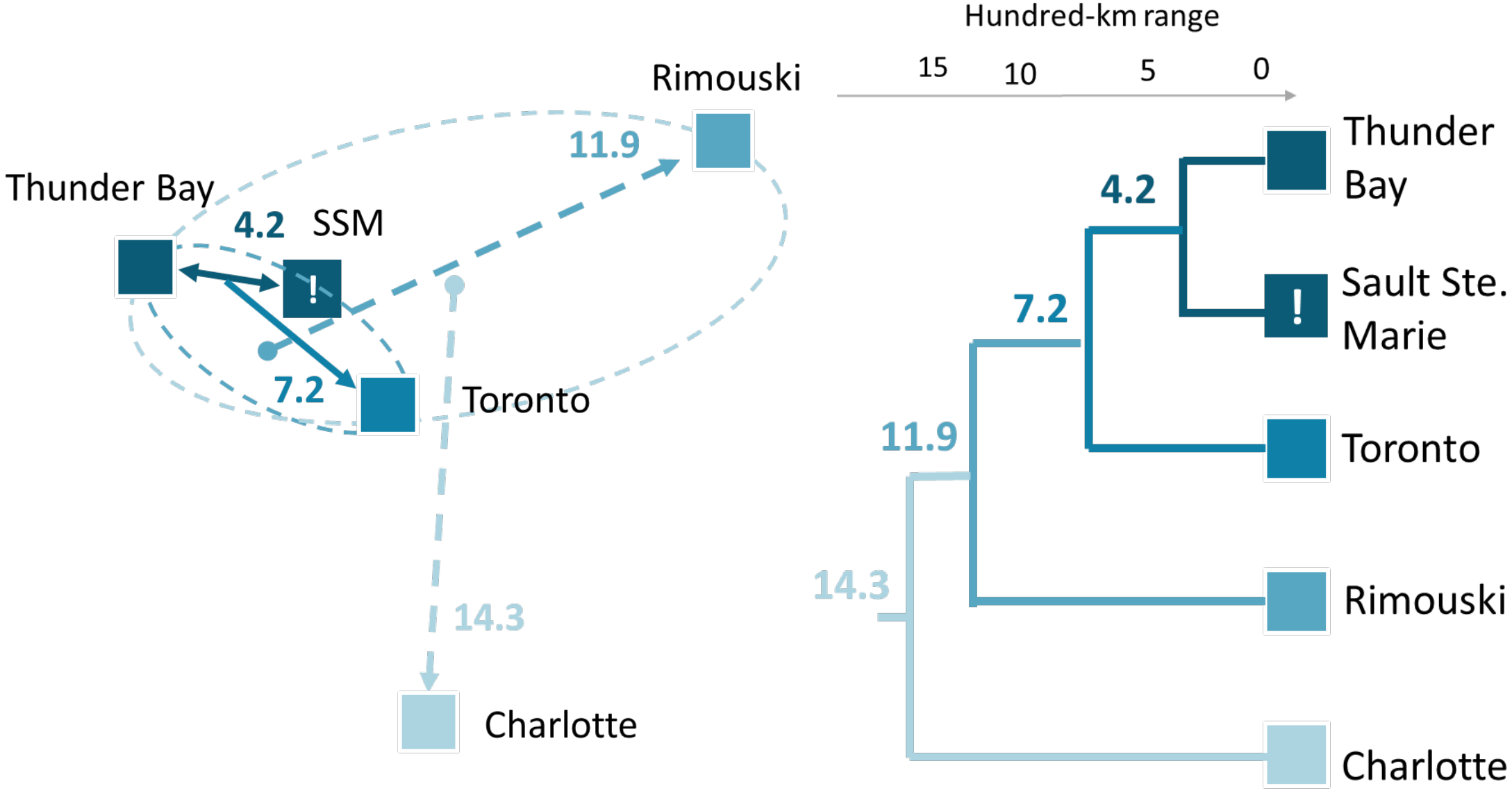
- The end-points (“leaves”) represent isolates
  - Here listed as A, B, C, and D
- The horizontal line (or "branch") length represents the relative number of differences between an isolate and its next closest relative neighbour(s) on the tree.



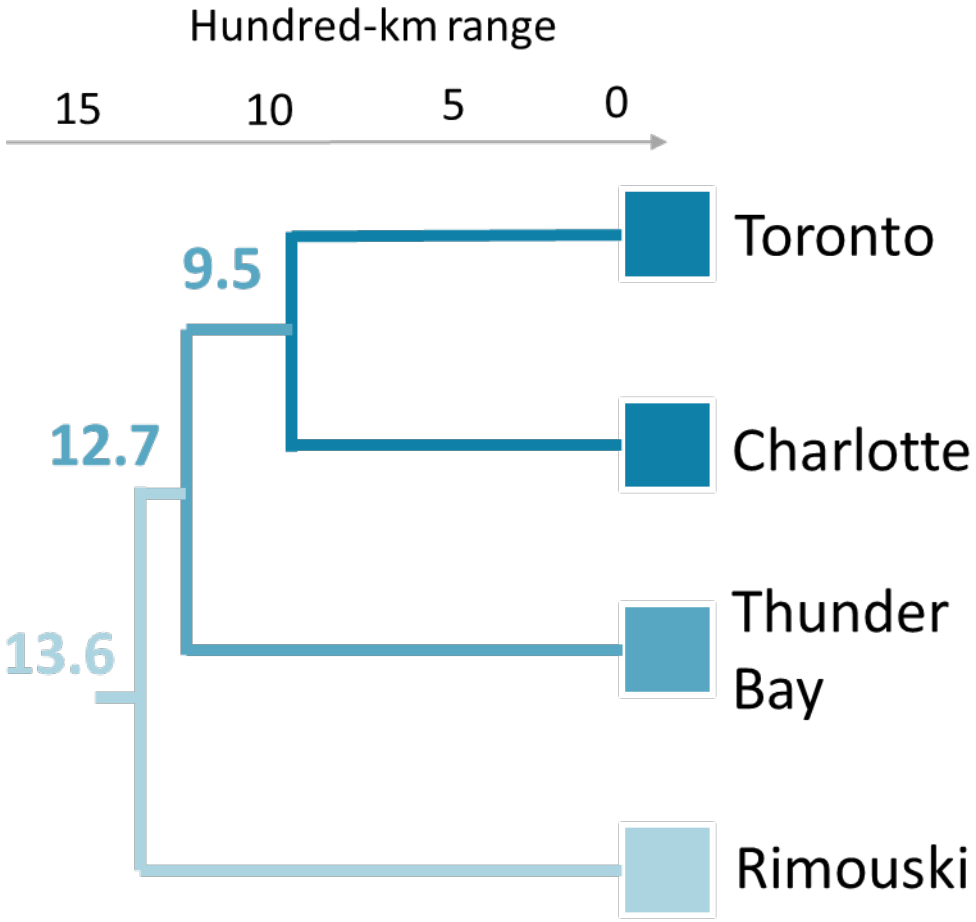
Analogy Example: Physical Clustering (1/2)

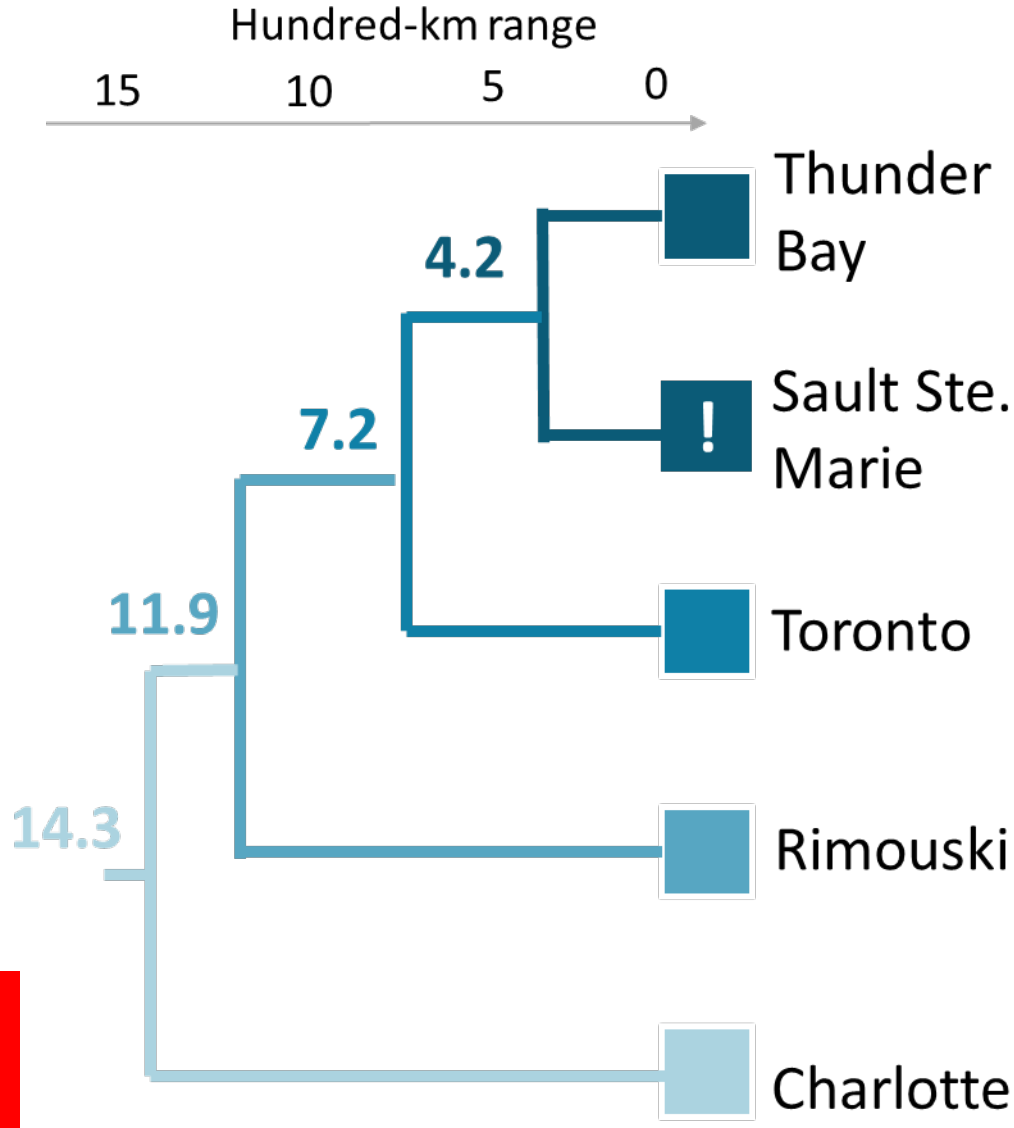


Analogy Example: Physical Clustering (2/2)



Versus





Cluster relatedness depends on the samples included – importance of finding untested links!

The PulseNet Screening Criteria for Potential Cluster Identification

Salmonella Enteritidis, Typhimurium, Heidelberg, or 4,[5],12;i;-	Other *Salmonella* serotypes, *Shigella*, and STEC	*Listeria*
3 or more isolates **≤ 10 alleles** in the past 60 days (and at least 2 isolates ≤ 5 alleles)	2 or more isolates **≤ 10 alleles** in the past 60 days	2 or more isolates **≤ 10 alleles** in the past 120 days

Standardized PulseNet Naming of Suspected Clusters:

[YY][MM][organism]WGS-[cluster order if same MM][province of first case]-[MP(added if multi-provincial)]

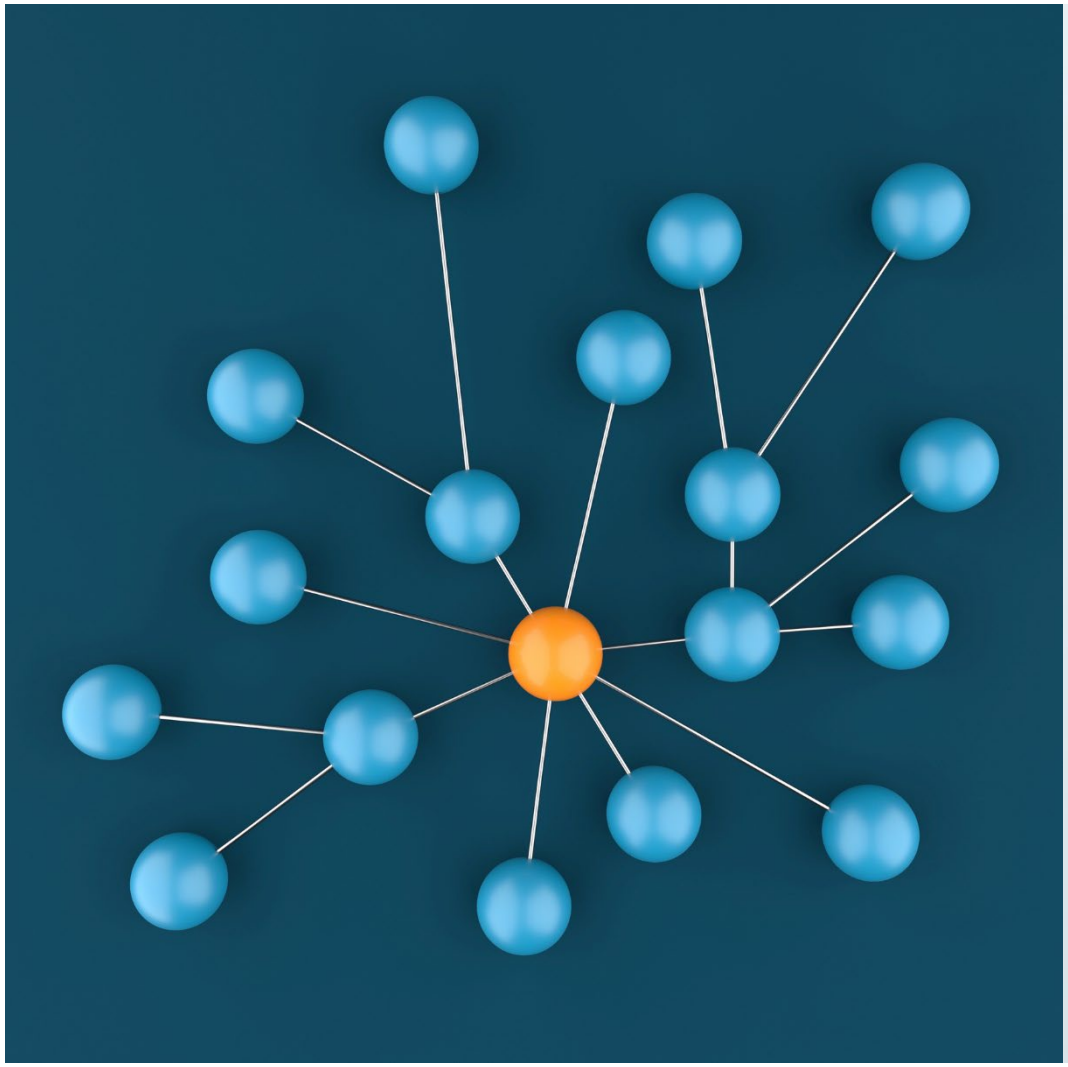
Example: 1906THWGS-1QC-MP

Salmonella Thompson cluster initially from June 2019 in QC but with multi-provincial cases.

Epidemiological Information is Essential Regardless of WGS Clustering

- Some outbreaks may be associated with a polyclonal contamination
  = may be different by WGS clustering
  but have the same source as per epidemiological information
- Some strains may have high mutation rate
  = expansion of the clustering cut-off point
  if cases are related per epidemiological information
- Some strains may have very low mutation rate
  = may be identical by WGS clustering
  but have different sources as per epidemiological information

How WGS Can Support Outbreak Investigations



Salmonella Infantis outbreak linked to unlicensed shredded pork rind and shredded pork skin sold to certain restaurants (1902SINWGS-1MP)

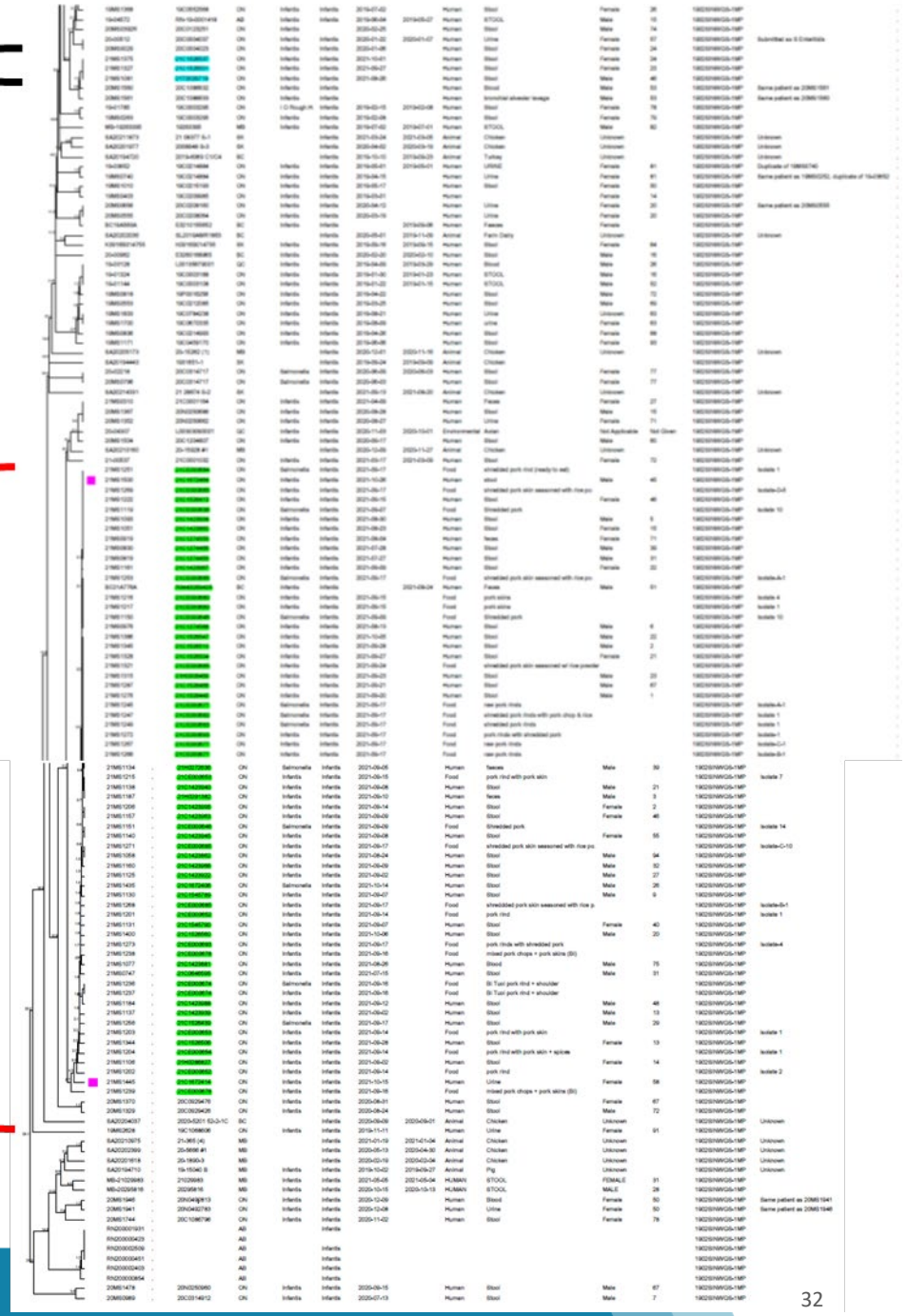
Always review WGS results in combination with epi data and food safety investigation findings!

Blue Isolates:
Non-Outbreak Cases

Reported either not eating pork, not eating shredded pork products, or ate pork but the location of purchase and supplier information are from an approved source

Green Isolates:
Outbreak Cases

≤ 7 allele differences among outbreak cases and food samples of the unlicensed pork rind and shredded pork skin



Variation in Alleles Range (1/2)

(Representative set of 12 outbreaks used to validate *Salmonella* WGS)

Salmonella Serotype	Suspect Outbreak Source	# of isolates included		# of allele differences among isolates
		Human	Non-human	
Chester	Head cheese	5	5	≤ 1
Litchfield	Fresh cantaloupes	6	0	≤ 2
Reading	Unknown	31	0	≤ 2
Braenderup	Mangos	10	0	≤ 2
Carrau	Melons	30	0	≤ 5
Enteritidis	Mail-order chicks	57	51	≤ 7
Newport, Saintpaul, and Hartford	Chia	62	46	N/A
Cubana	Sprouts	13	36	≤ 19
4,[5],12:i:-	Frozen reptiles and feeder mice	12	2	≤ 23
Typhimurium	Snakes/ Feeder rodents	46	1	≤ 107
Infantis	Raw chicken	110	21	≤ 119
Enteritidis	Frozen breaded chicken	56	20	≤ 200

Data source: Public Health Agency of Canada, Outbreak Management Division. Epidemiological validation of proposed PulseNetCanada whole-genome sequencing interpretation criteria [unpublished presentation]. Ottawa, ON: Government of Canada; 1 Oct 2018. Summary of wgMLST Allele Ranges.

Variation in Alleles Range (2/2)

(Representative set of 10 outbreaks used to validate STEC WGS)

STEC Serotype	Suspect Outbreak Source	# of isolates included		# of allele differences among isolates
		Human	Non-human	
Single strain	Walnuts	14	0	≤ 4
Single strain	Beef from national distributor	18	7	≤ 4
Single strain	Beef at restaurant and care home	5	2	≤ 5
Single strain	Romaine Lettuce	23	0	≤ 7
Single strain	Lettuce from fast food chains	31	0	≤ 8
Single strain	Raw milk cheese	29	15	≤ 9
Multiple strains	Leafy greens	13	0	≤ 8
Multiple strains	Frozen beef burgers	8	27	≤ 90
Multiple strains	Ground beef	15	18	≤ 99
Multiple strains	Various beef products	39	24	≤ 104

Data Source: Public Health Agency of Canada, Outbreak Management Division. Epidemiological validation of proposed PulseNetCanada whole-genome sequencing interpretation criteria [unpublished presentation]. Ottawa, ON: Government of Canada; 8 Jun 2018. Summary of findings. Adapted from: Rumore J, Tschetter L, Kearney A, Kandar R, McCormick R, Walker M, et al. Evaluation of whole-genome sequencing for outbreak detection of Verotoxigenic Escherichia coli O157:H7 from the Canadian perspective. BMC Genomics. 2018 Dec 4;19(1):870. Available from: https://doi.org/10.1186/s12864-018-5243-3

Collaboration Between Public Health Partners

- Epidemiological Evidence
- Food Safety Investigation
- Laboratory Evidence

Who Assesses and Leads Which Investigation

Type of WGS cluster codes	Who
Multi-provincial (MP) WGS clusters	PHAC
Ontario-only WGS clusters	PHO or Public Health Unit
Sub-cluster within MP or ON-only WGS clusters	PHAC or PHO or Public Health Unit

Number of WGS Clusters and Their Classification, 2017- Present

# of WGS Clusters by Pathogen	*Salmonella*	STEC	*Listeria*	*Shigella*
Multi-provincial	983	161	34	108
Ontario only	239	55	28	42
Total	1222	216	62	150

Data source: Public Health Agency of Canada, Outbreak Management Division. WGSSummaryExport September 16-20, 2024 [unpublished dataset]. Ottawa, ON: Government of Canada [producer]; 20 Sep 2024.
Ontario Agency for Health Protection and Promotion (Public Health Ontario). CNPHI CLSN data set [unpublished dataset]. Toronto, ON: King's Printer for Ontario [producer]; 20 Sep 2024.

Classification of Multi-Provincial Clusters by PHAC, 2017- Present

	Salmonella	STEC	Listeria	Shigella
Poultry	19%	0%	0%	0%
Travel	16%	7%	0%	14%
Sexual Transmission	0%	0%	0%	19%
Zoonotic	1%	<1%	0%	0%
Homeless/ Underhoused	0%	0%	0%	4%
Other	2%	4%	12%	0%
Unknown	61%	88%	88%	55%

Data source: Public Health Agency of Canada, Outbreak Management Division. Weekly WGS Summary September 16-20, 2024 [unpublished dataset]. Ottawa, ON: Government of Canada [producer]; 20 Sep 2024.

Examples of Criteria Used by PHO in Assessment of WGS Clusters

Laboratory Evidence

- Subtyping availability and frequency
- Relatedness by WGS

Epidemiologic

- Not travel-related
- Not poultry-related
- Temporal and geographic distribution
- Demographic profile
- Number of cases, especially new cases
- Common exposure
- Organisms with more severe outcome

Food

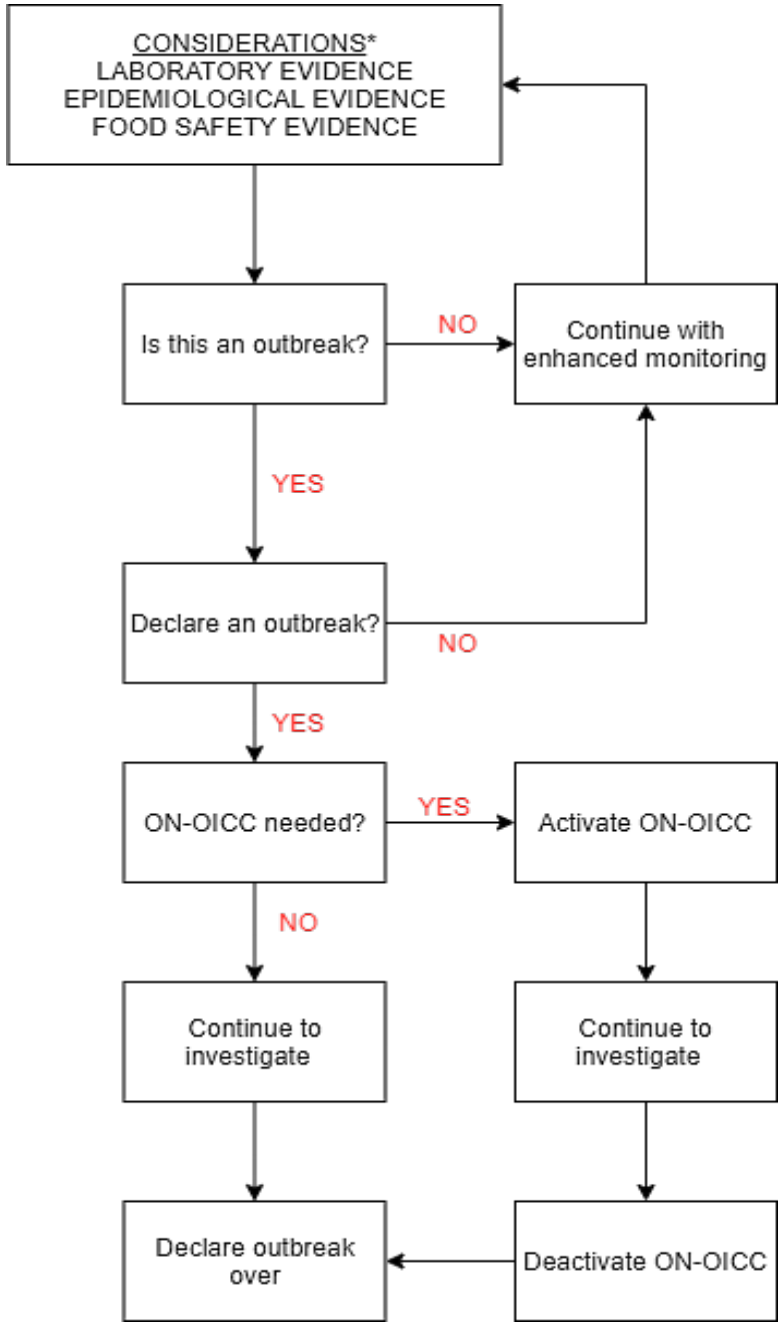
- Non-clinical matches
- Closed vs. open sample
- Same lot/production or not

Notification of WGS Results

- Public health units (PHUs) will be notified by email when:
  - There is a WGS cluster within a PHU, and
  - There are cases across PHUs and there is a need for further assessment by either PHO or PHAC. PHO may request additional information.
- PHO can also share WGS cluster code of outbreak cases with PHU for local outbreaks.
- Dendrogram for local outbreaks can be produced, upon request

For PHO or PHAC-Led Outbreaks

- iPHIS case IDs will be listed by health unit on Ontario Outbreak Central
  - Ontario Outbreak Central is on the secured Canadian Network for Public Health Intelligence (CNPHI) website
- Dendrograms are not routinely produced; only made upon request



Example: How CIDT and WGS work together for outbreak detection

- **Initial Report:** 4 cases in 1 PHU
  - 3 E. coli O157:H7 cases isolated by culture
  - 1 case with stx2 gene detected by CIDT but STEC not isolated by culture
  - All cases have a common ethnicity
  - PHO notified affected PHU
- **Final Count:** 6 cases in 3 PHUs
  - 5 E. coli O157:H7 cases isolated by culture related by WGS
  - 1 probable STEC case by CIDT
  - All six cases are reported consuming food at the same food premises

Summary (CIDT)

- CIDTs are a useful laboratory method for diagnostic purposes
- Community and hospital laboratories are expected to perform reflex culture following a positive CIDT result
- If there is no reflex culture or culture is negative, cases of bacterial enteric pathogens will remain as probable

  → ***Unable to determine if the case is part of an outbreak***

Summary (WGS)

- WGS offers greater accuracy in identifying potential genetic relatedness among bacterial isolates.
- A single genetic cluster and/or multiple genetic subclusters may occur (which may or may not be suggestive of different transmission events)
- WGS results need to be interpreted with epidemiological evidence and food safety investigation data:
  - Outbreaks may be due to either single or polyclonal strains/serotypes
  - The allele range and relative distances may vary by outbreaks and over time
- Collaboration among partners and good data quality can inform public health actions.

For more information, please contact

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