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

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Moderated by Dr. Christine Navarro, Public Health Physician

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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

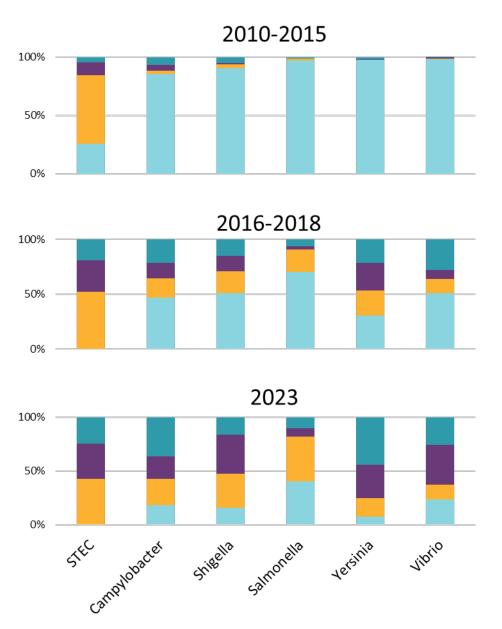
# **CIDT** = Culture-independent diagnostic test (i.e. any antigen or molecular test)

#### CIDTs

## **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 culture16% by CIDT only (culture not done)



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

47%

# **Components of Conventional Culture Panels**

#### Pathogens Routinely Isolated by Culture:

- Salmonella
- Shigella
- Campylobacter
- *E. coli* 0157
- 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* 0157
- Shiga toxin-producing E. coli Vibr
- 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

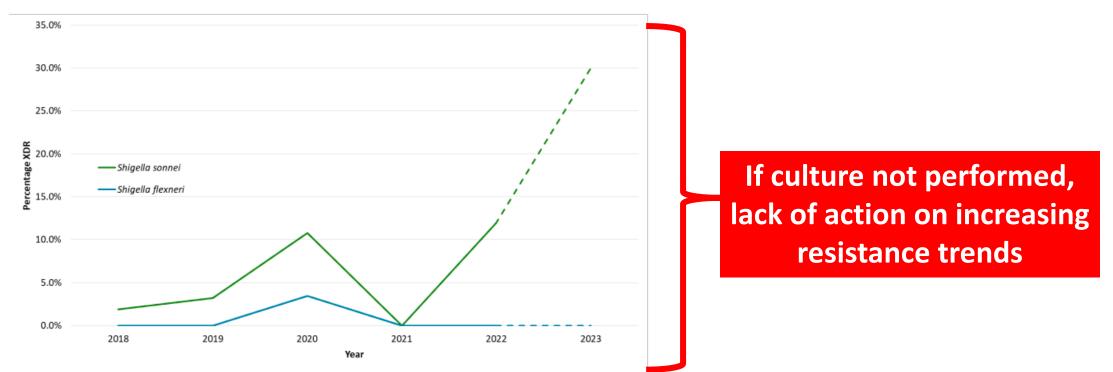
- Typhoidal and nontyphoidal Salmonella have different clinical and public health implications
- Lab would report as "Salmonella species detected"

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

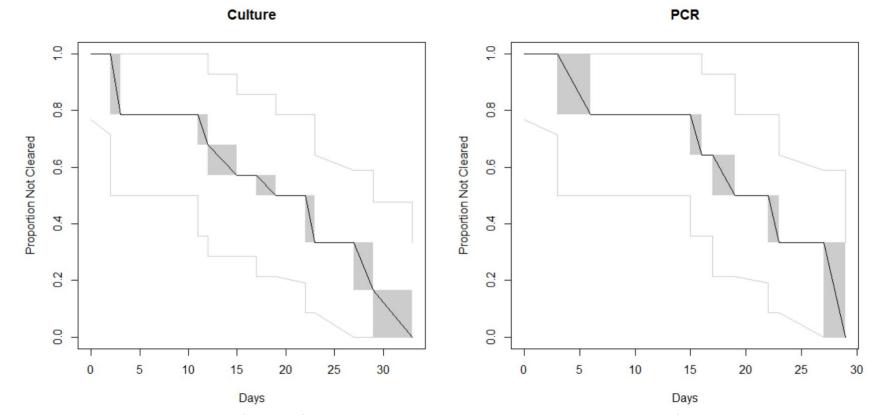


Note: Dashed line indicates a partial year including data up to March 31, 2023. *Shigella boydii* and *Shigella dysenteriae* were excluded due to low numbers. Excludes isolates where partial AST results were available (n=13). Data source: Public Health Ontario Laboratory Information Management System Ontario Agency for Health Protection and Promotion (Public Health Ontario). Shigella antimicrobial resistance. Toronto, ON: King's Printer for Ontario; 2023.

#### **CIDT Limitation 5: Detects Non-Infectious Residual Material**

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

 $\rightarrow$  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: <a href="https://doi.org/10.15585/mmwr.mm7326a1">https://doi.org/10.15585/mmwr.mm7326a1</a>

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

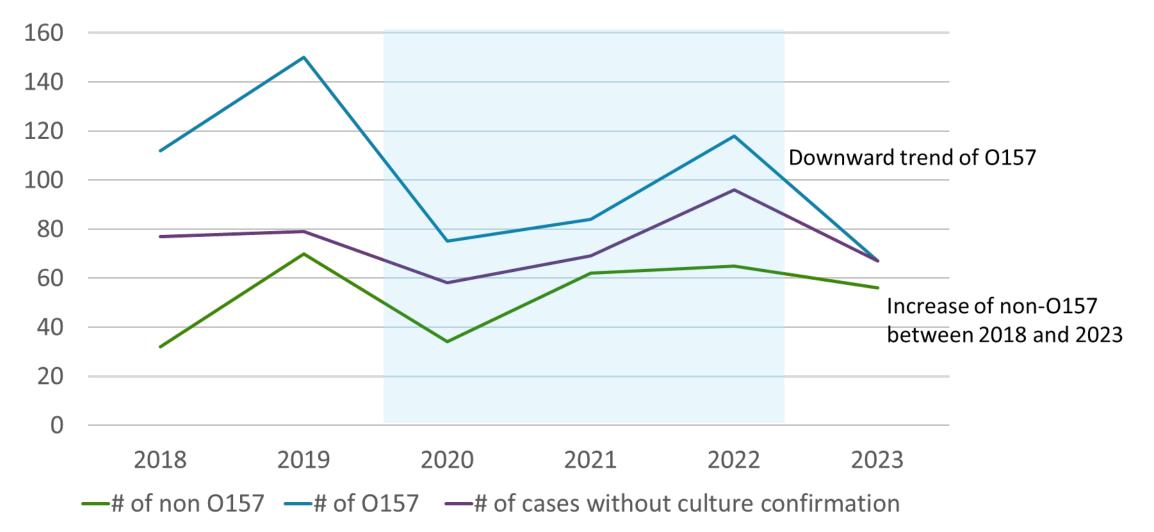
<b>CIDT Result</b> (e.g.: RT-PCR, PCR, NAAT, EIA, etc.)	Culture Result	<b>Case Classification</b>
	Isolated (positive)	Confirmed
Detected (positive)	Not isolated (negative) or not performed	Probable
Not detected	Isolated (positive)	Confirmed
(negative) or not performed	Not isolated (negative) or not 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 Salmonella = 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** = Network of public health labs conducting routine typing surveillance and outbreak investigations (including Salmonella, Shigella, Shiga toxin-producing E. coli, and *Listeria*)

PulseNet

#### 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")



• Or as DNA segments ("genes" or "loci")

Example:	Gene 1	Gene 2	Gene 3
Example:	1	2	3

#### WGS Analysis: Two Different Approaches

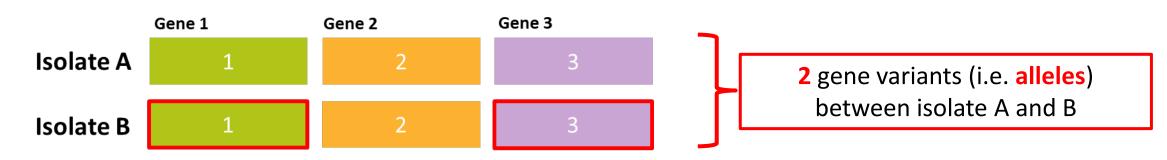
We can compare between two DNA sequences based on the number of...

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



B. Different DNA segments (called "alleles")

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

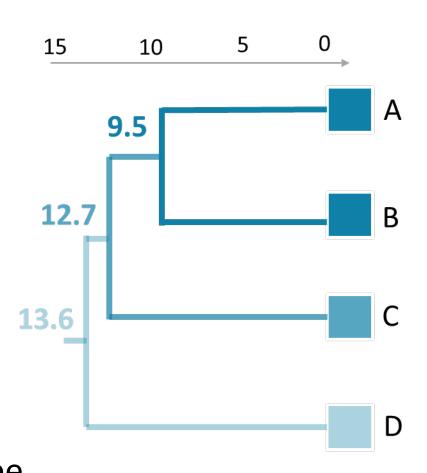


#### **Visualizing DNA Sequence Differences Using Evolutionary Trees**

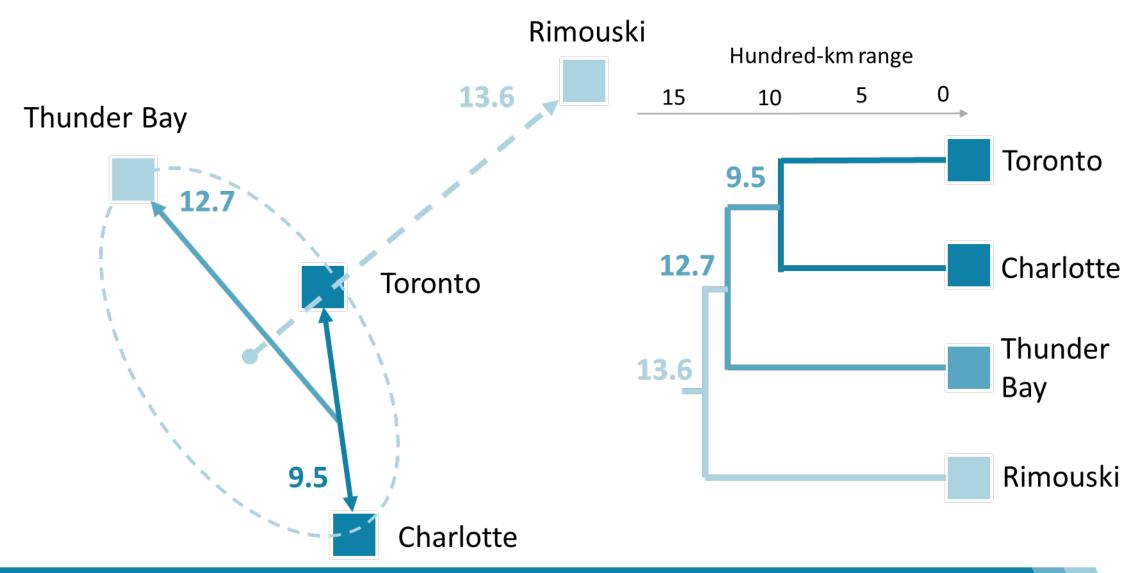
Genetic relatedness can be viewed on an evolutionary tree ("dendrogram")

- 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

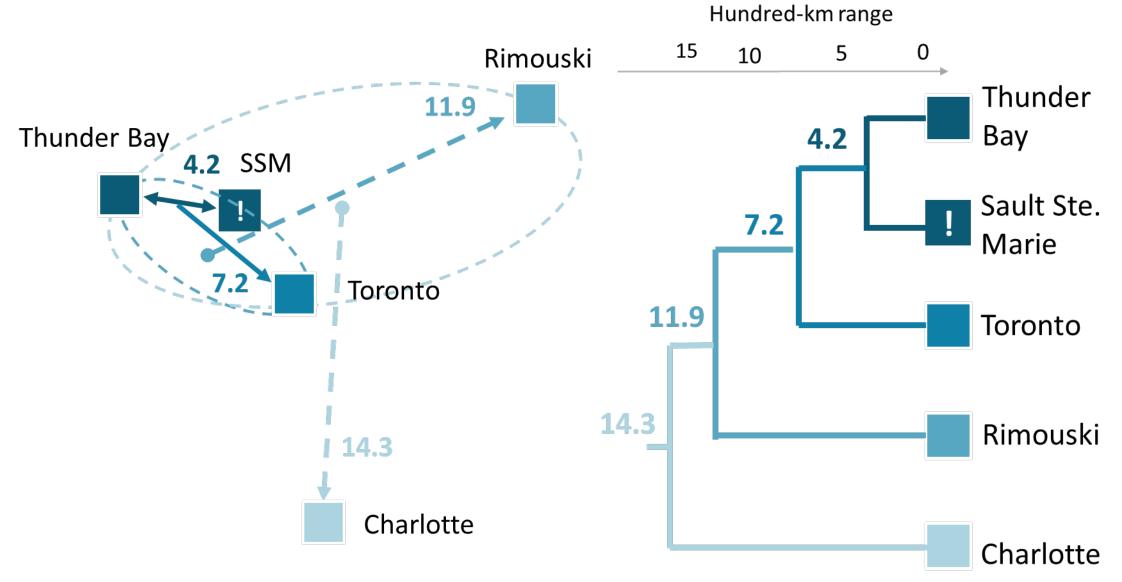
   between an isolate and its next
   closest relative neighbour(s) on the tree.



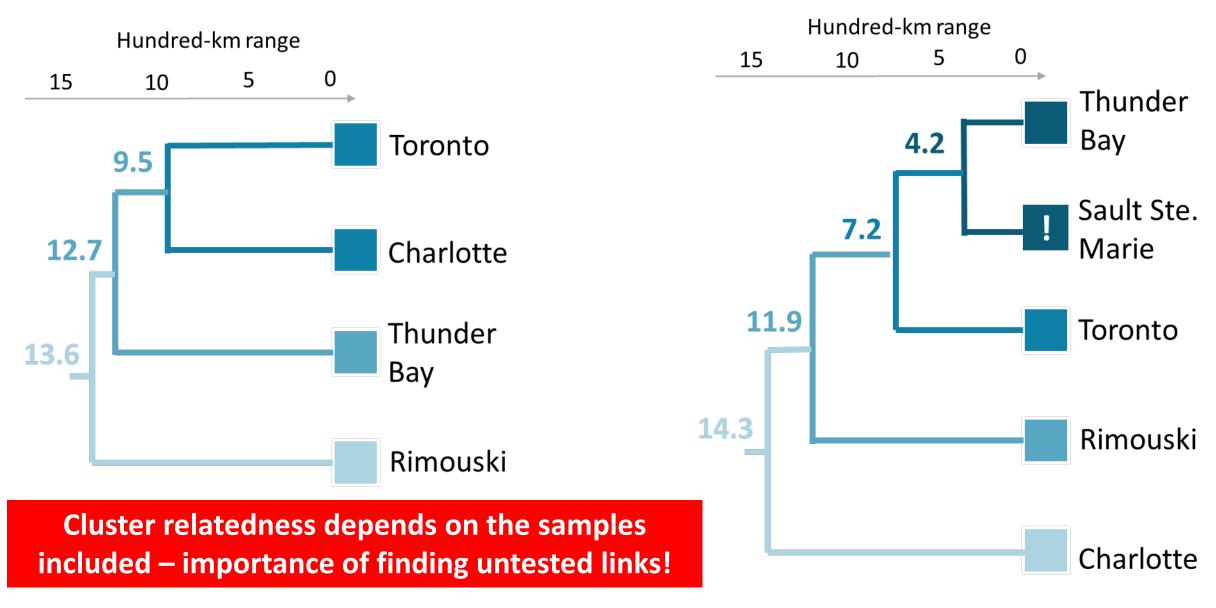
# **Analogy Example: Physical Clustering (1/2)**



#### **Analogy Example: Physical Clustering (2/2)**



#### Versus



# The PulseNet Screening Criteria for Potential Cluster Identification

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

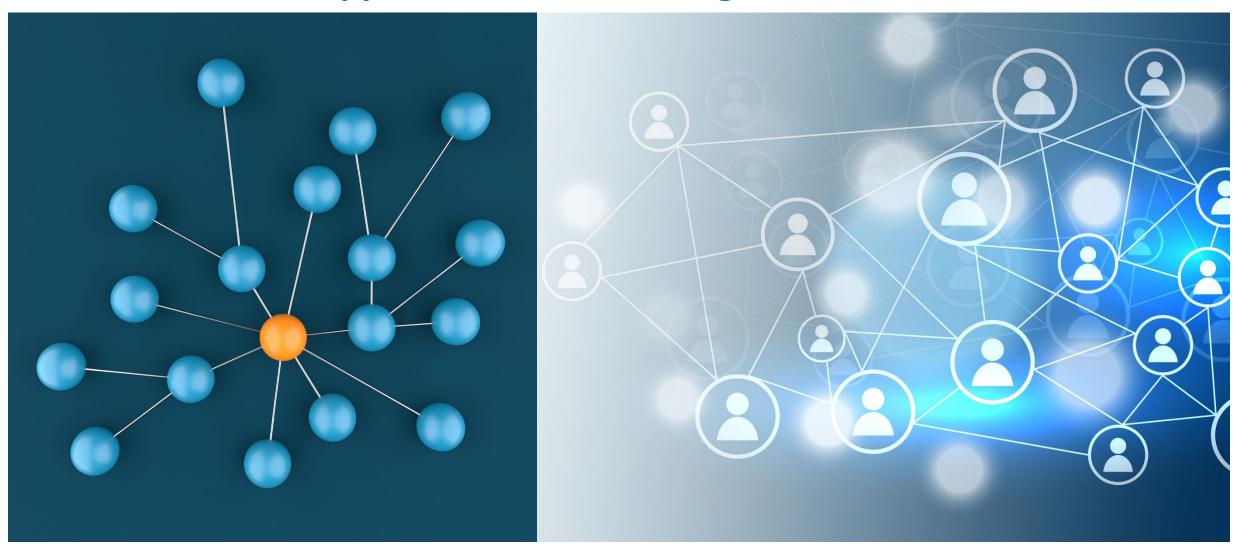
*Salmonella* Thompson cluster initially from June 2019 in QC but with multiprovincial cases.

#### **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 <u>not</u> eating pork, <u>not</u> eating shredded pork products, or <u>ate pork</u> but the location of purchase and supplier information are <u>from</u> <u>an approved</u> source

#### **Green Isolates**:

**Outbreak Cases** 

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

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	114	21M61297	anadappenter	ON	icheda Icheda	Infantia Infantia	2021-06-18		Food Human	Bi Tuoi pork rind + shoulder Biosi	Maie		18028/NW05-1MP	
-1	1115	21561137	010142808	ON	intentis	Infantia	2021-09-02		Human	Ghaoi	Male	13	100001WW05-1MP	
	1112	21M61258 . 21M61209 .	0101036430	ON ON	Salmonella Infantis	Infantia Infantia	2021-08-17 2021-06-14		Human Fool	fitted park rind with park skin	Male	29	18038/NW06-1MP	Include 1
		21M61344		ON ON	infantia Infantia	Infantia Infantia	2021-09-28		Human Food	Oboi poti mid with pork skin + spices	Female	59	180351W/06-1MP 180251W/06-1MP	insiste 1
	I IIF	21M01108		ON	Infanta	Infantia	2021-06-02		Human	thesi	Female	14	1828NW05-1MP	
		21M61202	0101070414	ON ON	inferda Inferda	Infantia Infantia	2021-09-14 2021-10-15		Food Human	pork rind Litre	Famile	58	1825NWQ5-1MP 1825NWQ5-1MP	Include 2
		21M61299 . 20M61370 .	200.0825478	ON ON	infanta Infanta	Infentia Infentia	2021-08-18		Food Human	mixed park drops + park skins (BI) Studi	Familie	47	18058NW06-1MP	
		20461329 . 8420204037	2000025428	ON .	intenta	infantia. Infantia	2020-06-04	2020-09-01	Human	65xxi Chicken	Male Unknown	72	1805NW05-1MP	Unitowen
		TOMICOTON .	1901068606	ON	Infertia	Infantia	2010-11-11		Human	Unine	Female	- 91	1825NW05-1MP	
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	_از	8A20201618 . 8A20104710 .	25-1890-3 15-15040 B	MD	intents	Infantia Infantia	2020-02-19 2019-10-02	2005-02-04	Animal	Chicken	Unknown		1803BNW05-1MP 1803BNW05-1MP	Unknown Unknown
	[L_	MB-21025543	21029043	NG ND	Indentia Indentia	Infantia.	2021-06-06 2025-10-15	2021-05-04	HUMAN	STOOL STOOL	FEMALE	81 28	180361W05-1MP	
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	140	20M61941 . 20M61744 .	20N0402783 2001066796	ON ON	Infantis Infantis	Infantia Infantia	2025-12-08		Human	Unine Bitaol	Female Female	50 78	180281W906-1MP	Same patient as 20M01548
I	-	Rh200001931 . Rh200000425		A0 A0		Infantia							1803BNW05-1MP	
		RN200002509		AB		Infantis							18098NW05-1MP	
4	L.T	RN20000451 . RN200002403 .		A0		infantia Infantia							18055NW05-1MP 18025NW05-1MP	
	L	RM200000854 . 20ME1478 .	201/0250960	All	Infants	Infantia Infantia	2020-09-15		Human	Stavi	Male	67	18035NW05-1MP	
	<u> </u>	20400089	2000014912	ON	Infandia	Infanta	2020-07-13		Human	tituoi	Male	1	1828/W/06-1MP	32

Variation in Alleles Range (1/2) (Representative set of 12 outbreaks used to validate *Salmonella* WGS)

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.

		# of isolate	s included	# of allele
Salmonella Serotype	Suspect Outbreak Source	Human	Non- human	differences among isolates
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

33

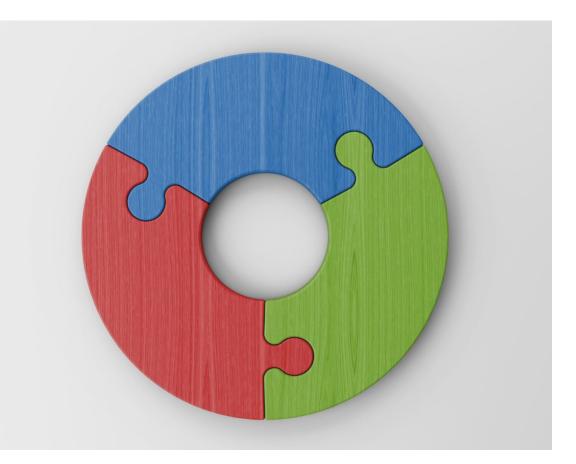
# Variation in Alleles Range (2/2) (Representative set of 10 outbreaks used to validate STEC WGS)

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: <u>https://doi.org/10.1186/s12864-018-5243-3</u>

		# of isolat	es included	# of allele
STEC Serotype	Suspect Outbreak Source	Human	Non- human	differences among isolates
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

#### **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

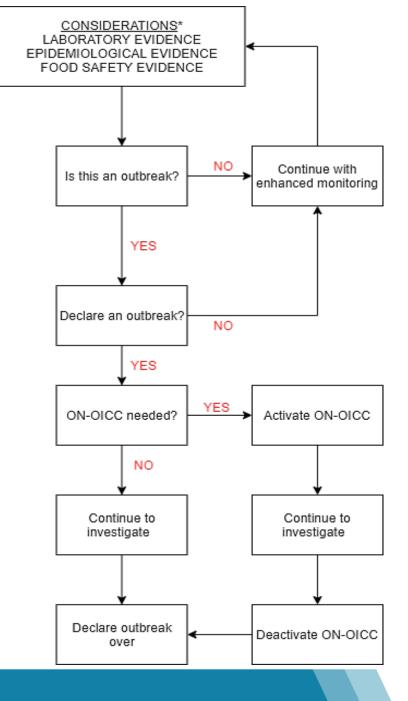
#### **Notification of WGS Results**

- 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

 $\rightarrow$  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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