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The Monthly Infectious Diseases Surveillance Report is produced by Public Health Ontario (PHO) for the public health community of Ontario. We welcome feedback by email to: cdepr@oahpp.ca. Past issues and additional information are available [online](#).

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IN FOCUS

Molecular typing of foodborne pathogens

Public health officials leverage many data sources when investigating cases of bacterial foodborne illness, such as case interviews, clinical data, food safety findings and laboratory results. Each type of data provides additional evidence to identify the source and understand the spread of infections, and ultimately to inform interventions that can prevent illness in the population. In the case of foodborne illness outbreaks associated with widely distributed food products, laboratory-derived molecular typing can confirm links among seemingly unrelated cases by differentiating background (or sporadic) cases from outbreak cases, as well as confirming linkages between cases and food sources where epidemiological evidence is scant.^{1,2,3} Numerous molecular typing methods exist, but they all aim to describe the relatedness among organisms for either surveillance or outbreak investigation purposes. The objective of this article is to provide a general understanding of:

- the current molecular typing tools available for bacterial foodborne outbreak investigations; and
- the microbiology of current molecular typing tools.

Phage typing (PT)

Phage typing is a subtyping method that was commonly used prior to the introduction of molecular typing methods. Phage typing continues to have some utility as an initial typing method and is routinely used for certain pathogens such as *Salmonella* Enteritidis. The microbiological basis of phage typing is found in bacteriophages, which are viruses that are able to infect specific bacteria. When cultured, the infected bacteria produce a unique visual pattern of lysis that is then interpreted as a numerical designation, such as PT4, PT8, PT13a, etc.

Pulsed-field gel electrophoresis (PFGE)

PFGE is the most common molecular typing method used in Ontario for bacterial foodborne pathogens. It is routinely used for *Listeria monocytogenes*, verotoxin-producing *Escherichia coli* and certain *Salmonella* serotypes.⁴ This method relies on properties of the bacteria's DNA that change over time at a rate that allows related strains to be grouped together by PFGE. Briefly, the bacterial DNA is "cut up" by enzymes that recognize specific sites in the DNA. The digested or "cut up" DNA is then placed on a gel with pulsed electrical charges for approximately 24 hours to separate the DNA based on the size of the fragments: the smaller, lighter fragments will travel farther on the gel than the heavier fragments. The gel is photographed and the number and relative position of fragments generate a pattern or molecular fingerprint for comparison to other isolates.

Multi-locus variable number of tandem repeat analysis (MLVA)

Compared to PFGE, MLVA is a newer typing method that is increasingly being used for characterizing *E. coli* O157:H7 isolates in Canada.⁵ Instead of relying on “cut sites” to generate patterns for comparison, MLVA looks at specific areas in a particular organism’s bacterial genome that contain repeating elements. The number of repeats in each of these areas varies with replication, which generates distinct patterns to assess for relatedness of strains. The advantage of MLVA over PFGE is that the repeating element counts are absolute and are easier to generate and compare than the more subjective visual patterns created by PFGE.⁶

Whole-genome sequencing (WGS)

WGS involves looking at the bacterial DNA in its entirety (the genome) and describing all of the differences that exist among the strains that are being compared. Characterizing the entire genome allows for examination in the highest detail of relatedness and can also yield information about other characteristics of the organism, such as antibiotic susceptibility and virulence.⁷ These additional traits are encoded by the DNA but are not routinely a part of the previously described typing methods.

The changing context of molecular typing

This report’s non-exhaustive and brief summary of different typing methods reflects two important underlying principles:

- 1) Technological advances drive the creation and adoption of new methods; and
- 2) Some typing methods are not optimal for certain organisms or specific purposes.

The first principle is analogous to advances made in forensic science whereby criminals were initially identified only by eye witness testimony. Incremental discoveries then led to the development of methods like hair strand comparison, fingerprinting and finally human DNA analysis. The foodborne pathogen molecular typing methods described above evolved in a similar fashion, resulting in greater discriminatory power.

The second principle is rooted in the diversity of DNA structure found in microorganisms. For example, organisms with DNA repetitive elements that change very quickly over time would be poor candidates for characterization by MLVA as there would be too much diversity to make meaningful matches. Moreover, some organisms’ DNA changes so slowly that one PFGE type may be associated with unrelated isolates collected across the entire province over many months.

The ongoing evolution of molecular typing will involve applying new technologies to describe new characteristics of different pathogens’ DNA that more accurately predict whether strains are related to transmission or a common food source. The ongoing assessment of existing methods and their ability to accurately predict relatedness is dependent on close collaboration between laboratories and public

health epidemiologists and investigators. These activities and collaboration are critical for guiding future method development to ensure that public health decisions are based on validated methods and a thorough understanding of the strengths and limitations of their application.

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SIGNIFICANT REPORTABLE DISEASE ACTIVITY

Table 1 provides a list of reportable diseases for which incidence in 2016 was found to be significantly higher ($p < 0.05$) than expected compared to the five-year historical average (2011–15). Both monthly and year-to-month (YTM) comparisons were made for each of the reportable diseases listed in [Appendix 1](#), with the exception of influenza, measles, rubella, and congenital rubella syndrome. Influenza surveillance data are regularly reported through the [Ontario Respiratory Pathogen Bulletin](#). Measles, rubella, and congenital rubella syndrome have been eliminated in Canada, although cases continue to occur related to travel importations. Statistical comparisons are not included for these diseases.

Table 1. Summary of statistically significant increases in reportable disease incidence: Ontario, January 1 to April 30, 2016

Reportable disease	2016				Historical comparisons						5-year avg annual count (2011–2015)
	Apr	Apr rate ‡	YTM	YTM rate ‡	Current month 5-year avg (2011–2015)	Current month 5-year avg (2011–2015) rate ‡	% difference in rates (current month minus 5-year avg)†	YTM 5-year avg (2011–2015)	YTM 5-year avg (2011–2015) rate ‡	% difference in rates (YTM 2016 minus YTM 5-year avg)†	
Chlamydial Infections ¹	3209	230.1	13533	970.2	3068.0	226.5	1.6	12175.2	899.0	7.9	36533.4
Gonorrhoea (All Types) ^{1,2}	553	39.6	2117	151.8	367.8	27.2	46.0	1543.8	114.0	33.1	4923.2
Listeriosis ^{1,2}	13	0.9	31	2.2	2.4	0.2	425.9	11.0	0.8	173.6	52.0
Pertussis (Whooping Cough) ^{1,2}	67	4.8	143	10.3	26.8	2.0	142.7	92.0	6.8	50.9	516.8
Salmonellosis ¹	206	14.8	1010	72.4	229.8	17.0	-13.0	882.4	65.2	11.1	2813.4
Shigellosis ¹	21	1.5	136	9.7	19.0	1.4	7.3	81.4	6.0	62.2	263.8
Syphilis, Infectious ¹	64	4.6	359	25.7	71.6	5.3	-13.2	278.0	20.5	25.4	856.2
Yersiniosis ¹	21	1.5	96	6.9	15.6	1.2	30.7	64.4	4.8	44.7	185.0

Ontario Cases: Ministry of Health and Long-Term Care (MOHLTC), integrated Public Health Information System (iPHIS) database, extracted by PHO [2016/05/11].

Ontario Population: Population estimates [2005–2011]: Statistics Canada, distributed by MOHLTC, received [2014/07/03]. Population estimates [2012–2014]: Statistics Canada, distributed by MOHLTC, received [2015/11/18]. Population projections [2015–2016]: MOHLTC, IntelliHEALTH Ontario, extracted by PHO [2015/03/13].

‡ Rates listed are cases per 1,000,000 population.

† Percent (%) difference is calculated using unrounded rates; numbers displayed in these columns may vary from calculations using rounded rates.

¹ Statistically significant difference ($p < 0.05$) in incidence reported in year-to-month (January 1 to April 30, 2016) compared to the five-year historical average (January 1 to April 30, 2011–15), using a likelihood ratio test.

² Statistically significant difference ($p < 0.05$) in incidence reported in current month (April 2016) compared to the five-year historical average (April 2011–15), using a likelihood ratio test.

Chlamydia

Compared to the five-year historical average, there was a statistically significant increase of 7.9% in the YTM incidence of laboratory-confirmed chlamydial infections reported between January 1, 2016 and April 30, 2016 (from 899.0 cases per 1,000,000 population to 970.2 cases per 1,000,000 population).

Over the past ten years, the incidence of chlamydial infections has increased with the exception of 2013, when a decrease in the incidence and testing was observed. For further details about this decrease, please refer to [Reportable Disease Trends in Ontario, 2013](#). PHO will continue to monitor provincial chlamydia epidemiology in subsequent months.

Gonorrhea

Since September 2013, there have been statistically significant increases in both the monthly and YTM incidence of gonorrhea in Ontario. The cause of this ongoing provincial increase is not yet well understood and is likely multifactorial. For a summary of the analyses conducted to date, please refer to the In Focus section of the [February 2015](#) issue of this report. Also, to download the slides and/or audio recording of a PHO Rounds presentation on gonorrhea (May 2015), please click [here](#).

Listeriosis

Incidence rates for listeriosis for the first four months of 2016 (January to April) were significantly higher in comparison to the monthly and YTM average incidence rates for the same period in the previous five years. From January to April 2016, 31 cases of listeriosis were reported compared to an expected average of 11 cases. The increase in 2016 can be attributed to two separate outbreaks that began in 2015. The source of one outbreak, which was part of a federally-led investigation, was linked to certain Dole and PC Organics brands of pre-packaged leafy green products that were [recalled](#) in January 2016. The second outbreak, which was confined to Ontario, has been linked to Neilson brand Partly Skimmed Chocolate Milk that was [recalled](#) in June 2016. Additional details about this outbreak are available on the PHO [listeriosis webpage](#).

Pertussis (Whooping Cough)

Increases in both the monthly and YTM incidence rates of pertussis were observed in April 2016. These increases can be attributed to a pertussis outbreak that has been ongoing in a well-defined community located in northeastern Ontario (Algoma Public Health Unit) since March 2016. To date, all disease transmission in relation to this outbreak has occurred within the boundaries of a single public health unit (PHU). As of May 12, 2016, 45.1% of all pertussis cases reported in Ontario since January 1, 2016 were associated with this single outbreak. In comparison, the PHU reporting the outbreak comprised 0.8% of Ontario's population in 2015. Pertussis is a disease with a cyclical pattern, with peaks occurring every two to five years. In Ontario, peaks in incidence occurred in 2006, 2012 and 2015.

Salmonellosis

A statistically significant increase in the YTM incidence rate of reported cases of salmonellosis was observed in April 2016. The cause of the increase is unknown at this time. PHO continues to monitor serotype-specific trends and investigate any increases in order to identify potential clustering.

Shigellosis

Incidence rates for shigellosis continue to be higher than expected in 2016. The YTM average incidence rate for 2016 was significantly higher than the average for the same period in the previous five years. From January to April 2016, 136 cases of shigellosis were reported compared to an expected average of 81 cases. The source of the increase was described in the [May 2016](#) issue of this report. PHO is collaborating with PHUs affected by this increase to identify risk factors, at-risk populations, and prevention and control measures for implementation.

Syphilis, Infectious

Between January 1 and April 30, 2016, 359 cases of infectious syphilis were reported for a YTM incidence of 25.7 cases per 1,000,000 population. This represents a statistically significant increase of 25.4% compared to the five-year historical average for the same time period (20.5 cases per 1,000,000 population).

Yersiniosis

A statistically significant increase in the YTM incidence rate of reported cases of yersiniosis was observed in April 2016. The cause of the increase is unknown at this time. PHO continues to monitor trends and investigate to identify potential clustering.

APPENDIX – REPORTABLE DISEASES

Appendix 1. Confirmed cases of reportable diseases, and probable cases of select reportable diseases, by month: Ontario, 2011–16*

Reportable disease	2016														Historical comparisons						5-year avg (2011-2015) annual count
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	YTM	YTM rate ‡	Current month 5-year avg (2011-2015)	Current month 5-year avg (2011-2015) rate ‡	% difference rates (current month minus 5-year avg)†	YTM 5-year avg (2011-2015)	YTM 5-year avg (2011-2015) rate ‡	% difference rates (YTM 2016 minus YTM 5-year avg)†	
Acute Flaccid Paralysis	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
AIDS	7	4	6	2	-	-	-	-	-	-	-	-	19	1.4	4.6	0.3	-57.8	29.2	2.2	-36.8	84.4
Amebiasis	55	70	76	71	-	-	-	-	-	-	-	-	272	19.5	75.2	5.6	-8.3	277.2	20.5	-4.7	798.2
Botulism	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.6	0.0	-100.0	0.8	0.1	-100.0	2.4
Brucellosis	0	0	1	0	-	-	-	-	-	-	-	-	1	0.1	0.4	0.0	-100.0	1.2	0.1	-19.1	5.4
Campylobacter enteritis	187	165	182	158	-	-	-	-	-	-	-	-	692	49.6	211.4	15.6	-27.4	825.2	60.9	-18.6	3689.4
Chlamydial Infections	3635	3251	3438	3209	-	-	-	-	-	-	-	-	13533	970.2	3068.0	226.5	1.6	12175.2	899.0	7.9	36533.4
Cholera	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.0	0.0	-	0.0	0.0	-	0.4
Cryptosporidiosis	20	23	21	22	-	-	-	-	-	-	-	-	86	6.2	14.4	1.1	48.3	64.6	4.8	29.3	331.2
Cyclosporiasis	1	0	1	1	-	-	-	-	-	-	-	-	3	0.2	9.0	0.7	-89.2	17.4	1.3	-83.3	135.0
Encephalitis	6	2	3	1	-	-	-	-	-	-	-	-	12	0.9	1.0	0.1	-2.9	5.8	0.4	100.9	21.4
Encephalitis/Meningitis	9	13	12	11	-	-	-	-	-	-	-	-	45	3.2	9.2	0.7	16.1	35.8	2.6	22.0	157.2
Food Poisoning, All Causes	4	1	2	1	-	-	-	-	-	-	-	-	8	0.6	4.6	0.3	-78.9	16.0	1.2	-51.5	76.6
Giardiasis	89	82	68	58	-	-	-	-	-	-	-	-	297	21.3	95.6	7.1	-41.1	411.6	30.4	-29.9	1333.6
Gonorrhoea (All Types)	561	472	531	553	-	-	-	-	-	-	-	-	2117	151.8	367.8	27.2	46.0	1543.8	114.0	33.1	4923.2
Group A Streptococcal Disease, Invasive	76	71	78	58	-	-	-	-	-	-	-	-	283	20.3	61.8	4.6	-8.9	266.6	19.7	3.1	640.8
Group B Streptococcal Disease, Neonatal	8	2	6	1	-	-	-	-	-	-	-	-	17	1.2	3.8	0.3	-74.5	16.2	1.2	1.9	52.8
Haemophilus Influenzae B Disease, Invasive	1	0	0	1	-	-	-	-	-	-	-	-	2	0.1	0.6	0.0	61.8	1.8	0.1	7.9	5.8
Hepatitis A	4	4	17	8	-	-	-	-	-	-	-	-	33	2.4	6.6	0.5	17.7	35.2	2.6	-9.0	96.8
Hepatitis B (Acute)	10	9	4	6	-	-	-	-	-	-	-	-	29	2.1	9.4	0.7	-38.0	38.0	2.8	-25.9	101.6
Hepatitis B (Chronic)	123	99	95	57	-	-	-	-	-	-	-	-	374	26.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Hepatitis C	379	373	377	314	-	-	-	-	-	-	-	-	1443	103.5	363.8	26.9	-16.2	1424.0	105.1	-1.6	4215.0
HIV	68	57	59	46	-	-	-	-	-	-	-	-	230	16.5	64.6	4.8	-30.9	255.8	18.9	-12.7	781.4
Influenza	743	3844	5181	1535	-	-	-	-	-	-	-	-	11303	810.3	754.6	55.7	97.5	5868.6	433.3	87.0	8386.6
Legionellosis	5	9	2	5	-	-	-	-	-	-	-	-	21	1.5	3.0	0.2	61.8	18.4	1.4	10.8	173.6
Leprosy	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.2	0.0	-100.0	1.2	0.1	-100.0	3.2
Listeriosis	8	5	5	13	-	-	-	-	-	-	-	-	31	2.2	2.4	0.2	425.9	11.0	0.8	173.6	52.0

Reportable disease	2016														Historical comparisons						5-year avg (2011-2015) annual count
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	YTM	YTM rate ‡	Current month 5-year avg (2011-2015)	Current month 5-year avg (2011-2015) rate ‡	% difference rates (current month minus 5-year avg)†	YTM 5-year avg (2011-2015)	YTM 5-year avg (2011-2015) rate ‡	% difference rates (YTM 2016 minus YTM 5-year avg)†	
Lyme Disease	0	0	3	1	-	-	-	-	-	-	-	-	4	0.3	6.2	0.5	-84.3	14.0	1.0	-72.3	255.4
Malaria	12	7	12	8	-	-	-	-	-	-	-	-	39	2.8	13.4	1.0	-42.0	45.4	3.4	-16.6	204.4
Measles	1	1	1	0	-	-	-	-	-	-	-	-	3	0.2	#	#	#	#	#	#	#
Meningitis	11	12	15	6	-	-	-	-	-	-	-	-	44	3.2	8.2	0.6	-29.0	28.2	2.1	51.5	128.6
Meningococcal Disease, Invasive	3	1	0	4	-	-	-	-	-	-	-	-	8	0.6	2.4	0.2	61.8	13.0	1.0	-40.3	31.4
Mumps	0	2	1	2	-	-	-	-	-	-	-	-	5	0.4	2.2	0.2	-11.7	7.2	0.5	-32.6	35.0
Ophthalmia neonatorum	0	1	0	2	-	-	-	-	-	-	-	-	3	0.2	0.4	0.0	385.4	1.4	0.1	108.0	2.8
Paralytic Shellfish Poisoning	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Paratyphoid Fever	3	7	1	2	-	-	-	-	-	-	-	-	13	0.9	5.6	0.4	-65.3	19.2	1.4	-34.3	42.0
Pertussis (Whooping Cough)	44	12	20	67	-	-	-	-	-	-	-	-	143	10.3	26.8	2.0	142.7	92.0	6.8	50.9	516.8
Q Fever	1	0	0	0	-	-	-	-	-	-	-	-	1	0.1	1.6	0.1	-100.0	4.4	0.3	-77.9	16.4
Rabies	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.2	0.0	-100.0	0.2	0.0	-100.0	0.2
Rubella	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	#	#	#	#	#	#	#
Rubella, Congenital Syndrome	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	#	#	#	#	#	#	#
Salmonellosis	279	252	273	206	-	-	-	-	-	-	-	-	1010	72.4	229.8	17.0	-13.0	882.4	65.2	11.1	2813.4
Shigellosis	34	33	48	21	-	-	-	-	-	-	-	-	136	9.7	19.0	1.4	7.3	81.4	6.0	62.2	263.8
Streptococcus Pneumoniae, Invasive	99	98	129	86	-	-	-	-	-	-	-	-	412	29.5	118.4	8.7	-29.5	465.0	34.3	-14.0	1135.4
Syphilis, Early Congenital	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.0	0.0	-	0.4	0.0	-100.0	1.6
Syphilis, Infectious	113	81	101	64	-	-	-	-	-	-	-	-	359	25.7	71.6	5.3	-13.2	278.0	20.5	25.4	856.2
Syphilis, Other	58	51	48	46	-	-	-	-	-	-	-	-	203	14.6	59.0	4.4	-24.3	231.4	17.1	-14.8	658.6
Tetanus	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.0	0.0	-	0.6	0.0	-100.0	2.0
Tuberculosis	44	38	48	44	-	-	-	-	-	-	-	-	174	12.5	56.4	4.2	-24.3	199.8	14.8	-15.4	619.2
Tularemia	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.0	0.0	-	0.0	0.0	-	0.4
Typhoid Fever	11	7	6	5	-	-	-	-	-	-	-	-	29	2.1	8.4	0.6	-42.2	32.0	2.4	-12.0	76.2
Verotoxin Producing E. coli Including HUS	4	5	9	8	-	-	-	-	-	-	-	-	26	1.9	9.8	0.7	-20.7	29.8	2.2	-15.3	176.4
West Nile Virus Illness	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.0	0.0	-	0.0	0.0	-	91.2
Yellow Fever	0	0	0	0	-	-	-	-	-	-	-	-	0	0.0	0.0	0.0	-	0.4	0.0	-100.0	1.2
Yersiniosis	28	24	23	21	-	-	-	-	-	-	-	-	96	6.9	15.6	1.2	30.7	64.4	4.8	44.7	185.0

Ontario Cases: MOHLTC, iPHIS database, extracted by PHO [2016/05/11].

Ontario Population: Population estimates [2005–2011]: Statistics Canada, distributed by MOHLTC, received [2014/07/03]. Population estimates [2012–2014]: Statistics Canada, distributed by MOHLTC, received [2015/11/18]. Population projections [2015-2016]: MOHLTC, IntelliHEALTH Ontario, extracted by PHO [2015/03/13].

* Appendix 1 is not an exhaustive list of all reportable diseases in Ontario. Case counts for amebiasis, Lyme disease, mumps, pertussis, and West Nile Virus illness are based on the sum of confirmed and probable cases as reported in iPHIS.

‡ Rates listed are cases per 1,000,000 population.

† Percent (%) difference is calculated using unrounded rates; numbers displayed in these columns may vary from hand calculations using rounded rates.

- #** Historical comparison data are not provided for measles, rubella, and congenital rubella syndrome because these diseases have been eliminated in Canada. However, as these diseases remain endemic in other countries, imported and import-related cases continue to occur in Ontario.
- n/a** Acute Flaccid Paralysis and Paralytic Shellfish Poisoning became reportable in Ontario in December 2013. No historical data are available for comparisons. Also, a provincial case definition for chronic hepatitis B was released in January 2012. Please note that chronic and acute hepatitis B case counts are not mutually exclusive and should not be added to obtain a total for hepatitis B cases in Ontario. Historical comparisons are not available as cases of chronic hepatitis B may have been entered using varying criteria prior to this time.
- Does not include cases for which the Ministry of Health and Long-Term Care was selected as the Diagnosing Health Unit or cases with a Disposition Description set to “DOES NOT MEET” or “ENTERED IN ERROR.”
 - Differentials in year-over-year comparisons are reflective of changes in disease incidence and changes in the size of the population.
 - Statistical tests comparing rates were not performed when the YTM rate in previous years was zero.
 - Case counts for tuberculosis and AIDS are based on diagnosis date and not episode date. HIV case counts are based on encounter date. Case counts for all other diseases are based on episode date.