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Mycobacterium tuberculosis Surveillance in Canada

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Introduction

The causative agent of human tuberculosis (TB), *Mycobacterium tuberculosis* (MTB), is a member of the MTB complex. The members of the MTB complex are given species designations based on host preference along with evolutionary and taxonomic status (1, 2, 3). TB was thought to be a disease prevalent in developing nations or in resource-poor settings. However, due to open borders and the predominance of immunological diseases such as HIV/AIDS, many parts of the world have experienced a resurgence of TB disease and more critically the emergence of multiple drug resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) (4, 5). Globally, in 2009, there were an estimated 9.4 million new cases of TB with 1.3 million deaths, with the majority occurring in developing countries (4). It has been estimated that each untreated TB case will infect, on average, another 10-15 people in a year (4). Therefore, the public health response to modern day TB control is to have an effective preventive strategy, early clinical and laboratory diagnosis, better treatment and an effective vaccine.

The total number of reported cases of active TB disease in Canada averages approximately 1,600 newly active and re-treatment cases annually. In 2008 there were 1,645 reported cases, yielding a case rate of 4.9 per 100,000 population. In 2008, British Columbia, Ontario and Quebec accounted for 69% of the all TB cases in Canada but Nunavut had the highest rate of TB at 186.6 per 100,000

Key Points

- The total number of reported cases of active tuberculosis (TB) in Canada averages approximately 1,600 newly active and re-treatment cases annually, with the highest rate of TB in Aboriginal populations.
- TB genotyping is a useful adjunct tool for TB surveillance and outbreak investigation, especially in cases where epidemiological data are inadequate or unavailable.
- The traditional gold standard TB genotyping method had been “Restriction Fragment Length Polymorphism” (RFLP); however, it has now been replaced by the newer, more reliable method of “Mycobacterial Interspersed Repeat Units-Variable Number Tandem Repeats” (MIRU-VNTR).
- The National Reference Centre for Mycobacteriology, within the National Microbiology Laboratory of the Public Health Agency of Canada (PHAC), provides reference and diagnostic services as well as technical assistance (e.g. technology transfer and personnel training) to provincial TB laboratories.
- An interactive TB genotyping reference database has been proposed for the national coordination of conventional and molecular epidemiology of TB. This proposal was endorsed by the Canadian Tuberculosis Committee (CTC).
- There is currently no established selection criterion in Canada to determine which TB samples should be genotyped.
- Surveillance data on TB cases and TB drug resistance patterns are collected and analyzed annually by PHAC. However, national TB reporting is hampered by the lack of in-depth case demographic and epidemiological information and inconsistency in the type of information collected.

population. Of all TB cases reported in Canada, foreign-born (FB) individuals account for 65% of the cases while Canadian-born non-Aboriginals (CB-NA) and Canadian-born Aboriginals (CB-A) account for 13% and 21% of the cases respectively. The rate of TB in FB, CB-NA and CB-A groups is 14.5, 0.9 and 28.6 per 100,000 population respectively,

demonstrating that the highest rate of TB is in the Canadian-born Aboriginal population (6, 7).

National Reference Centre for Mycobacteriology

The National Reference Centre for Mycobacteriology (NRCM) is a national TB reference laboratory within the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC), located in Winnipeg. The NRCM is a state-of-the-art containment level-3 laboratory that provides esoteric mycobacteriology testing services in Canada and provides support to all provincial mycobacteriology laboratories in MTB testing. The testing services not offered at the provincial level may either be due to the lack of capacity, resources or expertise. In addition, the NRCM also provides consultation services, offers proficiency testing panels to provincial counterparts, facilitates technology transfer, and validates new technologies. The NRCM conducts both basic and diagnostic research with its team of research scientists in both TB disease and TB-HIV co-infection. The NRCM also partners with other national and international organizations on research projects and provides outbreak support, laboratory capacity assessment and testing recommendations for laboratories.

MTB Genotyping

An extremely useful tool in the field of molecular epidemiology is bacterial strain typing which analyzes genetic information of the pathogen in infected individuals (1, 8). A database can be developed whereby strain patterns are used as clues to identify different lineages or members of the MTB complex, emerging antibiotic resistance, and most importantly, identification of an outbreak. TB Prevention and Control (TBPC), Centre for Communicable Diseases and Infection Control (CCDIC), PHAC, Ottawa and the NRCM, along with provincial counterparts work collaboratively in the comprehensive analysis of TB disease statistics in Canada. Molecular typing of MTB is an important adjunct tool in outbreak investigations, long term surveillance projects and in the identification of laboratory contamination, especially in cases where epidemiological data are inadequate or unavailable. In keeping with the international algorithms, the NRCM has now implemented a PCR- based 24

MIRU-VNTR loci method that has proven to be advantageous resulting in rapid test turnaround times and highly discriminatory genotyping results as compared to previous testing technologies. In cases where epidemiological data are unavailable or incomplete a high level of discriminatory power from typing data is of the utmost importance to help guide public health officials in the right direction for TB control with a reduction in a number of false leads.

Genotyping Methodologies

Within the microbial world, the genomes of the members of MTB complex are remarkably homologous (1, 9). However, current typing methods explore some of the unique aspects of the MTB complex genome with regard to the variable pattern of transposable insertion sequences, spacer regions between conserved direct repeats, as well as the number of allelic units within a specific locus (10, 11, 12, 13, 14).

The traditional gold standard typing method is a Restriction Fragment Length Polymorphism (IS6110-RFLP) method based on the 1,355 base pair transposable insertion sequence IS6110 (12). Like all transposons, this genetic element is mobile and thus capable of replicating and inserting itself in various locations throughout the genome in variable numbers. Based on positional and numerical polymorphism, strains are designated to be similar or different (12). While this traditional method has been highly discriminatory, it has major shortcomings. Major disadvantages are the labour-intensive nature of the technique, long test turnaround times and variation in both intra- and inter-laboratory interpretation of banding patterns. Finally, this method does not provide adequate discriminatory power for strains that have less than six IS6110 copy numbers (11, 14). In the latter case, a secondary method must be performed, further delaying the process. With such shortcomings, this traditional method is now deemed unsuitable in meeting the current epidemiological demands for rapidly tracking MTB outbreaks, as well as aiding in real-time outbreak management in affected communities.

The spacer oligonucleotide typing (spoligotyping) method is based on the direct repeat locus in the MTB complex genome where multiple, identical 36

base pair direct repeats are present and separated by unique spacer regions ranging from 35-41 base pairs (13). While the order of these unique spacers is conserved in all strains, they can be deleted due to homologous recombination between the direct repeat regions, disrupted by *IS6110* insertions, or can be polymorphic due to nucleotide mutations. To detect these polymorphisms, primers based on direct repeat regions are used to amplify the spacer regions. After amplification, the labelled spacer fragments are allowed to hybridize to each of the standard 43 unique spacer regions. A positive/negative hybridization pattern is generated that indicates which of the direct repeat spacer region have been changed (negative or '0') and which are conserved (positive or '1'). Although the spoligotyping method is rapid, reliable, and easily interpretable, it lacks the highly specific discriminatory power of *IS6110*-RFLP for strains that contain more than 6 copies of *IS6110*. Therefore, it is used in conjunction with other typing methods including *IS6110*-RFLP and MIRU-VNTR.

The PCR-based assay, Mycobacterial Interspersed Repeat Units-Variable Number Tandem Repeats (MIRU-VNTR), is based on the presence of loci throughout the genome that contain a variable number of 40-100 base pair repeat units (10, 11). Methodologies using 12, 15 or 24 of these VNTR loci have been developed for MTB genotyping. A 3 loci multiplex PCR assay with a total of 24 loci is the most commonly used to obtain highly discriminatory results (14, 15, 16). After amplification, fragment analysis is performed using capillary electrophoresis to analyze the size of each amplicon. Finally, a number is assigned to each locus based on the size of the fragment detected and a standardized sizing scheme (11). Through the generation of this 24 digit pattern, high-resolution strain typing can be achieved in a fast, reliable, and easily interpretable manner (11). Recent studies have shown that 24 loci MIRU-VNTR combined with spoligotyping can match or exceed the discriminatory power of *IS6110*-RFLP (14, 15, 16).

Technology Transfer

Semi-automated 24-loci MIRU can be used by any laboratory in Canada that has access to PCR equipment and a DNA sequencer for fragment analysis. The NRCM has offered assistance for technology transfer to its provincial counterparts

and some of the laboratories have sent personnel for training on-site at the NRCM laboratory. The capability of MIRU to be adopted by provincial PH laboratories exists and is in keeping with rapidly evolving international algorithms (11, 15).

Genotyping Algorithm

A multicentre genotyping study was conducted in Canada whereby four Canadian provinces participated and contributed to the development of a current genotyping algorithm for the NRCM (14). As per the Centers for Disease Control and Prevention (CDC) algorithm, 24-loci MIRU and spoligotyping should be used as the primary genotyping algorithm, with *IS6110*-RFLP being done on clustered cases (15). Clustered cases in this context are defined as indistinguishable patterns generated using 24-loci MIRU and spoligotyping. The NRCM also performs 24-loci MIRU and spoligotyping for primary MTB genotyping. Following the CDC genotyping standard, *IS6110*-RFLP is not conducted unless warranted, for example, when MIRU and spoligotyping identifies clusters and further differentiation is warranted as public health or epidemiological linkages between these cases are not apparent (15). This requires consultation with the testing laboratory.

The use of MIRU and other genotyping methods have immense advantages in routine surveillance of TB. It provides 'value-added' data to the traditional contact tracing data as circulating MTB strains have a tendency to exist for a number of years and reactivation can occur after many years. A major limitation is that data generated from all genotyped cases is not evaluated or assessed on a regular basis by public health departments due to the lack of expertise, understanding of the data utility or unavailability of data at the public health level.

Proposed National Genotyping Database

The National Canadian Tuberculosis Genotyping Reference Interactive Database (TB-GRID) is a collaborative effort to build a national team with the complementary strengths and capabilities required to create a national typing database based on currently existing international models. A five-year pilot project has been proposed to coordinate conventional and molecular epidemiology of tuberculosis in collaboration with the TBPC, the

NRCM/NML and Provincial TB Laboratories across Canada. The National Genotyping Database proposal was endorsed by the Canadian Tuberculosis Committee (CTC). This system will be interactive with the active user groups within the Canadian Network for Public Health Intelligence or through secure weblinks. Many other international, national and regional genotyping programs utilize a similar unified approach for data analysis. These studies aid in global epidemiological investigation of TB outbreaks, infection control and disease spread.

Test Request and Reporting Procedures at NRCM

The NRCM provides reference and diagnostic services as well as technical assistance to the provincial TB laboratories. The samples are submitted to the NRCM/NML and reports are sent directly to the client laboratories.

In Canada, there is currently no established selection criterion to determine which TB samples should be genotyped, As a result not all positive cultures are genotyped. Some of the provinces conduct their own genotyping testing, some refer all of their MTB samples for genotyping to the NRCM while some refer select investigational/query samples to be genotyped.

Formal Reports on TB in Canada

The Tuberculosis Prevention and Control program at PHAC publishes yearly statistics on TB cases as well as annual reports on the TB drug resistance pattern in Canada. All provincial and territorial TB control programs participate in the national surveillance systems for TB by voluntarily reporting all new and re-treatment cases of active TB disease that meet the Canadian case definition as defined in the Canadian Tuberculosis Standards, 6th edition. For those cases meeting this definition, selected non-nominal, demographic, clinical, radiographic, mycobacteriologic, treatment and outcome data are submitted to the TBPC.

At the federal level there are two systems used to track TB disease: the Canadian Tuberculosis Reporting System (CTBRS) and the Canadian Tuberculosis Laboratory Surveillance System (CTBLSS). The CTBRS collects, on an annual basis, demographic, clinical and treatment-related data

for all cases of active TB disease (not latent TB infection) reported within the preceding year. The data is analysed and the results are published in the annual *Tuberculosis in Canada* report (6). The CTBLSS was specifically designed to capture information on drug susceptibility. The drug sensitivity results for all isolates that are tested are voluntarily submitted to PHAC. This data is analysed and the results are published in the *Tuberculosis Drug resistance In Canada* annual report (7).

All members of the Canadian Tuberculosis Laboratories Technical Network (CTLTN) participate in the NRCM proficiency testing program. In addition to this national initiative, a number of laboratories also participate in other select external proficiency programs such as the College of American Pathologists and the Quality Management Program. The NRCM also participates in external proficiency testing program and is ISO 17025 certified.

Additional epidemiological information on the reported TB cases is desirable to examine more critical drug resistance patterns in Canada. However, challenges exist in the collection of this information as isolates are often submitted to the laboratories with only the sex and year of birth of the individual. As well this data cannot provide differentiation between primary and secondary/acquired drug resistance. With growing worldwide concern regarding resistance and with the emergence of XDR-TB, this surveillance system is vital and provides the necessary data in a timely fashion to monitor trends in TB drug resistance in Canada.

Correctional Service of Canada Report

The Infectious Disease Surveillance in Canadian Federal Penitentiaries report is prepared jointly by the TBPC and Correctional Service of Canada (CSC). This report brings together the surveillance data for a number of communicable diseases, including tuberculosis, among inmates in Canadian federal penitentiaries. All inmates are also offered ongoing assessment upon admission and throughout their incarceration. Staff assessments are conducted by Health Canada. Case reporting forms are submitted to the TBPC, PHAC and are entered into their database and statistics are published on the PHAC website (17).

Conclusion

Analysis of the distribution of TB among the Canadian population shows that the risks and impacts of MTB strains are not uniformly distributed within our boundaries. As a consequence, a national prevention and control program demands central coordination, monitoring and evaluation with each province and territory being responsible for developing and implementing control plans that are consistent with Canadian guidelines and protocols. The formal implementation of genotyping in epidemiological investigations in combination with surveillance data at the national and provincial levels could best provide a coherent approach to timely outbreak response and case management and strengthen the TB surveillance partnerships in Canada.

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NCCID Comments

Genotyping methodologies, such as RFLP, MIRU and spoligotyping, are an invaluable adjunct tool for TB surveillance and outbreak investigations.

Genotyping has the capability to accurately trace the lineage of the pathogen that lack conventional epidemiological links. MIRU, in particular, has achieved a critical level of sophistication and maturity that it could now be transferred, with complementary personnel training, to more laboratories. This is also supported by the development of a standardized national genotyping algorithm in Canada as a result of the collaboration among provinces and the NRCM. There are immense benefits for adopting these technologies for routine TB surveillance and outbreak investigations. To ensure proper, accurate and consistent usage of TB genotyping technologies nationwide, frontline public health practitioners should have a basic understanding of MIRU and other genotyping methods, and on how these technologies can enhance their practice. However, the onus of learning about and advocating for the adoption of new technologies does not fall on frontline public health practitioners alone; it requires communication and cooperation among

frontline practitioners, infectious disease physicians and laboratory scientists. In addition to putting in place the appropriate technical infrastructure, a successful and informative national surveillance program must be supported by the development of a unifying policy for TB reporting for all provinces and territories. There are currently no established genotyping selection criteria for TB samples and TB genotyping practice has been disparate across jurisdictions in Canada. National TB reporting and surveillance is further hampered by the lack of in-depth case demographic and epidemiological information and inconsistency in the type of information collected. This is an apparent gap that requires deliberation and agreement among public health practitioners and policy-makers in various levels of government. Moving forward, a national dialogue about the applicability and feasibility of implementing and coordinating TB genotyping efforts should be initiated. This discussion will form the basis for a plan to concretely address foreseeable challenges – such as the increased workload pertaining to genotyping efforts, the need for integrated and coordinated reporting of genotyping results at the provincial and national levels, and the use of genotyping results at the local level for local surveillance and outbreak investigations – in realizing a national TB genotypic surveillance program.

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