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Detection of pre-extensively drug-resistant tuberculosis by molecular testing

The use of molecular testing for tuberculosis aids early diagnosis and allows for the prompt initiation of effective antimycobacterial therapy and isolation precaution measures.

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ABSTRACT: A 20-year-old female student from India presented to the emergency department with a 3-week history of nonproductive cough, fever, and shortness of breath. On examination, she was febrile with a temperature of 38.2 °C and a heart rate of 124 and was saturating at 91% with room air. A chest X-ray showed bilateral innumerable nodules in her lungs, which was a concern for miliary tuberculosis. A sputum sample was tested using the GeneXpert MTB/RIF cartridge, which not only confirmed the *Mycobacterium tuberculosis* complex infection diagnosis but also identified rifampin-resistance-related genetic mutation. This triggered further molecular testing, which detected the presence of mutations and confirmed resistance to rifampin, isoniazid, pyrazinamide, and quinolones, which suggested the presence of pre-extensively drug-resistant tuberculosis. The patient's respiratory status deteriorated rapidly, which necessitated intubation and transfer to a high-acuity unit; however, as a result of rapid molecular diagnosis and drug resistance detection, effective therapy (an antimycobacterial regimen consisting of amikacin, bedaquiline, clofazimine, cycloserine, ethambutol, linezolid, and meropenem with amoxicillin/clavulanate) was promptly initiated and ultimately a positive outcome achieved. This case highlights the benefit of molecular mycobacterial resistance testing for appropriate early therapeutic management of drug resistance and disseminated tuberculosis.

Between 2009 and 2020, 20 546 people in Canada were reported to have tuberculosis.[1] However, multidrug-resistant (defined as resistance to isoniazid and rifampin) and pre-extensively drug-resistant/extensively drug-resistant tuberculosis were detected only 206 and 6 times, respectively.[1] In 2022, a World Health Organization report led to an update to the Canadian Tuberculosis Standards to revise the definitions of different categories of resistance **[Table 1]**.[2] Extensively drug-resistant tuberculosis is now divided into pre-extensively drug-resistant tuberculosis (defined as multidrug-resistant tuberculosis with additional resistance to any fluoroquinolone) and extensively drug-resistant tuberculosis (defined as pre-extensively drug-resistant tuberculosis with additional resistance to bedaquiline or linezolid).[2] The typical antimicrobial duration of treatment for tuberculosis is 6 months, even for disseminated tuberculosis, but extension may be considered with central nervous system involvement, immunocompromised patients, and drug-resistant tuberculosis, depending on medications

used.[2,3] The definition of disseminated tuberculosis is provided in **Table 2**.[3]

From 1989 to 1998, 3553 (77.1%) of 4606 notified cases of tuberculosis in British Columbia and Alberta were culture-positive.[4] Of those cases, 365 (10.3%) were drug resistant, and 24 (6.6%) of those drug-resistant cases were multidrug resistant.[4] Twenty (83%) of the multidrug-resistant patients were foreign-born, and five (21%) died.[4] In the past 2 decades, Western Canada has had the second-highest active tuberculosis incidence rates (5.6 to 6.4 cases per 100 000 population), behind the territories.[1] According to the 2020 annual report of the BC Centre for Disease Control (BCCDC), the rate of active tuberculosis in BC was 6.1 per 100 000 population (315 cases); 7.3% (23 cases) of all active tuberculosis cases had isoniazid resistance, including 2 cases of multidrug-resistant tuberculosis (0.6%).[5]

The microbiology and infectious diseases services at Surrey Memorial Hospital encountered a case of disseminated tuberculosis caused by a pre-extensively drug-resistant strain. With molecular testing, our teams promptly recognized the severity of the disease and initiated optimized therapeutic management to allow the timely implementation of infection control measures and improve the likelihood of a successful clinical outcome.

Case data

A 20-year-old female student visiting from India was seen in the emergency department for a 3-week history of nonproductive cough, fever, and increasing shortness of breath. She had arrived in Canada 6 months prior to presenting to care. Several months before coming to Canada, she was exposed to her uncle, who had been diagnosed with pulmonary tuberculosis. She herself denied hemoptysis, weight loss, and night sweats. She self-reported having a negative tuberculin skin test and a normal chest X-ray result on immigration screening 6 to 8 months prior to her arrival in Canada.

In Canada, she initially presented to her family physician with dyspnea and received a 1-week course of prednisone but no antibiotics or a clear diagnosis; the treatment provided no improvement in her symptoms. Later, she presented to a community hospital in BC, where, upon chest X-ray, she was found to have innumerable nodules in her lungs, which was a concern for miliary tuberculosis **[Figure]**. Airborne precautions were initiated. A sputum specimen was smear-negative for acid-fast bacilli, and *Mycobacterium tuberculosis* was not detected upon polymerase chain reaction molecular testing conducted at the BCCDC Public Health Laboratory.

In the past 2 decades, Western Canada has had the second-highest active tuberculosis incidence rates, behind the territories.

During her stay at the emergency department, the patient rapidly worsened, with fever (38.2 °C), tachypnea (respiratory rate of 40), tachycardia (heart rate of 124), and oxygen desaturation (91% on room air), and required up to 15 L of oxygen delivered via a nonrebreather mask. She was started on intravenous ceftriaxone and azithromycin for coverage of possible community-acquired pneumonia pathogens while awaiting respirology and infectious diseases consultation to determine further investigations and management of a working diagnosis of tuberculosis. The next day, she was transferred to a tertiary site at Surrey Memorial Hospital, where she was assessed by the respirology and infectious diseases services on day 2 and day 4 of her hospital presentation, respectively. The patient was started on a standard empiric regimen for tuberculosis consisting of weight-based rifampin, isoniazid/pyridoxine, pyrazinamide, and ethambutol

TABLE 1. Definitions of different types of drug-resistant tuberculosis, as per the 2022 Canadian Tuberculosis Standards.[2]

Type of tuberculosis (TB)	Definition
Mono-resistant TB	Resistance to only one of the four first-line drugs.*
Polydrug-resistant TB	Resistance to at least two first-line drugs without resistance to rifampin.
Multidrug-resistant TB	Resistance to isoniazid and rifampin with or without resistance to other first-line anti-TB drugs.
Pre-extensively drug-resistant TB	Multidrug-resistant TB with additional resistance to any fluoroquinolone.
Extensively drug-resistant TB	Pre-extensively drug-resistant TB with additional resistance to bedaquiline or linezolid.

*First-line drugs: isoniazid, rifampin, pyrazinamide, and ethambutol.

TABLE 2. Definitions of disseminated tuberculosis, as per the 2022 Canadian Tuberculosis Standards.[3]

Type of tuberculosis (TB)	Definition
Disseminated TB	Tuberculosis occurring in two or more noncontiguous organs or the isolation of *Mycobacterium tuberculosis* in blood, bone marrow, or liver biopsy.
Miliary TB	A distinct subset of disseminated TB. Hematogenous dissemination of TB causing formation of minute tubercles throughout multiple organs, often resulting in characteristic uniform micronodular (1–5 mm) changes on lung imaging and life-threatening systemic illness.

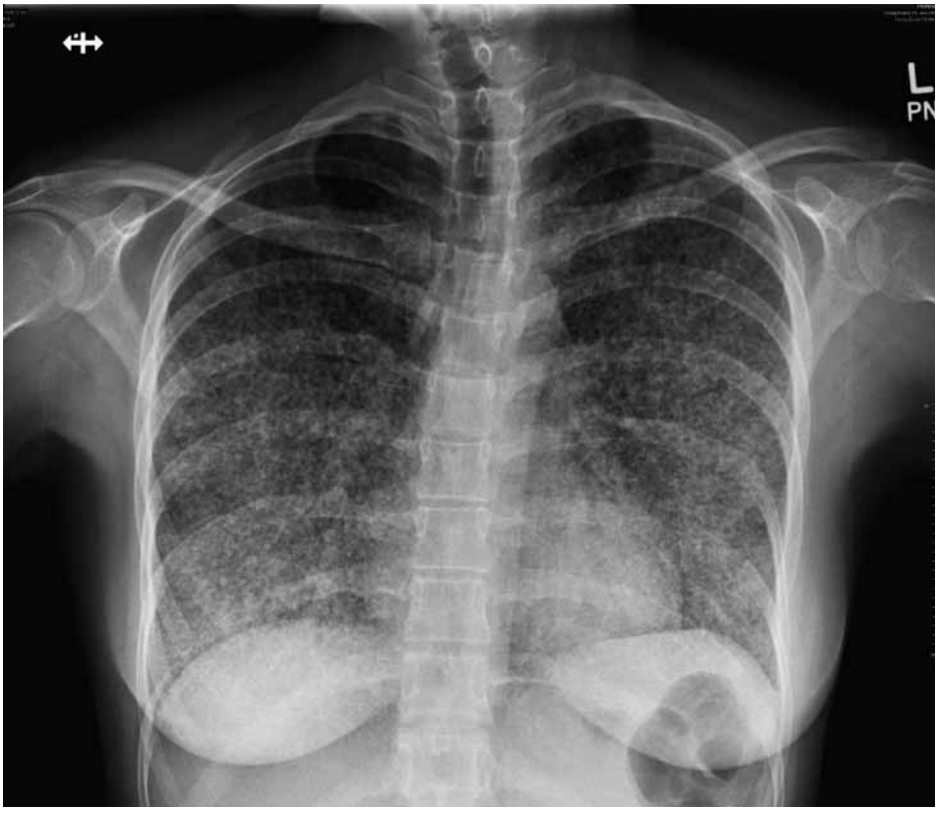

FIGURE. The chest X-ray of the patient on arrival to hospital showed innumerable nodules distributed throughout both lobes of the lungs.

TABLE 3. Phenotypic antimicrobial susceptibility results for first- and second-line drugs, at CLSI-recommended critical concentrations of the drugs.

Drug	Concentration	Susceptibility
Ethambutol	5.0 mg/L	Resistant
Isoniazid	0.4 mg/L	Resistant
Moxifloxacin	0.25 mg/L	Resistant
Pyrazinamide	100 mg/L	Resistant
Rifampin	1.0 mg/L	Resistant
Amikacin	0.1 mg/L	Susceptible
Capreomycin	2.5 mg/L	Susceptible
Ethionamide	5.0 mg/L	Resistant
Kanamycin	2.5 mg/L	Susceptible
Linezolid	1.0 mg/L	Susceptible
Ofloxacin	2.0 mg/L	Resistant
Para-aminosalicylic acid	4.0 mg/L	Susceptible
Rifabutin	0.5 mg/L	Resistant
Streptomycin	1.0 mg/L	Resistant
Cycloserine	60 mcg/mL	Susceptible*
Clofazimine	≤ 0.12 mcg/mL	Susceptible*
Bedaquiline	1.0 mcg/mL	Susceptible

CLSI = Clinical and Laboratory Standards Institute.

* The CLSI provides the minimum inhibitory concentrations for cycloserine and clofazimine only; the rest of the drugs are critical concentrations provided by the CLSI.

daily. Her course in hospital was as follows **[Table 3]**:

- On day 4 of the patient's presentation, CT imaging of her chest showed diffuse and extensive parenchymal opacification with innumerable nodular densities and confluence in the dependent lungs. Three more sputum samples for acid-fast bacilli smear, polymerase chain reaction testing, and mycobacteria culture were performed at the BCCDC. The infectious diseases service also ordered Xpert MTB/RIF assay using GeneXpert platform (Cepheid, Sunnyvale, California) molecular testing of her sputum specimen. The three sputum specimens were collected on day 4, day 7, and day 8 of her presentation. Of note, her HIV antibody and antigen testing was negative.
- The sputum collected on day 8 following presentation showed a positive result for *M. tuberculosis* complex DNA, in addition to rifampin-resistance-related genetic mutation, based on the GeneXpert MTB/RIF assay conducted at Surrey Memorial Hospital. The molecular testing conducted at the BCCDC also identified mutations that confirmed resistance to rifampin, isoniazid, pyrazinamide, and quinolones. This resistance pattern was consistent with pre-extensively drug-resistant tuberculosis. The infectious diseases service promptly requested special access to bedaquiline, clofazimine, and cycloserine, which are preferred medications for pre-extensively drug-resistant tuberculosis.
- On day 17 of her presentation, in consultation with the BCCDC's Tuberculosis Clinic, the patient was started on an antimycobacterial regimen consisting of amikacin, bedaquiline, clofazimine, cycloserine, ethambutol, linezolid, and meropenem with amoxicillin/clavulanate.
- On day 22 of the patient's presentation, due to clinical suspicion of central nervous system involvement by tuberculosis, a lumbar puncture was performed.

Her cerebrospinal fluid showed a white blood cell count of 264 × 10^6/L, 82% neutrophils, 1280 mg/L of protein, 1.2 mmol/L of glucose, and lactate of 79 U/L, with acid-fast bacilli seen on the smear; the GeneXpert MTB/RIF testing of her cerebrospinal fluid was also positive for *M. tuberculosis* complex DNA, which was indicative of central nervous system tuberculosis.

- On day 23 of the patient's presentation, CT imaging of her abdomen and pelvis showed mild multifocal confluent wedge-shaped hypoattenuation within the renal cortices bilaterally, which suggested renal involvement of disseminated tuberculosis.
- On day 36 of the patient's presentation, the BCCDC confirmed the growth of *M. tuberculosis* complex in her sputum (collected on day 4) by phenotypic culture methods. Subsequent phenotypic resistance testing confirmed the presence of pre-extensively drug-resistant tuberculosis, and second-line drug susceptibility testing was pursued.

The patient was subsequently extubated after being intubated for less than 1 month, and her multiple pneumothoraces gradually improved on chest radiography. She was discharged from hospital in stable condition after 148 days of inpatient stay and will be closely followed by the Vancouver Tuberculosis Clinic. She was prescribed the following antimycobacterial medications upon discharge:

- Bedaquiline: 200 mg orally every Monday, Wednesday, and Friday.
- Clofazimine: 100 mg orally once daily.
- Cycloserine: 250 mg orally twice daily.
- Delamanid: 100 mg orally twice daily.
- Linezolid: 600 mg orally once daily.

Benefits of molecular testing

Traditionally, mycobacterial culture can take up to 8 weeks to grow in specialized selective media.[6] During this waiting time when the diagnosis has not been established, clinicians are obliged to order multiple investigations to look for alternative diagnoses.[7,8] In Surrey Memorial Hospital, where the Fraser Health regional microbiology laboratory is located, GeneXpert MTB/RIF molecular assay has been implemented as the standard preliminary diagnostic test for samples on which tuberculosis testing is requested. It requires minimal laboratory processing and can provide results within 2 hours from the time of specimen arrival in the laboratory.[9] A second advantage is the simultaneous detection of *M. tuberculosis* complex and genotypic rifampin resistance markers.

The 2022 Canadian Tuberculosis Standards recommend that molecular detection of drug resistance be performed on all new diagnoses of tuberculosis.

However, there are arguments against overuse of molecular testing of tuberculosis. The GeneXpert MTB/RIF molecular assay may provide a false negative up to 11% of the time, failing to provide early diagnosis.[9] Molecular assays do not replace the need for mycobacterial culture using liquid broth and solid culture media, which is considered the criterion standard for diagnosis of tuberculosis and is required for complete phenotypic drug susceptibility testing.[10] Eckbo and colleagues found that of the 5484 acid-fast smear-negative specimens submitted to the BCCDC for tuberculosis testing in 1 year (1 October 2016 to 30 September 2017), only 36 (0.7%) were culture-positive.[11] The authors estimated that the annual cost of molecular testing of acid-fast smear-negative specimens was $247 000 (based on $45 per test) and questioned whether this special testing should be reserved only for physicians specialized in managing tuberculosis patients.[10] However, the 2022 Canadian Tuberculosis Standards recommend that molecular detection of drug resistance be performed on all new diagnoses of tuberculosis.[2]

Implications for therapeutic management

A rifampin-isoniazid-pyrazinamide-ethambutol regimen is the usual antimycobacterial therapy initiated for suspected and confirmed tuberculosis.[12] This regimen would need to be changed if drug resistance was suspected or confirmed. For instance, an initial regimen for multidrug-resistant tuberculosis may include bedaquiline, linezolid, clofazimine, cycloserine, and levofloxacin or moxifloxacin.[2] The regimen for pre-extensively drug-resistant and extensively drug-resistant tuberculosis may require five or more drugs selected based on the susceptibility and adverse effect profile of each of the antimycobacterials. As per the 2022 Canadian Tuberculosis Standards, for multidrug-resistant tuberculosis, a treatment duration of 18 to 20 months, guided by medications used and response to therapy, is recommended.[2] In Canada, novel and repurposed drug-resistant tuberculosis drugs (e.g., bedaquiline, cycloserine, clofazimine) are often available only several days to weeks after an application to Health Canada's Special Access Program has been submitted and drug procurement has been arranged. The application can be denied if it lacks strong evidence to support the indications of these special access medications, such as a lack of laboratory confirmation of drug resistance or susceptibility.[2]

Traditional phenotypic testing can take several weeks to complete. Without the quick turnaround time of molecular testing results, clinicians could unknowingly commit their patients to ineffective therapies, face delays accessing effective therapies, and ultimately delay the time to cure. These ineffective therapies could induce random mutations that lead to antimicrobial resistance and extrapulmonary complications.[2] Tuberculous meningitis, for example, has a global mortality rate of 20% to 40%; prompt initiation of effective antimycobacterial therapies may reduce short-term mortality to less than 10%.[3] Furthermore, rapid diagnostic testing has been proposed to aid antimicrobial stewardship through early discontinuation of

unnecessary antimicrobials,[13] which in turn may save drug costs and preserve patients' microbiome. On a health care system level, early molecular microbiological diagnosis prompts timely involvement by infectious diseases and respirology physicians, clinical pharmacists, microbiologists, and infection preventionists and can facilitate a multidisciplinary approach to the management of patients.

Summary

The management of tuberculosis involves a multidisciplinary approach that includes clinical pharmacology expertise from pharmacists, diagnostic support from microbiologists, procedural support from respirologists, consulting support from infectious diseases physicians, and day-to-day care from the admitting service and unit nurses. The availability of molecular testing for both detection and resistance markers of tuberculosis not only aids multidisciplinary teams in making an early diagnosis but also allows the prompt initiation of effective antimycobacterial therapy and isolation precaution measures. We acknowledge the cost of molecular testing and the rarity of rifampin-resistant tuberculosis in Canada. However, when used appropriately, under guidance of clinicians with tuberculosis-specific expertise and by experienced microbiology laboratory staff, these costs may be mitigated while providing the significant patient and health system benefits outlined herein. This case report highlights the beneficial role of molecular testing for tuberculosis, informs medical practitioners about the availability of this diagnostic tool, and encourages further development of local and regional algorithms to guide effective integration of molecular testing for tuberculosis as an early and cost-effective intervention. ■

The availability of molecular testing for detection and resistance markers of tuberculosis aids multidisciplinary teams in making an early diagnosis and allows the prompt initiation of effective antimycobacterial therapy and isolation precaution measures.

Competing interests

None declared.

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