TB in Canada: From Sea to Sea to Sea

Marcel A. Behr
Professor, McGill University
Director, McGill Int. TB Centre
marcel.behr@mcgill.ca
Disclosures

- I have no pharma funding
- When I tell a drug rep that I work on TB, s(he) politely thanks me, then leaves.

Royalties:
- US patent 6,291,190 B1: Molecular differences between species of the *Mycobacterium tuberculosis* complex.
  » Exploited in IGRA testing

Generic myopia:
- I generally know McGill literature better
- I apologize to those working on TB in other parts of Canada whose work I have overlooked
Some microbiology

Genomic epidemiology
  - Atlantic
  - Arctic
  - Pacific

Can we use Whole Genome Sequencing in clinical TB?
  - E.g. prediction of MDR-TB?
Mycobacteria genus by multi-locus sequence analysis

M. tb. complex (MTC)

M. tuberculosis

M. bovis
**M. tb. complex (MTC)**

- *M. tuberculosis*
- *M. microti*
- Oryx bacillus
- *M. caprae*
- *M. mungi*
- *M. suricattae*
- Dassie bacillus
- *M. africanum*
- *M. pinnipedii*
Genome-based phylogeny of the MTC

Mostowy et al., J. Bacteriology, 2005
Host-associated ecotypes of the MTC

- Molecular markers for robust subspeciation
  - All *M. pinnipedii* has same deletion pattern, regardless of provenance

- Human TB did not obviously come from cows
  - Did we give TB to goats and cows?

- Global TB control vs. One Health

- If host-pathogen association across species, what about within?
Human-associated lineages of *Mycobacterium tuberculosis*
Patient/strain associations

Montreal
60% of TB cases
- 7 countries

San Francisco
71% of TB cases
- 5 countries

Reed, JCM, 2009
M.\textit{tb} strains & place of birth: Montreal (n = 798)

Most diversity within Africa (like humans)
Several waves of TB dispersal

Reed M et al, J. Clin Micro, 2009
....but Canada is a big place
Clinical observation: Pyrazinamide mono-resistance

- Classically *M. tb* is PZA-susceptible
  - PZA-R seen with *M. bovis*
  - Due to non-synonymous SNP in *pncA*
- In Quebec, 7% of *M. tb sensu stricto* found to be PZA mono-resistant
  - A unique 8 bp deletion in *pncA* gene
- Is there a common strain?
- Any evidence of transmission?
RFLP in Quebec

Background diversity of sensitive strains

Mono-pyrazinamide resistant strains

Nguyen et al., J. Clin Micro, 2003
‘Quebec strain’ of *M. tuberculosis* (within Euro-American lineage)

Nguyen D et al., J. Clin Micro, 2004
‘Quebec strain’ of *M. tuberculosis* (within Euro-American lineage)

Nguyen D et al., J. Clin Micro, 2004

Similar, but non-identical RFLP patterns
‘Quebec strain’ of *M. tuberculosis* (within Euro-American lineage)

Nguyen D et al., J. Clin Micro, 2004
Quebec strain of *M. tb* spread west with the Fur Trade

Pepperell C et al., PNAS, 2011
Quebec strain of *M. tb*: two phases of spread

◆ Dispersal:
  – Quebec diversity of *M.tb* strains seen in west
  – Prolonged contact between traders (French) and natives (wives)

◆ Expansion:
  – Epidemic TB in western Canada after 1880
  – After Quebec-West trade mostly done

◆ The epidemic did NOT happen when the bacteria arrived; the epidemic happened when conditions changed

Pepperell C et al., PNAS, 2011
Public Health Observation:
Epidemic TB in Nunavik, 2012

Village K, Population = 933
Cases of active TB: 92 (incidence ~10%)
Culture-confirmed cases: 50 (5%)
Genotyping Kangiqsualujjuaq: MIRU

- MIRU, 2012:
  - All 24 the same across 49/50 isolates (one outlier)
Nunavik Question 1: Was it 1 outbreak?

- MIRU showed one pattern
  - Consistent with 1 outbreak

- Goal:
  - Look at public health links
  - Perform Whole Genome Sequencing (WGS) of bacteria to identify links
  - Combine the two (epi + WGS)
Village K: Contact data

+++ Epi links
“Everyone knows everyone”
Three strains: I, II, III (clonal replacement)

Strain III had 3 variants (IIIA, IIIB, IIIC)
Was it 1 event?
Combining WGS with public health files

‘outbreak’ was 6 (or more) events
WGS and a TB outbreak: Major findings

- **Scale of decades:**
  - Strain replacement
    » Twice gone / Third time unlucky

- **Scale of years:**
  - Outbreak was multiple outbreaks
    » Strain introduced 2007, diversified by 2012
  - No single ‘culprit’

- **WGS > resolution than RFLP/MIRU**

- **Highest resolution: WGS + Epi**

Lee et al., JID, 2015
Nunavik Question 2: Was it hypervirulent *M. tb*?

- 2012 outbreak due to a bacterium introduced in 2007
  - Where did 2007 strain come from?
- Goal:
  - Look at all villages of Nunavik
  - Perform Whole Genome Sequencing (WGS) of bacteria to infer ancestor
  - Assess bacterial evolution since its arrival in region for clues re: virulence
WGS of Nunavik 1991-2013, 163 sequences

- All Inuit isolates are Lineage 4 (Euro-American)
- NOT Quebec strain found out West
- Inuit did not bring TB from East-Asia (lineage 2)
- 153/163 descend from a single introduction
WGS of Nunavik
A century of evolution

- *M. tuberculosis* came to region circa 1919
  - From Europe or ‘European’
- Transmission primarily within villages
  - Between-village strains similar due to founder strain, not transmission
- No suggestion of hypervirulence
  - ‘lucky’ rather than ‘burly’ strain in a high-transmission environment

Lee et al, 2015, PNAS
- Is there a transmission advantage to being a ‘wimpy’ bug?
M. tb. spread through the ages

- *M. tuberculosis* came from Africa (all major lineages present)
- *M. tuberculosis* ‘walked’ out of Africa with the paleo-migration
- *M. tuberculosis* then ‘sailed’ out of Europe to the Americas
- *M. tuberculosis* ‘canoed’ and ‘kayaked’ across Canada
- *M. tuberculosis* has co-existed with us for a LONG time
\textit{M. \textit{tb}.: pathogen and symbiont}

\begin{itemize}
\item \textit{M. tuberculosis} is a pathogen
  \begin{itemize}
  \item Biomedical construct: causes disease
  \item N.B. Disease is part of transmission cycle
  \end{itemize}
\item \textit{M. tuberculosis} is a symbiont
  \begin{itemize}
  \item Biological construct: symbiosis is divergent organisms that live together
  \end{itemize}
\item \textit{M. tb.} successfully navigates the balance between these two missions
  \begin{itemize}
  \item Too virulent: kill host, ↓ transmission
  \item Too benign: ↓ pathology, ↓ transmission
  \end{itemize}
\end{itemize}

Veyrier, Dufort, Behr; Trends in Micro, 2011
M.t.b. is an educated pathogen

Legionella pneumophila
Diffuse, fast pathology
Uneducated Pathogen
sick host, no transmission

Mycobacterium tuberculosis
Localized, chronic pathology
Educated Pathogen
+/- sick host, transmission
M. tb strains & place of birth:

[Map showing global distribution of M. tb strains]
TB in the New World

- High incidence and mortality rates
  - “Virgin soil epidemic”
- Yet, fossil evidence of Pott’s disease
  - E.g. pre-Colombian mummies in Peru
  - Molecular confirmation of *M. tb* DNA by PCR
- *Was M. tuberculosis* brought to Americas across Bering strait (like *Helicobacter*)?
- If not, how did people get TB 1000 years ago?
TB and the Pacific

- **B.C. TB outbreak**
  - Resolved by Whole Genome Sequencing
    - Gardy, NEJM, 2011
  - Isolates also belong to Euro-American lineage in secondary analysis
    - Ford, Nature Genetics, 2013

- What about older samples?
- Paleo-DNA studies have done PCR
- Now: hybrid-capture, followed by Whole Genome Sequence (prev. used for leprosy)
  - Select for MTC DNA
  - Let the sequence result tell you what you have
LETTER

Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis

Kirsten I. Bos1*, Kelly M. Harkins2*, Alexander Herbig1,3*, Mireia Coscolla4,5*, Nico Weber2, Iñaki Comas6,7, Stephen A. Forrest1, Josephine M. Bryant8, Simon R. Harris8, Verena J. Schuenemann1, Tessa J. Campbell9, Kerttu Majander1, Alicia K. Wilbur2, Ricardo A. Guichon10, Dawnie L. Wolfe Steadman11, Della Collins Cook12, Stefan Niemann13,14, Marcel A. Behr15, Martin Zumarraga16, Ricardo Bastida17, Daniel Huson3, Kay Nieselt3, Douglas Young18,19, Julian Parkhill8, Jane E. Buikstra2, Sebastien Gagneux4,5, Anne C. Stone2 & Johannes Krause1,20,21
TB and the Pacific

Bos, Nature, 2014
Outline - revisited

◆ Epidemiology of TB in Canada
  – TB previously from Europe
    » Dissemination, then expansion
    » TB exploits social opportunities
  – TB presently from sites of immigration

◆ Methods
  – Whole Genome Sequencing is clearly best for epidemiology
  – WGS can be used to diagnose cause of TB in pre-Colombian mummies
  – But can it be clinically useful?
WGS visits the clin TB lab

Smear  LJ/MGIT  Molecular resistance
PCR    Accuprobe  Phenotypic DST

Where could WGS be useful?
WGS visits the clin TB lab

Smear  LJ/MGIT  Molecular resistance
PCR    Accuprobe  Phenotypic DST

WGS on sample
Lots of negatives
Lots of other DNA
Need $$$ enrichment

Reviewed in Lee & Behr, Therapeutic Advances in ID, 2016
WGS visits the clinical TB lab

- Smear
- PCR
- LJ/MGIT
- Accuprobe
- Molecular resistance
- Phenotypic DST

WGS on + culture
Speciation – nice
More rapid DST?

Reviewed in Lee & Behr, Therapeutic Advances in ID, 2016
DST prediction by WGS

- Operating parameters
  - Sensitivity/Specificity:
    » 83% / 98.5% - Bradley, Nature Comm, 2015
  - Overall accuracy:
The emergence of drug-resistant strains of *Mycobacterium tuberculosis* is a challenge to global tuberculosis (TB) control. Although culture-based methods have been regarded as the gold standard for drug susceptibility testing (DST), molecular methods provide rapid information on mutations in the *M. tuberculosis* genome associated with resistance to anti-tuberculosis drugs. We ascertained consensus on the use of the results of molecular DST for clinical treatment decisions in TB patients. This document has been developed by TBNET and RESIST-TB groups to reach a consensus about reporting standards in the clinical use of molecular DST results. Review of the available literature and the search for evidence included hand-searching journals and searching electronic databases. The panel identified single nucleotide mutations in genomic regions of *M. tuberculosis* coding for *katG*, *inhA*, *rpoB*, *embB*, *rrs*, *rpsL* and *gyrA* that are likely related to drug resistance in vivo. Identification of any of these mutations in clinical isolates of *M. tuberculosis* has implications for the management of TB patients, pending the results of in vitro DST. However, false-positive and false-negative results in detecting resistance-associated mutations in drugs for which there is poor or unproven correlation between phenotypic and clinical drug resistance complicate the interpretation. Reports of molecular DST results should therefore include specific information on the mutations identified and provide guidance for clinicians on interpretation and on the choice of the appropriate initial drug regimen.

**KEY WORDS**: clinician guidance; interpretation; molecular methods

*Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement

So is WGS faster than DST?

“Unadjusted median time from MGIT positivity to DST reporting was 25 days (IQR 14–32)…similarly, full WGS based reports were available in 31 days (IQR 21-60)”

“WGS processing delays were driven by sample batching for sequencing and delays in sharing sequencing data….The time delay would be minimised in high-throughput labs” Potentially 8 days

If MGIT takes 10 days to flag:

- Full DST is on day 35
- WGS-based prediction feasible by day 18

WGS visits the clin TB lab

- Smear
- PCR
- LJ/MGIT
- Accuprobe
- WGS report
- Phenotypic DST
WGS visits the clin TB lab

Smear  LJ/MGIT  Adjusted WGS report
PCR  Accuprobe  Phenotypic DST

What does the MD prescribe here?
Can the MD wait 18 days to start TB Rx?
WGS and global MDR-TB

WHO: 480,000 MDR-TB cases (210,000 deaths)
DST: 12% new cases, 58% prev. treated

Canada: 19 cases in 1376, 2014
WGS for clinical TB

- Proof-of-concept here
- Reasonably good for calling resistance
  - Not reliable enough yet for calling susceptible
- If sensitivity does not reach 100%, we will still need phenotypic DST
  - WGS as added, not replacement test
- Canada might be a hard place to prove relevance
Concluding thoughts

- **MTC: Mammalian mycobacteria**
  - New names good for challenging fellows on Royal College exams

- **Molecular epidemiology**
  - TB in Canada mostly reactivation
  - Pockets of ongoing spread due to bacteria here for 1-3 centuries

- **WGS in clinical lab**
  - Technology almost ready
  - Operating parameters? Need?
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