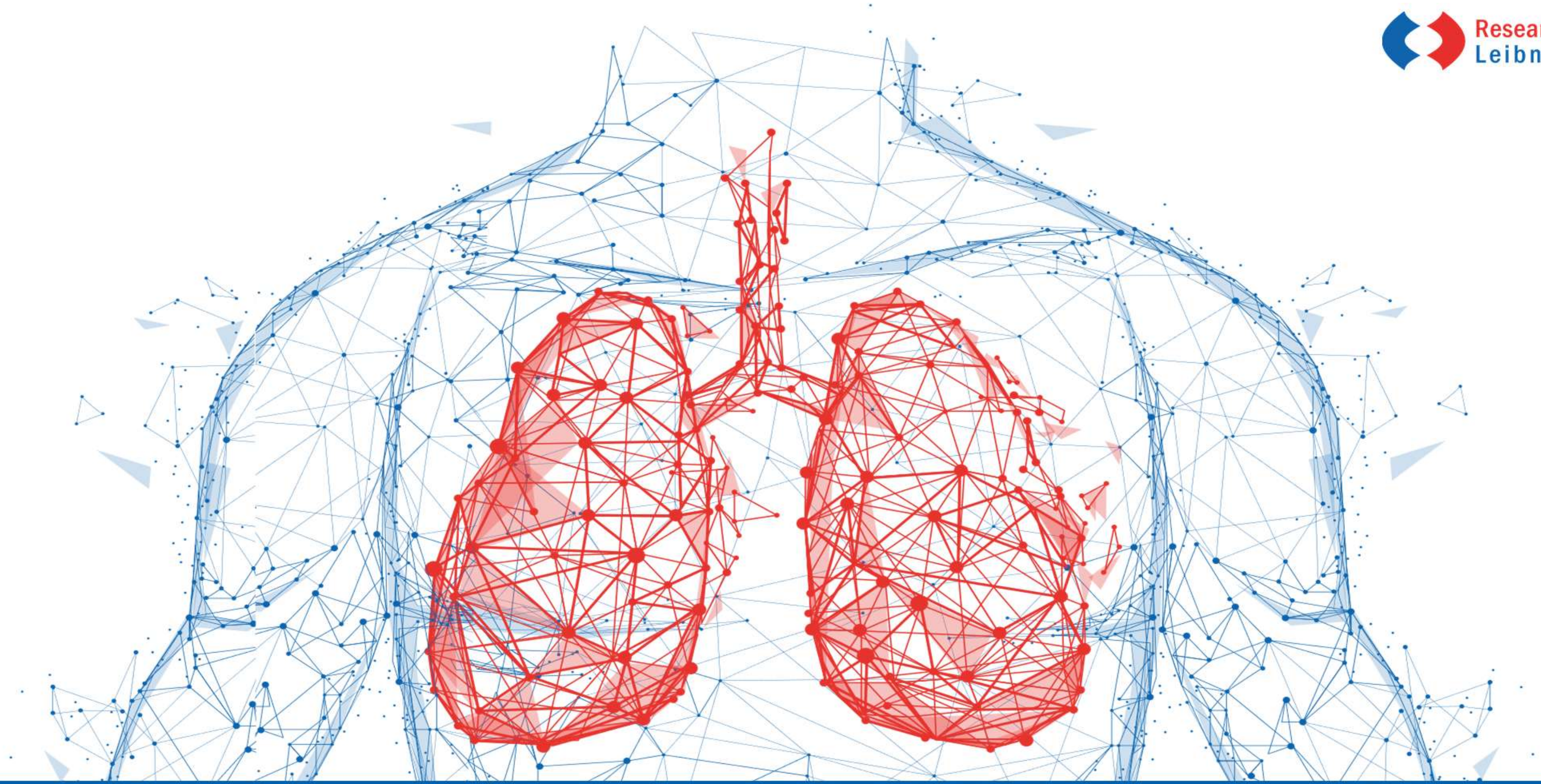
Leibniz
Association

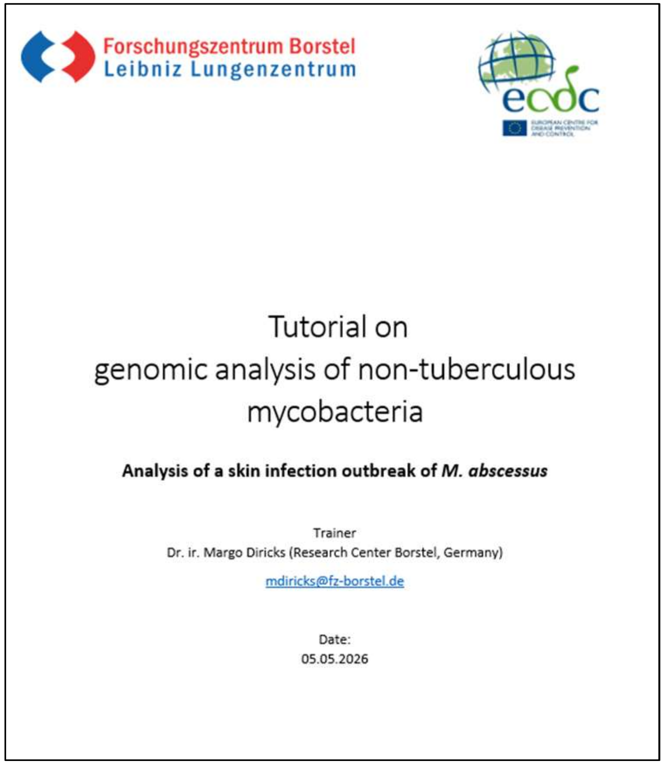
Research Center Borstel
Leibniz Lung Center



Genomic analysis of non-tuberculous mycobacteria (Tutorial)

OBJECTIVES

- Getting hands-on experience with analysis of genomic data from non-tuberculous mycobacteria
  - Analysis of a skin infection outbreak of *M. abscessus*
    - *Task: identify source of the outbreak*
  - Step-by-step instructions for analysis of WGS data using GUI-based tools
    - Pathogenwatch
    - pubMLST
    - NTM-profiler
    - Ridom Typer
  - Alternatively, CLI tools can be used (e.g. NTMprofiler, NTMseq)



Forschungszentrum Borstel
Leibniz Lungenzentrum

Tutorial on
genomic analysis of non-tuberculous
mycobacteria

Analysis of a skin infection outbreak of *M. abscessus*

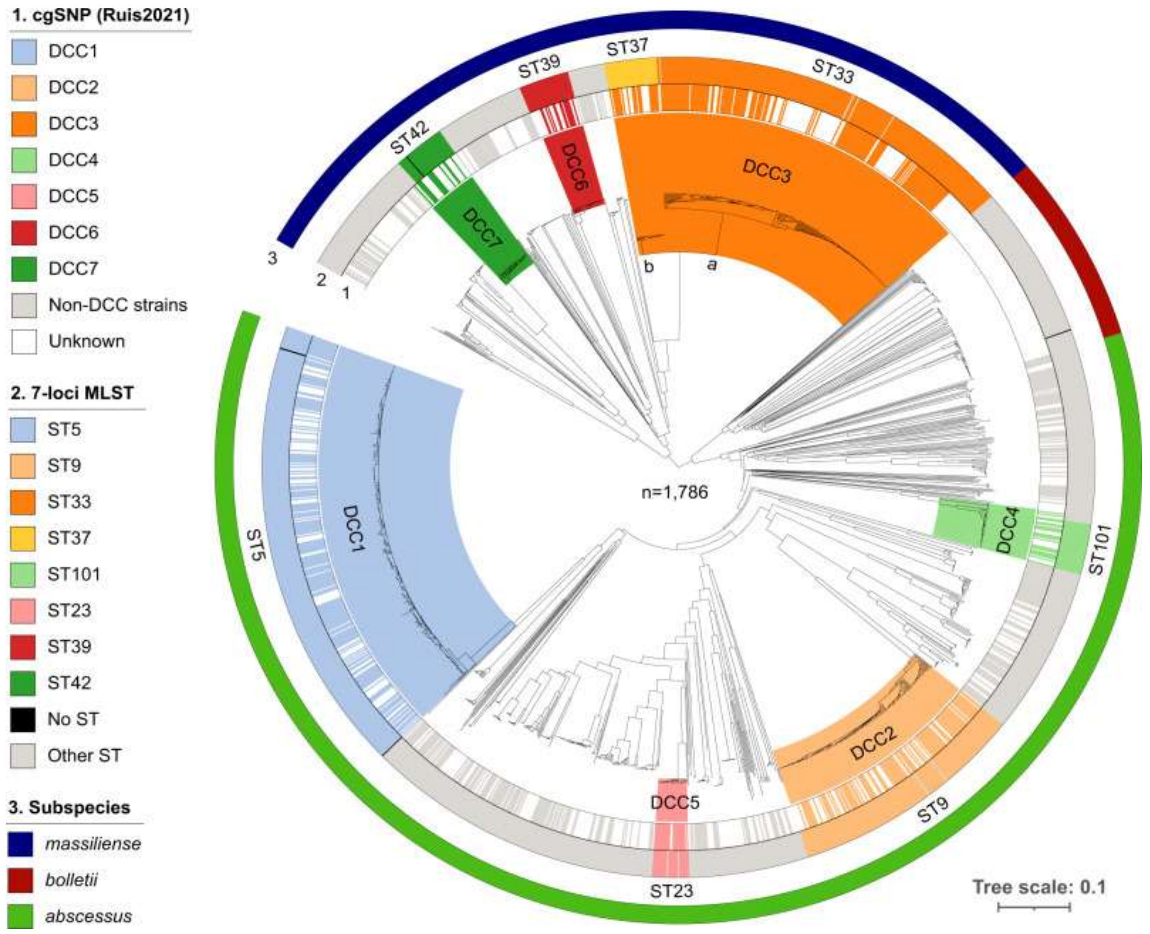
Trainer
Dr. ir. Margo Diricks (Research Center Borstel, Germany)
mdiricks@fz-borstel.de

Date:
05.05.2026

Tutorial and WGS data can be found in the ECDC learning portal

M. ABSCESSUS

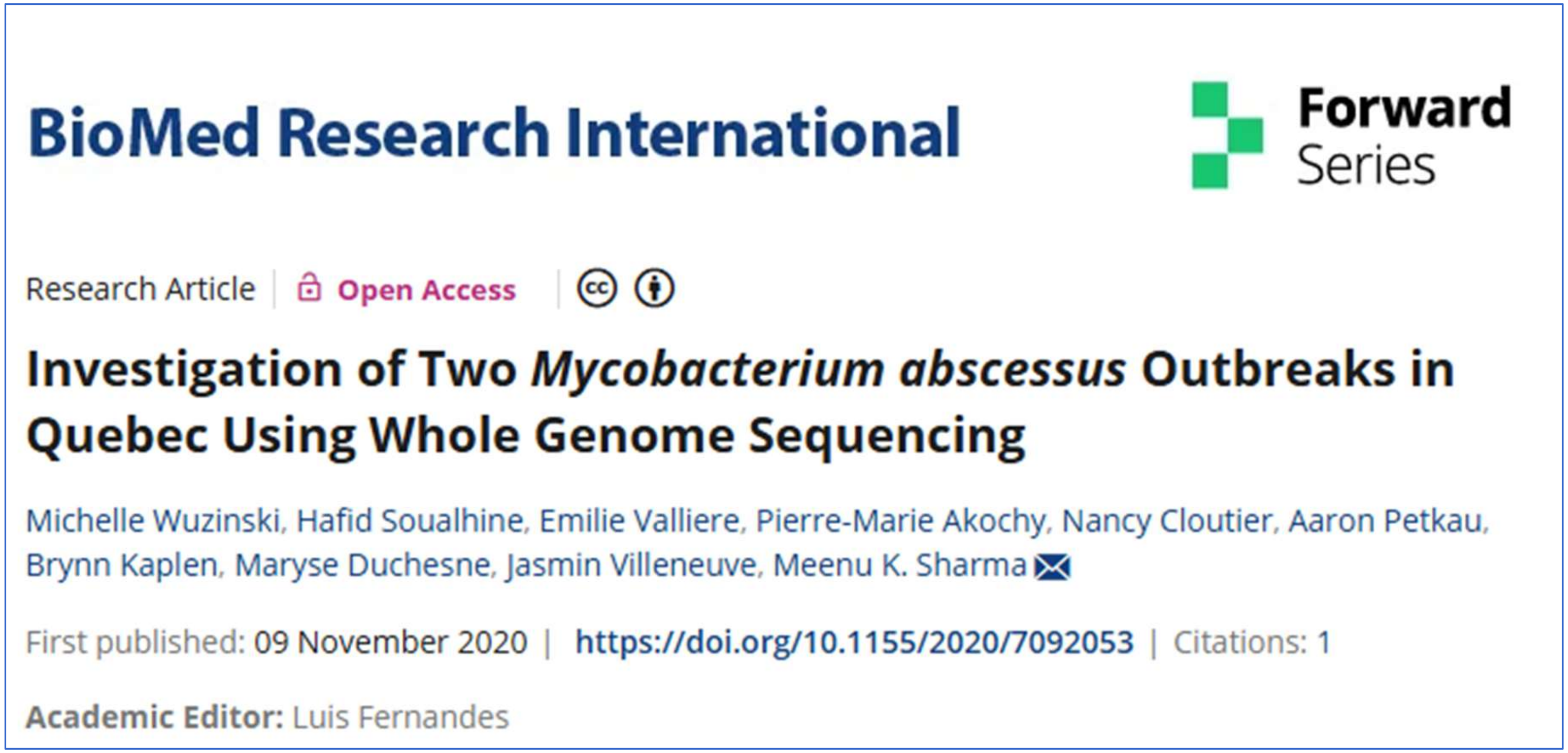
- Rapidly growing NTM
- Pulmonary and extra-pulmonary infections
- Notoriously difficult to treat
- 3 subspecies
    - massiliense
    - abscessus
    - bolletii
- 7 Dominant circulating clones
    - Most often found in patients (and the environment)
    - More virulent
    - Greater antibiotic resistance
    - Worse clinical outcomes
    - Lower mutation rate



Diricks et al 2022

SOURCE

- Tutorial based on real data from Wuzinski et al. 2020



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Investigation of Two *Mycobacterium abscessus* Outbreaks in Quebec Using Whole Genome Sequencing

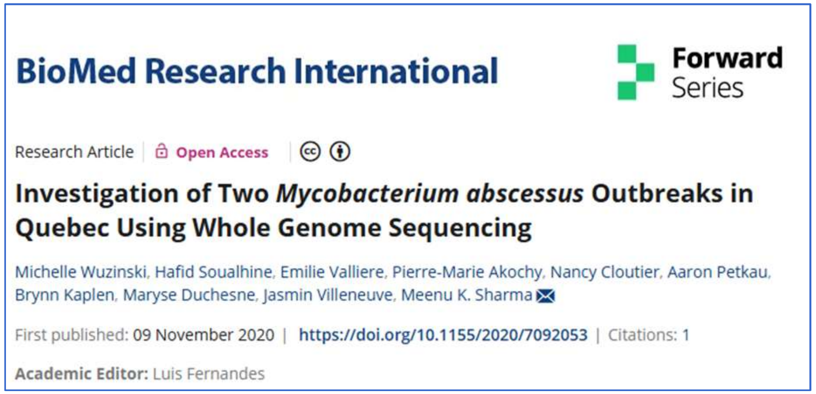
Michelle Wuzinski, Hafid Soualhine, Emilie Valliere, Pierre-Marie Akochy, Nancy Cloutier, Aaron Petkau, Brynn Kaplen, Maryse Duchesne, Jasmin Villeneuve, Meenu K. Sharma ✉

First published: 09 November 2020 | https://doi.org/10.1155/2020/7092053 | Citations: 1

Academic Editor: Luis Fernandes

CASE STUDY

- Within 6 weeks: 4 patients presented with skin infections around tattoo
- Biopsies: + for mycobacteria
- *M. abscessus subsp. abscessus* (MLST, 16S/hsp65)
- Same tattoo studio
- Public Health Laboratory in Quebec suspected outbreak
- Samples sent to National Microbiology Laboratory
- WGS: close genetic similarity (3-11 SNPs) → outbreak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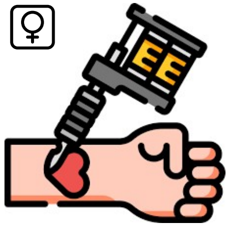
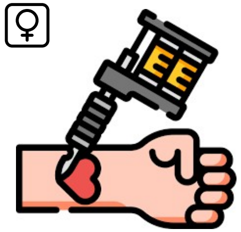
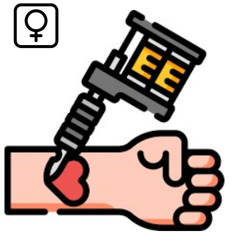


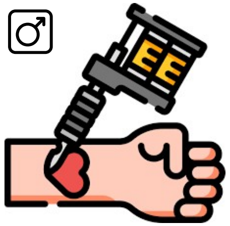
Patient 1
Sample H



Patient 2
Sample I



Patient 3
Sample J



Patient 4
Sample K

**Wu 2017*

CASE STUDY

♀

Patient 1
Sample H

♀

Patient 2
Sample I

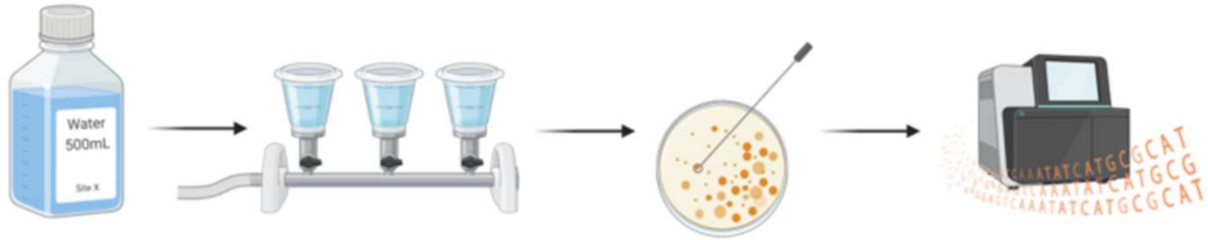
♀

Patient 3
Sample J

♂

Patient 4
Sample K

What is the source of the outbreak?



Collect sample

Preprocessing: Decontamination and filtration

Culturing: 7H10 37°C

DNA extraction and sequencing

Hypothetical environmental investigation in tattoo shop

Negative

Sample M

Sample O

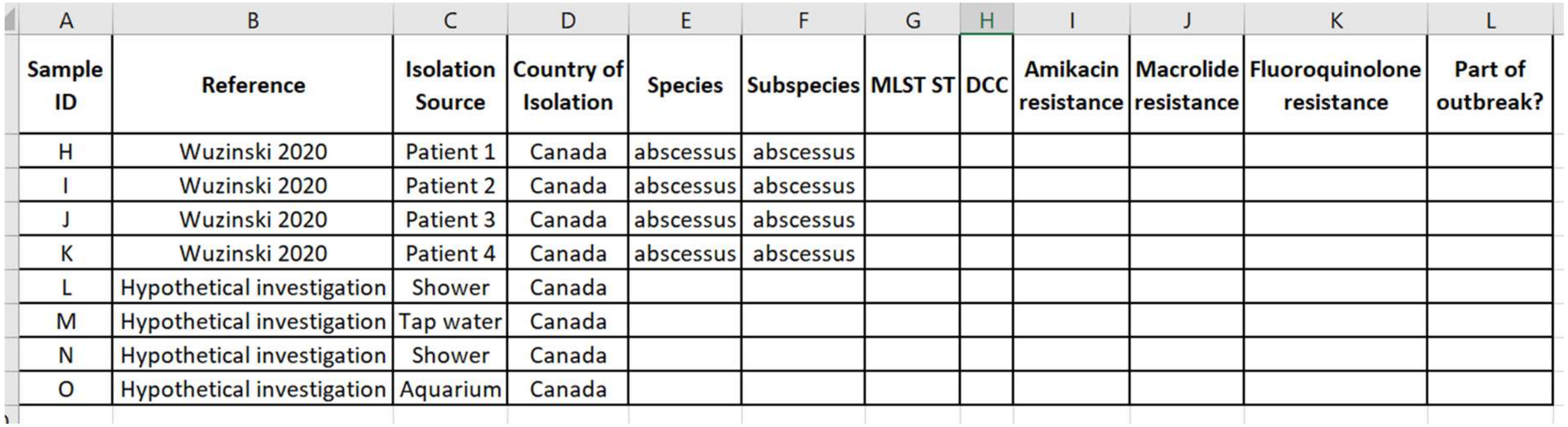
Sample L (SGM)
Sample N (RGM)

TASKS

- Goal: identify the source of the skin infection outbreak
  - Task 1: Determine which NTM (sub)species were isolated from the environmental samples (L-O)
  - Task 2: Determine the 7-loci MLST Sequence Type (ST) of the *M. abscessus* isolates
  - Task 3: Determine whether the *M. abscessus* isolates belong to known dominant circulating clones (DCCs)
  - Task 4: Determine the genetic distance (expressed as allele differences) between outbreak isolates
  - Task 5: Determine the genetic resistance profiles of the isolates

→ Complete all results in **Task_Exercise2026_NTM.xlsx**

Task 6: Formulate a hypothesis regarding the transmission route

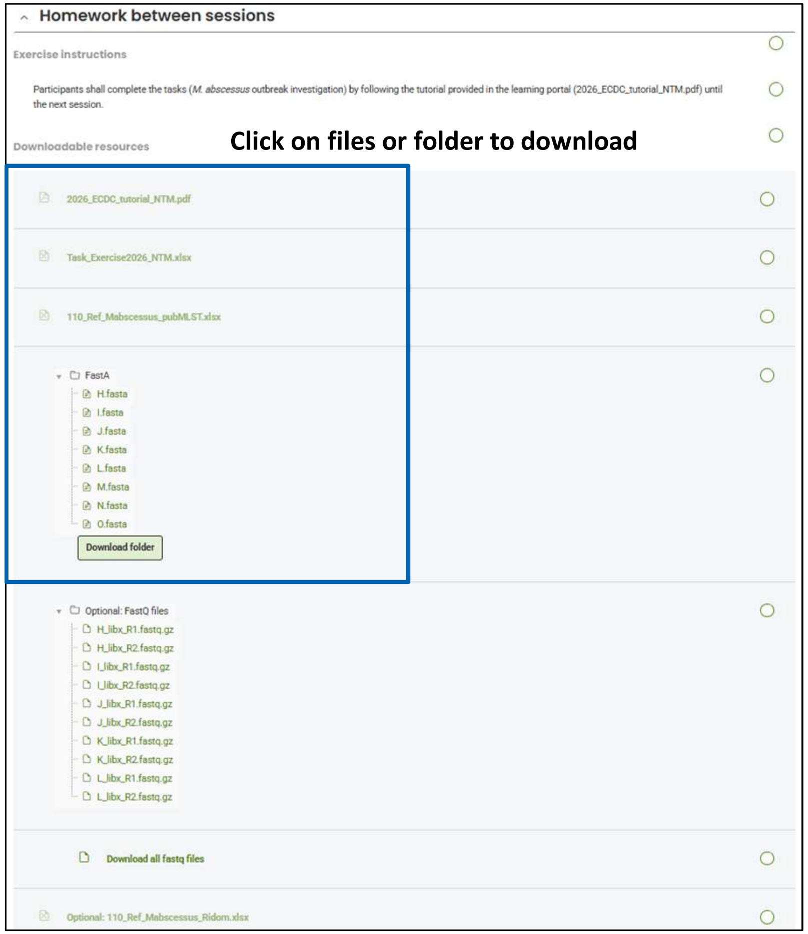


Sample ID	Reference	Isolation Source	Country of Isolation	Species	Subspecies	MLST ST	DCC	Amikacin resistance	Macrolide resistance	Fluoroquinolone resistance	Part of outbreak?
H	Wuzinski 2020	Patient 1	Canada	abscessus	abscessus						
I	Wuzinski 2020	Patient 2	Canada	abscessus	abscessus						
J	Wuzinski 2020	Patient 3	Canada	abscessus	abscessus						
K	Wuzinski 2020	Patient 4	Canada	abscessus	abscessus						
L	Hypothetical investigation	Shower	Canada								
M	Hypothetical investigation	Tap water	Canada								
N	Hypothetical investigation	Shower	Canada								
O	Hypothetical investigation	Aquarium	Canada								

NEXT STEPS

- Go to the ECDC learning portal and download the required files from section „**Homework between sessions**“
  - **2026_ECDC_tutorial_NTM.pdf** → step-by-step instructions
  - **Task_Exercise2026_NTM.xlsx** → To store results
  - **110_Ref_Mabscessus_pubMLST.xlsx** → To determine DCC
  - **Training FastA files** (draft assembled genomes)
- Optional
  - 110_Ref_Mabscessus_Ridom.xlsx → To determine DCC
    Only for current Ridom Typer/SeqSphere+ users with active license!!
  - FastQ files → Only for those with CLI experience

https://learning.ecdc.europa.eu/course/view.php?id=1239



Homework between sessions

Exercise instructions ○

Participants shall complete the tasks (*M. abscessus* outbreak investigation) by following the tutorial provided in the learning portal (2026_ECDC_tutorial_NTM.pdf) until the next session. ○

Downloadable resources ○

Click on files or folder to download

2026_ECDC_tutorial_NTM.pdf ○

Task_Exercise2026_NTM.xlsx ○

110_Ref_Mabscessus_pubMLST.xlsx ○

FastA ○
  - H.fasta
  - I.fasta
  - J.fasta
  - K.fasta
  - L.fasta
  - M.fasta
  - N.fasta
  - O.fasta

Download folder

Optional: FastQ files ○
  - H_libx_R1.fastq.gz
  - H_libx_R2.fastq.gz
  - I_libx_R1.fastq.gz
  - I_libx_R2.fastq.gz
  - J_libx_R1.fastq.gz
  - J_libx_R2.fastq.gz
  - K_libx_R1.fastq.gz
  - K_libx_R2.fastq.gz
  - L_libx_R1.fastq.gz
  - L_libx_R2.fastq.gz

Download all fastq files ○

Optional: 110_Ref_Mabscessus_Ridom.xlsx ○