
DTU

# Simulated exercise on CPO outbreak – *Pseudomonas aeruginosa*

EURGen-RefLabCap
Virtual multidisciplinary training workshop
September 2023
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# Simulated exercises - background

- Series of multidisciplinary training workshops
  - Sept/Oct 2022 – introduction to SNP analysis and cgMLST for cluster analysis (WS1)
  - May 2023 – Simulated exercise on outbreak analysis (*Klebsiella pneumoniae*; WS1)
  - Sept 2023– Simulated exercise on outbreak analysis (*Pseudomonas aeruginosa*; WS2)
  - 2024: Simulated exercises on outbreak analysis (WS1 and WS2)

WS1: CCRE/ *E. coli* and *Klebsiella* spp.

WS2: CPO/ *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

# Purpose of the workshop

- To build capacity to work with outbreak investigations
  - background information about bacterial subtyping and cluster analysis
  - Web-based bioinformatics tools to get started on bacterial phylogenetics and outbreak detection

- To work with larger sets of sequencing data and metadata and analyse outputs from SNP analyses

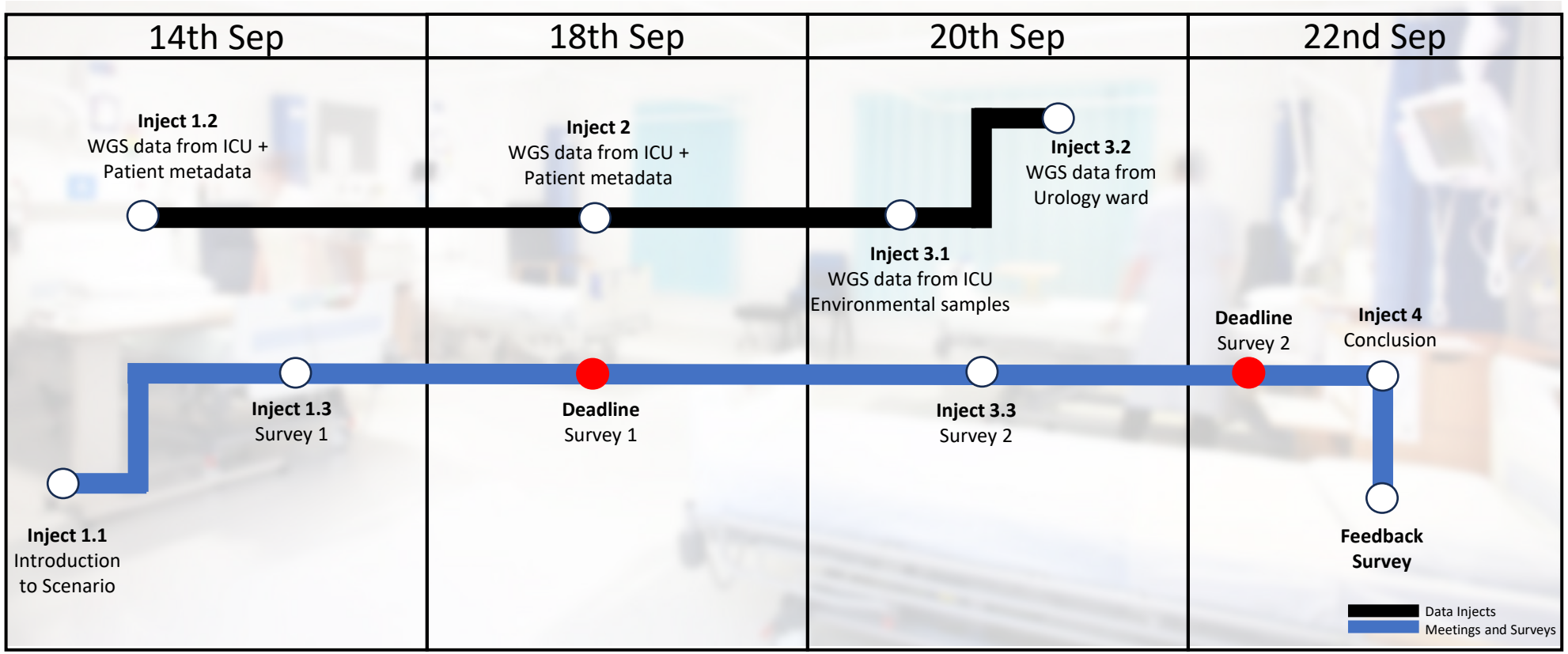
**The workshops build on the previous workshops**

**– video recordings of previous workshops are available**

# Learning objectives

- To be able to perform a **cluster analysis of bacteria** to look into possible genetic relatedness in a dataset
- To learn about other relevant **tools for sequence analysis** and work with a **real outbreak data set** to characterise and analyse
- Hands-on work on using the results from the cluster analysis, bacterial typing, and simulated patient metadata to elucidate a possible nosocomial outbreak

# Exercise overview



# Outbreak scenario background

*Location: Hospital X, Europe*

- Increased detection of carbapenem resistant *P. aeruginosa* (CRPa) in the ICU was observed.
- The infection prevention and control team (IPCT) has been alerted and initiated the screening of ICU for the multidrug resistant organisms (MDRO) upon admission, discharge, and twice weekly for patients in the ICU.
- There was an endemic problem with *Stenotrophomonas maltophilia* in the ICU detected in 2021 with a water reservoir as suspected source.
- Since *P. aeruginosa* and *S. maltophilia* are closely related environmental species and commonly associated with nosocomial infections, screening of environmental surfaces is being carried out in the ICU.

Fictitious

# Scenario-Roles

## Outbreak Management Team (OMT)

- Inter-department communication
- Patient health records
- Environmental assessment (e.g. identification of contaminated food or food handling equipment, infection control breaches, cleaning, environmental sampling)
- epidemiological data (e.g. movements and contacts of cases)
- Laboratory data (e.g. whole genome sequencing)

## Participant's Role

The role of exercise participants is to support OMT in the analyses of epidemiology and laboratory data (including WGS data) to generate a hypothesis of the most likely exposure that has caused the outbreak.

# Injects 1.1 to 1.3

**Sep 14, 2023**

-Introduction to outbreak scenario

-Tools for plasmid replicon typing

**Inject 1.1 (Introduction)**

10 carbapenem resistant *P. aeruginosa* isolates from different patients at ICU were identified in Hospital X

MLST, cluster analysis, AMR, Plasmid replicon analysis

**Inject 1.2**

Survey 1

Submit answers by Sep 18 12:00 CET

**Inject 1.3**

# Inject 2

**Sep 18, 2023**

Further 08 *P. aeruginosa* identified at ICU, Hospital X

cluster analysis, MLST, Plasmid replicon analysis

**Inject 2**

Deadline for Survey 1
12:00 CET

# Injects 3.1 to 3.3

**Sep 20, 2023**

09 *P. aeruginosa* identified from sinks, pipes, etc, **ICU**, Hospital X

cluster analysis, AMR genes, plasmid analysis

**Inject 3.1**

02 Cases from **urology ward** in were identified.

cluster analysis, AMR genes, plasmid analysis

**Inject 3.2**

Survey 2

Submit answers by Sep 22 09:00 CET

**Inject 3.3**

# Inject 4 – Results/discussion

**Sep 22, 2023**

Deadline for Survey 2
09:00 CET

-Results and conclusion of the exercise

-Additional analysis for plasmid typing – MOB-Recon

Questions

**Inject 4**

**A total of 29 *P. aeruginosa* isolates**

# Tasks - analyses

**Analyses**

- Characterisation:
  - Carbapenem resistance mechanism?
  - Plasmid replicons
- Subtyping:
  - MLST
- Cluster analysis

**Bioinformatics Tools**

- ResFinder/CARD/AmrFinderPlus
- MOB-Suite (Galaxy server)
- MyDbFinder with custom replicon database
- MLST at CGE (or Pasteur)
- CSIPhylogeny

No need characterize all isolates! Analyse isolates that are relevant to answer the survey questions.

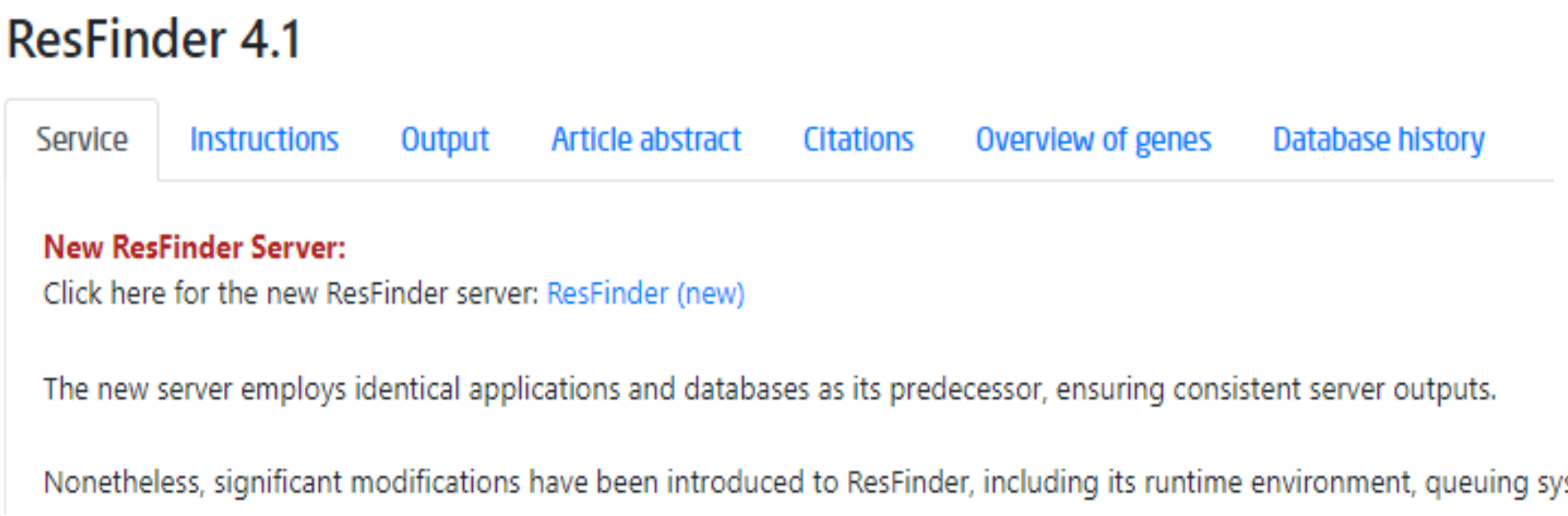
(Disclaimer: In the real outbreak, you need to characterize all the suspected case isolates)

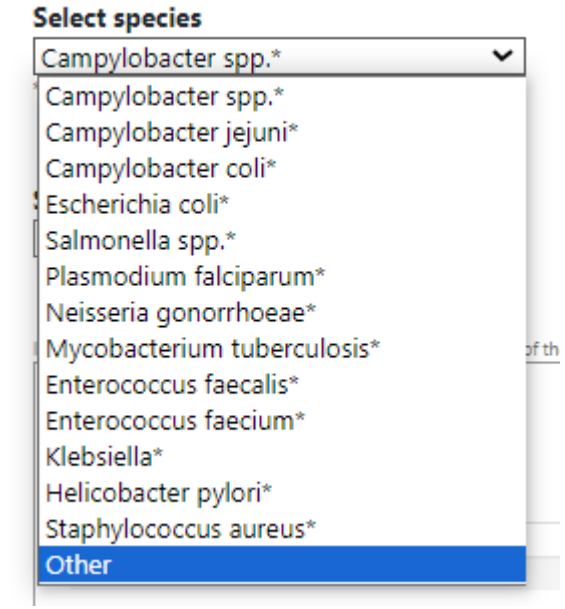
# Survey questions

- AMR genes (carbapenemase gene and gene variant?)
- MLST's?
- How many clusters?
- Part of a cluster?
  - How many SNPs difference within core-cluster?
- Plasmid carrying carbapenemase gene?
- Possible source of the outbreak?
  - Environmental vs clinical isolates

PDFs of the survey questions (Inject 1.3 + 3.3) available

# New ResFinder server & entry




Old:

https://cge.food.dtu.dk/services/ResFinder/

New:

http://genepi.food.dtu.dk/resfinder

**Both are working and run-time should be faster!**

**Be aware – ResFinder does not have a point mutation scheme for Pseudomonas**

**– you can only search for acquired genes**

# Carbapenem resistance in *P. aeruginosa*

- Several mechanisms of resistance in action
  - Acquired carbapenemase genes
  - Point mutations
  - Porin modification/deletion
  - Efflux pumps

- Acquired genes are often on plasmids
  - Plasmid content is continuously evolving
  - High volume of small mobile genetic elements (MGE) on plasmids
  - Other MGEs can also carry resistance genes

# Detection of plasmids from WGS data

- Several bioinformatics tools and databases are available for plasmid identification

- Usually, only the plasmid replicon is detected
- Numerous variants of some replicons

- Limited analysis possible using Illumina short reads!
  - Plasmid DNA can be assembled into several contigs
  - Hybrid plasmids are hard to detect

  - ONT MinION or other long read sequence data typically used for confirmation and full assembly/characterisation of plasmids

# Co-localisation of plasmid replicons and resistance genes on same contig

- For *E. coli, Klebsiella* and other *Entrobacterales* there are several suitable tools
  - We have available CGE tools
    - **Limited** to Gram Positive & *Enterobacterales* plasmids

- It is more challenging to analyse other pathogens like:
  - *Pseudomonas aeruginosa*
  - *Acinetobacter baumannii*


https://cge.food.dtu.dk/services/MobileElementFinder/

https://cge.food.dtu.dk/services/PlasmidFinder/

# MobileElementFinder: *E. coli* vs. *P. aeruginosa*

## MobileElementFinder Results

Sample name: *E. coli*
Date:
MGEfinder version: 1.0.3
MGEdb version: 1.0.2

Displaying: **21** of **166** mobile elements

| Contig | Plasmid | #MGEs | Resistance |
|---|---|---|---|
| NODE_57_length_12689_cov_1.779... | | 1 | sul2 |
| NODE_83_length_3482_cov_1.5576... | | 0 | dfrA17, aadA5 |
| NODE_45_length_31502_cov_5.278... | IncX4 | 0 | mcr-1.26 |
| NODE_79_length_3955_cov_2.1724... | | 0 | tet(A) |
| NODE_41_length_41695_cov_5.106... | IncX1 | 1 | blaTEM-32, blaTEM-40 |
| NODE_35_length_44591_cov_1.855... | | 1 | blaCTX-M-1 |
| NODE_6_length_182344_cov_3.375... | | 2 | |
| NODE_22_length_84699_cov_3.299... | | 2 | |
| NODE_70_length_7174_cov_2.9338... | | 0 | |
| NODE_31_length_49762_cov_2.263... | IncFII(pSE11) | 0 | |
| NODE_60_length_10308_cov_3.487... | | 0 | |
| NODE_16_length_115775_cov_3.17... | | 0 | |
| NODE_61_length_10163_cov_2.768... | | 0 | |
| NODE_12_length_137967_cov_3.19... | | 0 | |

## MobileElementFinder Results

Sample name: *P. aeruginosa*
Date:
MGEfinder version: 1.0.3
MGEdb version: 1.0.2

Displaying: **4** of **38** mobile elements

| Contig | Plasmid | #MGEs | Resistance |
|---|---|---|---|
| contig00005 len=433304 cov=38.... | | 0 | fosA |
| contig00029 len=5266 cov=40.7 ... | | 0 | tet(G) |
| contig00001 len=743927 cov=38.... | | 0 | blaPAO, aph(3')-IIb |
| contig00028 len=5992 cov=37.8 ... | | 0 | rmtB |
| contig00022 len=84655 cov=38.1... | | 2 | qacE, qnrVC1, ... |
| contig00010 len=272534 cov=39.... | | 1 | blaOXA-395 |
| contig00051 len=930 cov=78.0 c... | | 0 | sul1 |
| contig00023 len=83036 cov=39.1... | | 0 | catB7 |
| contig00039 len=1569 cov=39.4 ... | | 0 | blaNDM-1 |
| contig00003 len=517527 cov=38.... | | 0 | crpP |
| contig00004 len=472663 cov=38.... | | 1 | |

# Plasmid replicon typing: alternative tools

- MOB-Typer is a tool from MOB-suite
  - Command-line tool and has no official web-based application

- Two ways we can still use MOB-suite without command-line interface
  - CGE tools: MyDBFinder (https://cge.food.dtu.dk/services/MyDbFinder/)
    - Simple tool for BLASTing your query sequence (.fa) against your specified database
      - Use the MOB Suite database to search for plasmid replicons
      - one isolate at a time!

  - European Galaxy server (https://usegalaxy.eu/)
    - MOB-Suite incorporated
      - Can handle batch-upload

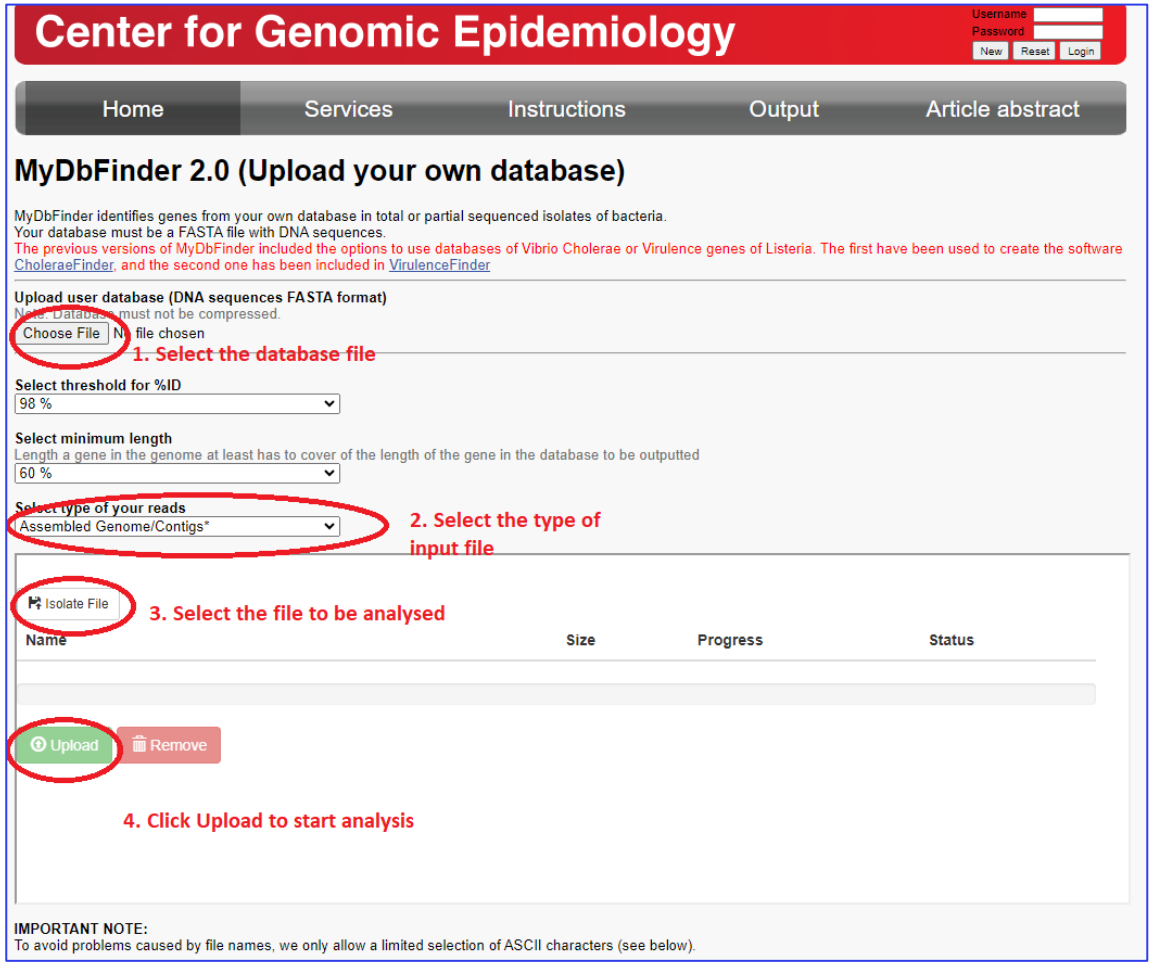
**MOB-suite: Software tools for clustering, reconstruction and typing of plasmids from draft assemblies**

https://github.com/phac-nml/mob-suite

# Finding plasmids using MyDBFinder

- Choose the MOB Suite database (text file) as user data base (located in Inject 1.1 folder on ScienceData)
- Adjust threshold for ID if you like
- Analyse one strain per upload

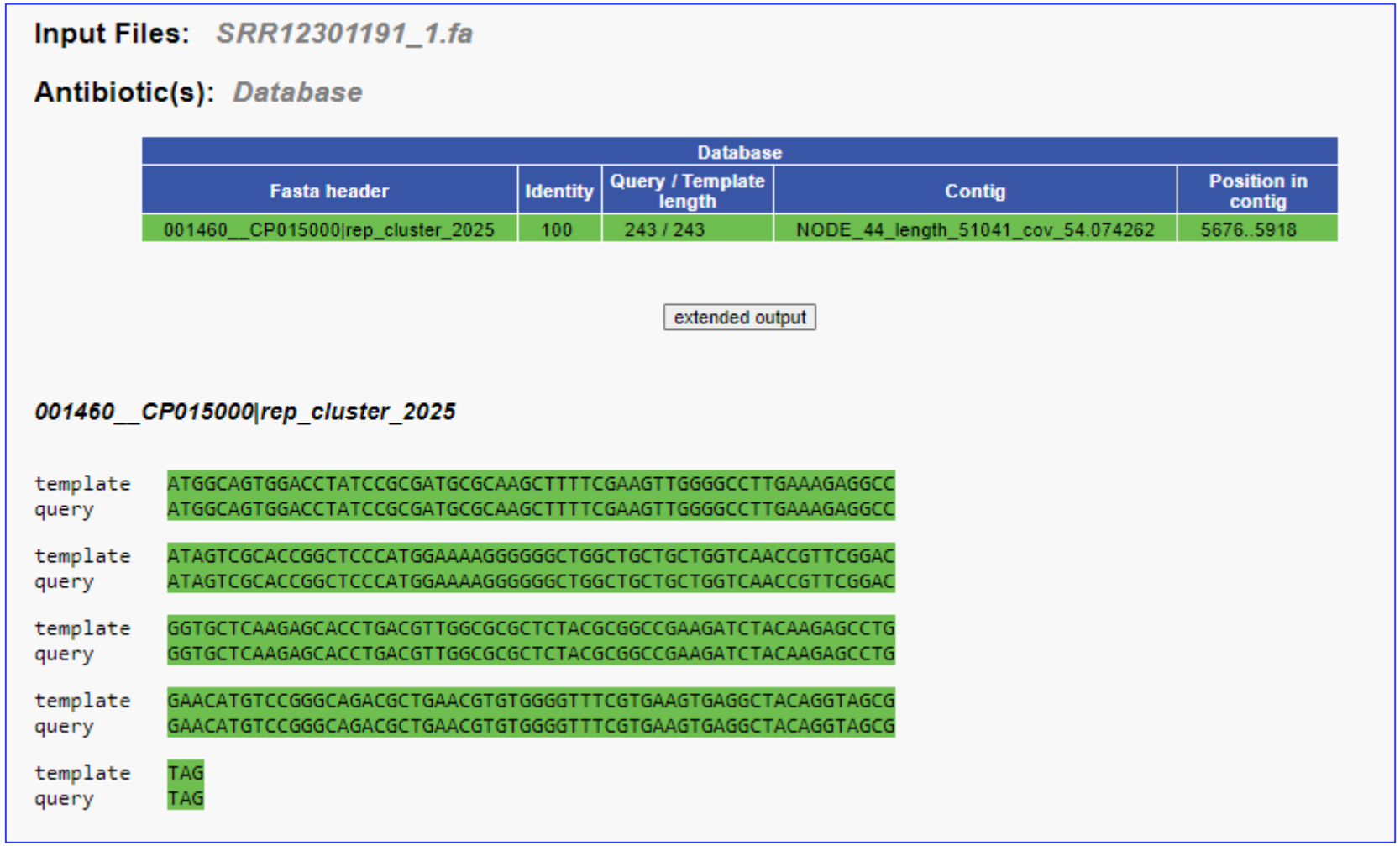
Database file: *rep.dna.fas*




Latest database can also be downloaded from MOB-Suite github page. See Annex. 3 in Inject 1.1

# Output from MyDBFinder

- MOB Suite DB fasta header
  - Accession number: CP15000
  - Rep_cluster_#: 2025
  - Identity %
  - Length of replicon
  - Which contig (NODE_44)
  - Length of contig (51,041 bp)
  - Position in contig
- Extended output
  - Variations in bases



**Input Files:** *SRR12301191_1.fa*

**Antibiotic(s):** *Database*

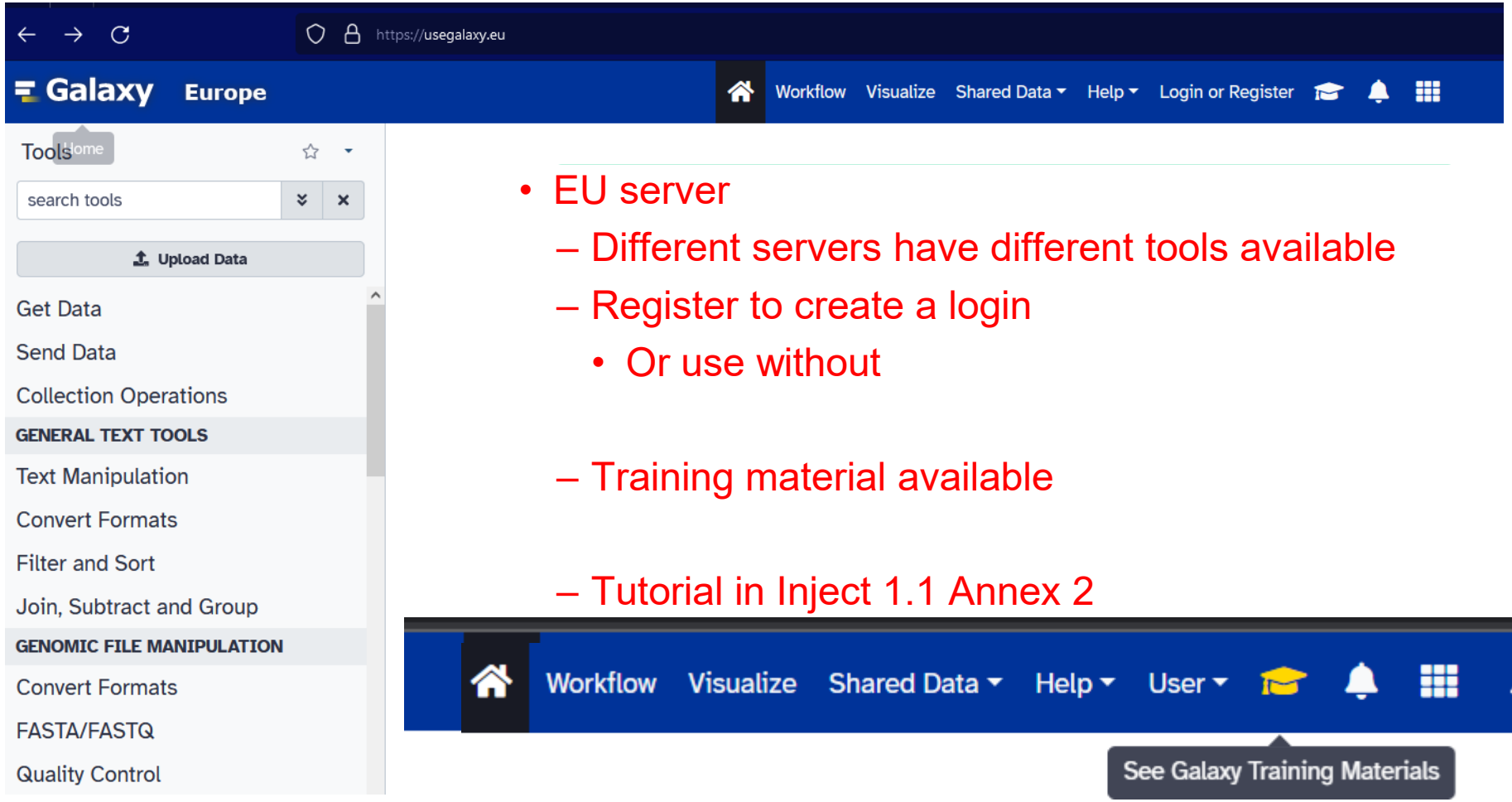
| Database | | | | |
|---|---|---|---|---|
| Fasta header | Identity | Query / Template length | Contig | Position in contig |
| 001460__CP015000\|rep_cluster_2025 | 100 | 243 / 243 | NODE_44_length_51041_cov_54.074262 | 5676..5918 |

extended output

***001460__CP015000|rep_cluster_2025***

```
template  ATGGCAGTGGACCTATCCGCGATGCGCAAGCTTTTCGAAGTTGGGGCCTTGAAAGAGGCC
query     ATGGCAGTGGACCTATCCGCGATGCGCAAGCTTTTCGAAGTTGGGGCCTTGAAAGAGGCC

template  ATAGTCGCACCGGCTCCCATGGAAAAGGGGGGCTGGCTGCTGCTGGTCAACCGTTCGGAC
query     ATAGTCGCACCGGCTCCCATGGAAAAGGGGGGCTGGCTGCTGCTGGTCAACCGTTCGGAC

template  GGTGCTCAAGAGCACCTGACGTTGGCGCGCTCTACGCGGCCGAAGATCTACAAGAGCCTG
query     GGTGCTCAAGAGCACCTGACGTTGGCGCGCTCTACGCGGCCGAAGATCTACAAGAGCCTG

template  GAACATGTCCGGGCAGACGCTGAACGTGTGGGGTTTCGTGAAGTGAGGCTACAGGTAGCG
query     GAACATGTCCGGGCAGACGCTGAACGTGTGGGGTTTCGTGAAGTGAGGCTACAGGTAGCG

template  TAG
query     TAG
```

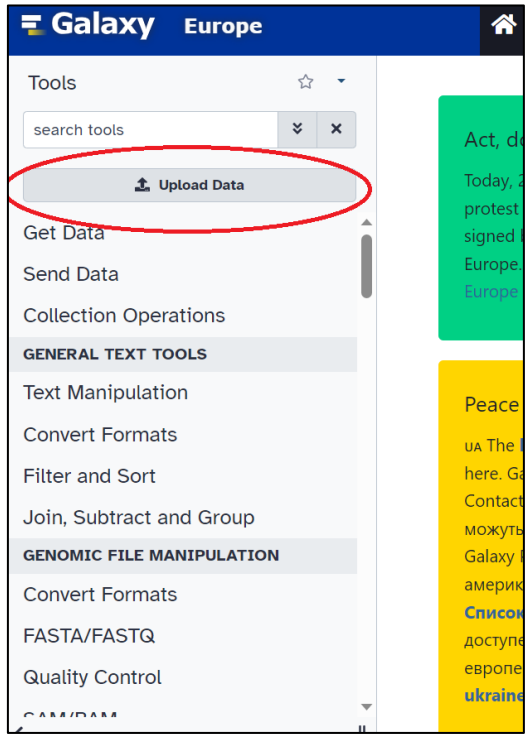
# Use Galaxy – https://usegalaxy.eu/

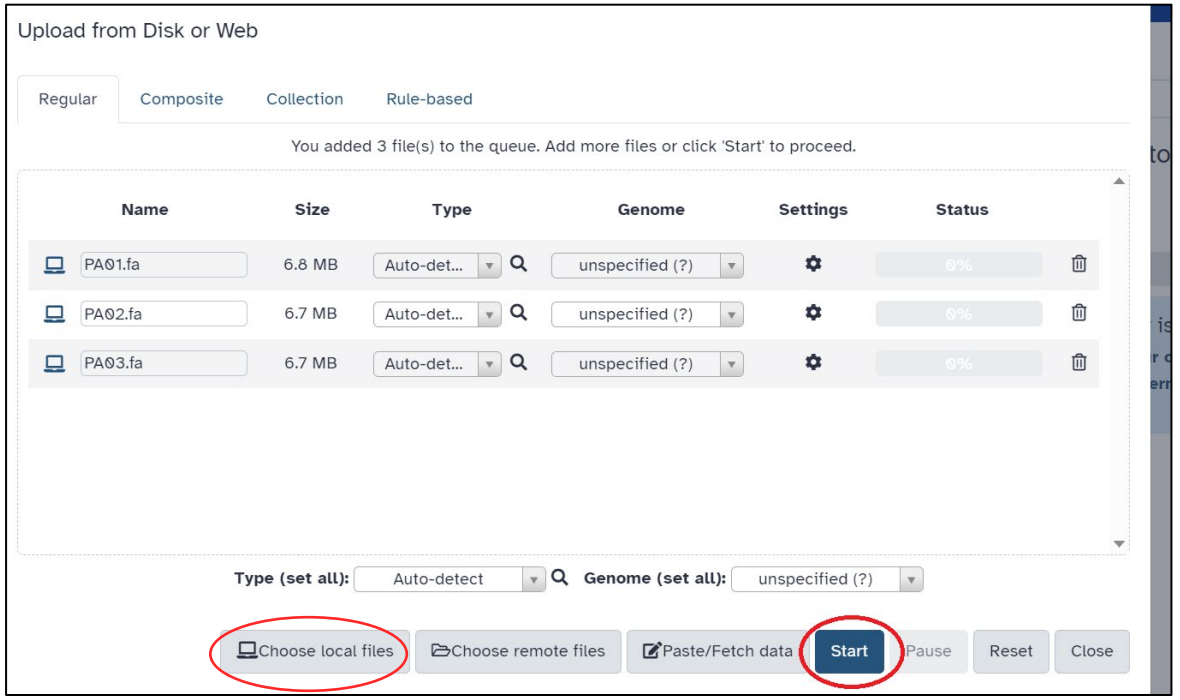


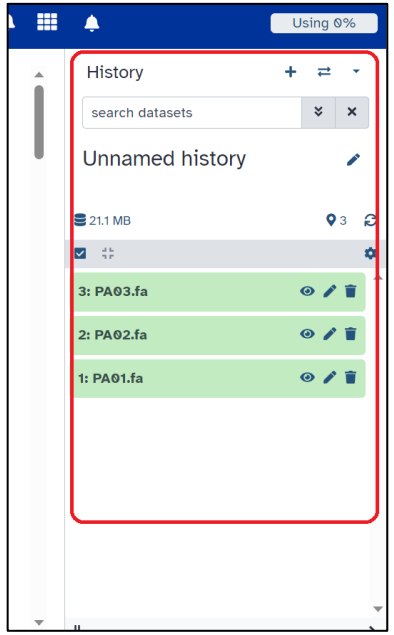

- EU server
  - Different servers have different tools available
  - Register to create a login
    - Or use without
  - Training material available
  - Tutorial in Inject 1.1 Annex 2

# Galaxy: MOB typer

- Upload the genomes (fasta files) that you want to analyse with MOB-typer.

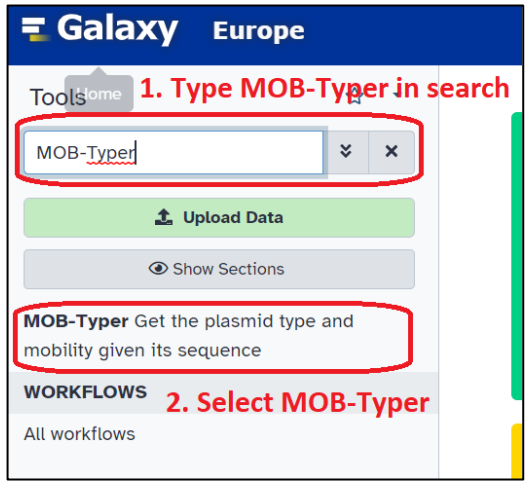


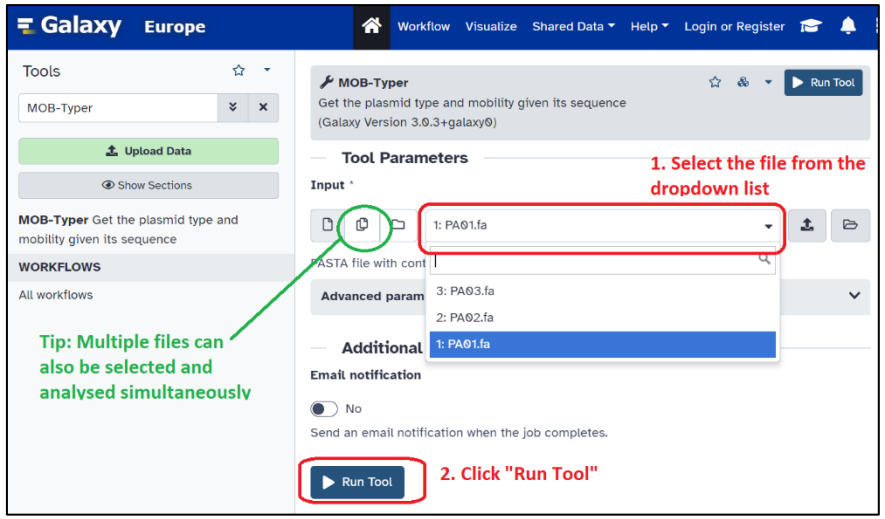



# Galaxy: MOB typer

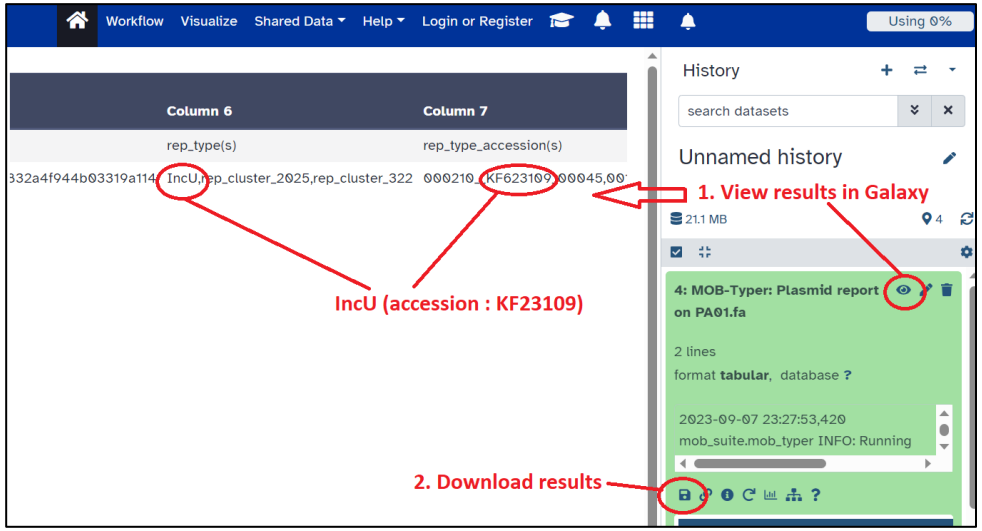
- Run **MOB-typer** from Galaxy Toolshed




Search MOB-Typer




Run the analysis




Interpret the results

# Co-localisation of plasmid replicons and resistance genes on same contig?

- Use the Contig outputs from fasta file analysis
  - ResFinder or other AMR detection tool
  - MyDbFinder on plasmid database

  - Keep in mind that lack of consensus does not necessarily mean that the gene is not on a plasmid

| Beta-lactam | | | | | |
|---|---|---|---|---|---|
| Resistance gene | Identity | Alignment Length/Gene Length | Position in reference | Contig or Depth | Position in contig |
| blaNDM-1 | 100.0 | 813/813 | 1..813 | contig00039 len=1569 cov=39.4 corr=0 origname=NODE_39_length_1569_cov_39.422869 sw=shovill-spades/1.1.0 date=20230316 | 208..1020 |

| Database | | | | |
|---|---|---|---|---|
| Fasta header | Identity | Query / Template length | Contig | Position in contig |
| 001460__CP015000\|rep_cluster_2025 | 100 | 243 / 243 | NODE_44_length_51041_cov_54.074262 | 5676..5918 |

# Resources

- Slack
  - Injects (except sequence data)
  - Video recordings
  - Link to the surveys

- ScienceData folder: https://sciencedata.dk/shared/dd60004f4cccc3452d49f24cfb938af3
  - Sequence data
  - MOB-suite database (text file)
  - Inject text
  - Link to the surveys and PDF file of survey questions

- Data Injects: both fasta and fastq files for each isolate (Illumina)
  - No suitable MinION data available

# Next Session (Friday, 22nd September 13:00-14:30 CET)

- Walkthrough of results and survey questions
- Discussion about cluster analysis
  – Input from participants
- Discussion about replicon typing tools
  – Input from participants
- MOB-Recon tool
- Feedback about the exercise

# Questions/Comments?