GenEpi-BioTrain – Virtual Training

Whole-genome sequence-based detection of antimicrobial resistance in bacteria

September 2023

Outline

#	Topic	Time
1	Introduction	12:30 - 12:40
2	Brush-up antimicrobial resistance (AMR)	12:40 - 13:10
3	Fundamentals of WGS-based detection of AMR	13:10 - 13:40
4	Q&A	13:40 - 13:45
5	*Break*	13:45 - 14:00
6	Tools for WGS-based detection of AMR	14:00 – 14:45
7	WGS-based detection of AMR in Enterobacterales	14:45 – 15:00
8	*Break*	15:00 – 15:10
9	WGS-based detection of AMR in *Staphylococcus aureus* and *Enterococcus* spp.	15:10 – 15:30
10	Explanation of the exercise	15:30 – 15:35
11	Q&A, conclusions and session evaluation	15:35 – 16:00

Meeting rules

- Camera and microphones are automatically de-activated
- Recording ●REC
- Use the **Q&A** for questions

Active participation is key!

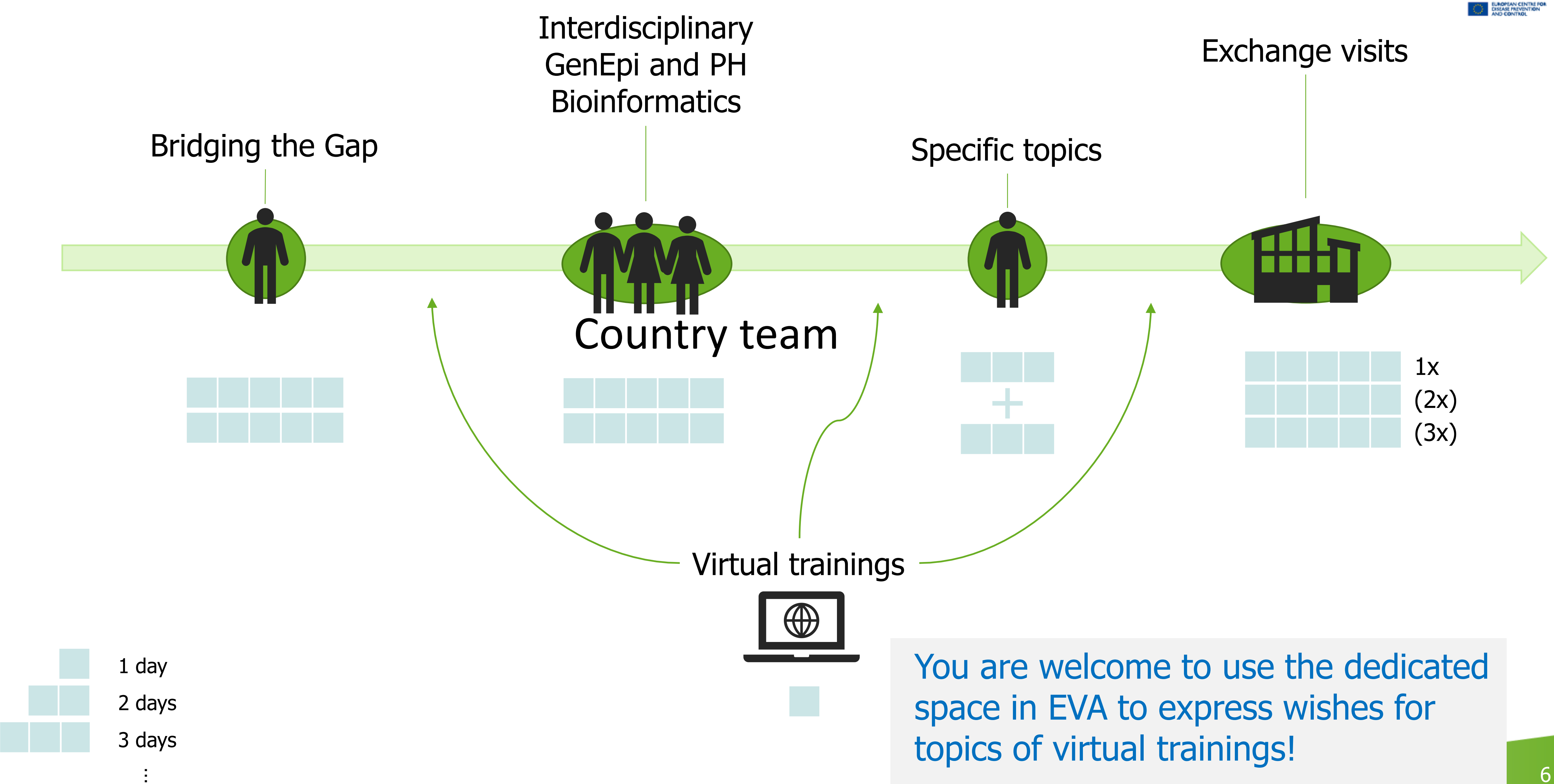
- Ask questions
- Share your knowledge
- Exchange your experiences
- Raise hands
- Participate in Slido polls and quizzes
- Interact after the session using the discussion forum in EVA ←

Let's try Slido! (Q1)

GenEpi-BioTrain

- 4-year contract (2023-2026)
- Funded by ECDC
- Managed by DTU and SSI
- Executed within a consortium (DTU, SSI, IP, KUH, RCB, THL)

Training activities in GenEpi-BioTrain



You are welcome to use the dedicated space in EVA to express wishes for topics of virtual trainings!

]

A few words about myself

- Working at Statens Serum Institut (SSI), Denmark
- DVM from University of Pisa, Italy
- PhD in AMR and zoonoses from University of Copenhagen, Denmark
- Working with several aspects of AMR
  - Research (evolution and transfer of ESBLs and carbapenemases)
  - Advisory services (EFSA, WHO, UNIDO)
  - Capacity building (EURL-AR, EURGen-RefLabCap, GenEpi-BioTrain)

…and a few words about you

Slido Q2 and Q3

Objectives

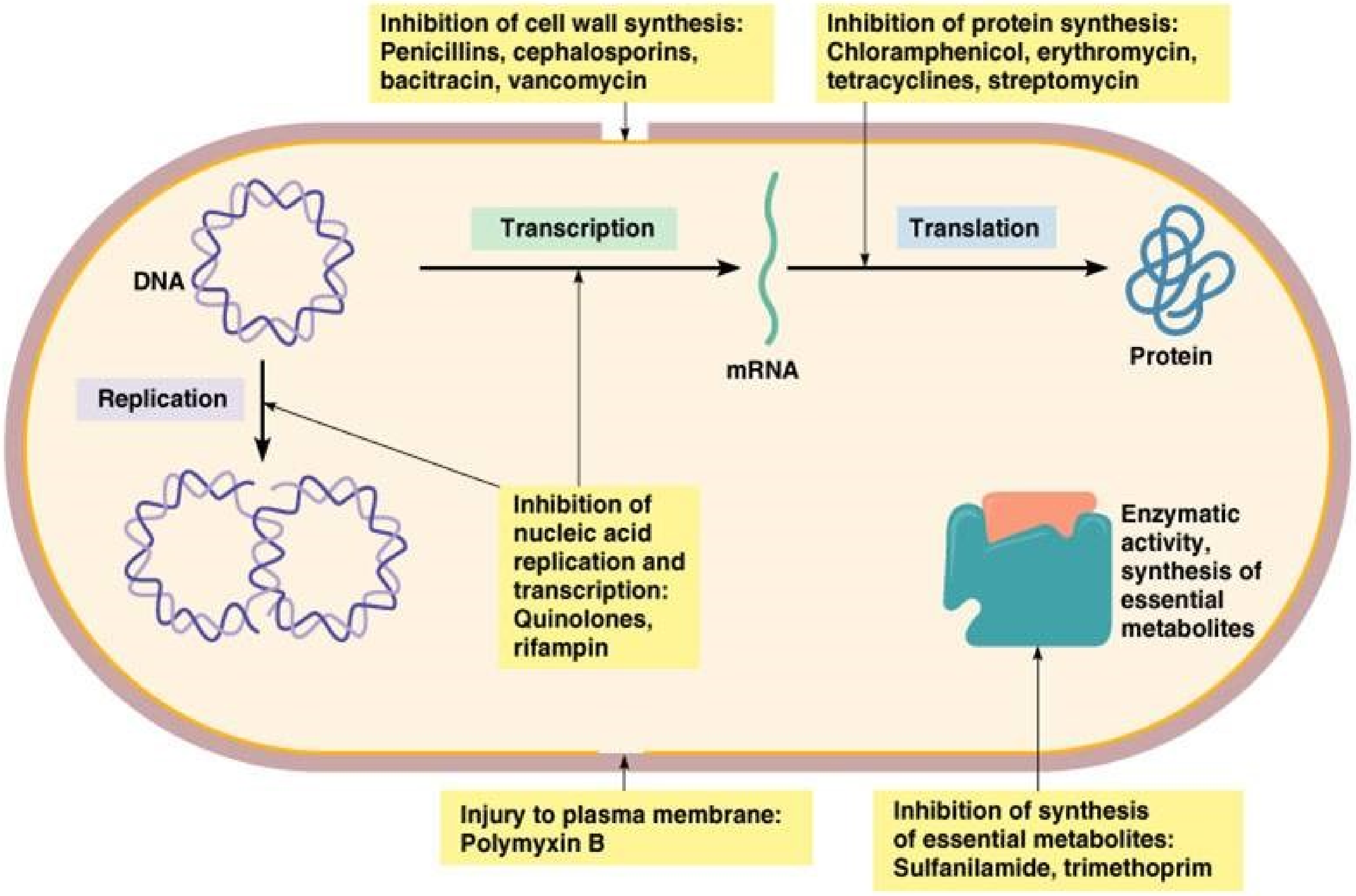
Specific objectives of this session:

1. Brush-up concepts of AMR: what is it and how do we detect it
2. Examine widely used tools for WGS-based detection of AMR
3. Introduce species-specific concepts of AMR for Enterobacterales, *S. aureus* and *Enterococcus* spp.

Related to the course goals to enable trainees to:

- Describe available open-source tools for detection of AMR in WGS data
- Describe the impact of different sequencing technologies on the detection of AMR
- Evaluate the advantages and disadvantages of AMR prediction based on phenotypic and genotypic methods in the context of surveillance

Antimicrobials target vital parts of bacterial cells



Inhibition of cell wall synthesis: Penicillins, cephalosporins, bacitracin, vancomycin

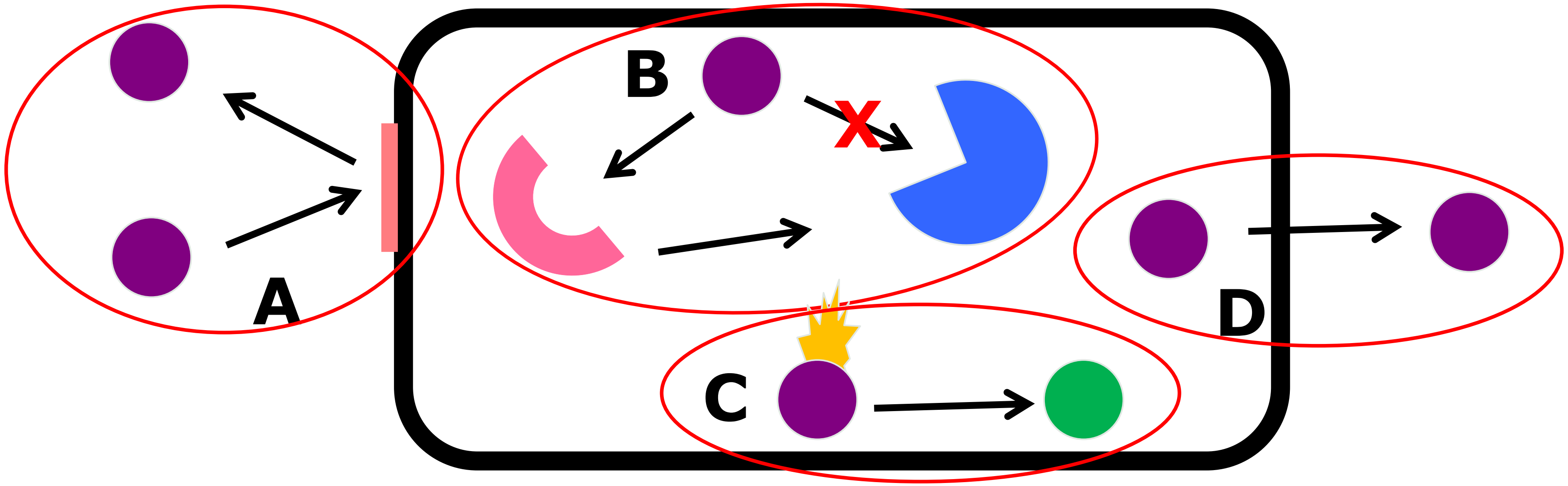
Inhibition of protein synthesis: Chloramphenicol, erythromycin, tetracyclines, streptomycin

Inhibition of nucleic acid replication and transcription: Quinolones, rifampin

Injury to plasma membrane: Polymyxin B

Inhibition of synthesis of essential metabolites: Sulfanilamide, trimethoprim

Bacteria natively have/can acquire mechanisms to avoid that antimicrobials reach their target



A, decreased cell permeability

B, alteration, replacement or protection of the target

C, enzyme inactivation

D, active export out of the cell

Antimicrobial Resistance

Intrinsic (expected resistant phenotypes):

due to structural or functional trait allowing tolerance by all members of a group (species, genus, etc…)

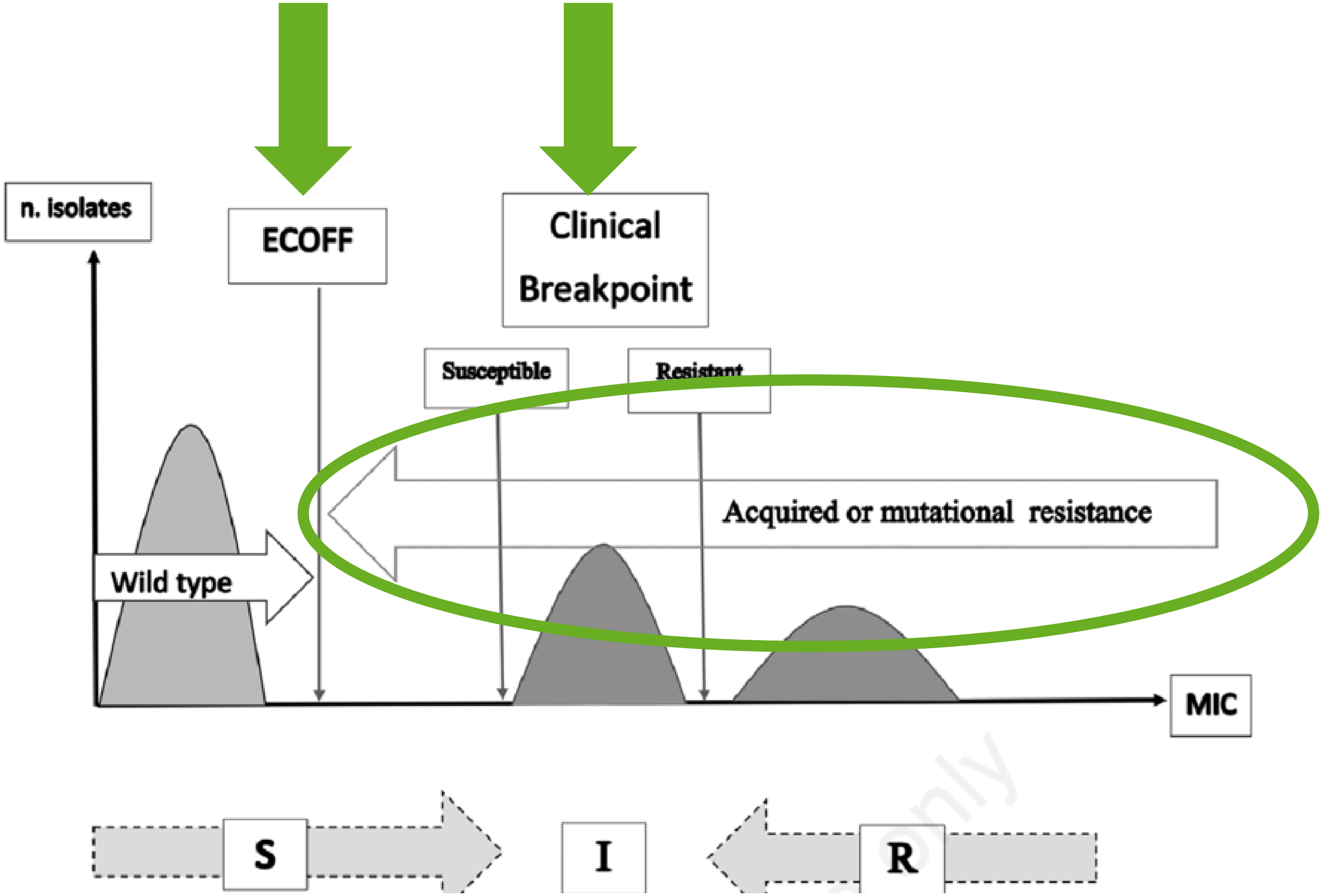
- Inaccessibility of the drug into the bacterial cell
- Innate production of enzymes that inactivate the drug
- Lack of affinity of the drug for the bacterial target

Antimicrobial Resistance

Acquired:

due to changes within an initially susceptible population

Key AMR concepts



Microbiological definition

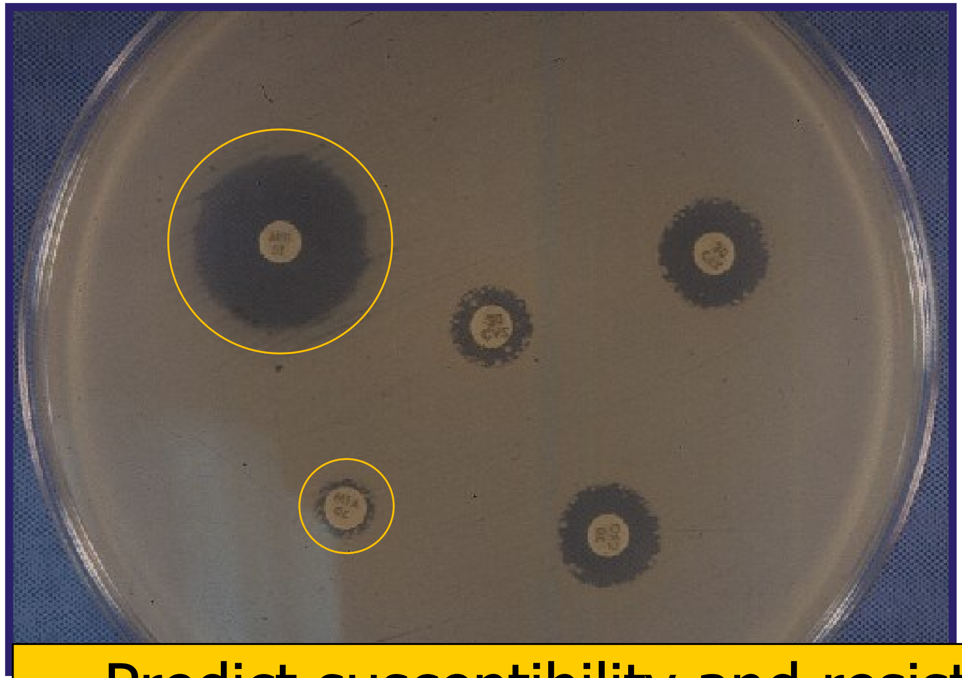
Resistance is the property of bacterial strains to survive at higher antibiotic concentrations compared with the wild-type population

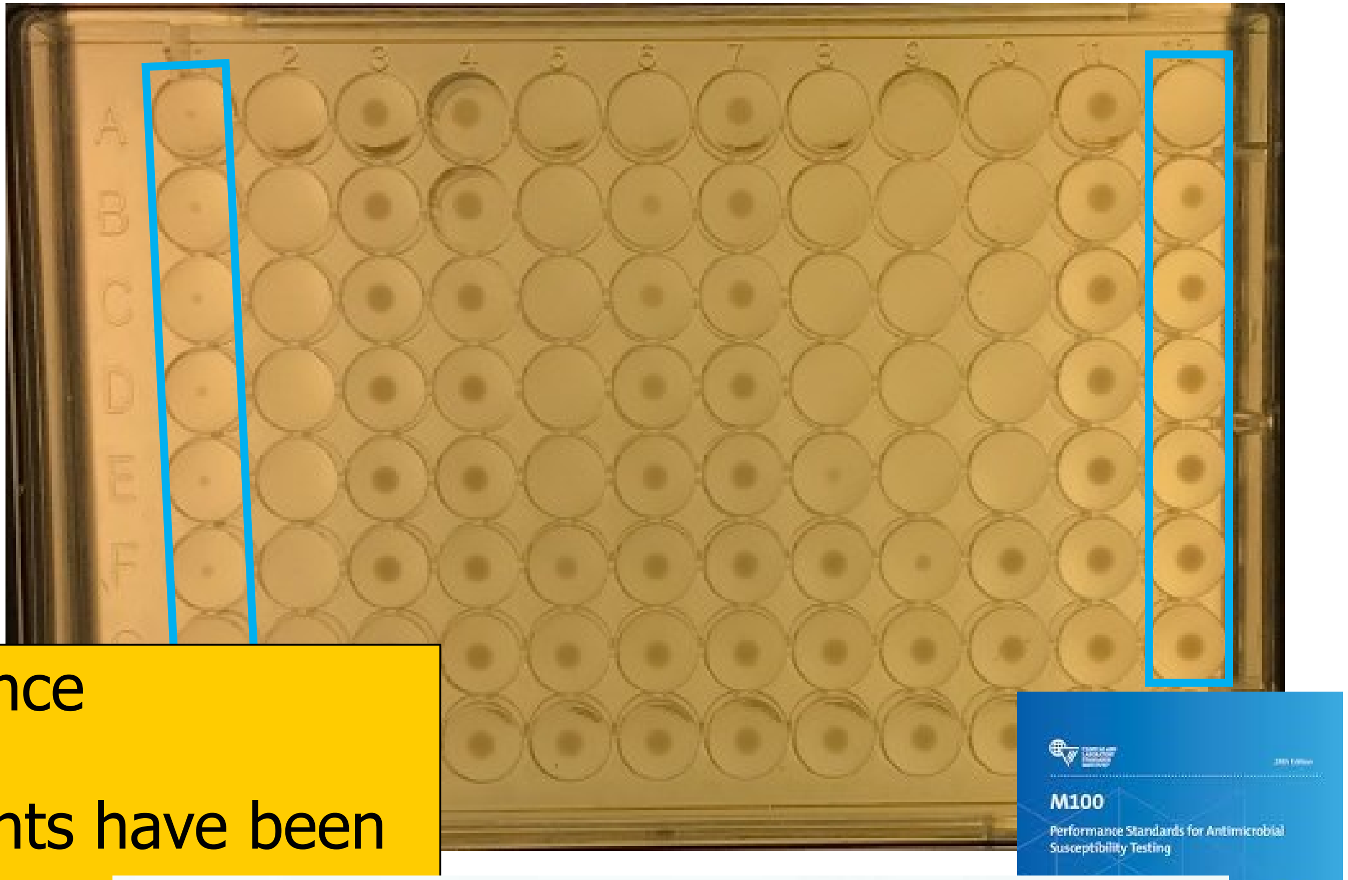
Clinical definition

Resistance is the bacterial ability to survive antimicrobial therapy

Tascini et al., 2016

Phenotypic methods for detection of AMR





M100

Performance Standards for Antimicrobial Susceptibility Testing

- Predict susceptibility and resistance
- Quantifiable
- Standard methods and breakpoints have been established
- Not always straightforward to read

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Genotypic methods for detection of AMR

- PCR
- Microarrays
- WGS

Outcome:

- Presence/Absence of genes/chromosomal mutations mediating AMR
- Caveats in making “Presence-Resistance” and “Absence-Susceptibility” correlations
- For WGS: lots of additional information useful for epidemiological purposes

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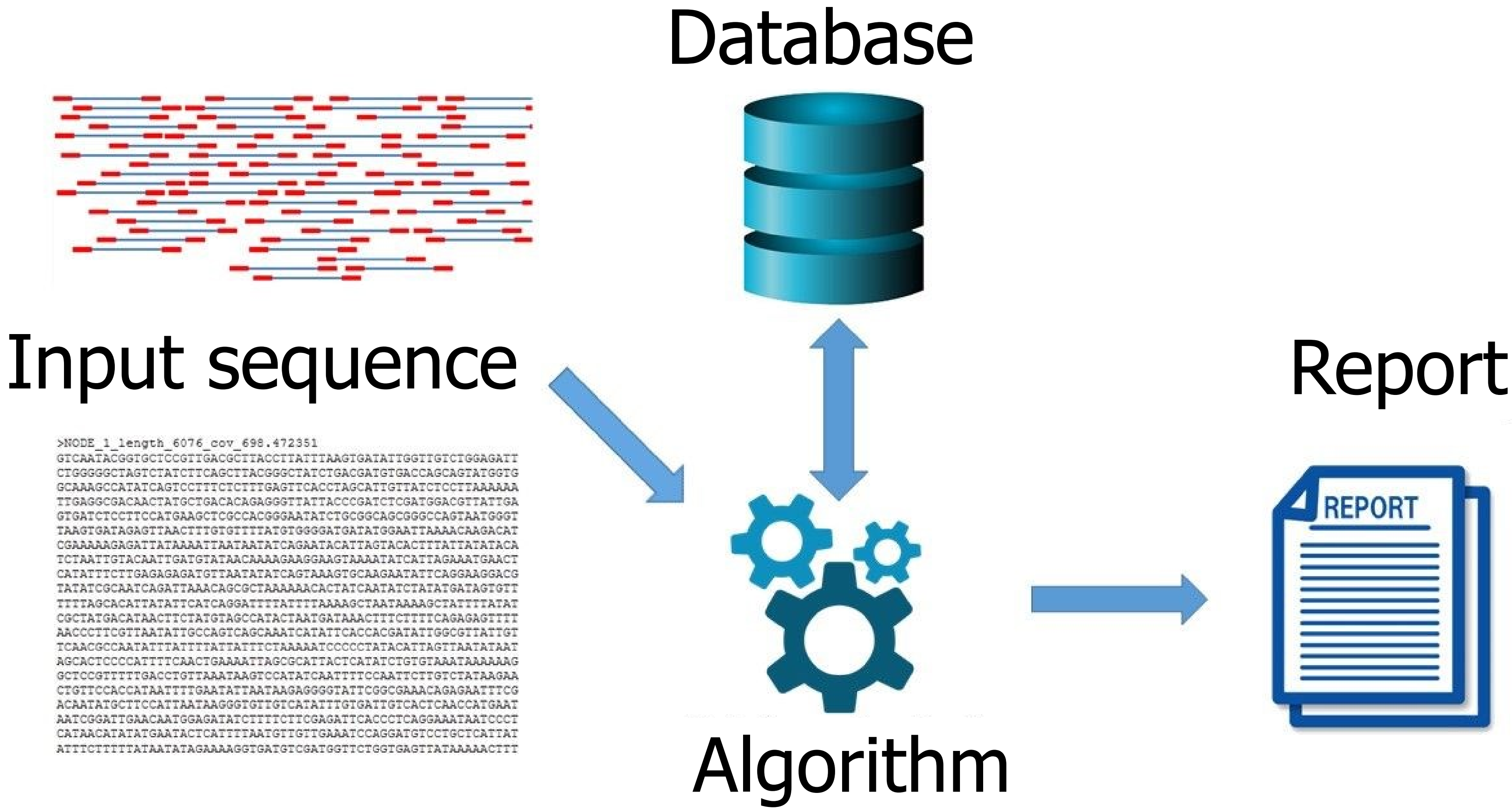
Fundamentals of WGS-based detection of AMR



Database

Input sequence

Report

Algorithm

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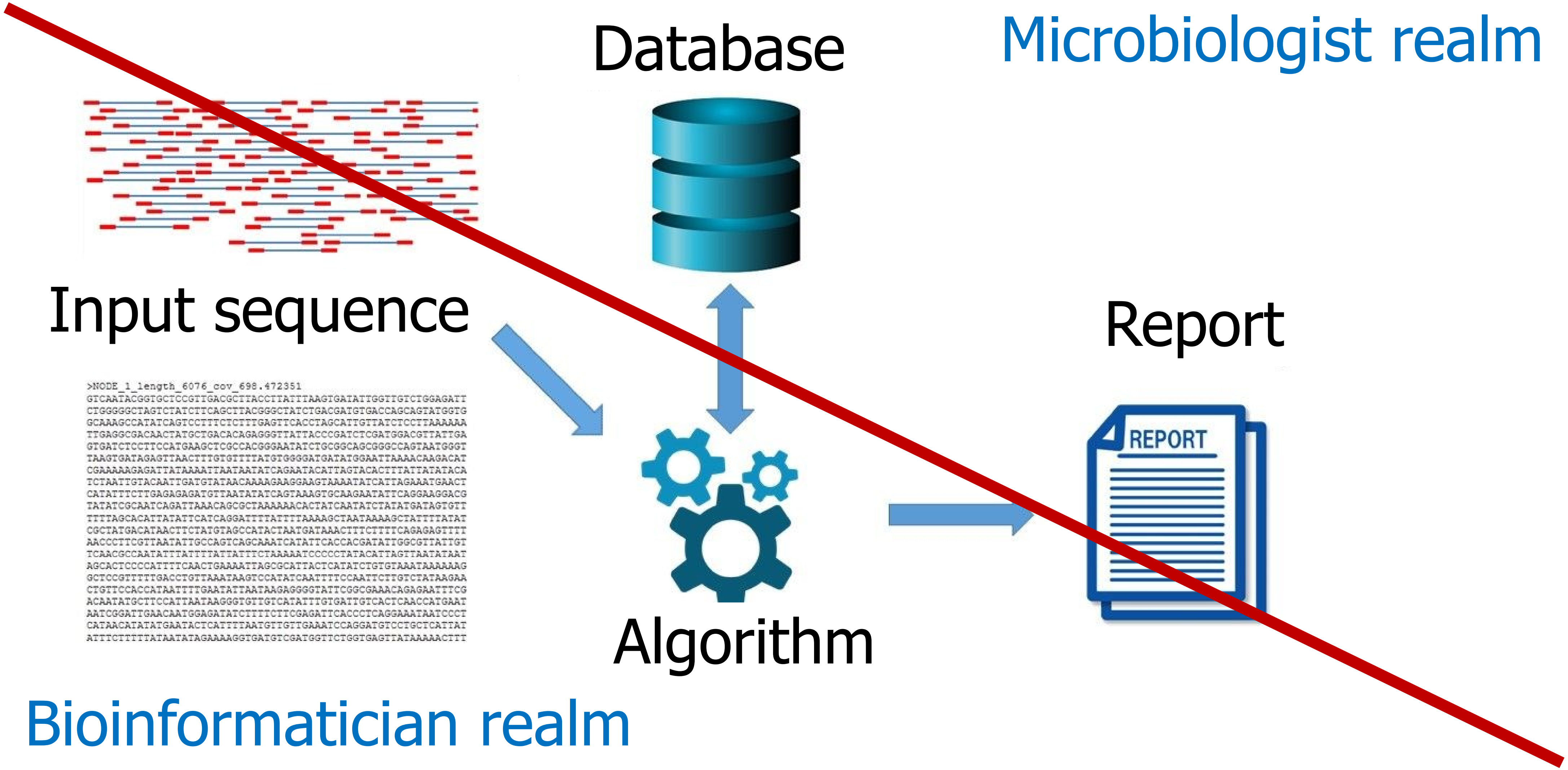
Modified from Hendriksen *et al.*, 2019

Fundamentals of WGS-based detection of AMR



Database

Microbiologist realm

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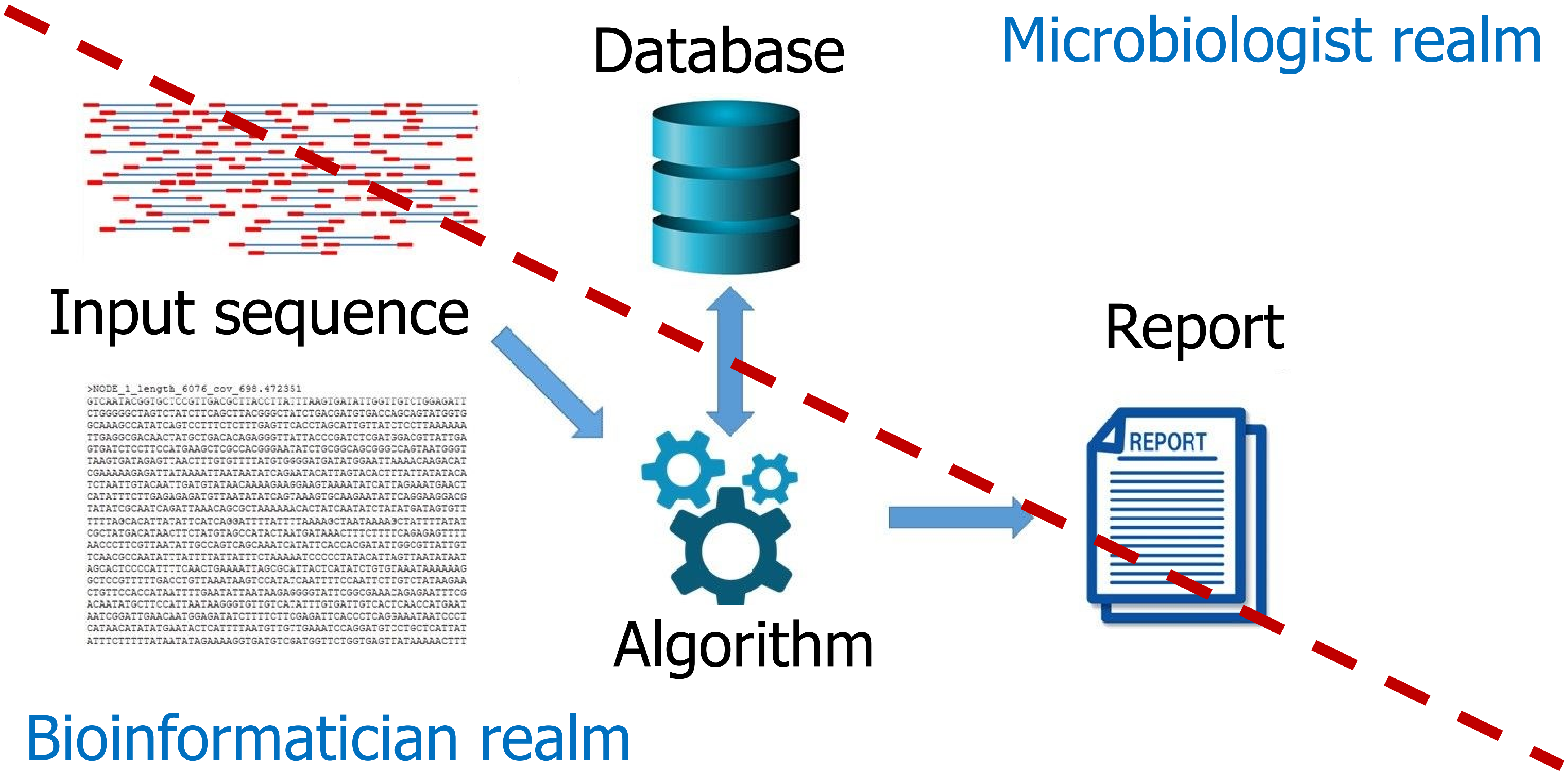
Report

Algorithm

Bioinformatician realm

Modified from Hendriksen *et al.*, 2019

Fundamentals of WGS-based detection of AMR



Microbiologist realm

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```

Algorithm

Bioinformatician realm

Modified from Hendriksen *et al.*, 2019

Slido 4

Input

- Nucleotide sequences
  - Raw reads (FASTQ files)
    - produced by the sequencer
  - Assembled sequence data aka contigs (FASTA files)
    - produced processing raw reads through assemblers
- Amino acid sequences

Users must ensure good quality of input sequences

(bad sequence quality = missing data)

Input – Nucleotide sequences

- Raw reads (FASTQ files)
  - Data of sub-optimal quality affect results
  - No information on location of the AMR genes
  - (!) some pipelines may perform assembly of the input raw reads

- Assembled sequences (FASTA files)
  - Poor assemblies (due to sub-optimal quality of WGS data) affect results
  - Different assemblers may lead to different results

GenEpi-BioTrain Virtual training **"From sequencer to polished reads for bacteria"** on **12 and 14 September 2023, at 9:00-13:00**
The participants will gain insights into interpreting quality control (QC) parameters as well as handling bad quality reads and contaminated data.

Input – Amino acid sequences

- (!) Some tools translate nucleotide sequence input into proteins
- Different annotation software may have an impact on the outcome of the analysis
- The AMR function is determined by the protein sequence and even single amino-acid changes can affect the phenotype
- A nucleotide approach can miss nonsense mutations
- Working in protein space also enables the use of protein-based HMMs that have been informed by biological data to discover potentially novel AMR genes

Algorithms

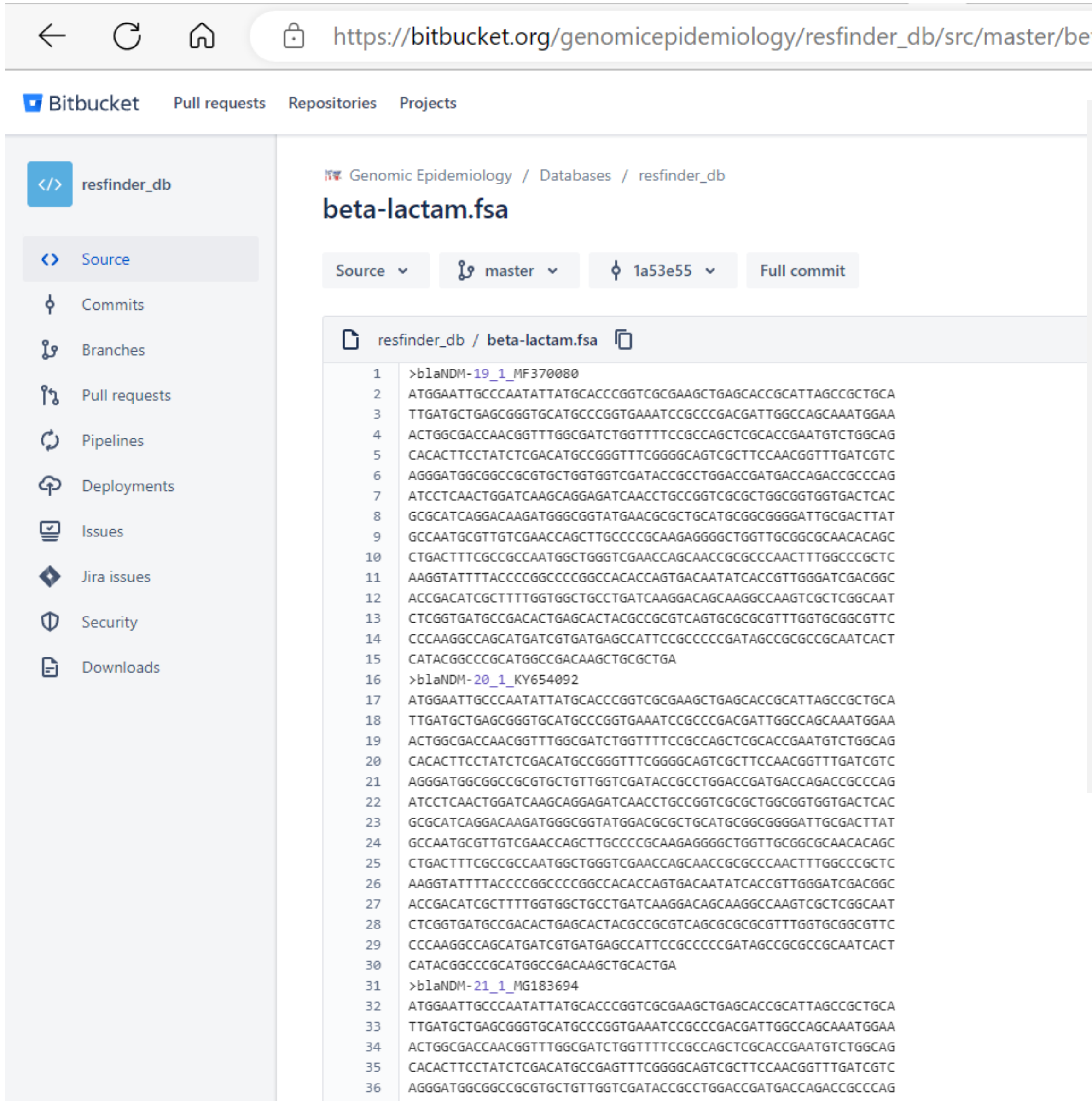
- BLAST
  - Applied to assembled data and amino acid sequence inputs
- *k*-mers
  - Applied to raw reads inputs
  - Rapid
- HMMs
  - Applied to amino acid sequence inputs

Algorithms are specific to each tool. For some algorithms, identity and coverage thresholds can be arbitrarily selected by the users

Databases

- They heavily influence the performance of tools
- They differ for structure, content and curation

A database of genes is a collection of sequences in FASTA format



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```

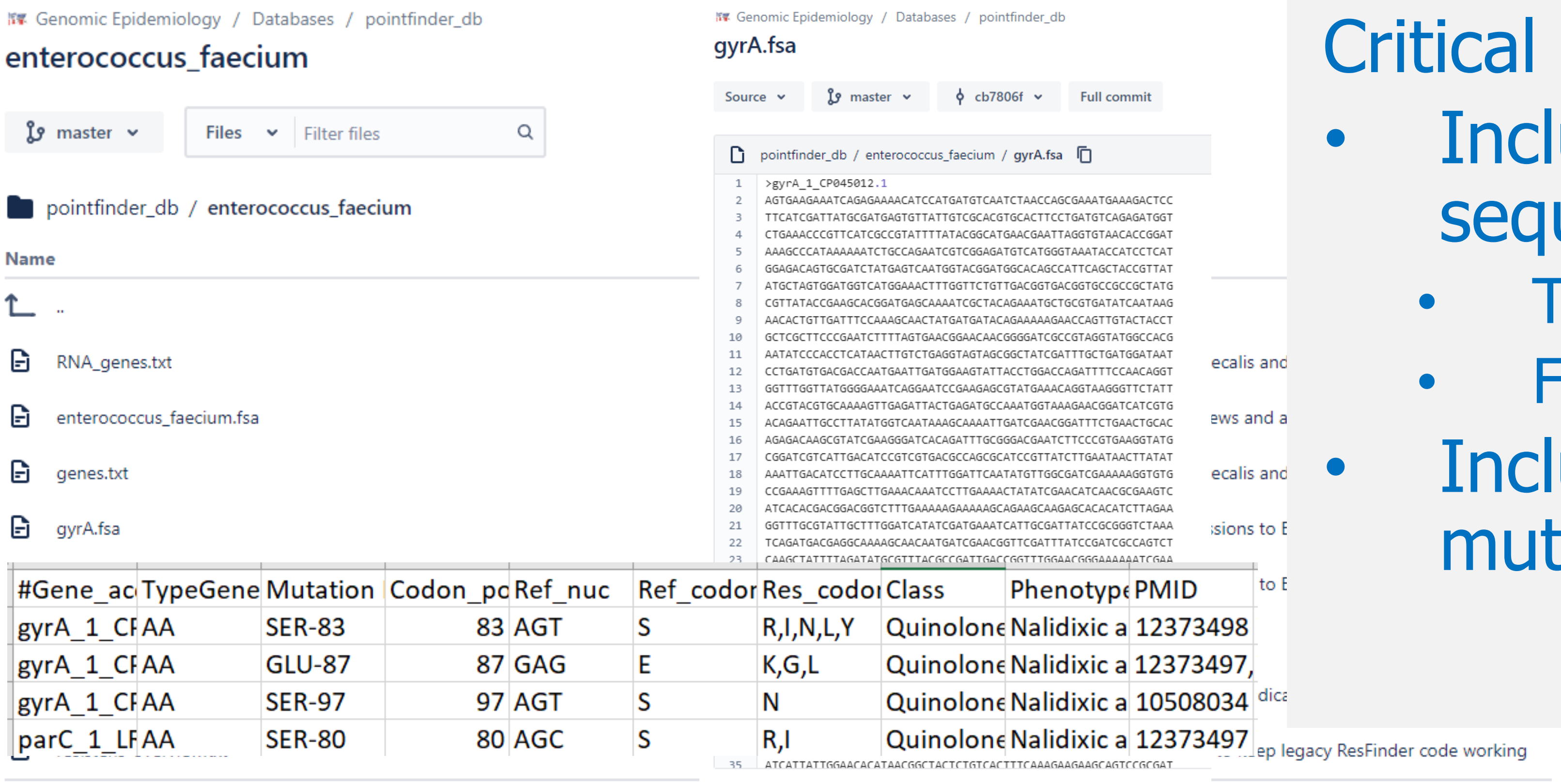
Critical steps:

- Include the correct sequences
  - True AMR genes (literature searches, retrieve sequences from curated repositories)
  - Full-length sequences
- Include the correct header

There are also AMR databases of protein sequences

A database of chromosomal mutations contains two key elements

- Sequence of each wild-type (WT) gene that is used for comparison (it is species-specific!)
- A list of mutations mediating resistance



Genomic Epidemiology / Databases / pointfinder_db

enterococcus_faecium

pointfinder_db / enterococcus_faecium

Name
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gyrA.fsa

Genomic Epidemiology / Databases / pointfinder_db

gyrA.fsa

pointfinder_db / enterococcus_faecium / gyrA.fsa

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gyrA_1_CF	AA	SER-97	97	AGT	S	N	Quinolone	Nalidixic a	10508034
parC_1_LF	AA	SER-80	80	AGC	S	R,I	Quinolone	Nalidixic a	12373497

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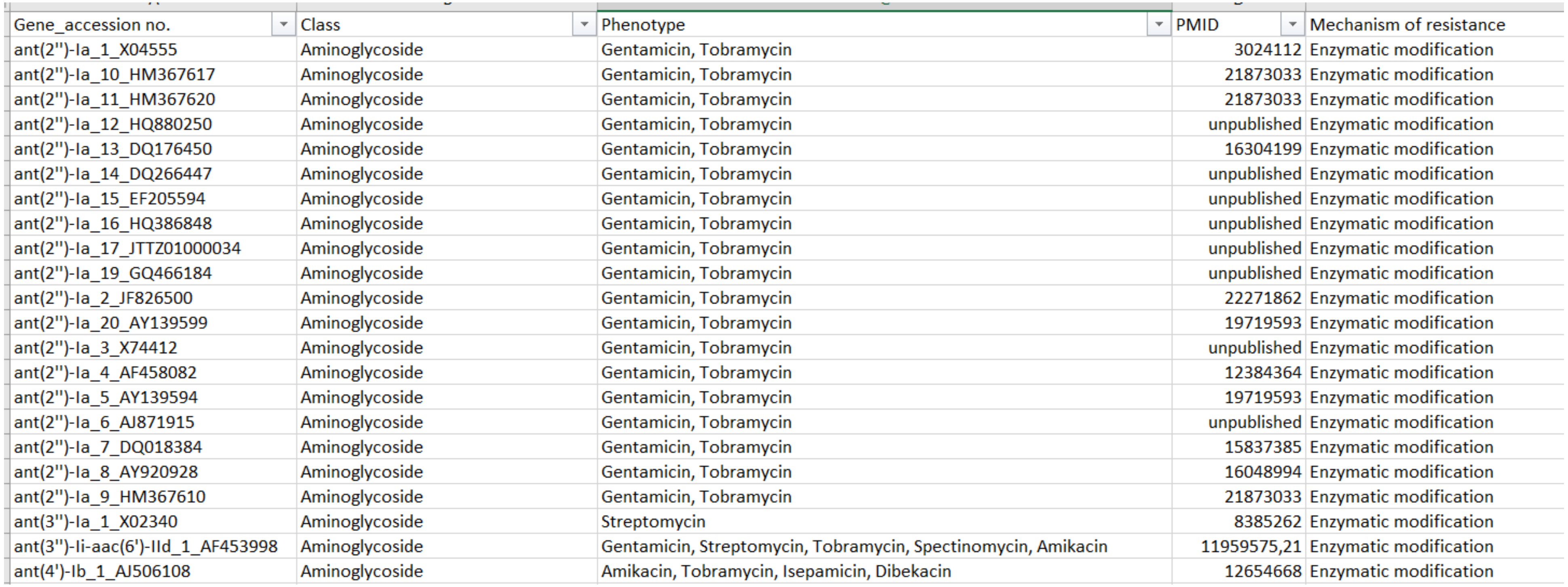
Critical steps:

- Include the correct WT sequences
  - True WT
  - Full-length sequences
- Include the correct mutations and positions

A database of phenotypes is a list of resistance phenotypes associated to specific genes/chromosomal mutations



Gene_accession no.	Class	Phenotype	PMID	Mechanism of resistance
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ant(2'')-Ia_10_HM367617	Aminoglycoside	Gentamicin, Tobramycin	21873033	Enzymatic modification
ant(2'')-Ia_11_HM367620	Aminoglycoside	Gentamicin, Tobramycin	21873033	Enzymatic modification
ant(2'')-Ia_12_HQ880250	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification
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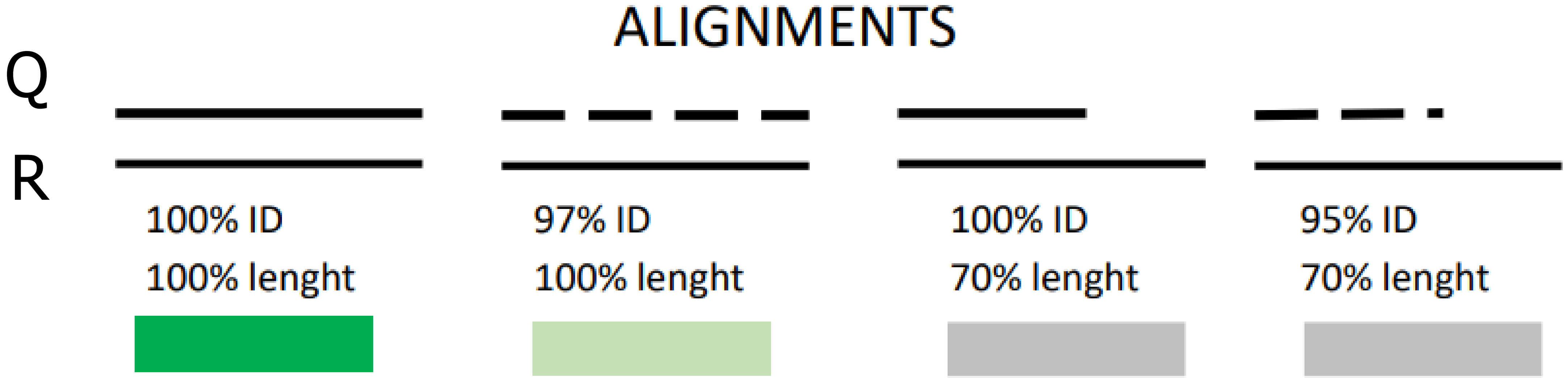
ant(2'')-Ia_3_X74412	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification
ant(2'')-Ia_4_AF458082	Aminoglycoside	Gentamicin, Tobramycin	12384364	Enzymatic modification
ant(2'')-Ia_5_AY139594	Aminoglycoside	Gentamicin, Tobramycin	19719593	Enzymatic modification
ant(2'')-Ia_6_AJ871915	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification
ant(2'')-Ia_7_DQ018384	Aminoglycoside	Gentamicin, Tobramycin	15837385	Enzymatic modification
ant(2'')-Ia_8_AY920928	Aminoglycoside	Gentamicin, Tobramycin	16048994	Enzymatic modification
ant(2'')-Ia_9_HM367610	Aminoglycoside	Gentamicin, Tobramycin	21873033	Enzymatic modification
ant(3'')-Ia_1_X02340	Aminoglycoside	Streptomycin	8385262	Enzymatic modification
ant(3'')-Ii-aac(6')-IId_1_AF453998	Aminoglycoside	Gentamicin, Streptomycin, Tobramycin, Spectinomycin, Amikacin	11959575,21	Enzymatic modification
ant(4')-Ib_1_AJ506108	Aminoglycoside	Amikacin, Tobramycin, Isepamicin, Dibekacin	12654668	Enzymatic modification

Database warnings!

- Familiarize with inclusion criteria of databases
- Nomenclature can be tricky: same gene but different name in different databases
- Be critical about the content of databases

Report - identity and coverage



ALIGNMENTS

Q

R

100% ID

100% lenght

97% ID

100% lenght

100% ID

70% lenght

95% ID

70% lenght

Coming next… Tools for WGS-based detection of AMR

We will focus on:

- AMRFinderPlus
- CARD/RGI
- PathogenWatch
- ResFinder

Several other tools exist

- See "Resources and further reading" on EVA

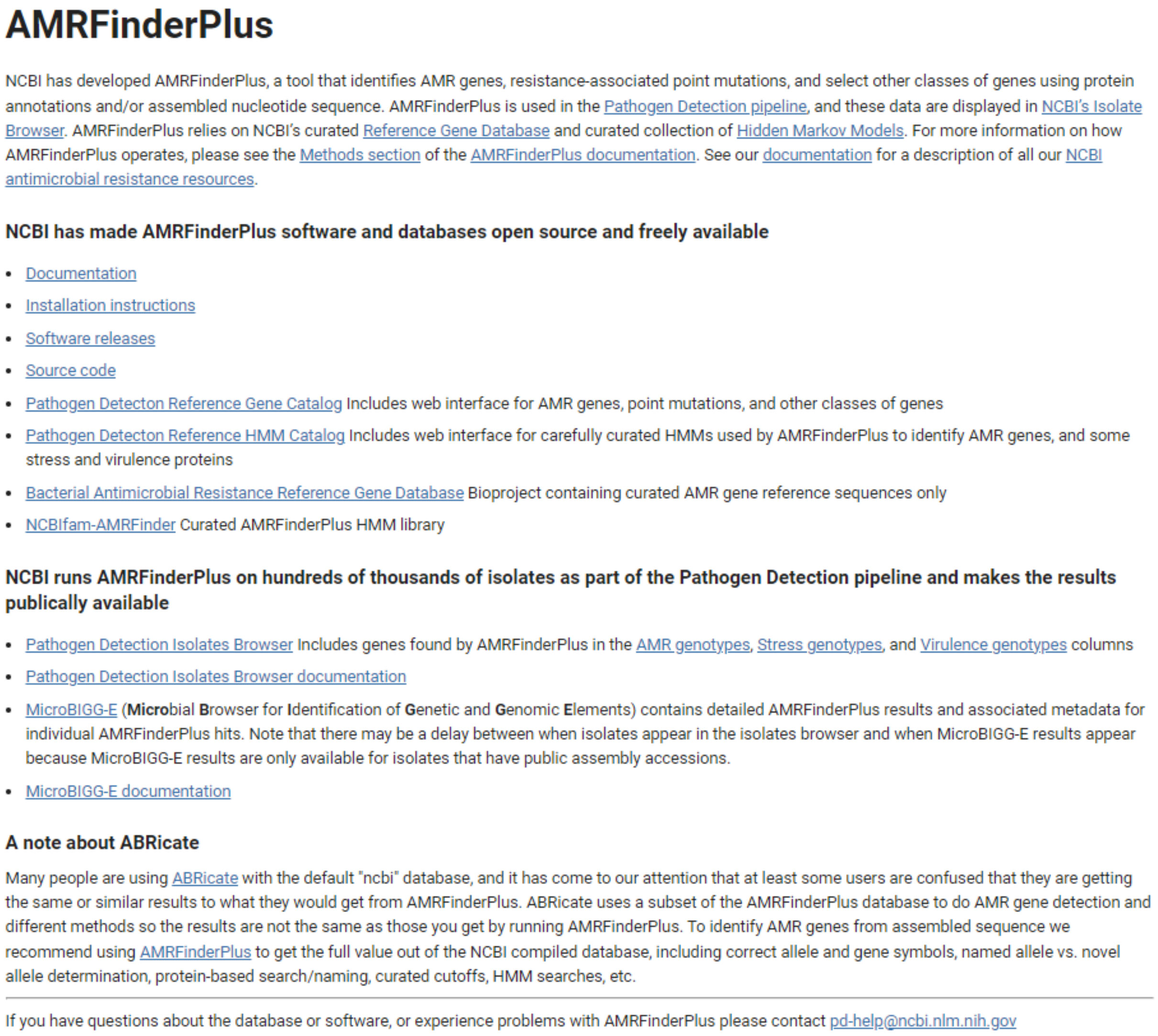
Q&A

Outline

Slido Q5

#	Topic	Time
1	Introduction	12:30 - 12:40
2	Brush-up antimicrobial resistance (AMR)	12:40 - 13:10
3	Fundamentals of WGS-based detection of AMR	13:10 - 13:40
4	Q&A	13:40 - 13:45
5	*Break*	13:45 - 14:00
6	Tools for WGS-based detection of AMR	14:00 – 14:45
7	WGS-based detection of AMR in Enterobacterales	14:45 – 15:00
8	*Break*	15:00 – 15:10
9	WGS-based detection of AMR in *Staphylococcus aureus* and *Enterococcus* spp.	15:10 – 15:30
10	Explanation of the exercise	15:30 – 15:35
11	Q&A, conclusions and session evaluation	15:35 – 16:00

AMRFinderPlus - NCBI



AMRFinderPlus

NCBI has developed AMRFinderPlus, a tool that identifies AMR genes, resistance-associated point mutations, and select other classes of genes using protein annotations and/or assembled nucleotide sequence. AMRFinderPlus is used in the Pathogen Detection pipeline, and these data are displayed in NCBI's Isolate Browser. AMRFinderPlus relies on NCBI's curated Reference Gene Database and curated collection of Hidden Markov Models. For more information on how AMRFinderPlus operates, please see the Methods section of the AMRFinderPlus documentation. See our documentation for a description of all our NCBI antimicrobial resistance resources.

NCBI has made AMRFinderPlus software and databases open source and freely available

- Documentation
- Installation instructions
- Software releases
- Source code
- Pathogen Detecton Reference Gene Catalog Includes web interface for AMR genes, point mutations, and other classes of genes
- Pathogen Detecton Reference HMM Catalog Includes web interface for carefully curated HMMs used by AMRFinderPlus to identify AMR genes, and some stress and virulence proteins
- Bacterial Antimicrobial Resistance Reference Gene Database Bioproject containing curated AMR gene reference sequences only
- NCBIfam-AMRFinder Curated AMRFinderPlus HMM library

NCBI runs AMRFinderPlus on hundreds of thousands of isolates as part of the Pathogen Detection pipeline and makes the results publically available

- Pathogen Detection Isolates Browser Includes genes found by AMRFinderPlus in the AMR genotypes, Stress genotypes, and Virulence genotypes columns
- Pathogen Detection Isolates Browser documentation
- MicroBIGG-E (**Micro**bial **B**rowser for **I**dentification of **G**enetic and **G**enomic **E**lements) contains detailed AMRFinderPlus results and associated metadata for individual AMRFinderPlus hits. Note that there may be a delay between when isolates appear in the isolates browser and when MicroBIGG-E results appear because MicroBIGG-E results are only available for isolates that have public assembly accessions.
- MicroBIGG-E documentation

A note about ABRicate

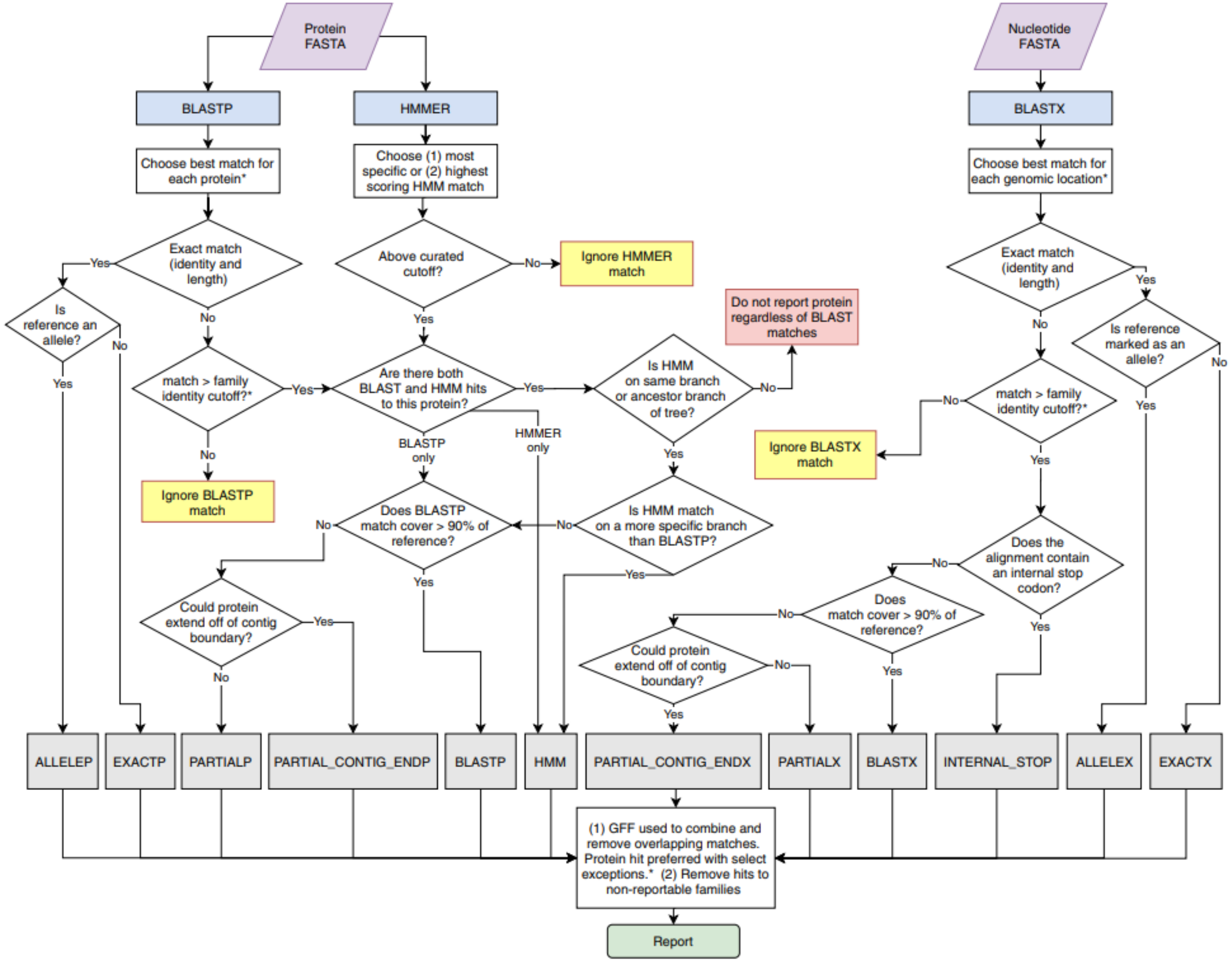
Many people are using ABRicate with the default "ncbi" database, and it has come to our attention that at least some users are confused that they are getting the same or similar results to what they would get from AMRFinderPlus. ABRicate uses a subset of the AMRFinderPlus database to do AMR gene detection and different methods so the results are not the same as those you get by running AMRFinderPlus. To identify AMR genes from assembled sequence we recommend using AMRFinderPlus to get the full value out of the NCBI compiled database, including correct allele and gene symbols, named allele vs. novel allele determination, protein-based search/naming, curated cutoffs, HMM searches, etc.

If you have questions about the database or software, or experience problems with AMRFinderPlus please contact pd-help@ncbi.nlm.nih.gov

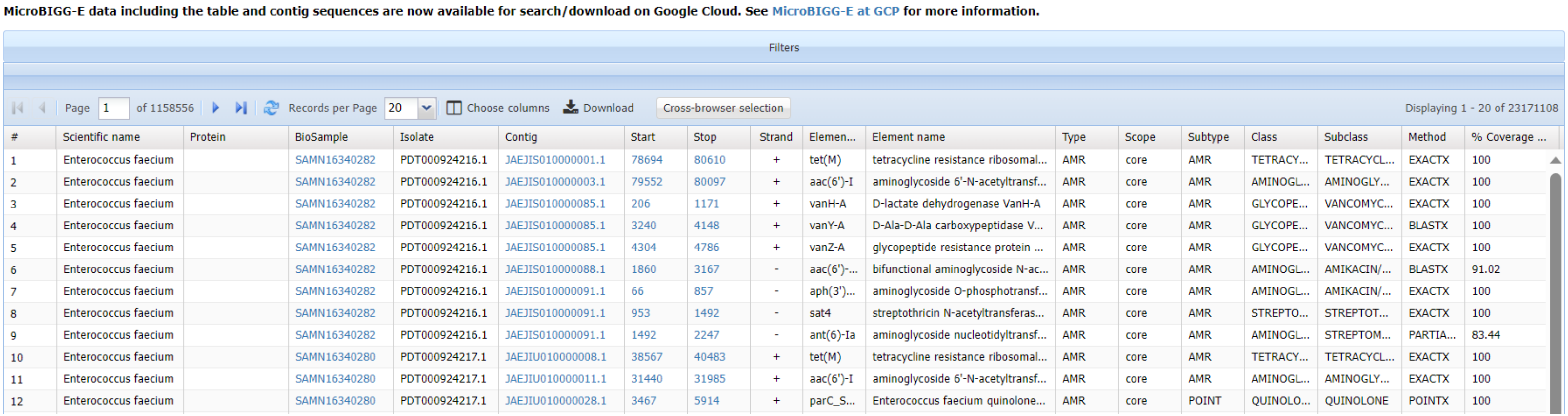
Website

AMRFinderPlus - NCBI

www.nature.com/scientificreports/


Protein FASTA
BLASTP
HMMER
Choose best match for each protein*
Choose (1) most specific or (2) highest scoring HMM match
Exact match (identity and length)
Yes
No
Is reference an allele?
Yes
No
match > family identity cutoff?*
Yes
No
Ignore BLASTP match
Above curated cutoff?
No
Ignore HMMER match
Yes
Are there both BLAST and HMM hits to this protein?
Yes
BLASTP only
HMMER only
Is HMM on same branch or ancestor branch of tree?
No
Do not report protein regardless of BLAST matches
Yes
Is HMM match on a more specific branch than BLASTP?
No
Yes
Does BLASTP match cover > 90% of reference?
No
Yes
Could protein extend off of contig boundary?
Yes
No
Nucleotide FASTA
BLASTX
Choose best match for each genomic location*
Exact match (identity and length)
Yes
No
Is reference marked as an allele?
Yes
No
match > family identity cutoff?*
No
Ignore BLASTX match
Yes
Does the alignment contain an internal stop codon?
No
Yes
Does match cover > 90% of reference?
No
Yes
Could protein extend off of contig boundary?
No
Yes
ALLELEP
EXACTP
PARTIALP
PARTIAL_CONTIG_ENDP
BLASTP
HMM
PARTIAL_CONTIG_ENDX
PARTIALX
BLASTX
INTERNAL_STOP
ALLELEX
EXACTX
(1) GFF used to combine and remove overlapping matches. Protein hit preferred with select exceptions.* (2) Remove hits to non-reportable families
Report

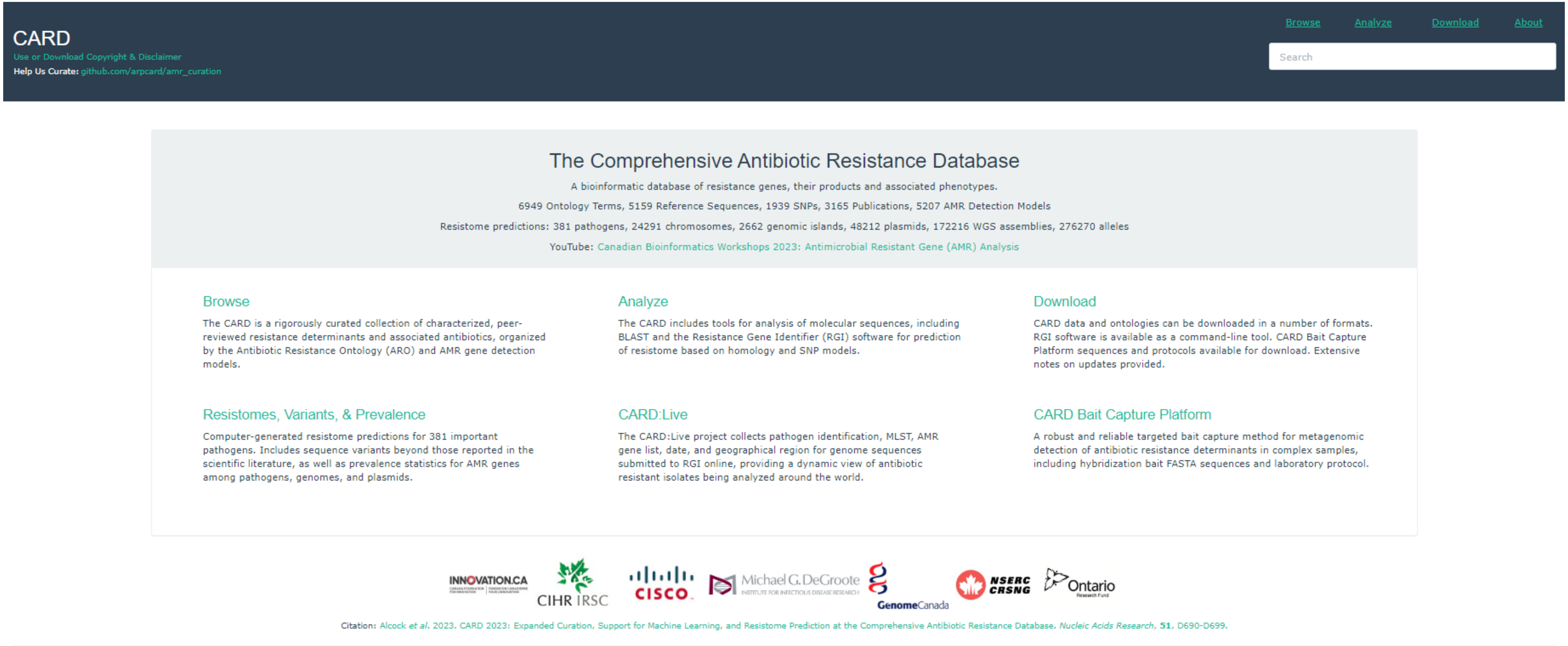
AMRFinderPlus output



MicroBIGG-E data including the table and contig sequences are now available for search/download on Google Cloud. See MicroBIGG-E at GCP for more information.

#	Scientific name	Protein	BioSample	Isolate	Contig	Start	Stop	Strand	Elemen...	Element name	Type	Scope	Subtype	Class	Subclass	Method	% Coverage ...
1	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000001.1	78694	80610	+	tet(M)	tetracycline resistance ribosomal...	AMR	core	AMR	TETRACY...	TETRACYCL...	EXACTX	100
2	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000003.1	79552	80097	+	aac(6')-I	aminoglycoside 6'-N-acetyltransf...	AMR	core	AMR	AMINOGL...	AMINOGLY...	EXACTX	100
3	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000085.1	206	1171	+	vanH-A	D-lactate dehydrogenase VanH-A	AMR	core	AMR	GLYCOPE...	VANCOMYC...	EXACTX	100
4	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000085.1	3240	4148	+	vanY-A	D-Ala-D-Ala carboxypeptidase V...	AMR	core	AMR	GLYCOPE...	VANCOMYC...	BLASTX	100
5	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000085.1	4304	4786	+	vanZ-A	glycopeptide resistance protein ...	AMR	core	AMR	GLYCOPE...	VANCOMYC...	EXACTX	100
6	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000088.1	1860	3167	-	aac(6')-...	bifunctional aminoglycoside N-ac...	AMR	core	AMR	AMINOGL...	AMIKACIN/...	BLASTX	91.02
7	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000091.1	66	857	-	aph(3')...	aminoglycoside O-phosphotransf...	AMR	core	AMR	AMINOGL...	AMIKACIN/...	EXACTX	100
8	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000091.1	953	1492	-	sat4	streptothricin N-acetyltransferas...	AMR	core	AMR	STREPTO...	STREPTOT...	EXACTX	100
9	Enterococcus faecium		SAMN16340282	PDT000924216.1	JAEJIS010000091.1	1492	2247	-	ant(6)-Ia	aminoglycoside nucleotidyltransf...	AMR	core	AMR	AMINOGL...	STREPTOM...	PARTIA...	83.44
10	Enterococcus faecium		SAMN16340280	PDT000924217.1	JAEJIU010000008.1	38567	40483	+	tet(M)	tetracycline resistance ribosomal...	AMR	core	AMR	TETRACY...	TETRACYCL...	EXACTX	100
11	Enterococcus faecium		SAMN16340280	PDT000924217.1	JAEJIU010000011.1	31440	31985	+	aac(6')-I	aminoglycoside 6'-N-acetyltransf...	AMR	core	AMR	AMINOGL...	AMINOGLY...	EXACTX	100
12	Enterococcus faecium		SAMN16340280	PDT000924217.1	JAEJIU010000028.1	3467	5914	+	parC_S...	Enterococcus faecium quinolone...	AMR	core	POINT	QUINOLO...	QUINOLONE	POINTX	100

CARD



CARD

Use or Download Copyright & Disclaimer

Help Us Curate: github.com/arpcard/amr_curation

The Comprehensive Antibiotic Resistance Database

A bioinformatic database of resistance genes, their products and associated phenotypes.

6949 Ontology Terms, 5159 Reference Sequences, 1939 SNPs, 3165 Publications, 5207 AMR Detection Models

Resistome predictions: 381 pathogens, 24291 chromosomes, 2662 genomic islands, 48212 plasmids, 172216 WGS assemblies, 276270 alleles

YouTube: Canadian Bioinformatics Workshops 2023: Antimicrobial Resistant Gene (AMR) Analysis

Browse

The CARD is a rigorously curated collection of characterized, peer-reviewed resistance determinants and associated antibiotics, organized by the Antibiotic Resistance Ontology (ARO) and AMR gene detection models.

Analyze

The CARD includes tools for analysis of molecular sequences, including BLAST and the Resistance Gene Identifier (RGI) software for prediction of resistome based on homology and SNP models.

Download

CARD data and ontologies can be downloaded in a number of formats. RGI software is available as a command-line tool. CARD Bait Capture Platform sequences and protocols available for download. Extensive notes on updates provided.

Resistomes, Variants, & Prevalence

Computer-generated resistome predictions for 381 important pathogens. Includes sequence variants beyond those reported in the scientific literature, as well as prevalence statistics for AMR genes among pathogens, genomes, and plasmids.

CARD:Live

The CARD:Live project collects pathogen identification, MLST, AMR gene list, date, and geographical region for genome sequences submitted to RGI online, providing a dynamic view of antibiotic resistant isolates being analyzed around the world.

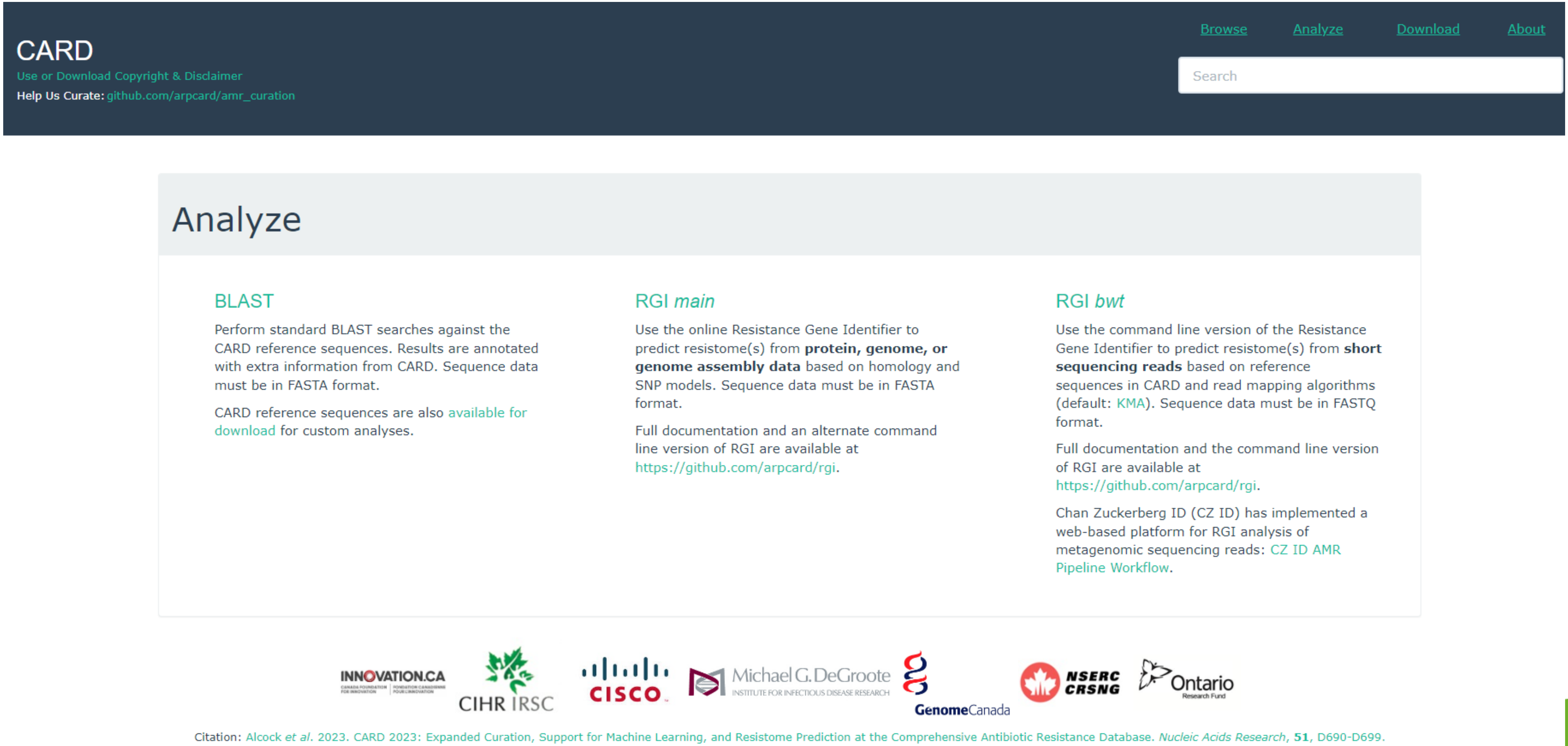
CARD Bait Capture Platform

A robust and reliable targeted bait capture method for metagenomic detection of antibiotic resistance determinants in complex samples, including hybridization bait FASTA sequences and laboratory protocol.

Citation: Alcock *et al*. 2023. CARD 2023: Expanded Curation, Support for Machine Learning, and Resistome Prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Research*, **51**, D690-D699.

Website

CARD



CARD

Use or Download Copyright & Disclaimer

Help Us Curate: github.com/arpcard/amr_curation

Analyze

BLAST

Perform standard BLAST searches against the CARD reference sequences. Results are annotated with extra information from CARD. Sequence data must be in FASTA format.

CARD reference sequences are also available for download for custom analyses.

RGI *main*

Use the online Resistance Gene Identifier to predict resistome(s) from **protein, genome, or genome assembly data** based on homology and SNP models. Sequence data must be in FASTA format.

Full documentation and an alternate command line version of RGI are available at https://github.com/arpcard/rgi.

RGI *bwt*

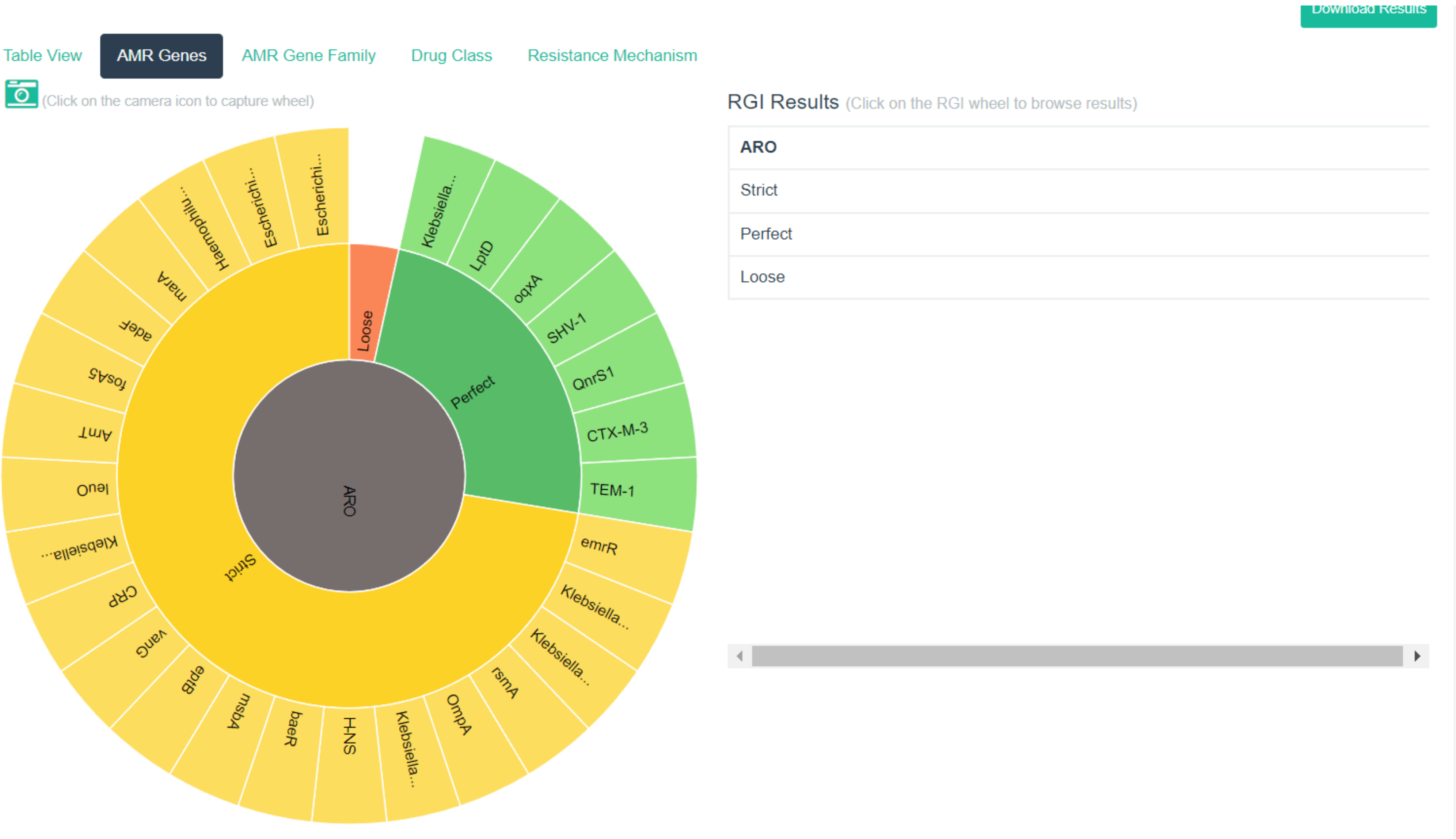
Use the command line version of the Resistance Gene Identifier to predict resistome(s) from **short sequencing reads** based on reference sequences in CARD and read mapping algorithms (default: KMA). Sequence data must be in FASTQ format.

Full documentation and the command line version of RGI are available at https://github.com/arpcard/rgi.

Chan Zuckerberg ID (CZ ID) has implemented a web-based platform for RGI analysis of metagenomic sequencing reads: CZ ID AMR Pipeline Workflow.

Citation: Alcock *et al.* 2023. CARD 2023: Expanded Curation, Support for Machine Learning, and Resistome Prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Research*, **51**, D690-D699.

CARD/RGI output



RGI Results (Click on the RGI wheel to browse results)

ARO
Strict
Perfect
Loose

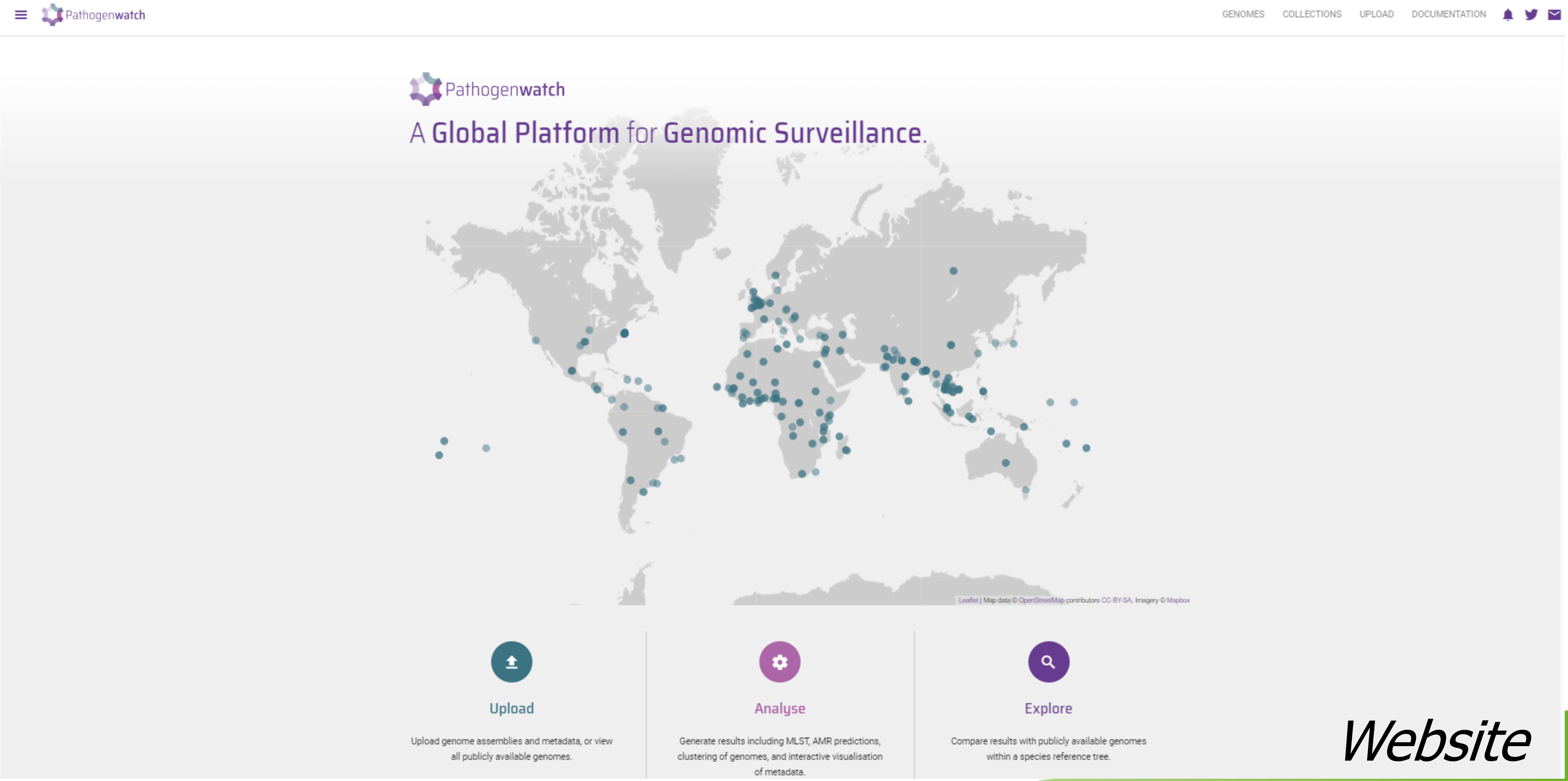
CARD/RGI output

Results (all Loose hits shown)

Search:

RGI Criteria	ARO Term	SNP	Detection Criteria	AMR Gene Family	Drug Class	Resistance Mechanism	% Identity of Matching Region	% Length of Reference Sequence
Perfect	Klebsiella pneumoniae KpnF		protein homolog model	small multidrug resistance (SMR) antibiotic efflux pump	macrolide antibiotic, aminoglycoside antibiotic, cephalosporin, tetracycline antibiotic, peptide antibiotic, rifamycin antibiotic, disinfecting agents and antiseptics	antibiotic efflux	100.0	100.00
Perfect	LptD		protein homolog model	ATP-binding cassette (ABC) antibiotic efflux pump	peptide antibiotic, aminocoumarin antibiotic, rifamycin antibiotic	antibiotic efflux	100.0	100.00
Perfect	oqxA		protein homolog model	resistance-nodulation-cell division (RND) antibiotic efflux pump	fluoroquinolone antibiotic, glycylcycline, tetracycline antibiotic, diaminopyrimidine antibiotic, nitrofuran antibiotic	antibiotic efflux	100.0	100.00
Perfect	SHV-1		protein homolog model	SHV beta-lactamase	carbapenem, cephalosporin,	antibiotic inactivation	100.0	100.00

Pathogenwatch



A Global Platform for Genomic Surveillance.

Upload

Upload genome assemblies and metadata, or view all publicly available genomes.

Analyse

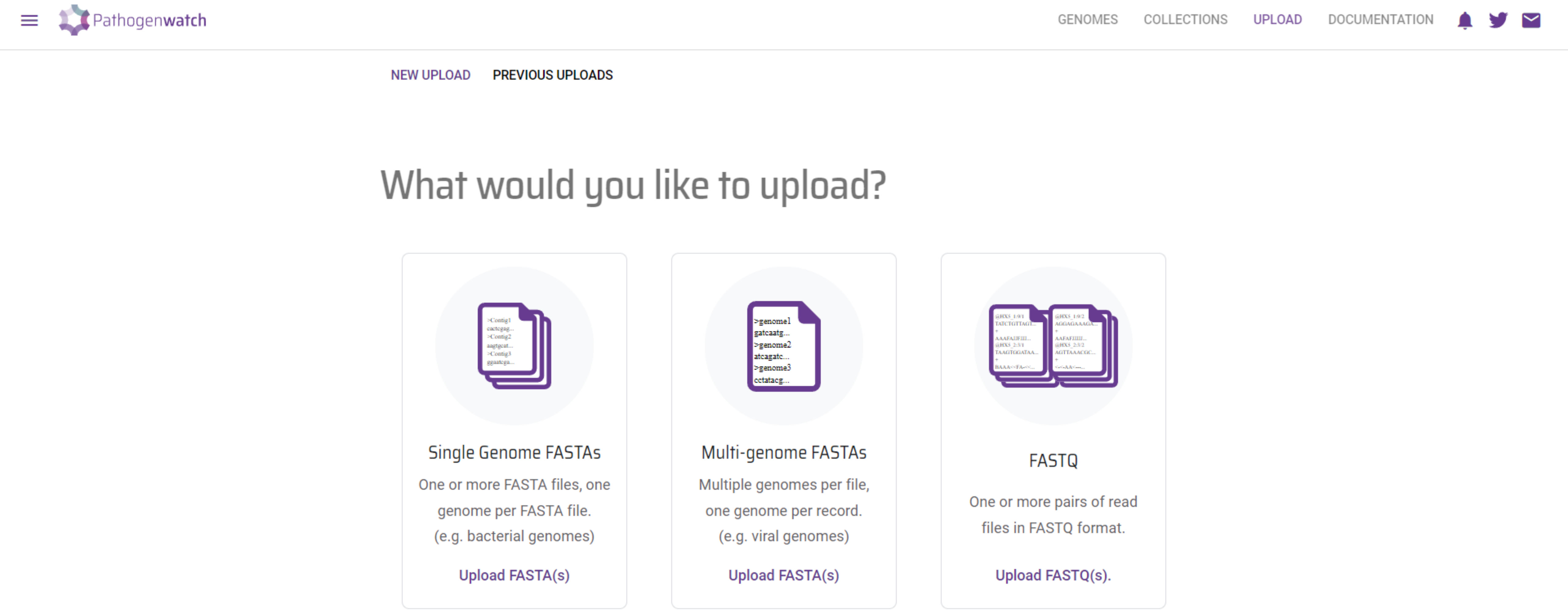
Generate results including MLST, AMR predictions, clustering of genomes, and interactive visualisation of metadata.

Explore

Compare results with publicly available genomes within a species reference tree.

Website

Pathogenwatch



What would you like to upload?

Single Genome FASTAs

One or more FASTA files, one genome per FASTA file. (e.g. bacterial genomes)

Upload FASTA(s)

Multi-genome FASTAs

Multiple genomes per file, one genome per record. (e.g. viral genomes)

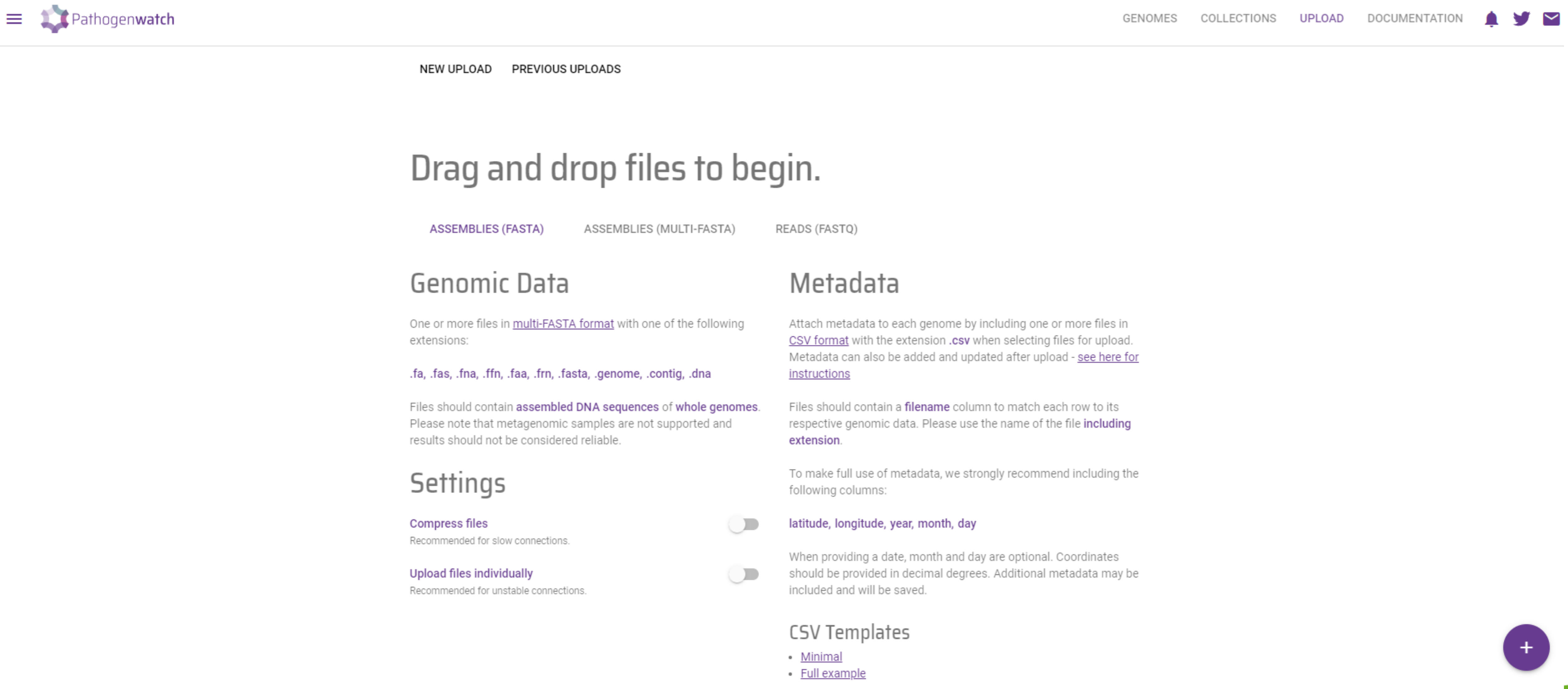
Upload FASTA(s)

FASTQ

One or more pairs of read files in FASTQ format.

Upload FASTQ(s).

Pathogenwatch



Drag and drop files to begin.

Genomic Data

One or more files in multi-FASTA format with one of the following extensions:

.fa, .fas, .fna, .ffn, .faa, .frn, .fasta, .genome, .contig, .dna

Files should contain **assembled DNA sequences** of **whole genomes**. Please note that metagenomic samples are not supported and results should not be considered reliable.

Settings

Compress files ○
Recommended for slow connections.

Upload files individually ○
Recommended for unstable connections.

Metadata

Attach metadata to each genome by including one or more files in CSV format with the extension **.csv** when selecting files for upload. Metadata can also be added and updated after upload - see here for instructions

Files should contain a **filename** column to match each row to its respective genomic data. Please use the name of the file **including extension**.

To make full use of metadata, we strongly recommend including the following columns:

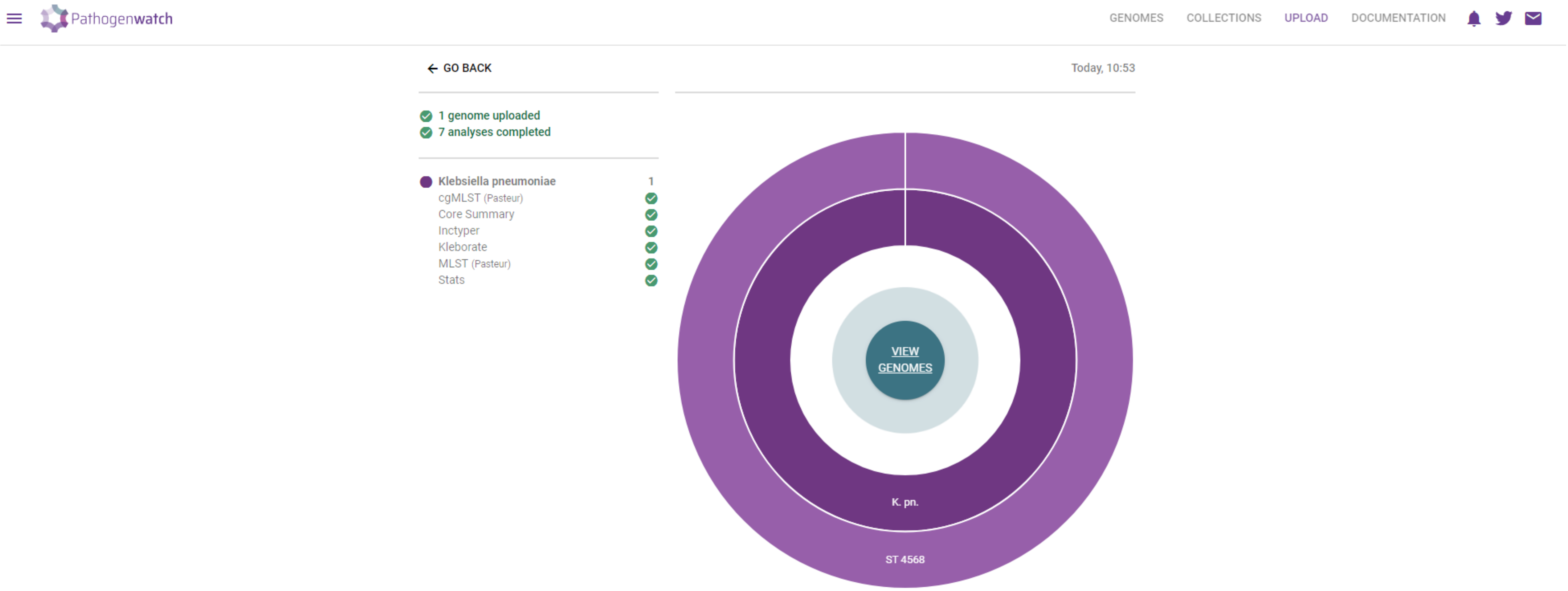
latitude, longitude, year, month, day

When providing a date, month and day are optional. Coordinates should be provided in decimal degrees. Additional metadata may be included and will be saved.

CSV Templates

- Minimal
- Full example

Pathogenwatch output

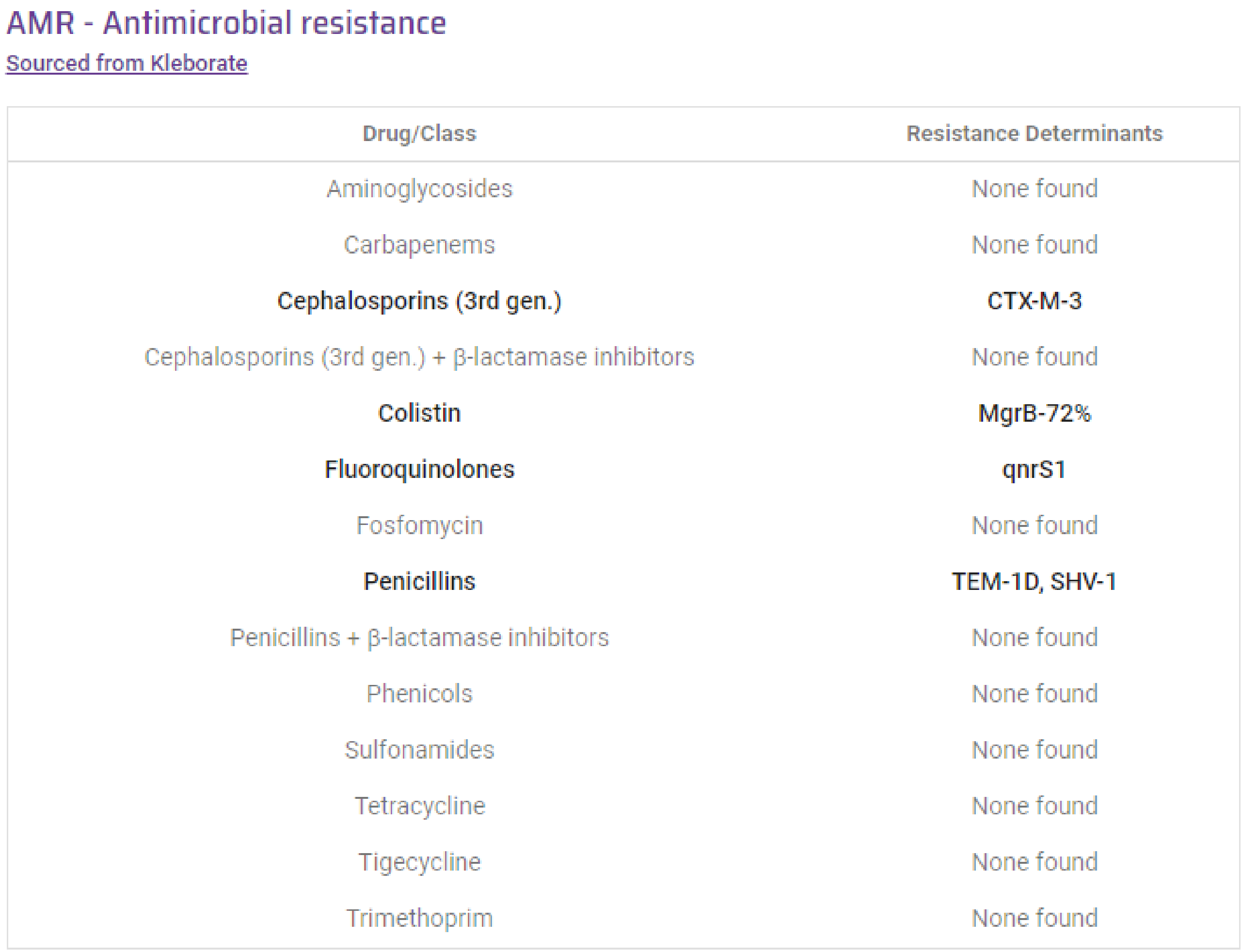


Today, 10:53

✓ 1 genome uploaded
✓ 7 analyses completed

● **Klebsiella pneumoniae**	1
cgMLST (Pasteur)	✓
Core Summary	✓
lnctyper	✓
Kleborate	✓
MLST (Pasteur)	✓
Stats	✓

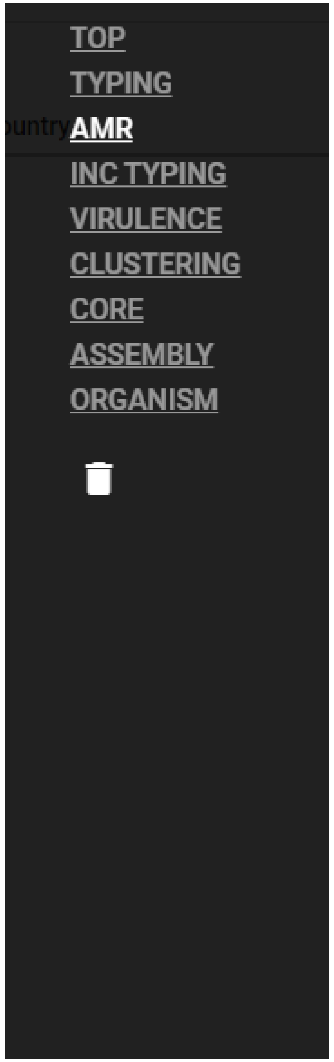
Pathogenwatch output



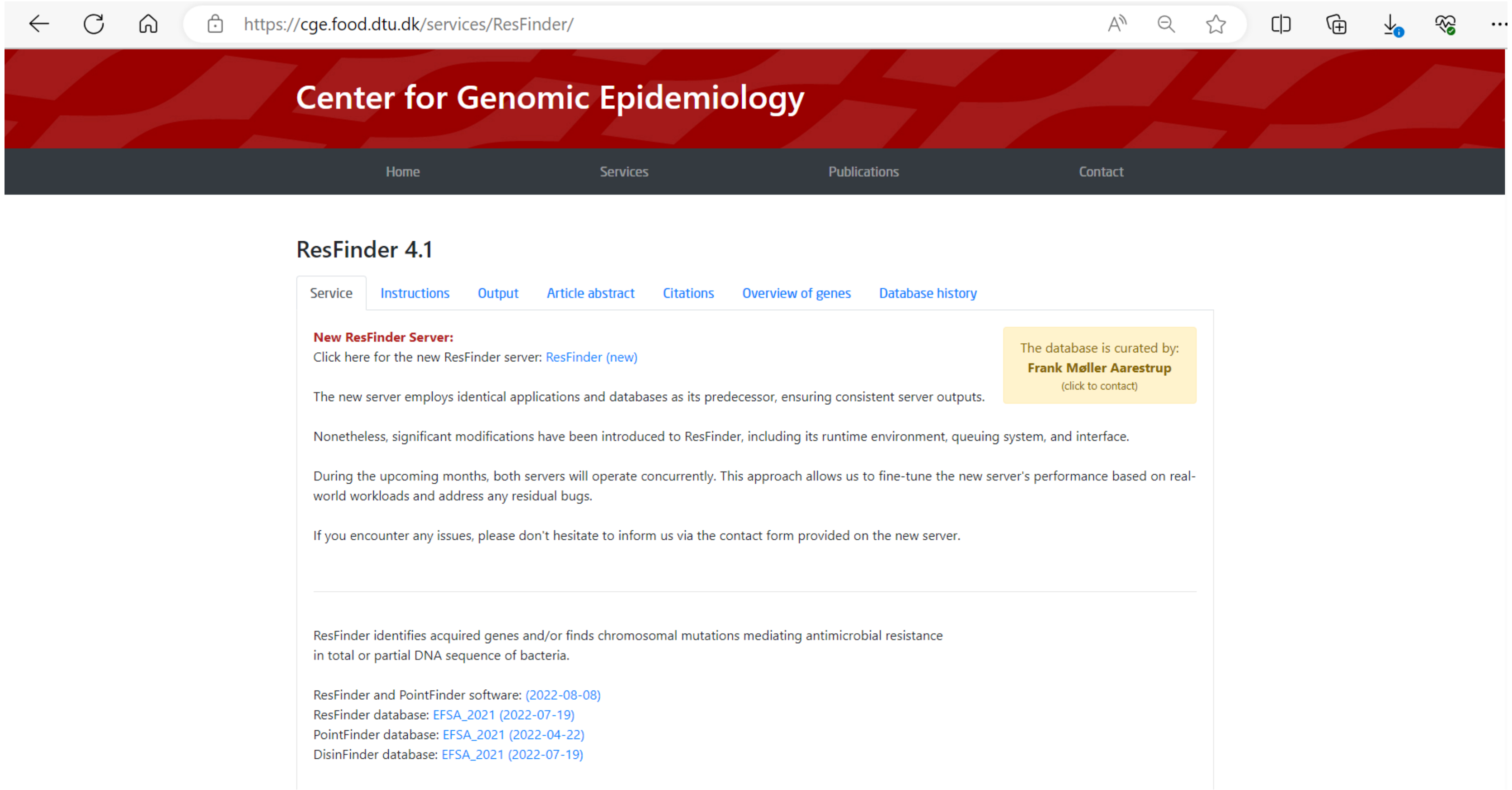
AMR - Antimicrobial resistance

Sourced from Kleborate

Drug/Class	Resistance Determinants
Aminoglycosides	None found
Carbapenems	None found
Cephalosporins (3rd gen.)	**CTX-M-3**
Cephalosporins (3rd gen.) + β-lactamase inhibitors	None found
Colistin	**MgrB-72%**
Fluoroquinolones	**qnrS1**
Fosfomycin	None found
Penicillins	**TEM-1D, SHV-1**
Penicillins + β-lactamase inhibitors	None found
Phenicols	None found
Sulfonamides	None found
Tetracycline	None found
Tigecycline	None found
Trimethoprim	None found



ResFinder



ResFinder 4.1

New ResFinder Server:
Click here for the new ResFinder server: ResFinder (new)

The database is curated by:
Frank Møller Aarestrup
(click to contact)

The new server employs identical applications and databases as its predecessor, ensuring consistent server outputs.

Nonetheless, significant modifications have been introduced to ResFinder, including its runtime environment, queuing system, and interface.

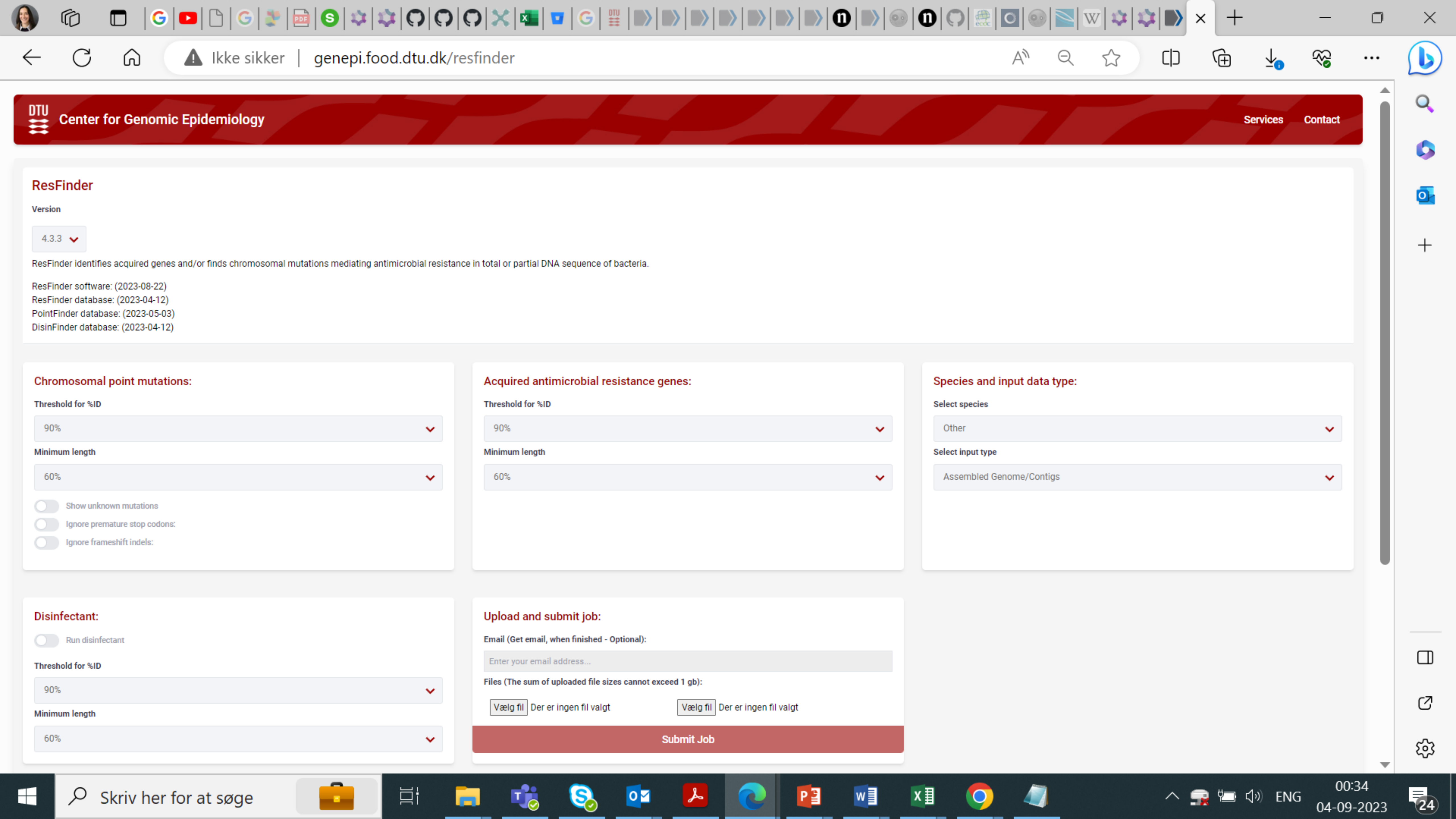
During the upcoming months, both servers will operate concurrently. This approach allows us to fine-tune the new server's performance based on real-world workloads and address any residual bugs.

If you encounter any issues, please don't hesitate to inform us via the contact form provided on the new server.

ResFinder identifies acquired genes and/or finds chromosomal mutations mediating antimicrobial resistance in total or partial DNA sequence of bacteria.

ResFinder and PointFinder software: (2022-08-08)
ResFinder database: EFSA_2021 (2022-07-19)
PointFinder database: EFSA_2021 (2022-04-22)
DisinFinder database: EFSA_2021 (2022-07-19)

Website



ResFinder

Version

4.3.3

ResFinder identifies acquired genes and/or finds chromosomal mutations mediating antimicrobial resistance in total or partial DNA sequence of bacteria.

ResFinder software: (2023-08-22)
ResFinder database: (2023-04-12)
PointFinder database: (2023-05-03)
DisinFinder database: (2023-04-12)

Chromosomal point mutations:

Threshold for %ID: 90%

Minimum length: 60%

○ Show unknown mutations
○ Ignore premature stop codons:
○ Ignore frameshift indels:

Acquired antimicrobial resistance genes:

Threshold for %ID: 90%

Minimum length: 60%

Species and input data type:

Select species: Other

Select input type: Assembled Genome/Contigs

Disinfectant:

○ Run disinfectant

Threshold for %ID: 90%

Minimum length: 60%

Upload and submit job:

Email (Get email, when finished - Optional):

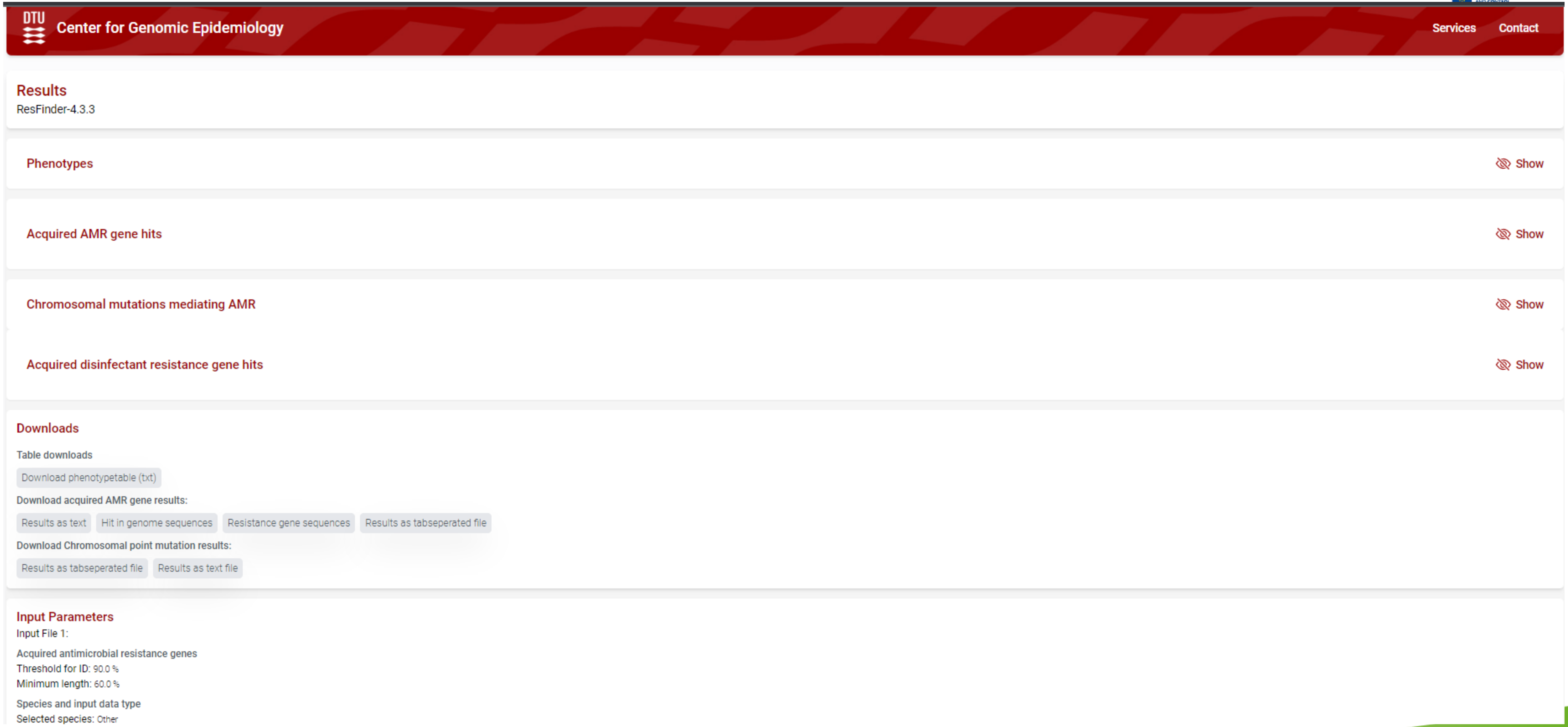
Enter your email address...

Files (The sum of uploaded file sizes cannot exceed 1 gb):

Vælg fil Der er ingen fil valgt   Vælg fil Der er ingen fil valgt

Submit Job

ResFinder output



Results

ResFinder-4.3.3

Phenotypes Show

Acquired AMR gene hits Show

Chromosomal mutations mediating AMR Show

Acquired disinfectant resistance gene hits Show

Downloads

Table downloads

Download phenotypetable (txt)

Download acquired AMR gene results:

Results as text | Hit in genome sequences | Resistance gene sequences | Results as tabseperated file

Download Chromosomal point mutation results:

Results as tabseperated file | Results as text file

Input Parameters

Input File 1:

Acquired antimicrobial resistance genes

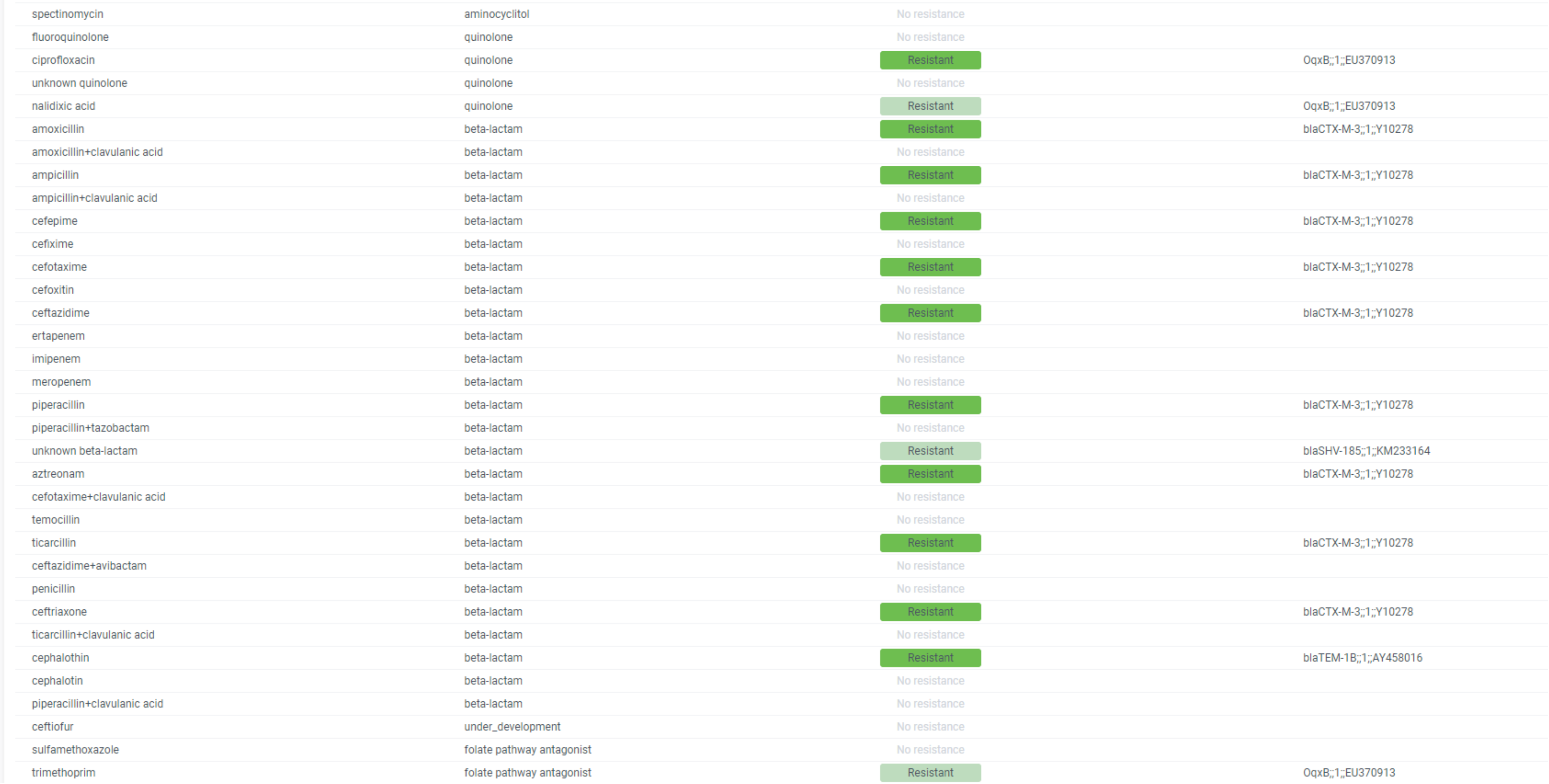
Threshold for ID: 90.0 %

Minimum length: 60.0 %

Species and input data type

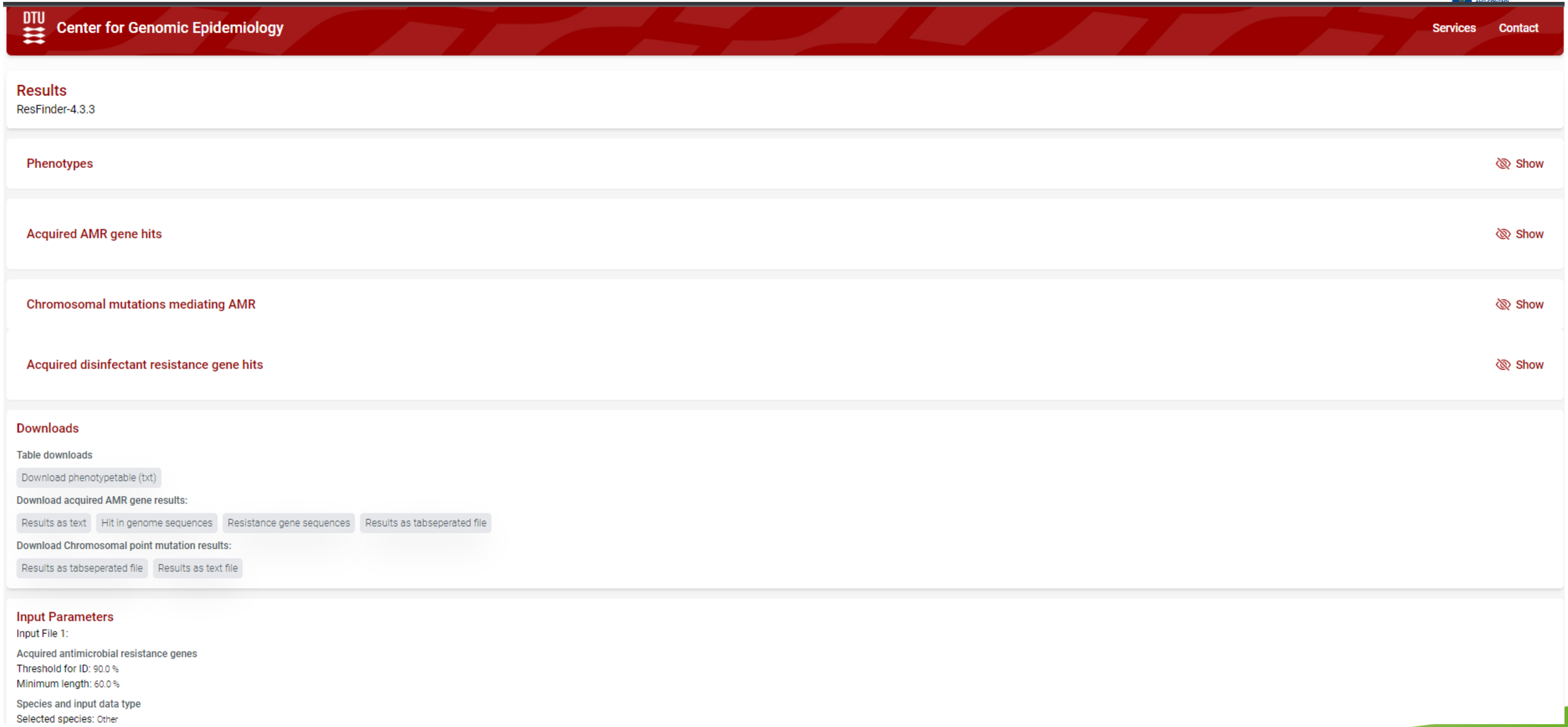
Selected species: Other

ResFinder output



spectinomycin	aminocyclitol	No resistance	
fluoroquinolone	quinolone	No resistance	
ciprofloxacin	quinolone	Resistant	OqxB;;1;;EU370913
unknown quinolone	quinolone	No resistance	
nalidixic acid	quinolone	Resistant	OqxB;;1;;EU370913
amoxicillin	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
amoxicillin+clavulanic acid	beta-lactam	No resistance	
ampicillin	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
ampicillin+clavulanic acid	beta-lactam	No resistance	
cefepime	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
cefixime	beta-lactam	No resistance	
cefotaxime	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
cefoxitin	beta-lactam	No resistance	
ceftazidime	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
ertapenem	beta-lactam	No resistance	
imipenem	beta-lactam	No resistance	
meropenem	beta-lactam	No resistance	
piperacillin	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
piperacillin+tazobactam	beta-lactam	No resistance	
unknown beta-lactam	beta-lactam	Resistant	blaSHV-185;;1;;KM233164
aztreonam	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
cefotaxime+clavulanic acid	beta-lactam	No resistance	
temocillin	beta-lactam	No resistance	
ticarcillin	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
ceftazidime+avibactam	beta-lactam	No resistance	
penicillin	beta-lactam	No resistance	
ceftriaxone	beta-lactam	Resistant	blaCTX-M-3;;1;;Y10278
ticarcillin+clavulanic acid	beta-lactam	No resistance	
cephalothin	beta-lactam	Resistant	blaTEM-1B;;1;;AY458016
cephalotin	beta-lactam	No resistance	
piperacillin+clavulanic acid	beta-lactam	No resistance	
ceftiofur	under_development	No resistance	
sulfamethoxazole	folate pathway antagonist	No resistance	
trimethoprim	folate pathway antagonist	Resistant	OqxB;;1;;EU370913

ResFinder output



Results

ResFinder-4.3.3

Phenotypes Show

Acquired AMR gene hits Show

Chromosomal mutations mediating AMR Show

Acquired disinfectant resistance gene hits Show

Downloads

Table downloads

Download acquired AMR gene results:

Download Chromosomal point mutation results:

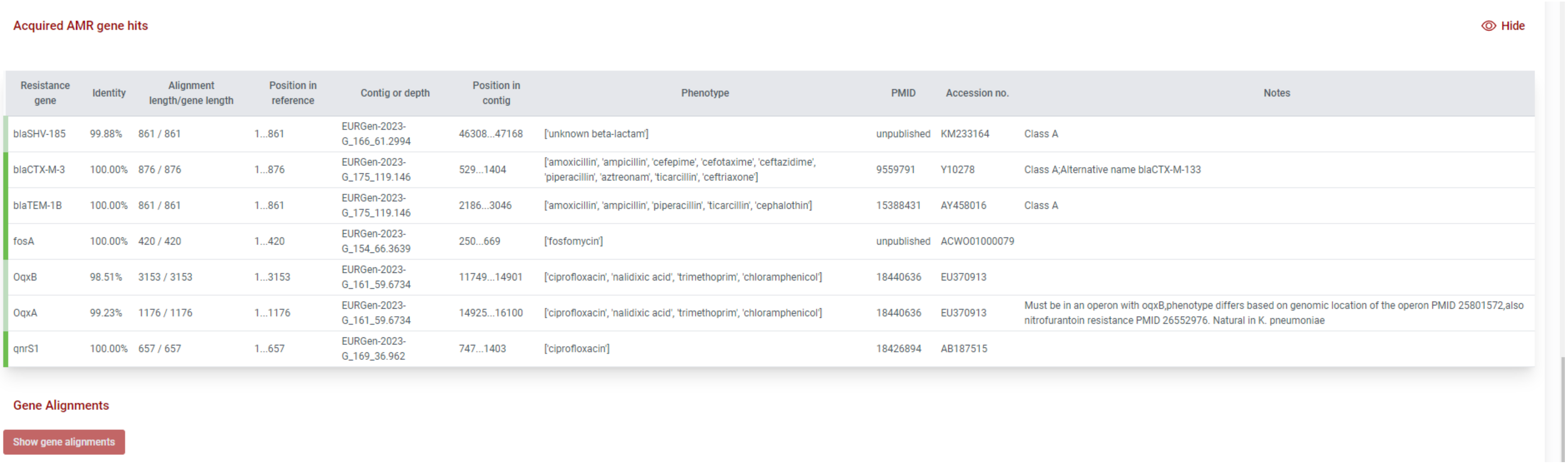
Input Parameters

Input File 1:

Acquired antimicrobial resistance genes
Threshold for ID: 90.0 %
Minimum length: 60.0 %

Species and input data type
Selected species: Other

ResFinder output

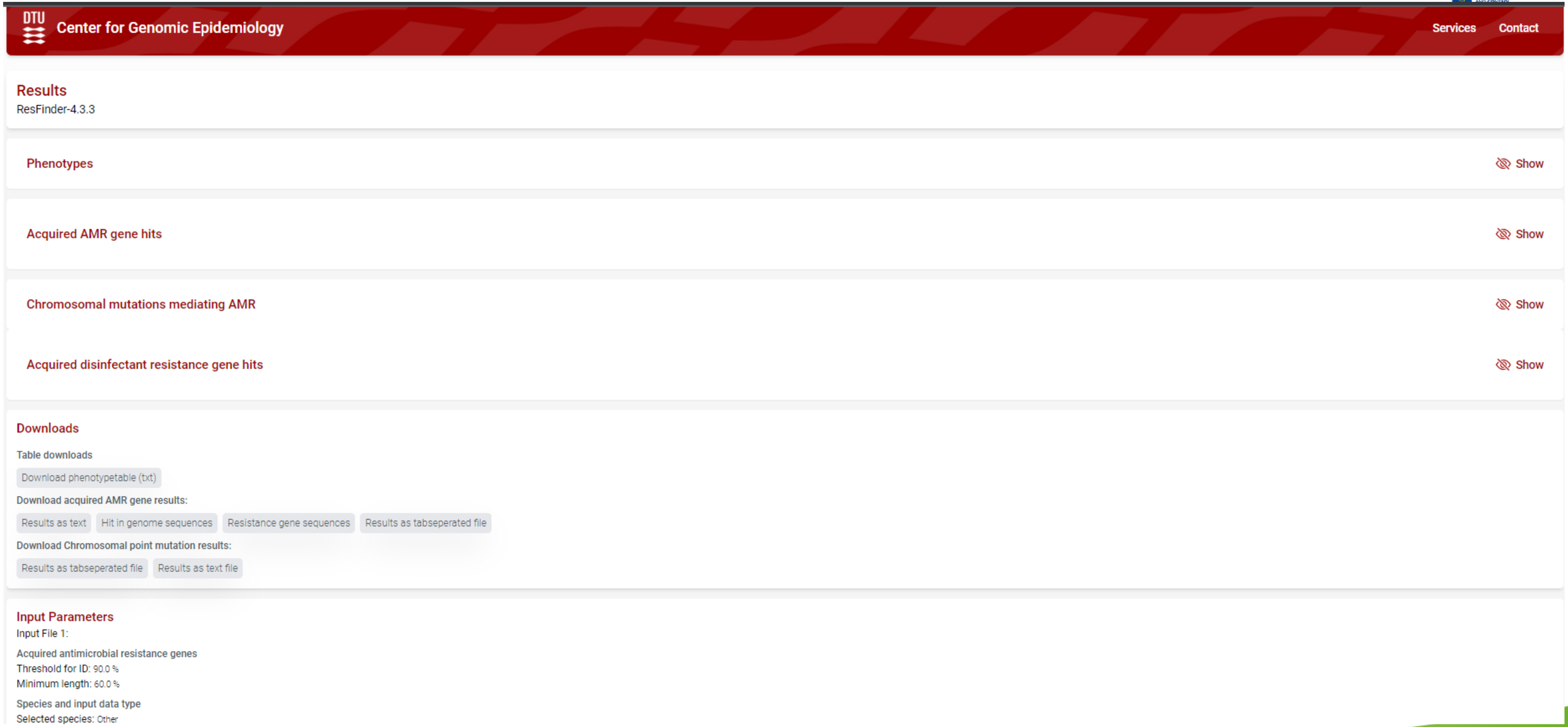


Acquired AMR gene hits

Resistance gene	Identity	Alignment length/gene length	Position in reference	Contig or depth	Position in contig	Phenotype	PMID	Accession no.	Notes
blaSHV-185	99.88%	861 / 861	1...861	EURGen-2023-G_166_61.2994	46308...47168	['unknown beta-lactam']	unpublished	KM233164	Class A
blaCTX-M-3	100.00%	876 / 876	1...876	EURGen-2023-G_175_119.146	529...1404	['amoxicillin', 'ampicillin', 'cefepime', 'cefotaxime', 'ceftazidime', 'piperacillin', 'aztreonam', 'ticarcillin', 'ceftriaxone']	9559791	Y10278	Class A;Alternative name blaCTX-M-133
blaTEM-1B	100.00%	861 / 861	1...861	EURGen-2023-G_175_119.146	2186...3046	['amoxicillin', 'ampicillin', 'piperacillin', 'ticarcillin', 'cephalothin']	15388431	AY458016	Class A
fosA	100.00%	420 / 420	1...420	EURGen-2023-G_154_66.3639	250...669	['fosfomycin']	unpublished	ACWO01000079	
OqxB	98.51%	3153 / 3153	1...3153	EURGen-2023-G_161_59.6734	11749...14901	['ciprofloxacin', 'nalidixic acid', 'trimethoprim', 'chloramphenicol']	18440636	EU370913	
OqxA	99.23%	1176 / 1176	1...1176	EURGen-2023-G_161_59.6734	14925...16100	['ciprofloxacin', 'nalidixic acid', 'trimethoprim', 'chloramphenicol']	18440636	EU370913	Must be in an operon with oqxB,phenotype differs based on genomic location of the operon PMID 25801572,also nitrofurantoin resistance PMID 26552976. Natural in K. pneumoniae
qnrS1	100.00%	657 / 657	1...657	EURGen-2023-G_169_36.962	747...1403	['ciprofloxacin']	18426894	AB187515	

Gene Alignments

ResFinder output



Results
ResFinder-4.3.3

Phenotypes

Acquired AMR gene hits

Chromosomal mutations mediating AMR

Acquired disinfectant resistance gene hits

Downloads

Table downloads

Download acquired AMR gene results:

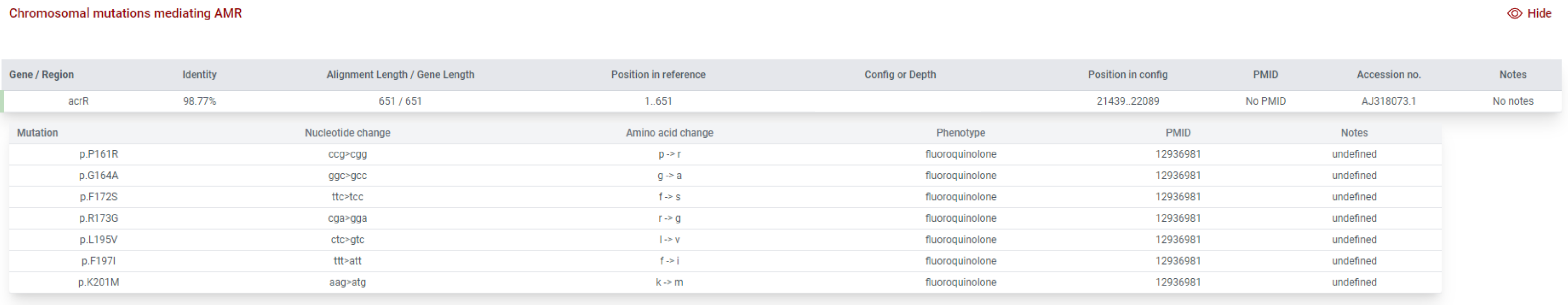
Download Chromosomal point mutation results:

Input Parameters
Input File 1:

Acquired antimicrobial resistance genes
Threshold for ID: 90.0 %
Minimum length: 60.0 %

Species and input data type
Selected species: Other

ResFinder output



Chromosomal mutations mediating AMR

Gene / Region	Identity	Alignment Length / Gene Length	Position in reference	Config or Depth	Position in config	PMID	Accession no.	Notes
acrR	98.77%	651 / 651	1..651		21439..22089	No PMID	AJ318073.1	No notes

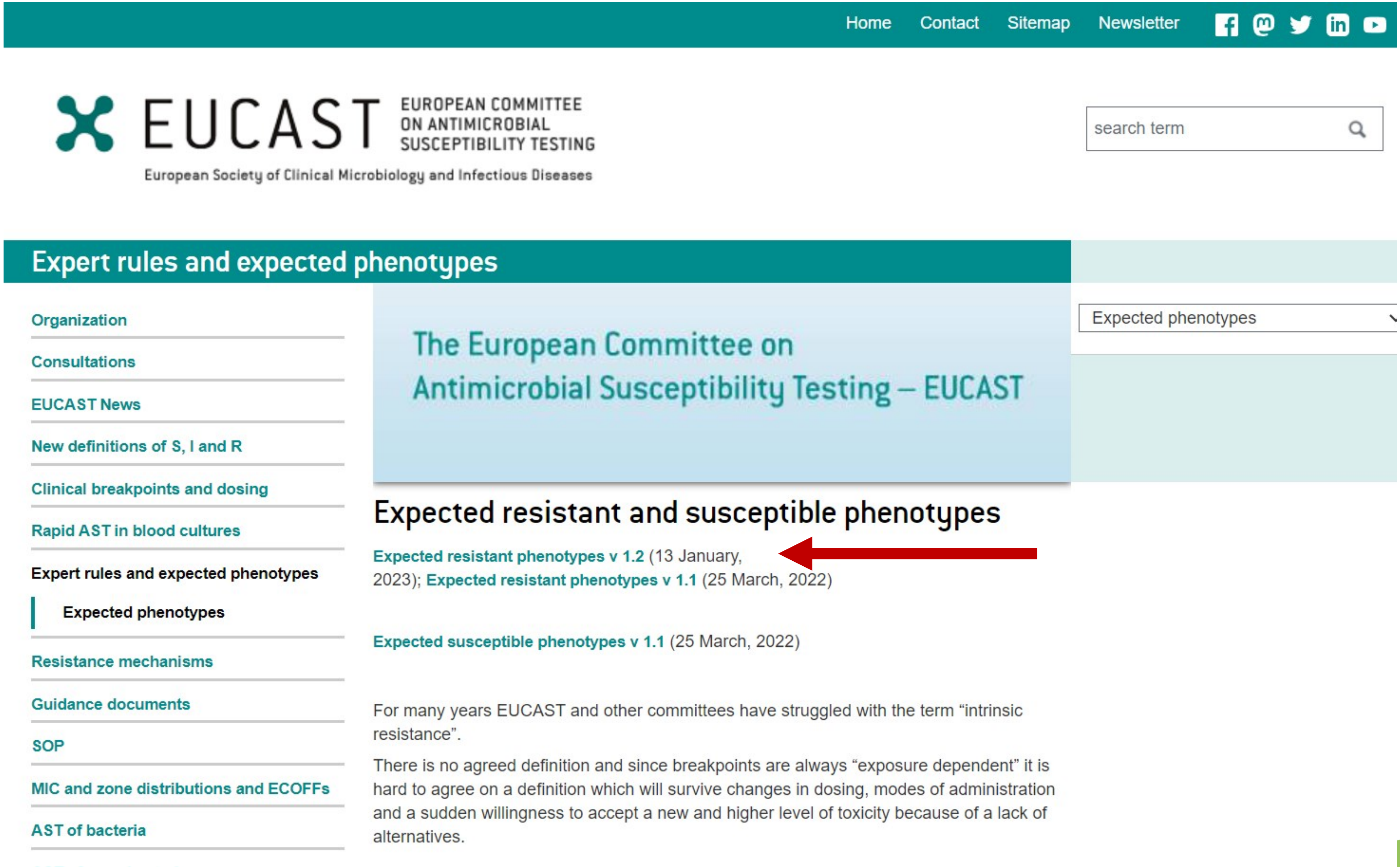
Mutation	Nucleotide change	Amino acid change	Phenotype	PMID	Notes
p.P161R	ccg>cgg	p -> r	fluoroquinolone	12936981	undefined
p.G164A	ggc>gcc	g -> a	fluoroquinolone	12936981	undefined
p.F172S	ttc>tcc	f -> s	fluoroquinolone	12936981	undefined
p.R173G	cga>gga	r -> g	fluoroquinolone	12936981	undefined
p.L195V	ctc>gtc	l -> v	fluoroquinolone	12936981	undefined
p.F197I	ttt>att	f -> i	fluoroquinolone	12936981	undefined
p.K201M	aag>atg	k -> m	fluoroquinolone	12936981	undefined

Which drug/bug combinations should we monitor in different bacterial species?

↓

Which resistance genes/chromosomal mutations can we detect with the available tools/current knowledge?

Expected phenotypes



Expected resistant and susceptible phenotypes

Expected resistant phenotypes v 1.2 (13 January, 2023); **Expected resistant phenotypes v 1.1** (25 March, 2022)

Expected susceptible phenotypes v 1.1 (25 March, 2022)

For many years EUCAST and other committees have struggled with the term “intrinsic resistance”.

There is no agreed definition and since breakpoints are always “exposure dependent” it is hard to agree on a definition which will survive changes in dosing, modes of administration and a sudden willingness to accept a new and higher level of toxicity because of a lack of alternatives.

WGS-based detection of AMR in Enterobacterales

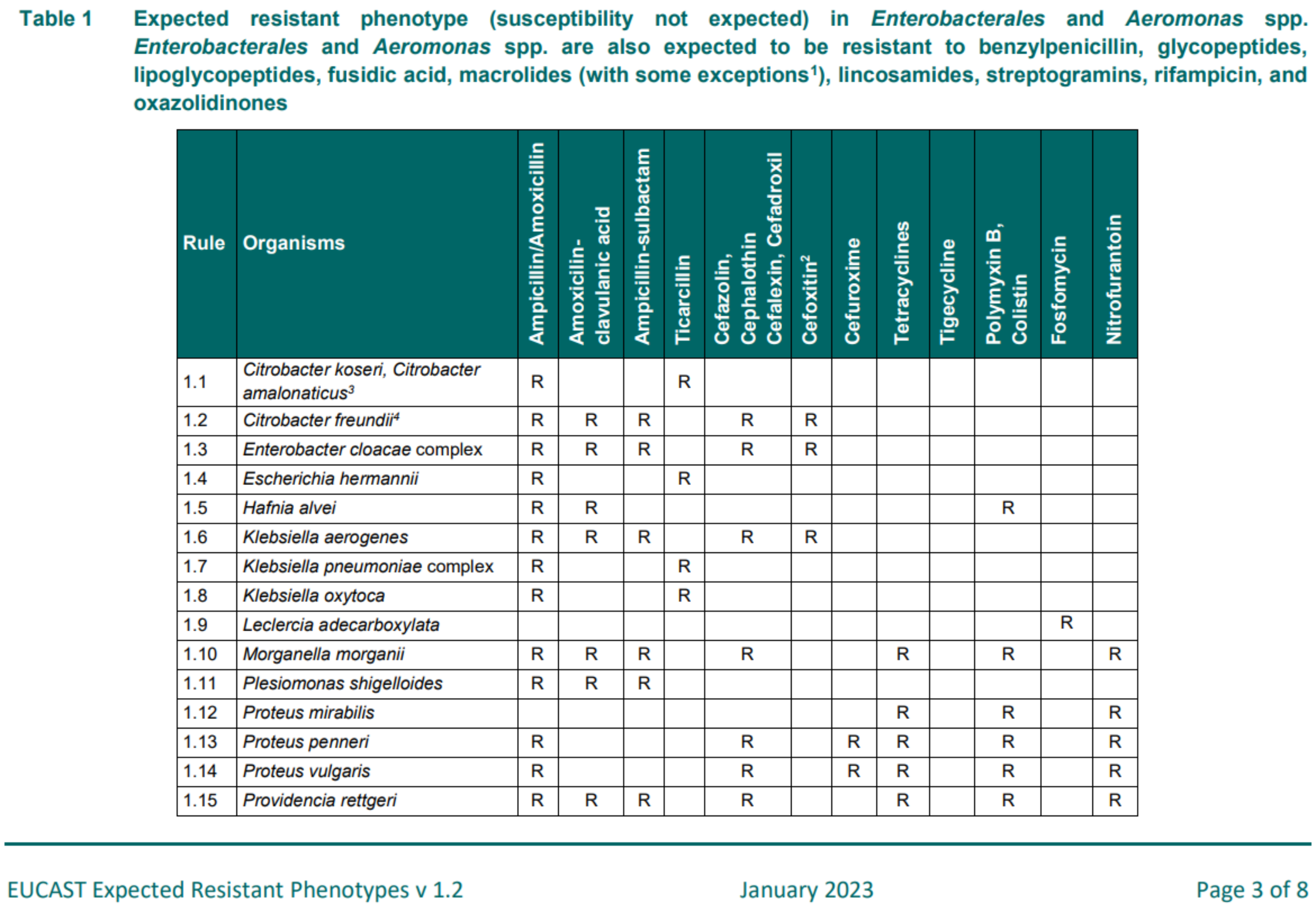


Table 1 **Expected resistant phenotype (susceptibility not expected) in *Enterobacterales* and *Aeromonas* spp. *Enterobacterales* and *Aeromonas* spp. are also expected to be resistant to benzylpenicillin, glycopeptides, lipoglycopeptides, fusidic acid, macrolides (with some exceptions[1]), lincosamides, streptogramins, rifampicin, and oxazolidinones**

Rule	Organisms	Ampicillin/Amoxicillin	Amoxicillin-clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Cefazolin, Cephalothin Cefalexin, Cefadroxil	Cefoxitin[2]	Cefuroxime	Tetracyclines	Tigecycline	Polymyxin B, Colistin	Fosfomycin	Nitrofurantoin
1.1	*Citrobacter koseri, Citrobacter amalonaticus*[3]	R			R								
1.2	*Citrobacter freundii*[4]	R	R	R		R	R						
1.3	*Enterobacter cloacae* complex	R	R	R		R	R						
1.4	*Escherichia hermannii*	R			R								
1.5	*Hafnia alvei*	R	R								R		
1.6	*Klebsiella aerogenes*	R	R	R		R	R						
1.7	*Klebsiella pneumoniae* complex	R			R								
1.8	*Klebsiella oxytoca*	R			R								
1.9	*Leclercia adecarboxylata*											R	
1.10	*Morganella morganii*	R	R	R		R			R		R		R
1.11	*Plesiomonas shigelloides*	R	R	R									
1.12	*Proteus mirabilis*								R		R		R
1.13	*Proteus penneri*	R				R		R	R		R		R
1.14	*Proteus vulgaris*	R				R		R	R		R		R
1.15	*Providencia rettgeri*	R	R	R		R			R		R		R

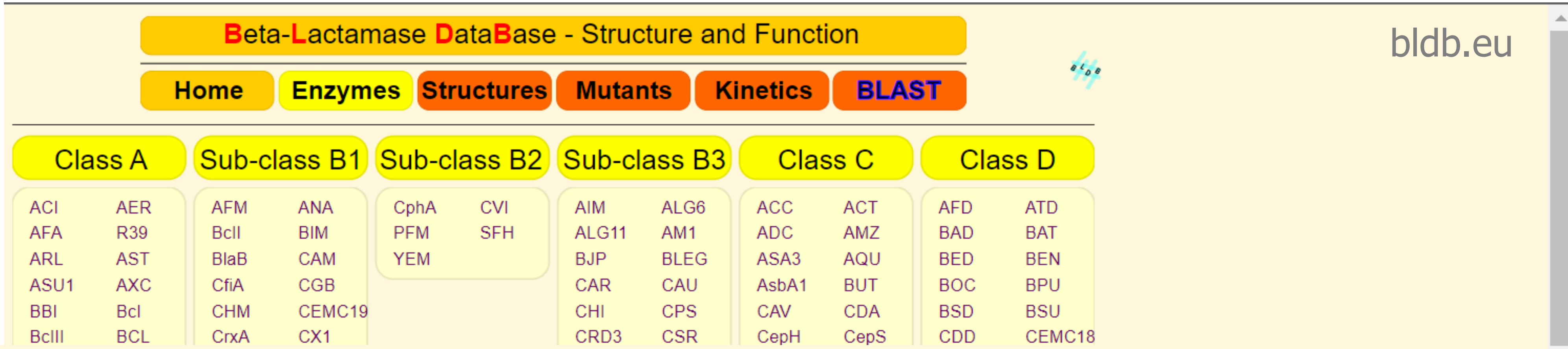
EUCAST Expected Resistant Phenotypes v 1.2 January 2023 Page 3 of 8

Introduction to AMR genes/mutations in Enterobacterales

Antimicrobial class	AMR genes/mutations (list not exhaustive)
Beta-lactams	• *bla* genes • specific mutations in promoter of intrinsic genes (e.g. *ampC*) • specific mutations in porin-encoding genes
Fluoroquinolones	• specific mutations in *gyrA, parC, gyrB, parE* • PMQR genes (*oqxAB, aac(6')-Ib-cr, qnr, qep*)
Aminoglycosides	• *aac(1), aac(3), aac(6’), aac(2’)* • *ant(2”), ant(3”), ant(4’)* • *aph(3’), aph(3”), aph(4), aph(6)*
Tetracyclines	• *tet* genes
Colistin	• specific mutations in *pmrCAB, phoPQ, mgrB, crrAB, yciM, lpxM* • *mcr* genes ((!) *mcr-9*)
Fosfomycin	• *fos* genes
Folate pathway antagonists	• *sul* genes, *dfr* genes • specific *folP* mutations

Understanding beta-lactamases for interpreting *bla* genes findings

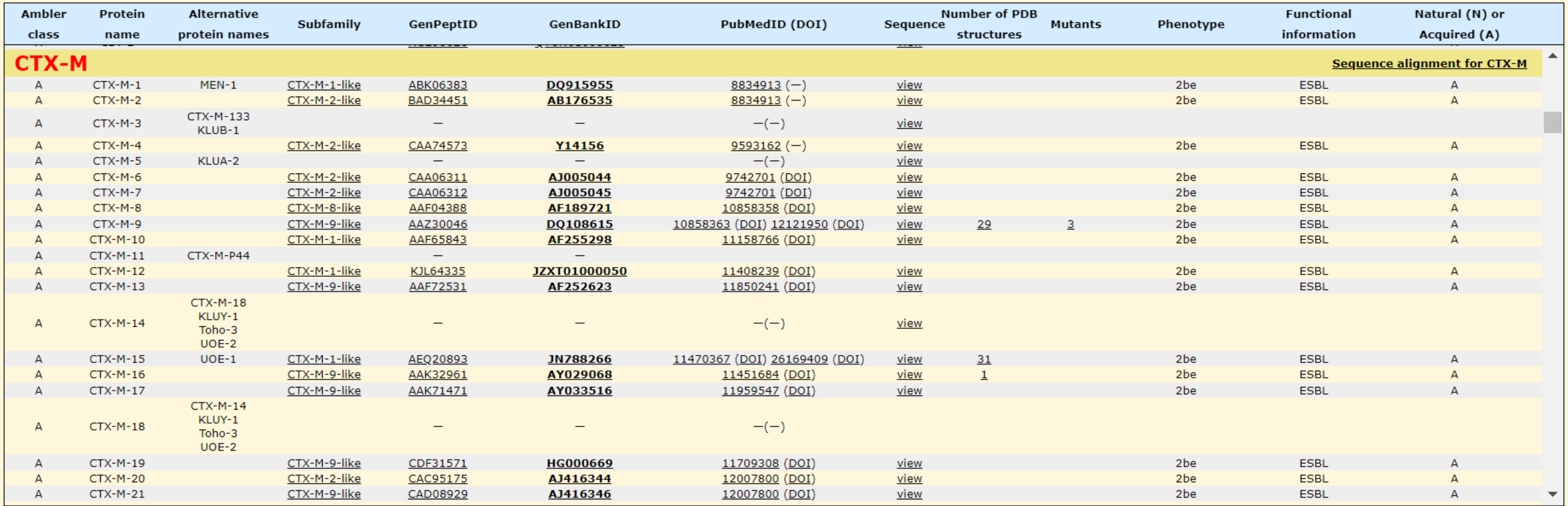
Beta-lactamases are enzymes that degrade beta-lactams



Beta-Lactamase DataBase - Structure and Function

bldb.eu

Class A		Sub-class B1		Sub-class B2		Sub-class B3		Class C		Class D	
ACI	AER	AFM	ANA	CphA	CVI	AIM	ALG6	ACC	ACT	AFD	ATD
AFA	R39	BcII	BIM	PFM	SFH	ALG11	AM1	ADC	AMZ	BAD	BAT
ARL	AST	BlaB	CAM	YEM		BJP	BLEG	ASA3	AQU	BED	BEN
ASU1	AXC	CfiA	CGB			CAR	CAU	AsbA1	BUT	BOC	BPU
BBI	BcI	CHM	CEMC19			CHI	CPS	CAV	CDA	BSD	BSU
BcIII	BCL	CrxA	CX1			CRD3	CSR	CepH	CepS	CDD	CEMC18



Ambler class	Protein name	Alternative protein names	Subfamily	GenPeptID	GenBankID	PubMedID (DOI)	Sequence	Number of PDB structures	Mutants	Phenotype	Functional information	Natural (N) or Acquired (A)
CTX-M												Sequence alignment for CTX-M
A	CTX-M-1	MEN-1	CTX-M-1-like	ABK06383	**DQ915955**	8834913 (—)	view			2be	ESBL	A
A	CTX-M-2		CTX-M-2-like	BAD34451	**AB176535**	8834913 (—)	view			2be	ESBL	A
A	CTX-M-3	CTX-M-133 KLUB-1		—	—	—(—)	view					
A	CTX-M-4		CTX-M-2-like	CAA74573	**Y14156**	9593162 (—)	view			2be	ESBL	A
A	CTX-M-5	KLUA-2		—	—	—(—)	view					
A	CTX-M-6		CTX-M-2-like	CAA06311	**AJ005044**	9742701 (DOI)	view			2be	ESBL	A
A	CTX-M-7		CTX-M-2-like	CAA06312	**AJ005045**	9742701 (DOI)	view			2be	ESBL	A
A	CTX-M-8		CTX-M-8-like	AAF04388	**AF189721**	10858358 (DOI)	view			2be	ESBL	A
A	CTX-M-9		CTX-M-9-like	AAZ30046	**DQ108615**	10858363 (DOI) 12121950 (DOI)	view	29	3	2be	ESBL	A
A	CTX-M-10		CTX-M-1-like	AAF65843	**AF255298**	11158766 (DOI)	view			2be	ESBL	A
A	CTX-M-11	CTX-M-P44		—	—							
A	CTX-M-12		CTX-M-1-like	KJL64335	**JZXT01000050**	11408239 (DOI)	view			2be	ESBL	A
A	CTX-M-13		CTX-M-9-like	AAF72531	**AF252623**	11850241 (DOI)	view			2be	ESBL	A
A	CTX-M-14	CTX-M-18 KLUY-1 Toho-3 UOE-2		—	—	—(—)	view					
A	CTX-M-15	UOE-1	CTX-M-1-like	AEQ20893	**JN788266**	11470367 (DOI) 26169409 (DOI)	view	31		2be	ESBL	A
A	CTX-M-16		CTX-M-9-like	AAK32961	**AY029068**	11451684 (DOI)	view	1		2be	ESBL	A
A	CTX-M-17		CTX-M-9-like	AAK71471	**AY033516**	11959547 (DOI)	view			2be	ESBL	A
A	CTX-M-18	CTX-M-14 KLUY-1 Toho-3 UOE-2		—	—	—(—)						
A	CTX-M-19		CTX-M-9-like	CDF31571	**HG000669**	11709308 (DOI)	view			2be	ESBL	A
A	CTX-M-20		CTX-M-2-like	CAC95175	**AJ416344**	12007800 (DOI)	view			2be	ESBL	A
A	CTX-M-21		CTX-M-9-like	CAD08929	**AJ416346**	12007800 (DOI)	view			2be	ESBL	A

Beta-lactamase types and variants definitions

- Each type is further divided into variants: e.g. CTX-M-1, CTX-M-2,…. CTX-M-265
- Correct nomeclature:

Gene	Protein
$\mathit{bla}_{\text{CTX-M-1}}$	CTX-M-1
$\mathit{bla}_{\text{TEM-52}}$	TEM-52
etc.	etc.

Important! Variants are defined **based on amino acid sequences**

which should be taken into account when using AMR detection tools working in the nucleotide space and allowing for <100% identity and coverage

Example (hypothetical ESBL):

a. ”ATG TTC CC**G**” is ”MFP”

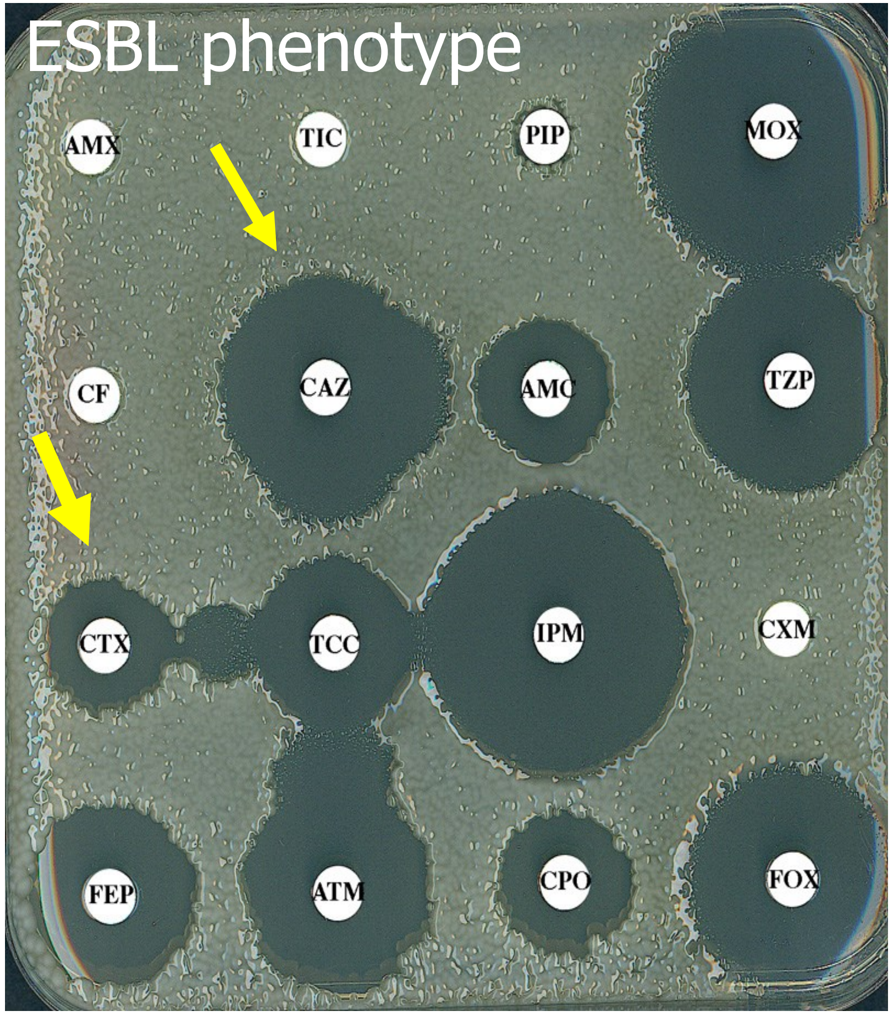
b. ”ATG TTC CC**A**” is ”MFP”

They are the same ESBL!
They can be defined as $\mathit{bla}_{\text{ESBLa}}$ and $\mathit{bla}_{\text{ESBLb}}$

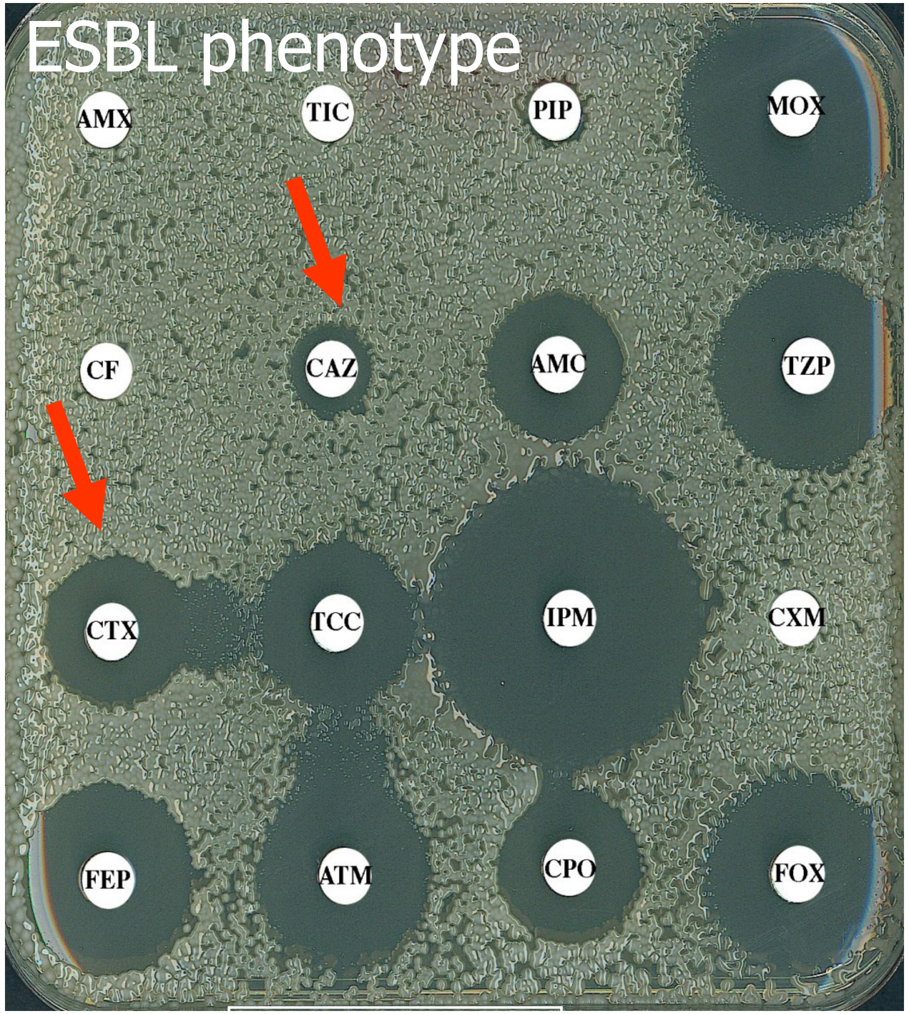
c. ”ATG TTC CTA” is ”MFL”

It is a different ESBL!

Highly-related beta-lactamase may encode diverse beta-lactam resistance phenotypes



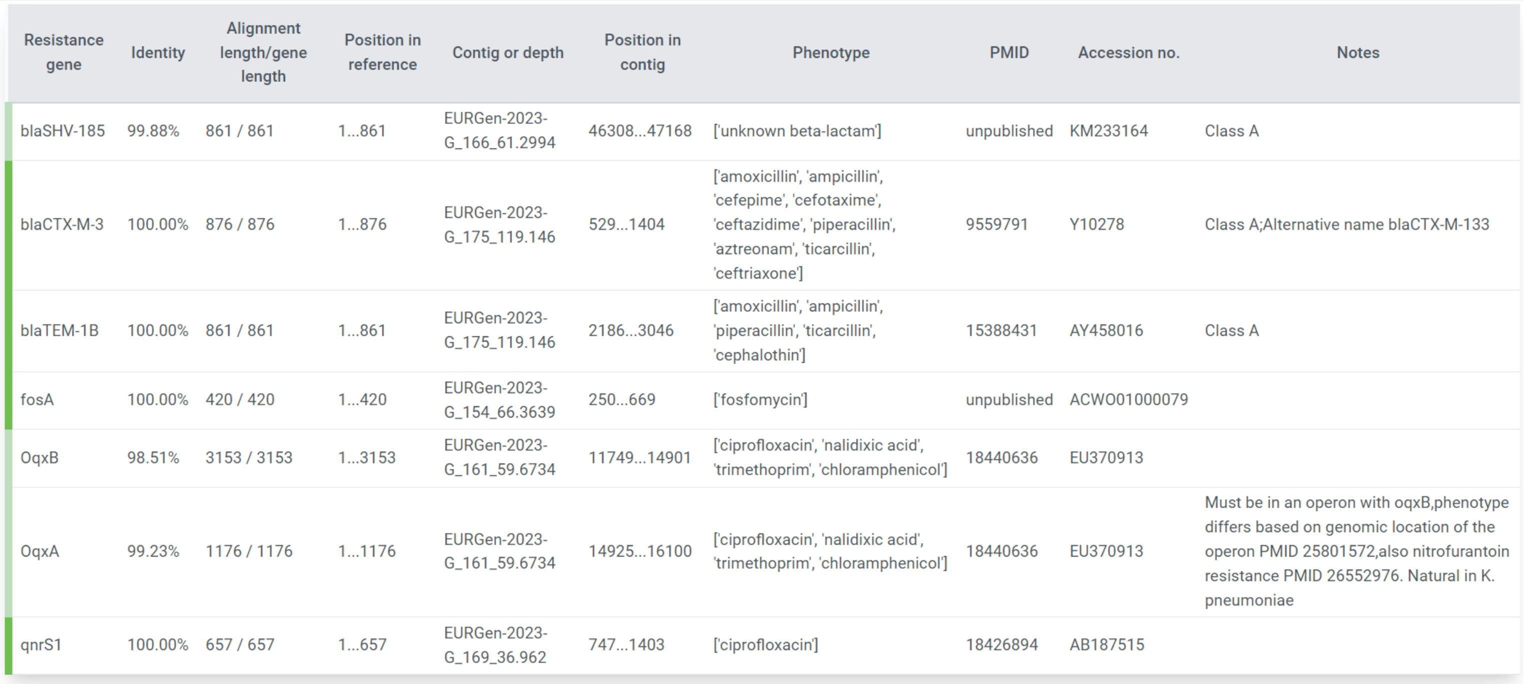
CTX-M-3



CTX-M-15

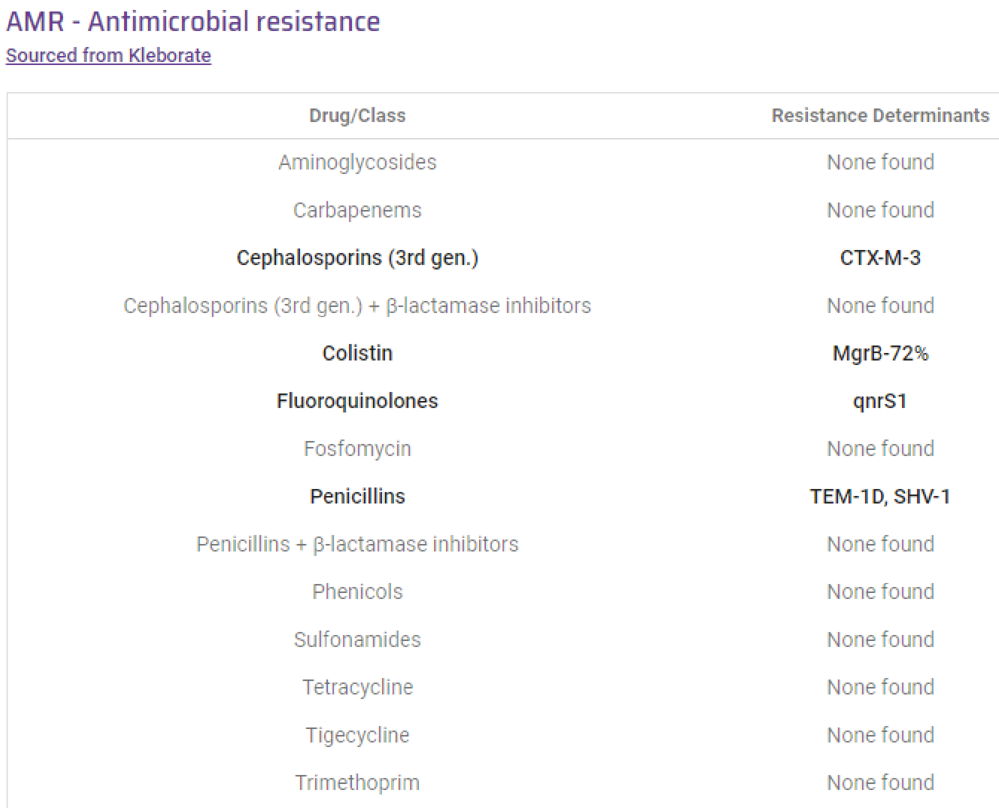
Pictures: Patrice Nordmann

Interpretation of reports requires knowledge of tools and bacteria



Resistance gene	Identity	Alignment length/gene length	Position in reference	Contig or depth	Position in contig	Phenotype	PMID	Accession no.	Notes
blaSHV-185	99.88%	861 / 861	1...861	EURGen-2023-G_166_61.2994	46308...47168	['unknown beta-lactam']	unpublished	KM233164	Class A
blaCTX-M-3	100.00%	876 / 876	1...876	EURGen-2023-G_175_119.146	529...1404	['amoxicillin', 'ampicillin', 'cefepime', 'cefotaxime', 'ceftazidime', 'piperacillin', 'aztreonam', 'ticarcillin', 'ceftriaxone']	9559791	Y10278	Class A;Alternative name blaCTX-M-133
blaTEM-1B	100.00%	861 / 861	1...861	EURGen-2023-G_175_119.146	2186...3046	['amoxicillin', 'ampicillin', 'piperacillin', 'ticarcillin', 'cephalothin']	15388431	AY458016	Class A
fosA	100.00%	420 / 420	1...420	EURGen-2023-G_154_66.3639	250...669	['fosfomycin']	unpublished	ACWO01000079	
OqxB	98.51%	3153 / 3153	1...3153	EURGen-2023-G_161_59.6734	11749...14901	['ciprofloxacin', 'nalidixic acid', 'trimethoprim', 'chloramphenicol']	18440636	EU370913	
OqxA	99.23%	1176 / 1176	1...1176	EURGen-2023-G_161_59.6734	14925...16100	['ciprofloxacin', 'nalidixic acid', 'trimethoprim', 'chloramphenicol']	18440636	EU370913	Must be in an operon with oqxB,phenotype differs based on genomic location of the operon PMID 25801572,also nitrofurantoin resistance PMID 26552976. Natural in K. pneumoniae
qnrS1	100.00%	657 / 657	1...657	EURGen-2023-G_169_36.962	747...1403	['ciprofloxacin']	18426894	AB187515	

ResFinder v4.3.3 (web)



AMR - Antimicrobial resistance

Sourced from Kleborate

Drug/Class	Resistance Determinants
Aminoglycosides	None found
Carbapenems	None found
Cephalosporins (3rd gen.)	**CTX-M-3**
Cephalosporins (3rd gen.) + β-lactamase inhibitors	None found
Colistin	**MgrB-72%**
Fluoroquinolones	**qnrS1**
Fosfomycin	None found
Penicillins	**TEM-1D, SHV-1**
Penicillins + β-lactamase inhibitors	None found
Phenicols	None found
Sulfonamides	None found
Tetracycline	None found
Tigecycline	None found
Trimethoprim	None found

Pathogenwatch v21.0.0

Outline

#	Topic	Time
1	Introduction	12:30 - 12:40
2	Brush-up antimicrobial resistance (AMR)	12:40 - 13:10
3	Fundamentals of WGS-based detection of AMR	13:10 - 13:40
4	Q&A	13:40 - 13:45
5	*Break*	13:45 - 14:00
6	Tools for WGS-based detection of AMR	14:00 – 14:45
7	WGS-based detection of AMR in Enterobacterales	14:45 – 15:00
8	*Break*	15:00 – 15:10
9	WGS-based detection of AMR in *Staphylococcus aureus* and *Enterococcus* spp.	15:10 – 15:30
10	Explanation of the exercise	15:30 – 15:35
11	Q&A, conclusions and session evaluation	15:35 – 16:00

WGS-based detection of AMR in *Staphylococcus aureus*

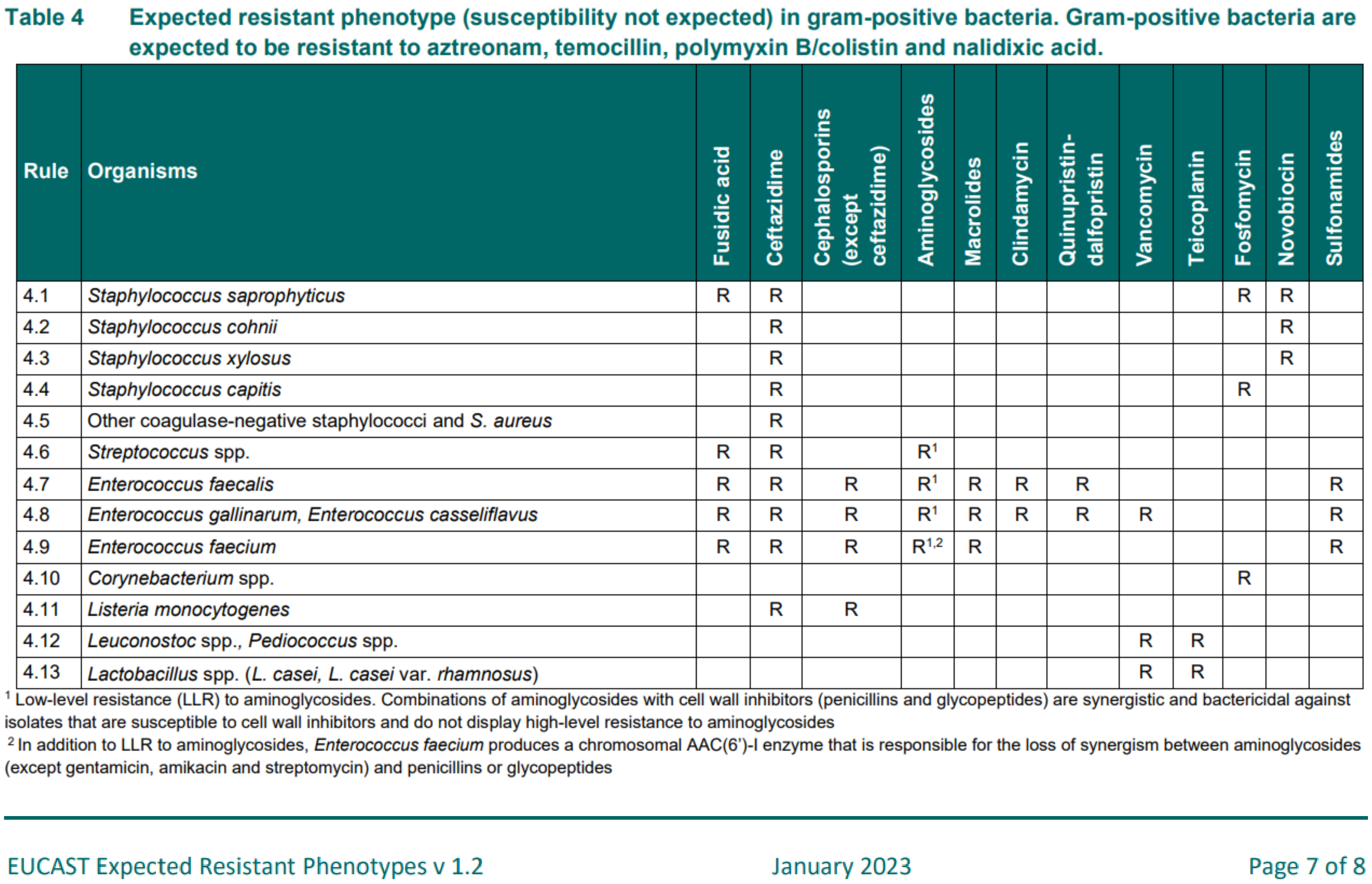


Table 4 **Expected resistant phenotype (susceptibility not expected) in gram-positive bacteria. Gram-positive bacteria are expected to be resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid.**

Rule	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *S. aureus*		R										
4.6	*Streptococcus* spp.	R	R		R^1								
4.7	*Enterococcus faecalis*	R	R	R	R^1	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R^1	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R^{1,2}	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides

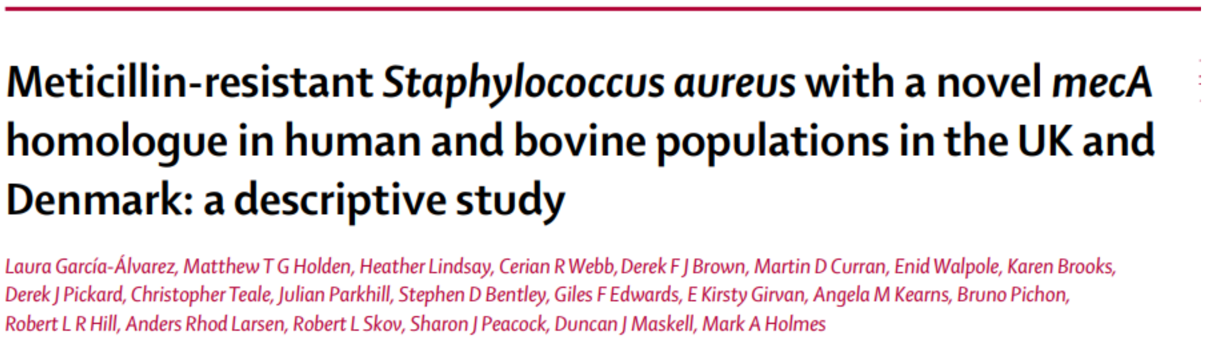
[2] In addition to LLR to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6')-I enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin and streptomycin) and penicillins or glycopeptides

EUCAST Expected Resistant Phenotypes v 1.2 January 2023 Page 7 of 8

Introduction to AMR genes/mutations in *S. aureus*

Antimicrobial class	AMR genes/mutations (list not exhaustive)
Beta-lactams	• *blaZ* • *mecA, mecC*
Fluoroquinolones	• specific *gyrA, gyrB, grlA, grlB* mutations
Aminoglycosides	• aac(6’), *aac(6’)-aph(2’’)* • *ant(4’), andt(6), ant(9)* • *aph(3’)*
Glycopeptides	• *vanA* operon • specific *rpoB* mutations
Macrolides, lincosamide, streptogramins	• *ere, mef, erm* genes
Tetracyclines	• *tet* genes
Oxazolidinones	• specific *rplC, rplD* mutations • *cfr*
Daptomycin	• specific *mprF* mutations • specific *rpoB* mutations
Fosfomycin	• *fos* genes • (chromosomal mutations)
Fusidic acid	• specific *fusA* mutations • *fusB, fusC, fusD*
Folate pathway antagonists	• specific *dfrB* mutations • *dfr* genes

Existing tools can be used to detect novel AMR genes (with some degree of similarity to known AMR genes)



Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study

Laura García-Álvarez, Matthew T G Holden, Heather Lindsay, Cerian R Webb, Derek F J Brown, Martin D Curran, Enid Walpole, Karen Brooks, Derek J Pickard, Christopher Teale, Julian Parkhill, Stephen D Bentley, Giles F Edwards, E Kirsty Girvan, Angela M Kearns, Bruno Pichon, Robert L R Hill, Anders Rhod Larsen, Robert L Skov, Sharon J Peacock, Duncan J Maskell, Mark A Holmes

Lancet Infect Dis 2011

- Meticillin-resistant *S. aureus*
- No *mecA*
- Analysis of WGS data reveals presence of a novel meticillin resistance gene with 70% identity to *mecA* (now known as *mecC*)

WGS-based detection of AMR in *Enterococcus* spp.

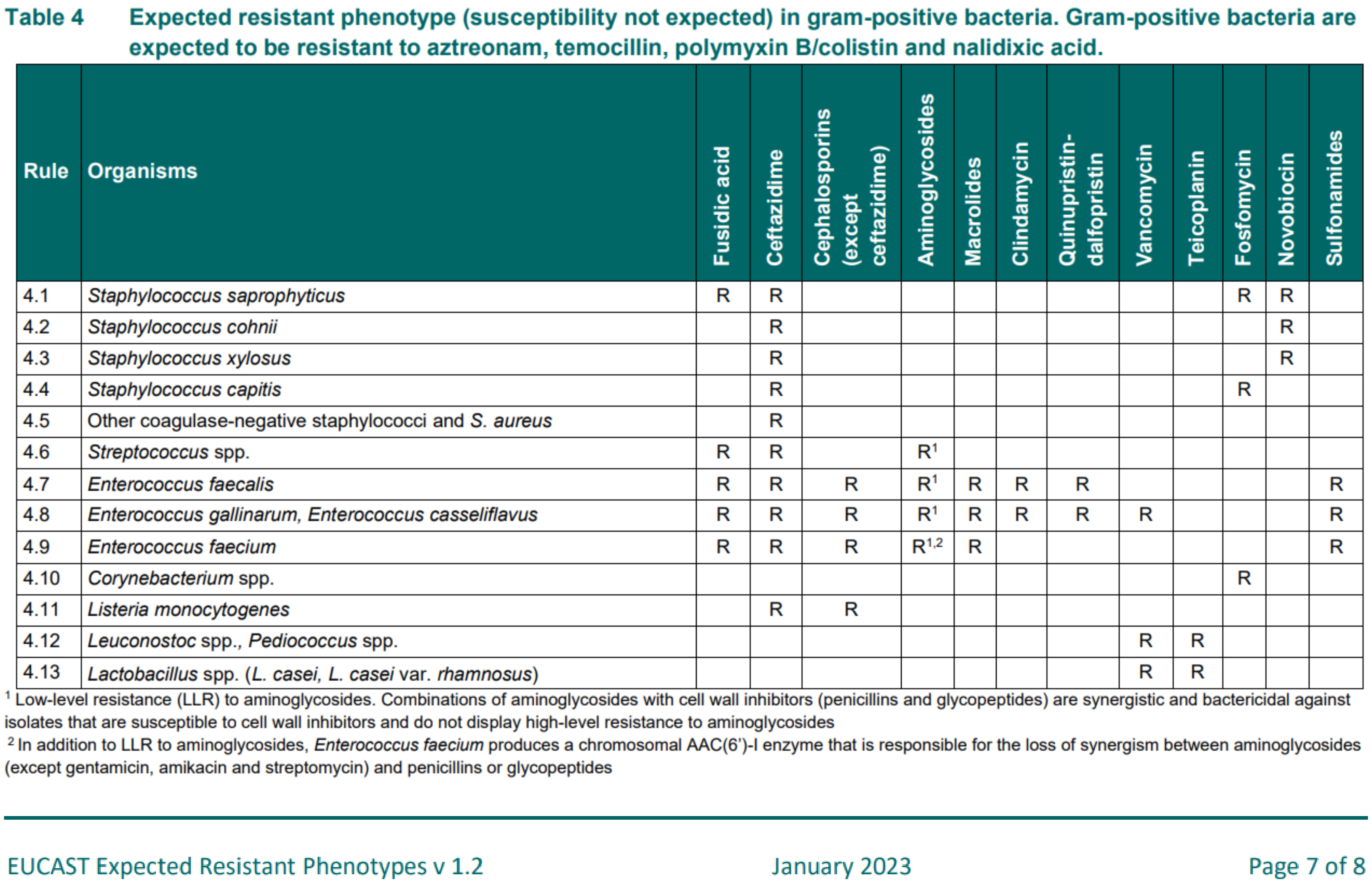


Table 4 **Expected resistant phenotype (susceptibility not expected) in gram-positive bacteria. Gram-positive bacteria are expected to be resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid.**

Rule	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *S. aureus*		R										
4.6	*Streptococcus* spp.	R	R		R[1]								
4.7	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides

[2] In addition to LLR to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6')-I enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin and streptomycin) and penicillins or glycopeptides

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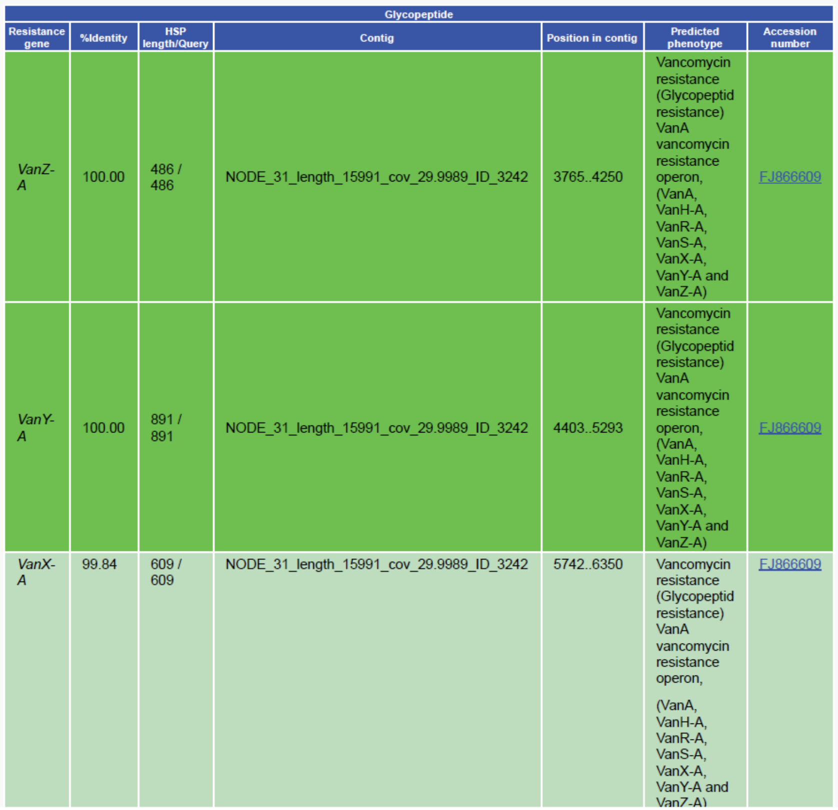
Introduction to AMR genes/mutations in *Enterococcus* spp.

Antimicrobial class	AMR genes/mutations (list not exhaustive)	
	E. faecium	**_E. faecalis*_**
Beta-lactams	• Specific mutations in *pbp5*	• Specific mutations in *pbp4*
Fluoroquinolones	• specific *gyrA, parC* mutations	• specific *gyrA, parC* mutations
Aminoglycosides	• aac(6’), *aac(6’)-aph(2”)* • *ant(3”), ant(4’), ant(6)* • *aph(2”), aph(3’), aph(6)*	• aac(6’), *aac(6’)-aph(2”)* • *ant(3”), ant(4’), ant(6)* • *aph(2”), aph(3’), aph(6)*
Glycopeptides	• *vanA, vanB* operons	• *vanA, vanB* operons
Macrolides, lincosamide, streptogramins	• *eat, erm, mefA, lsa, lnu, vat, vga* genes	• (Expected R to streptogramins) • *eat, erm, mefA, lsa, lnu, vat, vga* genes
Tetracyclines	• *tet* genes • specific *rpsJ* mutations	• *tet* genes
Oxazolidinones	• specific *rplC, rplD, rplV*, 23S RNA gene mutations • *cfr, optrA, poxtA*	• specific *rplC, rplD, rplV*, 23S RNA gene mutations • *cfr, optrA, poxtA*
Daptomycin	• specific *cls, liaF, liaS, liaR* mutations	• *liaF, liaS, liaR* mutations

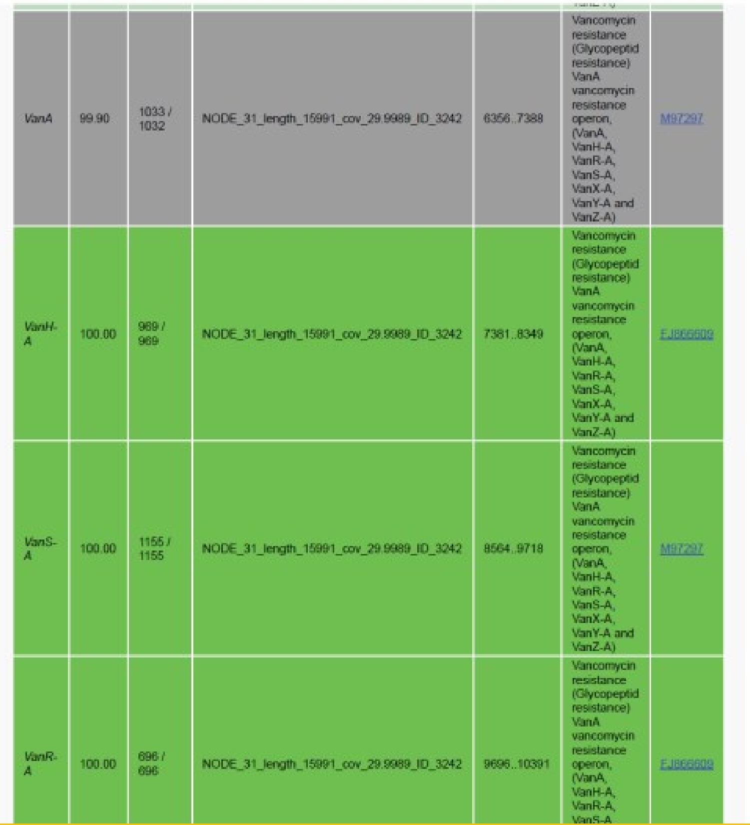
Phenotype & WGS data complement each other

E. faecium isolate

no van resistance!



Glycopeptide						
Resistance gene	**%Identity**	**HSP length/Query**	**Contig**	**Position in contig**	**Predicted phenotype**	**Accession number**
VanZ-A	100.00	486 / 486	NODE_31_length_15991_cov_29.9989_ID_3242	3765..4250	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A, VanX-A, VanY-A and VanZ-A)	FJ866609
VanY-A	100.00	891 / 891	NODE_31_length_15991_cov_29.9989_ID_3242	4403..5293	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A, VanX-A, VanY-A and VanZ-A)	FJ866609
VanX-A	99.84	609 / 609	NODE_31_length_15991_cov_29.9989_ID_3242	5742..6350	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A, VanX-A, VanY-A and VanZ-A)	FJ866609



VanA	99.90	1033 / 1032	NODE_31_length_15991_cov_29.9989_ID_3242	6356..7388	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A, VanX-A, VanY-A and VanZ-A)	M97297
VanH-A	100.00	969 / 969	NODE_31_length_15991_cov_29.9989_ID_3242	7381..8349	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A, VanX-A, VanY-A and VanZ-A)	FJ866609
VanS-A	100.00	1155 / 1155	NODE_31_length_15991_cov_29.9989_ID_3242	8564..9718	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A, VanX-A, VanY-A and VanZ-A)	M97297
VanR-A	100.00	696 / 696	NODE_31_length_15991_cov_29.9989_ID_3242	9696..10391	Vancomycin resistance (Glycopeptid resistance) VanA vancomycin resistance operon, (VanA, VanH-A, VanR-A, VanS-A	FJ866609

The entire vancomycin resistance (*vanA*) operon is present:
vanR, *vanS*, *vanY*, *vanZ*: 100% id
vanA: 99.9% id
vanX: 99.8% id

Slido Q8 and Q9

Introduction to the practical exercise

- In this exercise, you will detect AMR determinants in four bacterial isolates based on whole genome sequence (WGS) data.
- As there are many bioinformatics tools available for this purpose, and due to the lack of a gold standard, you will have to familiarize yourself with as many tools as possible and understand which one(s) you can or cannot use based on your expertise.
- You will also have to assess if results differ when using different tools, and you should try to understand and explain the underlying reasons.
- Finally, you will have to predict a phenotype for the isolates under investigation based on the results of the WGS analysis and, for a subset of isolates, compare it with the phenotype obtained in the laboratory, and troubleshoot in case of discordances.

Stage 1: Exploring tools for WGS-based detection of AMR

- Instructions are available on EVA – “Practical exercise”
- Submission of answers: from 4 September at 16:00 to 5 September at 23:00 (CEST)

Stage 2: Using tools for WGS-based detection of AMR

- WGS data and instructions will be available on EVA on 6 September at 9:00 CEST
- Submission of answers: from 6 September at 12:00 to 7 September at 14:00 (CEST)

In summary

List of learning points in this session:

1. In relation to “Brush-up concepts of AMR”, we rehearsed how antimicrobials affect bacteria, how bacteria can survive the presence of antimicrobials and how we define AMR
2. In relation to “Tools for WGS-based detection of AMR”, we dissected the fundamentals of bioinformatics tools and examined AMRFinderPlus, RGI/CARD, Pathogenwatch and ResFinder
3. In relation to “Species-specific concepts of AMR for Enterobacterales, *S. aureus* and *Enterococcus* spp.”, we summarised the most common AMR determinants and exemplified situations in which use of WGS was key to advance knowledge of AMR

If you combine the above, you should now be able to:

- Describe available open-source tools for detection of AMR in WGS data
- Describe the impact of different sequencing technologies on the detection of AMR
- Evaluate the advantages and disadvantages of AMR prediction based on phenotypic and genotypic methods in the context of surveillance

Further reading

Please refer to “Resources and further reading” file that will be uploaded on EVA

Acknowledgements

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