

Computational Practical 6: Detecting antimicrobial resistance from bacterial genomes using web-based tools

6. AMR detection using Pathogenwatch

Pathogenwatch (<https://pathogen.watch/>) is one of most intuitive and easy-to-use web-based platforms for the analysis of bacterial genomes, developed by The Centre for Genomic Pathogen Surveillance (CGPS), UK, that can be used to detect AMR in the genomes of many bacterial pathogens (but not all). You will be provided with pre-generated genome assemblies that can be directly uploaded as input to this tool. Once uploaded, Pathogenwatch performs strain identification, multi-locus sequence typing (MLST) and resistance prediction in an automated manner. Recently, the website was upgraded with the option to upload raw sequencing reads (those obtained directly from sequencing machines without further bioinformatic processing), but as the upload and analysis of raw reads takes much longer, we will be uploading the genome assemblies provided instead.

Open the website (<https://pathogen.watch/>) on a new Firefox tab. Click on the “upload” button at the top right corner as indicated by the arrow in **Figure 1**.

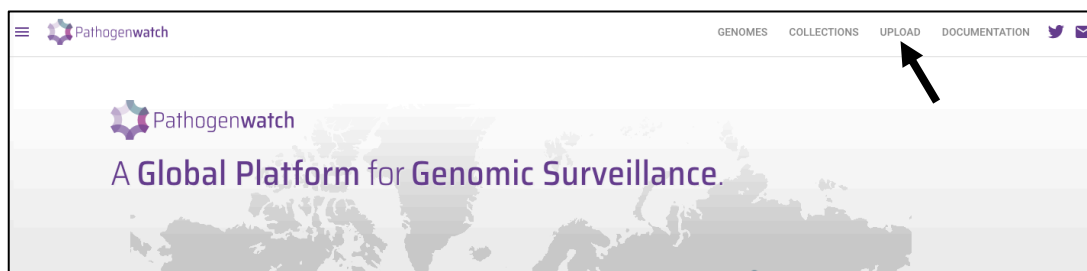


Figure 1: Pathogenwatch home page

You will need to sign in using one of the available options (**Figure 2**) before you can upload any genomes.

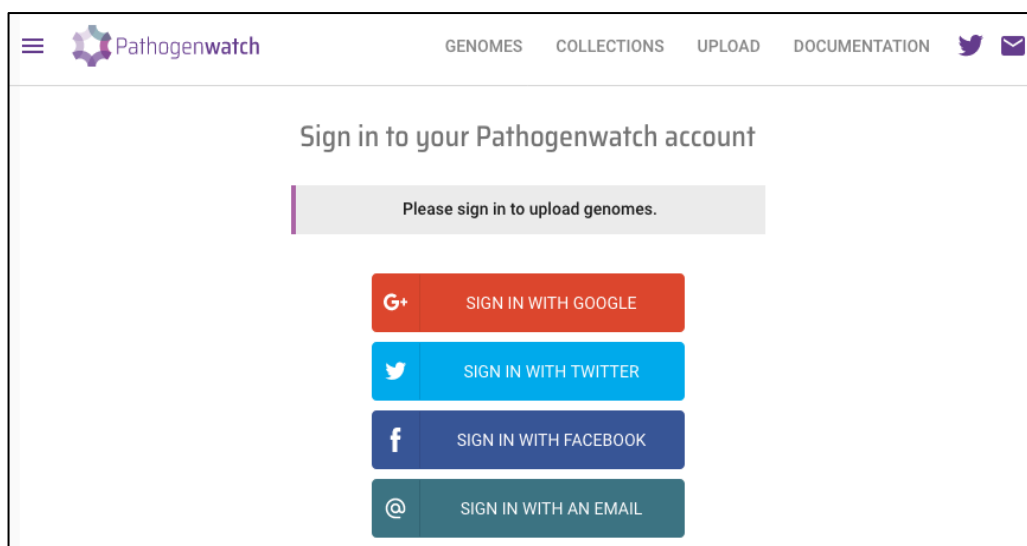


Figure 2: Pathogenwatch log in options

Once logged in, a new window with genome upload options will appear as shown in **Figure 3**.

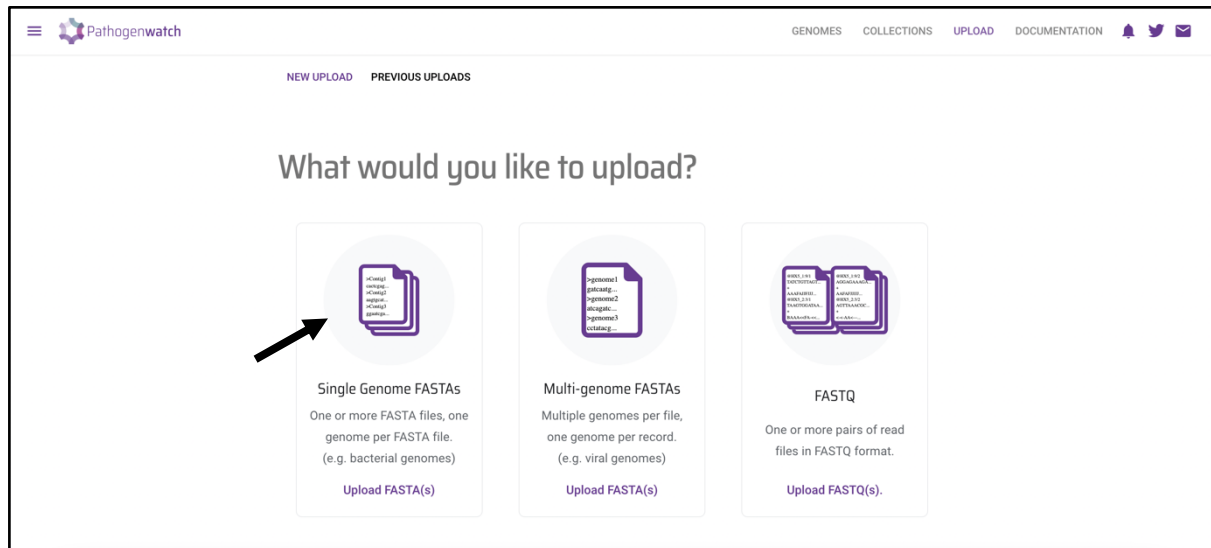


Figure 3. Pathogenwatch genome upload options

Click on the 'Single Genome FASTAs' option and select the genome assembly file of the *Klebsiella pneumoniae* strain **cpe004**. Next, a new window with upload information will appear (**Figure 4**). Click on the 'Add files' button to open the file browser and select the file *cpe004_Kpn-ST78-NDM1.fasta*. The file will be uploaded and the analysis will begin automatically.

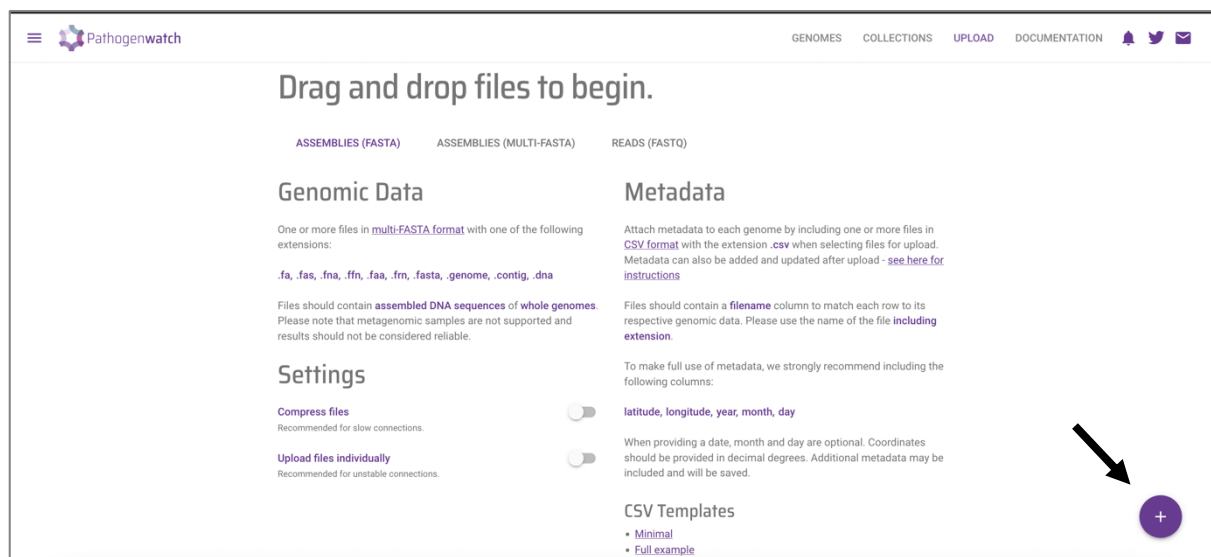


Figure 4. Pathogenwatch genome upload information

The new page (**Figure 5**) will show the status of the different genome analyses being conducted by Pathogenwatch on the background. Click on “**View Genomes**” once all analyses have finalised as pointed by the arrow. In the next window, click on “**View report**”.

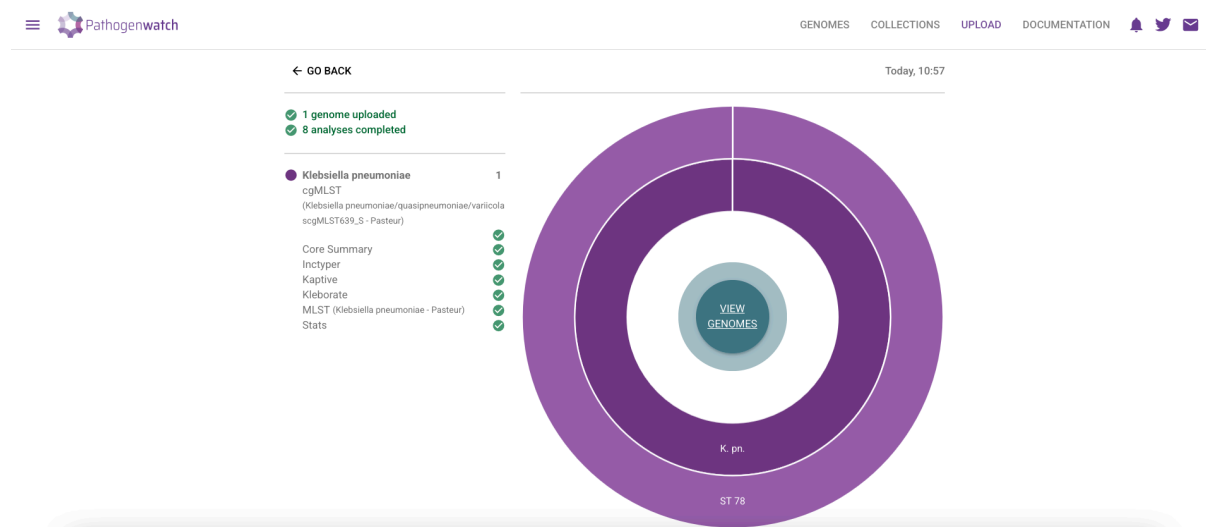


Figure 5. Pathogenwatch page on analysis status

The Pathogenwatch genome report (**Figure 6**) contains information on multi-locus sequence typing (MLST) at the top (TYPING) followed by antimicrobial resistance (AMR), and quality control (QC) stats. Spend some time familiarising yourself with the sections and content of the report and ask your instructor for clarifications if needed.

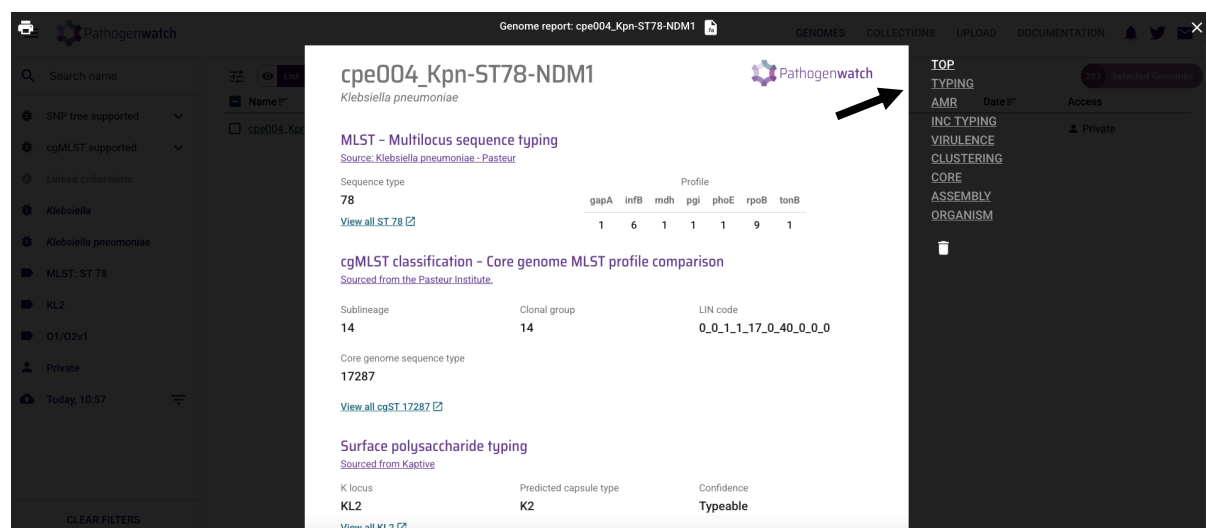


Figure 6. Pathogenwatch genome report for *Klebsiella pneumoniae* strain cpe004

Now click on the AMR section of the report (arrow in Figure 7). Scroll down and pay particular attention to the ‘Resistance determinants’, that is, the AMR genes and mutations detected in the genome assembly of our strain of interest. Spend some time exploring the AMR report, compare these results with those obtained with AMRFinder and ResFinder for strain cpe004.

Genome report: cpe004_Kpn-ST78-NDM1

AMR - Antimicrobial resistance

Sourced from Kleborate

Drug/Class	Resistance Determinants
Aminoglycosides	aac(3)-IId, aac(6)-Ib', aac(6)-Ib-cr, aac(6)-Ib-cr, aadA2, aph(3)-VI, armA, sat-2
Carbapenems	NDM-1, OmpK36GD
Cephalosporins (3rd gen.)	CTX-M-15, CTX-M-15
Cephalosporins (3rd gen.) + β -lactamase inhibitors	None found
Colistin	None found
Fluoroquinolones	qnrB1, GyrA-83Y, GyrA-87G, ParC-80I
Fosfomycin	None found
Penicillins	OXA-1, OXA-1, TEM-10, TEM-10, SHV-11, SHV-28
Penicillins + β -lactamase inhibitors	None found
Phenicol	CatB4, CatB4
Sulfonamides	sul1
Tetracycline	tet(D)
Tigecycline	None found
Trimethoprim	dfrA1, dfrA12, dfrA14 (homolog)

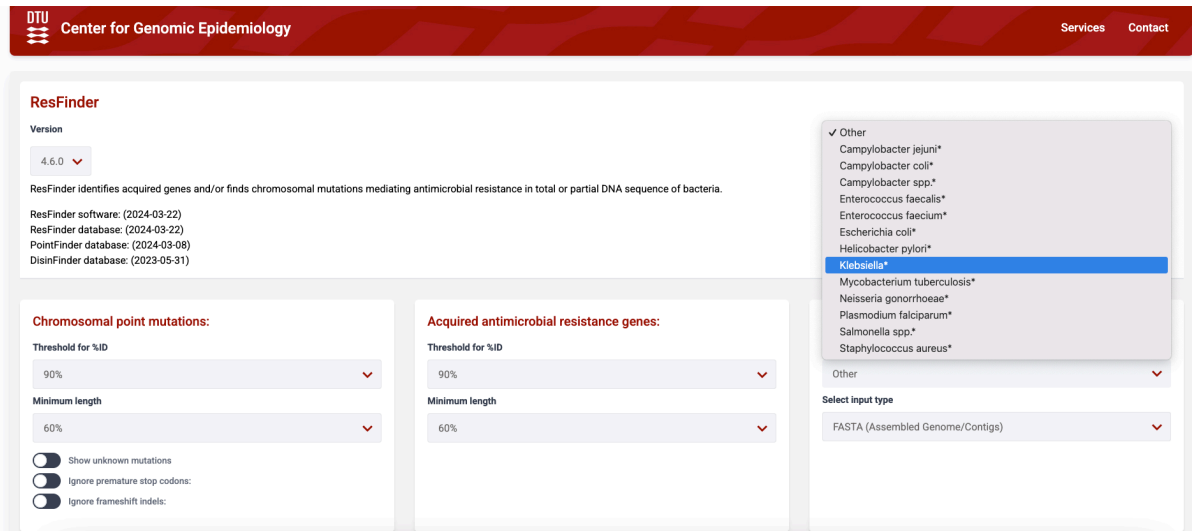
Navigation menu (right): TOP, TYPING, AMR, INC. TYPING, VIRULENCE, CLUSTERING, CORE, ASSEMBLY, ORGANISM.

Finally, upload the genome assembly of **your assigned outbreak strain** by repeating all previous steps (**Figures 3 to 6**).

Additionally, if time allows, obtain the Pathogenwatch report for the two additional *S. aureus* (from genome assembly files HO50960412.fa & ERR017261.assembly.fa) and the two *S. typhi* strains (from genome assembly files ERR2093245.assembly.fa & ERR2093329.assembly.fa).

5. AMR detection using ResFinder – Optional

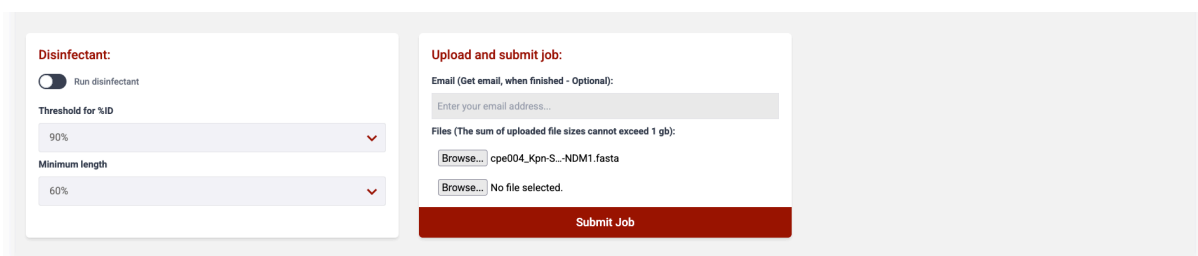
Open up a new tab on Firefox and go to <http://genepi.food.dtu.dk/resfinder>. We will upload the genome assemblies of the same strains to ResFinder. Make sure you select the right bacterial species under the option 'Species and input data type'. Here, keep 'FASTA' as the input type.



The screenshot shows the ResFinder web interface. At the top is a red header with the DTU logo and 'Center for Genomic Epidemiology', and links for 'Services' and 'Contact'. The main content area is titled 'ResFinder' and includes a version dropdown set to '4.6.0'. Below this, it states: 'ResFinder identifies acquired genes and/or finds chromosomal mutations mediating antimicrobial resistance in total or partial DNA sequence of bacteria.' It also lists database update dates: 'ResFinder software: (2024-03-22)', 'ResFinder database: (2024-03-22)', 'PointFinder database: (2024-03-08)', and 'DisinFinder database: (2023-05-31)'. There are two main configuration panels. The left panel, 'Chromosomal point mutations:', has a 'Threshold for %ID' dropdown at 90%, a 'Minimum length' dropdown at 60%, and three toggle switches for 'Show unknown mutations', 'Ignore premature stop codons', and 'Ignore frameshift indels'. The right panel, 'Acquired antimicrobial resistance genes:', has similar 'Threshold for %ID' (90%) and 'Minimum length' (60%) dropdowns. To the right of these panels is a dropdown menu for 'Species and input data type' with a list of bacterial species including 'Klebsiella*', 'Mycobacterium tuberculosis*', 'Neisseria gonorrhoeae*', 'Plasmodium falciparum*', 'Salmonella spp.*', and 'Staphylococcus aureus*'. Below this is a 'Select input type' dropdown set to 'FASTA (Assembled Genome/Contigs)'. At the bottom right is a 'Submit Job' button.

Figure 8. ResFinder genome upload options

Select the file `cpe004_Kpn-ST78-NDM1.fasta` after that.



The screenshot shows the 'Upload and submit job:' section of the ResFinder web interface. It includes a 'Disinfectant:' section with a toggle switch for 'Run disinfectant'. Below this are 'Threshold for %ID' and 'Minimum length' dropdowns, both set to 90% and 60% respectively. The 'Upload and submit job:' section has an 'Email (Get email, when finished - Optional):' field with a placeholder 'Enter your email address...'. Below this is a 'Files (The sum of uploaded file sizes cannot exceed 1 gb):' section with two 'Browse...' buttons. The first button is next to the file 'cpe004_Kpn-S...-NDM1.fasta' and the second is next to 'No file selected.'. At the bottom right is a red 'Submit Job' button.

Figure 9. ResFinder genome upload options

Figure 10 shows the ResFinder results page with predicted genotypic AMR for *Klebsiella pneumoniae* strain cpe004.

Take a note of the AMR genetic determinants identified by ResFinder compared to the ones detected by Pathogenwatch.

DTU Center for Genomic Epidemiology			
Results			
ResFinder-4.6.0			
Phenotypes			
<div>Klebsiella</div> <div>All</div>			
Antimicrobial	Class	WGS-predicted phenotype	Genetic background
gentamicin	aminoglycoside	Resistant	armA ₁ :AY220558
tobramycin	aminoglycoside	Resistant	armA ₁ :AY220558
streptomycin	aminoglycoside	Resistant	aadA2 ₂ :JQ364967
amikacin	aminoglycoside	Resistant	aph(3)-VI ₁ :KC170992
isepamicin	aminoglycoside	Resistant	armA ₁ :AY220558
dibekacin	aminoglycoside	Resistant	aac(6)-Ib-cr ₁ :DQ303918
kanamycin	aminoglycoside	No resistance	
neomycin	aminoglycoside	No resistance	
lividomycin	aminoglycoside	No resistance	
paromomycin	aminoglycoside	No resistance	
ribostamycin	aminoglycoside	No resistance	

Figure 10. ResFinder results page

6. AMR detection using CARD – Optional

Open up a new tab on Firefox and go to <https://card.mcmaster.ca/>. We will upload the genome assemblies of the same two strains to The Comprehensive Antibiotic Resistance Database (CARD), a commonly used AMR database (Figure 11).

CARD

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The Comprehensive Antibiotic Resistance Database

A bioinformatic database of resistance genes, their products and associated phenotypes.
8526 Ontology Terms, 6442 Reference Sequences, 4542 SNPs, 3328 Publications, 6490 AMR Detection Models
Resistome predictions: 414 pathogens, 24291 chromosomes, 2662 genomic islands, 48212 plasmids, 172216 WGS assemblies, 279120 alleles
YouTube: Canadian Bioinformatics Workshops 2024: 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, including lists of mutations and molecules with corresponding metadata and citations. RGI software is available as a command-line tool. CARD Bait Capture Platform sequences and protocols available for download. Extensive notes on updates provided.

Figure 11. CARD home page

We will specifically use the Resistance Gene Identifier (RGI) tool. Click on “**Analyze**” (arrow in Figure 11) and select “**RGI main**” in the next window (Figure 12).

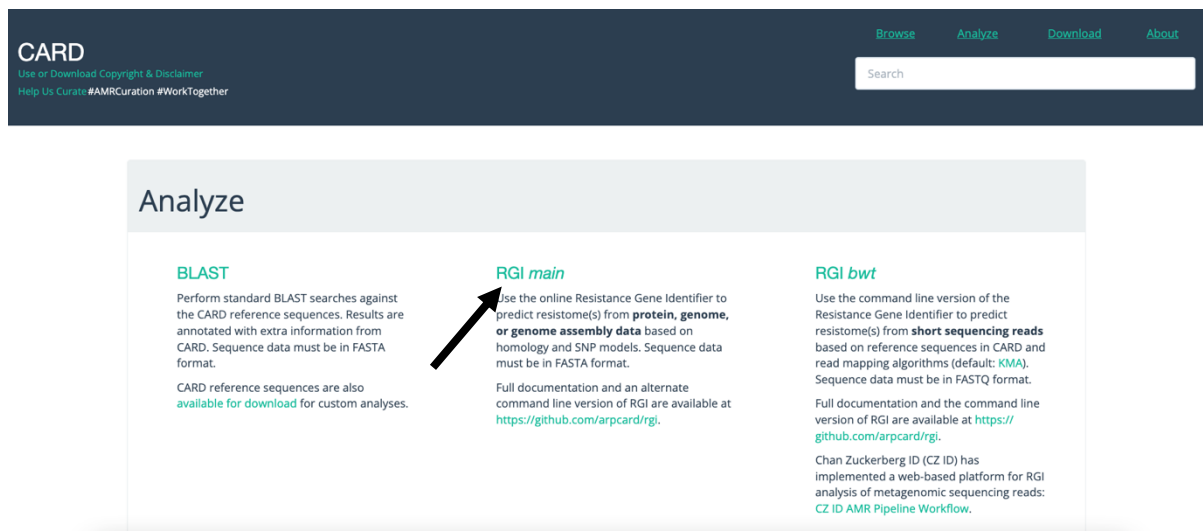


Figure 12. CARD genome analysis options

Click on the button “**Browse**” in the next window (**Figure 13**) to select the file `cpe004_Kpn-ST78-NDM1.fasta` previously submitted to Pathogenwatch and ResFinder. Scroll down to click on “**Submit**”.

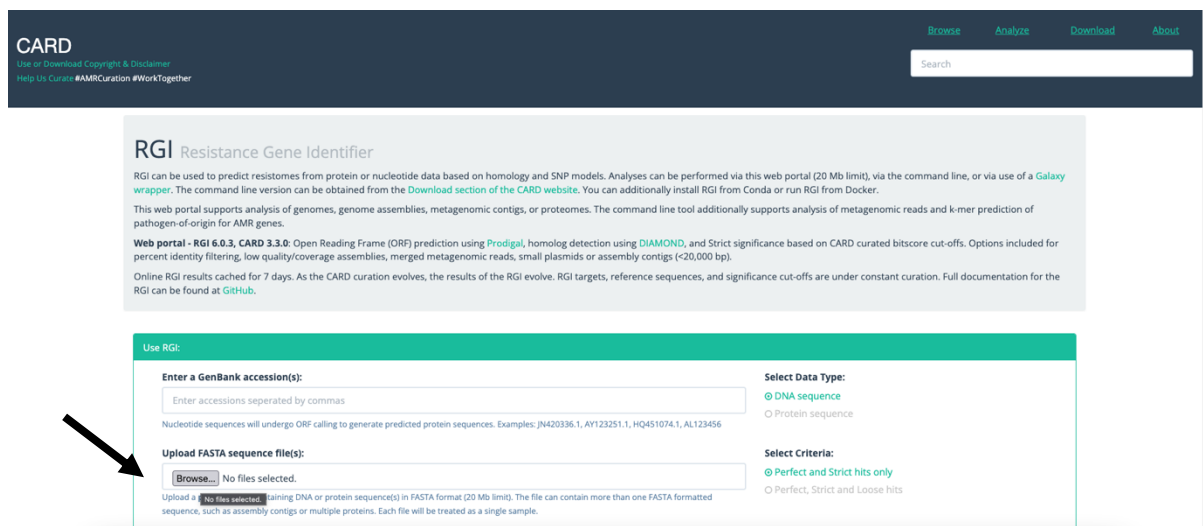


Figure 13. CARD RGI genome selection options

Take your time to familiarise yourself with the format and content of the CARD RGI reports (**Figure 14**). In the Result table, pay particular attention to the fields ‘RGI Criteria’, ‘ARO Term’ (which contains the names of AMR genes also used by other tools), ‘Drug class’ (the antibiotic class AMR genes confer resistance to) and ‘Resistance Mechanisms’.

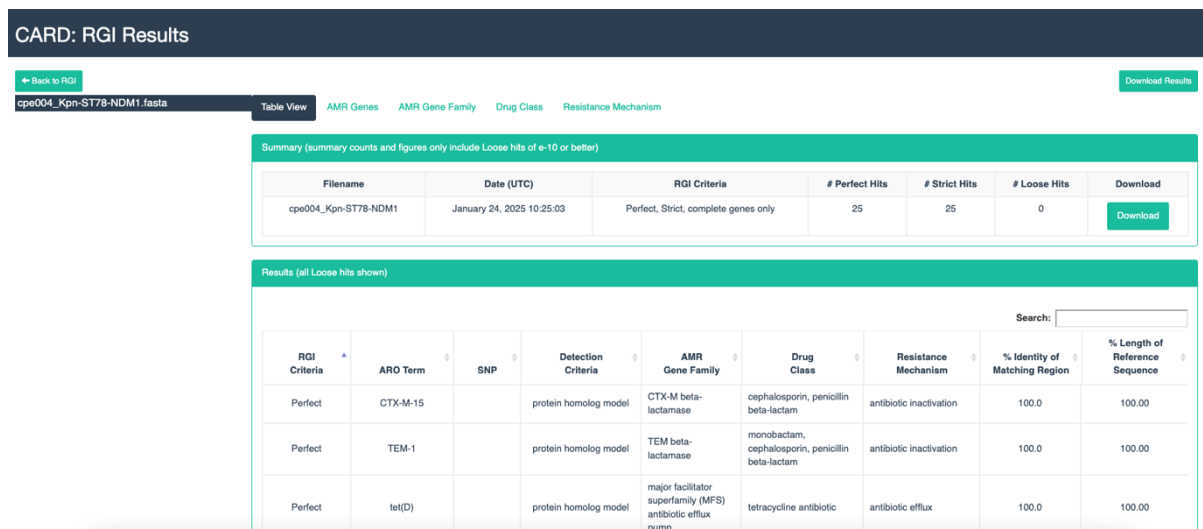


Figure 14. CARD RGI report

Next, click on the “**AMR Genes**” tab of the report to obtain a visual representation of all AMR genes detected (**Figure 15**). Here you can click on individual genes to display information on what antibiotics they confer resistance to and the specific mechanisms of resistance (**Figure 16**).

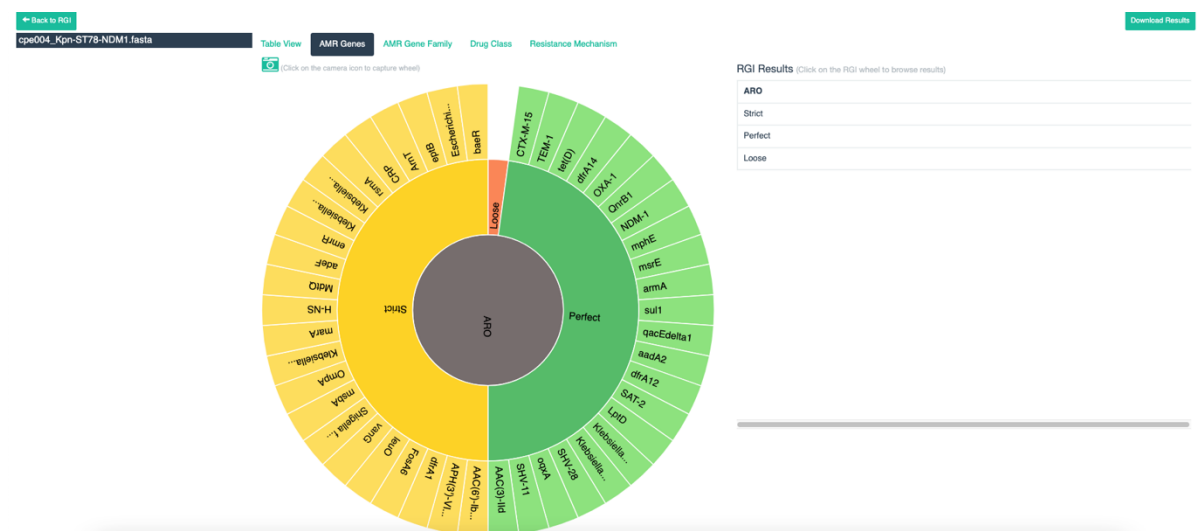


Figure 15. CARD RGI visual report

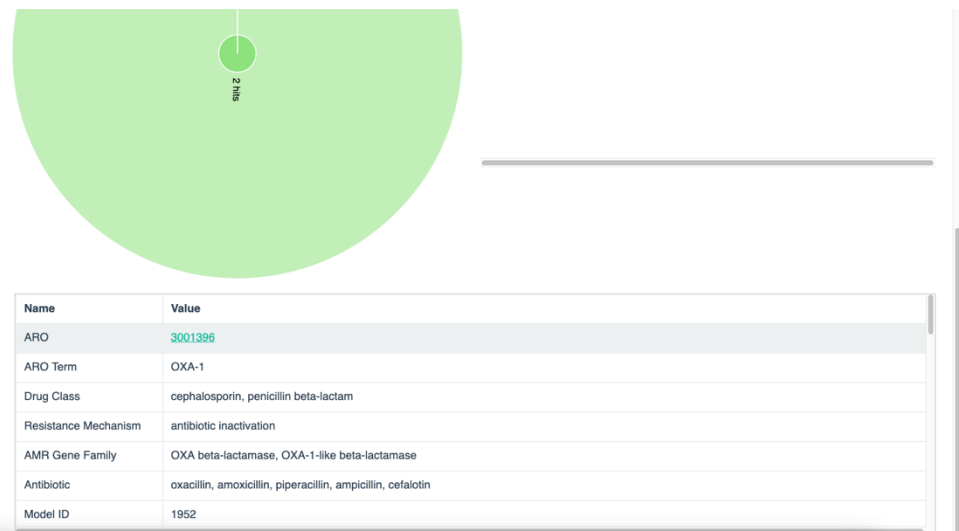


Figure 16. CARD RGI information on individual AMR genes

As done for the reports generated by Pathogenwatch and ResFinder, take note of the genes identified by CARD RGI from the genome assemblies. Finally compare the antibiotic resistance profile (antibiogram) predicted by different tools. You can make use of the summary tables in previous pages to annotate the AMR genes and mutations identified by each tool.