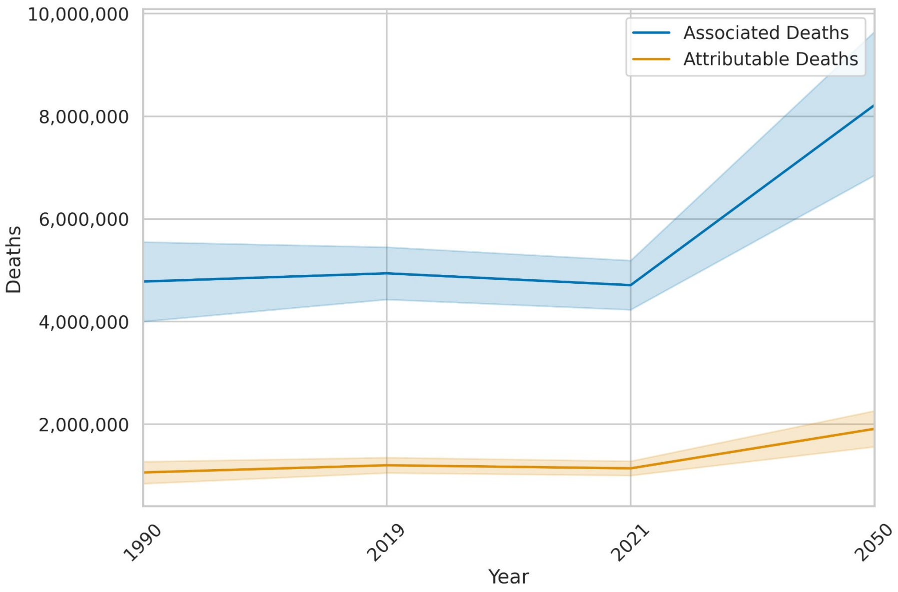
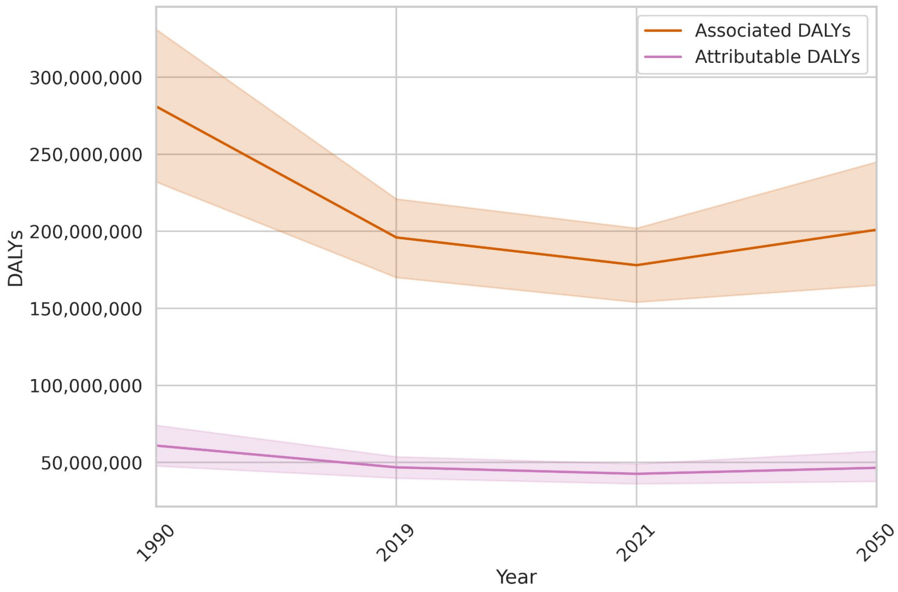
CARD tricks: harmonising and interpreting genomic AMR analyses

Finlay Maguire
Dalhousie University
Shared Hospital Laboratory
Public Health Alliance for Genomic Epidemiology

Considerable global burden of antimicrobial resistance

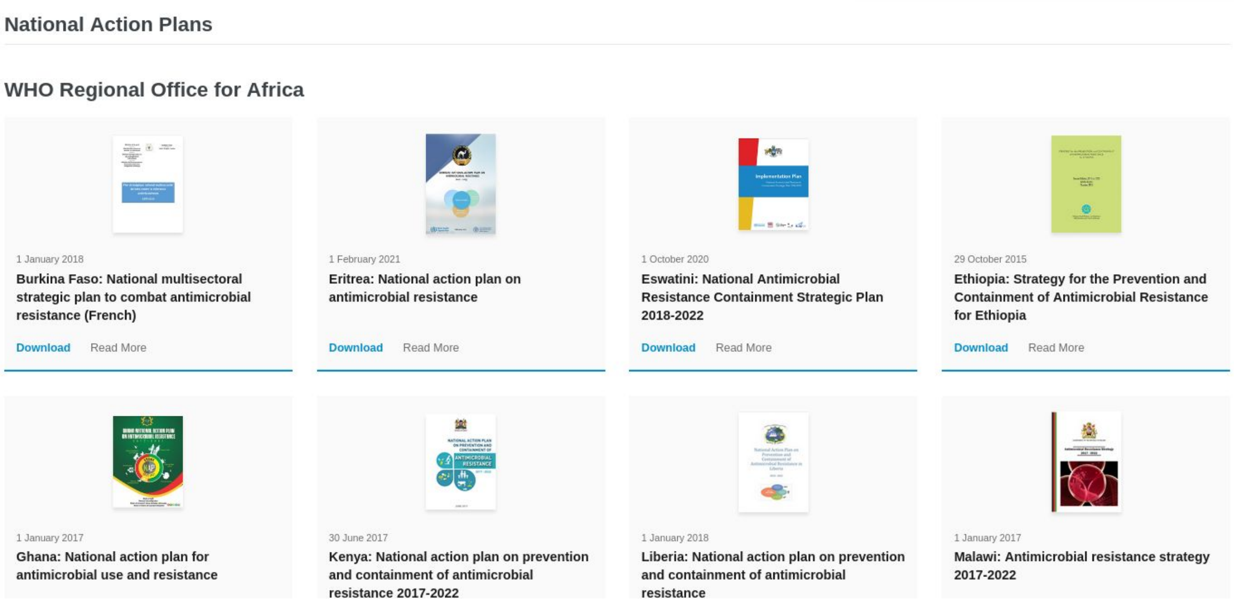




Data from: Naghavi, Mohsen, et al. "Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050." The Lancet 404.10459 (2024): 1199-1226.

What can we do about this?

National and international AMR action plans



National Action Plans

WHO Regional Office for Africa

1 January 2018

Burkina Faso: National multisectoral strategic plan to combat antimicrobial resistance (French)

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Eritrea: National action plan on antimicrobial resistance

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Ghana: National action plan for antimicrobial use and resistance

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Kenya: National action plan on prevention and containment of antimicrobial resistance 2017-2022

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Liberia: National action plan on prevention and containment of antimicrobial resistance

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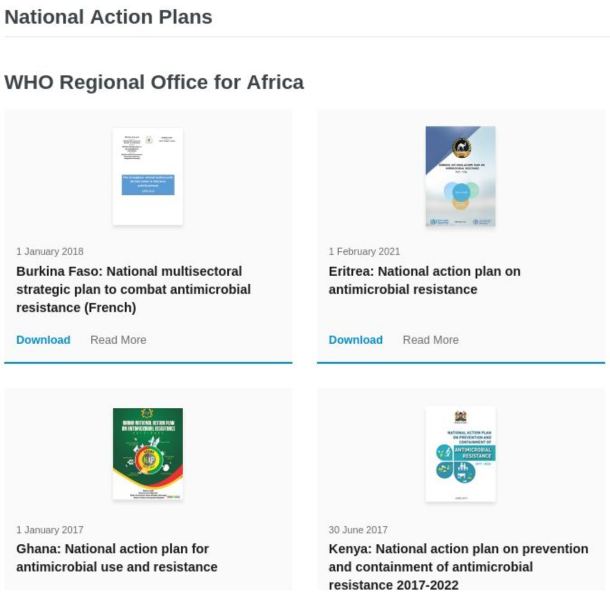
Malawi: Antimicrobial resistance strategy 2017-2022

Pan-Canadian Action Plan on Antimicrobial Resistance

National and international AMR action plans



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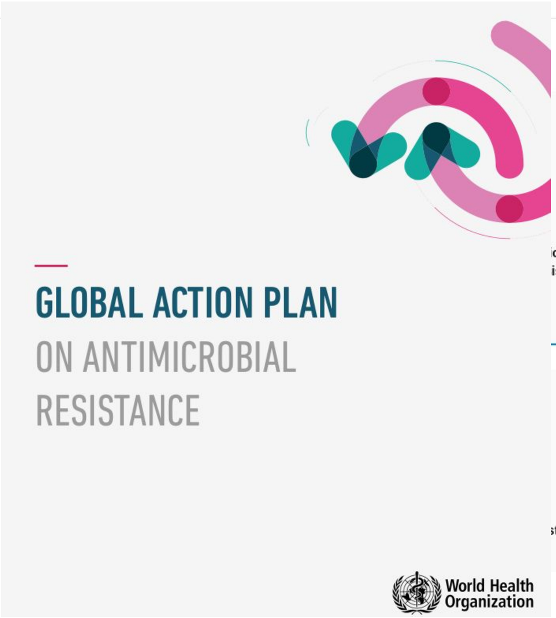
ion and
istance

strategy

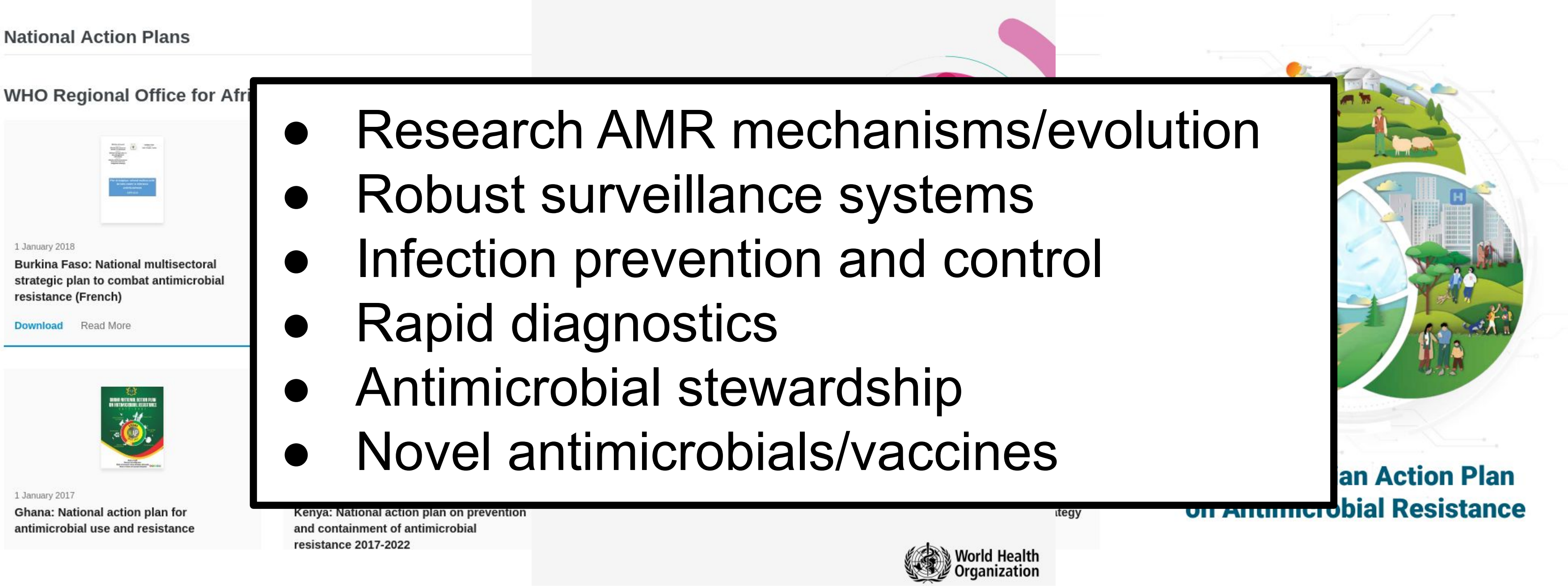


GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

World Health Organization

Pan-Canadian Action Plan on Antimicrobial Resistance

National and international AMR action plans



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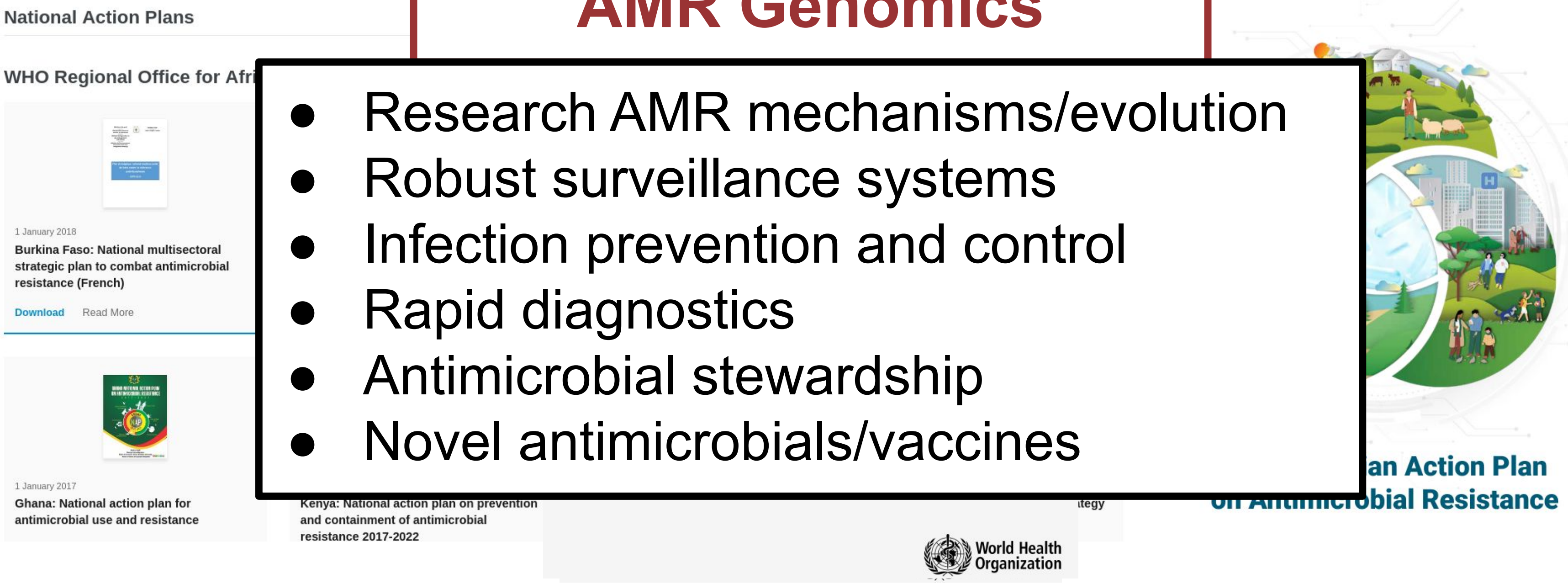
itegy

World Health Organization

an Action Plan
on Antimicrobial Resistance

- Research AMR mechanisms/evolution
- Robust surveillance systems
- Infection prevention and control
- Rapid diagnostics
- Antimicrobial stewardship
- Novel antimicrobials/vaccines

AMR genomics underscores most action plan priorities



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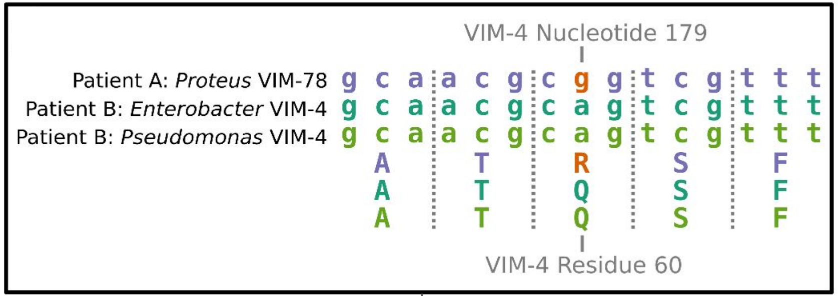
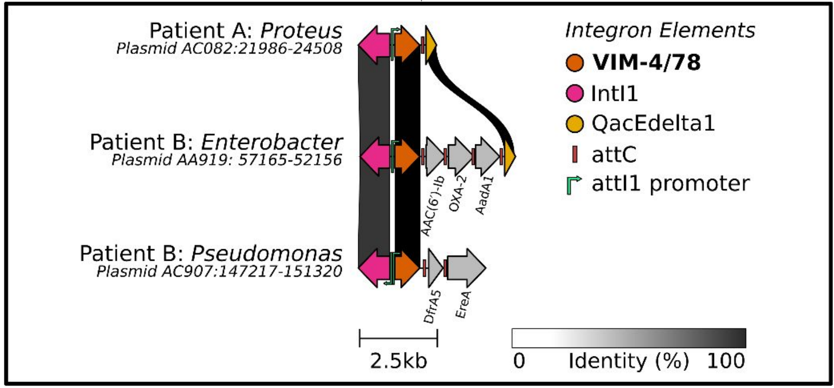
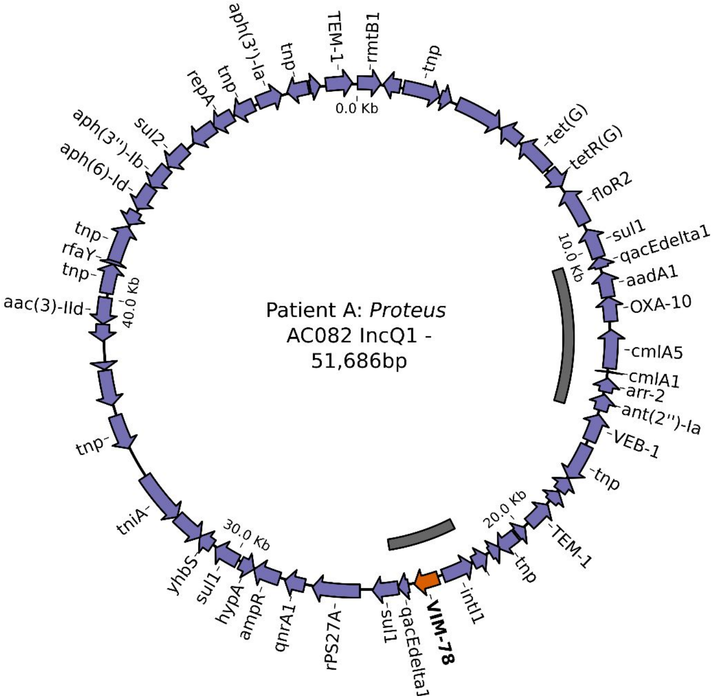
AMR Genomics

- Research AMR mechanisms/evolution
- Robust surveillance systems
- Infection prevention and control
- Rapid diagnostics
- Antimicrobial stewardship
- Novel antimicrobials/vaccines

Genomics needed to track spread & evolution of AMR

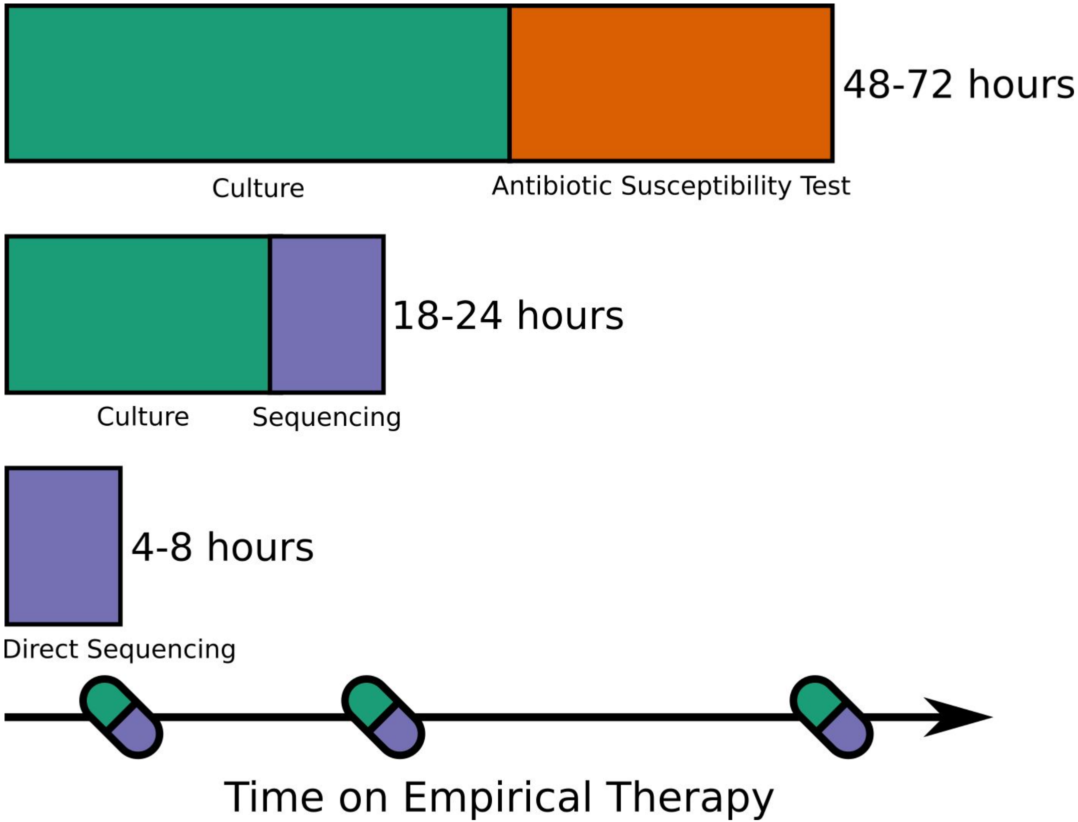






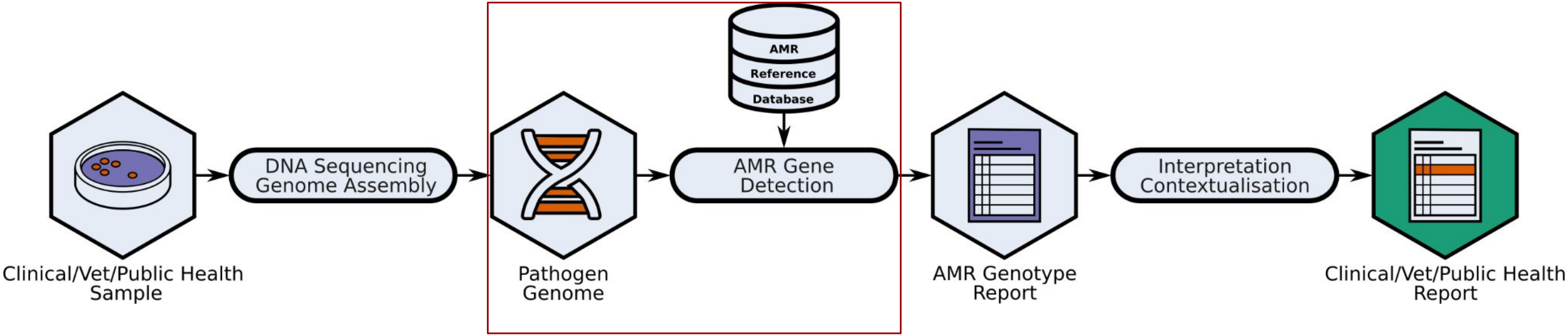
Mahoney, David Burke James, et al. "Utility of hybrid whole genome sequencing in assessing potential nosocomial VIM transmission." Antimicrobial Stewardship & Healthcare Epidemiology 4.1 (2024): e106.

Genomics enables rapid clinical diagnostics

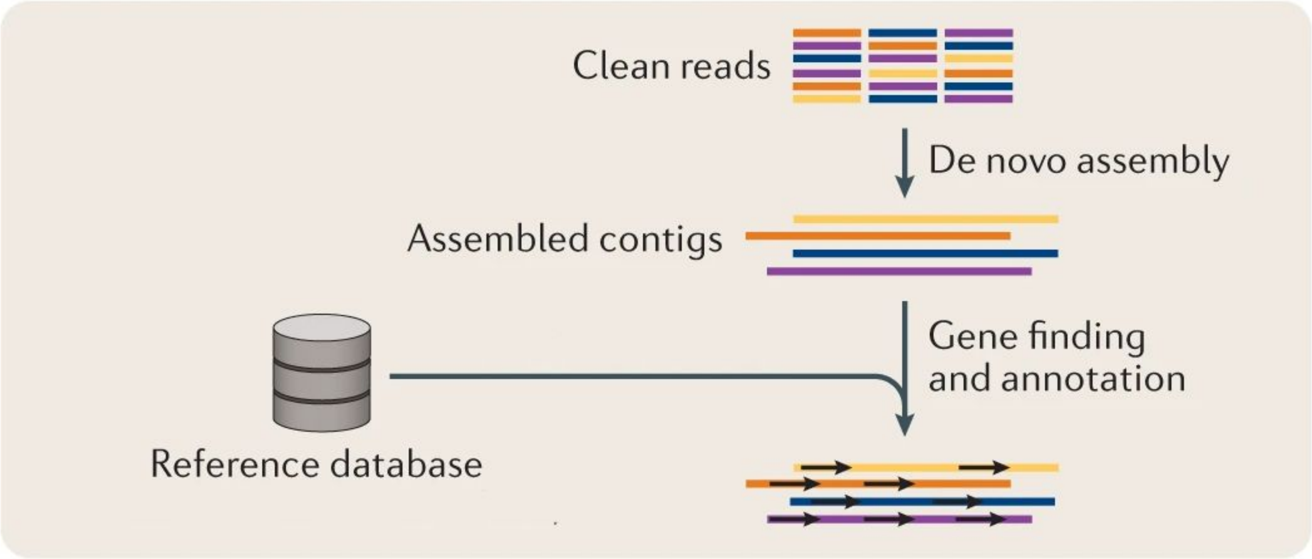


How do we identify AMR determinants using genomic data?

AMR genomics involves several analytical tasks



AMR genes identified by comparison to reference databases



Modified from: Boolchandani, Manish, Alaric W. D'Souza, and Gautam Dantas. "Sequencing-based methods and resources to study antimicrobial resistance." Nature Reviews Genetics 20.6 (2019): 356-370.

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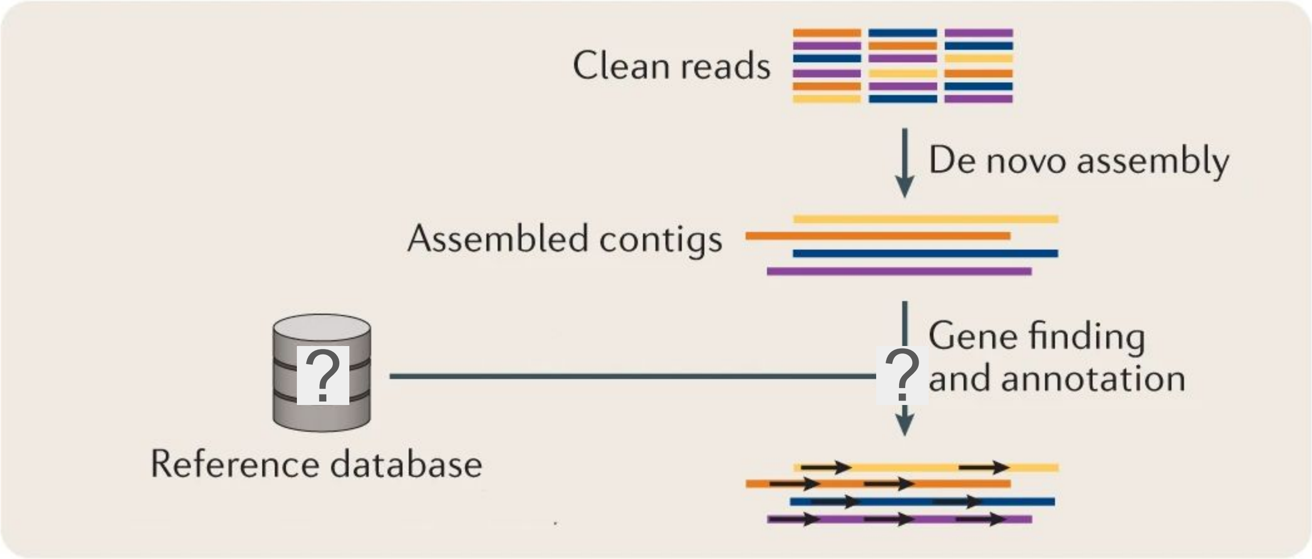

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Many databases: 3 species-agnostic actively curated options

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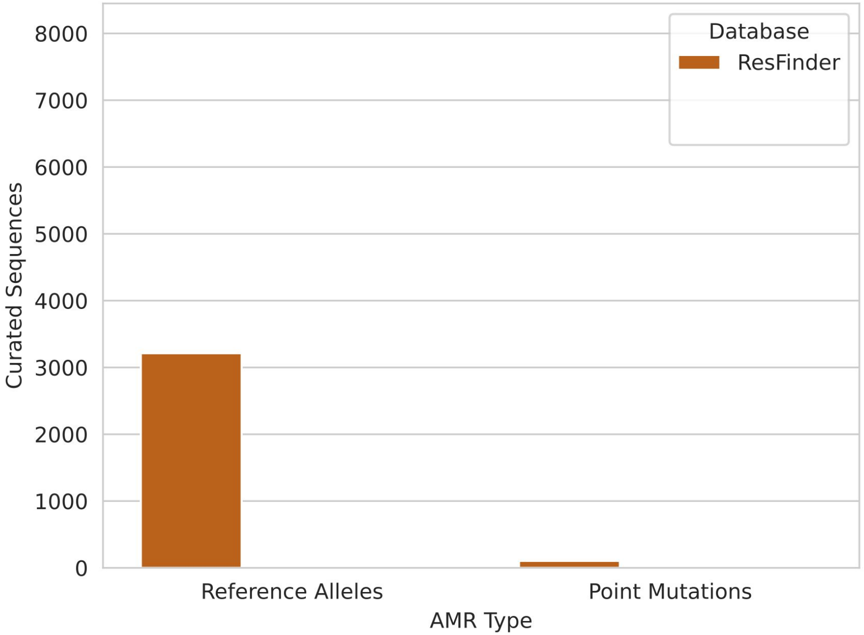

Florensa, Alfred Ferrer, et al. "ResFinder–an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes." Microbial genomics 8.1 (2022): 000748.

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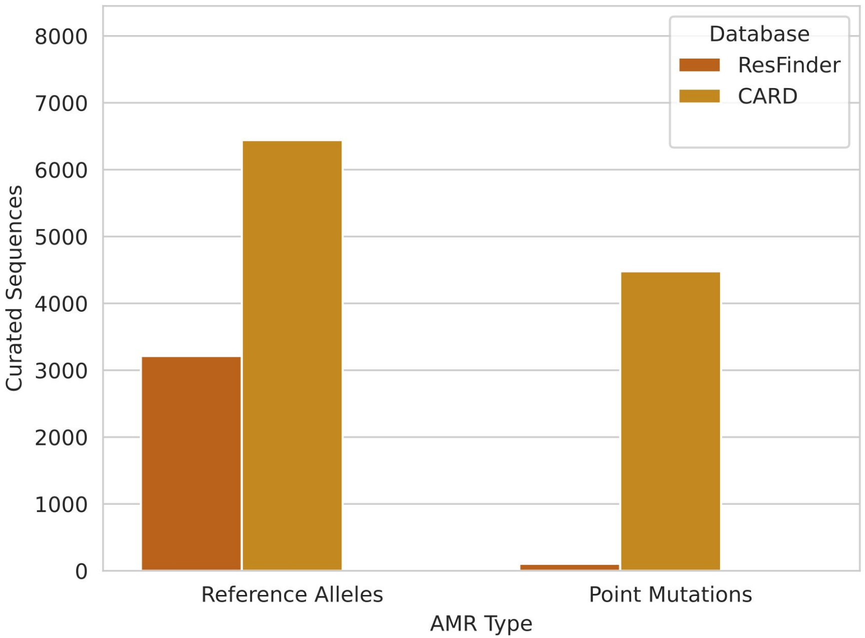

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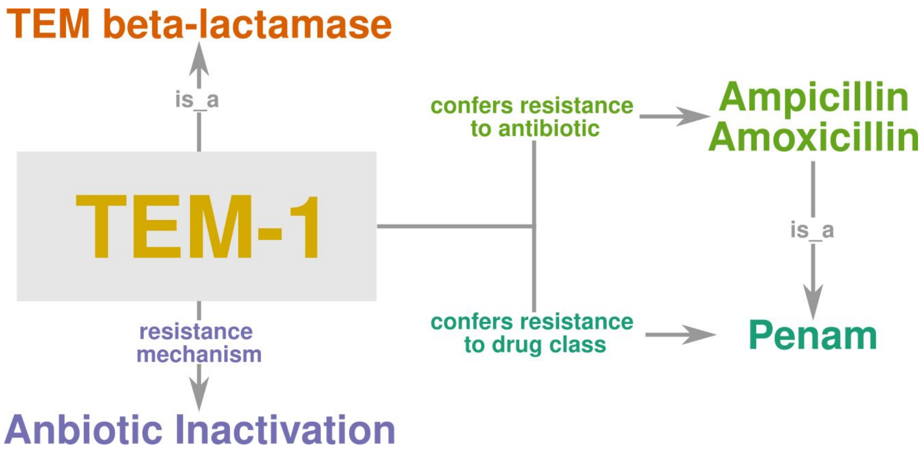

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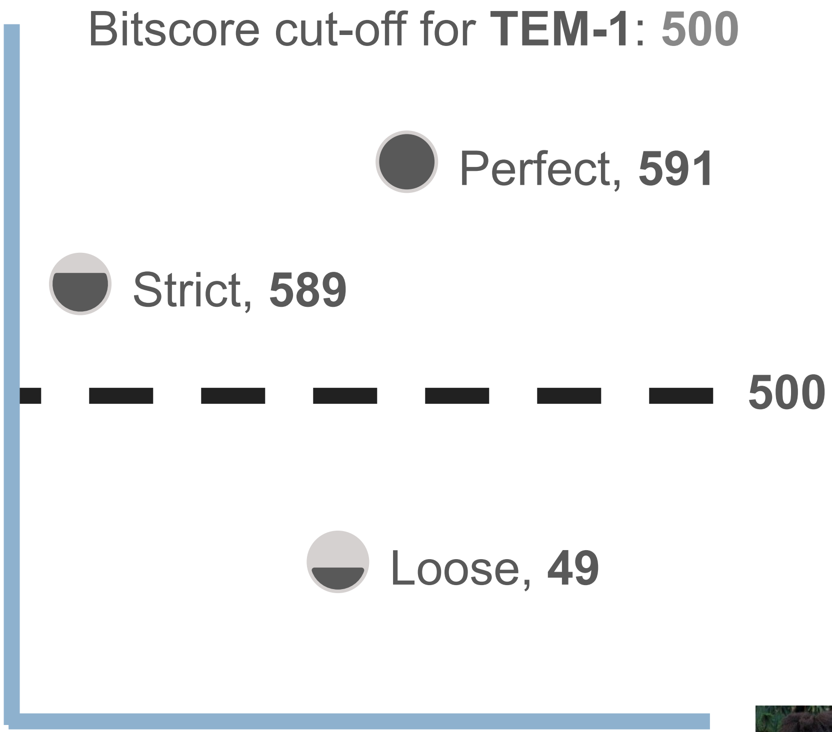
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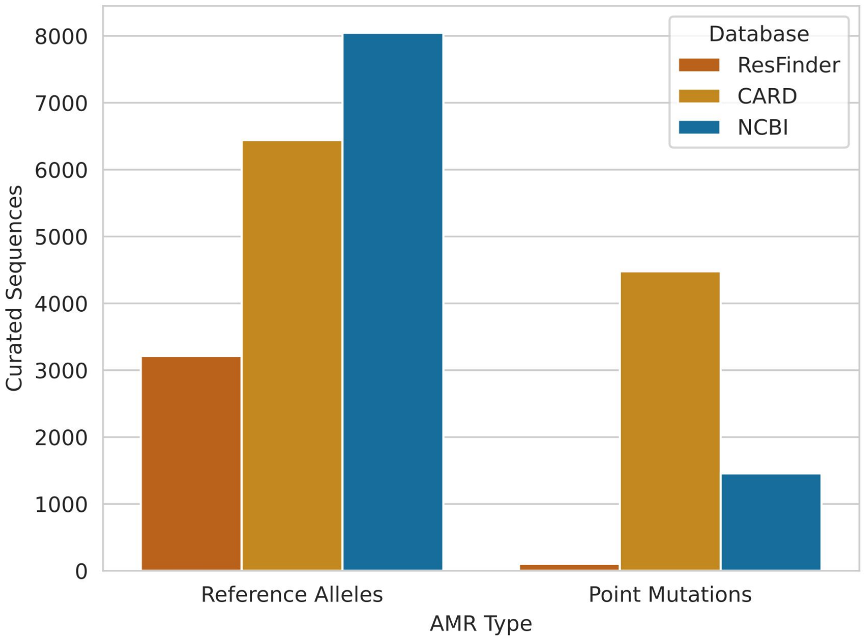
NCBI 2018	*Public Health & Clinical (Accuracy)*	*Reference Gene Hierarchy*	*AMRFinderPlus (Curated Cut-offs)*



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Feldgarden, Michael, et al. "Curation of the AMRFinderPlus databases: applications, functionality and impact." Microbial Genomics 8.6 (2022): 000832.

Many AMR tools each with own strengths/weaknesses

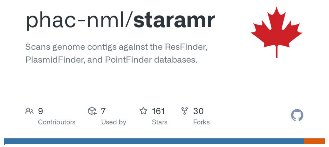
Which tool to use?

- Database-specific tools to handle nuances of reference data
- The tool & database whose underlying motivation matches yours

ABRicate
AMRFinderPlus
AMRplusplus
ARIBA
c-SSTAR
DeepARG
fARGene
GROOT
KmerResistance
Mykrobe
PointFinder
ResFams
ResFinder
RGI
SraX
SRST2
StarAMR
TBProfiler
AMR Gene Detection

MYKROBE

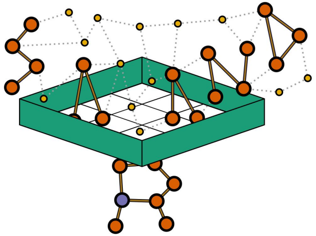
KLEBORATE



phac-nml/**staramr**

Scans genome contigs against the ResFinder, PlasmidFinder, and PointFinder databases.

9 Contributors
7 Used by
161 Stars
30 Forks



Many AMR tools each with own strengths/weaknesses

Which tool to use?

- Database-specific tools to handle nuances of reference data
- The tool & database whose underlying motivation matches yours

- Standard clinical/public health workflow:
  - ***NCBI - AMRFinderPlus***

- Research into novel/divergent AMR genes:
  - ***CARD - RGI***

- Quick analyses:
  - ***CARD or ResFinder web-portals***

- Don’t know?
  - ***NCBI - AMRFinderPlus***

- Unusual data-type or specific organism:
  - Specialised tools (e.g., MEGARes/AMR++, CZID-RGI-bwt, TBProfiler, Kleborate)

ABRicate
AMRFinderPlus
AMRplusplus
ARIBA
c-SSTAR
DeepARG
fARGene
GROOT
KmerResistance
Mykrobe
PointFinder
ResFams
ResFinder
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SraX
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AMR Gene Detection

MYKROBE

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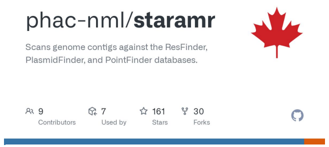

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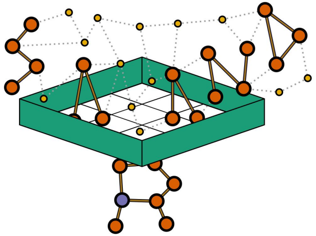


But - swapping tool selection to fit a given situation can be challenging

Most tools have incompatible output formats

AMRFinderPlus

Contig id

Gene Symbol

% Coverage of Reference Sequence

…

ResFinder

contig_name

gene

coverage

…

RGI

Contig

Best_Hit_ARO

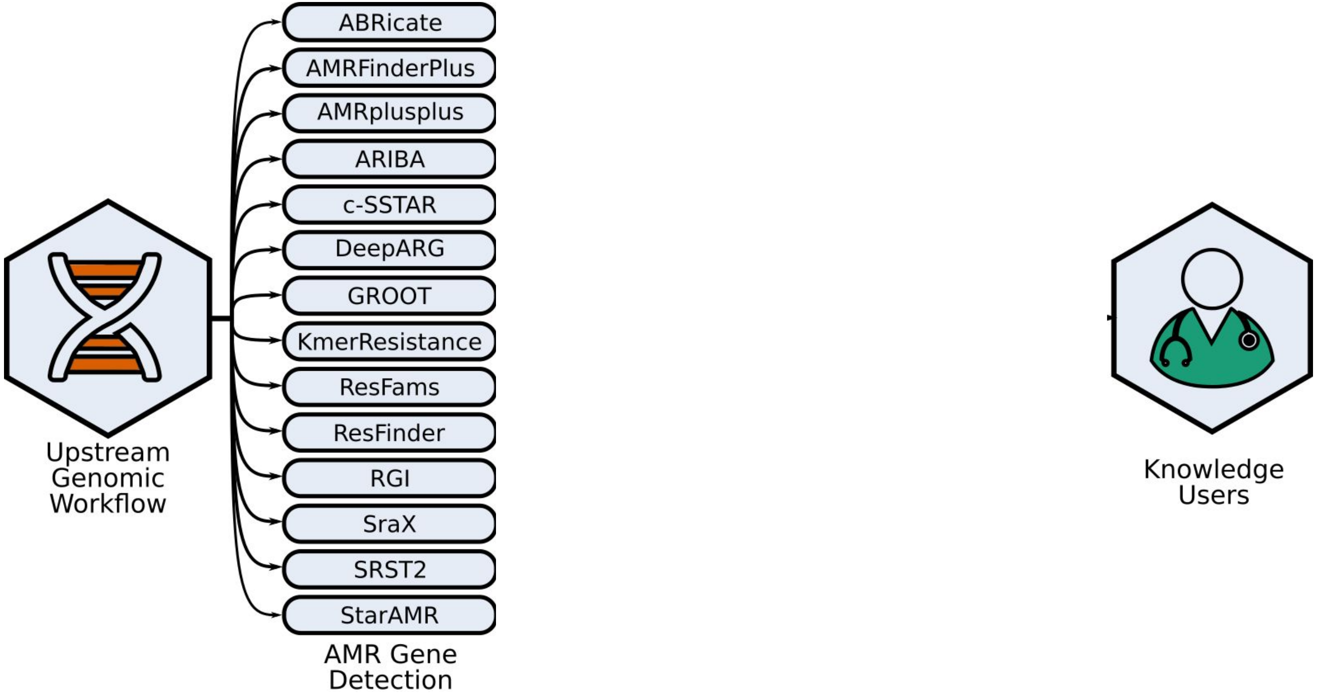
Percentage Length of Reference Sequence

…

https://github.com/pha4ge/hAMRonization

Mendes, Inês, et al. "hAMRonization: Enhancing antimicrobial resistance prediction using the PHA4GE AMR detection specification and tooling." bioRxiv (2024): 2024-03.

Barrier to implementation and reporting



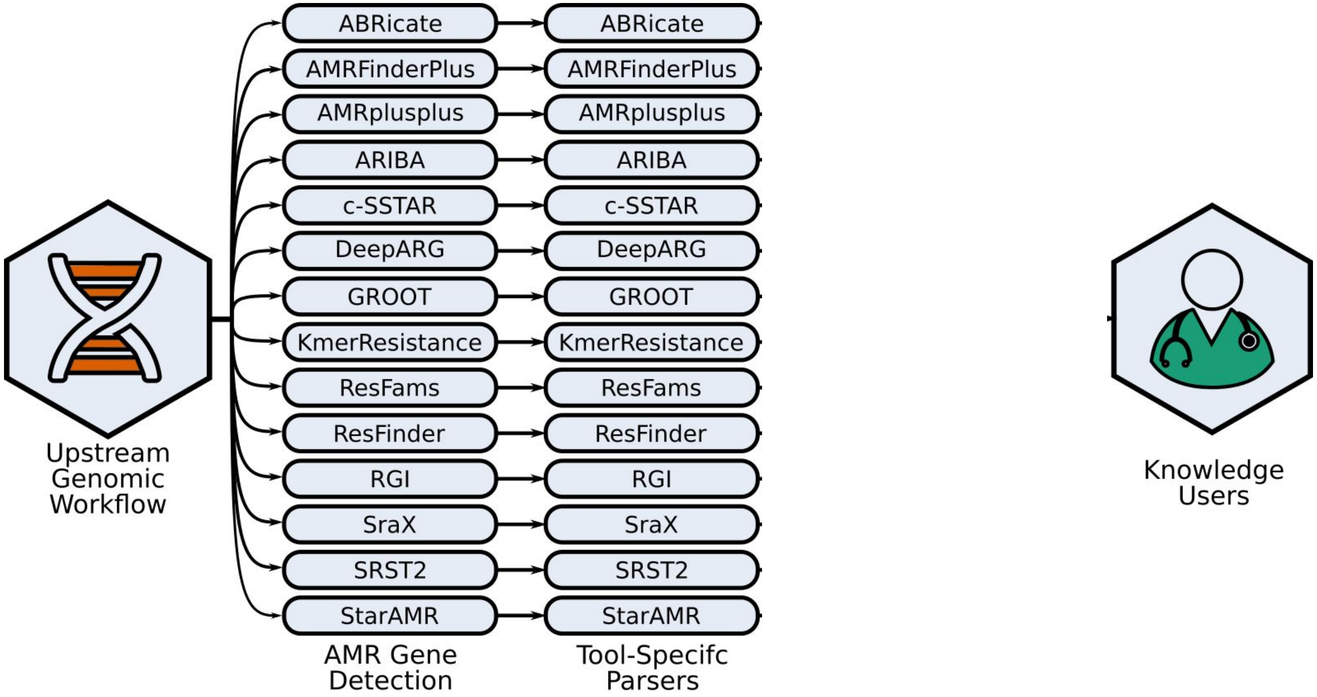
Upstream Genomic Workflow

- ABRicate
- AMRFinderPlus
- AMRplusplus
- ARIBA
- c-SSTAR
- DeepARG
- GROOT
- KmerResistance
- ResFams
- ResFinder
- RGI
- SraX
- SRST2
- StarAMR

AMR Gene Detection

Knowledge Users

Barrier to implementation and reporting



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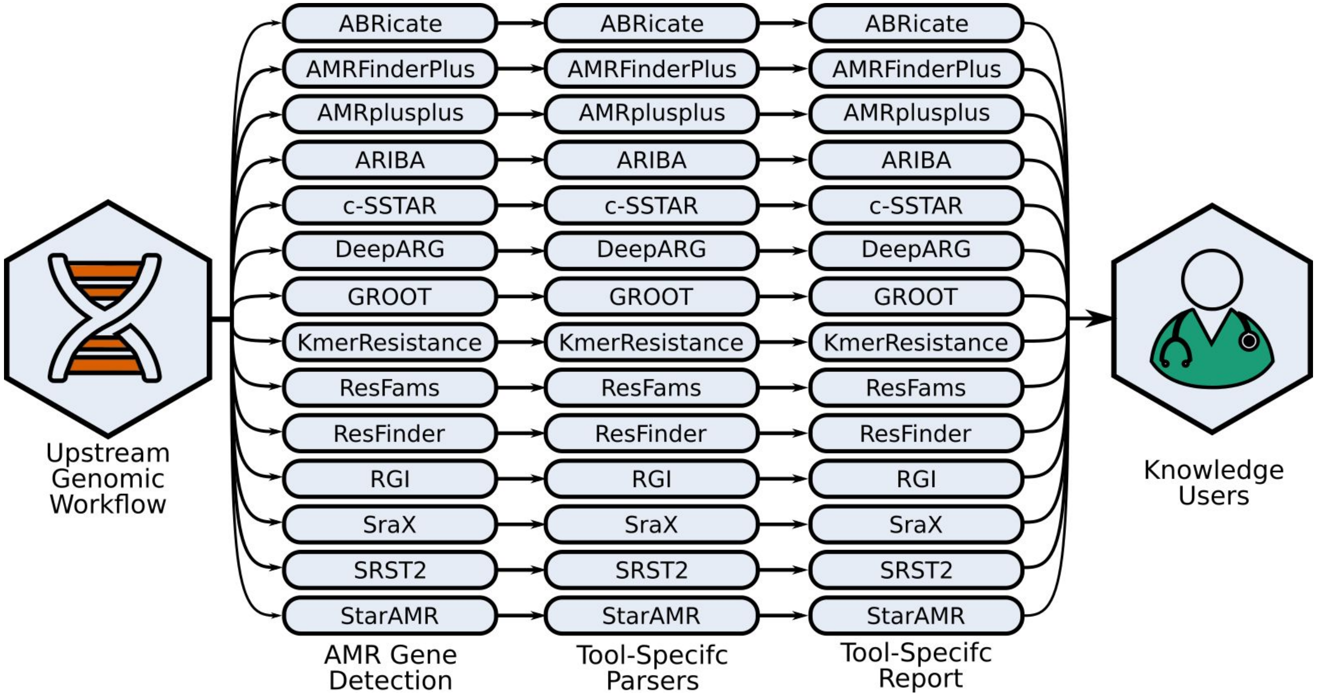
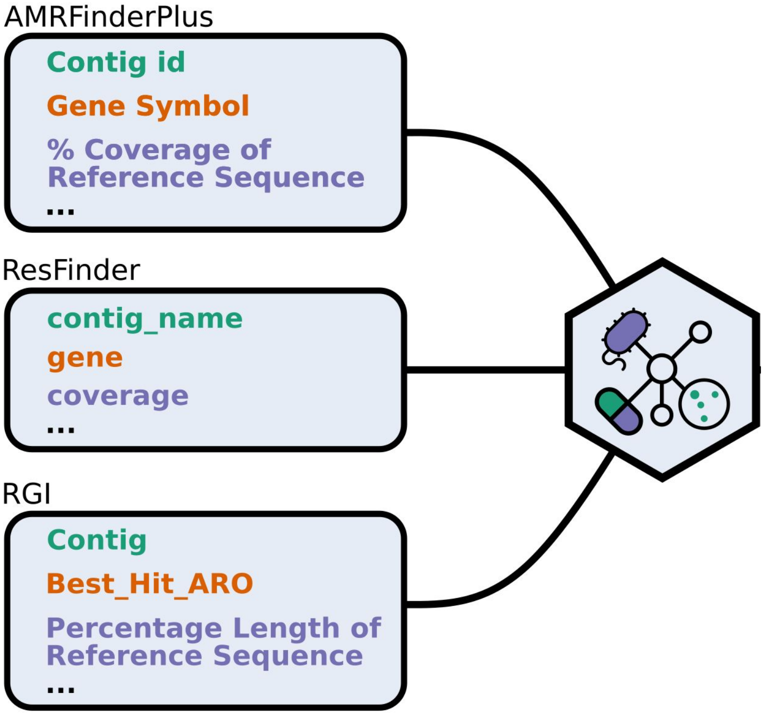


hAMRonization: common AMR annotation specification



AMRFinderPlus

Contig id
Gene Symbol
% Coverage of Reference Sequence
...

ResFinder

contig_name
gene
coverage
...

RGI

Contig
Best_Hit_ARO
Percentage Length of Reference Sequence
...

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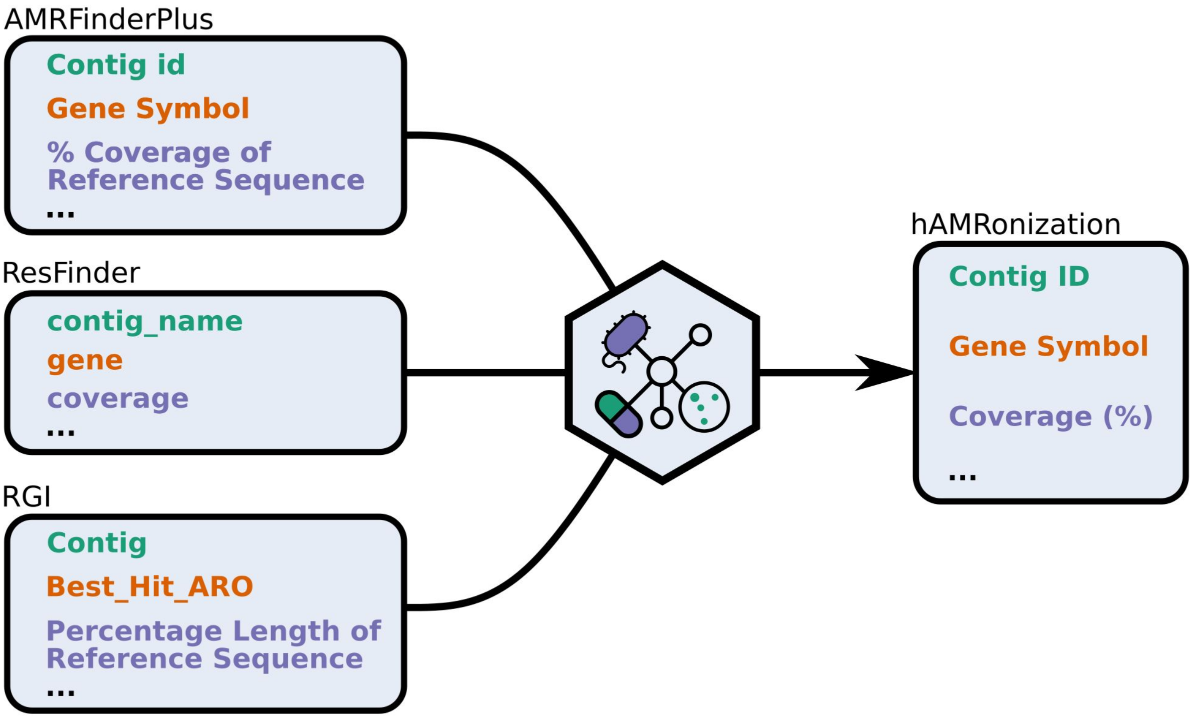


AMRFinderPlus

Contig id
Gene Symbol
% Coverage of Reference Sequence
…

ResFinder

contig_name
gene
coverage
…

RGI

Contig
Best_Hit_ARO
Percentage Length of Reference Sequence
…

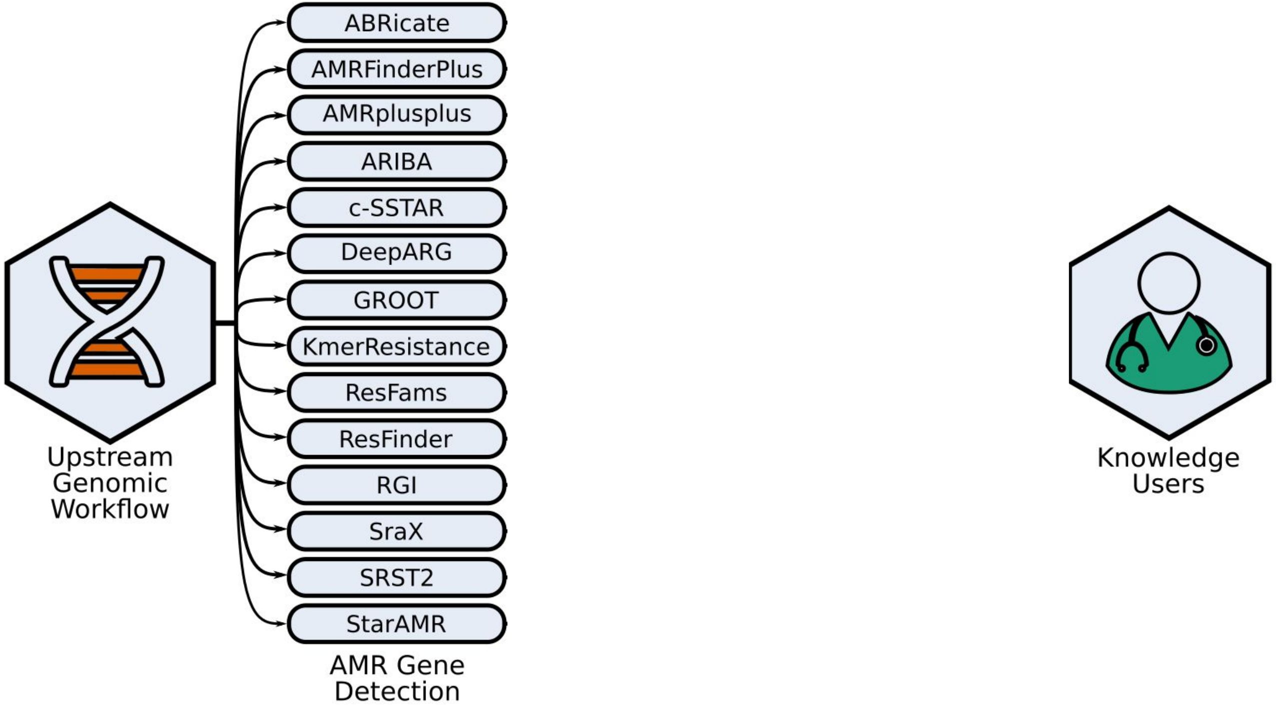
hAMRonization

Contig ID
Gene Symbol
Coverage (%)
…

https://github.com/pha4ge/hAMRonization

Mendes, Inês, et al. "hAMRonization: Enhancing antimicrobial resistance prediction using the PHA4GE AMR detection specification and tooling." bioRxiv (2024): 2024-03.

Operationalise specification with hAMRonization tool



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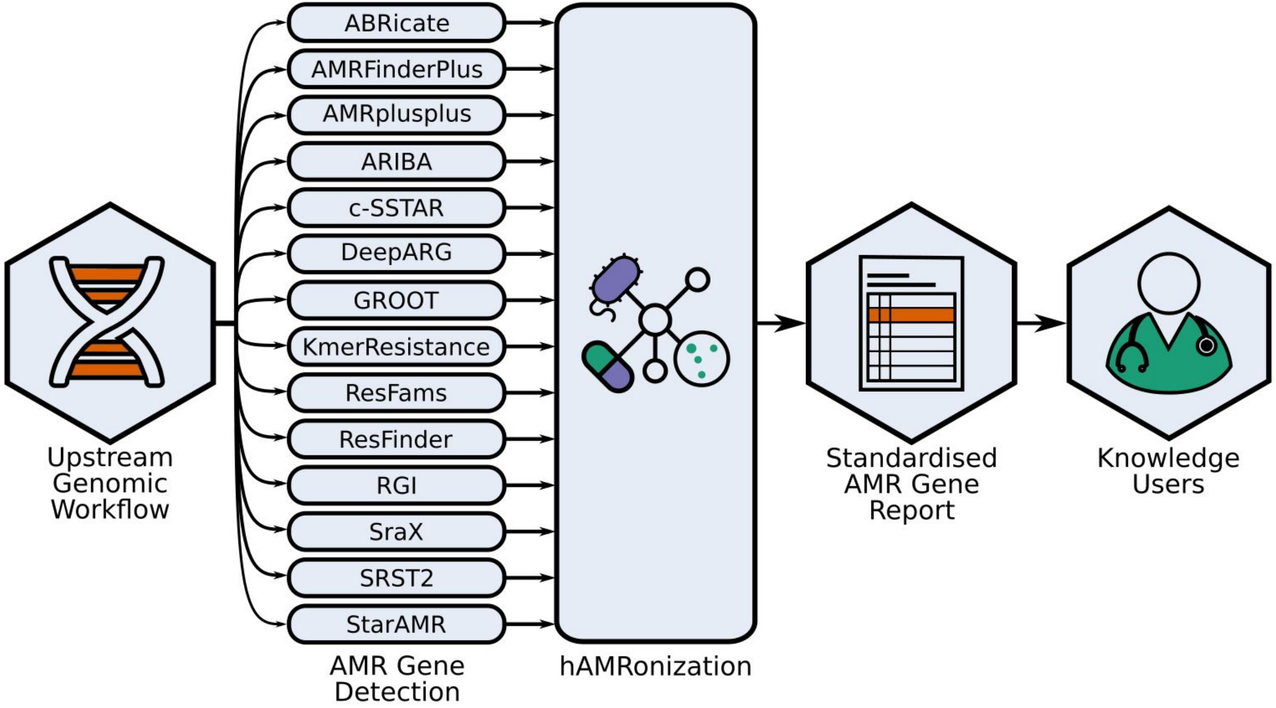

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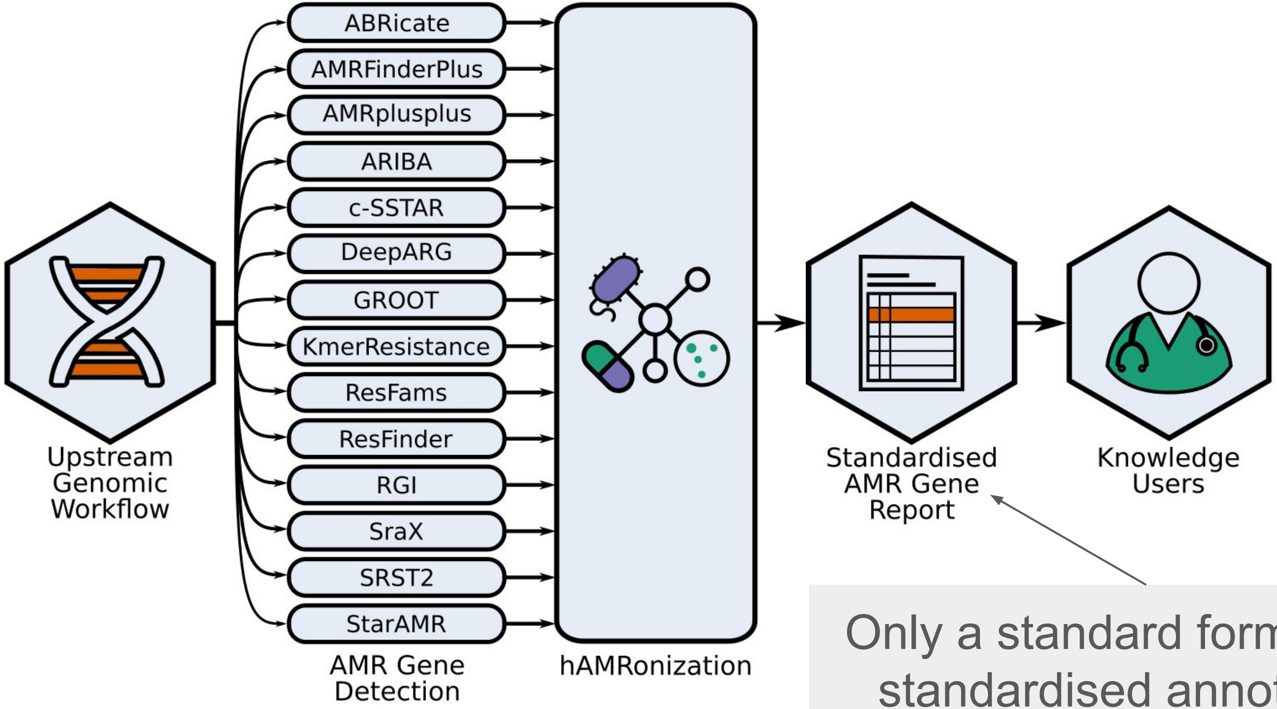

Only a standard format **NOT** standardised annotations

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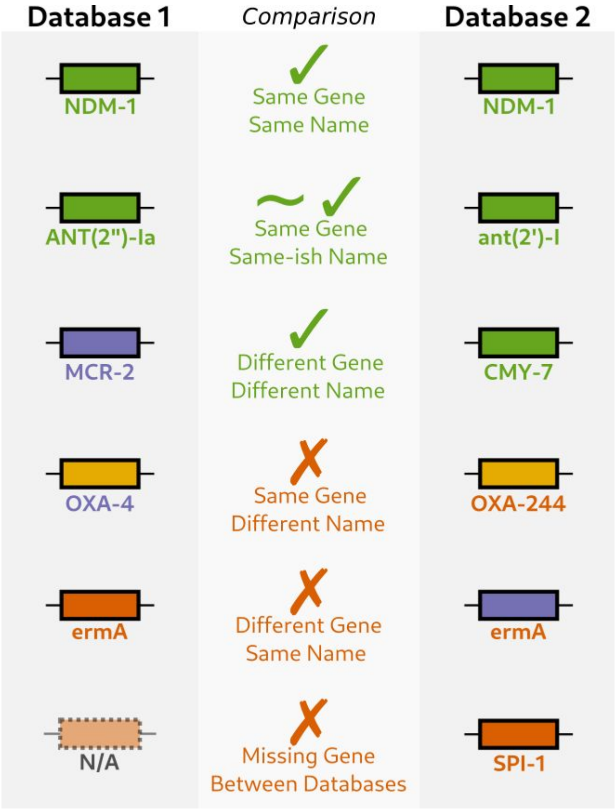
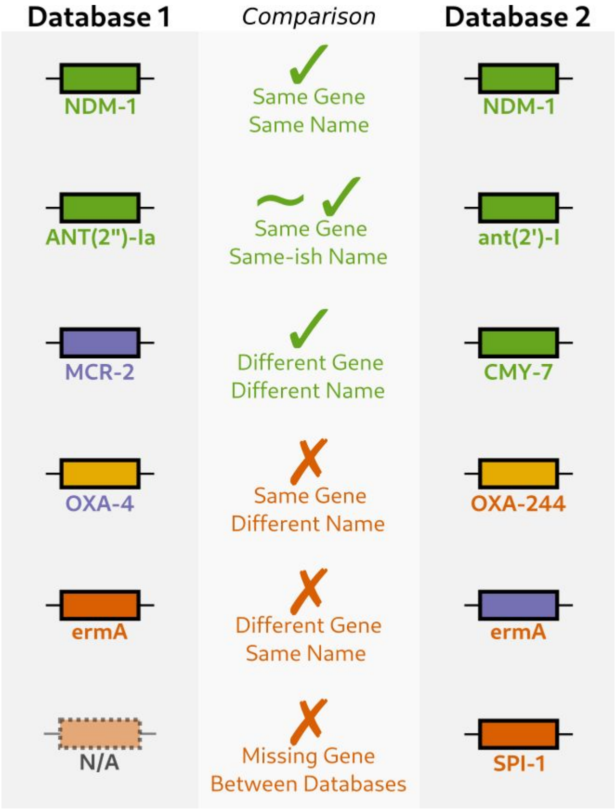
How can we compare annotations between databases?

Comparing AMR genes across databases is challenging



Database 1	*Comparison*	**Database 2**
NDM-1	✓ Same Gene Same Name	NDM-1
ANT(2")-Ia	~ ✓ Same Gene Same-ish Name	ant(2')-I
MCR-2	✓ Different Gene Different Name	CMY-7
OXA-4	✗ Same Gene Different Name	OXA-244
ermA	✗ Different Gene Same Name	ermA
N/A	✗ Missing Gene Between Databases	SPI-1

Comparing AMR genes across databases is challenging



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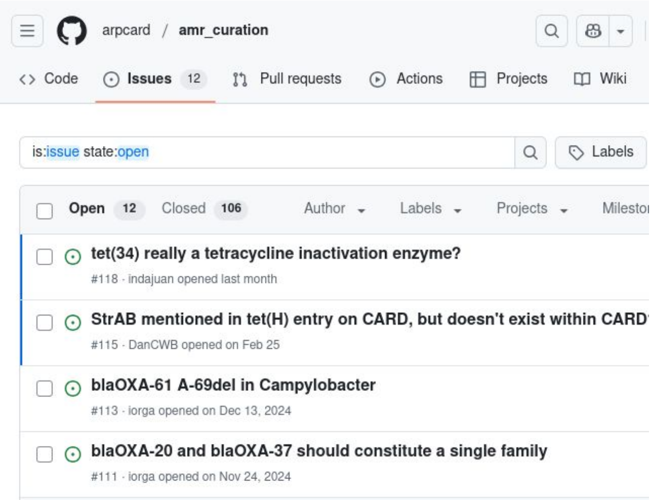
- Manual curation and reconciliation



is:issue state:open

Open 12 Closed 106

tet(34) really a tetracycline inactivation enzyme?
#118 · indajuan opened last month

StrAB mentioned in tet(H) entry on CARD, but doesn't exist within CARD
#115 · DanCWB opened on Feb 25

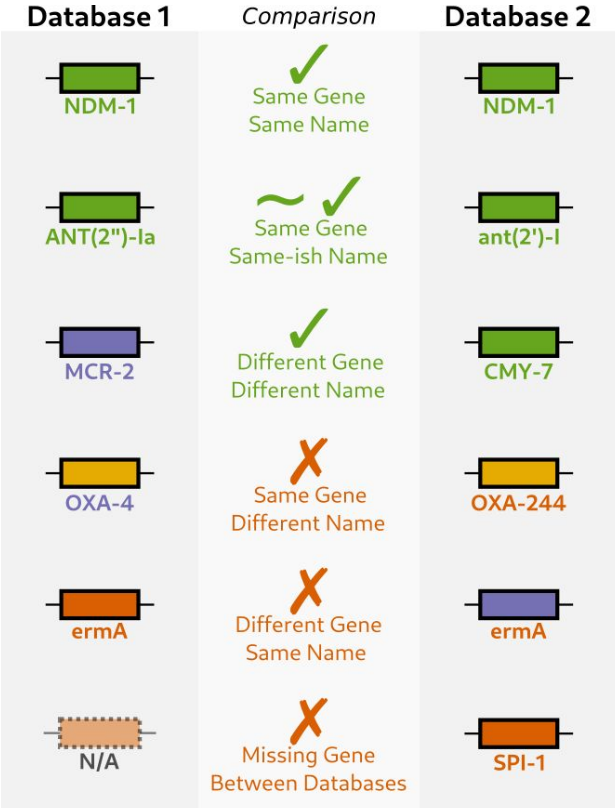
blaOXA-61 A-69del in Campylobacter
#113 · iorga opened on Dec 13, 2024

blaOXA-20 and blaOXA-37 should constitute a single family
#111 · iorga opened on Nov 24, 2024

https://github.com/arpcard/amr_curation

Lots of emails behind the scenes!

Comparing AMR genes across databases is challenging



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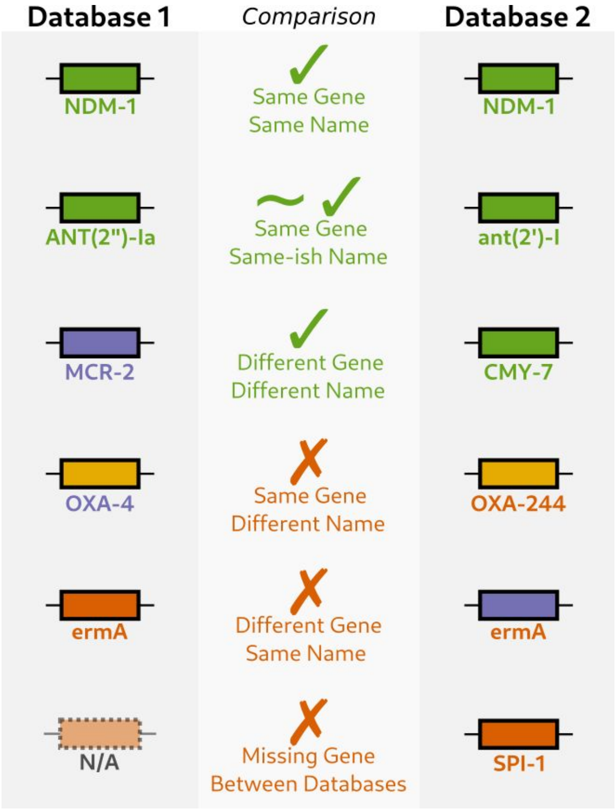
- Manual curation and reconciliation
- Cluster and collapse databases
  - Meaningful similarity differs across AMR genes
  - Easy to lose important curation
  - Hard to maintain merged database

Comparing AMR genes across databases is challenging



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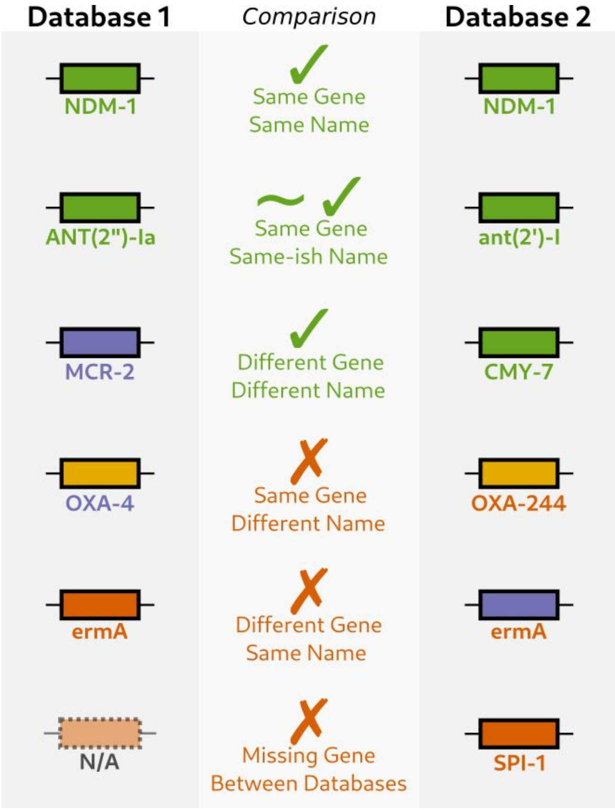
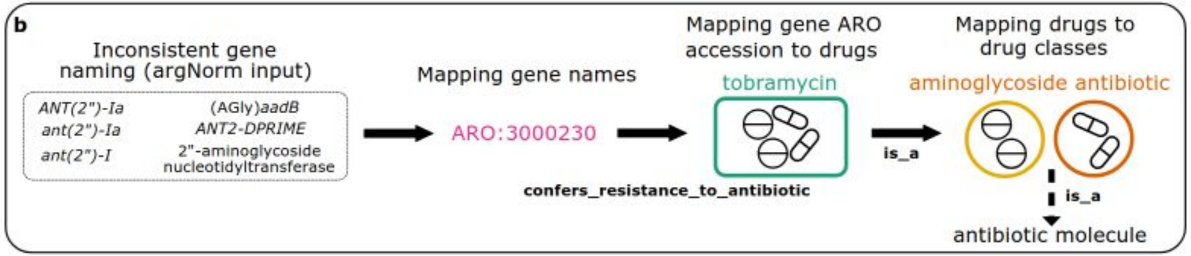
- Manual curation and reconciliation
- Cluster and collapse databases
- Explicitly mapping between databases
  - Caution required!

Comparing AMR genes across databases is challenging



- Manual curation and reconciliation
- Cluster and collapse databases
- Explicitly mapping between databases
  - Caution required!
  - argNorm

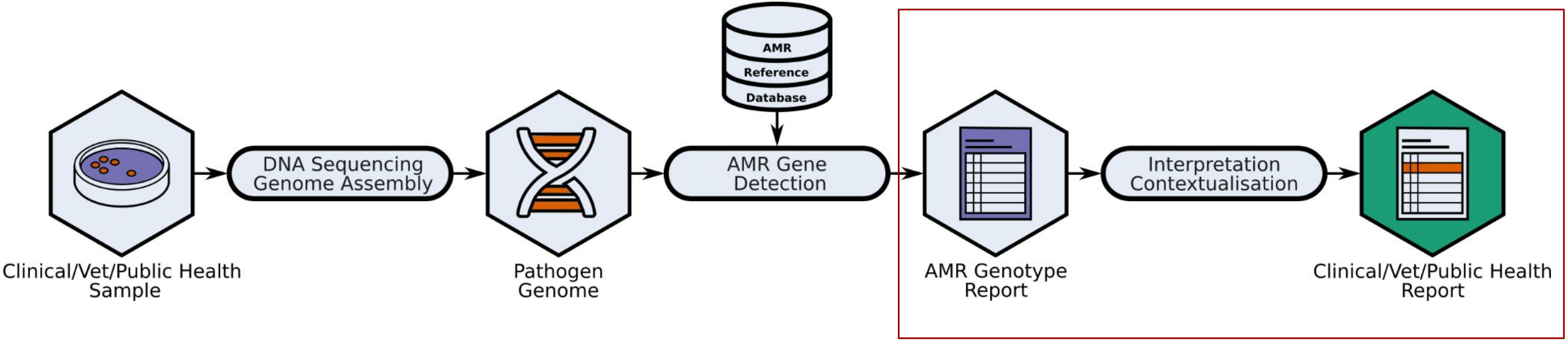


https://github.com/BigDataBiology/argNorm

Ugarcina Perovic, Svetlana, et al. "argNorm: normalization of antibiotic resistance gene annotations to the Antibiotic Resistance Ontology (ARO)." Bioinformatics 41.5 (2025): btaf173.

You’ve picked a AMR database and annotation tool and run them on your genome, what now?

Genotype not useful without interpretation & contextualisation



Interpreting AMR genotype data requires lots of expertise

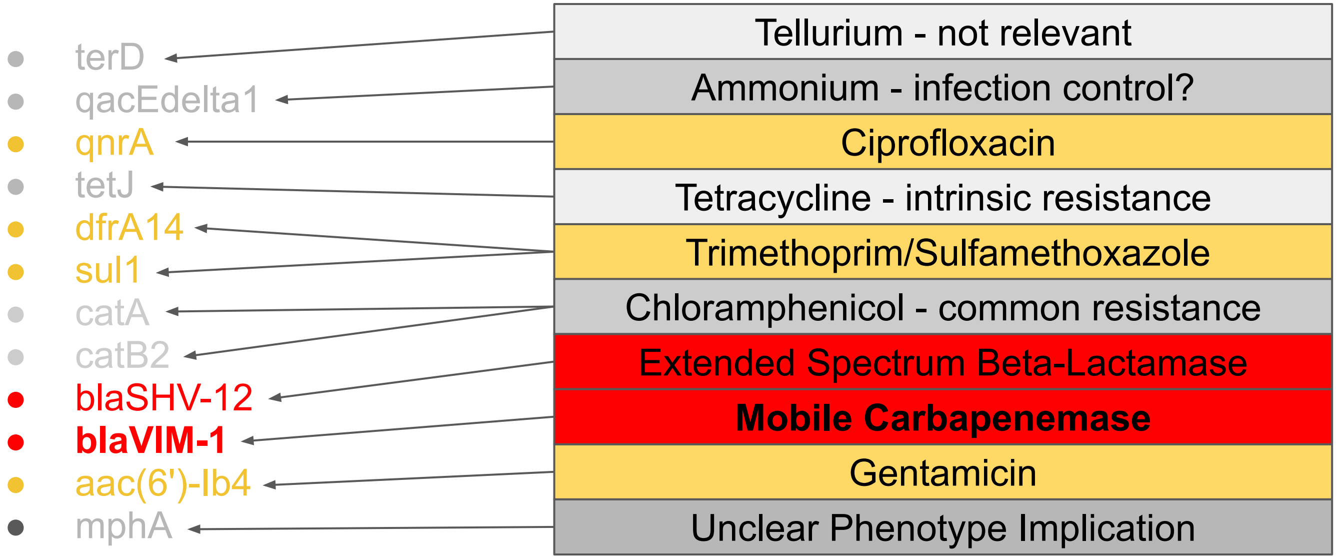
Proteus mirabilis isolate:

- terD
- qacEdelta1
- qnrA
- tetJ
- dfrA14
- sul1
- catA
- catB2
- blaSHV-12
- blaVIM-1
- aac(6')-Ib4
- mphA

- + 13 more AMR determinants

Interpreting AMR genotype data requires lots of expertise

Proteus mirabilis isolate:



- terD
- qacEdelta1
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- tetJ
- dfrA14
- sul1
- catA
- catB2
- blaSHV-12
- **blaVIM-1**
- aac(6')-Ib4
- mphA

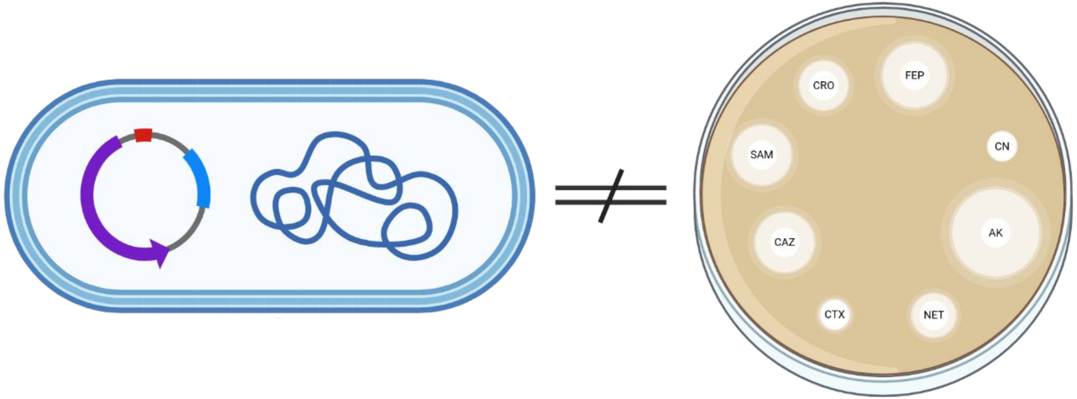
Tellurium - not relevant
Ammonium - infection control?
Ciprofloxacin
Tetracycline - intrinsic resistance
Trimethoprim/Sulfamethoxazole
Chloramphenicol - common resistance
Extended Spectrum Beta-Lactamase
Mobile Carbapenemase
Gentamicin
Unclear Phenotype Implication

- \+ 13 more AMR determinants

Ad-Hoc Analysis & Expert Knowledge:

- Clinical
- Surveillance
- Infection Control
- Genomic
- Evolutionary
- Microbiological

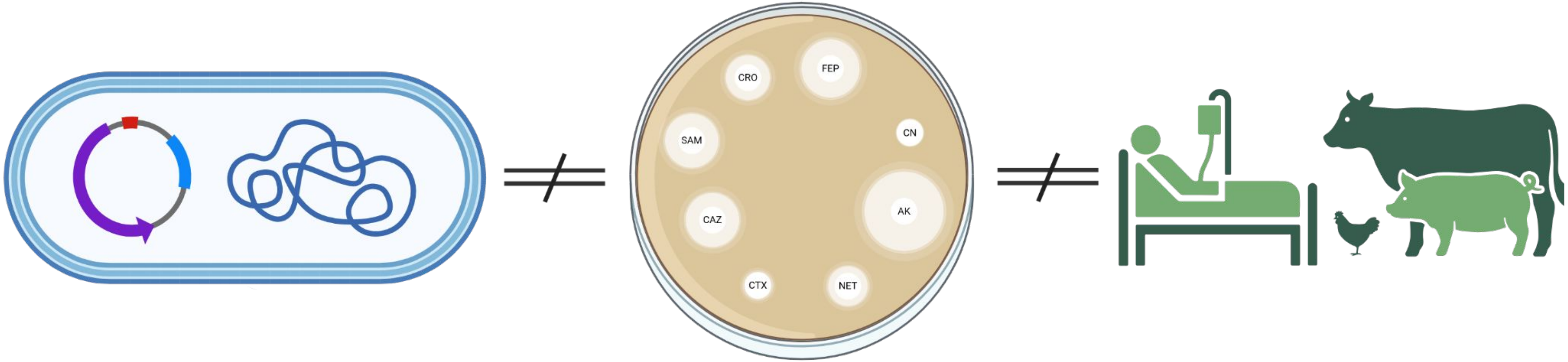
Clinical phenotype inference requires interpretative rules



AMR
Genotype

Lab
Phenotype

Clinical phenotype inference requires interpretative rules

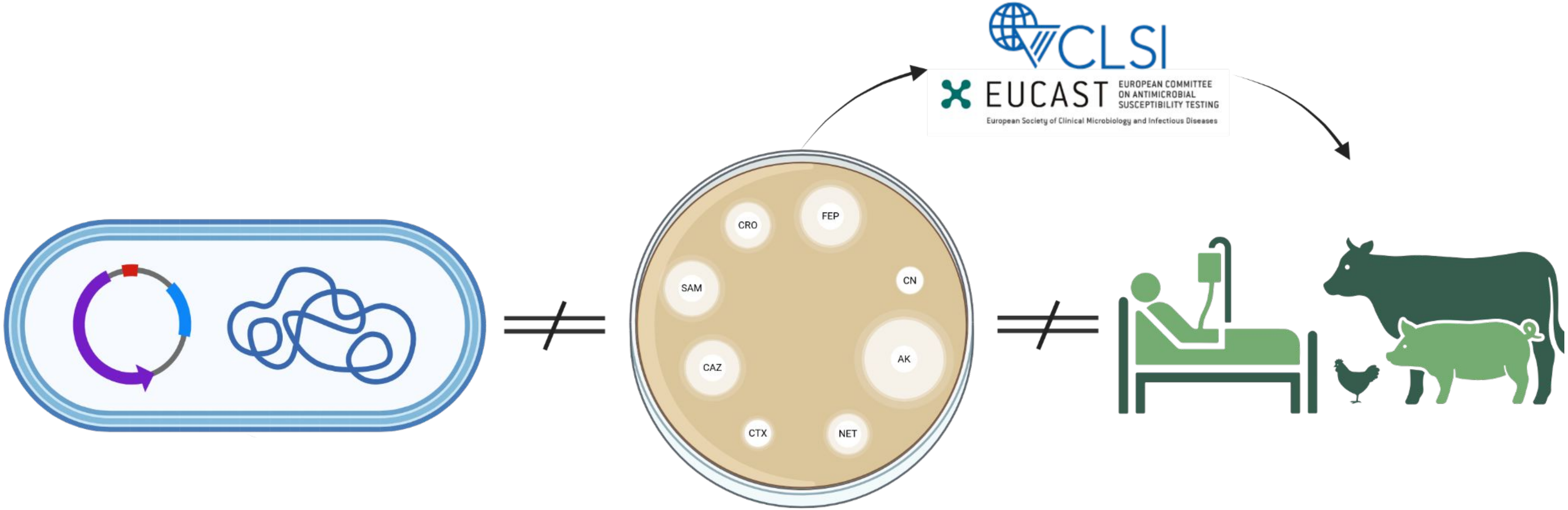


AMR
Genotype

Lab
Phenotype

Clinical
Phenotype

Clinical phenotype inference requires interpretative rules

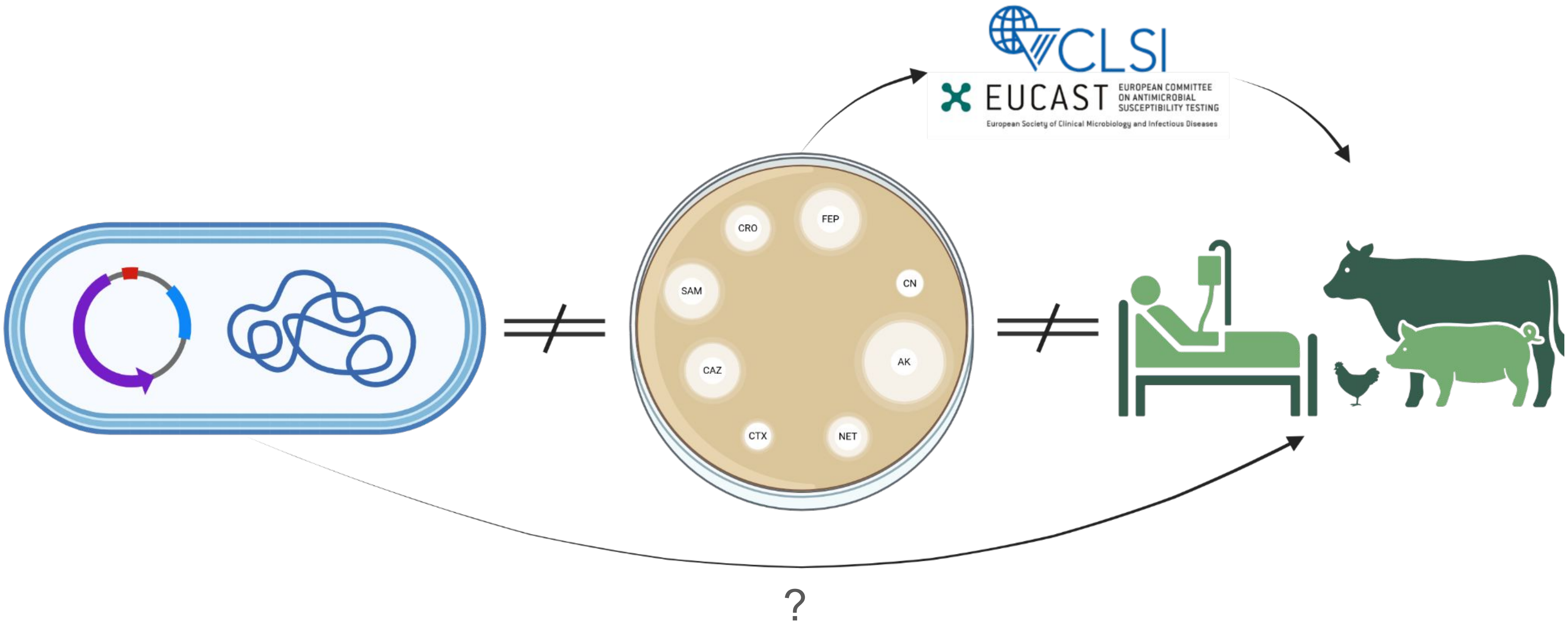


AMR Genotype

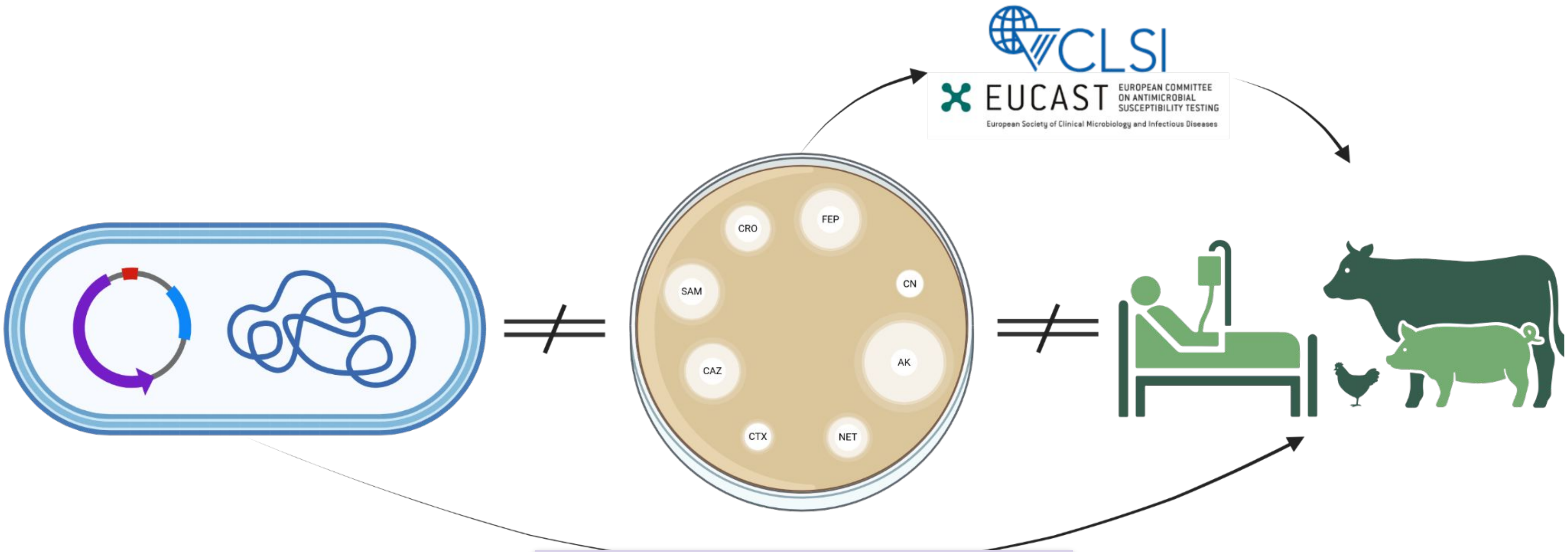
Lab Phenotype

Clinical Phenotype

Clinical phenotype inference requires interpretative rules



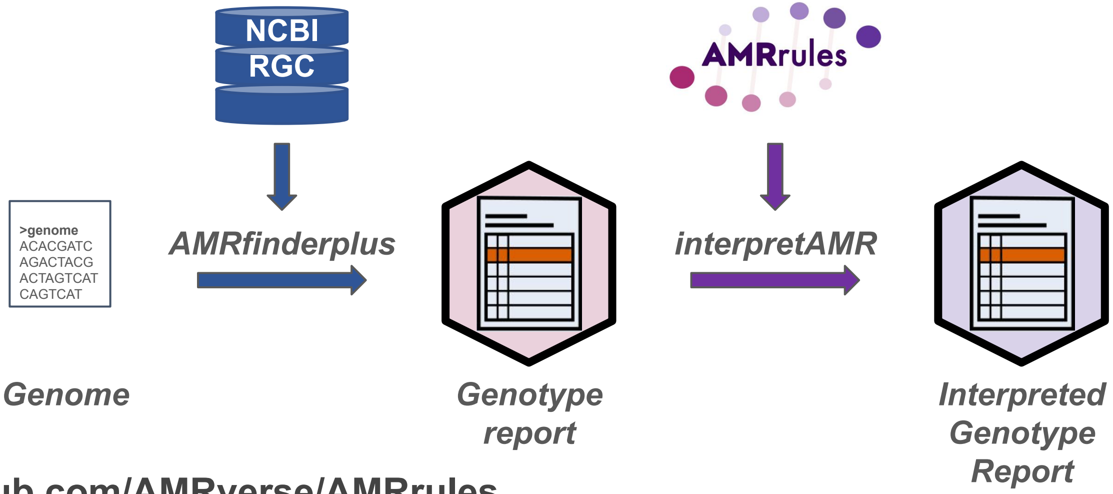
Clinical phenotype inference requires interpretative rules



Missing rules for interpretation

What does **gene X** in **species Y** mean for **drug Z?**

AMRrules: creating AMR genotype interpretive rules

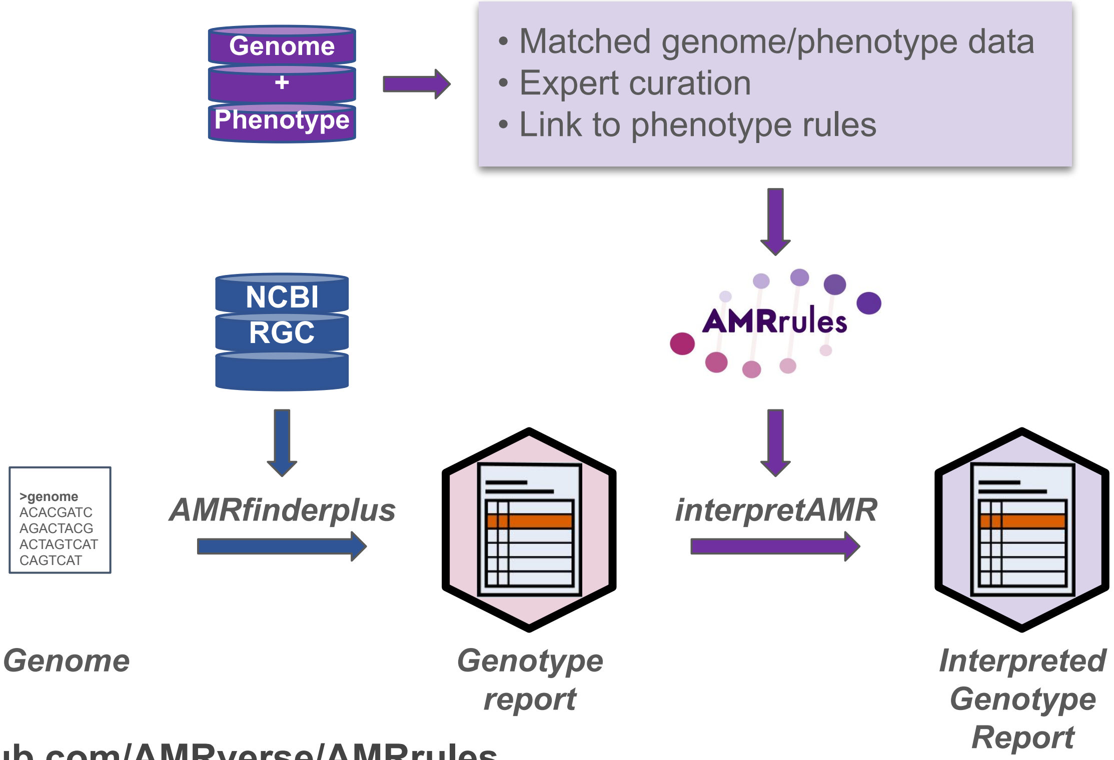


github.com/AMRverse/AMRrules

ESCMID
ESGEM-AMR
EUCAST
Public Health Alliance for Genomic Epidemiology

Dr. Kat Holt, Dr. Jane Hawkey, Dr. Natacha Couto

AMRrules: creating AMR genotype interpretive rules



Genome
+
Phenotype

- Matched genome/phenotype data
- Expert curation
- Link to phenotype rules

NCBI
RGC

AMRrules

>genome
ACACGATC
AGACTACG
ACTAGTCAT
CAGTCAT

AMRfinderplus

interpretAMR

Genome

Genotype report

Interpreted Genotype Report

github.com/AMRverse/AMRrules

ESCMID
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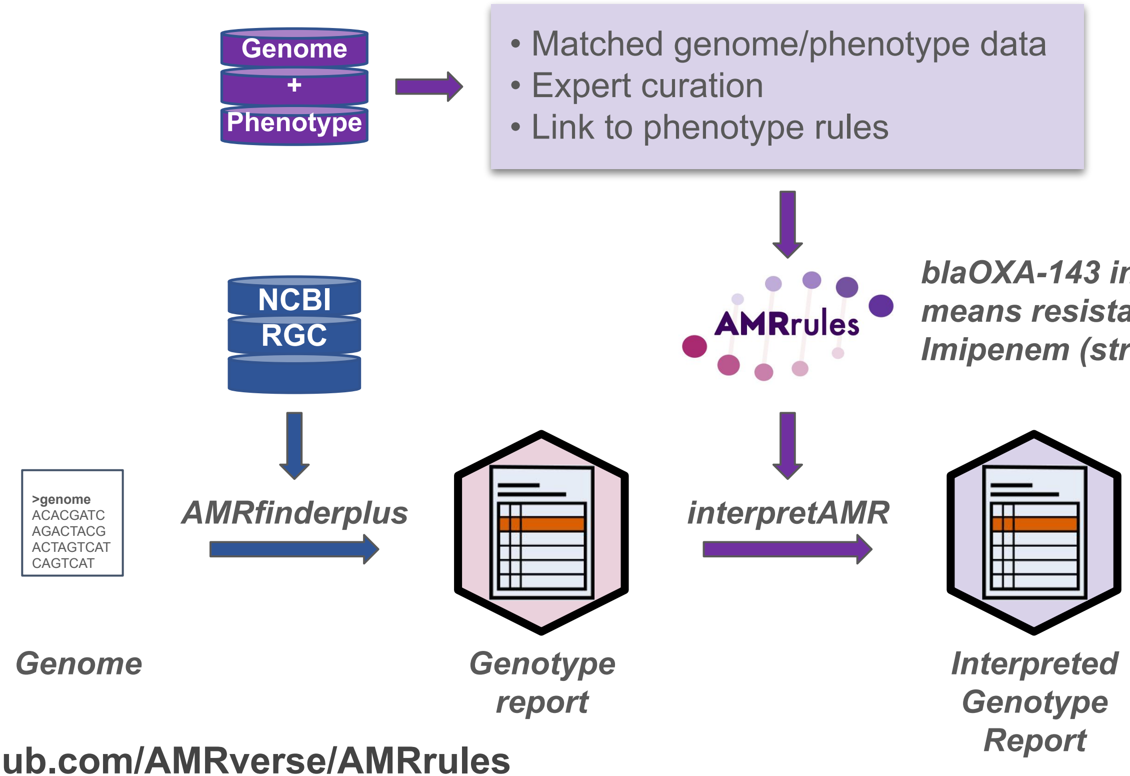
AMRrules: creating AMR genotype interpretive rules



Genome + Phenotype

- Matched genome/phenotype data
- Expert curation
- Link to phenotype rules

NCBI RGC

AMRrules

```
>genome
ACACGATC
AGACTACG
ACTAGTCAT
CAGTCAT
```

AMRfinderplus

interpretAMR

Genome

Genotype report

Interpreted Genotype Report

blaOXA-143 in A. baumanii means resistance to Imipenem (strong evidence)

A. baumanii - 170 rules
Enterobacter spp. - 20 rules
E. faecalis - 45 rules
E. faecium - 22 rules
E. coli - 83 rules
K. pneumoniae - 7 rules
N. gonorrhoeae - 123 rules
P. aeruginosa - 133 rules
Salmonella spp. - 2 rules
S. aureus - 42 rules
Yersinia spp. - 52 rules

ESCMID

ESGEM-AMR
EUCAST

Public Health Alliance for Genomic Epidemiology

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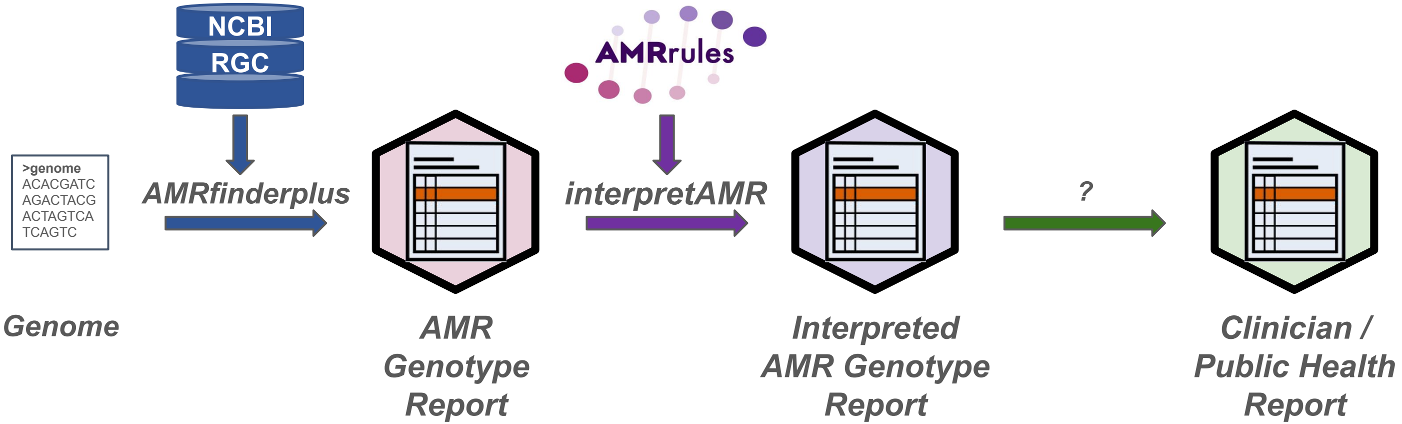
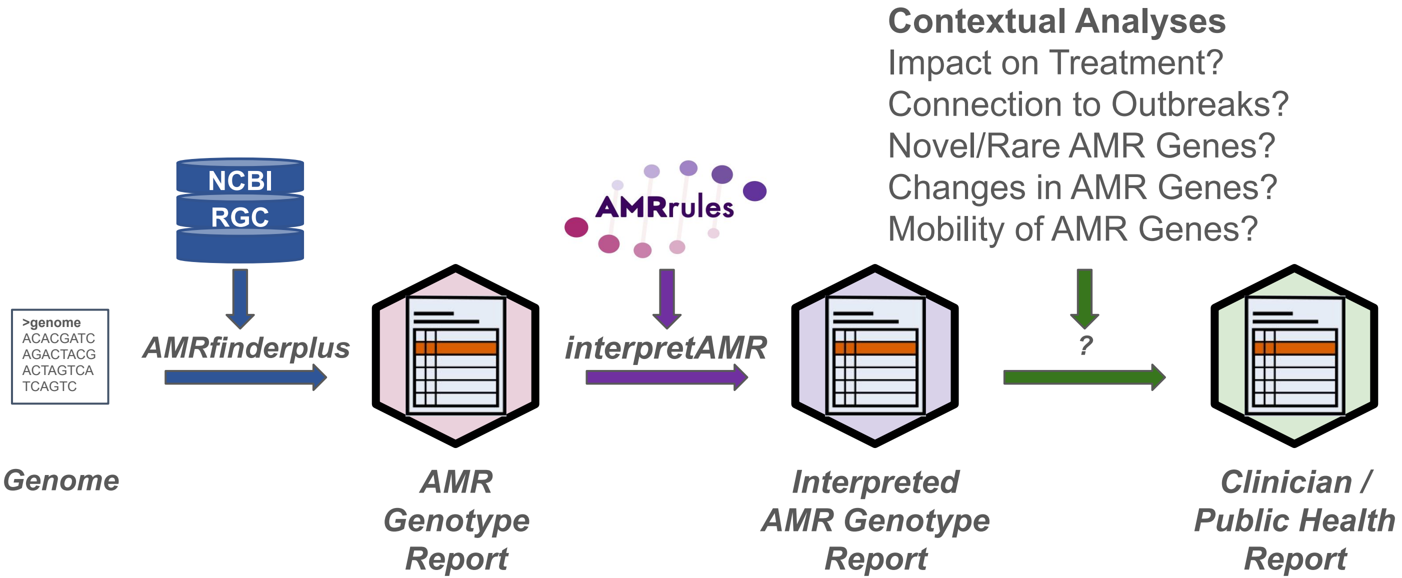
github.com/AMRverse/AMRrules

Contextualisation still needed after interpretation



Contextualisation still needed after interpretation



Contextual Analyses
Impact on Treatment?
Connection to Outbreaks?
Novel/Rare AMR Genes?
Changes in AMR Genes?
Mobility of AMR Genes?

AMRfinderplus

interpretAMR

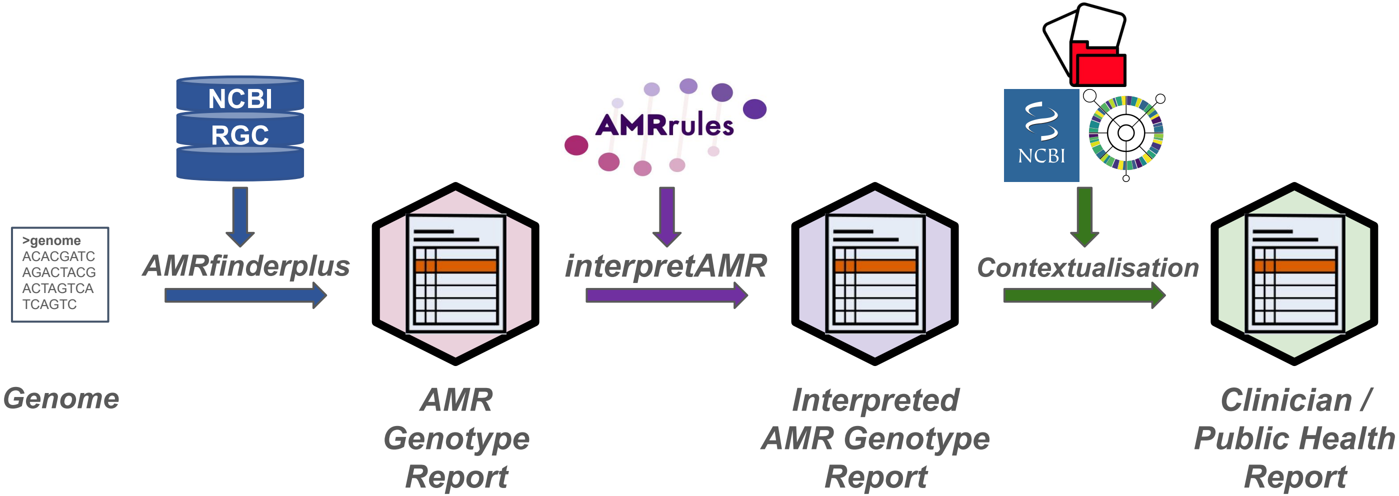
Genome

AMR Genotype Report

Interpreted AMR Genotype Report

Clinician / Public Health Report

Contextualisation involves comparison to representative data



>genome
ACACGATC
AGACTACG
ACTAGTCA
TCAGTC

NCBI
RGC

AMRrules

NCBI

AMRfinderplus

interpretAMR

Contextualisation

Genome

AMR Genotype Report

Interpreted AMR Genotype Report

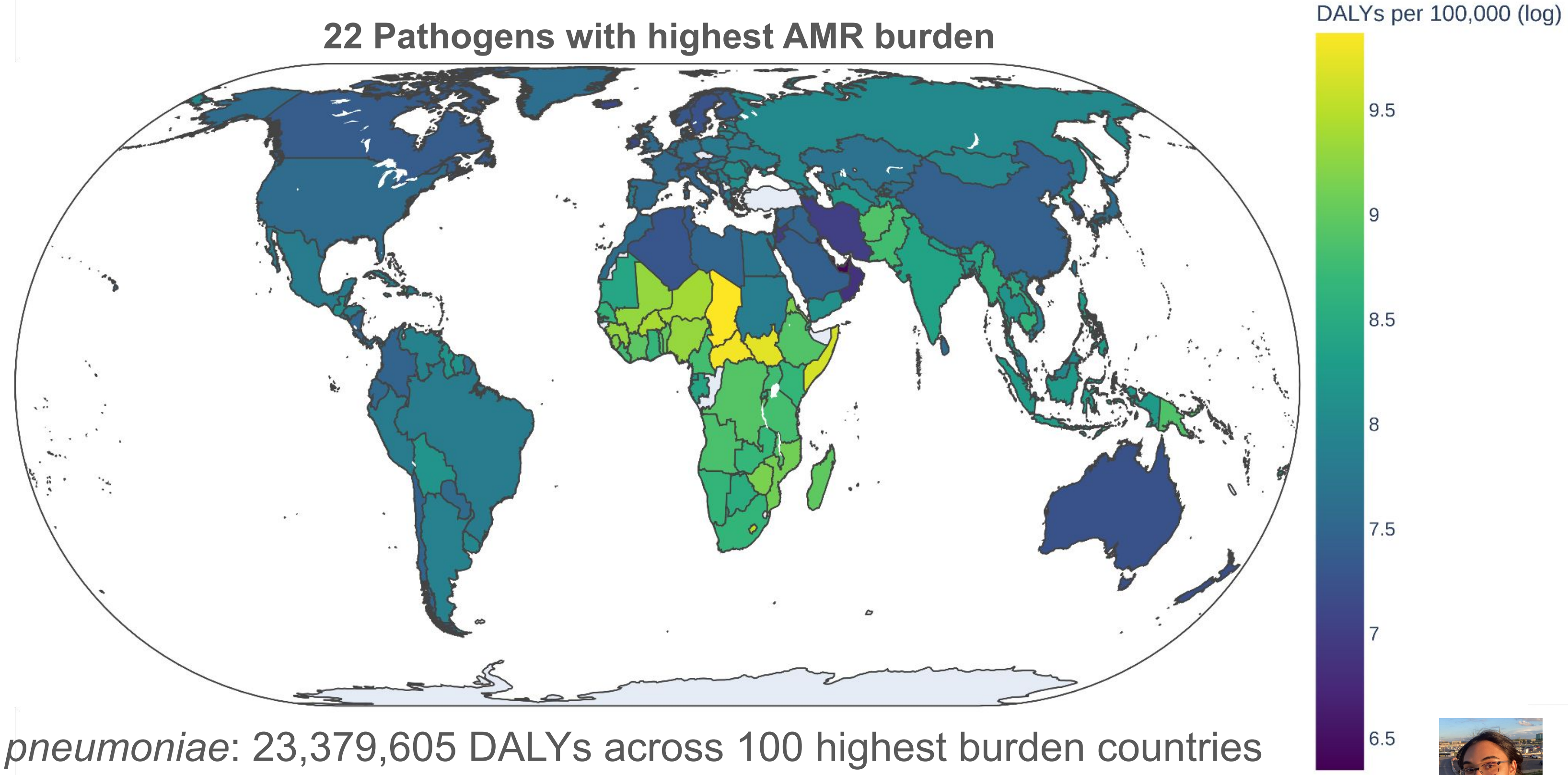
Clinician / Public Health Report

Currently working to automate parts of this process

So, what’s the problem?

Analyses are only as good as the available data!

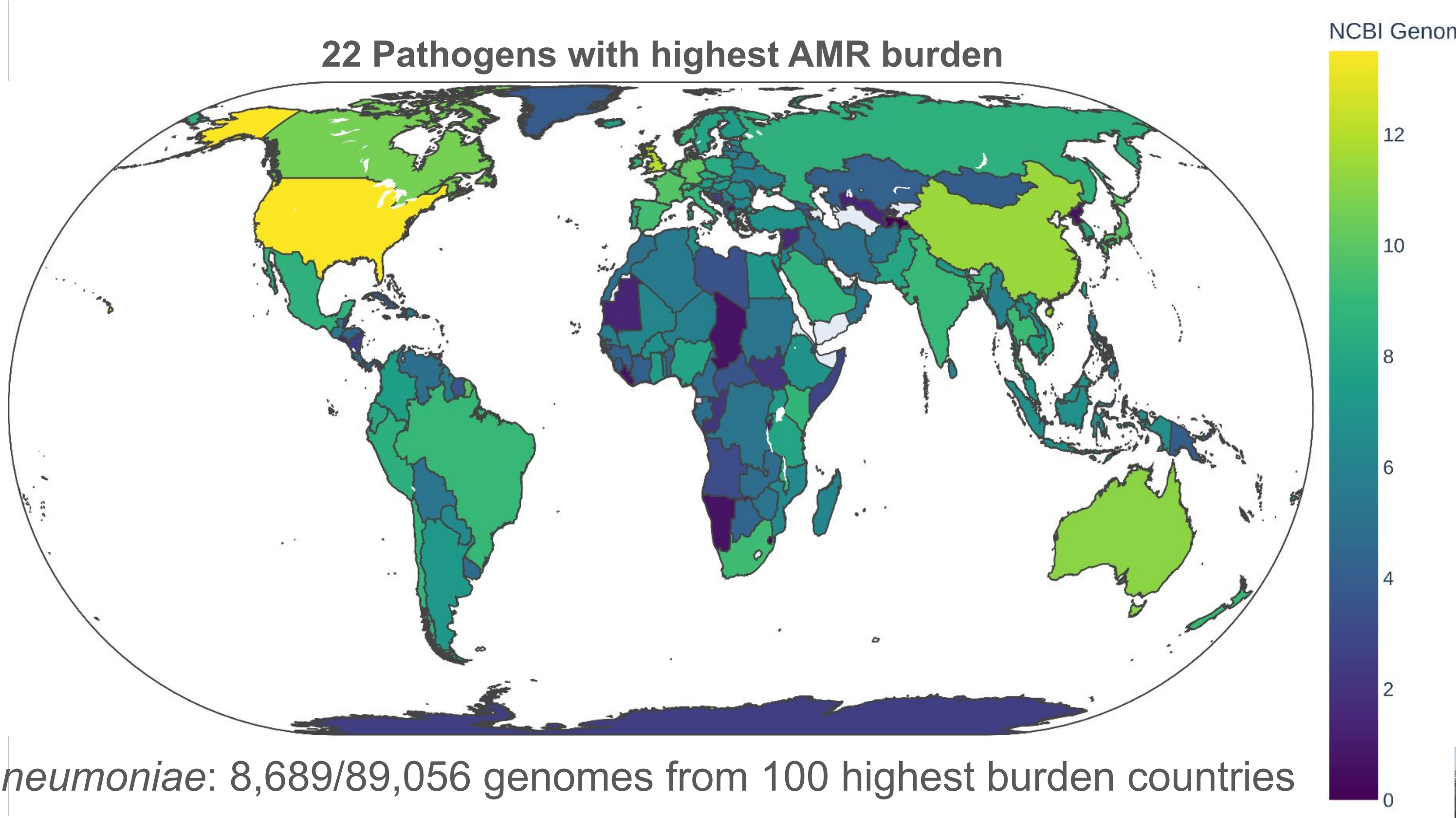
AMR burden (and incidence) not equally distributed



K. pneumoniae: 23,379,605 DALYs across 100 highest burden countries

Data from: Naghavi, Mohsen, et al. "Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050." The Lancet 404.10459 (2024): 1199-1226.

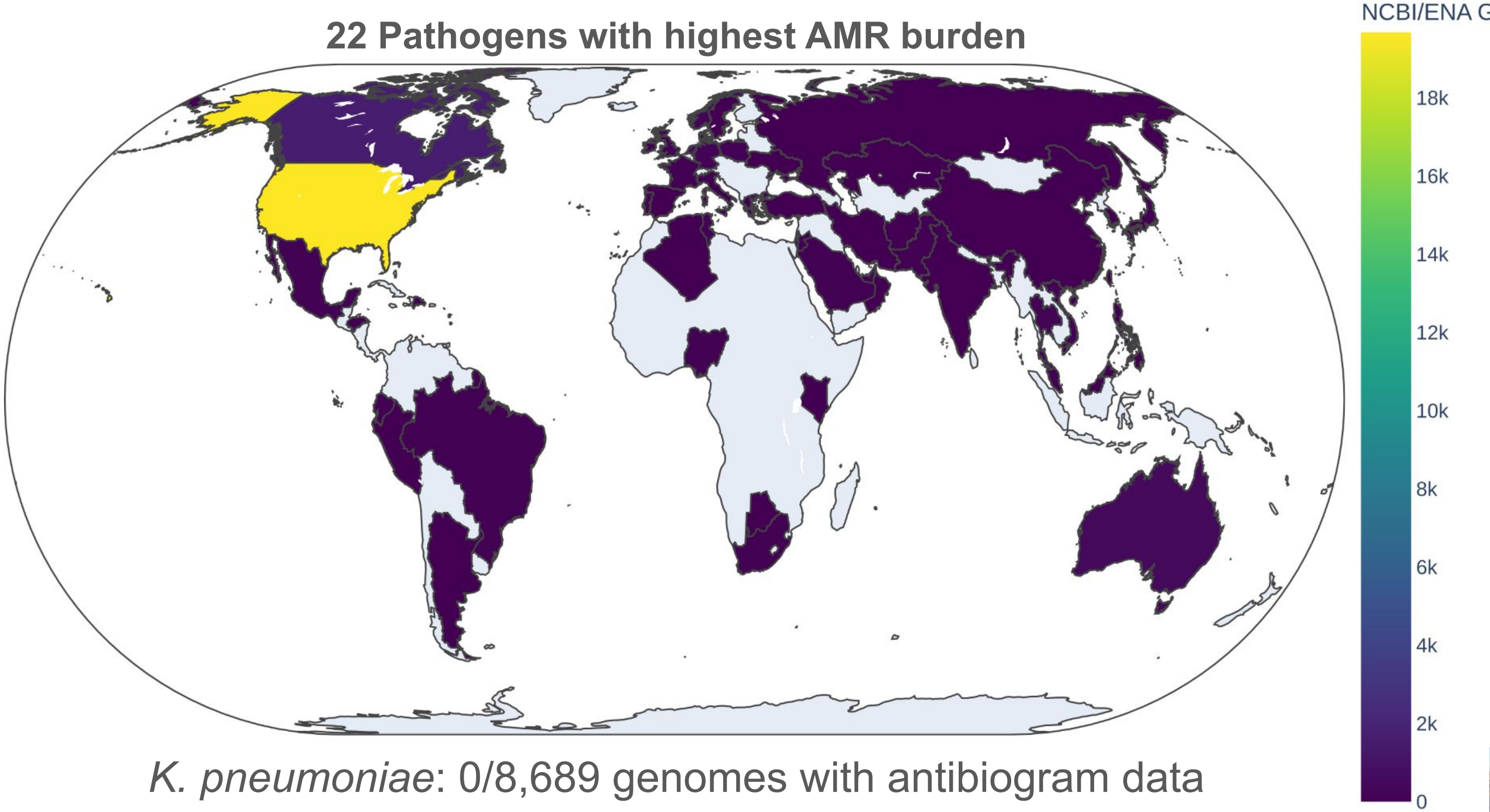
Genomic data is not equally available globally


22 Pathogens with highest AMR burden

K. pneumoniae: 8,689/89,056 genomes from 100 highest burden countries

Data from: NCBI Datasets 2025-06-15 - O'Leary, Nuala A., et al. "Exploring and retrieving sequence and metadata for species across the tree of life with NCBI Datasets." Scientific data 11.1 (2024): 732.

AMR genotype-phenotype data largely unavailable



K. pneumoniae: 0/8,689 genomes with antibiogram data

Data from: NCBI Datasets 2025-06-15 - O'Leary, Nuala A., et al. "Exploring and retrieving sequence and metadata for species across the tree of life with NCBI Datasets." Scientific data 11.1 (2024): 732.

What can we do about this?

Multi-faceted problem require multi-faceted solutions

- Make it easier to generate high quality data (standards, protocol, training, funding)
- Make it easier to collate and analyse data (data object model, norms, tools)
- Ensure access and benefits from data are shared globally (ethics, agreements, citation, IP reforms, WHO-IHR/PA)
- Strengthen international public health initiatives…
- Do **all** this in a community-led consensus manner!

PHA4GE Conference 2025

27 - 29 October 2025

Cape Town, South Africa

Data for Action: Overcoming Challenges and Seizing Opportunities in Public Health Genomics

IPSN International Pathogen Surveillance Network

WHO hosted network

Griffiths, Emma J., et al. "The PHA4GE Microbial Data-Sharing Accord: establishing baseline consensus microbial data-sharing norms to facilitate cross-sectoral collaboration." BMJ Global Health 9.10 (2024): e016474.

Timme, Ruth E., et al. "Putting everything in its place: using the INSDC compliant Pathogen Data Object Model to better structure genomic data submitted for public health applications." Microbial Genomics 9.12 (2023): 001145.

Griffiths, Emma J., et al. "PHA4GE quality control contextual data tags: standardized annotations for sharing public health sequence datasets with known quality issues to facilitate testing and training." Microbial Genomics 10.6 (2024): 001260.

Acknowledgements

Research Group

SHL
SHARED HOSPITAL LABORATORY

AMRrules

McArthur Lab

PUBLIC HEALTH ALLIANCE FOR GENOMIC EPIDEMIOLOGY

Data Structures Working Group
Co-Chaired with
Dr. Emma Griffiths

Funders

BILL & MELINDA GATES foundation

CIHR IRSC

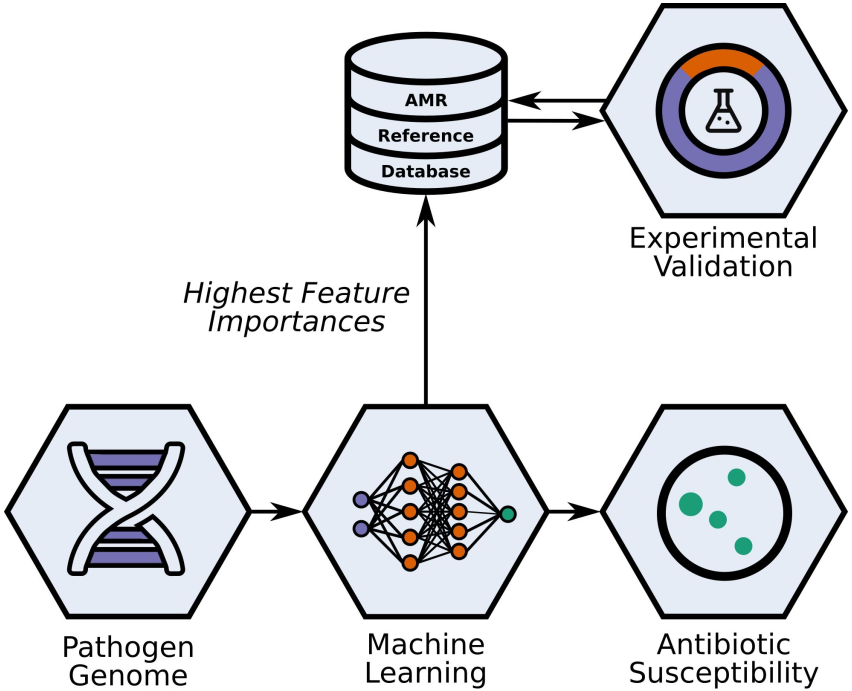
NSERC CRSNG

SSHRC CRSH

New Frontiers in Research Fund
Fonds Nouvelles frontières en recherche

Backup Slides

Inductive ML to find novel genotype-phenotype associations


AMR
Reference
Database

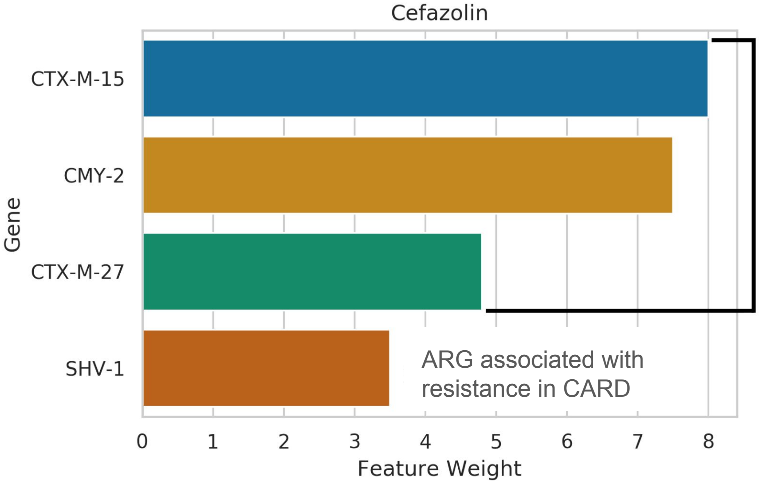
Experimental
Validation

Highest Feature Importances

Pathogen
Genome

Machine
Learning

Antibiotic
Susceptibility



ARGs not associated with resistance in CARD*

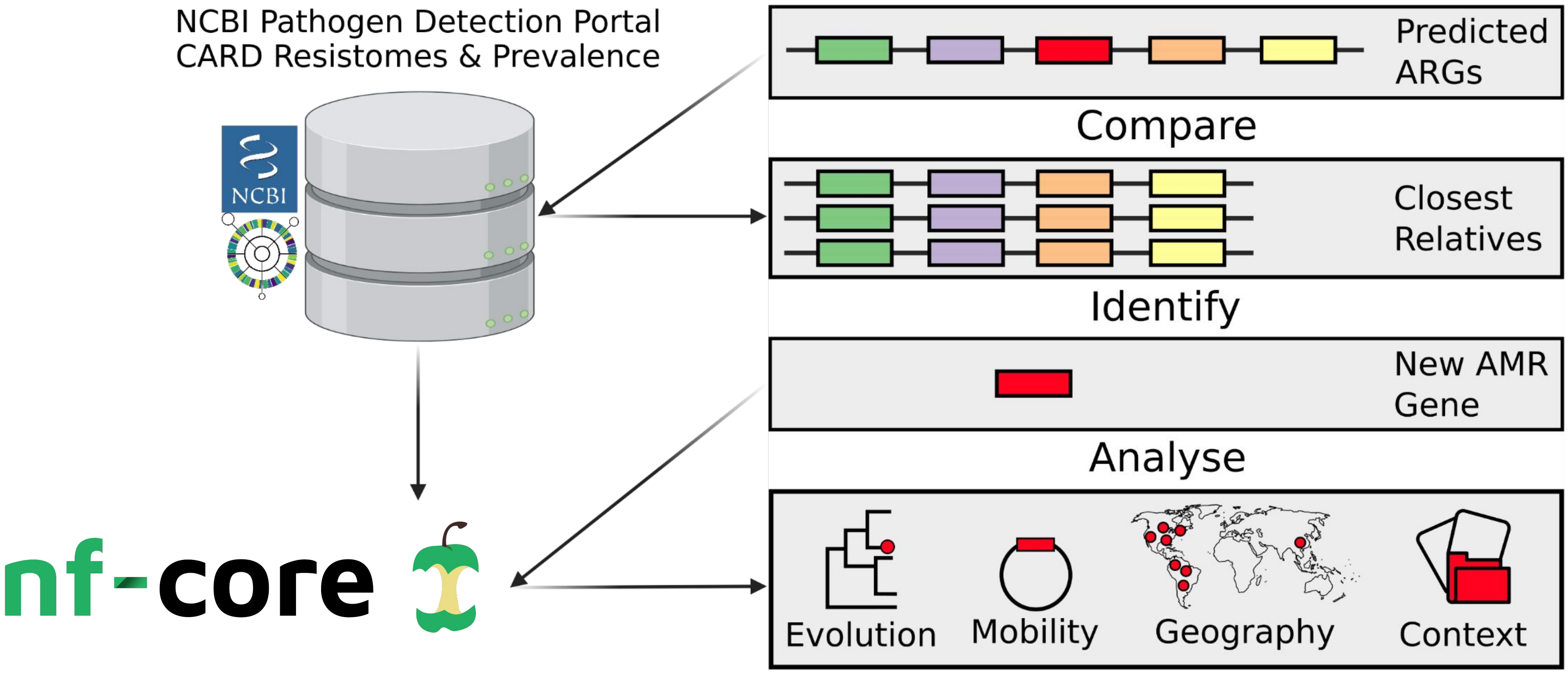
ARG associated with resistance in CARD

* at the time!

Tsang, Kara K., et al. "Identifying novel β-lactamase substrate activity through in silico prediction of antimicrobial resistance." Microbial genomics 7.1 (2021): 000500.

Kim, Jee In, et al. "Machine learning for antimicrobial resistance prediction: current practice, limitations, and clinical perspective." Clinical microbiology reviews 35.3 (2022): e00179-21.

Evolving Threat Detector: Automating contextual analyses



NCBI Pathogen Detection Portal
CARD Resistomes & Prevalence

nf-core

Predicted ARGs

Compare

Closest Relatives

Identify

New AMR Gene

Analyse

Evolution
Mobility
Geography
Context

Contextualisation requires comparison with databases

- Contextualisation involves comparing annotations to representative reference databases
- NCBI Pathogens
  - AMRFinderPlus - 101 species groups n=2,421,674)
- CARD-R Resistomes & Prevalence
  - RGI - 414 species n=244,719
- Amr.watch
  - AMRFinderPlus - 18 species n=1,117,800
- BV-BRC