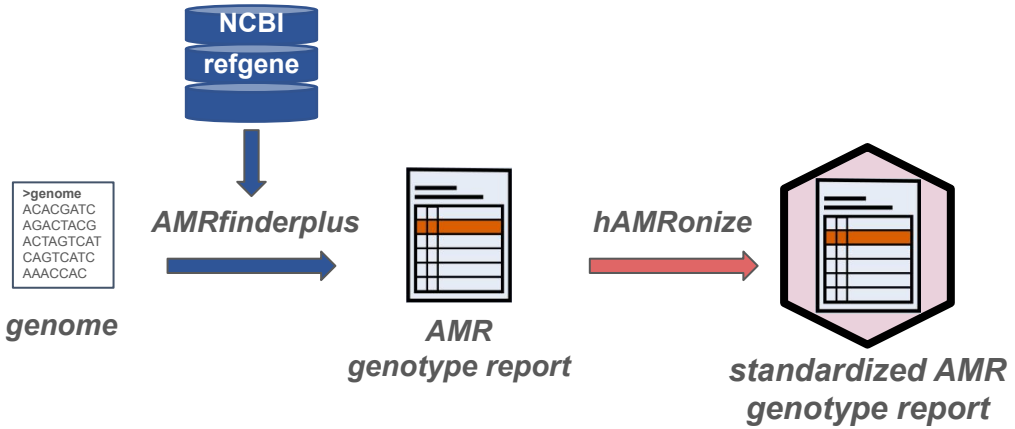

AMR rules
Interpretive Standards for AMR Genotypes

Time for expert rules for AMR genotype interpretation?

Genotyping AMR determinants in bacterial genomes is a fundamental task

Progress has been made on bioinformatics tools and resources

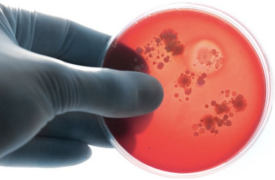
- ✓ Databases of AMR determinants - *NCBI, CARD, ResFinder*
- ✓ Tools for finding these in genomes - *AMRfinderplus, etc (hAMRonize to common format)*



Missing rules for interpretation

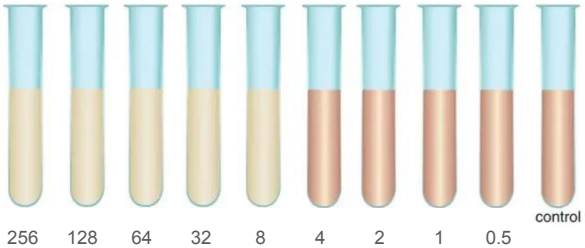
What does **gene X** in **species Y** mean for **drug Z?**

Interpretive standards



K. pneumoniae

Assay (microbroth dilution)



Imipenem concentration

Assay Result: MIC=8 mg/L

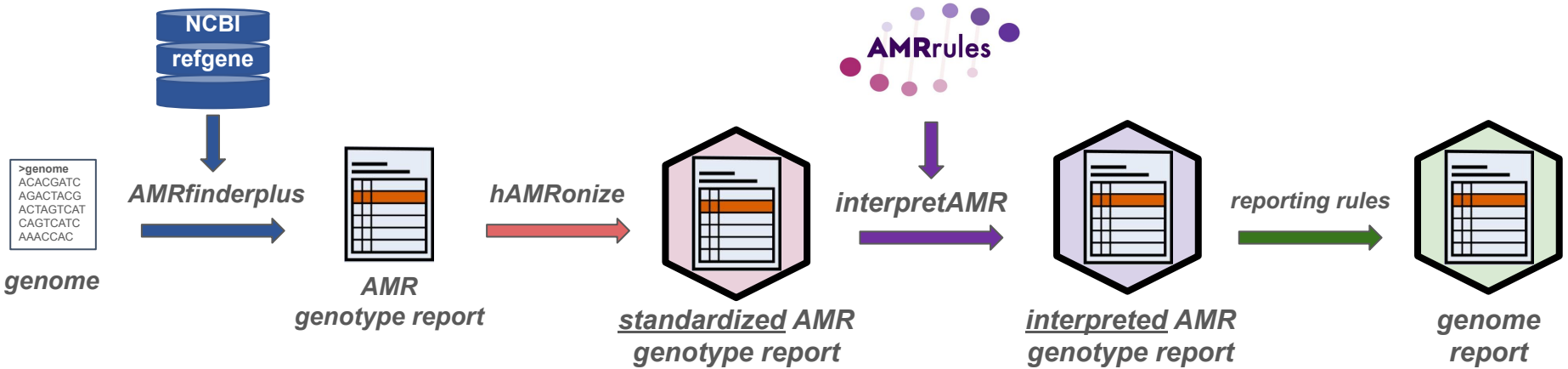
Interpretation: Resistant

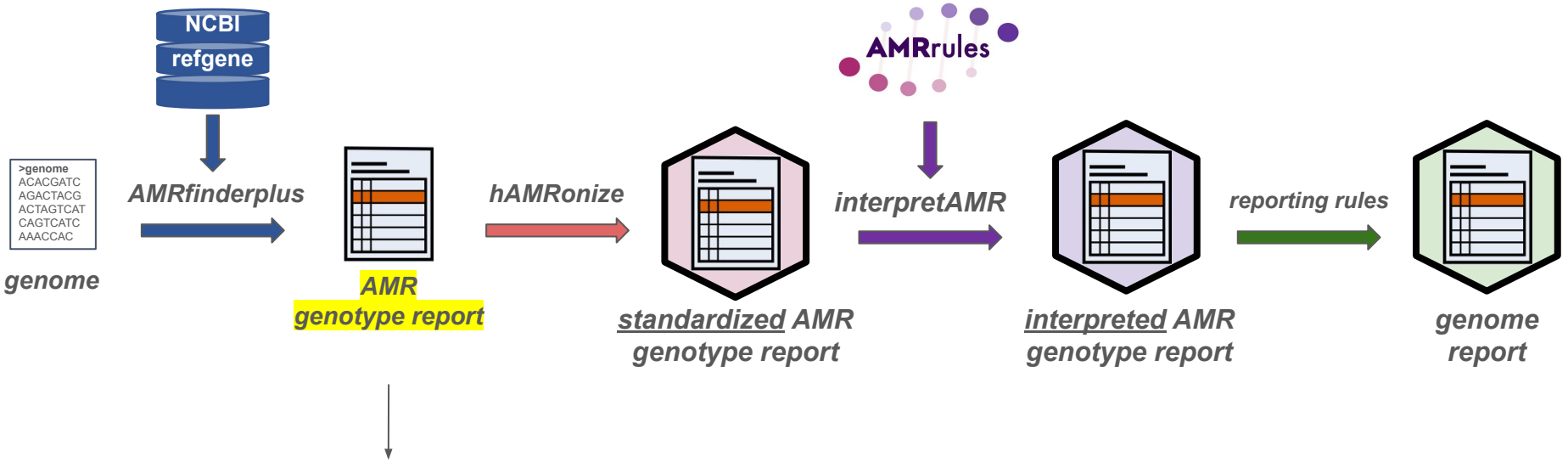
v_14.0_Breakpoint_Tables (EUCAST)

	Carbapenems[1]	MIC breakpoints (mg/L)		
61				
62		S ≤	R >	ATU
63	Doripenem	1	2	
64	Ertapenem	0.5	0.5	
65	Imipenem, *Enterobacterales* except *Morganellaceae*	2	4	
66	Imipenem[2], *Morganellaceae*	0.001	4	
67	Imipenem-relebactam, *Enterobacterales* except *Morganellaceae*	2[3]	2[3]	
68	Meropenem (indications other than meningitis)	2	8	
69	Meropenem (meningitis)	2	2	
70	Meropenem-vaborbactam	8[4]	8[4]	
71				

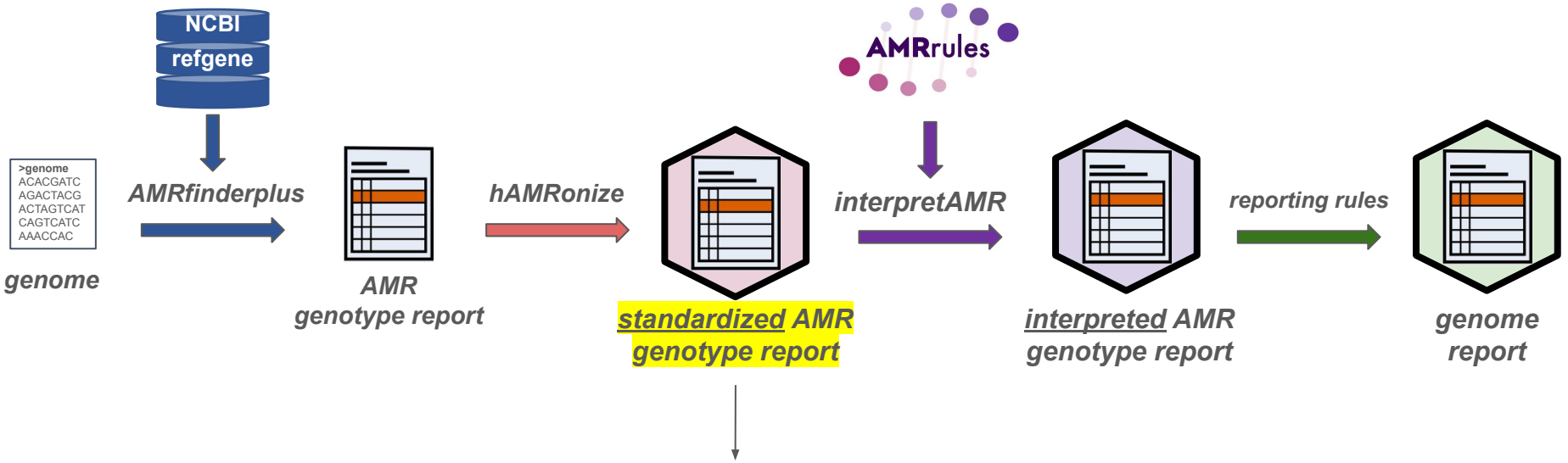
Technical uncertainty | Enterobacterales | Pseudomonas | S.maltophilia

Drug	Category
Amikacin	S
Ampicillin	R
Ceftriaxone	R
Imipenem	R

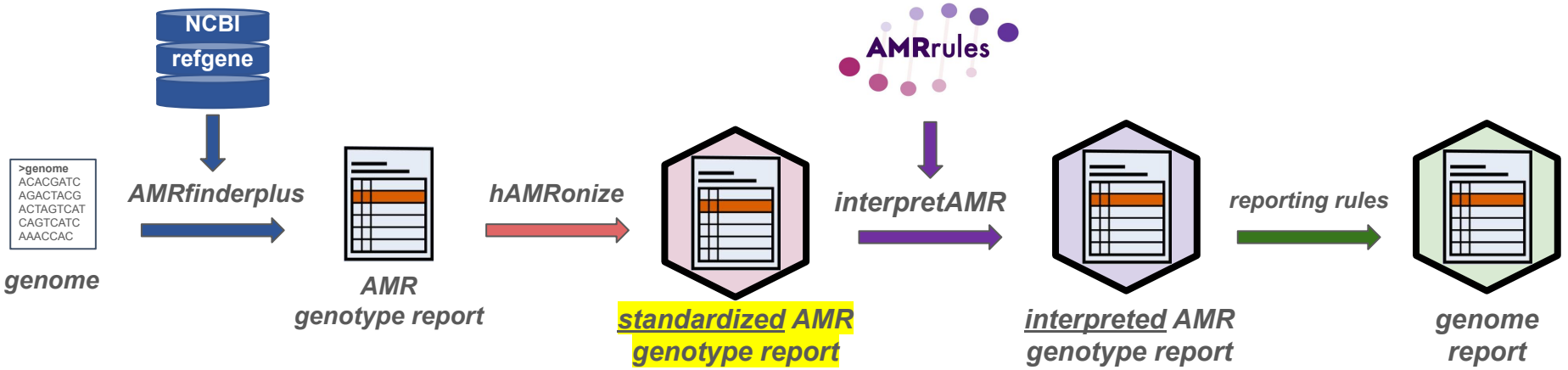

NCBI
refgene
>genome
ACACGATC
AGACTACG
ACTAGTCAT
CAGTCATC
AAACCAC
genome
AMRfinderplus
AMR
genotype report
hAMRonize
standardized AMR
genotype report
AMRrules
interpretAMR
interpreted AMR
genotype report
reporting rules
genome
report



Gene symbol	Class	Subclass
blaSHV-11	BETA-LACTAM	BETA-LACTAM
fosA	FOSFOMYCIN	FOSFOMYCIN
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN



Gene symbol	Class	Subclass	Analysis Software Name	Analysis Software Version	Genetic Variation Type
blaSHV-11	BETA-LACTAM	BETA-LACTAM	AMRFinderPlus	3.12.8	Gene presence detected
fosA	FOSFOMYCIN	FOSFOMYCIN	AMRFinderPlus	3.12.8	Gene presence detected
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	AMRFinderPlus	3.12.8	Gene presence detected
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	AMRFinderPlus	3.12.8	Gene presence detected
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN	AMRFinderPlus	3.12.8	Gene presence detected



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oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN

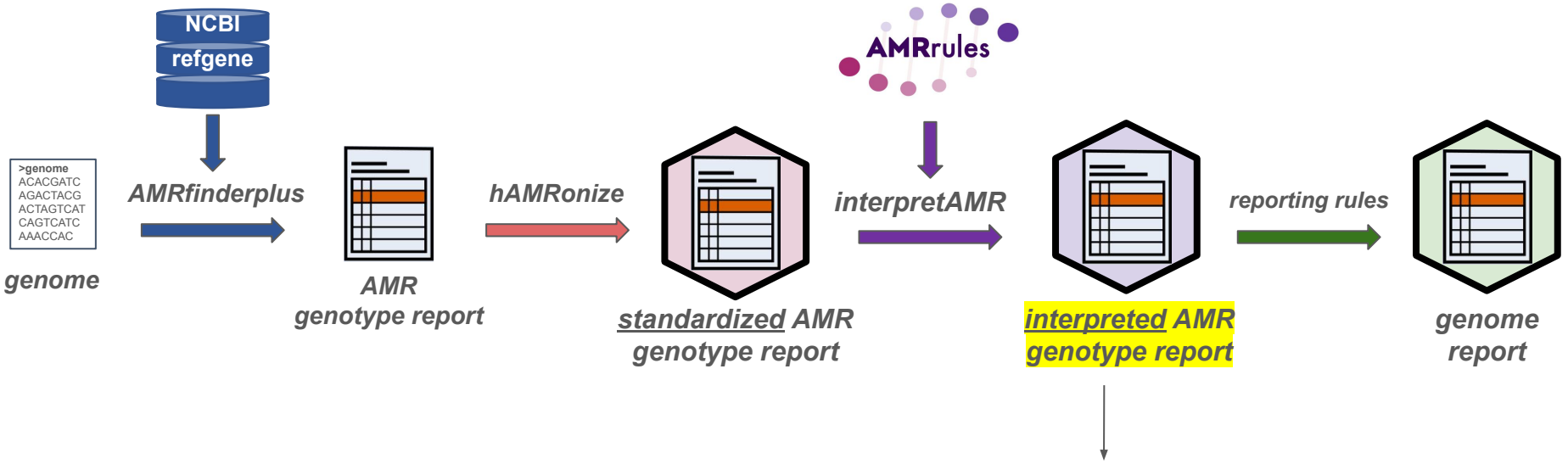
Interpreting results

Arjun Prasad edited this page on Sep 1, 2023 · 31 revisions

Note regarding Genotype vs. Phenotype

Users of AMRFinderPlus or its supporting data files are cautioned that presence of a gene encoding an antimicrobial resistance (AMR) protein or resistance causing mutation does not necessarily indicate that the isolate carrying the gene is resistant to the corresponding antibiotic. AMRFinderPlus does not predict phenotypic resistance. AMR genes must be expressed to confer resistance. Many AMR proteins reduce antibiotic susceptibility somewhat, but not sufficiently to cross clinical breakpoints. Meanwhile, an isolate may gain or lose resistance to an antibiotic by mutational processes, such as the loss of a porin required to allow the antibiotic into the cell. For some families of AMR proteins, especially those borne by plasmids, correlations of genotype to phenotype are much more easily deciphered, but users are cautioned against over-interpretation.

https://github.com/ncbi/amr/wiki/Interpreting-results



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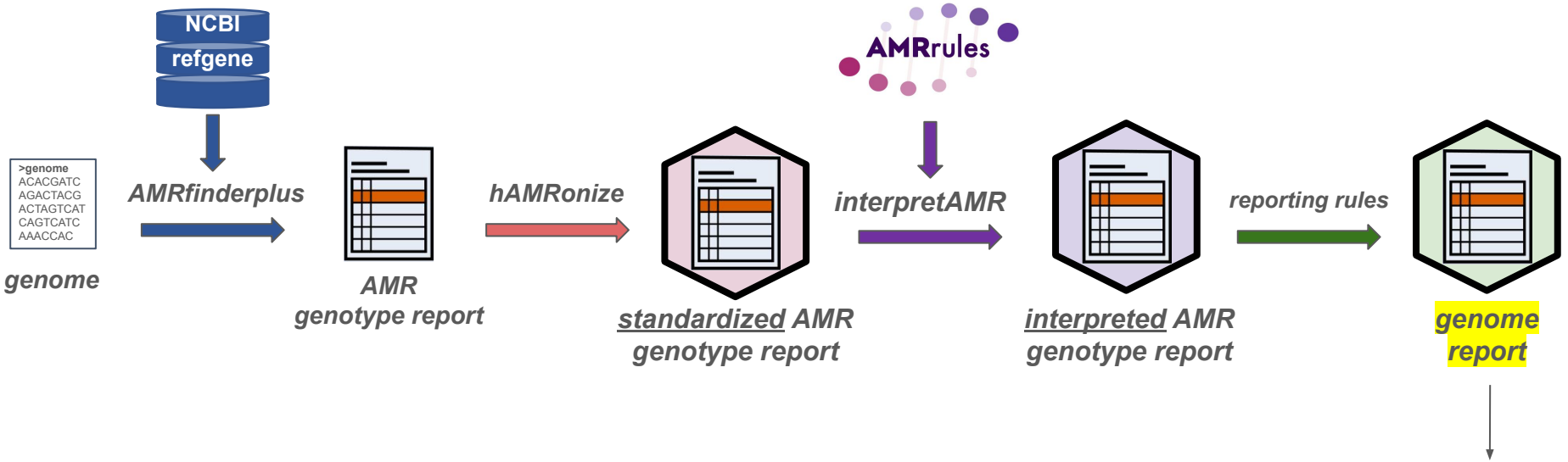
fosA	FOSFOMYCIN	FOSFOMYCIN	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	core	fosfomycin	wt S
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	core	ciprofloxacin	wt S
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	core	ciprofloxacin	wt S
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	acquired	ceftriaxone	nwt R



Local reporting rules

- Which drugs to include/suppress
- How much detail to include
- Format - e.g. ‘R’ or ‘Resistant’ or ‘predicted R’

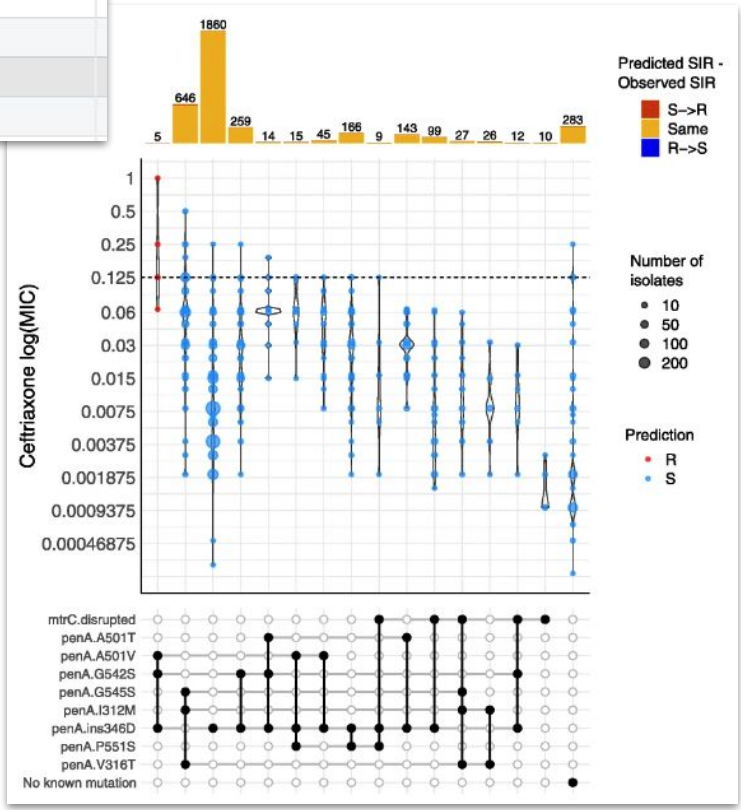
Drug	Predicted Category	Resistance Determinants
Amikacin	S	-
Ampicillin (expected R)	**R**	***bla*SHV-11 (core)**
Ceftriaxone	**R**	***bla*CTX-M-15**
Ciprofloxacin	S	-
Fosfomycin	S	-
Gentamicin	S	-
Imipenem	S	-
Trimethoprim	S	-

NAME	Azithromycin	Ceftriaxone	Cefixime	Ciprofloxacin	Penicillin	Sulfonamides	Spectinomycin	Tetracycline
ECDC_NL18_181113008902					●			
ECDC_ES18_8274					●			
ECDC_ES18_8442					●			
ECDC_ES18_8441					●			
ECDC_UK18_18BI179					●			
ECDC_ES18_8383			●		●			
ECDC_ES18_8304			●		●			

AMR - Antimicrobial resistance

PAARSNP AMR - Library 485 version 0.0.17

Agent	Inferred resistance	Known Determinants
Azithromycin	None	
Ceftriaxone	None	
Cefixime	**Resistant**	**penA_I312M/V316T/G545S**
Ciprofloxacin	None	
Penicillin	Intermediate	mtrR_A39T; penA_I312M/V316T/G545S
Sulfonamides	None	
Spectinomycin	None	
Tetracycline	None	mtrR_A39T

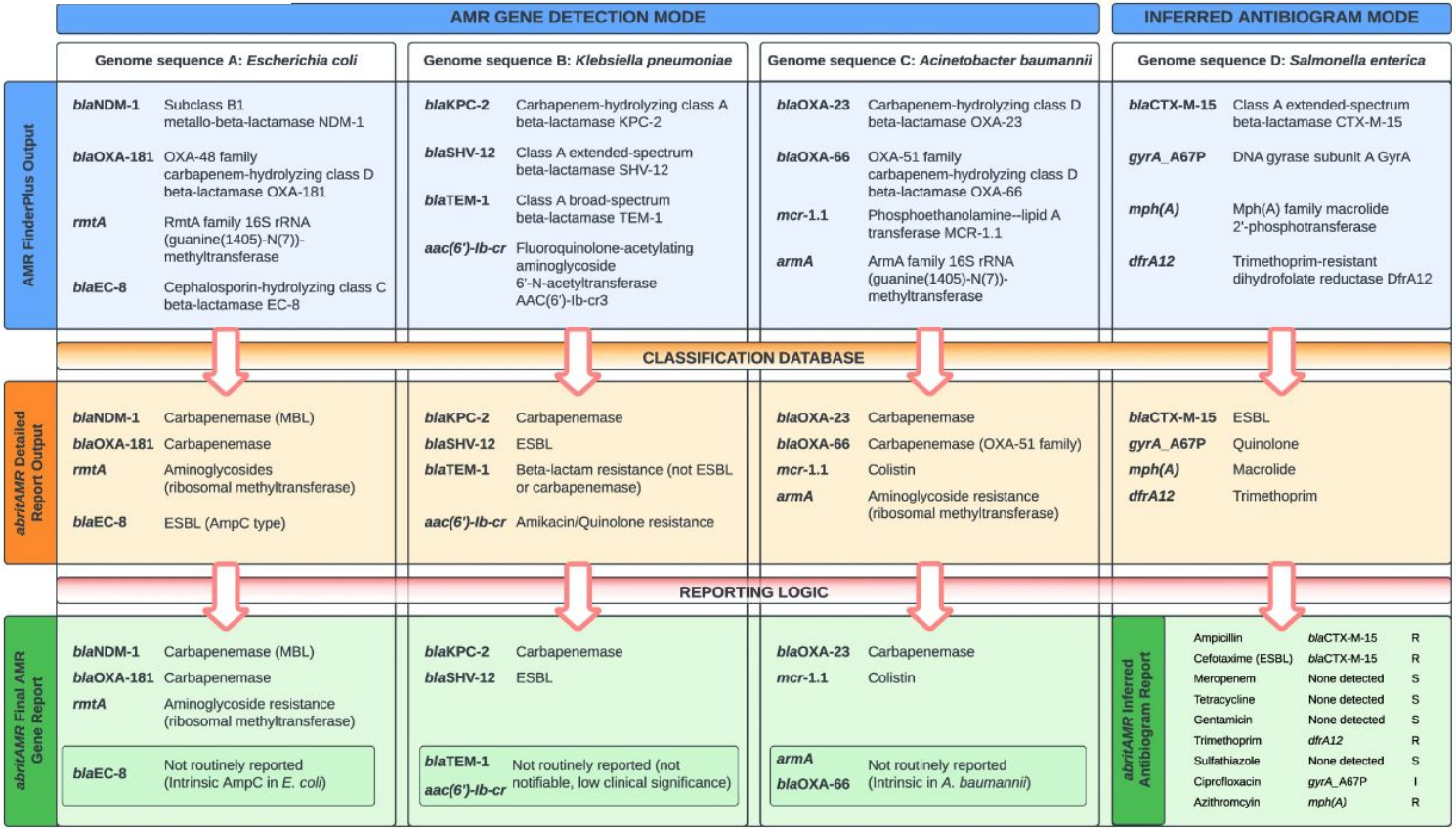


https://github.com/pathogenwatch-oss/amr-libraries

Sánchez-Busó *et al*, *Genome Med* 2021

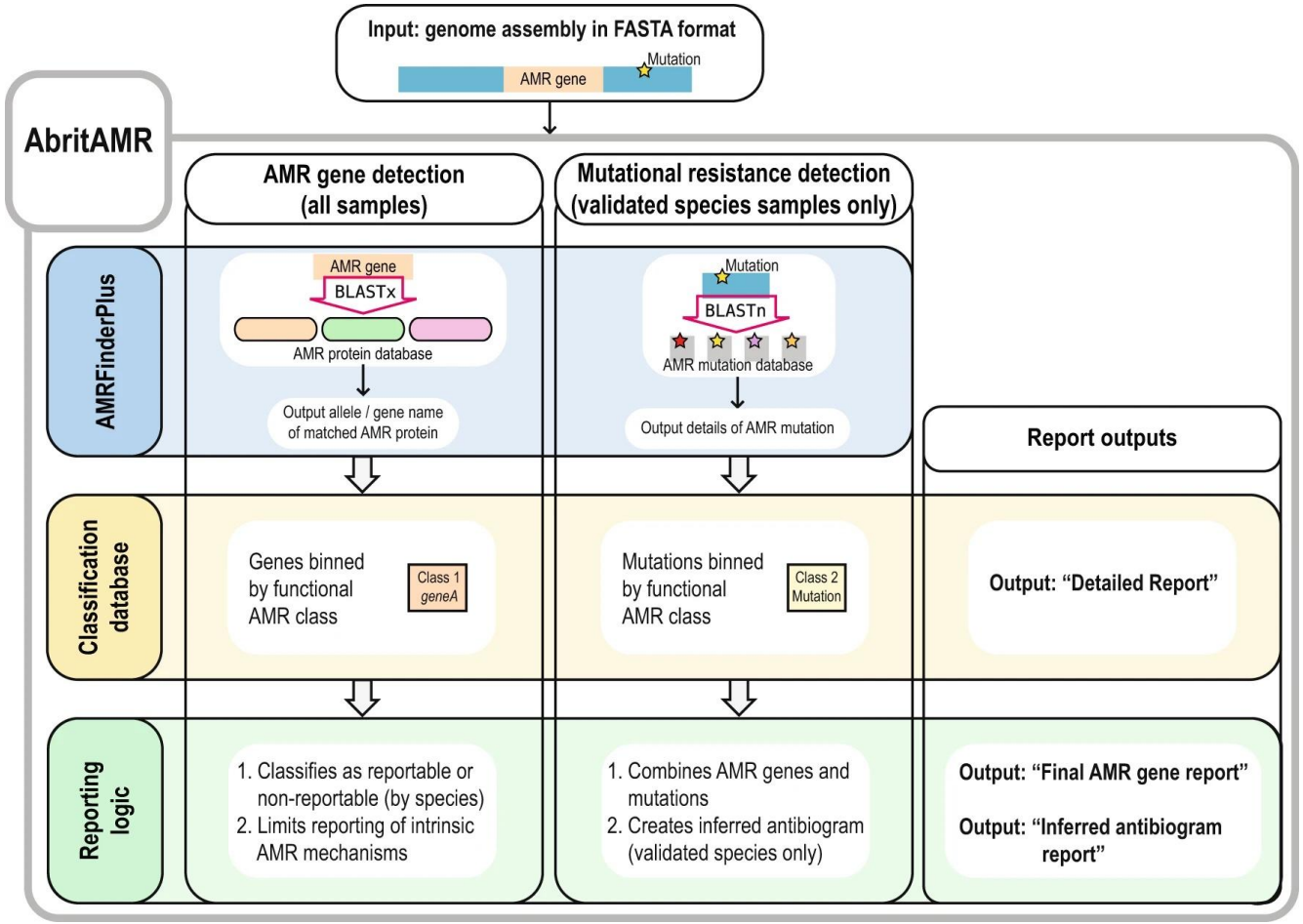
https://pathogen.watch

abriTAMR



Sherry *et al*, *Nat Comms* 2023

https://github.com/MDU-PHL/abritAMR



Sherry *et al*, *Nat Comms* 2023

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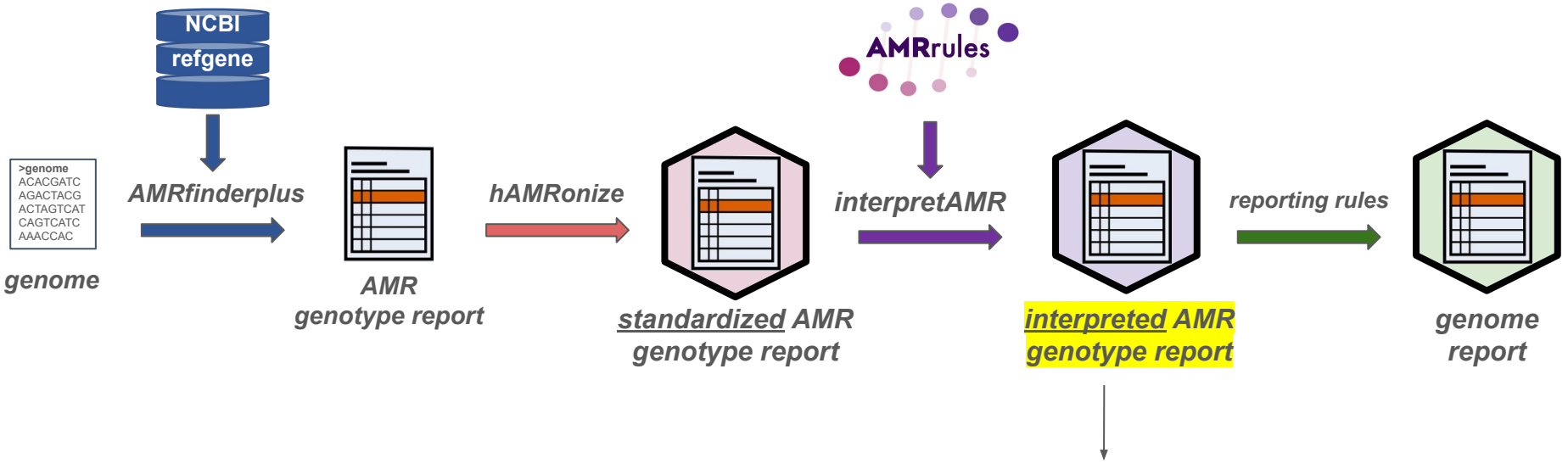

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oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	core	ciprofloxacin	wt S
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	core	ciprofloxacin	wt S
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	acquired	ceftriaxone	nwt R

Example rule set for *Klebsiella pneumoniae*

Rules for interpreting *Klebsiella pneumoniae* AMR genotypes

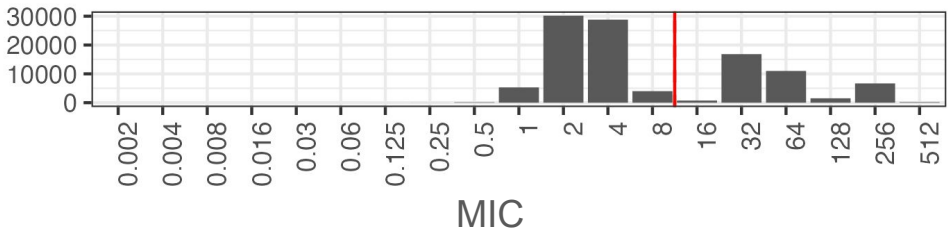
organism	gene	context	drug	category	PMID
s__Klebsiella pneumoniae	blaSHV	core	penicillins	wt R	32284385
s__Klebsiella pneumoniae	oqxA	core	ciprofloxacin	wt S	30834112
s__Klebsiella pneumoniae	oqxB	core	ciprofloxacin	wt S	30834112
s__Klebsiella pneumoniae	fosA5_fam	core	fosfomycin	wt S	27261267
s__Klebsiella pneumoniae	blaCTX-M-15	acquired	ceftriaxone	nwt R	12865392
...	...	...	...	...	...

expected phenotype category

Genotype vs phenotype vs clinical interpretation

Laboratory phenotype = minimum inhibitory concentration (MIC)

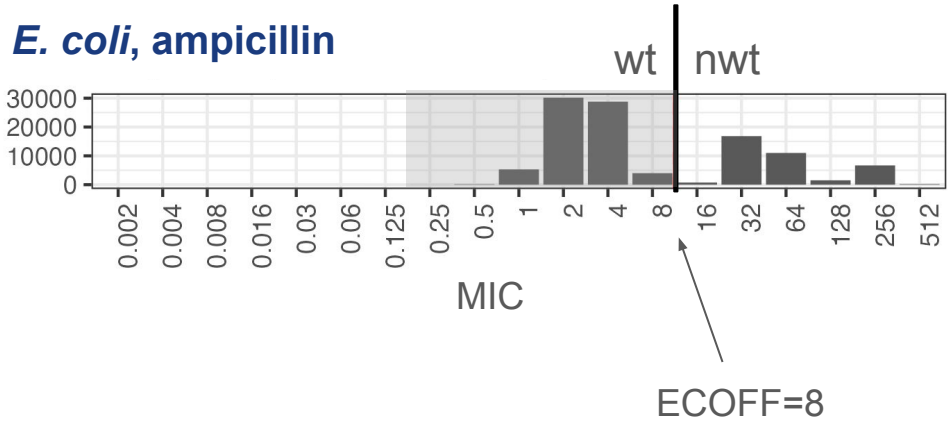
***E. coli*, ampicillin distribution**



Genotype vs phenotype vs clinical interpretation

Laboratory phenotype = minimum inhibitory concentration (MIC)

ECOFF = epidemiological cutoff, define wildtype (wt) phenotype

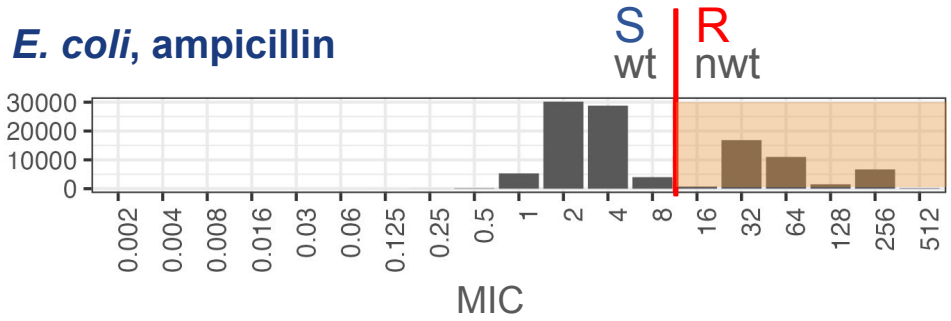


Genotype vs phenotype vs clinical interpretation

Laboratory phenotype = minimum inhibitory concentration (MIC)

ECOFF = epidemiological cutoff, define wildtype phenotype

Clinical categorization = interpretation of MIC based on breakpoints for S/R



R = high likelihood of **therapeutic failure** even with increased exposure

Genotype vs phenotype vs clinical interpretation

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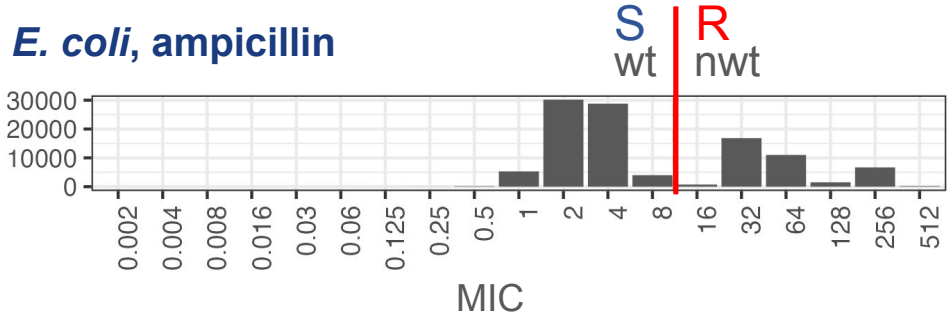
ECOFF = epidemiological cutoff, define wildtype phenotype

Clinical categorization = interpretation of MIC based on breakpoints for S/R



R = high likelihood of **therapeutic failure** even with increased exposure

Acquired beta-lactamase gene
shift **expected phenotype category** from **wt S** to **nwt R**

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington [1,†], O. Ekelund [2,†], F.M. Aarestrup [3], R. Canton [4], M. Doumith [1], C. Giske [5], H. Grundman [6], H. Hasman [7], M.T.G. Holden [8], K.L. Hopkins [1], J. Iredell [9], G. Kahlmeter [2], C.U. Köser [10], A. MacGowan [11], D. Mevius [12,13], M. Mulvey [14], T. Naas [15], T. Peto [16], J.-M. Rolain [17], Ø. Samuelsen [18], N. Woodford [1,*]

- Primary comparator should be **ECOFF** - i.e. WT/NWT
- Secondary comparator clinical **breakpoints** - i.e. S/I/R

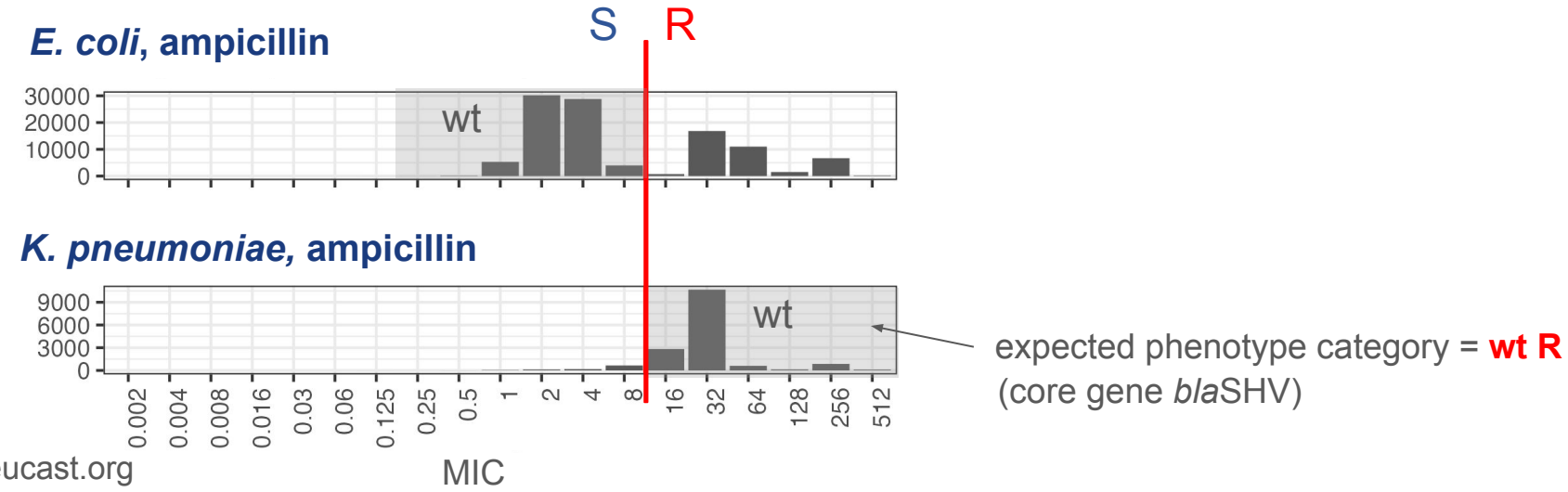
- Combining these gives 6 categories: S^{WT} I^{WT} R^{WT} S^{NWT} I^{NWT} R^{NWT}

Genotype vs phenotype vs clinical interpretation

Laboratory phenotype = minimum inhibitory concentration (MIC)

ECOFF = epidemiological cutoff, define wildtype phenotype

Clinical categorization = interpretation of MIC based on breakpoints for S/R



mic.eucast.org

EUCAST Expected Resistance Rules

Susceptibility testing is best avoided. A result which goes against the expected phenotype should be viewed with suspicion.

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								

www.eucast.org/expert_rules_and_expected_phenotypes/expected_phenotypes

Core genes can make wildtype genotypes look ‘resistant’

standardised genotype report

AMRfinderplus output for wildtype *Klebsiella pneumoniae* str SGH10

Gene symbol	Class	Subclass
blaSHV-11	BETA-LACTAM	BETA-LACTAM
fosA	FOSFOMYCIN	FOSFOMYCIN
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE

AMRfinderplus output for wildtype *Acinetobacter baumannii* str ARLG1933

Gene symbol	Class	Subclass
abaF	FOSFOMYCIN	FOSFOMYCIN
amvA	AMINOGLYCOSIDE	SPECTINOMYCIN/STREPTOMYCIN
blaADC-251	BETA-LACTAM	CEPHALOSPORIN
blaOXA-940	BETA-LACTAM	CARBAPENEM

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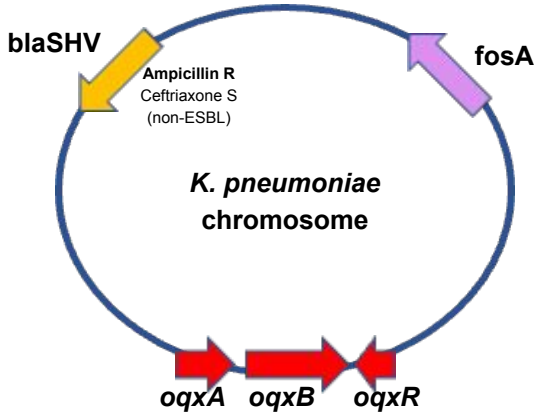
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE

makes all *K. pneumoniae* **resistant** to penicillins

all are **core genes** in this species



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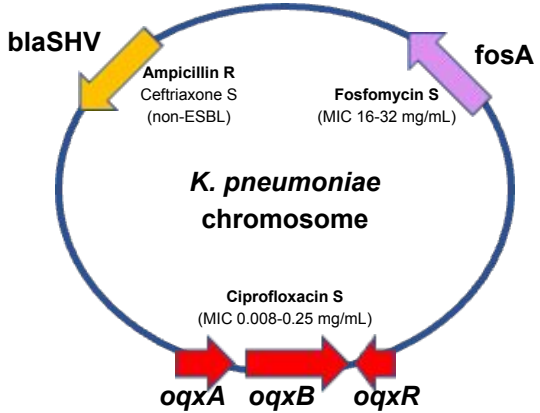
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE

increases fosfomycin MIC but remains **susceptible**

efflux pump, under normal regulation *K. pneumoniae* remain **susceptible**

all are **core genes** in this species



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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								

www.eucast.org/expert_rules_and_expected_phenotypes/expected_phenotypes

Example rule set for *Klebsiella pneumoniae* core genes

AMRrules

Rules for interpreting *Klebsiella pneumoniae* AMR genotypes

organism	gene	context	drug	category	PMID
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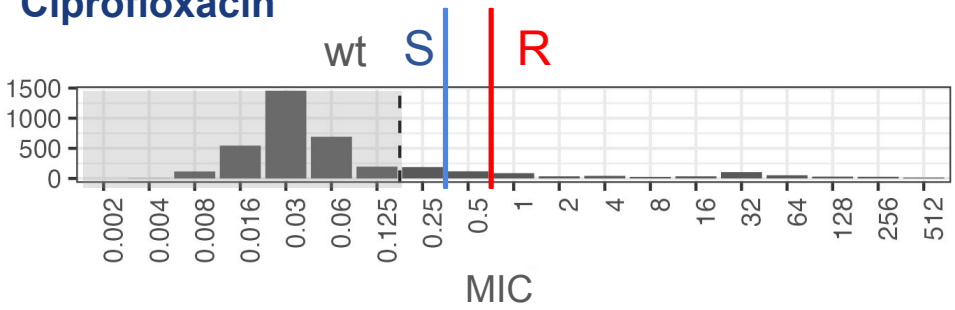
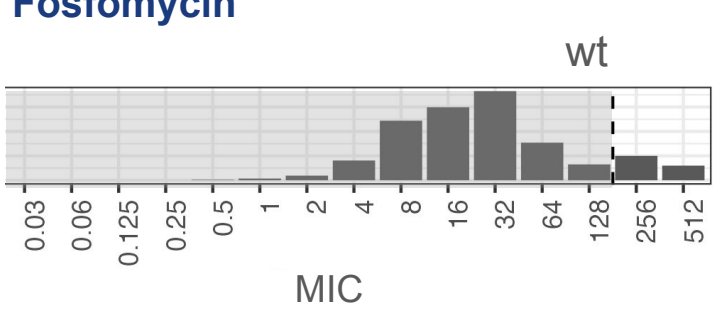
s__Klebsiella pneumoniae	oqxA	core	ciprofloxacin	wt S	30834112
s__Klebsiella pneumoniae	oqxB	core	ciprofloxacin	wt S	30834112
s__Klebsiella pneumoniae	fosA5_fam	core	fosfomycin	wt S	27261267

Ciprofloxacin



Fosfomycin



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Organism-specific interpretation of genotype report

standardised genotype report

AMRfinderplus output for wildtype *Klebsiella pneumoniae* str SGH10 + *interpretation*

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blaSHV-11	BETA-LACTAM	BETA-LACTAM	core	penicillins	wt R
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standardised genotype report

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fosA	FOSFOMYCIN	FOSFOMYCIN	core	fosfomycin	wt S
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	core	ciprofloxacin	wt S
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	core	ciprofloxacin	wt S
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN	acquired	ceftriaxone	nwt R

acquired gene
no special rule

assume nwt R by default

Specification of interpretive rules

organism	gene	context	drug	category	PMID	rule curation note
s__Klebsiella pneumoniae	blaSHV	core	penicillins	wt R	32284385	Specific alleles can also be ESBL, these are mostly mobile and are assigned nwt R for cephalosporins
s__Klebsiella pneumoniae	oqxA	core	ciprofloxacin	wt S	30834112	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promote
s__Klebsiella pneumoniae	oqxB	core	ciprofloxacin	wt S	30834112	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promote
s__Klebsiella pneumoniae	fosA5_fam	core	fosfomycin	wt S	27261267	Wildtype core gene, not expected to confer multiple drug resistance unless mobilised under strong promote
s__Klebsiella pneumoniae	blaCTX-M	acquired	ceftriaxone	nwt R	12865392	Acquired extended-spectrum beta-lactamase, demonstrated to confer resistance to third-generation cephalosporins in this species and others
...	...	...	...	...	...	...

GTDB: Genome Taxonomy DB

gtdb.ecogenomic.org

NCBI gene hierarchy

www.ncbi.nlm.nih.gov/pathogens/genehierarchy

WHO ATC Classification?

atcddd.fhi.no/atc_ddd_index

PubMed ID

pubmed.ncbi.nlm.nih.gov

Specification of interpretive rules

gene	breakpoint	breakpoint_standard	drug class	refseq accession	ARO accession	evidence level
blaSHV	-	Expected resistant phenotypes v 1.2 (13 January, 2023)	penicillins	NF000285.3	ARO:3000015	*
oqxA	MIC <=0.25 mg/L	EUCAST v14.0 (2024)	fluoroquinolones	NF000272.1	ARO:3003922	*
oqxB	MIC <=0.25 mg/L	EUCAST v14.0 (2024)	fluoroquinolones	NF000037.1	ARO:3003923	*
fosA5_fam	MIC >128 mg/L	ECOFF (January 2024)	phosphonic acid antibiotic	NF040540.1	-	*
blaCTX-M	MIC >2 mg/L	EUCAST v14.0 (2024)	3rd gen. cephalosporins	NF033089.1	ARO:3000016	*
...	...	...	...	...	...	...

Source of category definitions
e.g. EUCAST, CLSI

eucast.org
clsi.org

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oqxB	MIC <=0.25 mg/L	EUCAST v14.0 (2024)	fluoroquinolones	NF000037.1	ARO:3003923	*
fosA5_fam	MIC >128 mg/L	ECOFF (January 2024)	phosphonic acid antibiotic	NF040540.1	-	*
fosA5_fam	MIC >=256 mg/L	CLSI M100-Ed33 (May 2023)	phosphonic acid antibiotic	NF040540.1	-	*
blaCTX-M	MIC >2 mg/L	EUCAST v14.0 (2024)	3rd gen. cephalosporins	NF033089.1	ARO:3000016	*
...	...	...	...	...	...	...

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Antibiotic Resistance Ontology (ARO)

NCBI gene hierarchy

www.ncbi.nlm.nih.gov/pathogens/genehierarchy

Antibiotic Resistance Ontology (ARO)

card.mcmaster.ca

ESCMID | ESGEM-AMR

genome + phenotype

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

AMRrules

NCBI refgene

>genome
ACACGATC
AGACTACG
ACTAGTCAT
CAGTCATC
AAACCAC

genome

AMRfinderplus

AMR genotype report

hAMRonize

standardized genotype report

interpretAMR

interpreted genotype report

github.com/interpretAMR/AMRrules

What will the working group do?

ESCMID | ESGEM-AMR

- **Draft AMRrules** rule sets for organism/s according to expertise
  - Complete rule template (using expert knowledge & matched genome/AST data where available)
  - Submit to WG leads for review and testing (WG members may be asked to volunteer to review)
  - Guidance on formats and suggested protocols will be provided

    See: github.com/interpretAMR/AMRrulesCuration
- **Attend monthly meetings** to review progress and discuss issues arising
- **Contribute to initial publication in 2025**
- Consider drafting manuscripts describing the rationales and supporting data for individual focus organism/s

Working group model

ESCMID | ESGEM-AMR

May/June 2024

- Introductory webinars
- Register interest via online form
- Chairs to select working group members and assign to organism/s
  - Not too many per organism, may need to select based on expertise + data
  - For popular organisms, may need to nominate a leader to organise group
  - Members will be asked to sign an MOU and Code of Ethics to formalise their involvement

July 2024 through March 2025

- Draft **AMRrules** & attend meetings to discuss
- Priority = rules for **core genes & expected resistances**
  - If data available to fully interpret acquired resistances ✓
- Contribute to primary manuscript describing the **AMRrules** project

Principles guiding WG outputs

ESCMID | ESGEM-AMR

FAIR principles: **F**indable, **A**ccessible, **I**nteroperable, **R**eusable

- All materials freely available in GitHub repository
  - github.com/interpretAMR/AMRrules
  - github.com/interpretAMR/AMRrulesCuration
- Interoperable with NCBI refgene, hAMRonization, CARD tools and databases as far as possible
- Linked with EUCAST Expert Rules, guidance on WGS for AST prediction, and other materials as far as possible (EUCAST are partners)

Working group outputs

ESCMID | ESGEM-AMR

1. Interpretive standards in the form of **AMRrules** rule sets

2. **InterpretAMR** code to annotate genotype reports (hAMRonize compatible)
   - NOTE: code prototype only works with AMRFinderPlus output, need to work on this to be compatible with hAMRonize format, and identifiers from input databases besides refgene

3. Publication describing initial expert curation of **AMRrules** rule sets
   - ✓ Validated rules for core genes & expected resistances in key organisms
   - ✓ Discussion of data structure and issues
   - ✓ Requirements for systematic matched genome + AST data for acquired resistance

Working group priority organisms

ESCMID | ESGEM-AMR

1

ESKAPEE pathogens

- *Enterococcus faecium*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae* species complex*
- *Acinetobacter baumannii*
- *Pseudomonas aeruginosa*
- *Enterobacter cloacae* complex*
- *E. coli*

2

Other organisms on the WHO Priority Pathogens list

- *Salmonellae*, *Shigella* spp., other *Enterobacteriaceae*
- *Neisseria gonorrhoeae*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Helicobacter pylori*
- *Campylobacter* spp.

3

Other organisms of clinical relevance where sufficient expertise and data is available, prioritising those with **EUCAST Expected Resistant** phenotypes

AMRrules

AMRrules

Want to join?

ESCMID | ESGEM-AMR

Do I have to know a lot about organism-specific resistance?

✓ **YES, we are looking for at least one expert in each organism**
Please explain in the registration form what organism/s you have expertise in

Do I have to have matched genome/AST data?

× **NO, you do not need to have data to contribute, expertise is enough**
IF you able to contribute unpublished matched genome/AST data for the purpose of setting rules that would be very helpful, please note that in the registration form

Register interest:

bit.ly/AMRrules

Many issues to consider

ESCMID | ESGEM-AMR

- Which drugs should be included for a given organism?
- Standards of evidence?
- What if there's no breakpoint?
- What if there's multiple breakpoints?
- What if the breakpoint cuts the MIC distribution in half?
- Can/should we define a rule for a drug class rather than individual drugs?
- Can/should we define a rule for a taxonomic group other than species?
    - Species complex? Genus? Family?
- Can we assume some acquired genes have universal effects?
- How to define rules for combined effects of multiple genes/variants?
- How to handle combination drugs?
- How to ensure interoperability with multiple upstream databases & tools?

Some preliminary answers in Technical Guidance doc at:
github.com/interpretAMR/AMRrulesCuration

AMRrules

A Novel Data Structure for Prediction of Phenotypic Antimicrobial Resistance Based on Whole-Genome Sequencing

D. Brody Duncan[1,2]; Andrew G. McArthur[1,3]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University; [2]Department of Pathology and Molecular Medicine, McMaster University
[3]Michael G. DeGroote Institute for Infectious Disease Research, McMaster University

1. "Antibiotic first"
2. Lists every antibiotic↔gene combination
3. Conserves data and metadata

antibiotic	interpretation	gene_symbol	explanation	sequence_identity	reference_database	analysis_software_version
ampicillin	resistant	TEM-1	TEM confers resistance to aminopenicillins	100	CARD	6.0.3
ampicillin	resistant	CTX-M-15	CTX-M is a class A ESBL	100	CARD	6.0.3
amoxicillin	resistant	TEM-1	TEM confers resistance to aminopenicillins	100	CARD	6.0.3
amoxicillin	resistant	CTX-M-15	CTX-M is a class A ESBL	100	CARD	6.0.3
cefazolin	resistant	CTX-M-15	CTX-M is a class A ESBL	100	CARD	6.0.3
ceftriaxone	resistant	CTX-M-15	CTX-M is a class A ESBL	100	CARD	6.0.3
ceftazidime	resistant	CTX-M-15	CTX-M is a class A ESBL	100	CARD	6.0.3
ciprofloxacin	resistant	Escherichia coli gyrA c	presence of at least two gyrA or parC mutations confers resistance to fluoroquinolones	99.66	CARD	6.0.3
ciprofloxacin	resistant	Escherichia coli parC (	presence of at least two gyrA or parC mutations confers resistance to fluoroquinolones	99.2	CARD	6.0.3
gentamicin	unknown	APH(6)-Id	AMEs can increase MICs for aminoglycosides	99.28	CARD	6.0.3
gentamicin	unknown	APH(3")-Ib	AMEs can increase MICs for aminoglycosides	99.63	CARD	6.0.3
gentamicin	unknown	AAC(3)-IId	AMEs can increase MICs for aminoglycosides	100	CARD	6.0.3
tobramycin	unknown	APH(6)-Id	AMEs can increase MICs for aminoglycosides	99.28	CARD	6.0.3
tobramycin	unknown	APH(3")-Ib	AMEs can increase MICs for aminoglycosides	99.63	CARD	6.0.3
tobramycin	unknown	AAC(3)-IId	AMEs can increase MICs for aminoglycosides	100	CARD	6.0.3

4. Modifiable to fit local practice or new evidence
5. Open-source for any tool

Fig. 3: Analyzed *E. coli* WGS data reported using novel data structure. Phenotypic prediction rules created as proof-of-concept for this data-structure and are not clinically validated. Independent *in vitro* phenotypic testing reported resistance to ampicillin, ceftriaxone, ciprofloxacin, and gentamicin but susceptibility to piperacillin-tazobactam.