

A microscopic image of several purple, rod-shaped bacteria with numerous thin, hair-like flagella extending from their surfaces. The bacteria are set against a dark blue background.

# **AMR rules**

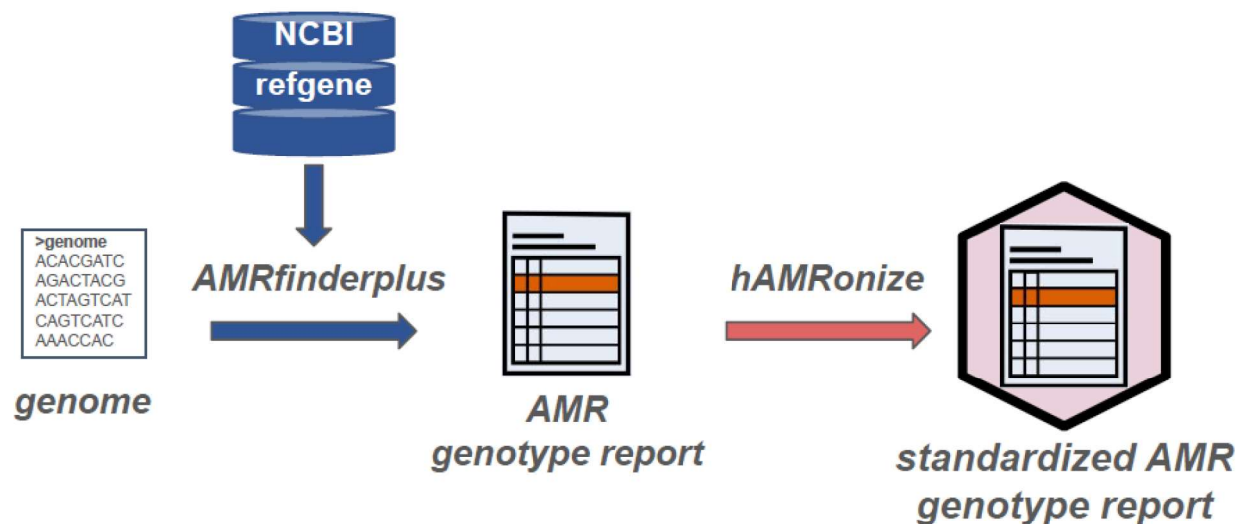
**Interpretive Standards for AMR Genotypes**

# Time for expert rules for AMR genotype interpretation?

Genotyping AMR determinants in bacterial genomes is a fundamental task

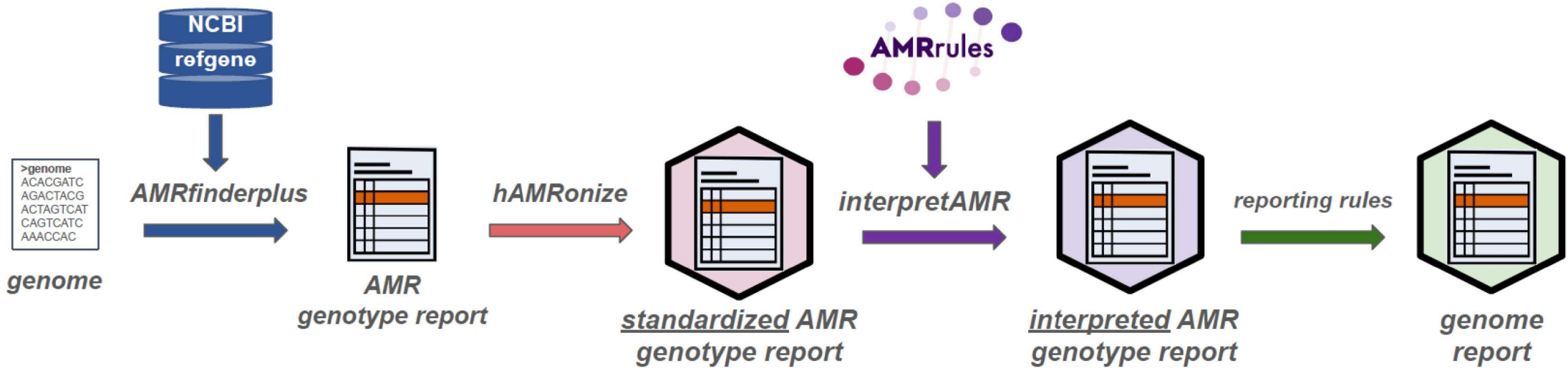
Progress has been made on bioinformatics tools and resources

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

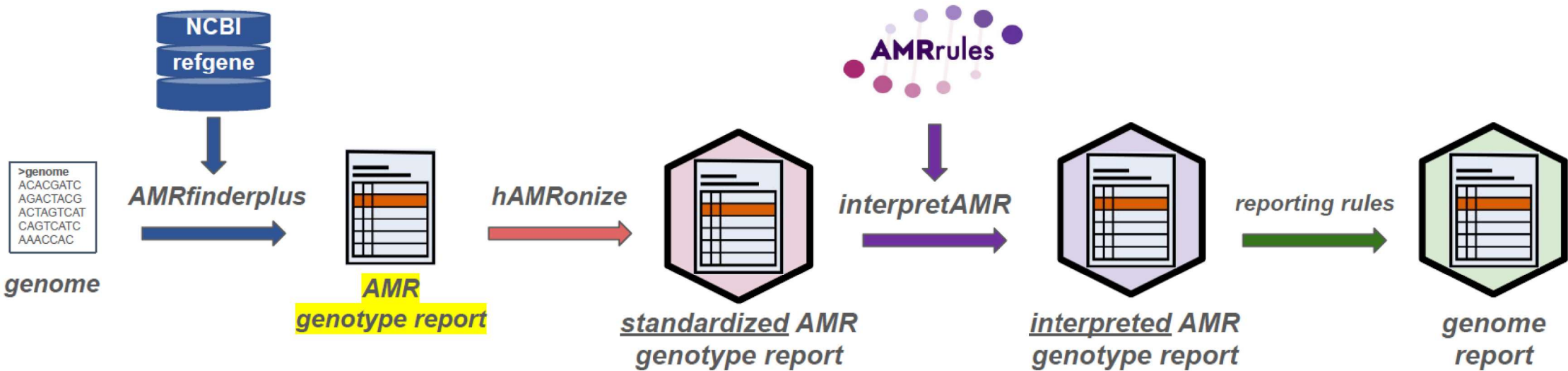


Missing rules for interpretation

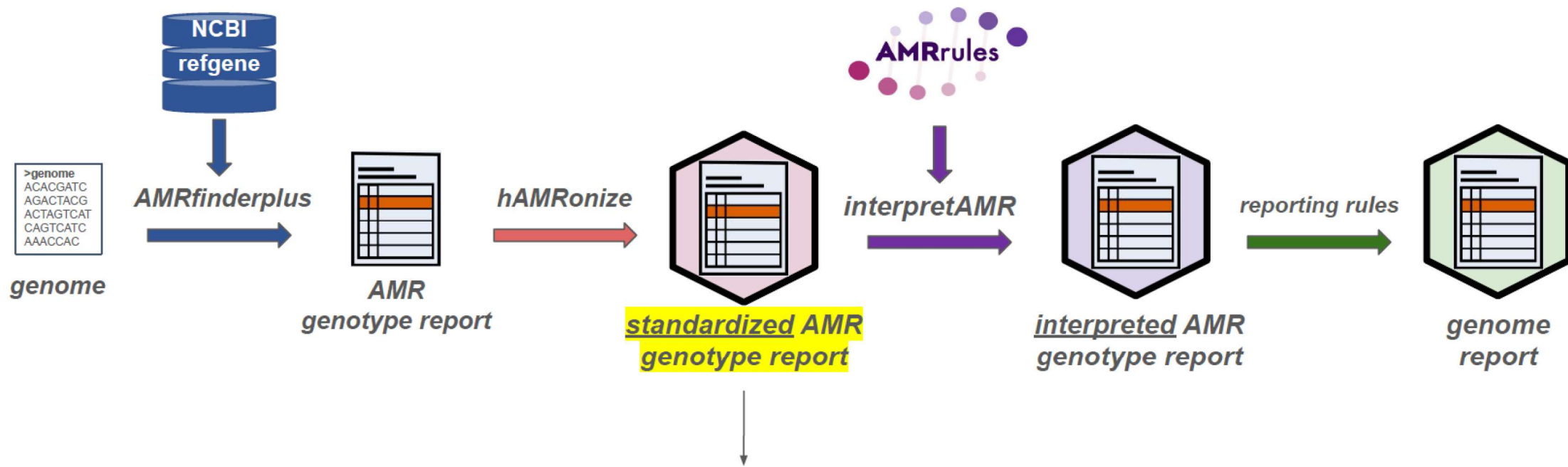
What does gene X in species Y mean for drug Z?



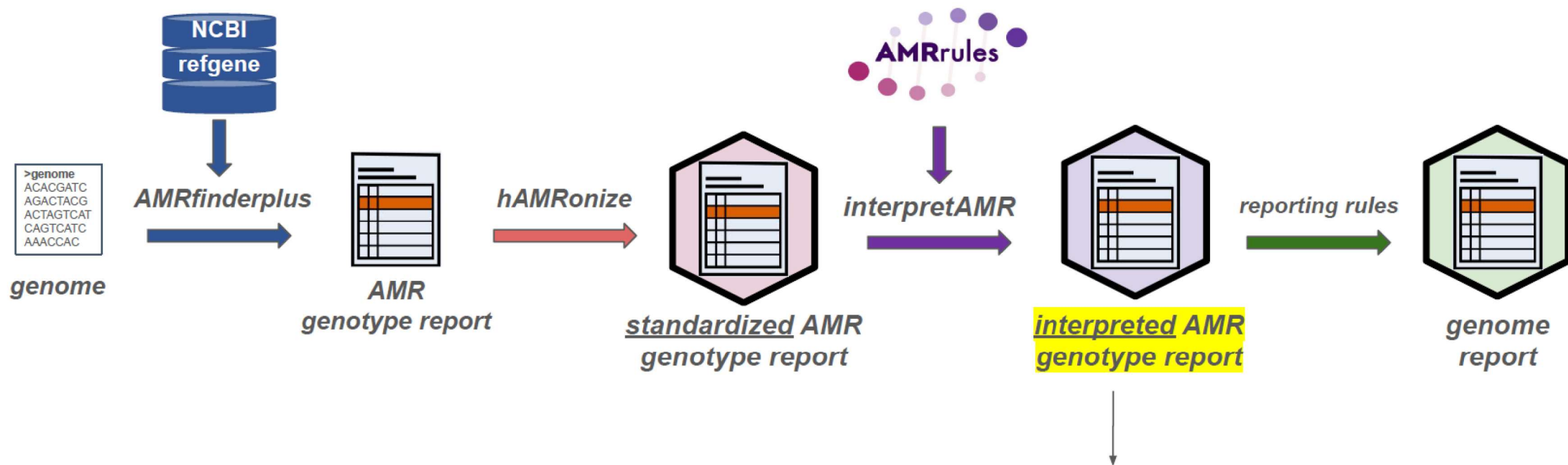




Gene symbol	Class	Subclass
blaSHV-11	BETA-LACTAM	BETA-LACTAM
fosA	FOSFOMYCIN	FOSFOMYCIN
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE
blaCTX-M-15	BETA-LACTAM	CEPHALOSPORIN



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



Gene symbol	Class	Subclass	Analysis Software Name	Analysis Software Version	Genetic Variation Type	Species interpretation	Context	Drug	Interpretation
blaSHV-11	BETA-LACTAM	BETA-LACTAM	AMRFinderPlus	3.12.8	Gene presence detected	Klebsiella pneumoniae; v1.1	core	penicillins	wt R
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

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

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

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

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

# Core genes can make wildtype genotypes look 'resistant'

AMRfinderplus output for wildtype *Klebsiella pneumoniae* str SGH10

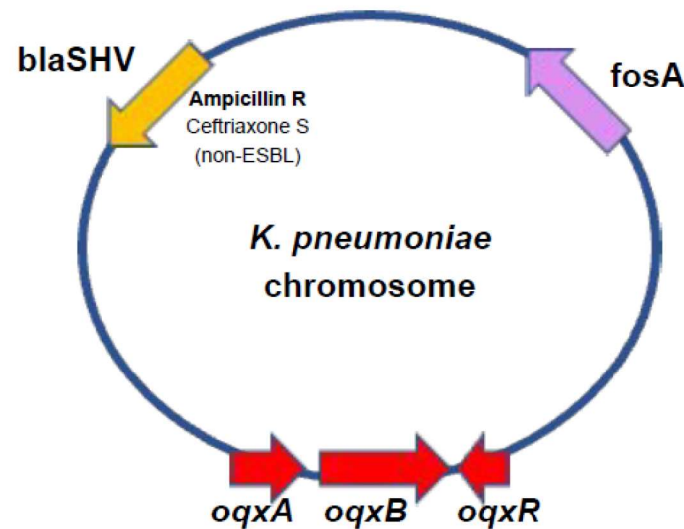


standardised genotype report

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

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

all are **core genes** in this species





# Core genes can make wildtype genotypes look 'resistant'

AMRfinderplus output for wildtype *Klebsiella pneumoniae* str SGH10



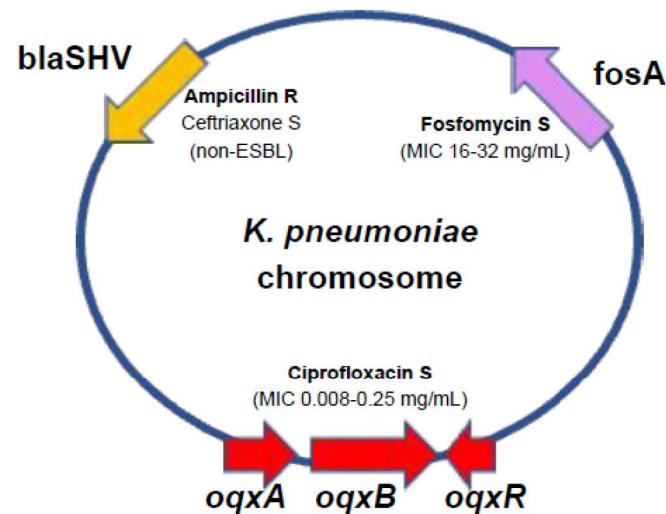
standardised genotype report

Gene symbol	Class	Subclass
blaSHV-11	BETA-LACTAM	BETA-LACTAM
fosA	FOSFOMYCIN	FOSFOMYCIN
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



# Organism-specific interpretation of genotype report



standardised  
genotype report

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

Gene symbol	Class	Subclass	Context	Drug	Interpretation
blaSHV-11	BETA-LACTAM	BETA-LACTAM	core	penicillins	wt R
fosA	FOSFOMYCIN	FOSFOMYCIN	core	fosfomicin	wt S
oqxA	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	core	ciprofloxacin	wt S
oqxB19	PHENICOL/QUINOLONE	PHENICOL/QUINOLONE	core	ciprofloxacin	wt S



# Organism-specific interpretation of genotype report

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

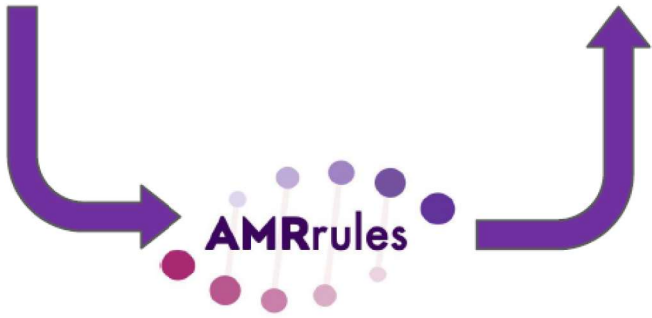


standardised genotype report

Gene symbol	Class	Subclass	Context	Drug	Interpretation
blaSHV-11	BETA-LACTAM	BETA-LACTAM	core	penicillins	wt R
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

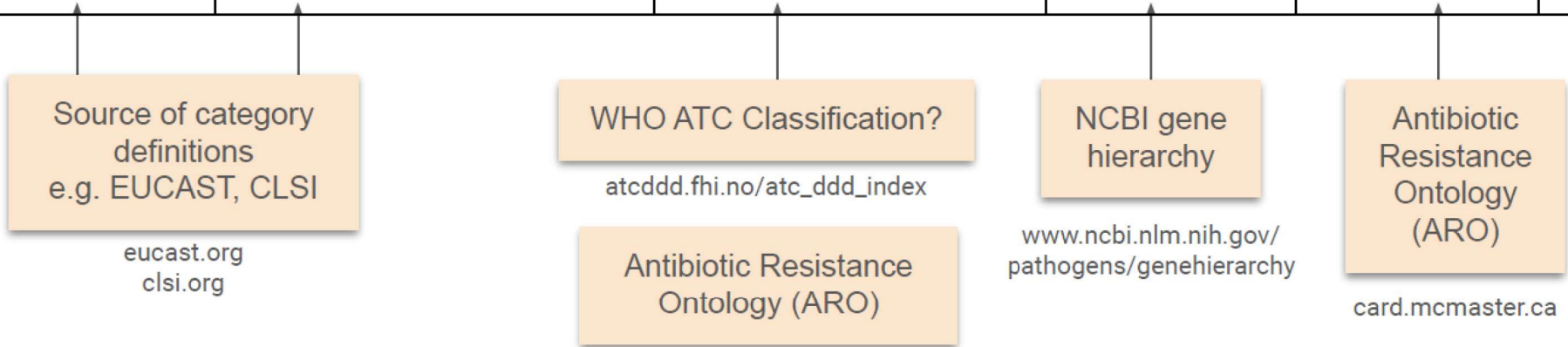
*assume nwt R  
by default*



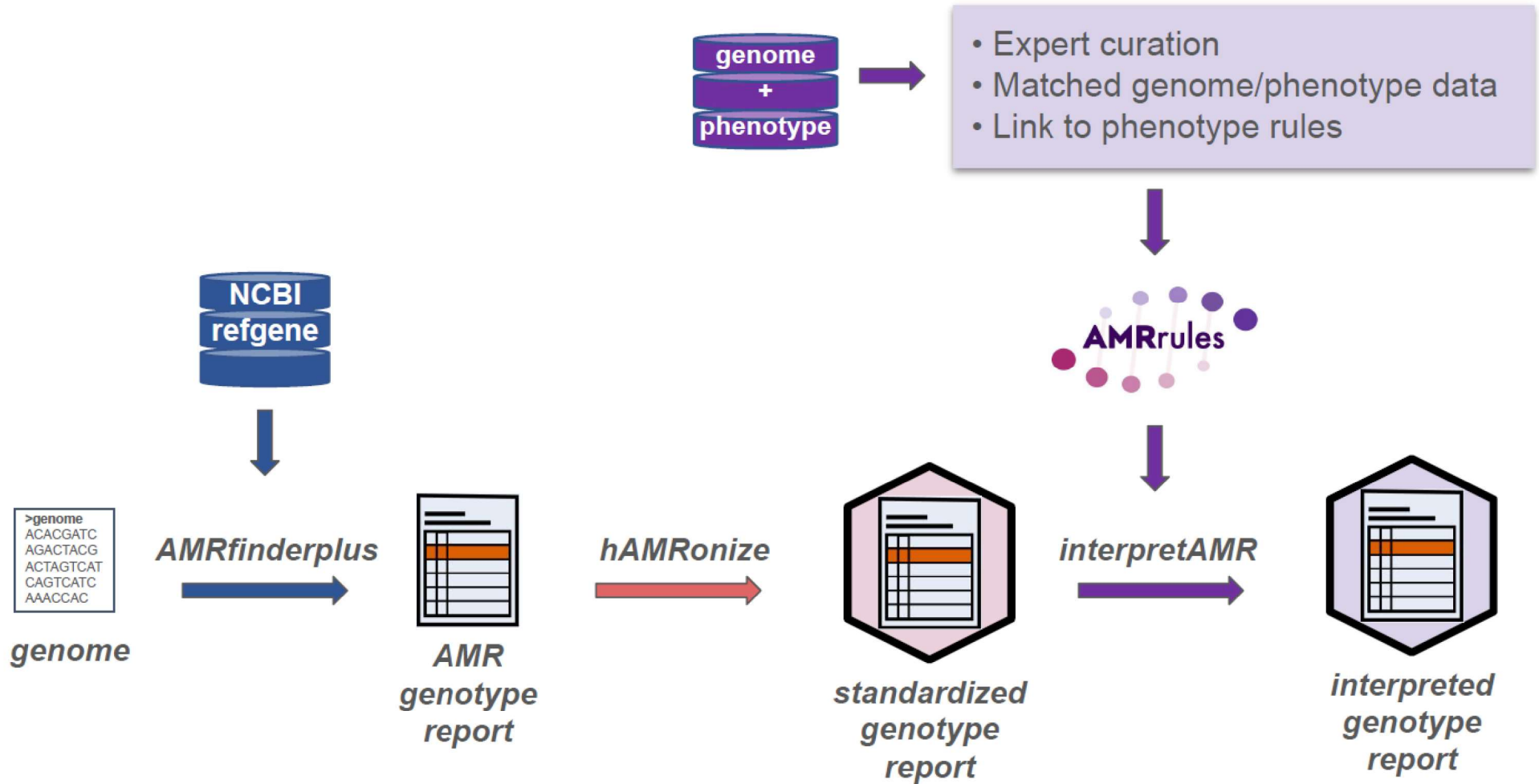


# Specification of interpretive rules

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	-	*
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	*
...	...	...	...	...	...	...







# What will the working group do?



- **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](https://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 priority organisms



1

## ESKAPEE pathogens

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

2

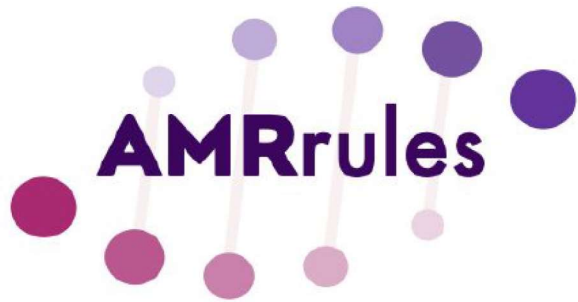
## Other organisms on the WHO Priority Pathogens list

- *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





## Want to join?



### 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](https://bit.ly/AMRRules)