ESGEM-AMR

ESCMID

AMRrules

Agenda

1. Summarise subgroup progress
2. Updates to the AMRrules specification (encoding complex variants)
3. Open issues & discussion
4. Future meetings
5. Any other business

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Organism Subgroups

1

ESKAPEE pathogens

- *Enterococcus*
- *Staphylococcus*
- *Klebsiella pneumoniae*
- *Acinetobacter baumannii*
- *Pseudomonas aeruginosa*
- *Enterobacter cloacae* complex
- *E. coli/Shigella*

2

Other WHO Priority Pathogens

- *Salmonella*
- *Serratia*
- *Neisseria gonorrhoeae*
- *Streptococcus*
- *Campylobacter* spp.
- *Mycobacterium tuberculosis*
- *Haemophilus influenzae*

3

Others

- *Achromobacter xylosoxidans*
- *Aeromonas*
- Anaerobes
- *Bordetella*
- *Brucella*
- *Burkholderia cepacia* complex
- *Burkholderia pseudomallei*
- *Chryseobacterium indologenes*
- *Corynebacterium diphtheriae*
- *Edwardsiella*
- *Legionella*
- *Listeria*
- *Mycoplasma pneumoniae*
- *Neisseria meningitidis*
- *Pasteurella*
- *Proteus mirabilis*
- *Shewanella*
- *Stenotrophomonas maltophilia*
- *Treponema*
- *Vibrio*
- *Yersinia*

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- *Proteus mirabilis*
- *Shewanella*
- *Stenotrophomonas maltophilia*
- *Treponema*
- *Vibrio*
- *Yersinia*

Have met
Scheduled a meeting
No scheduled meeting yet
Did not respond

How subgroups are prioritising work?

- Prioritisation of species (if multiple species within one genus)
- Prioritisation of antimicrobials (i.e., if EUCAST breakpoints exist or start with 1st line drugs)
- Divide and conquer (divide AMR mechanisms per member depending on expertise)

Encoding complex variants: Data & Tools + TB groups

Goal: to encode AMRrules for the following types of AMR variants:

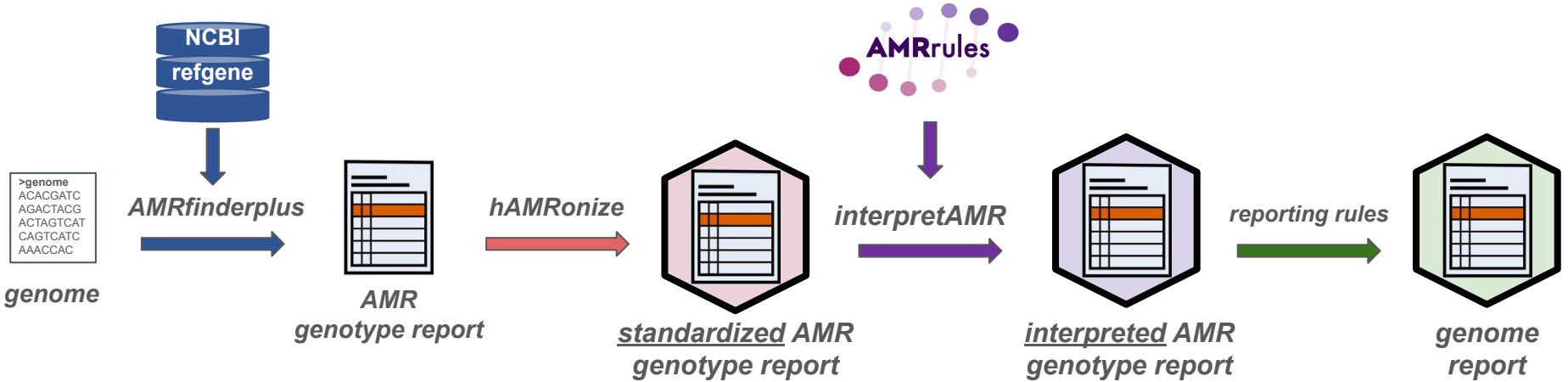
- Gene presence detected
- Amino acid substitution or insertion
- Nucleotide substitution or insertion
- Gene truncated (loss of function)
- Mutation in promoter region (substitution, deletion or insertion, including IS)
- Gene copy number changes
- Mutations in multi-copy genes (e.g. 23S rRNA)
- Low frequency variants (i.e. heterozygosity)

For mutations: use HGVS compliant standard where possible (https://hgvs-nomenclature.org/stable/)

Thanks to those who submitted examples for the group to consider!!
Yonatan Grad, Michael Feldgarden, Conor Meehan, Fernando Lázaro, Patrick Harris, Leonor Sánchez Busó (for *Neisseria* gono subgroup), Sylvain Brisse, Célia Rodrigues Betencourt

Concern for now is <u>how</u> to specify a variant that an AMRrule applies to

- Ultimately these need to be compatible / interoperable with upstream tools, including how variants are stored and labelled in **databases**, and how tools **identify** and **report** the variants.
- Data & Tools group members are involved in many of those upstream tools/DBs and will aim to **coordinate development of standards** across the ecosystem to improve interoperability generally (esp. PHA4GE, hAMRonization, CARD, NCBI).



github.com/interpretAMR/AMRrules

Specification of interpretive rules

Google sheet:
bit.ly/AMRrules_Spec03

organism	GTDB taxonomy
gene	NCBI AMR reference gene hierarchy
refseq accession	*Optional: NCBI RefSeq accession*
ARO accession	*Optional: CARD ARO accession*
mutation	Mutation within gene (may be '-')
variation type	Type of variant (e.g. gene detected, promoter variant)
context	Core or acquired
drug	Drug *OR* drug class *(WHO ATC Index)*
drug class	
category	**Expected phenotype category (wt/nwt and S/I/R)**
PMID	PubMed ID for supporting literature
rule curation note	*Optional: brief description of rule logic/mechanism*
breakpoint	Definition used to define category
breakpoint_standard	AST standard used (e.g. EUCAST 2024)
evidence level	*To be defined*
explanatory note	*To be defined*

All links at: github.com/interpretAMR/AMRrulesCuration/

Encoding simple variants

gene	mutation	variation type	drug	category
blaSHV	-	Gene presence detected	ampicillin	wt R
gyrA	p.Ser83Tyr	Protein variant detected	ciprofloxacin	nwt I
ompK36	c.25C>T	Nucleotide variant detected	meropenem	nwt S

Encoding simple variants

gene	mutation	variation type	drug	category
blaSHV	-	**Gene presence detected**	ampicillin	wt R
gyrA	p.Ser83Tyr	Protein variant detected	ciprofloxacin	nwt I
ompK36	c.25C>T	Nucleotide variant detected	meropenem	nwt S

Rule to interpret the presence of an acquired AMR gene

Variation type: 'Gene presence detected'

Mutation: '-'
(rule concerns presence/absence of gene, not a specific mutation within the gene)

Encoding simple variants

gene	mutation	variation type	drug	category
blaSHV	-	Gene presence detected	ampicillin	wt R
gyrA	p.Ser83Tyr	Protein variant detected	ciprofloxacin	nwt I
ompK36	c.25C>T	Nucleotide variant detected	meropenem	nwt S

Rule to interpret an amino acid substitution in the specified gene

Variation type: 'Protein variant detected'

Mutation: HGVS standard nomenclature, e.g. 'p.Ser83Tyr'

- 'p' indicates the specified mutation is in the protein sequence
- '83' indicates the mutated coordinate within the protein sequence
- 'Ser83Tyr' indicates a substitution of Serine with Tyrosine at amino acid 83

Encoding simple variants

gene	mutation	variation type	drug	category
blaSHV	-	Gene presence detected	ampicillin	wt R
gyrA	p.Ser83Tyr	Protein variant detected	ciprofloxacin	nwt I
ompK36	c.25C>T	Nucleotide variant detected	meropenem	nwt S

Rule to interpret an amino acid substitution in the specified gene

Variation type: Nucleotide variant detected'

Mutation: HGVS standard nomenclature, e.g. 'c.25C>T'

- 'c' indicates the specified mutation is in the coding DNA sequence
- '25' indicates the mutated coordinate within the coding DNA sequence
- 'C>T' indicates a substitution of Cytosine with Thymine at this coordinate

Encoding an in-frame insertion

gene	mutation	variation type	drug	category
ompK36	p.114_115insGlyAsp	Protein variant detected	meropenem	nwt I

Rule to interpret an in-frame insertion, specified at protein level

Variation type: 'Protein variant detected'

Mutation: HGVS standard nomenclature, e.g. 'p.114_115insGlyAsp'

- 'p' indicates the specified mutation is in the protein sequence
- '114_115' indicates the insertion is between amino acids 114 and 115
- 'insGlyAsp' indicates an insertion of amino acids Glycine and Aspartic acid

Note: in HGVS nomenclature, when specifying insertions, x_y indicates a range from x (inclusive) to y (exclusive)

Encoding a rule for inactivation / loss of a gene

gene	mutation	variation type	drug	category
mgrB	p.(1_100)	Inactivating mutation detected	colistin	nwt R

Rule to interpret inactivation or loss of a gene, regardless of the specific mechanism (e.g. could occur through deletion / frameshift / internal stop codon)

Variation type: 'Inactivating mutation detected' (not yet in hAMRonization, based on NCI Thesaurus Ontology: C178119)

Mutation: e.g. 'p.(1_100)'

- 'p' indicates the specified mutation is defined in relation to the protein sequence
- '(1_100)' indicates the inactivating mutation may occur anywhere within the first 100 amino acids (i.e., if the first 100 amino acids are not intact, the rule will apply)
    - E.g. if you know a particular domain must be intact, but a truncation after that has no effect, you could specify the end coordinates of the domain after which mutations can be tolerated

Note: in HGVS, x_y indicates a **specific range** from x to y at which the mutation occurs, (x_y) indicates uncertainty in the positions. We use this to specify a **wildcard range**, meaning the mutation may occur at any position within the range.

Encoding a rule for inactivation / loss of a gene

gene	mutation	variation type	drug	category
mgrB	-	Inactivating mutation detected	colistin	nwt R

1. If no mutation specified, implies that the rule applies to truncation/deletion of <u>any</u> part of the gene

Encoding a rule for inactivation / loss of a gene

gene	mutation	variation type	drug	category
mgrB	-	Inactivating mutation detected	colistin	nwt R
mgrB	p.Glu30*	Protein variant detected	colistin	nwt R

1. **If no mutation specified, implies that the rule applies to truncation/deletion of <u>any</u> part of the gene**

2. **Note rules should be applied hierarchically, from most specific to least specific**

- If there is a specific inactivating mutation in a gene, that has strong evidence to cause loss-of-function and consequence resistance, you may want to include this specifically.
- That way, the more specific rule can be linked to stronger evidence (codes to be decided) and specific references, that may differ from the evidence and literature support for the more general rule.

(Note * indicates stop codon; i.e. p.Glu30* indicates amino acid 30 replaced by a premature stop codon)

Encoding a promoter variant

gene	mutation	variation type	drug	category
ampC	c.-11C>T	Promoter variant detected	ceftriaxone	nwt R

Rule to interpret a substitution in the promoter region

Variation type: ‘Promoter variant detected’

Mutation: HGVS standard nomenclature, e.g. ‘c.-11C>T’

- ‘c’ indicates the mutation is specified relative to the coding DNA sequence
- ‘-11’ indicates the substitution occurs 11 bases upstream of the coding sequence
- ‘C>T’ indicates a substitution of Cytosine with Thymine at this coordinate

Encoding a promoter variant

gene	mutation	variation type	drug	category
ampC	c.-11C>T	Promoter variant detected	ceftriaxone	nwt R
ampC	c.-14_-13insGT	Promoter variant detected	ceftriaxone	nwt R

Rule to interpret an insertion in the promoter region

Variation type: 'Promoter variant detected'

Mutation: HGVS standard nomenclature, e.g. 'c.-14_-13insGT'

- 'c' indicates the mutation is specified relative to the coding DNA sequence
- '-14_-13' indicates the insertion occurs between 14 and 13 bases upstream of the coding sequence
- 'insGT' indicates an insertion of two bases, Guanine and Thymine, at this position

Encoding an IS insertion in the promoter

gene	mutation	variation type	drug	category
ampC	c.-11C>T	Promoter variant detected	ceftriaxone	nwt R
ampC	c.-14_-13insGT	Promoter variant detected	ceftriaxone	nwt R
blaOXA-58	c.(-35_1)ins[ISAba125:inv]	Promoter variant detected	ceftriaxone	nwt R

Mutation: HGVS syntax, e.g. 'c.(-35_1)ins[ISAba125:inv]'

- 'c' indicates the mutation is specified relative to the coding DNA sequence
- '(-35_1)' indicates the rule applies to an insertion occurring anywhere between 35 bases upstream of the coding sequence and the start of the coding sequence
- 'ins[ISAba125:inv]' indicates insertion of ISAba125, in reverse orientation
    - NOTE: the reference for IS names is ISfinder (https://isfinder.biotoul.fr/)

Encoding copy number variants

gene	mutation	variation type	drug	category
blaTEM	c.[3]	Gene copy number variant detected	piperacillin+ tazobactam	nwt R

Rule to interpret a copy number variant, i.e. when presence of multiple (≥N) copies is associated with a change in expected phenotype category

Variation type: 'Gene copy number variant detected'

Mutation: based on HGVS syntax for repeated sequence, e.g. 'c.[3]'

- 'c' indicates the mutation is specified relative to the coding DNA sequence
- '[3]' indicates at least 3 copies of the coding sequence are needed for the rule to apply

Encoding variants in multi-copy genes

gene	mutation	variation type	drug	category
23S rDNA	c.[2045A>G][3]	Nucleotide variant detected in multi-copy gene	azithromycin	nwt R

Rule to interpret a mutation in a multi-copy gene (e.g. 23S rRNA)

Variation type: ‘Nucleotide variant detected in multi-copy gene’

Mutation: based on HGVS syntax for heterozygous alleles, but specifying the minimum number of mutant alleles (rather than specifying the specific combination)

- ‘c’ indicates the mutation is specified relative to the coding DNA sequence
- ‘[2045A>G]’ indicates an allelic variant with substitution of Adenine with Guanine at coordinate 2045 within the coding DNA sequence
- ‘[3]’ indicates at least 3 copies of this allelic variant are needed for the rule to apply

Encoding low-frequency allelic variant

gene	mutation	variation type	drug	category
gyrA	p.[Ala94Gly][0.13]	Low frequency variant detected	ciprofloxacin	nwt R

Rule to interpret a low-frequency allelic variant, e.g. where reads data shows a mixed population of cells of which some fraction support the variant (TB only)

Variation type: ‘Low frequency variant detected’’

Mutation: based on HGVS syntax for heterozygous alleles, but specifying the minimum fraction of reads supporting the variant

- ‘p’ indicates the mutation is specified relative to the protein sequence
- ‘Ala94Gly’ indicates substitution of Alanine with Glycine at amino acid position 94
- ‘[0.13]’ indicates the rule applies when at least 13% of reads carry this variant

Encoding combinatorial rules

ruleID	gene	mutation	variation type	drug	category
KPN0002	oqxA	-	Gene presence detected	ciprofloxacin	wt S
KPN0008	gyrA	p.Ser83Tyr	Protein variant detected	ciprofloxacin	nwt I
KPN0009	parC	p.Ser80Ile	Protein variant detected	ciprofloxacin	nwt I
KPN0013	qnr	-	Gene presence detected	ciprofloxacin	nwt I
KPN0051	KPN0008 & KPN0009	-	Combination	ciprofloxacin	nwt R
KPN0052	(KPN0008 | KPN0009) & KPN0013	-	Combination	ciprofloxacin	nwt R

unique identifier for each rule (3-letter prefix per organism subgroup) then numbers (unique within subgroup)

Encoding combinatorial rules

ID	gene	mutation	variation type	drug	category
KPN0002	oqxA	-	Gene presence detected	ciprofloxacin	wt S
KPN0008	gyrA	p.Ser83Tyr	Protein variant detected	ciprofloxacin	nwt I
KPN0009	parC	p.Ser80Ile	Protein variant detected	ciprofloxacin	nwt I
KPN0013	qnr	-	Gene presence detected	ciprofloxacin	nwt I
KPN0051	KPN0008 & KPN0009	-	Combination	ciprofloxacin	nwt R
KPN0052	(KPN0008 | KPN0009) & KPN0013	-	Combination	ciprofloxacin	nwt R

- ‘gene’ column can be a logical expression defining combinations of variants
- ‘ruleIDs’ may be used as shorthand labels for variants in these logical expressions
  - e.g. KPN0008 is shorthand label for ‘gyrA:p.Ser83Tyr’
  - e.g. KPN0013 is shorthand label for ‘qnr (Gene presence detected)’

Encoding combinatorial rules - operons

ID	gene	mutation	variation type	drug	category
PSA0001	mexA	-	Gene presence detected	ampicillin	nwt S
PSA0002	mexB	-	Gene presence detected	ampicillin	nwt S
PSA0003	oprM	-	Gene presence detected	ampicillin	nwt S
PSA0013	PSA0001 & PSA0002 & PSA0003	-	Combination	ampicillin	wt R

The presence of any of these genes on their own should be interpreted as susceptible, as the full operon is required for resistance

Encoding combinatorial rules - operons

ID	gene	mutation	variation type	drug	category
PSA0001	mexA	-	Gene presence detected	ampicillin	nwt S
PSA0002	mexB	-	Gene presence detected	ampicillin	nwt S
PSA0003	oprM	-	Gene presence detected	ampicillin	nwt S
PSA0013	PSA0001 & PSA0002 & PSA0003	-	Combination	ampicillin	wt R

The presence of any of these genes on their own should be interpreted as susceptible, as the full operon is required for resistance

Encoding combinatorial rules - operons

ID	gene	mutation	variation type	drug	category
ENT0001	vanA	-	Gene presence detected	vancomycin	wt S
ENT0002	vanHA	-	Gene presence detected	vancomycin	wt S
ENT0003	vanRA	-	Gene presence detected	vancomycin	wt S
ENT0004	vanSA	-	Gene presence detected	vancomycin	wt S
ENT0005	vanXA	-	Gene presence detected	vancomycin	wt S
ENT0006	vanYA	-	Gene presence detected	vancomycin	wt S
ENT0007	vanZA	-	Gene presence detected	vancomycin	wt S
ENT0008	ENT0001 & ENT0002 & ENT0003 & (ENT0004 | ENT0005 | ENT0006 | ENT0007)		Combination	vancomycin	nwt R

Variant type

	The specified AMRrule applies if...
Gene presence detected	...the gene specified in the 'gene' column is detected as being present.
Protein variant detected	...the protein variant specified in the 'mutation' column is detected in the specified 'gene'.
Nucleotide variant detected	...the nucleotide variant specified in the 'mutation' column is detected in the specified 'gene'.
Promoter variant detected	...the promoter variant specified in the 'mutation' column is detected in the specified 'gene'.
Inactivating mutation detected	...the gene specified in the 'gene' column is inactivated by any type of mechanism (e.g. frameshift, internal stop, deletion, truncation), in the amino acid range specified in the 'mutation' column (or anywhere in the gene, if the 'mutation' column is blank i.e. '-').
Gene copy number variant detected	...the gene specified in the 'gene' column is detected in at least the minimum number of copies specified in the 'mutation' column.
Nucleotide variant detected in multi-copy gene	...the gene specified in the 'gene' column is a gene that is normally present in multiple copies (e.g. rRNA genes), and the nucleotide variant specified in the 'mutation' column is detected in at least the minimum number of alleles specified in the 'mutation' column.
Low frequency variant detected	...the reads data supports a mixed population, for which a minimum fraction specified in the 'mutation' column support the presence of the nucleotide variant specified in the 'mutation' column being present in the gene specified in the 'gene' column (currently intended for TB only).
Combination	...the logical expression in the 'gene' column, which expresses a combination of component rules identified by their 'ruleID', evaluates as true.

Examples: mutations in multi-copy 23S rRNA

organism	gene	mutation	variation type	drug	category	rule curation note	PMID
s__Neisseria_gonorrhoeae	23S rRNA	c.[2045A>G][3]	Nucleotide variant detected in multi-copy gene	azithromycin	nwt I	23S rRNA is present in multiple copies. This mutation in 3 or more copies is associated with higher MICs.	
s__Escherichia_coli	23S rRNA	c.[2059A>G][3]	Nucleotide variant detected in multi-copy gene	azithromycin	nwt I	23S rRNA is present in multiple copies. This mutation in 3 or more copies is associated with MIC >R.	
s__Escherichia_coli	23S rRNA	c.[2059A>G][5]	Nucleotide variant detected in multi-copy gene	azithromycin	nwt R	23S rRNA is present in multiple copies. This mutation in 5 or more copies is associated with MIC >R.	
s__Escherichia_coli	23S rRNA	c.[2611C>T][3]	Nucleotide variant detected in multi-copy gene	azithromycin	nwt I	23S rRNA is present in multiple copies. This mutation, or 2059A>G, in 3 or more copies is associated with higher MICs.	
s__Escherichia_coli	23S rRNA	c.[2059A>G ^ 2611C>T][3]	Nucleotide variant detected in multi-copy gene	azithromycin	nwt I	23S rRNA is present in multiple copies. If mutations of 2059A>G or 2611C>T are present in 3 or more copies, this is associated with MIC >I.	

“N. gonorrhoeae has 4 copies of the 23S rDNA gene, and from 1 to 4 can be mutated. More copies mutated is usually associated with higher MICs. Assemblies generated from Illumina sequencing usually collapse the four copies into one, so only strains with 3-4 copies mutated are usually properly called as having the 2045A>G (2059A>G in E. coli) or (2611C>T in E. coli) substitutions. Assemblies generated from long-read data do have the 4 23S rDNA copies and the number of mutated alleles could be called from them.”

Examples: inactivating mutations (typically of repressors)

organism	gene	mutation	variation type	drug	category	rule curation note	PMID
s__Neisseria_gonorrhoeae	mtrR	-	Inactivating mutation detected	azithromycin	nwt S	The disruption of the mtrR (repressor) causes an overexpression of the MtrCDE efflux pump and has been associated to a decreased susceptibility to different antimicrobials in N. gonorrhoeae	27432602
s__Mycobacterium_tuberculosis	mmpR	-	Inactivating mutation detected	bedaquiline	nwt I	Loss of function of the mmpl5 efflux pump repressor, mmpR, can greatly increase resistance to bedaquiline.	34460306
s__Mycobacterium_tuberculosis	mmpR	c.138_139insG	Nucleotide variant detected	bedaquiline	nwt I	Insertion causes loss of function of the mmpl5 efflux pump repressor, mmpR, which can greatly increase resistance to bedaquiline.	34460306

“The disruption of the mtrR (repressor) causes an overexpression of the MtrCDE efflux pump and has been associated to a decreased susceptibility to different antimicrobials in N. gonorrhoeae, such as macrolides and B-lactams. doi:10.1093/jac/dkw288.”

“Loss of function mutations have been found to influence resistance to Bedaquiline because it is mutations in the Mmpl5 efflux pump repressor which increase pump activity and cause resistance. LOF of the repressor can greatly increase resistance. The table 1 (page 6) in the WHO catalogue (https://www.who.int/publications/i/item/9789240082410) outlines the current LOF and other 'additional grading rules' applied for Mtb. There are additional epistatic rules where mutations and LOF interact to revert resistance to susceptibility. Some of these are in the table 1 as mentioned above and also a good example is this paper; https://pubmed.ncbi.nlm.nih.gov/34460306/”

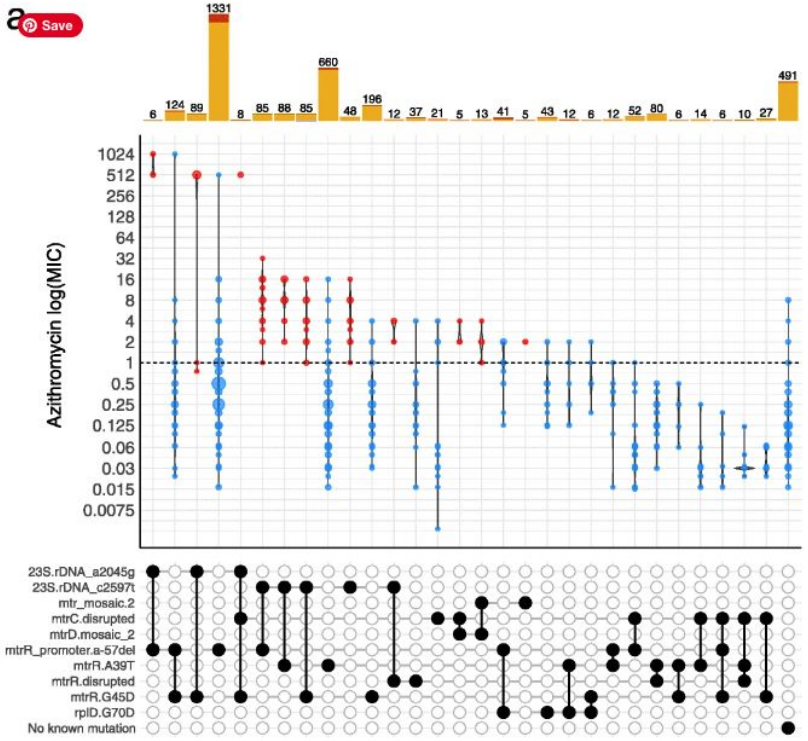
“Chromosomally encoded and inducible ampC beta-lactamase; induced by various beta-lactams giving rise to intrinsic resistance to Amp/Aug/Cephazolin (easy to predict) but resistance to 3rd gen cephs (e.g. ceftriaxone/ceftazidime) is dependent on mutations in regulatory genes, leading to AmpC de-repression and high-level expression”

Examples: combinations

ruleID	gene	mutation	variation type	drug	category
NGO1	mtrR	c.-57del	Promoter variant detected	azithromycin	nwt S
NGO2	mtrR	p.Ala39Tyr	Protein variant detected	azithromycin	wt S
NGO3	mtrR	p.Gly45Asp	Promoter variant detected	azithromycin	wt S
NGO4	23S rRNA	c.[2045A>G][1]	Nucleotide variant detected	azithromycin	?
NGO5	NGO1 & NGO4	-	Combination	azithromycin	nwt R
NGO6	NGO3 & NGO4	-	Combination	azithromycin	nwt R

“Multiple variations in the promoter of mtrR (repressor of the MtrCDE efflux pump) are associated with an overexpression of the pump and a decreased susceptibility to different antimicrobials. However, these do not have a big effect on their own, are usually additive. These are, e.g. -56A>C, -57delA, -131G>A (mtr120) and also some other insertions (+TT or +T) or deletions (-T).
https://doi.org/10.1186/s13073-021-00858-2
https://doi.org/10.1093/jac/dkw288
https://doi.org/10.1093/jac/47.5.651”

“The disruption of the mtrC gene increases susceptibility to azithromycin, so decreases MICs when some other azithromycin-resistance mutations are present.
https://doi.org/10.1038/s41467-020-17980-1.”



https://doi.org/10.1186/s13073-021-00858-2

Examples: minor shifts in MIC, in vitro evidence only

organism	gene	mutation	variation type	drug	category	rule curation note	PMID
s__Neisseria_gonorrhoeae	macAB	c.-48G>T	Promoter variant detected	azithromycin	nwt S	Identified in vitro, not yet reported in clinical strains.	16162665
s__Neisseria_gonorrhoeae	norM	c.-7A>G	Promoter variant detected	ciprofloxacin	nwt I	Associated with increased efflux, resulting in decreased susceptibility to ciprofloxacin in vitro, not yet reported in clinical strains.	12533487
s__Neisseria_gonorrhoeae	norM	c.-10C>T	Promoter variant detected	ciprofloxacin	nwt I	Associated with increased efflux, resulting in decreased susceptibility to ciprofloxacin in vitro, not yet reported in clinical strains.	12533487
s__Acinetobacter_baumannii	ampC	xxx	Protein variant detected	cefiderocol	nwt S		

“A particular mutation in the promoter of macAB (-48G>T) - efflux system - has been associated with a decreased susceptibility to azithromycin in vitro, although not in clinical strains. However, we usually keep the mutation in the databases just in case.
DOI: 10.1093/jac/dki333”

"Particular mutations in the promoter of norM (-7A>G, -104C>T) - efflux system - has been associated with a decreased susceptibility to ciprofloxacin in vitro, although not in clinical strains. However, we usually keep the mutation in the databases just in case.
doi: 10.1128/JB.185.3.1101-1106.2003"

“I have seen several mutations in the AmpC of A. baumannii that do not cause direct change in the susceptibility interpretation (remains as S) but do increase the MIC. These mutations are usually a first step to develope a second mutations that increases the MIC above the breakpoint.
So, to sum up, there are mutations that do not cause direct resistance to cefiderocol, but are clearly related with a high risk of selecting resistance mutants.”

Examples: upregulation of core beta-lactamase

organism	gene	mutation	variation type	drug	category	rule curation note	PMID
s__Campylobacter_jejuni	blaOXA-61	c.-57G>T	Promoter variant detected	ampicillin	nwt R	The resistant phenotype has been linked to a G to T point mutation in the promoter region of the blaOXA gene	24408987
s__Acinetobacter_baumannii	blaOXA-58	c.(-35_1)ins [ISAba125:inv]	Promoter variant detected	carbapenems	nwt R	Insertions of ISAba125 in reverse orientation, in the promoter region of core gene blaOXA-58,, result in increased expression of the enzyme resulting in carbapenem resistance.	24696435
s__Corynebacterium_diphtheriae	pbp2m	c.[3]	Gene copy number variant detected	meropenem	nwt I	Tandem repeats of Tn3503 carrying pbp2m are associated with reduced susceptibility. Isolates with 3 copies had low-level resistance.	32772111
s__Corynebacterium_diphtheriae	pbp2m	c.[16]	Gene copy number variant detected	meropenem	nwt R	Tandem repeats of Tn3503 carrying pbp2m are associated with reduced susceptibility. Isolates with 16 copies had MIC >32 mg/L.	32772111

“The presence of blaOXA genes alone, sich as blaOXA-61, in Campylobacter, does not necessarily mean ampicillin resistance. The resistant phenotype has been linked to a G to T point mutation in the promoter region of the blaOXA gene. This mutation restores the TATA box.
Publications:
https://pubmed.ncbi.nlm.nih.gov/24408987/ - original discovery
https://www.nature.com/articles/s41598-021-88318-0”

“When located upstream and in the opposite orientation to a blaOXA-58 family gene, the element provides a promoter that increases gene expression; PMID: 24696435”

“pbp2m (also called pbp2c) can be found duplicated/multiplied in tandem during treatment or in-vitro; stability of tandem repeats is poorly known and precise link with phenotype too (see e.g., https://pubmed.ncbi.nlm.nih.gov/32772111/)”

Other issues arising from the call for complex examples

"Mosaic sequences generated by recombination in the penA (penicillin-binding protein 2) gene are associated with decreased susceptibility to cephalosporins (cefixime and ceftriaxone). Mosaics are defined in the NG-STAR database https://ngstar.canada.ca/alleles/penA, however, not all mosaics are associated with resistance. Some mutations are indicators of mosaic, but not always of mosaic types."

"Mosaic sequences generated by recombination in genes encoding the MtrCDE efflux pump or its repressor (mtrR) are associated with decreased susceptibility to azithromycin. There is not a database of mosaic types in this case and not all mosaics are associated with resistance. Some mutations are indicators of mosaic but not always.
doi: 10.1128/mBio.01419-18"

Feedback from Data & Tools group discussion:

Could the *Neisseria gonorrhoeae* subgroup propose a method for calling and interpreting mosaic alleles?

- Tools/DBs are keen to incorporate these if the community can come to consensus.
- Model 1: Could labels be assigned to each allele, and these placed in a DB that can track their association with resistance? (similar to *pbp* alleles in Streptococcus, or *bla* genes)
- Model 2: Could the specific mutations of functional relevance be identified and tracked/typed? (similar to pbp4 mutations in Staphylococcus)

Reminder about Technical Guidance

- Updated to cover latest spec v0.3 including updates to specifying complex variants and combinatorial rules (discussed today)
- ‘Suggested Protocol section’
- ‘Existing curations’
- ‘Open Issues’ section

Technical Guidance: latest version (v0.3)
bit.ly/AMRrules_Tech

All links at: github.com/interpretAMR/AMRrulesCuration/

Reminder about using NCBI gene hierarchy

- The gene name specified in the ‘gene’ column for an AMRrule should be a leaf node or internal node in the NCBI Gene Hierarchy (https://www.ncbi.nlm.nih.gov/pathogens/genehierarchy)
- AMRFinderPlus uses these nodes internally but doesn’t report the node name by default, you need to switch this on using `--print_node`
- Example command for running AMRFinderPlus:

```
amrfinder -n genomeA.fasta --plus --print_node --organism Acinetobacter_baumannii --name genomeA
```

See Technical guidance (page 5):
bit.ly/AMRrules_Tech

All links at: github.com/interpretAMR/AMRrulesCuration/

Qs from Enterococcus group

- In the absence of known genetic mechanisms of expected resistance, can we still assume and predict expected resistance?
- Considering the remit of ESGEM-AMR is to make “clinical predictions”, we understand EUCAST clinical breakpoints need to be prioritised when defining rules. How do other subgroups plan to prioritise what breakpoints to use?
- In addition to MIC values, are we expected to use disk diffusion inhibition zones when defining rules?
- Should we leave aside antibiotics that are of less clinical value against enterococci (e.g., the aminoglycosides and tetracyclines) when defining rules? How do other subgroups plan to prioritise antibiotics?

Open issues to consider

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- **Evidence levels** - balance mechanistic vs correlation, extrapolation from other organisms
- Genome quality
- AST data quality - most data will be from automated platforms
- Definition of core genes? - minimum number, diversity, threshold proportion?
- Which drugs should be included for a given organism?
- What if there's no breakpoint?
- What if there's multiple breakpoints?
- What if the breakpoint cuts the MIC distribution in half?
- When 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?
- Quantitative rules? OR [95% CI]

AMRrules

Schedule for Update Meetings

ESCMID | ESGEM-AMR

September 12/13

- Focus: Specification of complex rules (v0.3)

October 23/24 (+ ASM NGS meetup in DC, Oct 14?)

- Focus: Evidence codes

November/December TBD

January TBD

February TBD

March TBD

April 11-15 - ESCMID Global (Vienna meetup?)

Standing Agenda for Update Meetings

- Updates from subgroups
- Issues emerging related to protocols, rule specification, etc
- Planning for outputs, conferences, funding

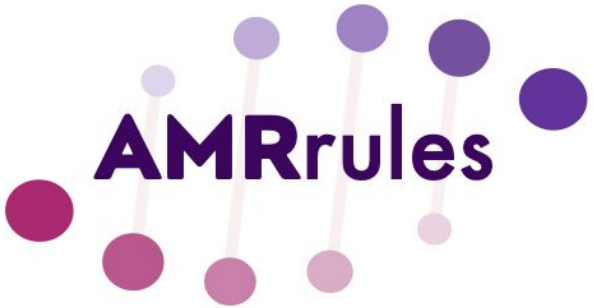
Attendees

- Open to all, but not compulsory
- One spokesperson per subgroup (lead or delegate)

Questions? / Any other business?

ESGEM-AMR

ESCMID



https://github.com/interpretAMR/AMRrulesCuration

Have you joined slack?

If you need the link, email esgem.amr@gmail.com

Requests to add members

- Any new additions need to be considered by the Chairs and the relevant subgroup lead
- We will consider a second call for volunteers for orphan bugs, and perhaps for more data for existing subgroups, after we see how things are progressing
- **All members need to be properly registered and sign the MOU**
- **Do not share the slack invite, or any Zoom links for ESGEM-AMR or its subgroups, with anyone who has not signed the MOU**