ESGEM-AMR

ESCMID

AMRrules

Agenda

1. **Data sharing, Public AST & AMRfinderplus data**
2. Gene identifiers
    a. Node hierarchy
    b. If there is no node?
3. Evidence codes
4. Group updates
5. Open issues & discussion
6. Next meeting

Data sharing

Susceptibility data: use NCBI's template available at https://www.ncbi.nlm.nih.gov/biosample/docs/antibiogram/

AMRfinderplus output (record version used)

Additional data (to ensure diversity):

- Typing (for example ST)
- Country of isolation
- Isolation type
- Short-read/Long-read sequencing and assembler

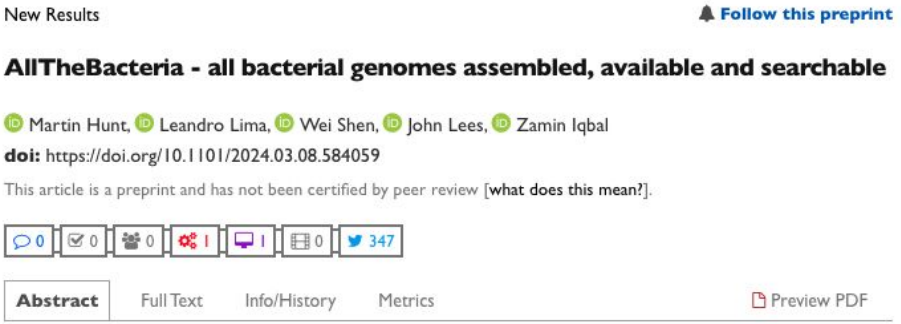
Public AST & genome resources

1. **AllTheBacteria** project has posted data for >2M public genomes (Aug 2024)

- **AMRfinderplus** (v3.12.8) results for all genomes (Jane Hawkey): https://osf.io/zgexh
- **Species calls** were made using GTDB (via tool sylph): https://ftp.ebi.ac.uk/pub/databases/AllTheBacteria/Releases/0.2/metadata/species_calls.tsv.gz

Source: https://github.com/AllTheBacteria/AllTheBacteria

Info: https://allthebacteria.readthedocs.io/en/latest/



Public AST & genome resources

2. Github repository showing how to match up AMRfinderplus and ATB species:

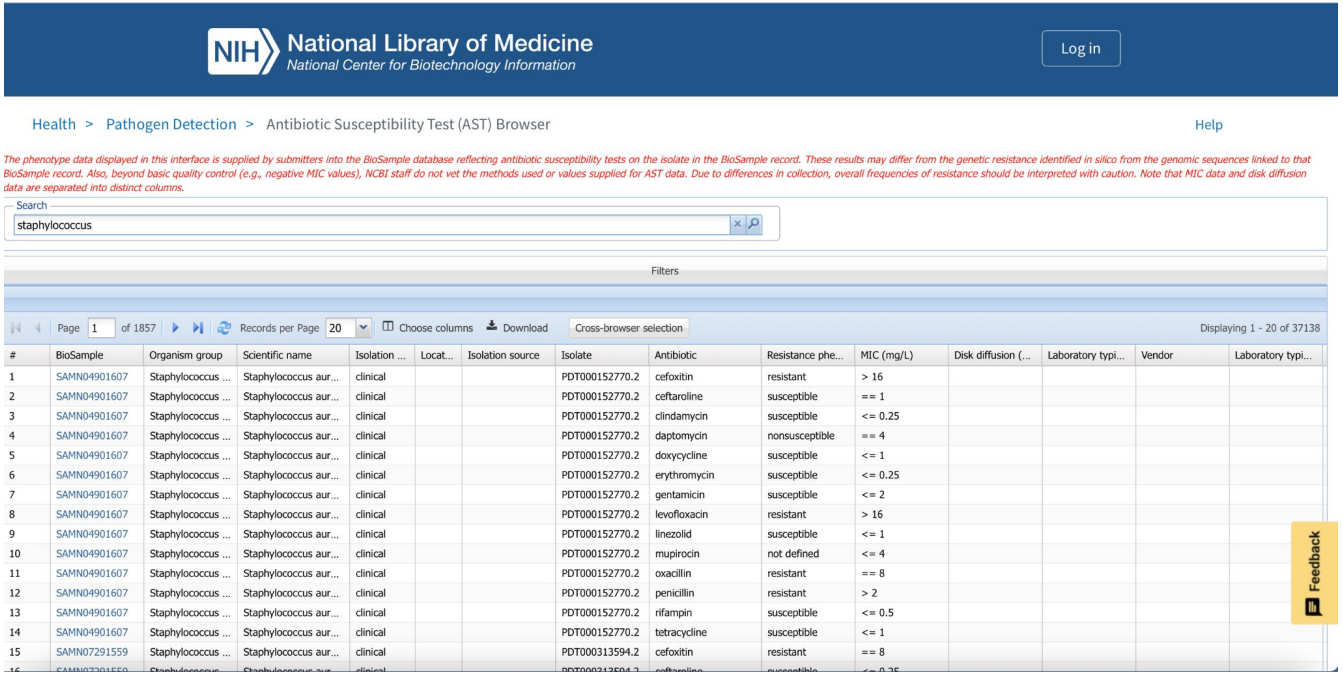
- https://github.com/interpretAMR/datacuration

```
# get list of E. coli samples
species_calls <- read_tsv("AllTheBacteria/ATB_species_calls.tsv.gz")
ecoli <- species_calls %>% filter(Species=="Escherichia coli") %>% pull(Sample)

# read in only those lines matching these samples
f <- function(x, pos) subset(x, Name %in% ecoli)
ecoli_AFP <- read_tsv_chunked("AMRFP_results.tsv.gz", DataFrameCallback$new(f), chunk_size = 1

# select key columns to keep output file size small-ish
ecoli_AFP %>% select(Name, `Gene symbol`, `Hierarchy node`, Class, Subclass, `% Coverage of re
  write_tsv(file="ATB_Ecoli_AFP.tsv.gz")
```

Or you can use the function `atb_amrfp_filter_by_taxa` in the file `AllTheBacteria_functions.R`

Public AST & genome resources

3. NCBI AST browser: https://www.ncbi.nlm.nih.gov/pathogens/ast/



#	BioSample	Organism group	Scientific name	Isolation ...	Locat...	Isolation source	Isolate	Antibiotic	Resistance phe...	MIC (mg/L)	Disk diffusion (...	Laboratory typi...	Vendor	Laboratory typi...
1	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	cefoxitin	resistant	> 16				
2	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	ceftaroline	susceptible	== 1				
3	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	clindamycin	susceptible	<= 0.25				
4	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	daptomycin	nonsusceptible	== 4				
5	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	doxycycline	susceptible	<= 1				
6	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	erythromycin	susceptible	<= 0.25				
7	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	gentamicin	susceptible	<= 2				
8	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	levofloxacin	resistant	> 16				
9	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	linezolid	susceptible	<= 1				
10	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	mupirocin	not defined	<= 4				
11	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	oxacillin	resistant	== 8				
12	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	penicillin	resistant	> 2				
13	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	rifampin	susceptible	<= 0.5				
14	SAMN04901607	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000152770.2	tetracycline	susceptible	<= 1				
15	SAMN07291559	Staphylococcus ...	Staphylococcus aur...	clinical			PDT000313594.2	cefoxitin	resistant	== 8				

Public AST & genome resources

4. Combine AST data with AMRfinderplus results:

- https://github.com/interpretAMR/datacuration

  R code with examples using public AST data, and AMRfinderplus results, to explore core genes and the association of AMR genotypes with phenotypes.

  `Enterobacter/Enterobacter.Rmd` (output in `Enterobacter/Enterobacter.html`)

  `Pseudomonas/Pseudomonas_aeruginosa.Rmd` (output in `Pseudomonas/Pseudomonas_aeruginosa.html`)

  `functions.R` (functions for comparing AST vs AMRfp genotype data, including assessing solo positive-predictive value per marker, and fitting and plotting logistic regression for a given drug and associated markers)

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Reminder about using NCBI gene hierarchy

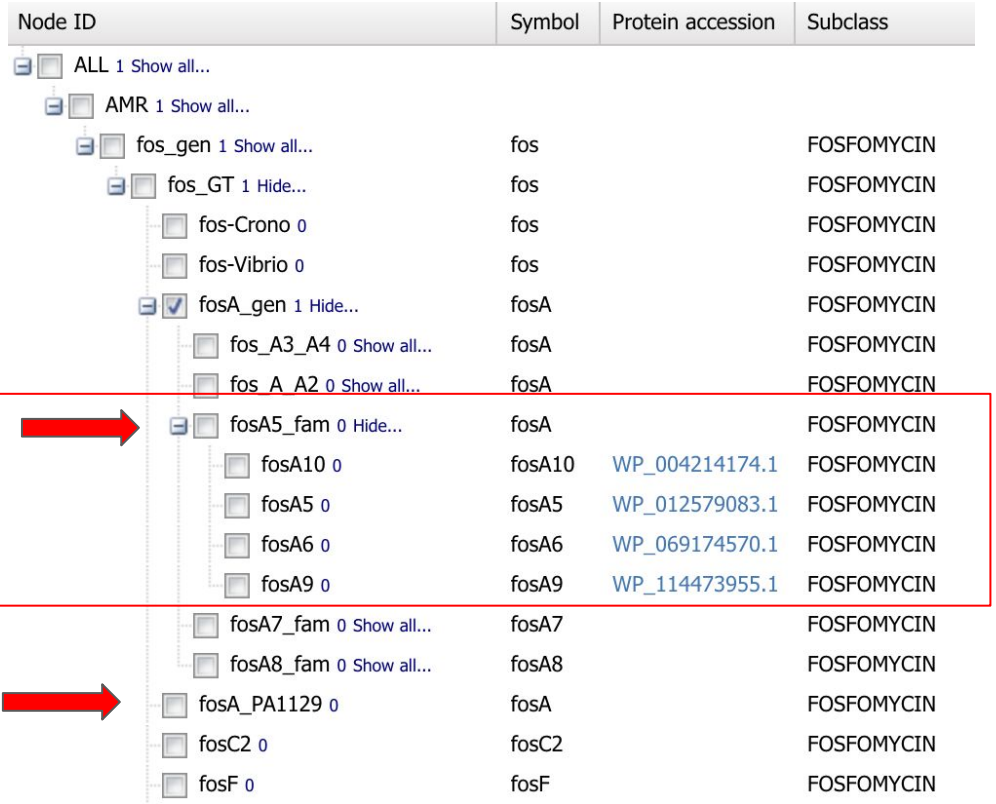
- The gene name specified in the ‘gene’ column for an AMRrule should be a leaf node or internal node in the NCBI Gene Hierarchy (https://www.ncbi.nlm.nih.gov/pathogens/genehierarchy)
- AMRFinderPlus uses these nodes internally but doesn’t report the node name by default, you need to switch this on using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

Gene identifiers: node hierarchy



Klebsiella pneumoniae core gene

Pseudomonas aeruginosa core gene

Gene identifiers: node hierarchy

organism	gene	variation type	context
required	required	required	required
s__Klebsiella pneumoniae	fosA5_fam	Gene presence detected	core
s__Pseudomonas aeruginosa	fosA_PA1129	Gene presence detected	core

Different hierarchy nodes for core fosA genes in *K. pneumoniae* vs *P. aeruginosa*

Gene identifiers: node hierarchy

Gene symbol	Class	Subclass	Hierarchy node	n	freq
mexA	EFFLUX	EFFLUX	mexA	24843	100%
fosA	FOSFOMYCIN	FOSFOMYCIN	fosA_PA1129	24690	99%
aph(3')-IIb	AMINOGLYCOSIDE	KANAMYCIN	aph(3')-IIb	24647	99%
mexE	EFFLUX	EFFLUX	mexE	24638	99%
mexX	EFFLUX	EFFLUX	mexX	24470	98%
catB7	PHENICOL	CHLORAMPHENICOL	catB7	24153	97%
crpP	FLUOROQUINOLONE	FLUOROQUINOLONE	crpP	15686	63%

Example: hierarchy nodes for the most common markers called by AMRfinderplus in n=24854 *Pseudomonas aeruginosa* (code in /datacuration repository)

Gene identifiers: node hierarchy

Example: hierarchy nodes for *fosA* alleles called in *Klebsiella pneumoniae* (n=57,070 genomes from AllTheBacteria)

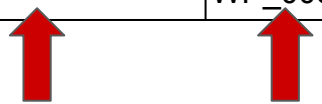
core	acquired	n
fosA5_fam	-	51223
fosA9	-	30
fosA10	-	1806
fosA5	-	789
fosA5_fam	fosA7	2311
fosA5_fam	fosA4	12
fosA5_fam	fosA3	664
fosA10	fosA3	12

Gene identifiers: what if there is no node in the hierarchy?

Update to spec v0.4:

- explicit nodeID field, distinct from gene
- alternative identifiers, must have at least one of node/refseq/genbank/HMM

organism	gene	nodeID	refseq accession	GenBank accession	HMM accession	ARO accession
required	required	require AT LEAST ONE OF: nodeID (preferred) or refseq or GenBank or HMM accession				optional (recommended)
s__Klebsiella pneumoniae	fosA5_fam	fosA5_fam	NF040540.1	-	-	-
s__Pseudomonas aeruginosa	fosA_PA1129	fosA_PA1129	WP_038415208.1	-	-	-
s__Pseudomonas aeruginosa	mexA	mexA	-	AAA74436.1	-	ARO:3000377
s__Pseudomonas aeruginosa	mexB	-	WP_003107312.1	-	-	ARO:3000378



AMRrules ESCMID | ESGEM-AMR

Rules Specification

Google sheet:
bit.ly/AMRrules_Spec04

ruleID	required
organism	required *(GTDB)*
gene	required *(NCBI node)*
nodeID	require AT LEAST ONE OF: nodeID (preferred) or refseq or GenBank or HMM accesion
refseq accession	
GenBank accession	
HMM accession	
ARO accession	Optional *(CARD)*
mutation	required *(HGVS+AMRrules)*
variation type	required *(AMRrules)*
context	required *(core/acquired)*
drug	require ONE OF drug or drug class *(WHO ATC Index)*
drug class	
category	required *(wt/nwt S/I/R)*
breakpoint	required *(definition used)*
breakpoint_standard	required *(reference standard)*
PMID	required *(pubmed)*
rule curation note	optional *(free text)*

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

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Recording evidence to support a rule

- **Evidence code**
  - What is the nature of the evidence supporting the assertion?
  - Evidence and Conclusion Ontology (ECO): www.evidenceontology.org
- **Evidence grade**
  - What is the overall strength of the evidence in supporting the assertion?

PMID	rule curation note	evidence_code	evidence_grading
required	optional (recommended)	required	required

Evidence codes - what evidence supports the rule?

Evidence description relevant to AMR	ECO code
Gene knockout	ECO:0001091 knockout phenotypic evidence
Complementation (e.g. of a knockout, or in a different strain/species)	ECO:0000012 functional complementation evidence
Gene is mutated to form the allelic variant and then shown to confer resistance to the drug	ECO:0001113 point mutation phenotypic evidence
Gene product extracted from that organism is shown to bind to, or have activity against, the drug	ECO:0000024 protein-binding evidence ECO:0001034 crystallography evidence ECO:0000005 enzymatic activity assay evidence
Gene has been transferred from one organism into another	ECO:0000042 gain-of-function mutant phenotypic evidence
Mutant library screen	ECO:0007000 high throughput mutant phenotypic evidence
Inferred from comparison of phenotypes between natural variants (e.g. statistical analysis of genotype vs AST)	ECO:0001103 natural variation mutant evidence

Note: further discussions to be had about the quantitative support for geno/pheno associations.

Evidence grade - how conclusive is the evidence?

Expert curators' overall assessment of the level of support provided by all evidence considered.

Qs to consider:

- Qualitative or quantitative?
- How granular should it be?
- How specific should definitions be?

Note that quantitative evidence of geno-pheno association will be dealt with separately.

This is about a subjective assessment of the total weight of evidence for the interpretation of gene/variant X for drug Y in species Z

Evidence grade - how conclusive is the evidence?

Expert curators' overall assessment of the level of support provided by all evidence considered.

Confidence scoring system currently employed for metabolic reconstructions

Evidence type	Confidence score	Examples
Biochemical data	4	Direct evidence for gene product function and biochemical reaction: protein purification, biochemical assays, experimentally solved protein structures and comparative gene-expression studies
Genetic data	3	Direct and indirect evidence for gene function: knockout characterization, knock-in characterization and overexpression
Physiological data	2	Indirect evidence for biochemical reactions based on physiological data: secretion products or defined medium components serve as evidence for transport and metabolic reactions
Sequence data	2	Evidence for gene function: genome annotation and SEED annotation
Modeling data	1	No evidence is available, but reaction is required for modeling. The included function is a hypothesis and needs experimental verification. The reaction mechanism may be different from the included reaction(s)
Not evaluated	0	

https://www.nature.com/articles/nprot.2009.203/tables/2

Evidence grade - how conclusive is the evidence?

Expert curators' overall assessment of the level of support provided by all evidence considered.

What is GRADE?

GRADE (Grading of Recommendations, Assessment, Development, and Evaluations) is a transparent framework for developing and presenting summaries of evidence and provides a systematic approach for making clinical practice recommendations.[1][2][3] It is the most widely adopted tool for grading the quality of evidence and for making recommendations with over 100 organisations worldwide officially endorsing GRADE.

Certainty	What it means
Very low	The true effect is probably markedly different from the estimated effect
Low	The true effect might be markedly different from the estimated effect
Moderate	The authors believe that the true effect is probably close to the estimated effect
High	The authors have a lot of confidence that the true effect is similar to the estimated effect

Table 1. GRADE certainty ratings

Evidence grade - how conclusive is the evidence?

Expert curators' overall assessment of the level of support provided by all evidence considered.

Option 1: Strong/Moderate/Weak - with explicit reason flags for 'weak'

Strong	Experimental evidence provides strong support for the interpretation of this gene/variant in this species for this drug; and no conflicting or missing evidence.
Moderate	There is good evidence to support the interpretation of this gene/variant ~~in this species~~ for this drug, but it is not conclusive (e.g. incomplete or conflicting evidence; protein-drug interaction is supported but there is a lack of direct evidence in this organism; or statistical geno/pheno evidence without experimental support).
Weak (lacks degree specificity)	There is evidence supporting a link between this gene/variant and this drug in this species, but the degree to which this impacts phenotype categorisation is unclear and the categorical interpretation may be wrong.
Weak (lacks species specificity)	There is evidence supporting a link between this gene/variant and this drug, but the degree to which this impacts phenotype categorisation in this species is unclear and the categorical interpretation may be wrong.
Weak (lacks genus specificity)	There is evidence supporting a link between this gene/variant and this drug, but the degree to which this impacts phenotype categorisation in this genus is unclear and the categorical interpretation may be wrong.
Weak (lacks drug specificity)	There is evidence supporting a link between this gene/variant and resistance to some/related drugs, but not direct evidence for this drug and the categorical interpretation may be wrong.

Option 2: Strong/Moderate/Weak - with explicit reasons for ‘moderate’ or ‘weak’

Strong	Experimental evidence provides strong support for the interpretation of this gene/variant in this species for this drug; and no conflicting or missing evidence.
Moderate	There is good evidence to support the interpretation of this gene/variant in this species for this drug, but it is not conclusive (e.g. incomplete or conflicting evidence; or statistical geno/pheno evidence without experimental support).
Moderate (lacks species specificity)	There is good evidence to support the categorical interpretation of this gene/variant for this drug in this genus, but not directly in this species.
Moderate (lacks genus specificity)	There is good evidence to support the categorical interpretation of this gene/variant for this drug, but not directly in this genus.
Weak (lacks degree specificity)	There is evidence supporting a link between this gene/variant and this drug in this species, but the degree to which this impacts phenotype categorisation is unclear and the categorical interpretation may be wrong.
Weak (lacks species specificity)	There is evidence supporting a link between this gene/variant and this drug, but the degree to which this impacts phenotype categorisation in this species is unclear and the categorical interpretation may be wrong.
Weak (lacks genus specificity)	There is evidence supporting a link between this gene/variant and this drug, but the degree to which this impacts phenotype categorisation in this genus is unclear and the categorical interpretation may be wrong.
Weak (lacks drug specificity)	There is evidence supporting a link between this gene/variant and resistance to some/related drugs, but not direct evidence for this drug and the categorical interpretation may be wrong.

AMR Rules Spec v0.4

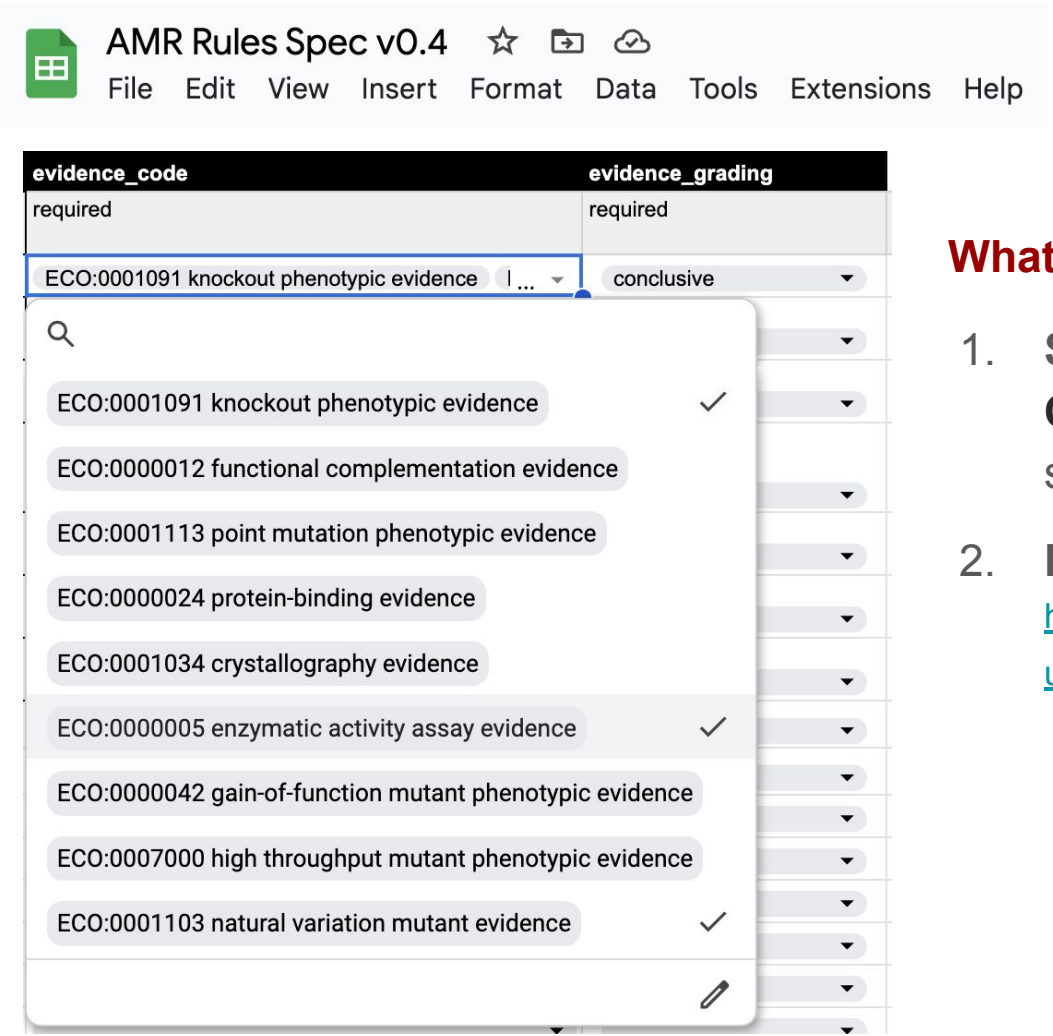
File Edit View Insert Format Data Tools Extensions Help

evidence_code	evidence_grading
required	required
ECO:0001091 knockout phenotypic evidence | ...	conclusive

- ECO:0001091 knockout phenotypic evidence ✓
- ECO:0000012 functional complementation evidence
- ECO:0001113 point mutation phenotypic evidence
- ECO:0000024 protein-binding evidence
- ECO:0001034 crystallography evidence
- ECO:0000005 enzymatic activity assay evidence ✓
- ECO:0000042 gain-of-function mutant phenotypic evidence
- ECO:0007000 high throughput mutant phenotypic evidence
- ECO:0001103 natural variation mutant evidence ✓

evidence_grading
required
weak (lacks species specificity)

- conclusive
- moderate
- **weak (lacks species specificity)**
- weak (lacks genus specificity)
- weak (lacks drug specificity)
- weak (lacks degree specificity)



What if these terms don't cover it?

1. **Search the Evidence & Conclusion Ontology (ECO)** to see if there is a suitable term www.evidenceontology.org
2. **Post an issue to the AMRrules github** https://github.com/interpretAMR/AMRrulesCuration/issues to let us know if
   a. There is a new ECO term you want to use,
   b. There is no term, we can discuss how to proceed e.g. proposing a new term to ECO or defining an interim term for AMRrules
   c. We can then add new terms to the specification sheet dropdown for others to use also

When there is no experimental evidence

Note that if no experimental evidence is available, the rule should NOT be recorded as 'strong', even if there is strong evidence of statistical association between genotype and phenotype in natural populations for this species.

evidence_code	evidence_grading
required	required
ECO:0001103 natural variation mutant evidence	moderate

Note: further discussions need to be had about the quantitative support for geno/pheno associations.

Future updates of the spec will include additional fields to record quantitative details of genotype/phenotype associations.

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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*
- *Mycobacterium tuberculosis*
- *Campylobacter spp.*
- *Haemophilus influenzae*

3

Others

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

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

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Issues arising from subgroups

Pseudomonas

- Some drugs not in WHO ATC classification
  - inhibitor combinations
  - tigecycline
- Key efflux components not in NCBI refgene
  - mexA but not mexB or oprM, which are required for efflux pump to function
- Core genes in refgene that are not phenotypically relevant
  - crpP, mexE, blaOXA-50, catB7
  - *added complexity when there are multiple core genes linked to a drug with expected resistance, but not all are relevant to that phenotype

- Chloramphenicol is an expected resistance in *P. aeruginosa*
- Key mechanism is efflux via *mexAB*, as evidenced by knockouts
- Other core genes (*catB7, mexXY* efflux pump) are reported as having activity for chloramphenicol, but evidence shows without *mexAB* there is no resistance

Drug	MIC (µg/ml)					
	Strain	PAO1	YM2	YM3	YM5	YM4	YM6
	Deficiency		ΔMexXY	ΔMexAB	ΔMexAB ΔOprM	ΔMexXY ΔMexAB	ΔMexXY ΔMexAB ΔOprM
Chloramphenicol		32	64	8	2	4	2

PMID 12483565

How to encode the interpretation of catB7 and mexXY for chloramphenicol?

gene	category	evidence_code	evidence_grade	rule curation note
mexA	**wt R**	ECO:0001091 knockout phenotypic evidence	Strong	MexAB knockout results in loss of expected chloramphenicol resistance, despite presence of catB7 and MexXY (PMID: 12483565). All components of the efflux-pump system need to be functional (MexA-MexB-OprM).
mexX	**?**	ECO:0001091 knockout phenotypic evidence	Strong	Species has expected resistance to chloramphenicol and MexXY efflux pump has some activity for efflux of this substrate. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of MexXY in the absence of MexAB is unlikely to confer resistance.
catB7	**?**	ECO:0000005 enzymatic activity assay evidence	Strong	Species has expected resistance to chloramphenicol, and catB7 has demonstrated activity as a chloramphenicol acetyltransferase. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of catB7 in the absence of MexAB is unlikely to confer resistance.

mexX

Download Sequences

Accession	ARO:3003034
CARD Short Name	*mexX*
Definition	MexX is the membrane fusion protein of the MexXY-OprM multidrug efflux complex.
AMR Gene Family	resistance-nodulation-cell division (RND) antibiotic efflux pump
Drug Class	aminoglycoside antibiotic, disinfecting agents and antiseptics, phenicol antibiotic, tetracycline antibiotic, carbapenem, fluoroquinolone antibiotic, macrolide antibiotic, penam, cephamycin, cephalosporin
Resistance Mechanism	antibiotic efflux
Efflux Component	efflux pump complex or subunit conferring antibiotic resistance

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mexX	**wt R** X	ECO:0001091 knockout phenotypic evidence	Strong	Species has expected resistance to chloramphenicol and MexXY efflux pump has some activity for efflux of this substrate. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of MexXY in the absence of MexAB is unlikely to confer resistance.
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mexX	**wt S** X	ECO:0001091 knockout phenotypic evidence	Strong	Species has expected resistance to chloramphenicol and MexXY efflux pump has some activity for efflux of this substrate. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of MexXY in the absence of MexAB is unlikely to confer resistance.
catB7	**wt S** X	ECO:0000005 enzymatic activity assay evidence ECO:0001091 knockout phenotypic evidence	Strong	Species has expected resistance to chloramphenicol, and catB7 has demonstrated activity as a chloramphenicol acetyltransferase. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of catB7 in the absence of MexAB is unlikely to confer resistance.

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How to encode the interpretation of catB7 and mexXY for chloramphenicol?

gene	category	evidence_code	evidence_grade	rule curation note
mexA	**wt R**	ECO:0001091 knockout phenotypic evidence	Strong	MexAB knockout results in loss of expected chloramphenicol resistance, despite presence of catB7 and MexXY (PMID: 12483565). All components of the efflux-pump system need to be functional (MexA-MexB-OprM).
mexX	**wt (R)**	ECO:0001091 knockout phenotypic evidence	Strong	Species has expected resistance to chloramphenicol and MexXY efflux pump has some activity for efflux of this substrate. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of MexXY in the absence of MexAB is unlikely to confer resistance.
catB7	**wt (R)**	ECO:0000005 enzymatic activity assay evidence ECO:0001091 knockout phenotypic evidence	Strong	Species has expected resistance to chloramphenicol, and catB7 has demonstrated activity as a chloramphenicol acetyltransferase. However MexAB knockout results in loss of expected resistance (MIC 8 mg/L), despite presence of catB7 and MexXY, therefore the presence of catB7 in the absence of MexAB is unlikely to confer resistance.

How to encode the interpretation of blaOXA-50 for ampicillin?

gene	category	evidence_code	evidence_grade	rule curation note
blaPDC_gen	**wt R**	ECO:0001091 knockout phenotypic evidence ECO:0000005 enzymatic activity assay evidence	Strong	AmpC (blaPDC) expression is inducible by certain β-lactams, induction requires a functional AmpR. Knockouts have dramatically reduced ampicillin MIC even in the presence of intact blaOXA-50, indicating blaPDC is responsible for intrinsic resistance.
blaOXA-50_fam	**wt**	ECO:0001091 knockout phenotypic evidence ECO:0000005 enzymatic activity assay evidence	Strong	blaOXA-50 is a chromosomally encoded β-lactamase, also known as PoxB. Increases carbapenem MICs when overexpressed in vitro, but contribution to intrinsic resistance, if any, is marginal, and poxB deletions failed to alter β-lactam sensitivities.

PMID: 26621621

	MIC[a] (μg/ml)				
β-Lactam	**PAO1**	**PAOΔ*poxA***	**PAOΔ*poxB***	**PAOΔ*ampC***	**PAOΔ*ampC*Δ*poxB***
Ampicillin	>256	>256	>256	16–24	24
Ampicillin-sulbactam	>256	>256	>256	16–32	16–32
Amoxicillin	>256	>256	>256	8–12	16–24
Amoxicillin-clavulanate	>256	>256	>256	8	8

Issues arising from subgroups

Staphylococcus

- What if the intrinsic mechanism of resistance is unknown?

Table 4 Expected resistant phenotype (susceptibility not expected) in gram-positive bacteria. Gram-positive bacteria are expected to be resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid.

Rule	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *S. aureus*		R										
4.6	*Streptococcus* spp.	R	R		R[1]								
4.7	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides

[2] In addition to LLR to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6')-I enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin and streptomycin) and penicillins or glycopeptides

Is it reported by AMRfinderplus?

Search

staphylococcus

You can now download the sequences directly from this reference gene catalog. Please see Help for details.

db version: 2024-07-22.1 Changelog

Bacterial Antimicrobial Resistance Reference Gene Database

Filters

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#	Allele	Whitelisted taxa	Gene f...	Product name	Scope	Type	Subtype	Class	Subclass	RefSeq protein	RefSeq...	GenBa...	GenBa...	Curate...	Links
1	gyrB_I...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
2	gyrB_G...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
3	gyrB_I...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
4	gyrB_S...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
5	gyrB_R...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
6	gyrB_R...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
7	gyrB_T...	Staphylococcus_aure	gyrB	DNA topoisomerase (ATP-hydrolyzing) subunit B GyrB	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_010956585.1	NC_00...	CAG39...	BX5718...	No	
8	parE_G...	Staphylococcus_aure	parE	DNA topoisomerase IV subunit B ParE	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_001837315.1	NZ_CP...	AVG68...	CP0269...	No	
9	parE_R...	Staphylococcus_aure	parE	DNA topoisomerase IV subunit B ParE	core	AMR	POINT	AMINO...	AMINOCOUMARIN	WP_001837315.1	NZ_CP...	AVG68...	CP0269...	No	
10			arsB	arsenite efflux transporter membrane subunit ArsB	plus	STRESS	METAL	ARSENIC	ARSENITE			AAA27...	M8056...	No	
11			arsB	arsenite efflux transporter membrane subunit ArsB	plus	STRESS	METAL	ARSENIC	ARSENITE			AAA25...	M8682...	No	
12	pbp2_S...	Staphylococcus_aure	pbp2	penicillin-binding protein PBP2	core	AMR	POINT	BETA-L...	CEPHALOSPORIN	WP_000138348.1		AAX13...	AY9204...	No	
13	pbp4_T...	Staphylococcus_aure	pbp4	D-alanyl-D-alanine carboxypeptidase PBP4	core	AMR	POINT	BETA-L...	CEPHALOSPORIN		NZ_RIX...		RIXR01...		
14	pbp4_E...	Staphylococcus_aure	pbp4	D-alanyl-D-alanine carboxypeptidase PBP4	core	AMR	POINT	BETA-L...	CEPHALOSPORIN	WP_123090655.1	NZ_RIX...	RNG97...	RIXR01...	No	
15	pbp4_...	Staphylococcus_aure	pbp4	D-alanyl-D-alanine carboxypeptidase PBP4	core	AMR	POINT	BETA-L...	CEPHALOSPORIN	WP_123090655.1	NZ_RIX...	RNG97...	RIXR01...	No	
16	pbp4_E...	Staphylococcus_aure	pbp4	D-alanyl-D-alanine carboxypeptidase PBP4	core	AMR	POINT	BETA-L...	CEPHALOSPORIN	WP_123090655.1	NZ_RIX...	RNG97...	RIXR01...	No	
17	pbp4_F...	Staphylococcus_aure	pbp4	D-alanyl-D-alanine carboxypeptidase PBP4	core	AMR	POINT	BETA-L...	CEPHALOSPORIN	WP_123090655.1	NZ_RIX...	RNG97...	RIXR01...	No	
18	glpT_F3I	Staphylococcus_aure	glpT	glycerol-3-phosphate transporter GlpT	core	AMR	POINT	FOSFO...	FOSFOMYCIN	WP_001010111.1	NC_00...	AAW38...	CP0000...	No	
19	glpT_L...	Staphylococcus_aure	glpT	glycerol-3-phosphate transporter GlpT	core	AMR	POINT	FOSFO...	FOSFOMYCIN	WP_001010111.1	NC_00...	AAW38...	CP0000...	No	
20	glpT_A...	Staphylococcus_aure	glpT	glycerol-3-phosphate transporter GlpT	core	AMR	POINT	FOSFO...	FOSFOMYCIN	WP_001010111.1	NC_00...	AAW38...	CP0000...	No	

Feedback

Issues arising from subgroups

Mycoplasma

- How do we deal with genes/proteins with no RefSeq/CARD accession numbers?
- What if EUCAST does not have guidelines for my species/genus?
- What if AMRFinder or CARD or ResFinder do not report any mechanism of intrinsic resistance in my species?

No EUCAST guidelines

- Use ECOFFs (Legionella subgroup)
- Alternative guidelines, for example CLSI
- Use evidence codes if using guidelines from other species (i.e., Mycoplasma genitalium)

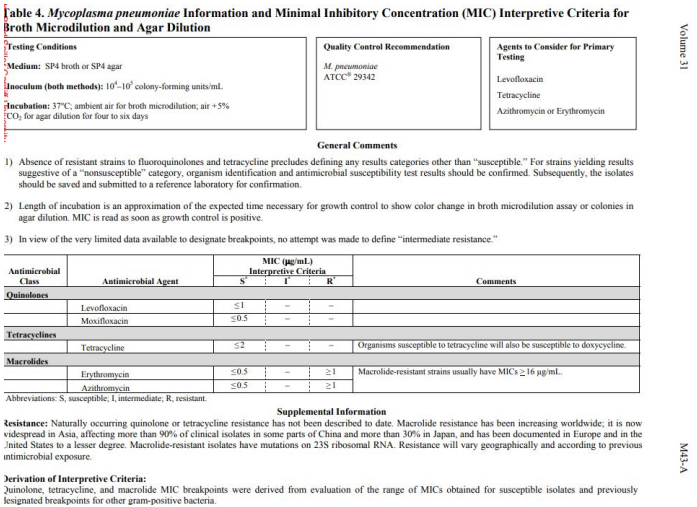

Table 4. *Mycoplasma pneumoniae* Information and Minimal Inhibitory Concentration (MIC) Interpretive Criteria for Broth Microdilution and Agar Dilution

Testing Conditions	Quality Control Recommendation	Agents to Consider for Primary Testing
Medium: SP4 broth or SP4 agar **Inoculum (both methods):** 10^4–10^5 colony-forming units/mL **Incubation:** 37°C; ambient air for broth microdilution; air + 5% CO_2 for agar dilution for four to six days	*M. pneumoniae* ATCC® 29342	Levofloxacin Tetracycline Azithromycin or Erythromycin

General Comments

1) Absence of resistant strains to fluoroquinolones and tetracycline precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory for confirmation.

2) Length of incubation is an approximation of the expected time necessary for growth control to show color change in broth microdilution assay or colonies in agar dilution. MIC is read as soon as growth control is positive.

3) In view of the very limited data available to designate breakpoints, no attempt was made to define "intermediate resistance."

Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria S[a]	I[a]	R[a]	Comments
Quinolones					
	Levofloxacin	≤1	–	–	
	Moxifloxacin	≤0.5	–	–	
Tetracyclines					
	Tetracycline	≤2	–	–	Organisms susceptible to tetracycline will also be susceptible to doxycycline.
Macrolides					
	Erythromycin	≤0.5	–	≥1	Macrolide-resistant strains usually have MICs ≥ 16 µg/mL.
	Azithromycin	≤0.5	–	≥1	

Abbreviations: S, susceptible; I, intermediate; R, resistant.

Supplemental Information

Resistance: Naturally occurring quinolone or tetracycline resistance has not been described to date. Macrolide resistance has been increasing worldwide; it is now widespread in Asia, affecting more than 90% of clinical isolates in some parts of China and more than 30% in Japan, and has been documented in Europe and in the United States to a lesser degree. Macrolide-resistant isolates have mutations on 23S ribosomal RNA. Resistance will vary geographically and according to previous antimicrobial exposure.

Derivation of Interpretive Criteria:
Quinolone, tetracycline, and macrolide MIC breakpoints were derived from evaluation of the range of MICs obtained for susceptible isolates and previously designated breakpoints for other gram-positive bacteria.

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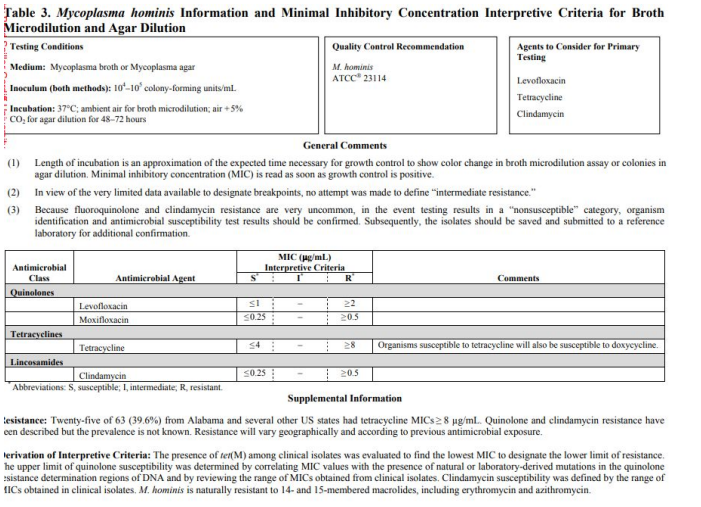

Table 3. *Mycoplasma hominis* Information and Minimal Inhibitory Concentration Interpretive Criteria for Broth Microdilution and Agar Dilution

Testing Conditions	Quality Control Recommendation	Agents to Consider for Primary Testing
Medium: Mycoplasma broth or Mycoplasma agar **Inoculum (both methods):** 10^4–10^5 colony-forming units/mL **Incubation:** 37°C; ambient air for broth microdilution; air + 5% CO_2 for agar dilution for 48–72 hours	*M. hominis* ATCC® 23114	Levofloxacin Tetracycline Clindamycin

General Comments

(1) Length of incubation is an approximation of the expected time necessary for growth control to show color change in broth microdilution assay or colonies in agar dilution. Minimal inhibitory concentration (MIC) is read as soon as growth control is positive.

(2) In view of the very limited data available to designate breakpoints, no attempt was made to define "intermediate resistance."

(3) Because fluoroquinolone and clindamycin resistance are very uncommon, in the event testing results in a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory for additional confirmation.

Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria S[a]	I[a]	R[a]	Comments
Quinolones					
	Levofloxacin	≤1	–	≥2	
	Moxifloxacin	≤0.25	–	≥0.5	
Tetracyclines					
	Tetracycline	≤4	–	≥8	Organisms susceptible to tetracycline will also be susceptible to doxycycline.
Lincosamides					
	Clindamycin	≤0.25	–	≥0.5	

Abbreviations: S, susceptible; I, intermediate; R, resistant.

Supplemental Information

Resistance: Twenty-five of 63 (39.6%) from Alabama and several other US states had tetracycline MICs ≥ 8 µg/mL. Quinolone and clindamycin resistance have been described but the prevalence is not known. Resistance will vary geographically and according to previous antimicrobial exposure.

Derivation of Interpretive Criteria: The presence of *tet*(M) among clinical isolates was evaluated to find the lowest MIC to designate the lower limit of resistance. The upper limit of quinolone susceptibility was determined by correlating MIC values with the presence of natural or laboratory-derived mutations in the quinolone resistance determination regions of DNA and by reviewing the range of MICs obtained from clinical isolates. Clindamycin susceptibility was defined by the range of MICs obtained in clinical isolates. *M. hominis* is naturally resistant to 14- and 15-membered macrolides, including erythromycin and azithromycin.

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Genes/proteins with no RefSeq/CARD accession numbers

- alternative identifiers, must have at least one of node/refseq/genbank/HMM

organism	gene	nodeID	refseq accession	GenBank accession	HMM accession	ARO accession
required	required	require AT LEAST ONE OF: nodeID (preferred) or refseq or GenBank or HMM accession				optional (recommended)
s__Mycoplasmoides pneumoniae	rpoB	-	-	VEU56940.1	-	-

Genes/proteins with no RefSeq/CARD accession numbers

- AMRFinder or CARD or ResFinder do not report any mechanism of intrinsic resistance
- Alternatively, use BLAST to identify core mutations/genes

organism	gene	nodeID	refseq accession	GenBank accession	HMM accession	ARO accession	mutation
required	required	require AT LEAST ONE OF: nodeID (preferred) or refseq or GenBank or HMM accession				optional (recommended)	required
s__Mycoplasmoides pneumoniae	rpoB	-	-	VEU56940.1	-	-	p.His526Asp

Reminder about Technical Guidance

- Updated to cover latest spec v0.4
- ‘Suggested Protocol section’
- ‘Existing curations’
- ‘Open Issues’ section

Technical Guidance: latest version (v1.4)
bit.ly/AMRrules_Tech

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

Schedule for Update Meetings

ESCMID | ESGEM-AMR

December (first week, 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

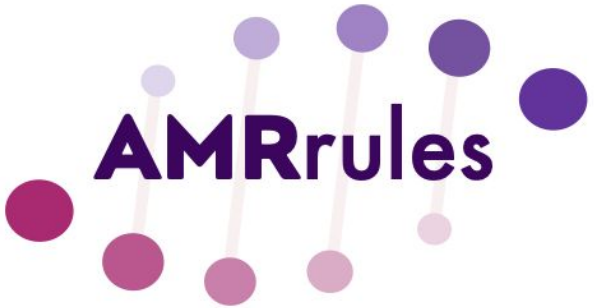
Attendees

- 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