Microbiological Diagnostic Unit
PUBLIC HEALTH LABORATORY

SeqAsia tutorial: Reporting genomic results on single isolates

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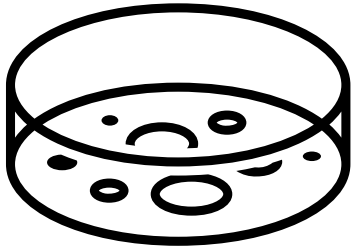
Doherty Institute
THE UNIVERSITY OF MELBOURNE
The Royal Melbourne Hospital

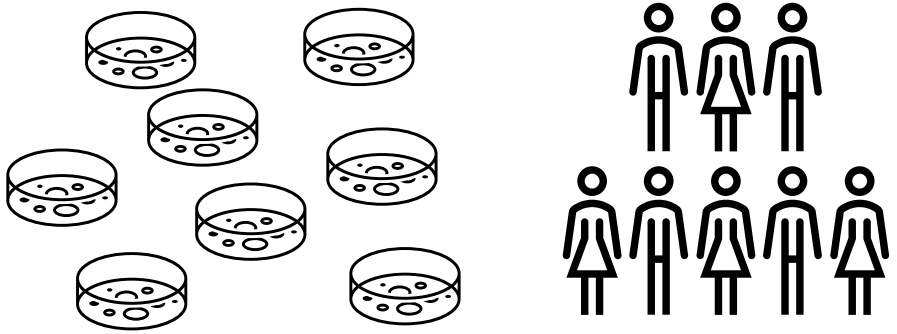
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Brainstorm: What types of genomic results do we report?



SINGLE ISOLATES

- **Speciation**
- **Typing**
  - Generic e.g. MLST, cgMLST
  - Species-specific typing schemes
    - *In silico* serotypes, capsule types
    - Others e.g. NG-MAST, *emm* typing
- **AMR**
  - Resistance mechanisms, inferred phenotype
- **Virulence factors**



MULTIPLE ISOLATES

- **Line list reports** – multiple independent results
  - Usually for surveillance/public health
- **Comparative genomics**
  - Compares multiple genomes to each other to establish genomic relationships

How do we report?

Depends which lab you're working at! Here are some general concepts:

- **Laboratory information management system (LIMS)** – track samples from receipt to report

- **Genomic results are generated – Isolate > Sequence > QC & analysis > Report**
  - Usually via LIMS for single isolates

- **Usual clinical microbiology reporting concepts still apply!**
  - Correct patient/sample, correct referrer/referring lab?
  - Do you have to report the accreditation status of test?

How do we report?

What are the steps to reporting a genomic result?

1. **Data entry / transcription**

2. **Check results are valid and make sense**
   - Are sequence QC results valid?
   - Are the results within expected limits – given everything else you know about the patient/isolate?
     - Does it fit with the species ID/expected species/sample type/clinical history?
     - Does it match with phenotypic results (if available)?

3. **Interpretation**
   - Add comments to help those receiving report to interpret results correctly (if required)

4. **Do you need to alert anyone to the result?**
   - Is this result a mandatory notifiable disease? Does the referring lab/clinician need to be notified for patient care?

What to consider when designing a report

1. What is the purpose of sequencing and reporting?
2. Who is the audience/client (and how much knowledge)?
3. What are the main question/s to be answered?
4. How long will they have to read the report?
5. Are you reporting on single isolates or multiple (line list)?
6. What are the uncertainties/caveats and comments required to accurately interpret the findings?
7. What are the obligations for reporting (e.g. NATA or equivalents, notifiable disease)?

Reporting AMR results

1. What is the purpose of sequencing and reporting? Clinical (patient) or surveillance?
2. Who is the audience/client (and how much knowledge)? Clinicians, IPC or public health
3. What are the main question/s to be answered? What can I treat this patient with, do I need to isolate them, and how frequent is this genotype/phenotype?
4. How long will they have to read the report? Clinicians & IPC: not long (<1min), PH: longer
5. Are you reporting on single isolates or multiple (line list)?
6. What are the uncertainties/caveats and comments required to accurately interpret the findings? Will vary by species/drug. More caveats for inferred phenotype, clinical reporting
7. What are the obligations for reporting (e.g. NATA)? Will depend how you design your validation and report. Genotype much easier, phenotype more complex (validation and comments). Consider notifiable diseases – jurisdictional, national, CARAlert. For inferred phenotypes, what can you validly infer from current geno-pheno knowledge?

Characteristics	Isolate sent from diagnostic lab for CPE testing	Routine *Shigella* AMR surveillance	Isolate from TB clinic for MTB AMR
Purpose	Detect CPE genes for individual isolates for infection control	Detect AMR genes for surveillance purposes	Detect AMR mutations for patient treatment
Audience	Lab, clinicians, IPC Assume limited knowledge of AMR genes and implications	Epidemiologists from PHU Assume moderate to high level of specific AMR knowledge	Usually specialist TB clinicians (increasingly genomics-literate)
Main question	Is this CPE? (Do we have to isolate? How to treat?)	What are the concerning AMR determinants? (Worry about increasing AMR/clusters?)	What can I treat my patient with? (inferred antibiogram)
Report length	Short	Moderate	Moderate
Single/multiple	Single isolate per report	Line-list of multiple isolates	Single isolate per report
Caveats and comments	Low level of uncertainty, but needs interpretation for audience	Low level of uncertainty, interpretation may not be needed	Varying uncertainty depending on drug; different mutations may also confer different levels of resistance Caution required for patient Rx

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How do we translate AMR genomics results for laboratory reports?

Clinicians

- Reporting presence/absence of resistance genes or mutations in most cases
- Most clinicians can't infer resistance from a given AMR gene (name or description)
- Specify antibiotic class/antibiotic – need to make sense clinically – **interpret for audience**

Epidemiologists

- Reporting presence/absence of resistance genes or mutations in most cases
- Generally like **more information** rather than less information
- Have more domain specific knowledge, likely to be integrated into PH databases

What don't they want to know?

- Reporting QC, % gene identity and coverage is usually not a reasonable option
- Make a call – is the gene/mutation likely to be there or not?
- Users unlikely to want to know about QC results – trust you only report is QC is good
- **Extraneous information not** usually wanted and can confuse reports (clinicians >> epis) e.g. intrinsic resistance mechanisms

How do we translate AMR genomics results for laboratory reports?

	START	END	GENE	COVERAGE	COVERAGE_MAP	GAPS	%COVERAGE	%IDENTITY	DATABASE	ACCESSION	PRODUCT
75_00031	48593	49726	blaEC-13	1-1134/1134	==========	0/0	100.00	99.91	ncbi	A7J11_00180	ncbi~~~blaEC-13~~~A7J11_00180 class C extended-spectrum beta-lactamase EC-13
75_00093	6376	7035	catA1	1-660/660	==========	0/0	100.00	99.85	ncbi	A7J11_00207	ncbi~~~catA1~~~A7J11_00207 type A-1 chloramphenicol O-acetyltransferase
75_00101	5332	6192	blaTEM-1	1-861/861	==========	0/0	100.00	100.00	ncbi	A7J11_00169	ncbi~~~blaTEM-1~~~A7J11_00169 class A broad-spectrum beta-lactamase TEM-1
75_00112	1917	2732	sul2	1-816/816	==========	0/0	100.00	99.88	ncbi	A7J11_00971	ncbi~~~sul2~~~A7J11_00971 sulfonamide-resistant dihydropteroate synthase Sul2
75_00112	2769	3596	aph(3'')-Ib	1-828/828	==========	0/0	100.00	100.00	ncbi	A7J11_05252	ncbi~~~aph(3'')-Ib~~~A7J11_05252 aminoglycoside O-phosphotransferase APH(3'')-Ib
75_00112	3596	4432	aph(6)-Id	1-837/837	==========	0/0	100.00	100.00	ncbi	A7J11_01109	ncbi~~~aph(6)-Id~~~A7J11_01109 aminoglycoside O-phosphotransferase APH(6)-Id
75_00115	3226	4147	mph(A)	1-921/921	========/======	1/1	100.00	99.67	ncbi	A7J11_00571	ncbi~~~mph(A)~~~A7J11_00571 Mph(A) family macrolide 2'-phosphotransferase
75_00119	2721	3581	aac(3)-IId	1-861/861	==========	0/0	100.00	100.00	ncbi	A7J11_00380	ncbi~~~aac(3)-IId~~~A7J11_00380 aminoglycoside N-acetyltransferase AAC(3)-IId
75_00120	928	1401	dfrA17	1-474/474	==========	0/0	100.00	99.79	ncbi	A7J11_04594	ncbi~~~dfrA17~~~A7J11_04594 trimethoprim-resistant dihydrofolate reductase DfrA17
75_00120	1532	2320	aadA5	1-789/789	==========	0/0	100.00	100.00	ncbi	A7J11_00675	ncbi~~~aadA5~~~A7J11_00675 ANT(3'')-Ia family aminoglycoside nucleotidyltransferase AadA5
75_00120	2526	2873	qacEdelta1	1-348/348	==========	0/0	100.00	100.00	ncbi	A7J11_00271	ncbi~~~qacEdelta1~~~A7J11_00271 quaternary ammonium compound efflux SMR transporter QacE delta 1
75_00120	2867	3457	sul1	1-591/882	==========....	0/0	67.01	100.00	ncbi	A7J11_02078	ncbi~~~sul1~~~A7J11_02078 sulfonamide-resistant dihydropteroate synthase Sul1
75_00121	1444	2649	tet(B)	1-1206/1206	==========	0/0	100.00	100.00	ncbi	A7J11_00078	ncbi~~~tet(B)~~~A7J11_00078 tetracycline efflux MFS transporter Tet(B)
75_00122	1910	2275	ble	1-366/366	==========	0/0	100.00	100.00	ncbi	A7J11_02663	ncbi~~~ble~~~A7J11_02663 bleomycin binding protein Ble-MBL
75_00122	2279	3091	blaNDM-4	1-813/813	==========	0/0	100.00	100.00	ncbi	A7J11_02790	ncbi~~~blaNDM-4~~~A7J11_02790 subclass B1 metallo-beta-lactamase NDM-4
75_00129	88	337	arr-2	1-250/453	=========.....	0/0	55.19	99.60	ncbi	A7J11_00651	ncbi~~~arr-2~~~A7J11_00651 NAD(+)--rifampin ADP-ribosyltransferase Arr-2
75_00129	422	1054	catB3	1-633/633	==========	0/0	100.00	100.00	ncbi	A7J11_00446	ncbi~~~catB3~~~A7J11_00446 type B-3 chloramphenicol O-acetyltransferase CatB3

Antimicrobial	Interpretation
Ampicillin	R
Amoxycillin-clavulanate	R
Piperacillin-tazobactam	R
Cefazolin	R
Ceftriaxone	R
Meropenem	R
Ciprofloxacin	R
Gentamicin	I
Amikacin	S
Tigecycline	S
Trimethoprim-sulfamethoxazole	R
Colistin	S

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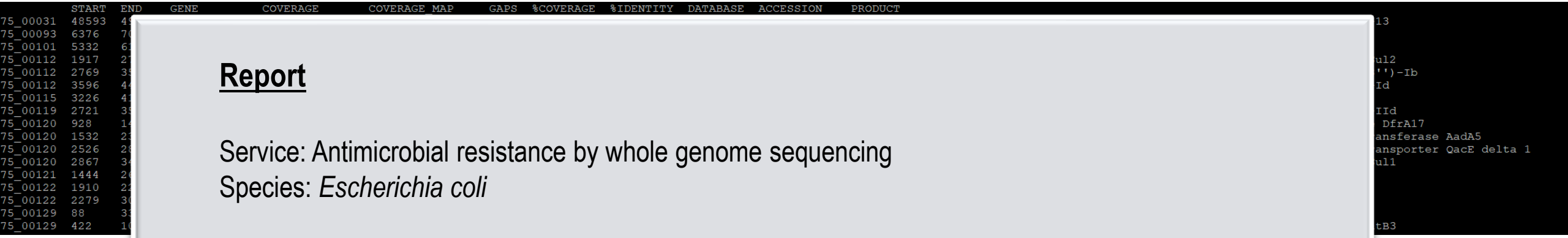

Report

Service: Antimicrobial resistance by whole genome sequencing
Species: *Escherichia coli*

AMR gene/mutation	Confers resistance to	Mechanism
NDM-4	Beta-lactams (carbapenems, penicillins, cephalosporins)	Carbapenemase (MBL-type)
sul2, dfrA17	Trimethoprim-sulfamethoxazole	
aac(3)-IId	Gentamicin	

This isolate produces a metallo-beta-lactamase (MBL)-type carbapenemase. Avibactam is ineffective against this type of carbapenemase.

AbriTAMR pipeline (GitHub) Kristy Horan

How do we express our confidence that we've found an AMR gene?

Identity – How similar is the gene to the reference?

100% = Identical to reference

95-99%

- Probably a variant allele that's not in the database
- OR an error in sequencing/assembly

<95% – lower confidence that a functional AMR gene is present

Coverage – how much of the gene is covered in sequence?

100% coverage = full gene present

95-99% coverage – usually assembly errors e.g. AMR gene crosses contigs

<95% - lower confidence that a functional AMR gene is present

ABRicate output

SEQUENCE	START	END	GENE	COVERAGE	COVERAGE_MAP	GAPS	%COVERAGE	%IDENTITY	ACCESSION	PRODUCT
2018-23862_00003	15269	16069	tet(M)	1-801/1920	=======.....	0/0	41.72	100	A7J11_01100	tetracycline resistance ribosomal protection protein Tet(M)
2018-23862_00003	18574	19071	dfrG	1-498/498	==========	0/0	100	100	A7J11_01202	trimethoprim-resistant dihydrofolate reductase DfrG
2018-23862_00003	19346	20473	tet(M)	793-1920/1920	.....=========	0/0	58.75	100	A7J11_01100	tetracycline resistance ribosomal protection protein Tet(M)
2018-23862_00004	80440	81918	msr(C)	1-1479/1479	==========	0/0	100	98.92	A7J11_00509	ABC-F type ribosomal protection protein Msr(C)
2018-23862_00109	231	839	vanX-A	1-609/609	==========	0/0	100	100	A7J11_00037	D-Ala-D-Ala dipeptidase VanX-A
2018-23862_00109	845	1876	vanA	1-1032/1032	==========	0/0	100	100	A7J11_00782	D-alanine--(R)-lactate ligase VanA
2018-23862_00109	1869	2837	vanH-A	1-969/969	==========	0/0	100	100	A7J11_00175	D-lactate dehydrogenase VanH-A
2018-23862_00109	3052	4206	vanS	1-1155/1155	==========	0/0	100	100	A7J11_00064	VanA-type vancomycin resistance histidine kinase VanS
2018-23862_00109	4184	4879	vanR	1-696/696	==========	0/0	100	100	A7J11_00955	VanA-type vancomycin resistance DNA-binding response regulator VanR
2018-23862_00135	217	469	sat4	291-543/543	.....=======	0/0	46.59	100	A7J11_00062	streptothricin N-acetyltransferase Sat4
2018-23862_00135	562	1356	aph(3')-IIIa	1-795/795	==========	0/0	100	100	A7J11_00801	aminoglycoside O-phosphotransferase APH(3')-IIIa
2018-23862_00135	2106	2851	erm(B)	1-747/747	========/======	1/1	99.87	99.47	A7J11_00404	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(B)

Strain ID
Contig number

NT number where gene starts and ends

Which parts of the gene are covered, any gaps

% Identity to reference

NCBI accession (look up papers/ phenotype)

NCBI description of function

ABRicate output

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Full copies of genes with 100% identity to references: *dfrG* (TMP R), *msr(C)* (macrolide R), *vanHAX* (*vanA* operon with regulators), *aph(3')-IIIa* (aminoglycoside R)

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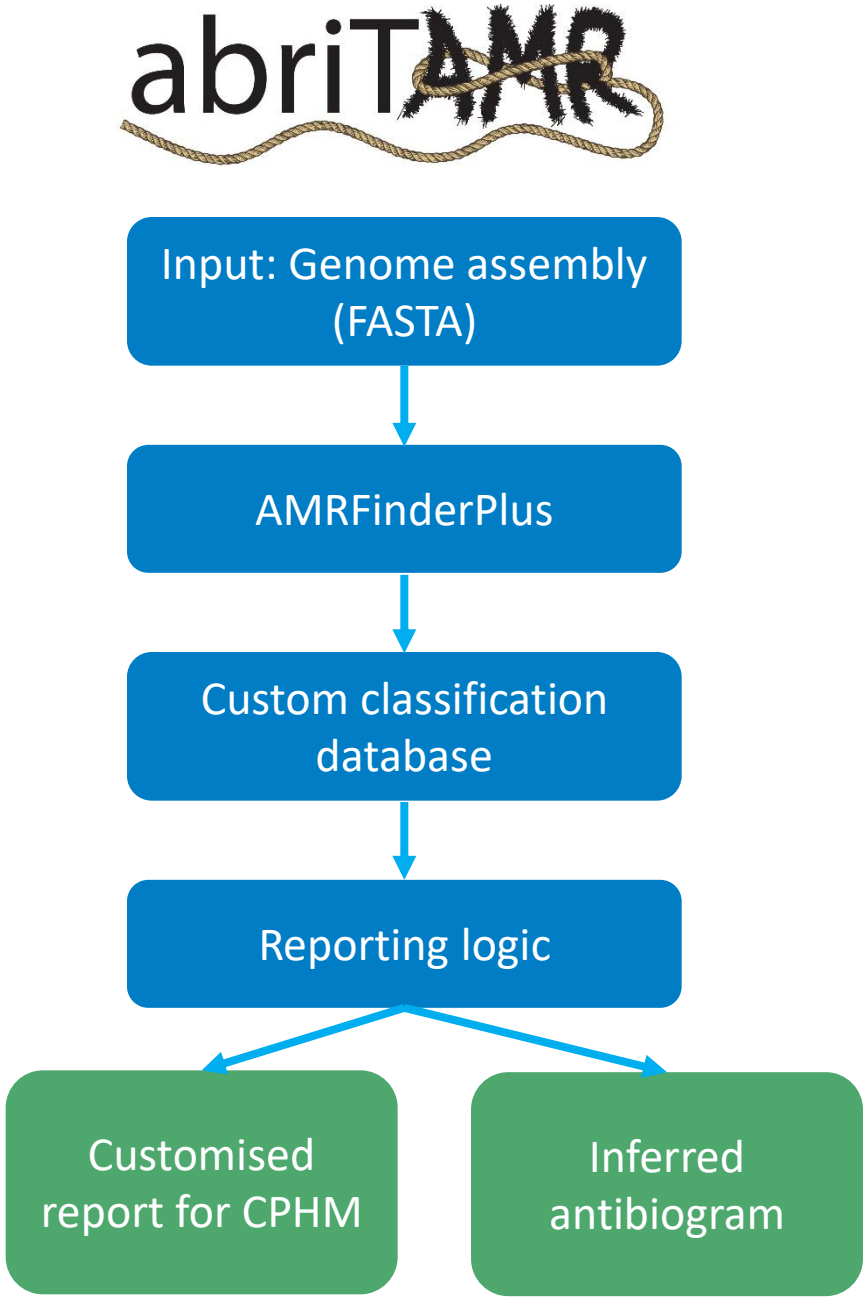
ermB gene (MLS_B resistance) with single gap
Sequencing/assembly error?
Or actual deletion causing frameshift mutation and non-functional protein?

Tailoring reporting of AMR determinants for CPHM

- **Aim to optimise pipeline for CPHM reporting**
  - Custom classification database
  - Reporting logic (by species)
  - Inferred antibiograms (limited species)
- **Examples:**
  - Exclude intrinsic AMR genes
  - Separate carbapenemase classes
  - Separate out cephalosporin resistance mechanisms
  - Tailor for local reporting requirements



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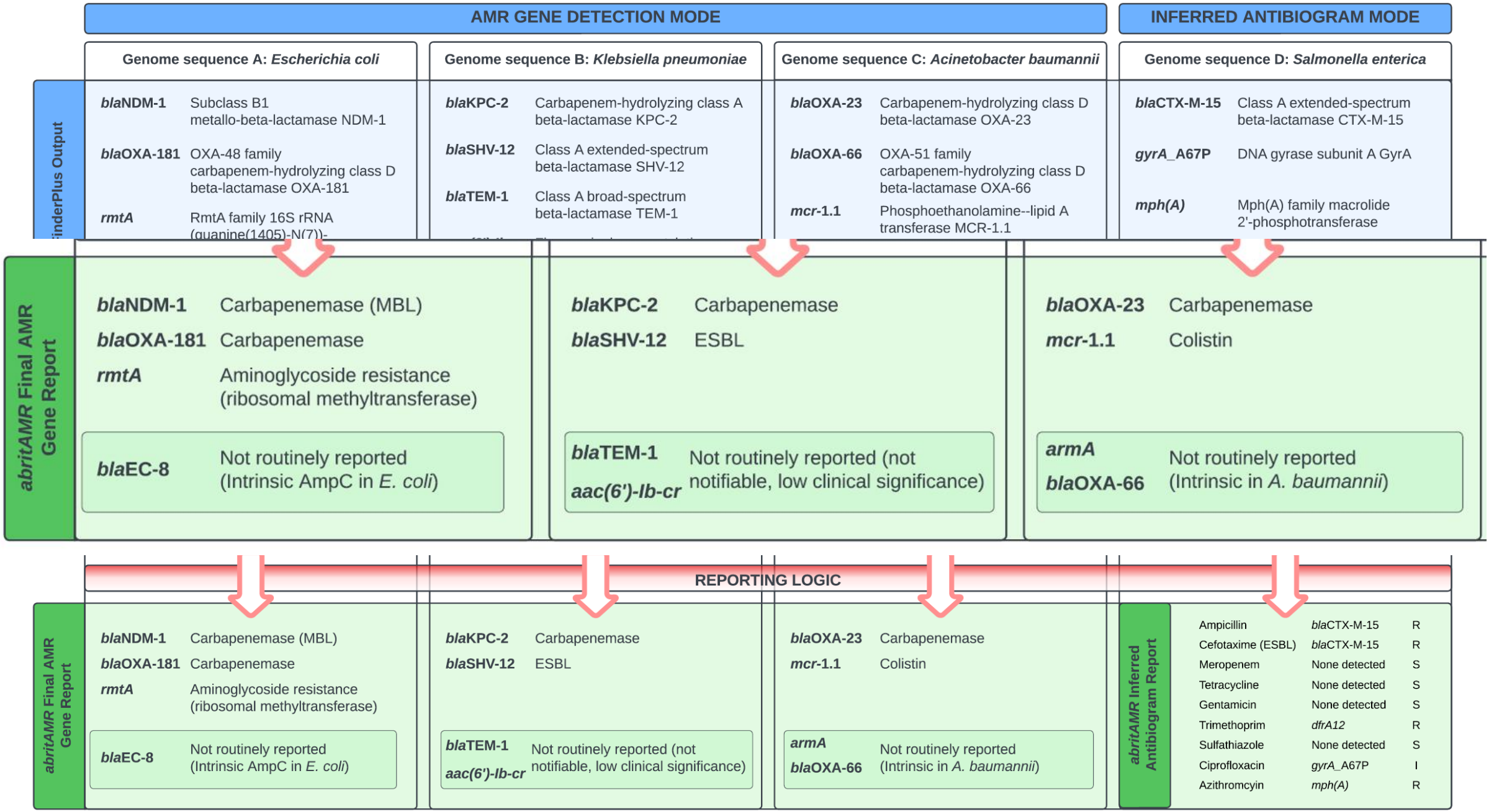
Code available on GitHub:
github.com/MDU-PHL/abritamr (Kristy Horan)



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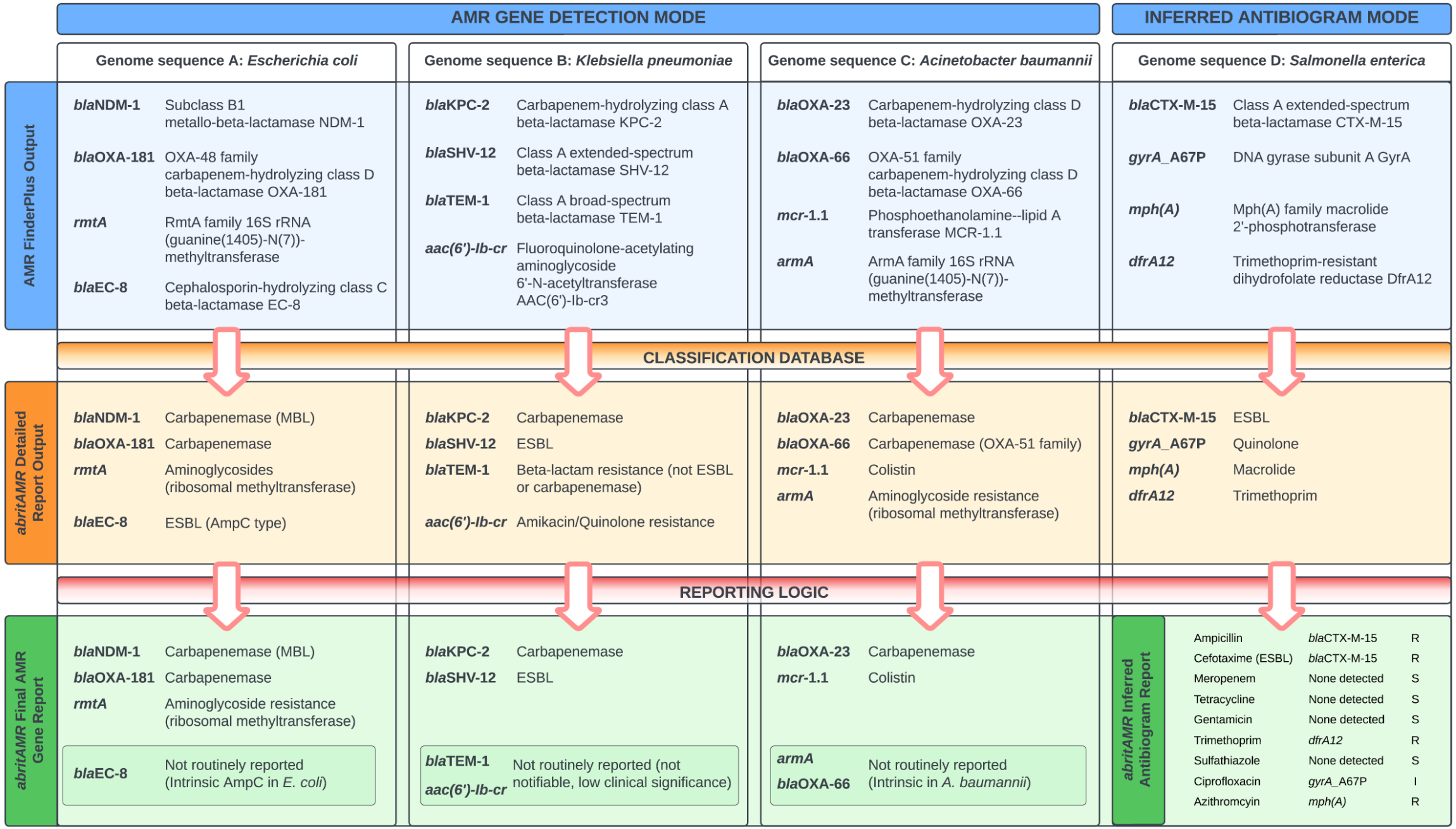

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abritAMR Reporting Logic

Minimise reporting of intrinsic AMR genes according to species

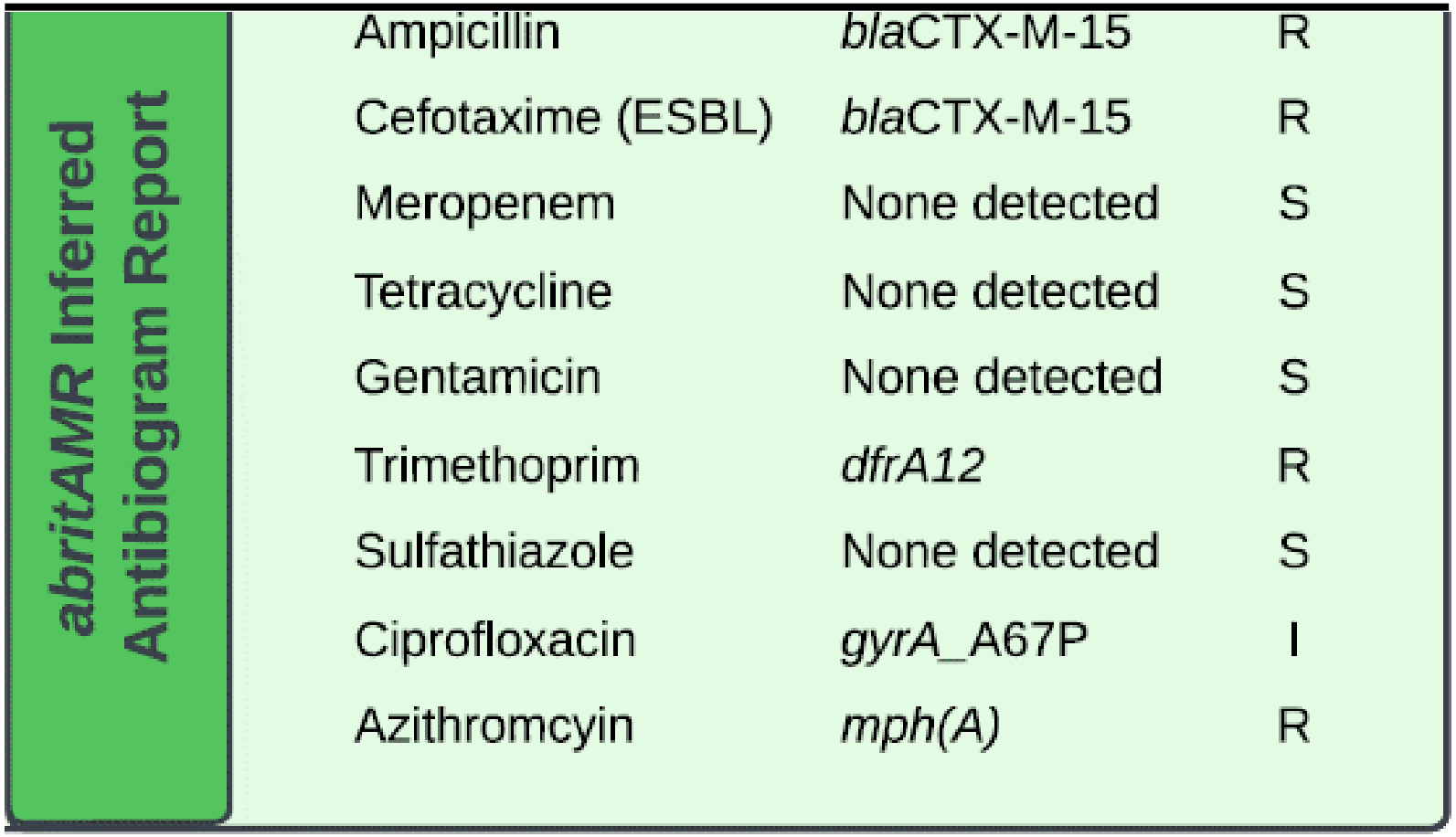
De-clutter reporting by focussing on high-priority AMR mechanisms

Could be tailored to local needs

Examples of genes not reported

- ***bla*EC** – intrinsic AmpC in *E. coli*
- **OXA-51 group (weak) carbapenemases** – intrinsic in *Acinetobacter baumannii* complex
- ***bla*TEM-1** – narrow spectrum, not of clinical relevance in most situations

Inferred antibiogram: *Salmonella spp.*



Note – for some classes, AMR determinants are additive
e.g. quinolones in *Salmonella*

- 1 mutation/gene – Intermediate
- >=2 mutations/genes – Resistant

Sherry, Horan *et al.* Nature Comms 2023

Another example: TB AMR

Complex, species-specific

Own database (WHO) with different parameters

Ideally integrate phenotype + genotype (+/- molecular results) to give an overall 'call' on AMR

- Evolving area

Integrated reports for clinicians

Drug	Xpert Ultra/XDR	Phenotypic AST	Genome sequencing	
			Resistance mechanism	Confidence level
Rifampicin	Resistance NOT detected	R	*rpoB* (1292 1294dupGCC)	Uncertain
Isoniazid	HL Resistance DETECTED	R	*katG* (Ser315Thr) *fabG1* (-15C>T)	High
Pyrazinamide	N/A	R	*pncA* (Gly24Arg)	Uncertain
Ethambutol	N/A	(S/R) pending	*emb*B (Met306Ile)	High
Moxifloxacin	LL Resistance DETECTED	LL R	*gyrA* (Asp94Ala)	High
Amikacin	Resistance DETECTED	R	*rrs* (1401A>G)	Uncertain
Ethionamide	Resistance DETECTED	S	*fabG1* (-15C>T)	High

Other reporting formats: Inferred antibiogram in HIV

STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

HIVdb: Genotypic Resistance Interpretation Algorithm

Summary Data

Sequence includes PR: codons: 1 - 99
Sequence includes RT: codons: 1 - 341

There are no insertions or deletions

Subtype and % similarity to closest reference isolate:

1. *PR: CRF02_AG (98.3%)*
2. *RT: CRF02_AG (95.4%)*

Drug Resistance Interpretation

PI Major Resistance Mutations:	None
PI Minor Resistance Mutations:	None
Other Mutations:	I13V, K14R, I15L, K20I, E35D, M36I, R41K, H69K, L89M

Protease Inhibitors	
ATV	Susceptible
DRV	Susceptible
FPV	Susceptible
IDV	Susceptible
LPV	Susceptible
NFV	Susceptible
SQV	Susceptible
TPV	Susceptible

Drug Resistance Interpretation

NRTI Resistance Mutations:	**M41L, M184V, T215Y**
NNRTI Resistance Mutations:	None
Other Mutations:	V35T, T39A, D123E, I135V, S162T, K173T, Q174K, D177E, T200A, Q207E, V245Q, T286A, E291D, V292I, I293V, P294V, S322A, I326V, Q334P, G335D

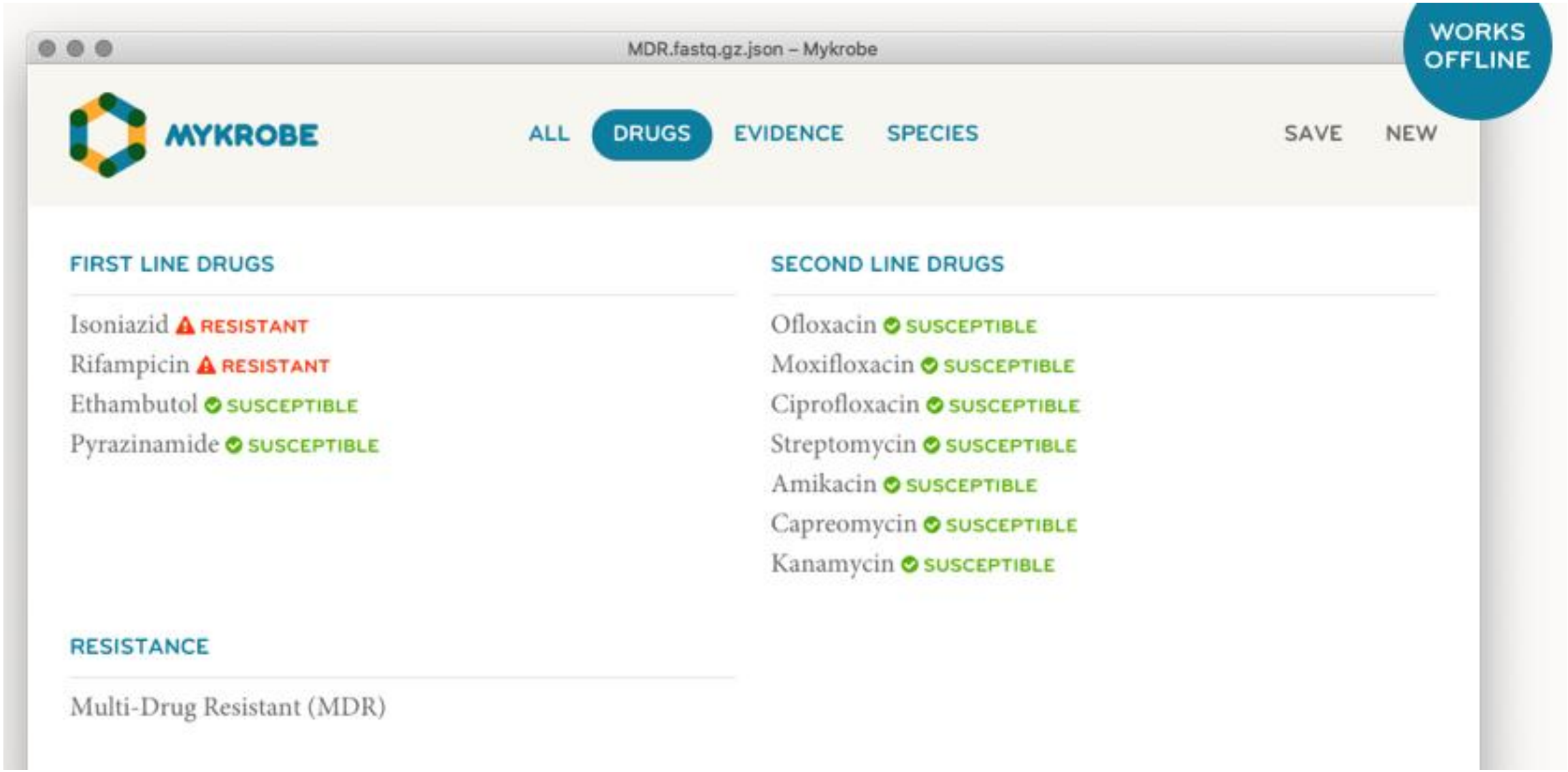
Nucleoside RTI		Non-Nucleoside RTI	
3TC	High-level resistance	**DLV**	Susceptible
ABC	Intermediate resistance	**EFV**	Susceptible
AZT	Intermediate resistance	**ETV**	Susceptible
D4T	Intermediate resistance	**NVP**	Susceptible
DDI	Intermediate resistance		
FTC	High-level resistance		
TDF	Low-level resistance		

RT Comments

- M41L usually occurs with T215Y. Together these mutations confer intermediate-to-high level resistan AZT and d4T and a lower level of resistance to ddI, ABC, and TDF.
- M184V/I cause high-level in vitro resistance to 3TC and FTC and low-level in vitro resistance to ddI a ABC. M184V/I increase susceptibility to AZT, TDF, and d4T.
- T215Y causes AZT and D4T resistance and reduces susceptibility to ABC, ddI, and TDF particularly it occurs in combination with M41L and L210W.

- M184V partially reverses AZT, d4T, and TDF resistance caused by other AZT mutations (TAMs). AZ mutations in this isolate include: M41L, T215Y.

- M184V partially reverses AZT, d4T, and TDF resistance caused by other AZT mutations (TAMs). AZ mutations in this isolate include: M41L, T215Y.



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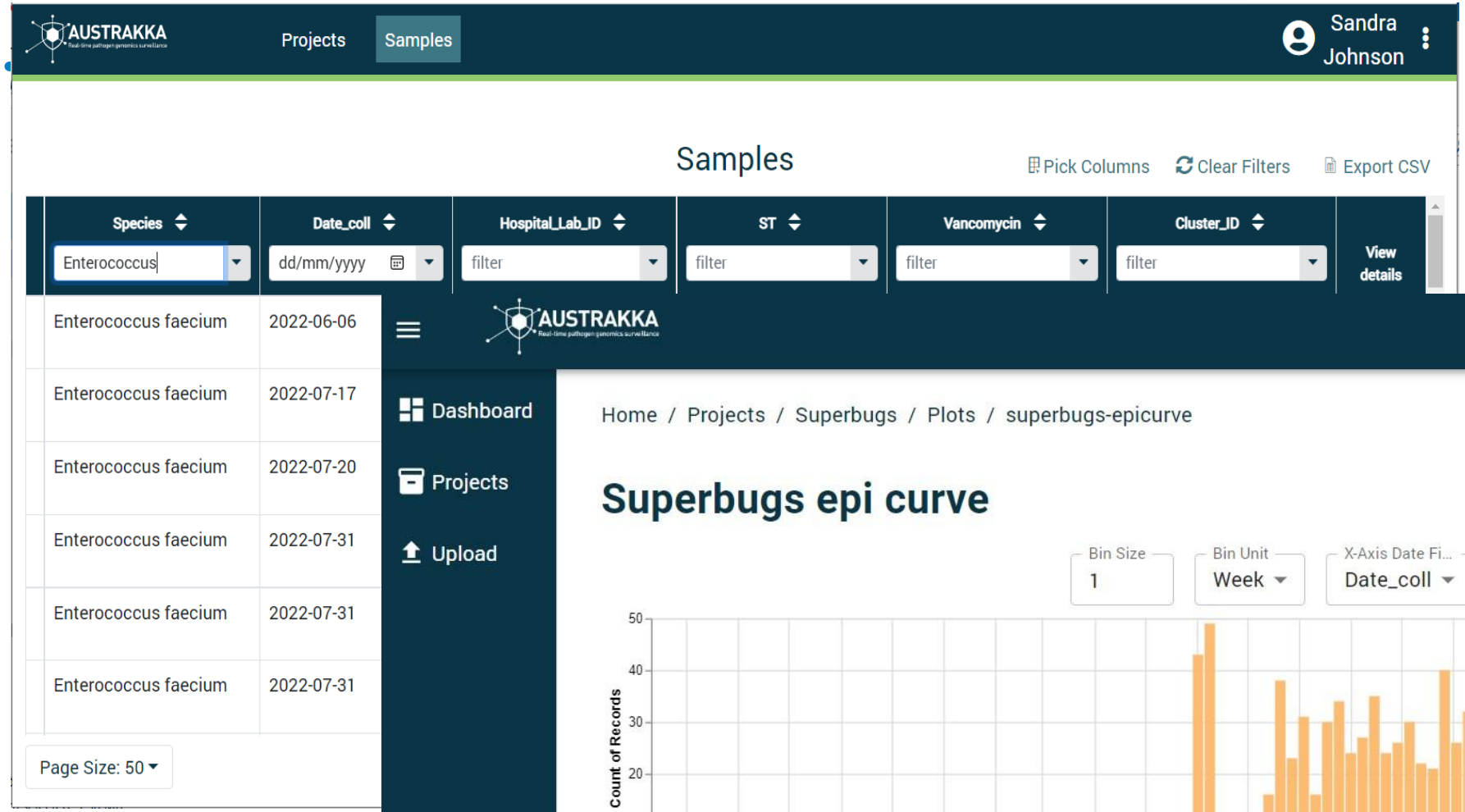
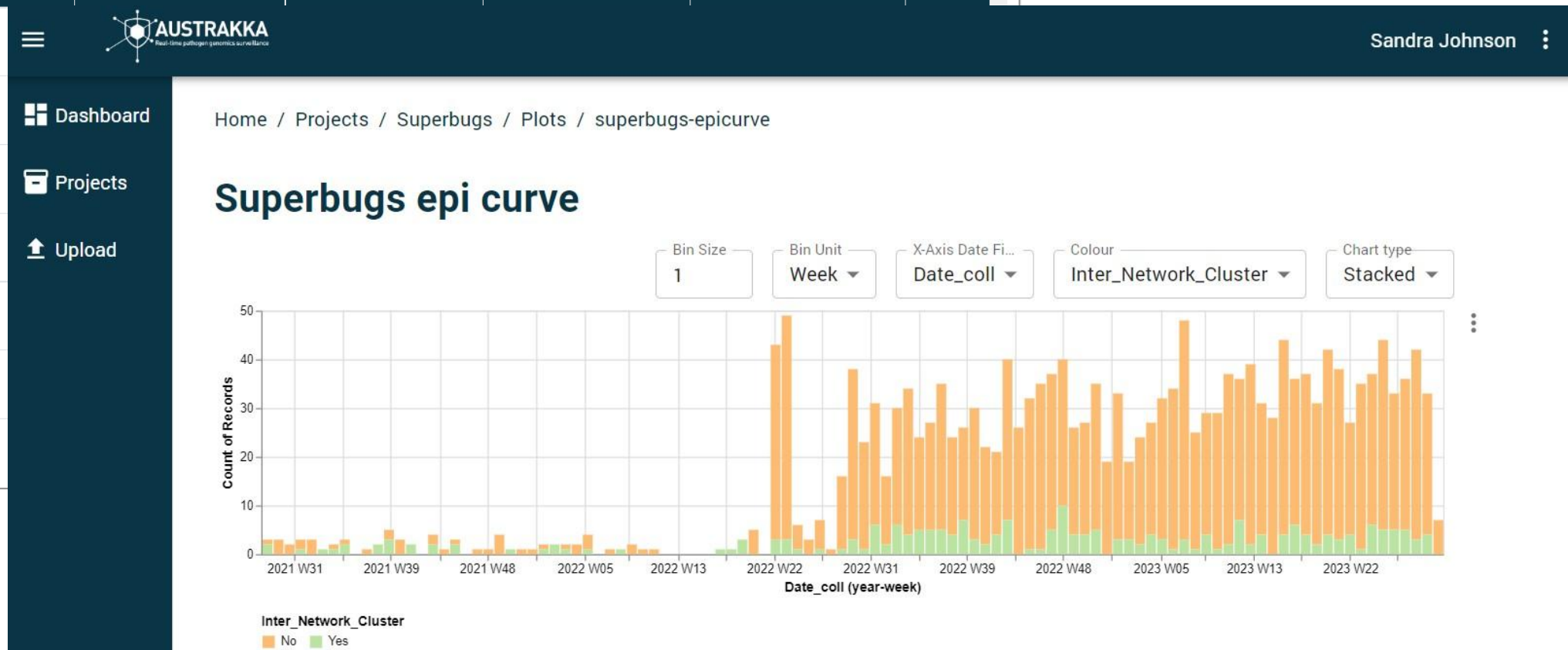
Evolving – dashboards for reporting?





Critical role of scientists reporting AMR

Unlike some other genomic tests, significant knowledge of AMR mechanisms, intrinsic resistance and phenotypic testing is required to integrate genomic AMR into reports

1. Check genotype fits with phenotype

e.g. CIM positive but no carbapenemase gene detected

2. Reporting requirements – notifiable (national or reportable to network)

3. Identify when further wet lab or dry lab work might be required

e.g. bioinformatic analysis identifies partial genes

e.g. plasmid dropout in culture

Reporting other results

Reporting typing results

Mostly straightforward, but 'typing' is quite variable

- Single isolate … >=1 typing result
- Important to ensure typing scheme used is clear

Examples:

- **MLST** – most clinically significant species
- **Other clustering** – cgMLST, (wgMLST), species & site specific e.g. Vic Mtb phylo clusters
- **Species-specific** – lineages, virulence factors, AMR profiles, strains, genotypes
  - Examples - Mtb lineages, *emm* types (GAS), NG-MAST (gono), *Salmonella* OH typing, HBV genotypes

Things to consider when reporting typing

Reporting scheme/versions of typing used – e.g. MLST scheme, database versions (where relevant). Note there are multiple MLST schemes for some species!

Make sure your local databases are updated regularly – esp relevant for global schemes (MLST is a common one that needs updating)

Dynamic phylo clustering schemes are complex e.g. cgMLST – clusters change based on what isolates are in the analysis, and will split & merge – good communication is needed to ensure that people receiving report understand this and can manage this tracking in their daily work

Speciation

- **Report methods – e.g. kmer ID, ANI**
- **Compare to phenotype (including MALDI/Vitek)**
  - And maybe expected species from referrer?
- **Think about if and how you might need to communicate uncertainty**
  - For difficult ID cases

Virulence factors

- First question – Is it needed?
- Do they (VFs) link to any disease phenotype, severity, or risk?

- If you report – which database, and interpretatiom

Next week

- Example results to report – some tricky ones!
- Design some reports
- (If you have any reports you need to design for your lab, let us know and we can all do it together!)