Inter-laboratory Study Comparing Bacterial AMR Predictions From WGS Data

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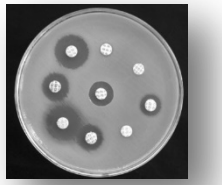
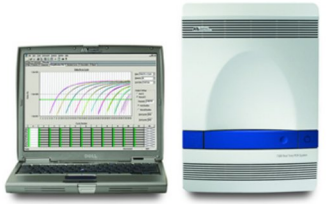
Current Methods For Antibiotic Sensitivity Testing

Culture



Molecular



WGS

Whole Genome Sequencing

- Where it works well…
  - Provides a single test of all known resistance determinants;
  - If genotype/phenotype relationship is well defined;
  - Slow growing organisms;
  - Can trace the spread of resistance;
  - Good results from *M. tuberculosis* and *S. aureus*.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

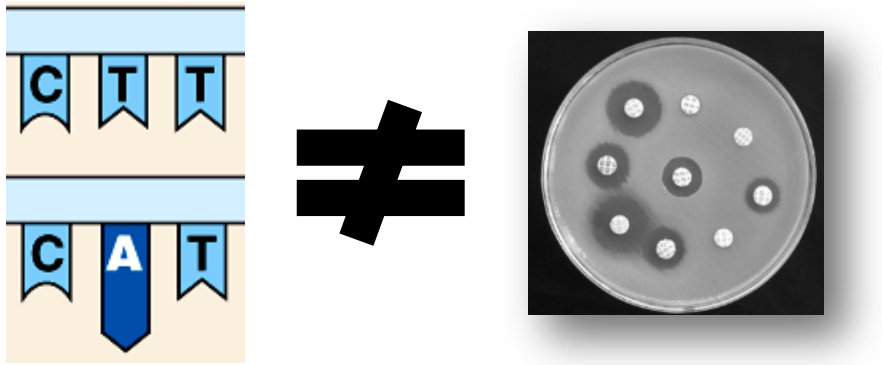
The CRyPTIC Consortium and the 100,000 Genomes Project

RESULTS

A total of 10,209 isolates were analyzed. The largest proportion of phenotypes was predicted for rifampin (9660 [95.4%] of 10,130) and the smallest was predicted for ethambutol (8794 [89.8%] of 9794). Resistance to isoniazid, rifampin, ethambutol, and pyrazinamide was correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity, respectively, and susceptibility to these drugs was correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity. Of the 7516 isolates with complete phenotypic drug-susceptibility profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted. Among the 4037 phenotypic profiles that were predicted to be pansusceptible, 3952 (97.9%) were correctly predicted.

Whole Genome Sequencing

- Where it doesn't work well…
  - WGS does not measure susceptibility. Resistance must inferred. WGS will not replace phenotypic AST.
  - Cost;
  - Speed compared to phenotypic AST for most organisms.



The Problem

- WGS methods developed independently;
- Tested internally;
- Methods not always published;
- Easy to produce confirmatory manuscripts showing WGS works well
  - Pick a bug, get a bunch of samples
  - Pick a drug
  - Train your bioinformatics pipeline to recognise likely resistance mechanisms in advance
  - Publish your high sensitivities and specificities with a couple of unexplained outlier samples

Inter-laboratory Study Plan

9 Laboratories

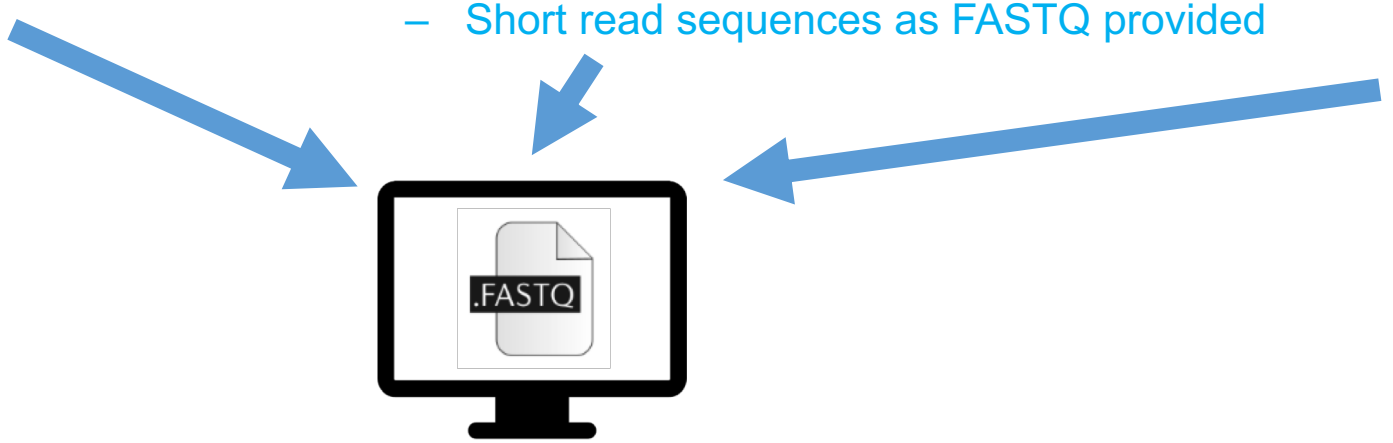
- Unique analysis pipelines.
- Mixture of research and clinical labs.

10 Samples

- Genuine clinical samples
- Carbapenem-resistant *Enterobacteriaceae*
- Cultured isolates – genomic DNA
- Short read sequences as FASTQ provided

4 Antimicrobials

- Ciprofloxacin
- Amikacin
- Gentamicin
- Cefotaxime



Reporting: Species ID, AMR associated genes, resistance prediction for 4 antimicrobials

10 Samples – AMRIL_1, AMRIL_2, AMRIL_3… etc

AMRIL_1 and AMRIL_6

- Exact duplicates
- Susceptible to majority of antibiotics
- Sequenced to very high depth
- Very few resistance associated genes

AMRIL_2 and AMRIL_5

- Duplicates
- AMRIL_5 a high depth sample
- AMRIL_2 an extremely low depth sample
- Extremely resistant

AMRIL_3 and AMRIL_9

- Both from same original isolate
- Sequenced using two different methods in two separate labs.

AMRIL_4, AMRIL_7 and AMRIL_8

- Variety of species, genes, resistance profiles and sequencing depths.

AMRIL_10

- Not an *Enterobacteriaceae - Acinetobacter baumanii*
- Highly resistant, multiple carbapenemase genes
- Intrinsically less susceptible to antimicrobials

Results – Taxonomic identification

Lab ID	AMRIL_1	AMRIL_2	AMRIL_3	AMRIL_4	AMRIL_5	AMRIL_6	AMRIL_7	AMRIL_8	AMRIL_9	AMRIL_10
Lab_1	*K. pneumoniae*	*E. cloacae*	*K. oxytoca*	*K. pneumoniae*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*
Lab_2	*K. pneumoniae*	*Shigella phage SflV*	*K. oxytoca*	*K. pneumoniae*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*
Lab_3	*K. pneumoniae*		*K. oxytoca*	*K. pneumoniae*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*Citrobacter sp.*	*K. oxytoca*	*A. baumannii*
Lab_4	*K. pneumoniae*	*E. cloacae*	*K. oxytoca*	*K. pneumoniae*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*Citrobacter sp.*	*K. oxytoca*	*A. baumannii*
Lab_5	*K. pneumoniae*	*E. cloacae complex*	*K. oxytoca*	*E. coli*	*K. pneumoniae*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*
Lab_6	*K. pneumoniae*	*E. cloacae complex*		*Klebsiella sp.*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*
Lab_7	*K. pneumoniae*	*E. cloacae complex*	*K. oxytoca*	*K. pneumoniae*	*E. cloacae complex*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*
Lab_8	*K. pneumoniae*	*E. cloacae complex*	*K. oxytoca*	*K. pneumoniae*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*
Lab_9	*K. pneumoniae*	*E. cloacae subsp. cloacae*	*K. oxytoca*	*K. pneumoniae*	*E. cloacae subsp. cloacae*	*K. pneumoniae*	*E. coli*	*C. freundii*	*K. oxytoca*	*A. baumannii*

Species identification made by each laboratory for each sample. Missing data represents results not reported

Results – Carbapenemase Genes

Lab ID	AMRIL_1	AMRIL_2	AMRIL_3	AMRIL_4	AMRIL_5	AMRIL_6	AMRIL_7	AMRIL_8	AMRIL_9	AMRIL_10
REF PCR	**OXA-48-like**	**OXA-48-like**	**OXA-48-like**	**NDM**	**OXA-48-like**	**OXA-48-like**	**IMP**	**VIM**	**OXA-48-like**	**OXA-23-like + OXA-51-like**
Lab_1a	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_1b	OXA-48	OXA-48	OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_2	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_3	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_4	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-34 + IMP-9	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_5	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23
Lab_6	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_7	OXA-48	OXA-48	OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_8	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_9	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66

Carbapenemase genes reported by each laboratory for each sample. Included for comparison are specific carbapenemase PCR results for each sample in the second row under the ID “REF PCR”. Lab_1 provided different results using two separate methods and so are included as Lab_1a and Lab_1b. Missing data represents results not reported.

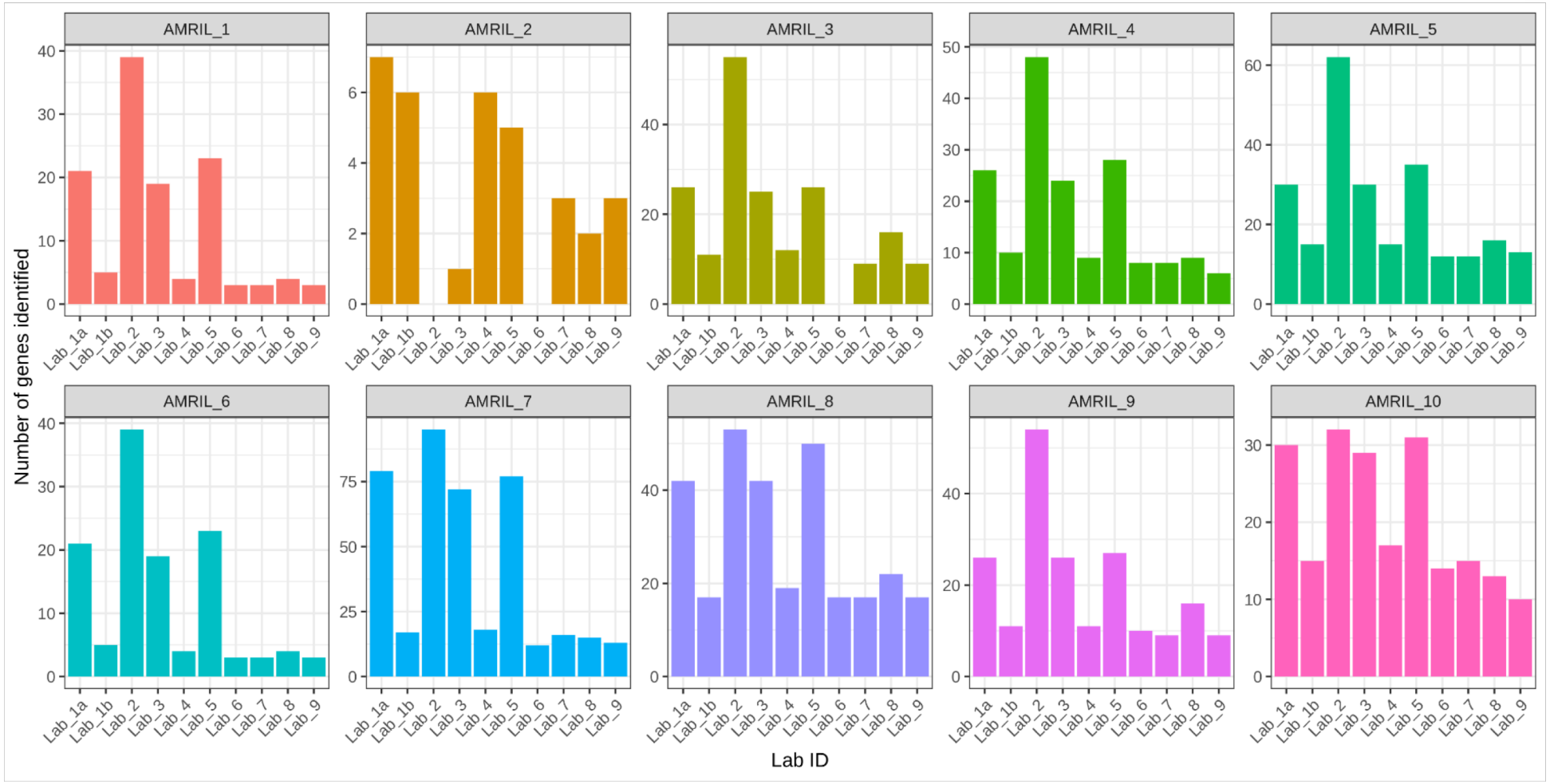
Results – Carbapenemase Genes

Database choice

Lab ID	AMRIL_1	AMRIL_2	AMRIL_3	AMRIL_4	AMRIL_5	AMRIL_6	AMRIL_7	AMRIL_8	AMRIL_9	AMRIL_10
REF PCR	OXA-48-like	OXA-48-like	OXA-48-like	NDM	OXA-48-like	OXA-48-like	IMP	VIM	OXA-48-like	OXA-23-like + OXA-51-like
Lab_1a	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_1b	OXA-48	OXA-48	OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_2	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_3	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_4	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-34 + IMP-9	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_5	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23
Lab_6	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_7	OXA-48	OXA-48	OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_8	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-34	VIM-4	OXA-181	OXA-23 + OXA-66
Lab_9	OXA-48		OXA-181	NDM-1	OXA-48	OXA-48	IMP-1	VIM-4	OXA-181	OXA-23 + OXA-66

Carbapenemase genes reported by each laboratory for each sample. Included for comparison are specific carbapenemase PCR results for each sample in the second row under the ID “REF PCR”. Lab_1 provided different results using two separate methods and so are included as Lab_1a and Lab_1b. Missing data represents results not reported.

Results – Number Of AMR Associated Genes



Results – Concordance Across Laboratories And Drug

Lab ID	Ciprofloxacin	Gentamicin	Amikacin	Cefotaxime
Lab_1a	70%	80%	50%	90%
Lab_1b	70%	80%	50%	90%
Lab_3	70%	70%	40%	60%
Lab_5	80%	60%	20%	70%
Lab_6	67%	67%	67%	67%
Lab_7	70%	90%	60%	60%
Lab_8	70%	80%	40%	60%
Lab_9	70%	90%	40%	80%

Results – Concordance Across Laboratories And Samples

High and low depth duplicate samples

Lab ID	AMRIL_1	AMRIL_2	AMRIL_3	AMRIL_4	AMRIL_5	AMRIL_6	AMRIL_7	AMRIL_8	AMRIL_9	AMRIL_10
Lab_1a	100%	50%	75%	100%	100%	100%	50%	75%	100%	0%
Lab_1b	100%	50%	75%	100%	100%	100%	50%	75%	100%	0%
Lab_3	50%	25%	50%	25%	75%	75%	75%	75%	50%	75%
Lab_5	25%	25%	75%	50%	100%	0%	75%	75%	50%	100%
Lab_6	75%	0%	0%	50%	100%	75%	25%	75%	75%	75%
Lab_7	75%	25%	75%	100%	75%	75%	50%	100%	50%	75%
Lab_8	75%	25%	50%	50%	100%	75%	50%	75%	50%	75%
Lab_9	100%	25%	50%	75%	100%	100%	50%	75%	50%	75%

Results – Concordance Across Laboratories And Samples

Same isolate sequenced with different methods

Lab ID	AMRIL_1	AMRIL_2	AMRIL_3	AMRIL_4	AMRIL_5	AMRIL_6	AMRIL_7	AMRIL_8	AMRIL_9	AMRIL_10
Lab_1a	100%	50%	75%	100%	100%	100%	50%	75%	100%	0%
Lab_1b	100%	50%	75%	100%	100%	100%	50%	75%	100%	0%
Lab_3	50%	25%	50%	25%	75%	75%	75%	75%	50%	75%
Lab_5	25%	25%	75%	50%	100%	0%	75%	75%	50%	100%
Lab_6	75%	0%	0%	50%	100%	75%	25%	75%	75%	75%
Lab_7	75%	25%	75%	100%	75%	75%	50%	100%	50%	75%
Lab_8	75%	25%	50%	50%	100%	75%	50%	75%	50%	75%
Lab_9	100%	25%	50%	75%	100%	100%	50%	75%	50%	75%

Results – Concordance Across Laboratories And Samples

The same sample!

Lab ID	AMRIL_1	AMRIL_2	AMRIL_3	AMRIL_4	AMRIL_5	AMRIL_6	AMRIL_7	AMRIL_8	AMRIL_9	AMRIL_10
Lab_1a	100%	50%	75%	100%	100%	100%	50%	75%	100%	0%
Lab_1b	100%	50%	75%	100%	100%	100%	50%	75%	100%	0%
Lab_3	50%	25%	50%	25%	75%	75%	75%	75%	50%	75%
Lab_5	25%	25%	75%	50%	100%	0%	75%	75%	50%	100%
Lab_6	75%	0%	0%	50%	100%	75%	25%	75%	75%	75%
Lab_7	75%	25%	75%	100%	75%	75%	50%	100%	50%	75%
Lab_8	75%	25%	50%	50%	100%	75%	50%	75%	50%	75%
Lab_9	100%	25%	50%	75%	100%	100%	50%	75%	50%	75%

Conclusions & Recommendations

- No two labs returned the same results
- Identification of carabapenemase genes and resistant isolates works well
- Nomenclature of identified resistance genes changes based on the reference database used
- Human interpretation of results still part of pipelines – open to human error in analysing identical samples
- Major errors from low read depth. Data must be of a certain quality or cannot be used
- Identity cut-off when comparing against databases key – too low and specificity drops

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