Module 1
From genotype to phenotype:
Prediction of AMR by WGS

Exercise

The Fleming Fund
Regional Grants

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Exercise in understanding ResFinder output

- Training in understanding the output of tools for AMR prediction by WGS
  - ResFinder, but many other tools available
  - Excel file with list of tools (not completely updated)

Table X. Open-access resources for *in silico* antimicrobial resistance detection in bacteria					
Name	**Target**		**Software**		**Database**
			Type	Downloadable[1]	Source
ABRES Finder	General AMR		Profile HMM	No	Own
ABRICATE	General AMR		BLAST	Yes	ResFinder, CARD, ARG-ANNOT, NCBI AMRFinder, EcOH, Plasmid
ARDB	General AMR		BLAST	Yes	Own
ARG-ANNOT	General AMR		–	–	Own
ARIBA	General AMR (single isolate sequences)		Minimap, Bowtie2	Yes	Derived from ARG-ANNOT, CARD, PlasmidFinder, ResFinder, VFI
CARD	General AMR		BLAST, RGI	Yes	Own
IRIDA plugin AMR detection	General AMR		RGI, staramr	Yes	CARD, PointFinder, PlasmidFinder and ResFinder
Kmer resistance	General AMR		KMA	Yes	ResFinder
MEGARes (AMRplusplus)	General AMR		BWA	Yes	Derived from ARG-ANNOT, CARD, NCBI Lahey Clinic beta-lactam
NCBI AMRFinder	General AMR		BLAST, HMMER	Yes	Own
Noradab	General AMR		BLAST	No	Derived from ARDB and CARD[2]
Patric	General AMR		BLAST	Yes	Own
ResFinder-3	General AMR		BLAST, KMA	Yes	Own

Exercise

- **Materials:**
- Six *E. coli* sequence analysis outputs from ResFinder
  - a list of links - to avoid bottle neck and delay on ResFinder
- Sequence data in the format of assemblies (fasta files), if you want to try other tools
- A list of open access resources for in-silico AMR detection (Excel).

Tasks

- Determine the presence of resistance genes and their corresponding predicted resistances within selected antimicrobial classes/antimicrobials based on ResFinder outputs with focus on aminoglycoside, macrolide, polymyxin and beta-lactam resisance

- Additional question on the beta-lactam resistance type

Evaluation:

- Report results by SurveyMonkey
https://www.surveymonkey.com/r/Gentophen_1

- For each isolate, you should report the resistance phenotypes and the beta-lactam resistance type

- The survey will give you an automated score after completion.

- We will upload a results sheet

* 2. Based on the sequence analysis outputs of strain 1 from Resfinder (or similar tool), which of the following groups or classes of antimicrobials did you detect resistance genes towards?

☐ Aminoglycosides

☐ Macrolides

☐ Beta-lactams

☐ Polymyxins (Colistin)

3. If the strain 1 is predicted to be beta-lactam resistant; can you determine which type of resistance

☐ ESBL (extended spectrum beta-lactam resistance)

☐ AmpC

☐ Carbapenem

☐ No beta-lactam resistance

☐ I'm unsure

ESBL, AmpC and carbapenemase phenotypes

- Classification of carbapenemase, ESBL (Extended spectrum beta-lactamase) and AmpC phenotypes according to the scheme provided by EFSA

- Carbapenemase (CPE) phenotype if **meropenem** MIC >0.12 μg/ml;

- Prediction: resistant to meropenem, imipenem and/or ertapenem

ESBL classification

- Classification of ESBL phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC <=8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime)

- Prediction: Resistant to cefotaxime and/or ceftazidime but not meropenem and not cefoxitin

AmpC phenotype

- AmpC phenotype if cefotaxime/ceftazidime MIC >1 μg/ml and meropenem MIC <=0.12 μg/ml and cefoxitin MIC >8 μg/ml and no synergy (clavulanic acid and cefotaxime/ceftazidime)

- Prediction: CMY-2 gene or AmpC-promoter upregulation (point mutation). Resistant to cefotaxime and/or ceftazidime and cefoxitin but not meropenem

ESBL-AmpC phenotype

- ESBL-AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime)
  - Often an artifact of shortcomings in MIC method

- Prediction: If both genes/mutations relevant for ESBL and AmpC phenotypes are present

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
amikacin	aminoglycoside	No resistance	
tobramycin	aminoglycoside	Resistant	aac(3)-IIa (aac(3)-IIa_X51534)
gentamicin	aminoglycoside	Resistant	aac(3)-IIa (aac(3)-IIa_X51534)
cefepime	beta-lactam	No resistance	
piperacillin+tazobactam	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
cefoxitin	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
ampicillin	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840), blaTEM-1B (blaTEM-1B_AY458016)
ampicillin+clavulanic acid	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
cefotaxime	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
imipenem	beta-lactam	No resistance	
ertapenem	beta-lactam	No resistance	
ceftazidime	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
temocillin	beta-lactam	No resistance	
meropenem	beta-lactam	No resistance	
ciprofloxacin	fluoroquinolone	Resistant	qnrB19 (qnrB19_EU432277) gyrA (p.S83L)
nalidixic acid	fluoroquinolone	Resistant	gyrA (p.S83L)
sulfamethoxazole	folate pathway antagonist	Resistant	sul3 (sul3_AJ459418)
trimethoprim	folate pathway antagonist	No resistance	
fosfomycin	fosfomycin	No resistance	
azithromycin	macrolide	Resistant	msr(E) (msr(E)_FR751518)
chloramphenicol	phenicol	Resistant	cmlA1 (cmlA1_M64556)
colistin	polymyxin	No resistance	
tigecycline	tetracycline	No resistance	
tetracycline	tetracycline	Resistant	tet(B) (tet(B)_AF326777)

Not all beta-lactam resistance genes are ESBL/AmpC nor Carba

Try it ☺

- Results of survey is only for your own evaluation
- Results sheet will be uploadet too

- Try out different databeses and compare the results