EUCIC Workshop II: Resistance profiling

Detection of antibiotic resistance

Bacterial genomes don't only contain information to reconstruct phylogenetic relationships from the core genome. They also carry additional DNA in form of plasmids that can contain antibiotic resistance as well as integrons and pathogenicity islands that reside on the chromosome.

These antibiotic resistance genes are manifold and often specific for a particular group of antibiotics. There are antibiotics that impact on bacterial protein synthesis by binding to ribosomal RNA and ribosomal proteins, for example macrolides, lincosamides, and tetracyclines. Other antibitics bind to proteins essential for bacterial metabolism such as fusidic acid, which binds to the elongation factor fusA, or the quinolones, which act on DNA gyrase. A large group of antibiotics aims at interfering with cell wall synthesis and thus inhibit bacterial growth; these are the betalactam antibiotics.

Betalactamase genes, for example blaZ, blaTEM-1, can split the betalactam ring that makes up penicillin for instance. ESBL genes – which stands for extended spectrum betalactamase – are effective not only against penicillins but also their improvements the expanded-spectrum cephalosporins cefotaxime, ceftriaxone, and ceftazidime. Examples for this are blaCTX-M-15 and blaSHV-5. Carbapenem antibiotics are the latest development, but even here there are carbapenemases that inactivate the antibiotic, such as blaOXA-48, blaVIM, blaNDM, and blaIMP.

Example table of laterally acquired resistance genes

against (antibitotic class): *example*	**Gene abbreviations:**
Aminoglycoside *Amikacin, Gentamycin, Neomycin, Streptomycin*	aac, aad, ant, aph, armA, npmA, rmt, spc, sph, str
Beta-Lactams *Penicillins (e.g. Amoxicillin, Ampicillin, Oxacillin)* *Cephalosporine/ESBL (5 Generations*)* *Carbapenems (Ertapenem, Doripenem, Imipenem, Meropenem)*	 blaTEM-1, blaSHV-1, mecA blaCTX-M-14+15, blaTEM-10, 12+26, blaSHV-5+12, blaPER, blaVEB blaOXA-48, BlaIMP, blaKPC, BlaNDM, blaVIM, blaCMY, blaSME
Chloramphenicol *Halemycin, Posifemicol*	cat, cfr, cml, cmr, cmx, flexA, floR, pexA
Colistin *also: Polymyxin E; Reserve antibiotic!*	mcr-1, mcr-2, mcr-3, mcr-4
Fluorchinolone *Ciprofloxacin*	Qnr, aac (6´)Ib-cr, norA, oqxA+B, qepA+A_2
Fosfomycin *Infectofos, Fosfuro, Monuril*	fosA, B +C
Fusidic acid *Fucidin, Fusicutan*	fusB, far1

Glycopeptide *Teicoplanin; Vancomycin; Reserve antibiotic!*	Van, dd1A2-Sc
Lipopetide** *Daptomycin*	mprF, yycFG
Macrolide/Lincosamide/Streptogramin B *Erythromycin, Clarithromycin / Clindamycin/ Pristinamycin IA*	car(A),cfr, ere, erm, lmr, lnu, lsa, mef, mph, msr, ole, srm, tlr, vat, vga, vgb
Nitroimidazole *Metronidazol*	nim
Oxazolidinone *Tedezolid; Reserve antibiotic!*	cfr
Rifampicin *Eremfat, Rifater, Rifinah*	ARR-2 bis -7
Sulfonamide *Cotrimoxazol*	sul 1 bis 3
Tetracycline *Doxycyclin, Tigecyclin*	ortB, otrA+B, tcr,tet
Trimethoprim *Motrim, Triprim, Bactrim (combination)*	dfr

*)1.Cafadroxil, 2. Cofoxitin, 3. Ceftriaxone, 4.Cefepime, 5. Cetaroline fosamil

source:
https://cge.cbs.dtu.dk/services/ResFinder/database.php **)
https://www.ncbi.nlm.nih.gov/pubmed/23215859

Data download

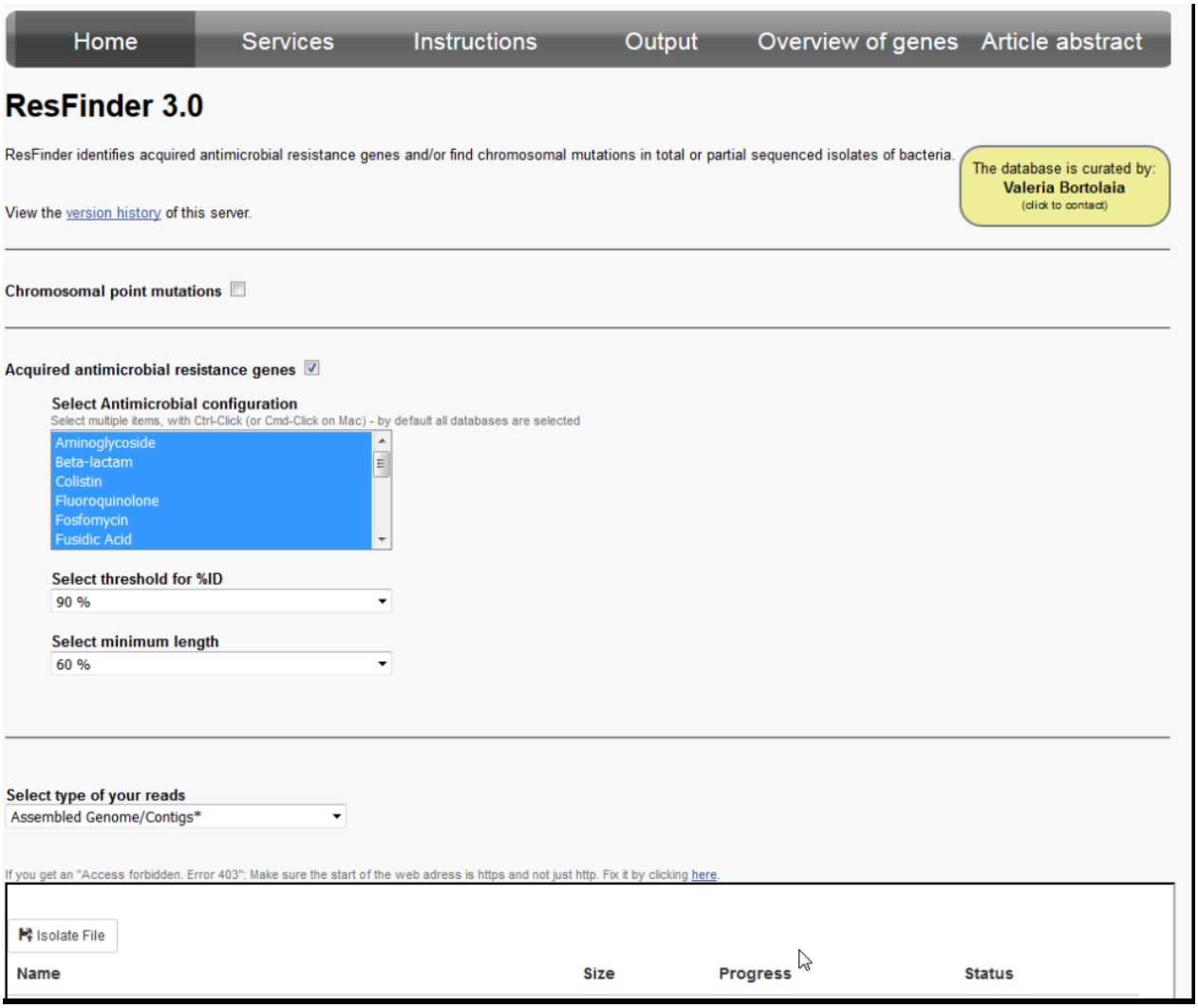
You will have downloaded the samples for this part of the practical this morning from this link:

https://we.tl/t-WskIk5ckDE

The files you need are:
Assembly of *Enterobacter kobei:* Ekobei_15682_8_87.contigs_velvet.fa
Assembly of *S. aureus EMRSA-15:* EMRSA15_7480_8_66.contigs_velvet.fa

Detection of resistance determinants with ResFinder

The sequencing data we obtain from a MiSeq run can be put back together – like a puzzle – with the help of programs such as velvet or SPAdes. This process is called assembly. An assembly usually reflects 95-99% of the complete genome but it does tend to have gaps. This is due to the repeated presence of IS elements, which cannot be bridged due to the short read length. However, we can still make use of these incomplete, draft assemblies to look for the presence of antibiotic resistance genes. There are databases such as ResFinder that contain a large number of genes to search for.



Go to the ResFinder webpage (https://cge.cbs.dtu.dk/services/ResFinder/) and upload the genome of *Enterobacter kobei* (Ekobei_15682_8_87.contigs_velvet.fa) to start with.

Which antibiotic resistance genes can you find and can you allocate them to the respective antibiotic in the antibiogram?

E. kobei Antibiogram

Antibiotic	Antibiotic class	Susceptibility / Resistance	Allocated gene
Ertapenem	Carbapenem	R	
Meropenem	Carbapenem	R	
Imipenem	Carbapenem	R	
Colistin	Polypeptide	S	
Ceftazidime	Cephalosporine	R	
Ciprofloxacin	(Fluoro-) Quinolone	R	
Amikacin	Aminoglycoside	S	
Gentamicin	Aminoglycoside	R	
Trimethoprim	Trimethoprim	S	

Can you find all genes? Are there things you cannot allocate? What could be a possible explanation?

Upload the second genome now – *S. aureus* EMRSA-15 (EMRSA15_7480_8_66.contigs_velvet.fa). What can you see when you compare the ResFinder results with the antibiogram?

S. aureus EMRSA-15 Antibiogram

Antibiotic	Antibiotic class	Susceptibility / Resistance	Allocated gene – ResFinder	Allocated gene – pathogen.watch
Gentamicin	Aminoglycoside	S		
Penicillin	Penicillin	R		
Oxacillin / Methicillin	Penicillin	R		
Fusidic acid	Fusidic acid	R		
Vancomycin	Glycopeptide	S		
Teicoplanin	Glycopeptide	S		
Erythromycin	Macrolide	R		
Clindamycin	Lincosamide	S		
Daptomycin	Lipopeptide	S		
Mupirocin	Mupirocin	S		
Linezolid	Oxazolidinone	S		
Ciprofloxacin	(Fluoro-) Quinolone	R		
Rifampicin	Rifampicin	S		
Tetracycline	Tetracycline	S		
Trimethoprim	Trimethoprim	S		

Detection of resistance determinants with pathogen.watch

Upload the *S. aureus* genome to a second database – pathogen.watch. You will need to register – either with an email address or using an existing account like google or facebook. Then upload the sample by clicking on „upload (create your own collection)“ and then dragging & dropping the genome. For the analysis click „View Genomes“. Afterward select the genome by ticking the box next to the name. Then go to the purple button at the top and click „Create Collection“. It is not necessary to enter any other information, you can directly click on „Create Now“. Your progress will be displayed: the webserver will estimate a simple tree based on the core genome, it will determine the MLST, and under PAARSNP the antibiotic resistance prediction.
Sophia David will explain more functionality tomorrow!

You will find the general information under typing and stats.

Click in „Antibiotics“ (1), to get to the antibiotic resistance prediction.

Under the header „SNPs“ (2) you can find the respective mutations in the housekeeping genes.

And under the header „Genes“ (3) you can find the horizontally acquired genes that were found.

Pathogen.Watch is under active development, and *S. aureus* was one of the first bugs to be implemented, to show simple evolutionary relationships and predict antibiotic resistance. What differences to ResFinder can you see?

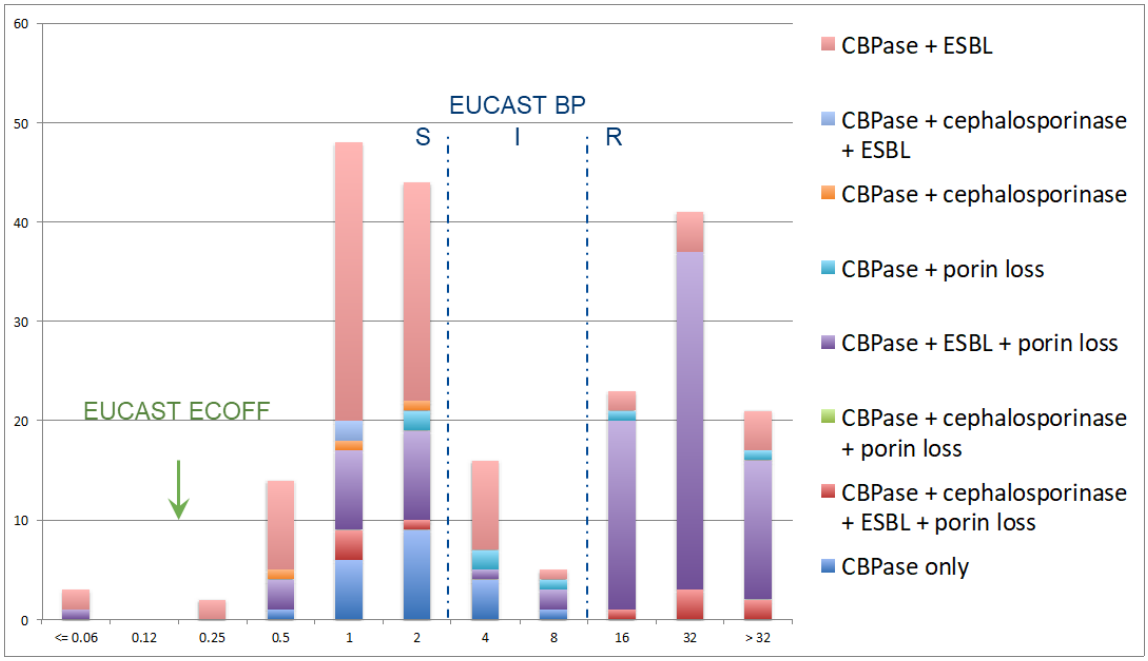
Pathogen.Watch was not only developed for the analysis of single sequences but for larger datasets as well. Additionally to genomes, tables containing metadata may be uploaded, for example latitudes and longitudes for samples that are then displayed on a map. This way you may be able to monitor the spread of antibiotic resistance bugs. Sophia David will be showcasing more of this tomorrow!

Things to keep in mind in antibiotic resistance prediction (some ramblings from personal experience)

How good is the phenotypic testing?
If your phenotyping testing is unreliable, it will be difficult to find clear genomic markers. Thus prediction of resistance is difficult as well. This applies quite often for intermediate phenotypes such as vancomycin intermediate resistance in MRSA. The isolates showing this phenotype are also known as VISA. The phenotype results from a thickening of the cell wall and has been attributed to central regulators walKR, however no specific mutations are known, and not hotspot for mutations could be identified in these genes. As the phenotype is also near the breakpoint it depends on your phenotypic method – Vitek, disk diffusion, dilution – on how well you are able to detect it.

What contributes to a phenotype?
In Gram negatives, carbapenem resistance may be conferred by carbapenemases, obviously. However, you can achieve similar levels of resistance if the bacterium carries an extended-spectrum beta-lactamase (ESBL) or cephalosporinase in combination with a porin loss. This is an example of a combination of factors contributing to resistance. An ESBL or a cephalosporinase or a porin loss on its own do not confer resistance, it is the combination that makes it effective.
From a treatment point of view, this makes no difference.
From an infection control point of view, this is important as carbapenemases are carried on plasmids, which may be spread independent of the organism, making the resistance component itself transferrable. (see also keynote lecture later today)

What is the basis of your phenotype and how strong is the conferred phenotype?
Some carbapenemases are better than others. KPC (*Klebsiella pneumoniae* carbapenemase) for example confers high levels of resistance to all the common carbapenem antibiotics ertapenem, meropenem, and imipenem.
However, OXA-48 is quite variable in terms of resistance levels. This gene works fairly well for ertapenem but not so well with imipenem, and for meropenem the actual MIC distribution looks like this:



So far, we have not identified any combination of genes or mutations that makes some OXA-48 more resistant than others. Preliminary tests in the lab have shown, however, that even those isolates with low MIC levels may revert to high levels relatively quickly under exposure to the antibiotic. In this case, the presence of a gene would overrule phenotypic testing, as reliance on the phenotypic test might lead to treatment failure.

Critical use of databases

Antibiotic resistance databases are a good starting point, however quite often they have multiple problems:

- They contain only genes that are horizontally acquired. There are resistance that arise from mutations of the target gene, so that the antibiotic cannot bind any longer and thus is non-effective. This is quite often the case for quinolones and fusidic acid. These genes are always present in the genome; a general search for presence is therefore pointless. It is more important to identify the mutated positions, however from a bioinformatics point of view this is a different process.
- They describe genes that may be acquired horizontally but that may not automatically confer resistance in the original organisms. Only the presence in a new organism, together with a strong promoter that activates the expression of the gene, can lead to resistance.
- Generally, databases are only as good as the data that is entered into them. There is the saying „shit in – shit out". If a database contains rubbish as only partial genes were deposited or because the annotation is wrong, then you will unfortunately end up with a wrong result as well.

Therefore take not when interpreting data such as antibiotic resistance prediction. It is also worth keeping in mind that you can only predict the presence of a gene, not if it is actually transcribed and expressed or if it is functionally whole.
One can only draw conclusions about potentially impaired functionality if an obvious mutation in a gene is present, such as a deletion, insertion, or a frameshift. If mutations are present in regulators or promoters however, these might be much more difficult to detect.
You can also only exclude which resistance is present, not to which antibiotics a bacterium might be susceptible as you cannot exclude new, unknown resistance mechanisms or mutations. In some organisms we know a lot about resistance and which hotspots might be affected, also for unknown mutations; this is the case in *S. aureus*, for example. In the realm of Gram-negatives, *E. coli, Klebsiella pneumoniae, Acinetobacter baumannii* a lot of unknowns still exist.