Whole Genome Sequencing for Antimicrobial Resistance Detection and Surveillance

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Background

Antimicrobial resistance poses a growing threat to public health as bacterial infections are becoming increasingly challenging to treat successfully. It primarily arises in one of three ways:

- Acquisition of anti-microbial resistance genes
- Accumulation of SNPs in genes encoding proteins targeted by antibiotics
- Insertion of a transposon inactivating or altering the expression of genes related to resistance

The spread of resistant bacteria and transmission of resistance mechanisms is alarming. Detection and surveillance is key to preventing and controling infection.

Whole genome sequencing (WGS) has gained acceptance as a tool for the prediction of antimicrobial resistance patterns, because it offers a sensitive and reproducible alternative to antimicrobial susceptibility testing. The advantage over alternative approaches is the potential to reveal the full complement of resistance determinants, including resistance towards compounds that are not routinely tested. In addition, WGS data offer insight to the mechanisms by which antimicrobial resistance is transmitted.

Aim

Our aim is to demonstrate the application of WGS for the detection of resistance genes and resistance-causing mutations using tools from the QIAGEN Microbial Genomics Pro Suite.

This poster shows the application of resistance detection in three different settings: *Klebsiella pneumoniae, Actinobacillus pleuropneumoniae* and *Mycobacterium tuberculosis*.

Resistance Detection in *Klebsiella pneumoniae* (1)

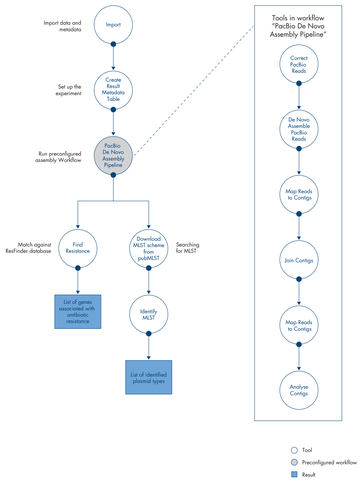
Background

Carbapenem-resistant *Enterobacteriaceae*, such as *K. pneumoniae*, are a major public health threat with an associated economic burden due to the lack of efficient antibiotics, extensive transmission and high mortality rate. Carbapenem resistance arises from several mechanisms. One is the production of carbapenemases, which hydrolyse almost all beta-lactams. These are encoded by genes that reside on plasmids or transposons and are thus easily transferrable.

Detection and tracking of plasmid-encoded resistance is difficult using short read technologies due to mobile elements and the repetitive nature of plasmids. Long read technologies have the potential to resolve and fully assemble genomes and accompanying plasmids.

Methods

Conlan et al. (2014) described carbapenem resistance in isolates of Enterobacteriaceae in a hospital setting. Using their data we demonstrate the assembly of single-molecule real-time sequencing (SMRT) reads to discriminate and resolve plasmid from chromosomally encoded resistance genes in *K. pneumoniae*.



Resistance Detection in *Klebsiella pneumoniae* (2)

Error correction of SMRT sequencing

The primary obstacles when using SMRT sequencing are the high rate of sequencing errors, the presence of chimeric reads and untrimmed adapters. However, if coverage is sufficiently high, reads can be corrected and assembled into high-quality contigs. Error-correction is part of the PacBio de Novo Assembly Pipeline found in the QIAGEN Microbial Genomics Pro Suite.

Detecting plasmid-encoded resistance genes

We detected carbapenemase-encoding genes in all five isolates analyzed. In four isolates, the gene was located on plasmids. In one isolate, it was chromosomally encoded.

Isolate	Plasmid type	bla_{KPC} gene
KP29	IncN	bla_{KPC-9}
KP30	IncF	bla_{KPC-2}
KP31	Chromosomal	bla_{KPC-2}
KP32	IncF	bla_{KPC-3}
KP33	IncF	bla_{KPC-3}

Plasmid no.	Plasmid type	Resistance gene	Predicted antimicrobial resistance
1/3	IncFII(K)	bla_{KPC-2}	Beta-lactam resistance
1/3	IncFII(K)	bla_{OXA-9}	Beta-lactam resistance
2/3	IncFIB	bla_{OXA-9}	Beta-lactam resistance
3/3	IncN	*sul1*	Sulphonamide resistance
3/3	IncN	*aadA1*	Aminoglycoside resistance
3/3	IncN	*dfrA1*	Trimethoprim resistance

Additional plasmid-encoded antimicrobial resistance mechanisms were detected in isolate KP30.

Resistance Detection in *Actinobacillus pleropneumoniae* (1)

Background

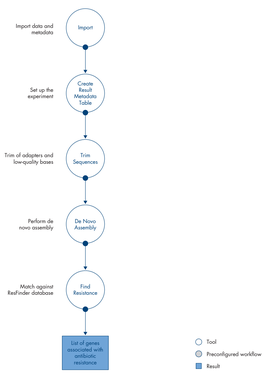
Antimicrobial resistance in animals destined for food production is of increasing concern. Because farm animals may serve as a reservoir from which antibiotic-resistant bacteria can spread to humans, there is increasing concern about antimicrobial resistance in this setting. *A. pleuropneumoniae* is a major contributor to swine respiratory disease, and antimicrobials are routinely used to limit disease severity and transmission. Resistance profiling is important for treatment decisions and surveillance purposes.

Methods

We combined the tools *Trim Sequences*, *De Novo Assembly* and *Find Resistance* in a custom-built workflow to streamline the data processing of the 94 isolates.

ResFinder database

The *Find Resistance* tool uses the ResFinder database, a curated database of acquired resistance genes, to detect antimicrobial resistance (Zankari *et al.* 2012). The output is a table dosplaying detected resistance gene and supplementary data.



Resistance Detection in *Actinobacillus pleropneumoniae* (2)

Detecting resistance genes

Using the data of Bossé et al. (2017), which describes antimicrobial resistance genes in 94 isolates of *A. pleuropneumoniae*, we demonstrate the detection of antimicrobial resistance genes from whole genome data.

Resistance makers were identified in 65 out of 94 screened isolates. Compared to the original study, we detected an additional nine resistance genes in seven isolates.

Congruence between the detected antimicrobial resistance genes and the measured MIC values was high. In only nine (6%) cases did the detection of a resistance gene not correlate with a resistant phenotype. This corresponds to a positive predictive value of 89%. In three (2%) cases, we did not detect a resistance gene in a phenotypically resistant isolate.

Antimicrobial	Gene	Resistant by genotype	Resistant by phenotype	Congruence between genotype and phenotype	MIC breakpoint
Tetracycline	*tetB, tetH*	54	56	96%	≥ 4 mg/l
Ampicillin	bla^{ROB-1}	21	19	90%	≥ 4 mg/l
Sulfoxazole	*sul2*	49	44	90%	≥ 256 mg/l
Trimthoprim	*dfrA14*	16	15	94%	≥ 32 mg/l

Resistance Detection in *Mycobacterium tuberculosis* (1)

Background

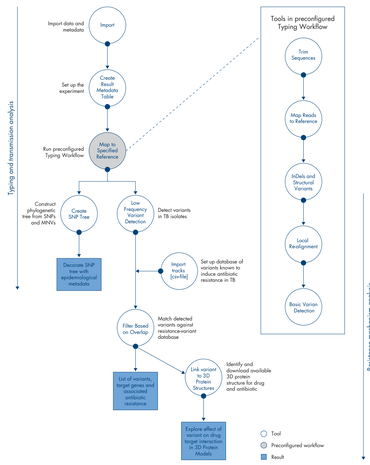
Antimicrobial resistance in *M. tuberculosis* is predominantly caused by mutations that interrupt drug–target interaction rather than by uptake of mobile elements that carry resistance genes.

Methods

Custom variant database

Our database contained variants described by Coll et al. 2014 (TBDreaM, MUBII-TB-DB and recent litterature), Miotto et al., 2014, and Allana et al. 2017.

The resulting custom database contained nearly **1500** variants in **31** loci conferring resistance towards **15** different anti-TB drugs.

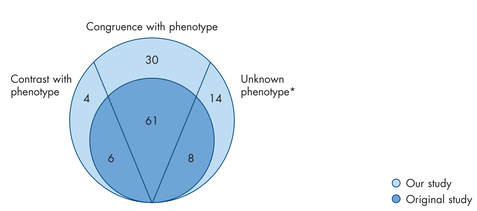


Resistance Detection in *Mycobacterium tuberculosis* (2)

Detecting resistance-causing variants

Using the data of Fiebig et al. (2017), which describes an outbreak of multi-drug-resistant TB, we demonstrate detection of resistance-causing variants in M. tuberculosis. By applying our optimized variant detector, we detected 123 resistance-causing variants the genomes of 13 analyzed isolates of *M. tuberculosis*. Of these variants, 75 were described in the original paper, while the remaining 48 were novel.

Compared to the original study, our more sensitive approach to variant detection resulted in increased congruence between detected genotypic resistance and the independently obtained results of antimicrobial susceptibility testing, increasing the positive predictive value from 63% to 90%.

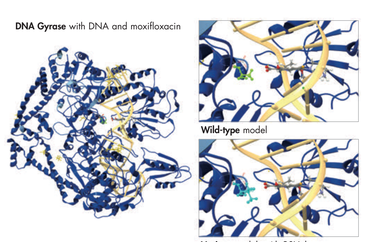


* Antimicrobial susceptibility was not tested for all drugs for all isolates

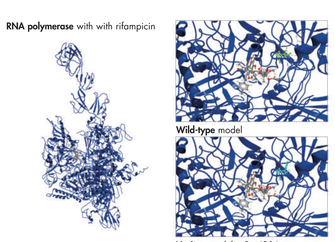
Resistance Detection in *Mycobacterium tuberculosis* (3)

Exploring and qualifying variants in 3D models

3D visualisation of variants can be an advantageous method for predicting and qualifying the effect of previously undescribed variants on antimicrobial susceptibility.



DNA gyrase is the target for fluoroquinolones. DNA gyrase is involved in supercoiling of DNA, binding DNA and introducing double-stranded breaks. Fluoroquinolones bind and lock the gyrase–DNA complex. Mutation of the target region of the gyrase alter the binding affinity for the drug, resulting in resistance.



RNA polymerase is the target for rifamycin antimicrobials, which bind inside the RNA/DNA channel, physically blocking elongation. Antimicrobial resistance arises from amino acid alterations in the binding channel, decreasing the affinity for the drug. The lower panel shows the variant located in the drug-binding site.

Conclusions

The tools of QIAGEN Microbial Genomics Pro Suite allow accurate detection of antimicrobial resistance markers. Workflows and built-in access to public databases streamline data analysis and help users get started easily.

References

1. Allana et al. (2017) pncA gene mutations associated with pyrazinamide resistance in drug-resistant tuberculosis, South Africa and Georgia. Emerg Infect Dis **23(3)**, 491–495.
2. Coll et al. (2015) Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med **7(1)**, 51.
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4. Miotto et al. 2014. *Mycobacterium tuberculosis* pyrazinamide resistance determinants: a multicenter study. M Bio. **5(5)**.
5. Zankari et al. (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother **67(11)**, 2640–2644.

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