

Beyond ordinary molecular diagnostics: next generation sequencing analytic pipeline for understanding antibiotic resistance mechanisms in clinical diagnostics

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Monitoring of drug resistance conferring genes

- rising numbers of drug resistant bacteria
 - the landscape of resistance genes is changing
 - new emerging resistance genes: *e.g. mcr-1***
 - various carbapenemases and ESBL
- epidemiology of resistance needs to be monitored in clinical setting

** Liu et al. 2016 Lancet ID

Antibiotic resistance detection pipeline: Experience in clinical practice

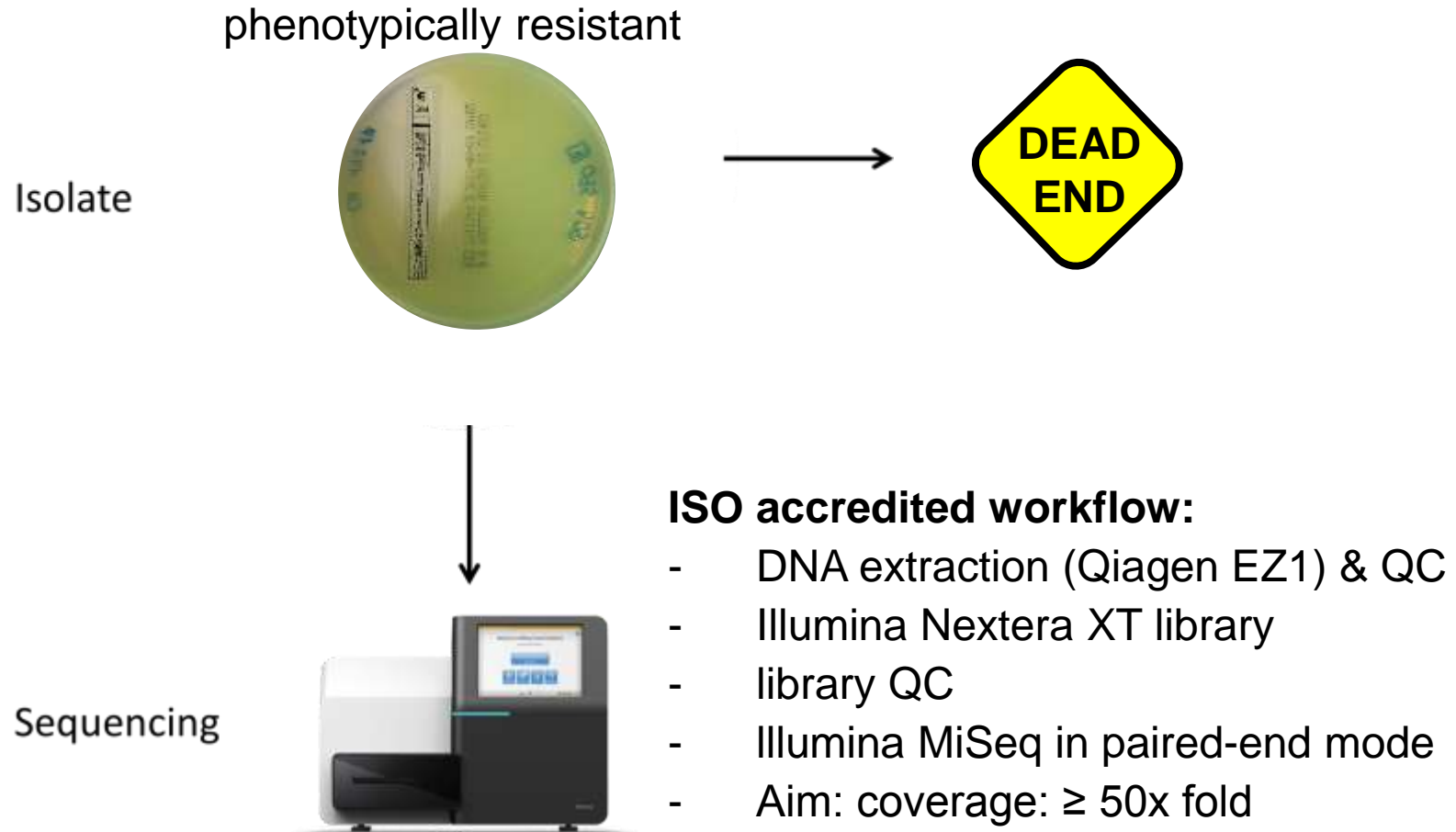
phenotypically resistant

Isolate



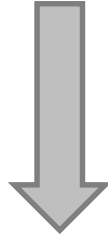
standard molecular assays:
no clear determination of
mechanism by e.g. Genexpert®
(Cepheid), easyplex® (Amplex),...

Antibiotic resistance detection pipeline: Experience in clinical practice



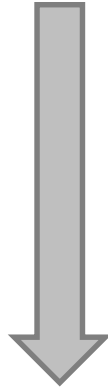
Analytical Pipeline

data preprocessing



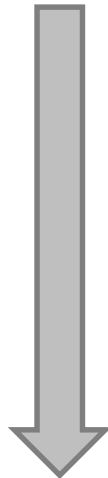
- quality control of raw reads
- trimming of reads
- assembly

gene acquisitions



- based on Zankari et al 2012 & 2013
- BLAST vs **2154** resistance conferring genes
- reporting by similarity and coverage
- covers only gene acquisitions
 - Suitable for a wide range of bacteria

resistance mutations

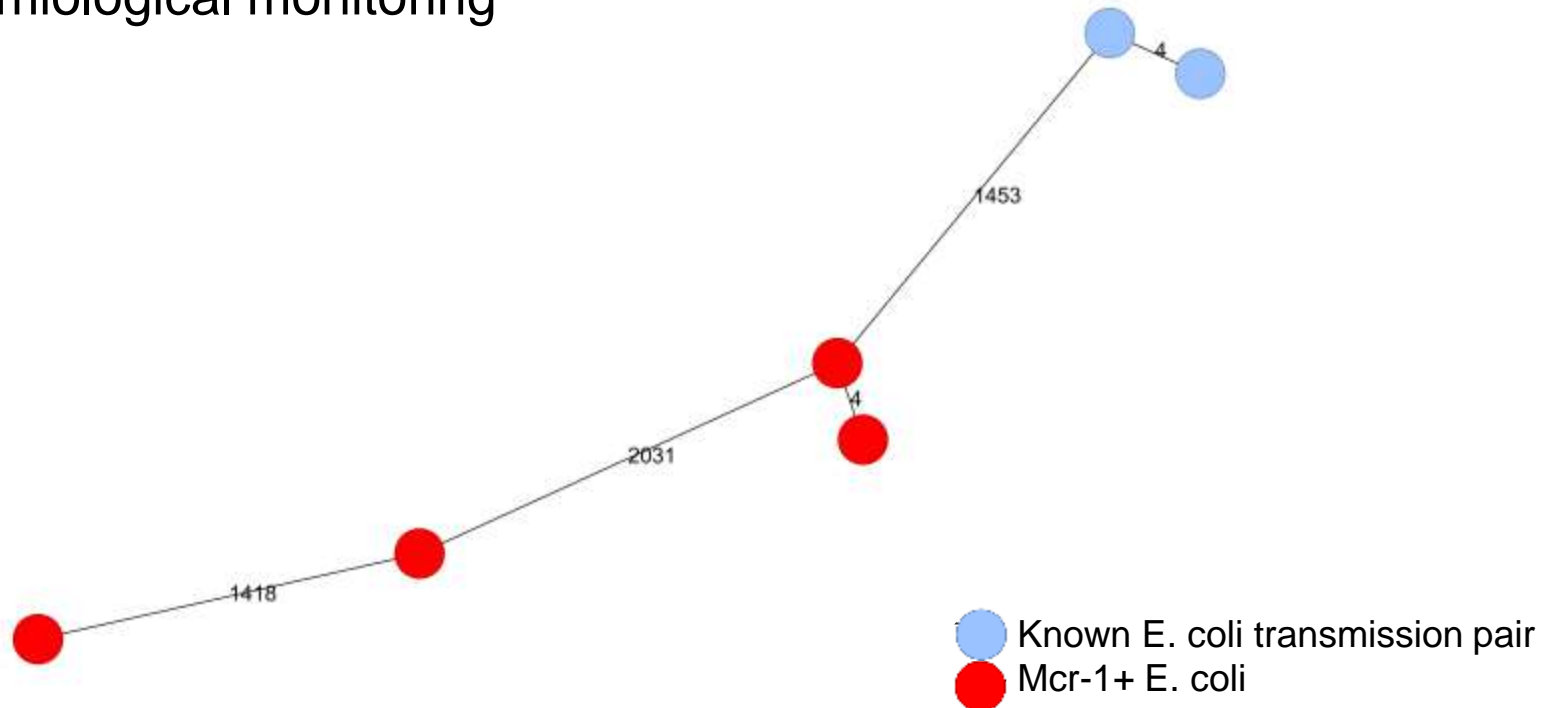


- activation of
 - *ampC*
 - efflux pumps
- inactivation
 - porins (*oprD*)
- mutations
 - *gyrA*, *parC*
- species specific

Clinical examples 1:

E. coli

- slightly above MIC cutoff for Colistin
- low level resistance can be caused by plasmid mcr1:
- *E. coli* carried mcr-1 gene
- epidemiological monitoring



Clinical examples 2:

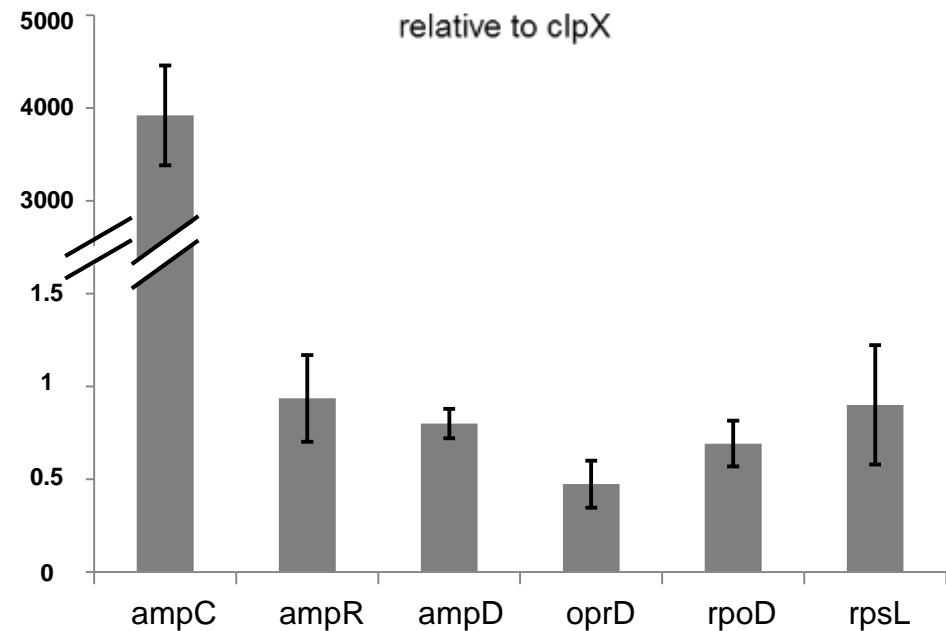
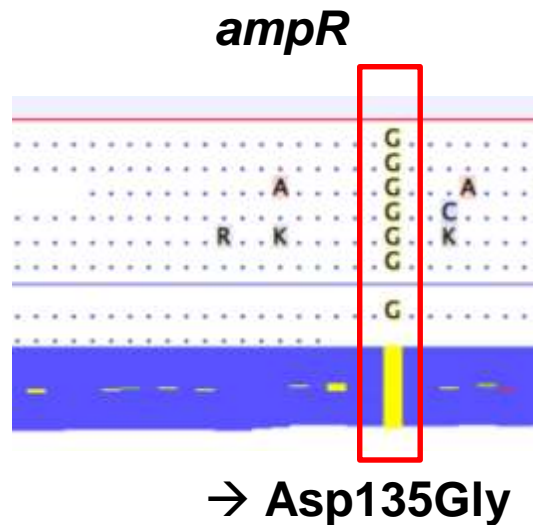
Pseudomonas aeruginosa

Piperacillin – Tazobactam	R
Ceftazidim	R
Imipenem	R
Meropenem	R
Amikacin	S
Ciprofloxacin	S
Levofloxacin	S
Colisitin	S

→ No *Carbapenamase* detectable....

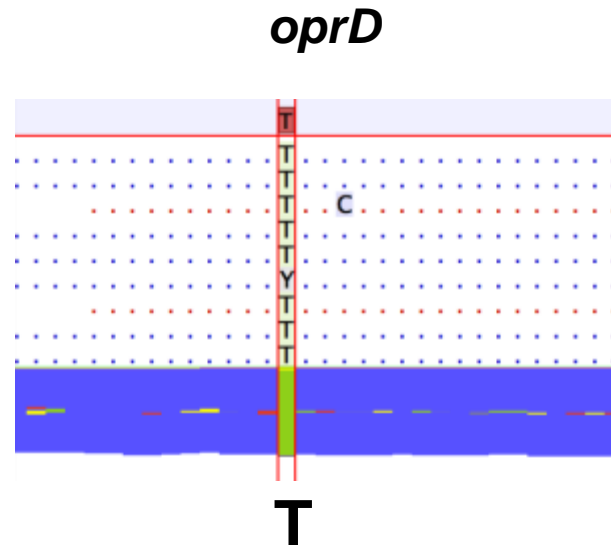
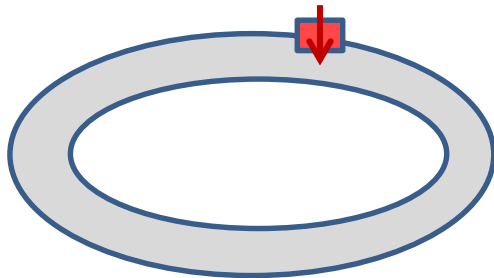
Resistance to Piperacillin – Tazobactam & Ceftazidim

- Piperacillin-Tazobactam and Ceftazidim resistance: hyperactivation of ampC production:



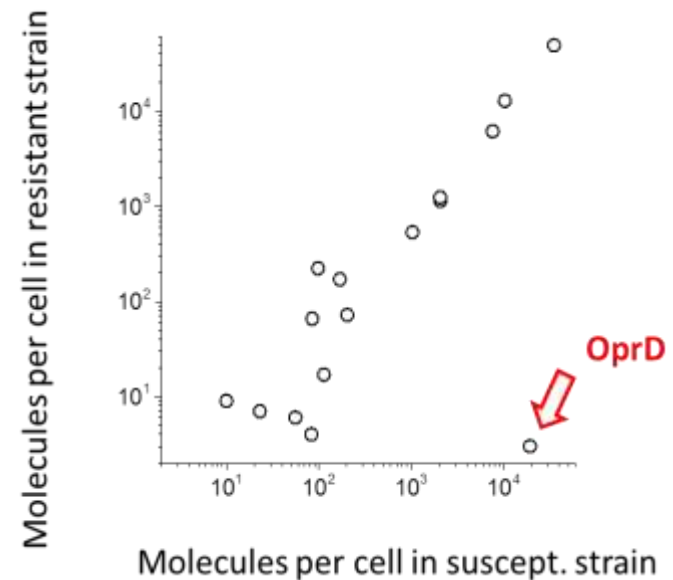
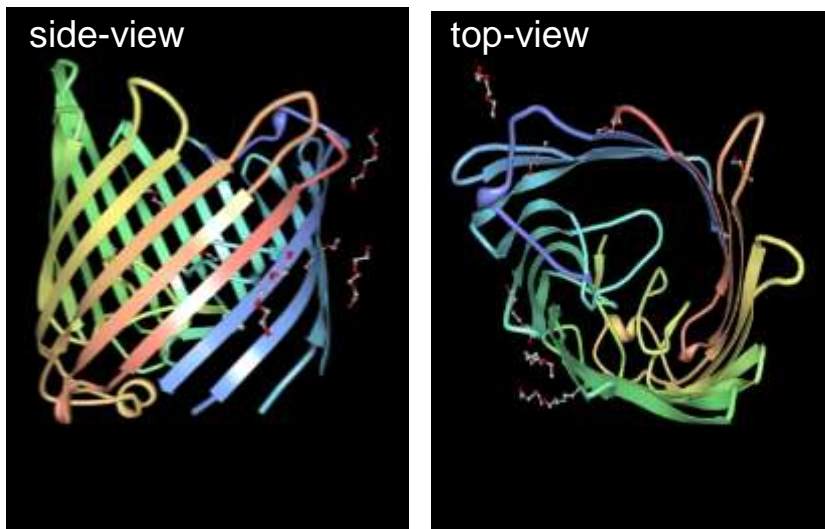
Resistance to Meropenem and Imipenem

- regulation of Influx and Efflux can have major impact on resistance pattern



→ Gln422* → deletion of 19 aa of oprD

OprD-mutation leads to Imipenem & Meropenem Resistance



- β strand is deleted
- instable membran insertion

Tandem-MS:
Wt: app. 20.000 copies
Res: < 10 copies

RCSB PDB: 3SY7

Conclusion: NGS is a powerful tool for understanding antibiotic resistance

+ Advantages

- unclear resistance mechanisms can be identified by
 - gene acquisition
 - resistance conferring mutations
- molecular epidemiology of resistance mechanisms and surveillance for transmission in the same data set
- for fastidious bacteria: antibiotic prediction faster as phenotypic
 - M. tuberculosis poster Tuesday (12:30-13:30), P1636

- Disadvantages

- costly
- long turn around times vs. phenotypic tests
- need for comprehensive resistance catalogue

Acknowledgements:

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- Prof. Dirk Bumann, Biocenter University Basel, Switzerland
- Infectious Diseases and Hospital Epidemiology, University Hospital Basel: Prof. Manuel Battegay, Prof. Andreas Widmer, PD Dr. Sarah Tschudin
- SNF: NRP72, Antimicrobial resistance



Clinical examples 2:

several **gram negative bacteria**

- with phenotypic resistance
- but no molecular verification:
- problem: resistance pattern to carbapenems and cephalosporins in such cases sometimes not conclusive

- NGS-result

Enterobacter cloacae

imi1

Pseudomonas aeruginosa

imp18

Klebsiella pneumoniae

shv1

Molecular assays

- Genie II (Amplex)
 - KPC
 - NDM
 - Oxa-48, Oxa-181
 - VIM
 - CTX-M1
 - CTX-M9

- OXA-23
- OXA-40

- Xpert Carba-R (Cepheid):
 - KPC
 - NDM
 - Oxa-48, Oxa-181
 - VIM
 - IMP-1

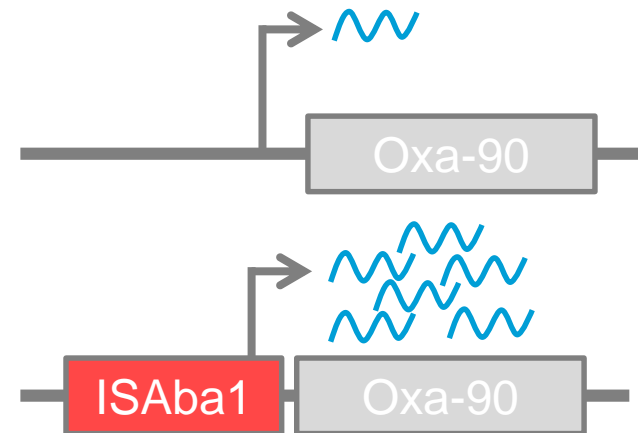
Difficulties in interpreting pipeline results....

Acinetobacter baumannii:

Meropenem	R
Imipenem	R
Tigecyclin	R
Colistin	S

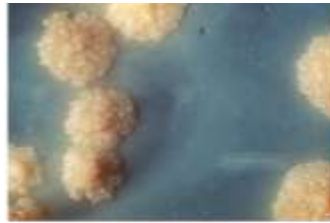
- molecular carbapenemase assay (Amplex eazyplex®): None detected
- NGS: only two β -Lactamases found:
 - *ampC*: cephalosporinase
 - *oxa-90*: *oxa-51*-variant

→ Integration of IS-Element upstream (ISAba1):
→ Hyperactivation of *oxa-90*!

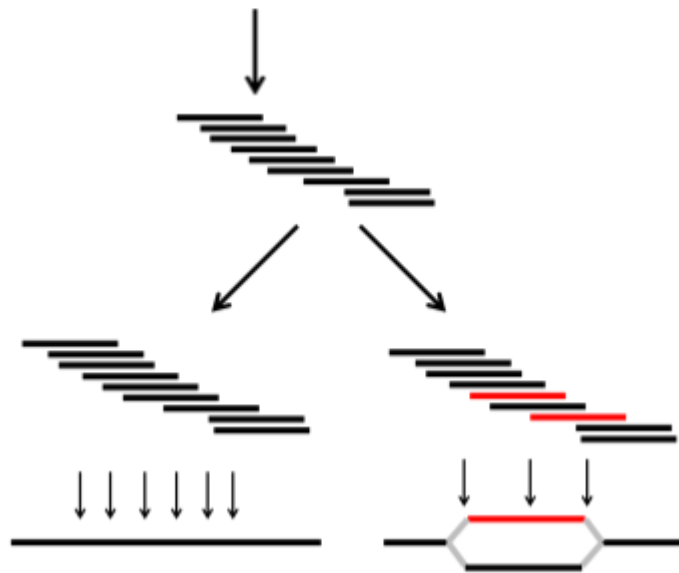


*Turton et al 2006: The role of ISAba1 in expression of OXA carbapenemase genes in *A. baumannii*

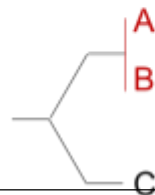
Specialized pipeline for M. tuberculosis



Sequencing
(3.5 d)



Dataanalysis (<2h)



Rifampicin S

Isoniazid R

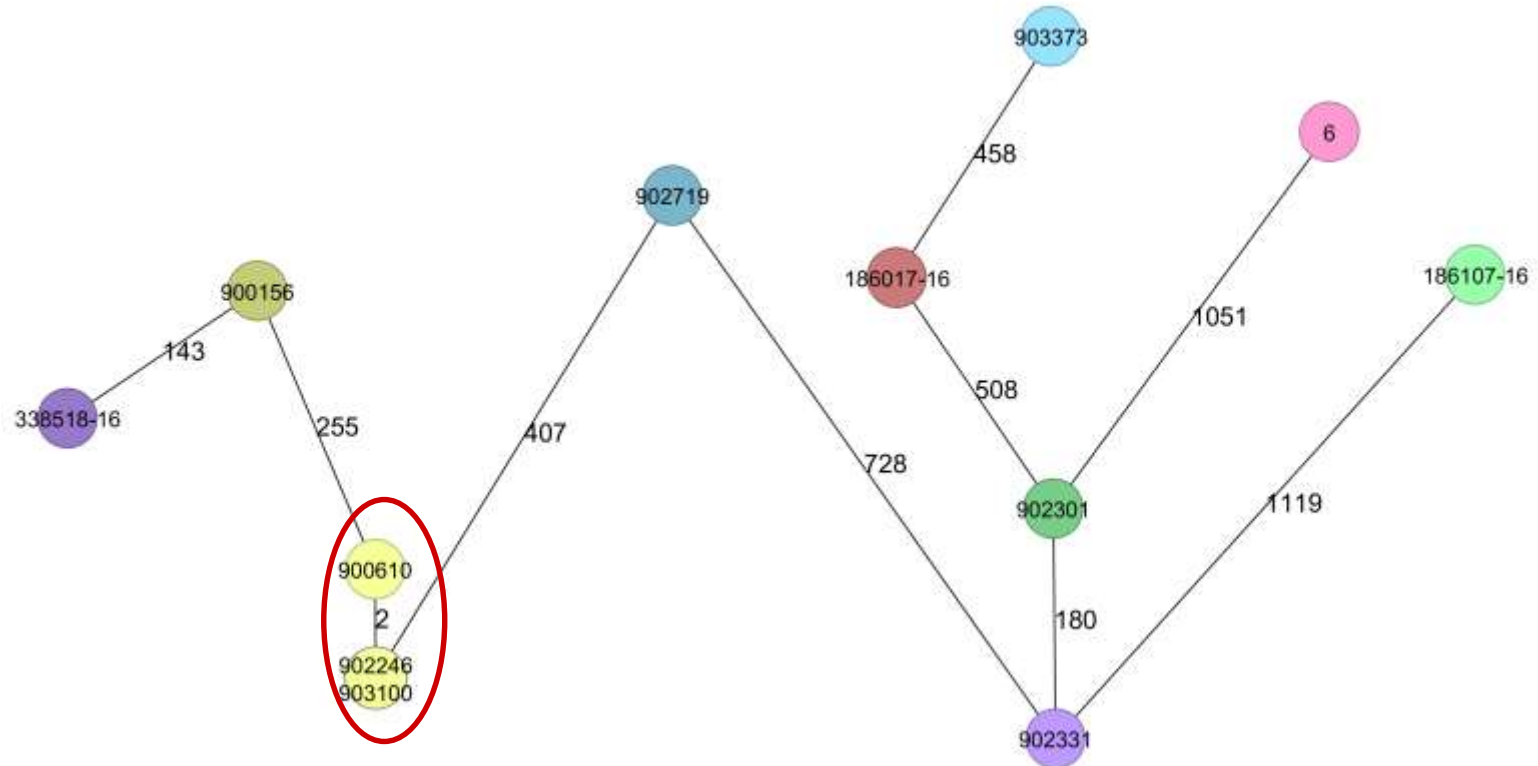
Quinolones S

Tuberculosis in a school for further education Basel

Three cases of tuberculosis in a school for further education within 8 months.

Can we use the pipeline for **transmission** analysis and **phenotypic prediction**?

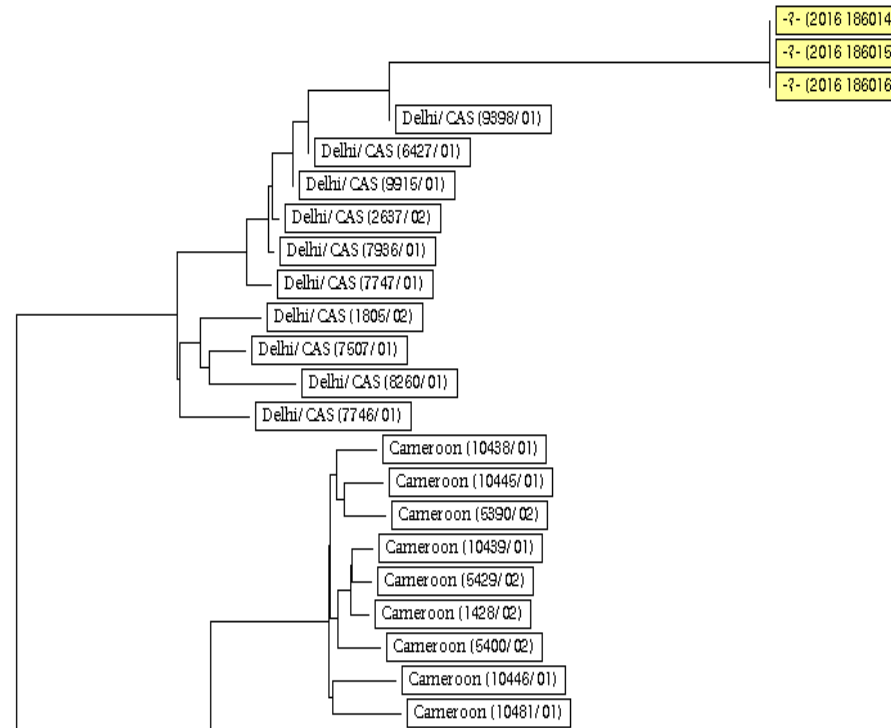
Specialized pipeline for M. tuberculosis



lineage Delhi / Central Asia

Specialized pipeline for M. tuberculosis: verification by MIRU-VNTR

NJ-Tree, MIRU-VNTR [24]: Categorical (1), Spoligo: Categorical (1), RD: Categorical (1), SNP: Categorical (1)



MIRU VNTR by Peter Keller at the Reference Center for Mycobacteria in Zurich

Specialized pipeline for *M. tuberculosis*

24 *M. tuberculosis* isolates subjected to phenotypic/genotypic resistance testing:

		Resistance mutations:
Completely susceptible strains:	12	
Isoniazid resistant strain:	1	(S135X in <i>katG</i>)
Isoniazid/Streptomycin resistant	1	(S135X in <i>katG</i> & K43G in <i>rpsL</i>)
MDR (>Rifampicin & Isoniazid)	8	(several in <i>rpoB</i> , <i>katG</i> , etc.)

→ 100% concurrence on preliminary data set to phenotypic testing

→ NGS for *M. tuberculosis* fast compared to culture methods

→ NGS will be assessed for it`s value in routine diagnostics