

Rapid inference of antibiotic resistance and susceptibility by genomic neighbor typing

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Antibiotic resistance is a major threat to public health



Antimicrobial resistance

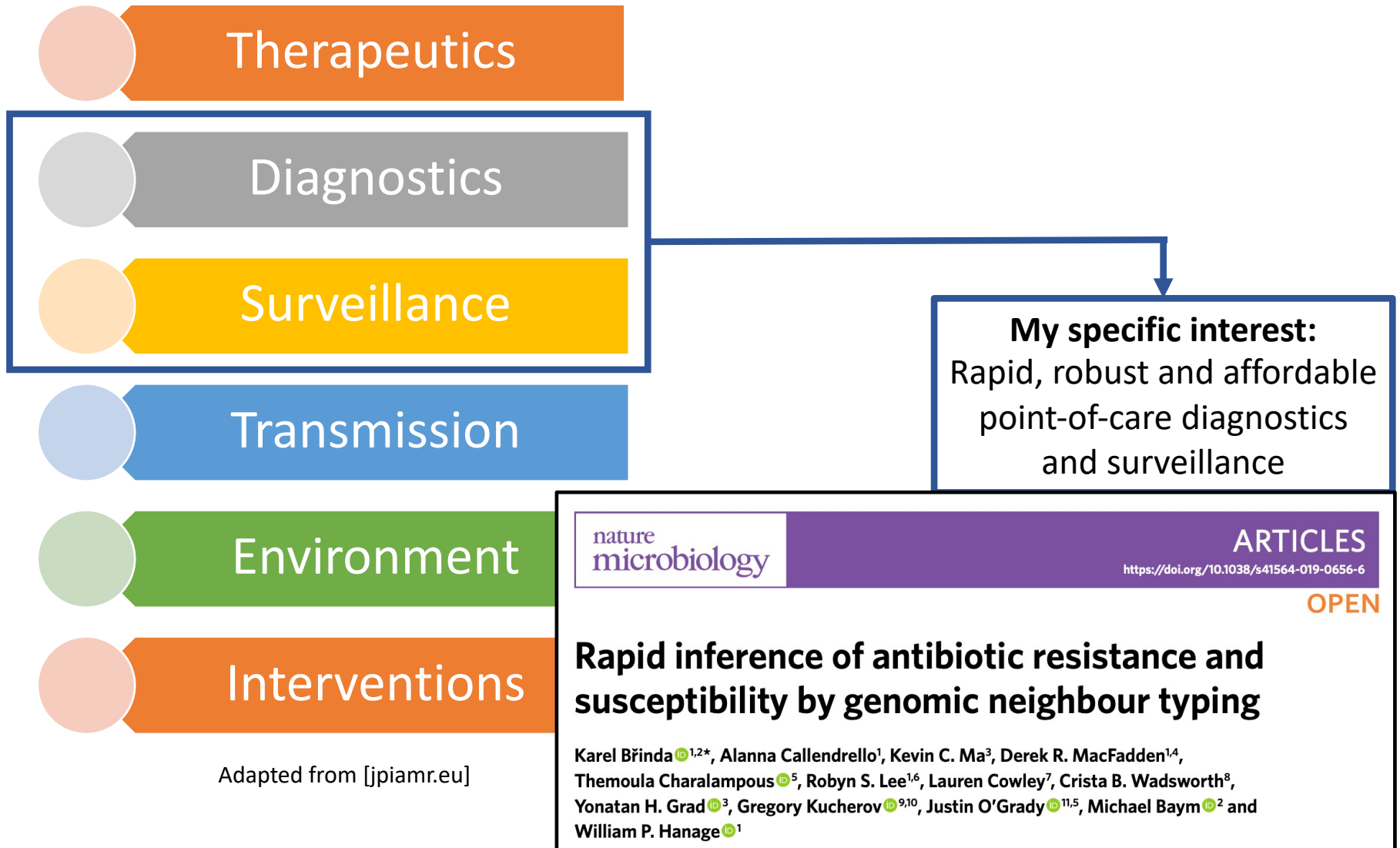
The development of antibiotics, antivirals and antimalarials are some of modern medicine's greatest successes. Now, time with these drugs is **running out**. Antimicrobial resistance – the ability of bacteria, parasites, viruses and fungi to resist these medicines – threatens to send us back to a time when we were unable to easily treat infections such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis. The inability to prevent infections could seriously compromise surgery and procedures such as chemotherapy.

Resistance to **tuberculosis** drugs is a formidable obstacle to fighting a disease that causes around 10 million people to fall ill, and 1.6 million to die, every year. In 2017, around 600 000 cases of tuberculosis were resistant to rifampicin – the most effective first-line drug – and 82% of these people had multidrug-resistant tuberculosis.

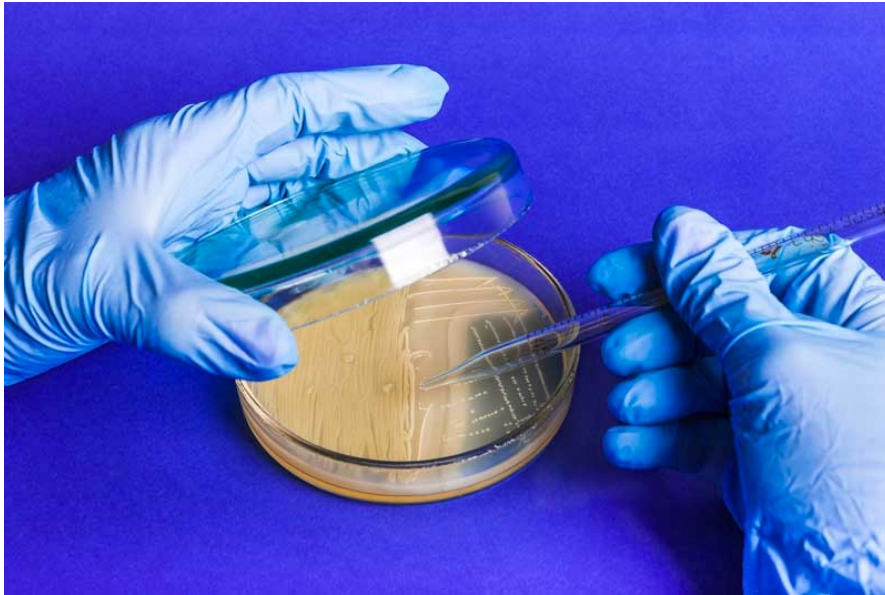


WHO/Q. Mattingly
ANTIMICROBIAL RESISTANCE - Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. WHO is supporting countries with development of national action plans and strengthening of health and surveillance systems to help prevent and manage antimicrobial resistance.

Combating antibiotic resistance

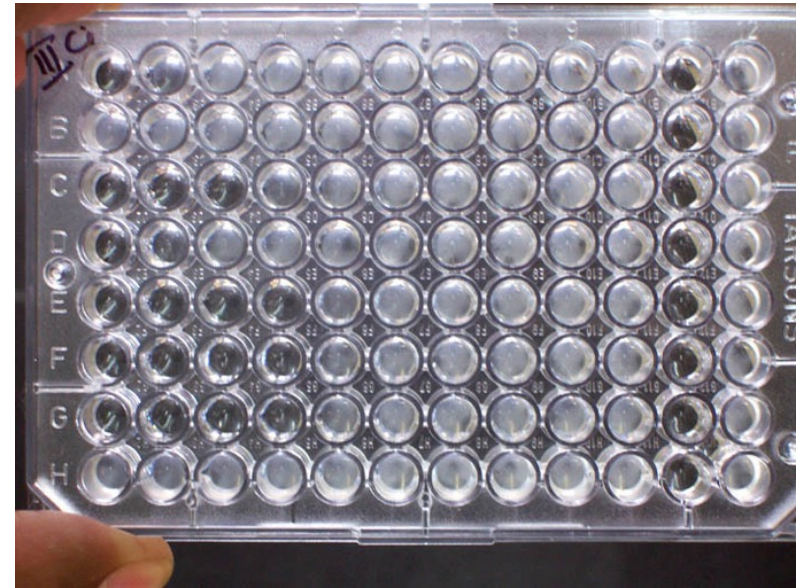


Traditional diagnostics are slow



Culturing bacteria

[\[www.promeconnections.com\]](http://www.promeconnections.com)

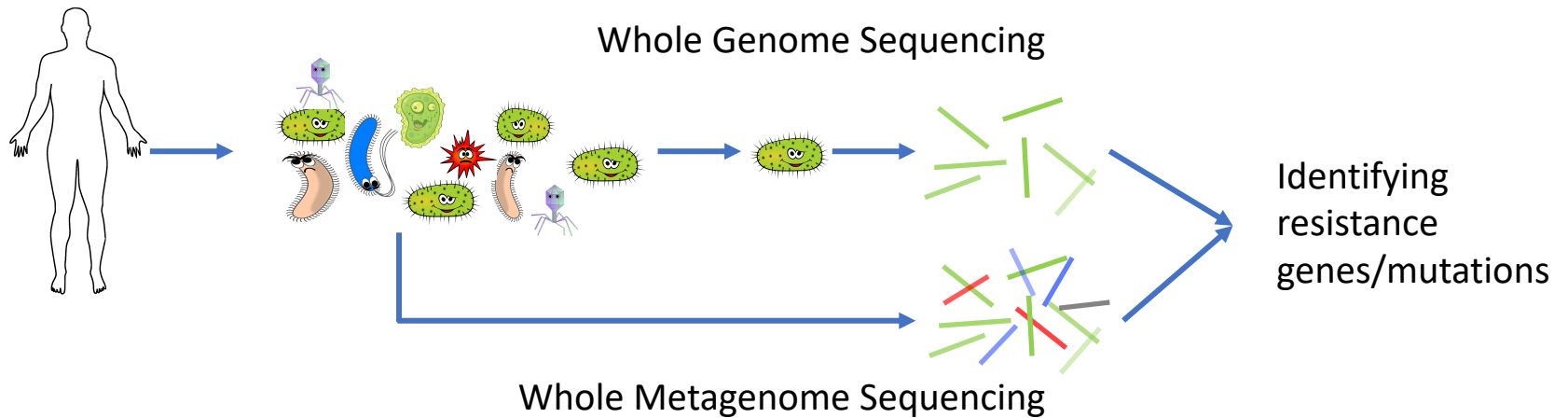


Microbroth dilution method

[\[https://courses.lumenlearning.com\]](https://courses.lumenlearning.com)

Rapid diagnostics requires avoiding culture

DNA sequencing getting faster



Analogy:



≈ Genome / Metagenome

Like looking for specific words/typos
in a shredded library

≈ Sequencing reads

Current paradigm: Gene-based diagnostics

- General databases and tools – RESFINDER, CARD, NDARO, ...
- Specialized – e.g., Mykrobe predictor for *Mtb* and *S.aureus*



Review Article | Published: 18 March 2019

Sequencing-based methods and resources to study antimicrobial resistance

Manish Boolchandani, Alaric W. D'Souza & Gautam Dantas 

Limits of gene-based diagnostics

October 11, 2018

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

1) Resistance still well understood only for few pathogen-drug combinations (e.g., first-line drugs for Mtb)

2) SNP calling slow as it requires deep coverage (e.g., for Mtb no faster than 16 hours)



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MICROBIOLOGY

Journal of
Clinical Microbiology®

Same-Day Diagnostic and Surveillance Data for Tuberculosis via Whole-Genome Sequencing of Direct Respiratory Samples

Antonina A. Votintseva,^a Phelim Bradley,^b Louise Pankhurst,^a
Carlos del Ojo Elias,^b Matthew Loose,^c Kayzad Nilgiriwala,^d Anirvan Chatterjee,^d
E. Grace Smith,^{e,f} Nicolas Sanderson,^a Timothy M. Walker,^a Marcus R. Morgan,^g
David H. Wyllie,^{a,f,h} A. Sarah Walker,^{a,i} Tim E. A. Peto,^{a,i} Derrick W. Crook,^{a,f,h}
Zamin Iqbal,^b

3) Difficult to confidently reject a gene presence

Genome-search-based diagnostics

Observation:

Predicting susceptibility \neq Identifying resistance mutations/genes

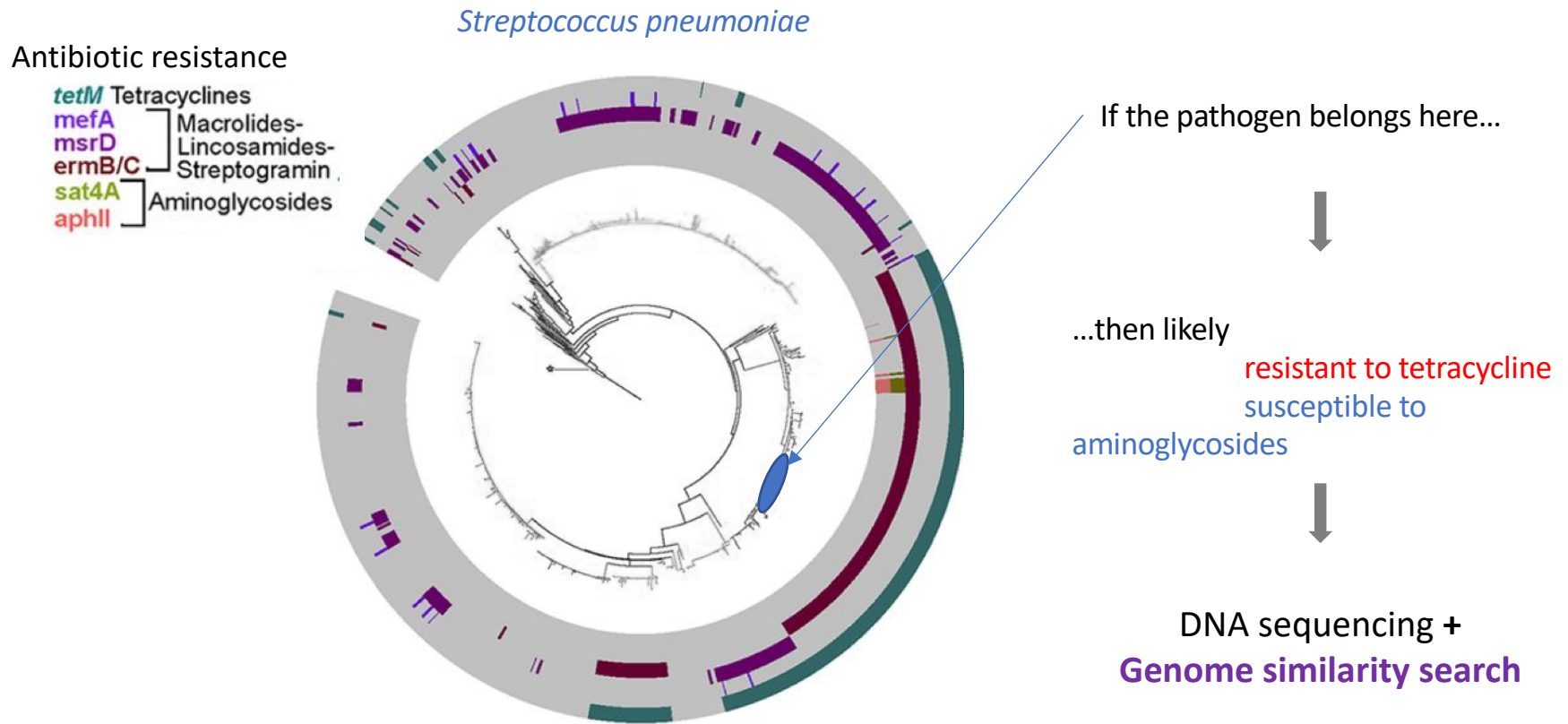
Trick:

Causation \rightarrow Correlation

How:

Mutation/gene identification \rightarrow Genome similarity search

Resistance diagnostics using the population structure



Andam & al., *J. of Clin. Microbiology*, 2017

Genomic Neighbor Typing

1. **Zooming** into the population structure of a given species
2. **Predicting** resistance and susceptibility based on the closest known relatives

Nanopore sequencing

3 key features for rapid diagnostics:

1. Portable
2. Streaming
3. Long reads



Short- vs. long-read sequencing



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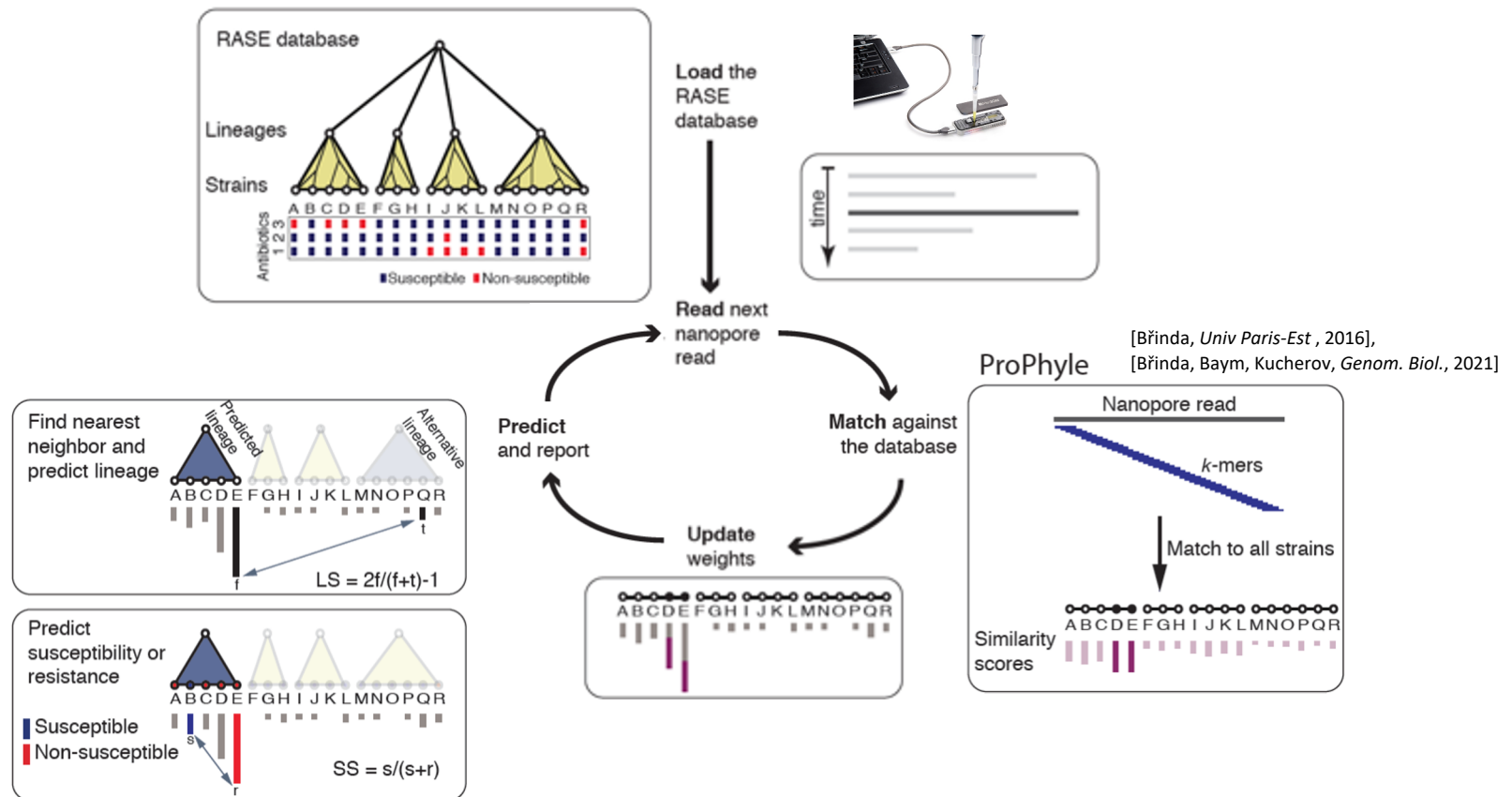


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[<https://www.seattlepi.com/seattlenews/article/Northwest-icons-under-the-microscope-7229960.php>]

RASE: an implementation of Genomic Neighbor Typing



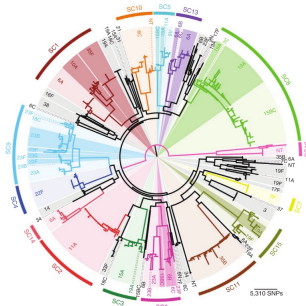
RASE databases

Streptococcus pneumoniae

616 isolates from MA

Resistance data for **5 antibiotics**

[Croucher et al., *Nat. Gen.*, 2013;
Croucher et al., *Sci. Data*, 2015]

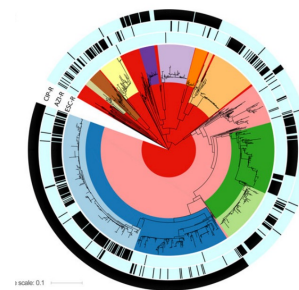


Neisseria gonorrhoeae

1,102 isolates from USA

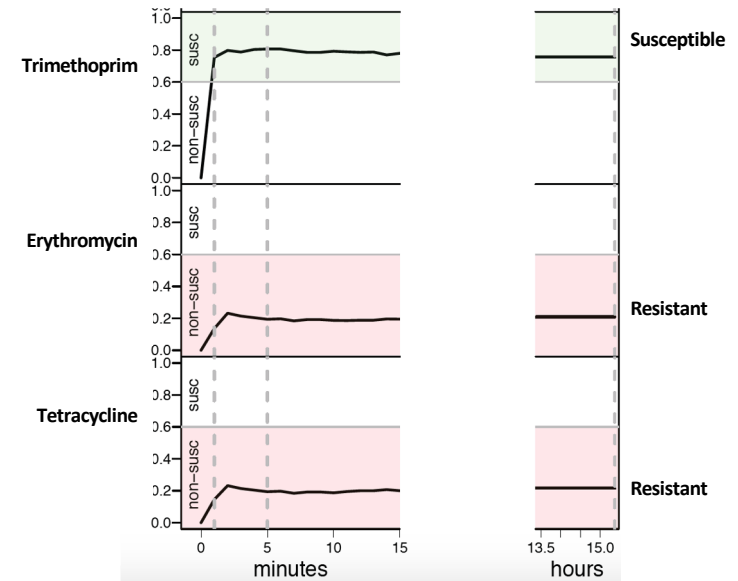
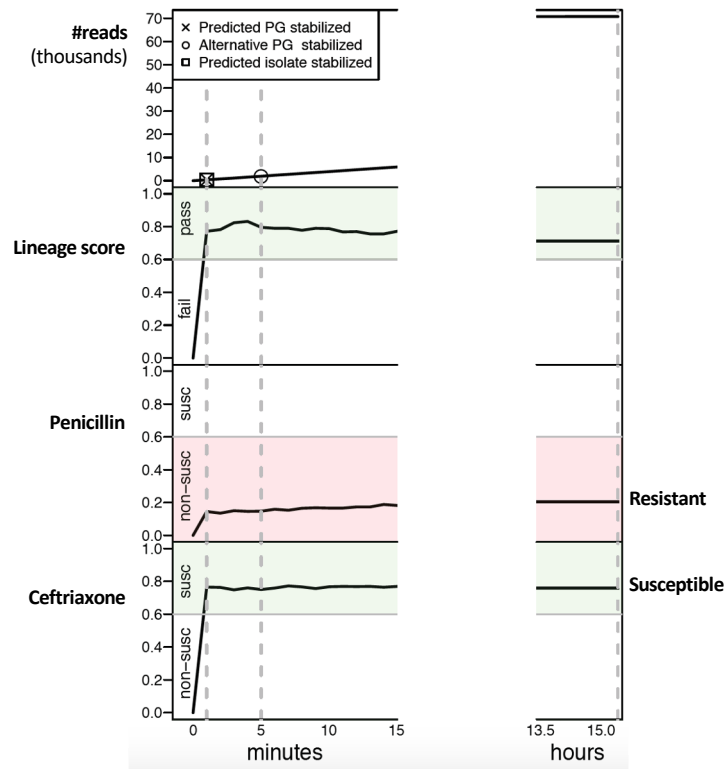
Resistance data for **4 antibiotics**

[Grad et al., *J. of Inf. Dis.*, 2016]

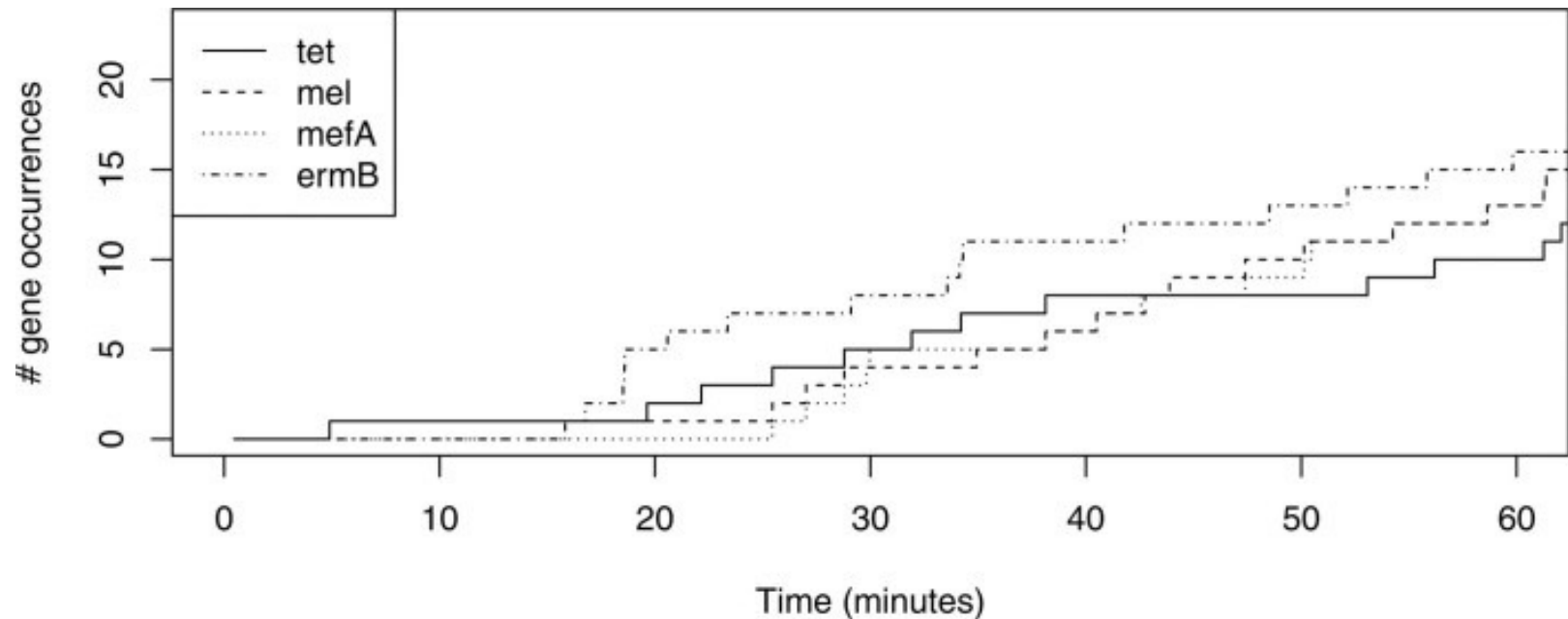


Results: Resistance diagnostics within 10 minutes of nanopore sequencing

Example: *S. pneumoniae*, a sputum sample



Resistance & susceptibility predicted even before resistance genes are sequenced



A multidrug-resistant isolate of *S. pneumoniae*

Results: overview

- ***S. pneumoniae* isolates** – sens/spec **91%/100%** (n=30), 5 min of seq
 - 5 antibiotics; 2 db isolates + 4 isolates from another state (Philadelphia)
- ***N. gonorrhoeae* isolates** – sens/spec **81%/100%** (n=76), 10 mins of seq
 - 4 antibiotics, 5 db isolates + 14 isolates from a different continent (Switzerland)
- ***S. pneumoniae* clinical metagenomic sputum samples**
 - sens/spec **75%/100%** (n=16), 4 hours of sample collection
 - 3 antibiotics
 - 6 metagenomes from LRI patients from a different continent (Norwich, UK)

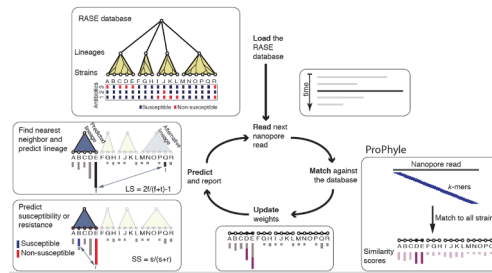
Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection

Themoula Charalampous^{1,8}, Gemma L. Kay^{1,2,8}, Hollian Richardson^{1,8}, Alp Aydin², Rossella Baldan^{1,3}, Christopher Jeanes⁴, Duncan Rae⁴, Sara Grundy⁴, Daniel J. Turner⁵, John Wain^{1,2}, Richard M. Leggett⁶, David M. Livermore^{1,7} and Justin O'Grady^{1,2*}

The framework is easy to extend

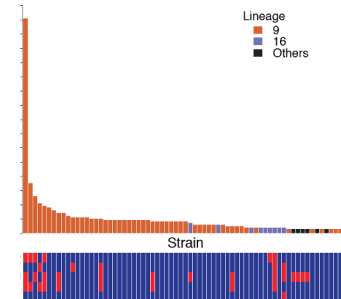
RASE-core

<https://github.com/c2-d2/rase>



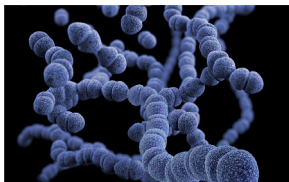
RASE-pipeline

<https://github.com/c2-d2/rase-pipeline>



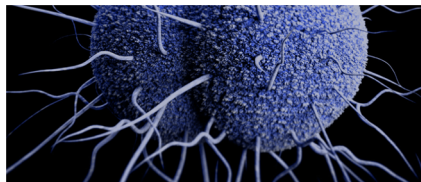
***S. pneumoniae* DB**

<http://github.com/c2-d2/rase-db-spneumoniae-sparc>



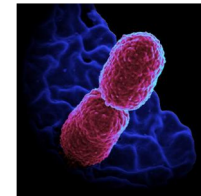
***N. gonorrhoeae* DB**

<http://github.com/c2-d2/rase-db-ngonorrhoeae-gisp>



Skeleton for new DBs

<http://github.com/c2-d2/rase-db-skeleton>



Limitations of Genomic Neighbor Typing

- Not all species-drugs combinations will work
- Database must be representative
- The species must be sufficiently diverse
- It still 4+ hours

Could we scale the method to millions of genomes?

Several comprehensible microbial corpuses recently published:

Article | [Published: 04 February 2019](#)

Ultrafast search of all deposited bacterial and viral genomic data

[Phelim Bradley](#), [Henk C. den Bakker](#), [Eduardo P. C. Rocha](#), [Gil McVean](#) & [Zamin Iqbal](#) 

[Nature Biotechnology](#) **37**, 152–159 (2019) | [Cite this article](#)

425 thousand cleaned de Bruijn graphs of microbes from ENA (16.7 TB mccortex)

New Results

 [Follow this preprint](#)

Exploring bacterial diversity via a curated and searchable snapshot of archived DNA sequences

 [Grace A. Blackwell](#),  [Martin Hunt](#),  [Kerri M. Malone](#),  [Leandro Lima](#),  [Gal Horesh](#),  [Blaise T.F. Alako](#),  [Nicholas R Thomson](#),  [Zamin Iqbal](#)

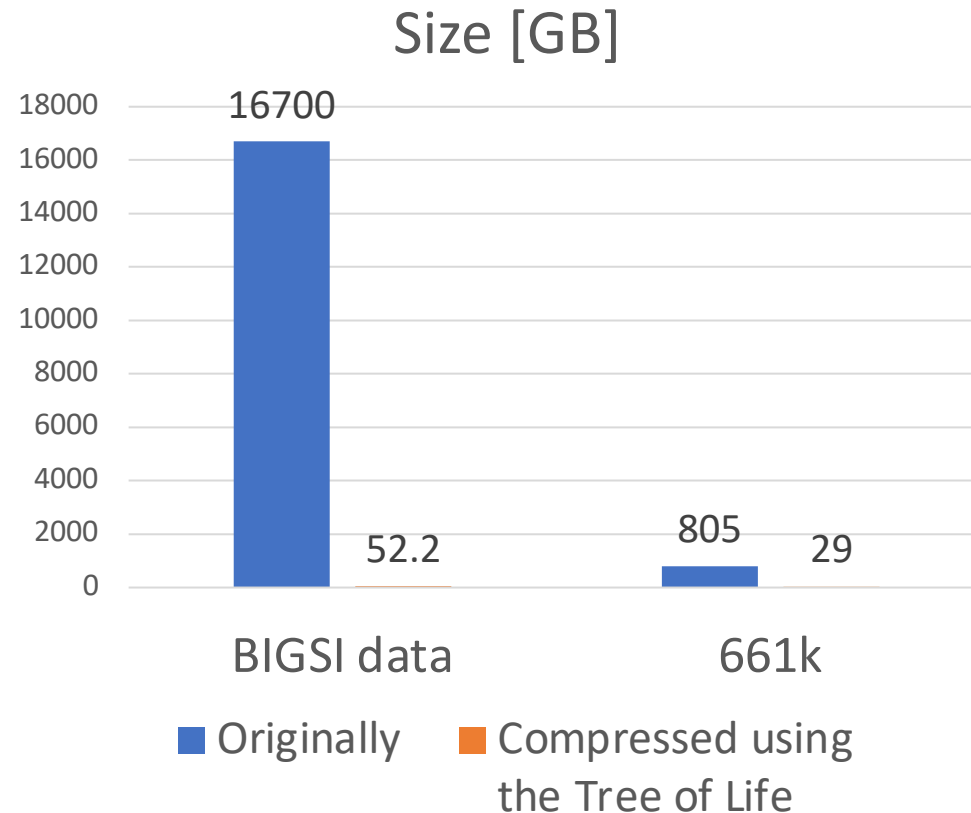
doi: <https://doi.org/10.1101/2021.03.02.433662>

661 thousand consistently assembled bacteria from ENA (805 GB gzip)

How to even get these huge data into your laptop??

Results: All microbes on a flash drive

Key idea: Using **the tree of life** as a biological **prior** for **lossless compression**



(joint work with M. Baym, G. Kuchеров, Z. Iqbal, R. Chikhi, *et al.*)

Will continue working on this and other projects in Inria (starting from January 2022)



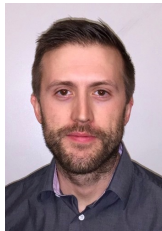
Conclusions

- RASE can predict antibiotic resistance within minutes of nanopore sequencing
- Current patient-to-result time: 4 hours
 - Approx. 4 hours of sample prep + 10 minutes of sequencing
- The RASE method can scale across continents

Thank you for your attention



Alanna
Callendrello



Derek
MacFadden



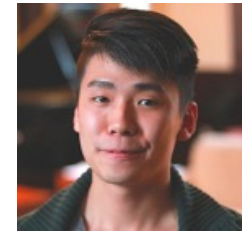
Robyn S.
Lee



Themoula
Charalampous



Lauren
Cowley



Kevin C.
Ma



Yonatan
Grad



Gregory
Kuchеров



Justin
O'Grady



Michael
Baym



Bill
Hanage