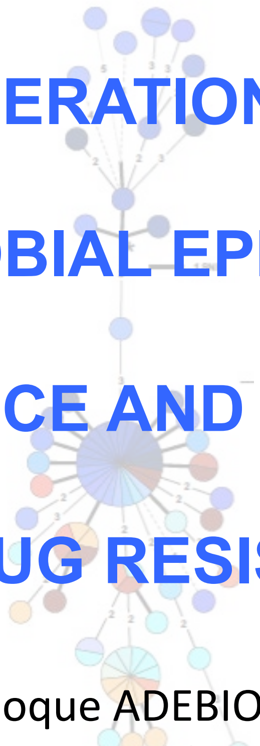
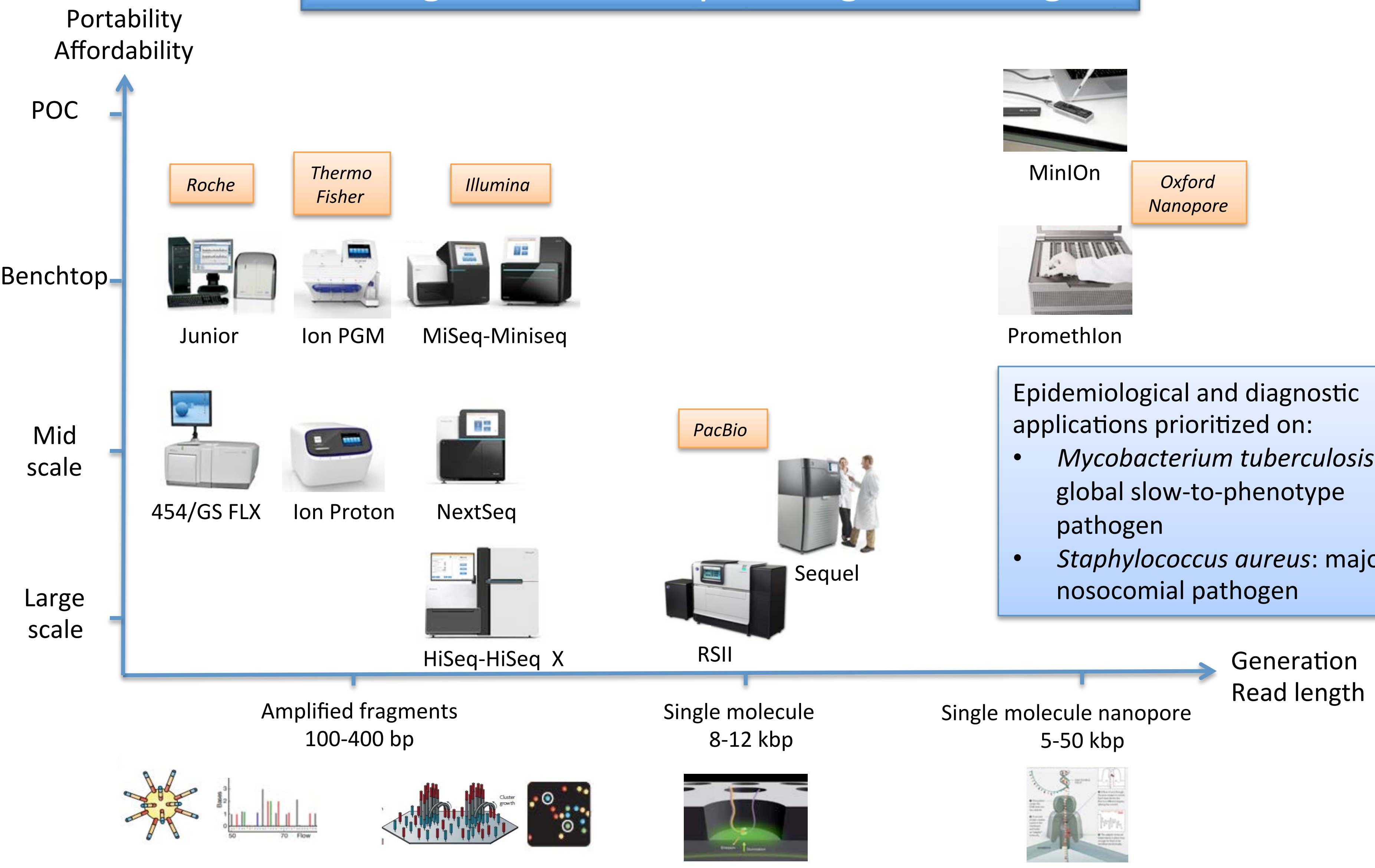


NEXT-GENERATION SEQUENCING FOR MICROBIAL EPIDEMIOLOGICAL SURVEILLANCE AND DIAGNOSTICS OF DRUG RESISTANCE

Colloque ADEBIOTECH
Mars 2016

Philip Supply, CNRS-Genoscreen
philip.supply@ibl.cnrs.fr/philip.supply@genoscreen.fr

Next-generation sequencing technologies



Portability
Affordability

POC

Benchtop

Mid
scale

Large
scale

Roche

Thermo Fisher

Illumina

Junior

Ion PGM

MiSeq-Miniseq

454/GS FLX

Ion Proton

NextSeq

HiSeq-HiSeq X

PacBio

Sequel

RSII

MinIOn

Oxford Nanopore

PromethIon

Generation
Read length

Amplified fragments
100-400 bp

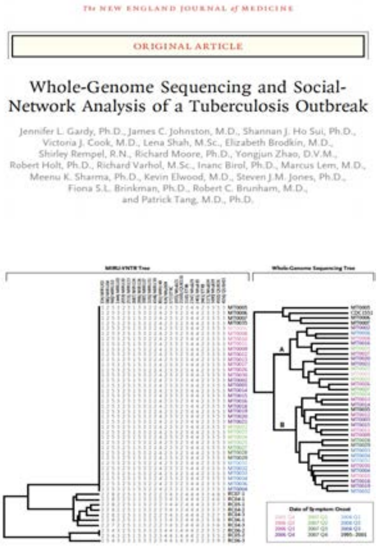
Single molecule
8-12 kbp

Single molecule nanopore
5-50 kbp

Epidemiological and diagnostic applications prioritized on:

- *Mycobacterium tuberculosis*: global slow-to-phenotype pathogen
- *Staphylococcus aureus*: major nosocomial pathogen

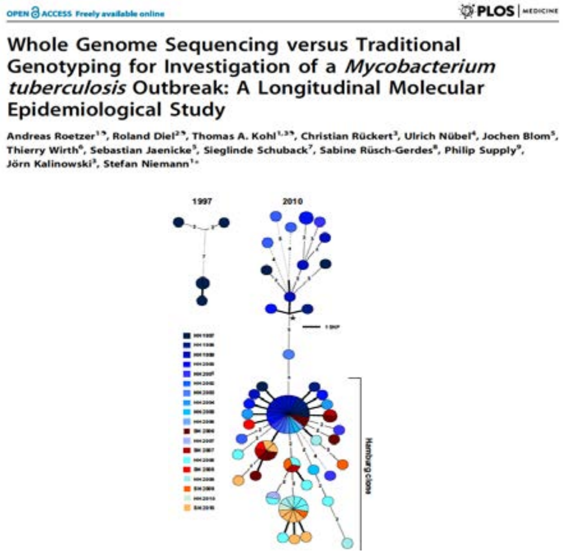
WGS for surveillance and outbreak investigation


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Whole-Genome Sequencing and Social-Network Analysis of a Tuberculosis Outbreak

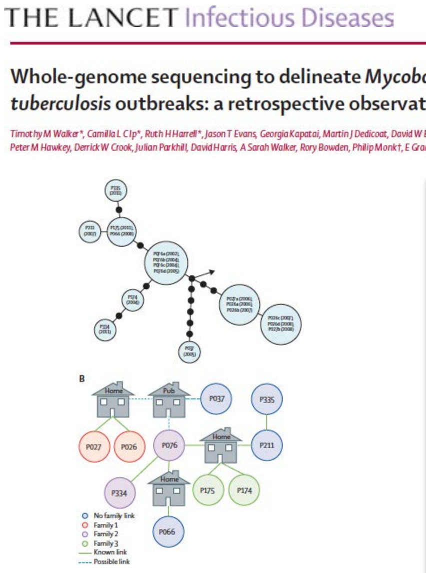
Jennifer L. Gardy, Ph.D., James C. Johnston, M.D., Shannan J. Ho Sui, Ph.D., Victoria J. Cook, M.D., Lena Shah, M.Sc., Elizabeth Brodkin, M.D., Shirley Rempel, R.N., Richard Moore, Ph.D., Yongjun Zhao, D.V.M., Robert Holt, Ph.D., Richard Varhol, M.Sc., Inanc Birol, Ph.D., Marcus Lem, M.D., Meenu K. Sharma, Ph.D., Kevin Elwood, M.D., Steven J.M. Jones, Ph.D., Fiona S.L. Brinkman, Ph.D., Robert C. Brunham, M.D., and Patrick Tang, M.D., Ph.D.


OPEN ACCESS Freely available online

PLOS | MEDICINE

Whole Genome Sequencing versus Traditional Genotyping for Investigation of a *Mycobacterium tuberculosis* Outbreak: A Longitudinal Molecular Epidemiological Study

Andreas Roetzer[1,9], Roland Diel[2,9], Thomas A. Kohl[1,3,9], Christian Rückert[3], Ulrich Nübel[4], Jochen Blom[5], Thierry Wirth[6], Sebastian Jaenicke[5], Sieglinde Schuback[7], Sabine Rüsch-Gerdes[8], Philip Supply[9], Jörn Kalinowski[3], Stefan Niemann[1*]

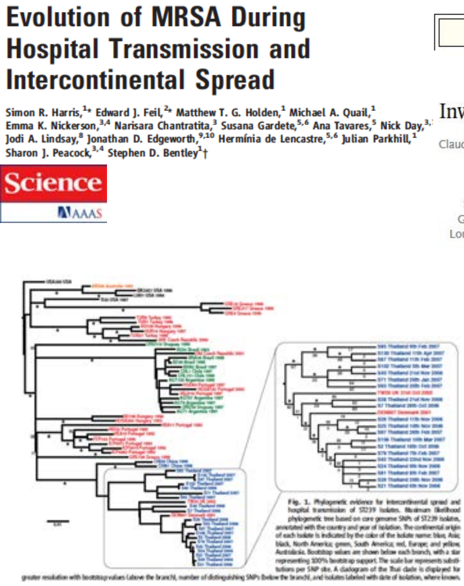

THE LANCET Infectious Diseases

Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study

Timothy M Walker*, Camilla L C Ip*, Ruth H Harrell*, Jason T Evans, Georgia Kapatai, Martin J Dedicoat, David W Eyre, Daniel J Wilson, Peter M Hawkey, Derrick W Crook, Julian Parkhill, David Harris, A Sarah Walker, Rory Bowden, Philip Monk†, E Grace Smith†, Tim E A Peto†

Superior resolution vs classical typing:
Internationally adopted for outbreak investigation

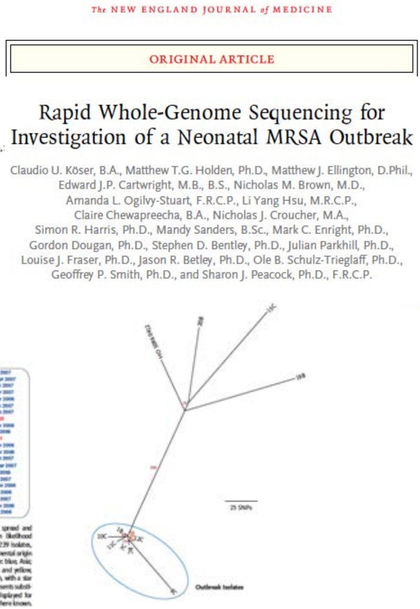
BUT bioinformatic analysis component of WGS not standardized
Accuracy incompletely determined


Evolution of MRSA During Hospital Transmission and Intercontinental Spread

Simon R. Harris,[1*] Edward J. Feil,[2*] Matthew T. G. Holden,[1] Michael A. Quail,[1] Emma K. Nickerson,[3,4] Narisara Chantratita,[3] Susana Gardete,[5,6] Ana Tavares,[5] Nick Day,[3,4] Jodi A. Lindsay,[8] Jonathan D. Edgeworth,[9,10] Hermínia de Lencastre,[5,6] Julian Parkhill,[1] Sharon J. Peacock,[3,4] Stephen D. Bentley[1]†

Science AAAS

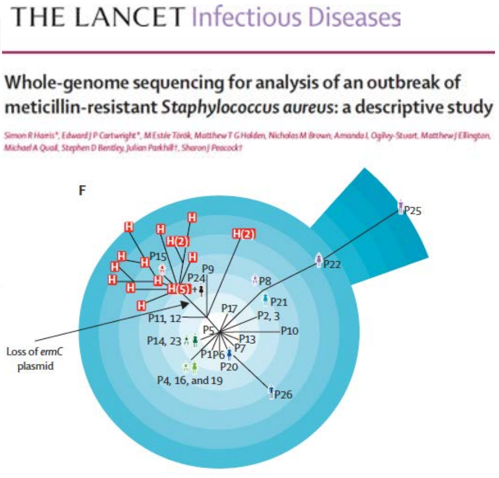
Fig. 1. Phylogenetic evidence for intercontinental spread and hospital transmission of ST239 isolates. Maximum likelihood phylogenetic tree based on core genome SNPs of ST239 isolates, annotated with the country and year of isolation. The continental origin of each isolate is indicated by the color of the isolate name: blue, Asia; black, North America; green, South America; red, Europe; and yellow, Australasia. Bootstrap values are shown below each branch, with a star representing 100% bootstrap support. The scale bar represents substitutions per SNP site. A cladogram of the Thai clade is displayed for greater resolution with bootstrap values (above the branch), number of distinguishing SNPs (below the branch), and isolates labeled with date of isolation, where known.


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

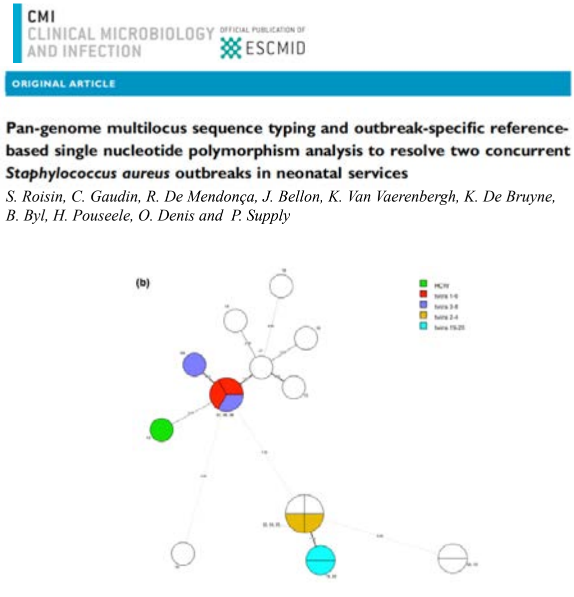
Rapid Whole-Genome Sequencing for Investigation of a Neonatal MRSA Outbreak

Claudio U. Köser, B.A., Matthew T.G. Holden, Ph.D., Matthew J. Ellington, D.Phil., Edward J.P. Cartwright, M.B., B.S., Nicholas M. Brown, M.D., Amanda L. Ogilvy-Stuart, F.R.C.P., Li Yang Hsu, M.R.C.P., Claire Chewapreecha, B.A., Nicholas J. Croucher, M.A., Simon R. Harris, Ph.D., Mandy Sanders, B.Sc., Mark C. Enright, Ph.D., Gordon Dougan, Ph.D., Stephen D. Bentley, Ph.D., Julian Parkhill, Ph.D., Louise J. Fraser, Ph.D., Jason R. Betley, Ph.D., Ole B. Schulz-Trieglaff, Ph.D., Geoffrey P. Smith, Ph.D., and Sharon J. Peacock, Ph.D., F.R.C.P.


THE LANCET Infectious Diseases

Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study

Simon R Harris*, Edward J P Cartwright*, M Estée Török, Matthew T G Holden, Nicholas M Brown, Amanda L Ogilvy-Stuart, Matthew J Ellington, Michael A Quail, Stephen D Bentley, Julian Parkhill†, Sharon J Peacock†


CMI CLINICAL MICROBIOLOGY AND INFECTION OFFICIAL PUBLICATION OF ESCMID

ORIGINAL ARTICLE

Pan-genome multilocus sequence typing and outbreak-specific reference-based single nucleotide polymorphism analysis to resolve two concurrent *Staphylococcus aureus* outbreaks in neonatal services

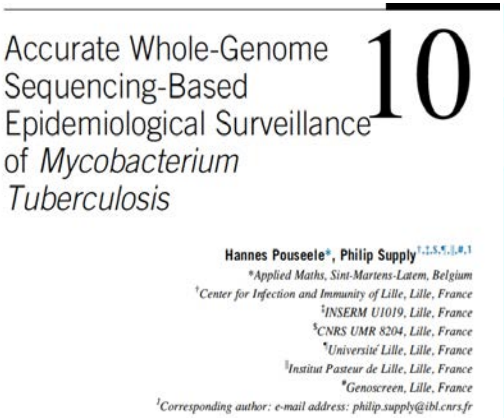
S. Roisin, C. Gaudin, R. De Mendonça, J. Bellon, K. Van Vaerenbergh, K. De Bruyne, B. Byl, H. Pouseele, O. Denis and P. Supply

WGS for epi surveillance and outbreak investigation

European Commission

SEVENTH FRAMEWORK PROGRAMME

PATHoNGenTrace

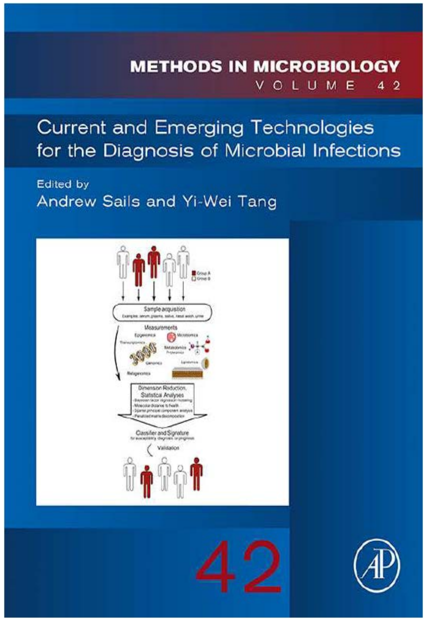


10

Accurate Whole-Genome Sequencing-Based Epidemiological Surveillance of *Mycobacterium Tuberculosis*

Hannes Pouseele[*], **Philip Supply**[†,‡,§,¶,‖,#,1]

[*]Applied Maths, Sint-Martens-Latem, Belgium
[†]Center for Infection and Immunity of Lille, Lille, France
[‡]INSERM U1019, Lille, France
[§]CNRS UMR 8204, Lille, France
[¶]Université Lille, Lille, France
[‖]Institut Pasteur de Lille, Lille, France
[#]Genoscreen, Lille, France
[1]Corresponding author: e-mail address: philip.supply@ibl.cnrs.fr



METHODS IN MICROBIOLOGY
VOLUME 42

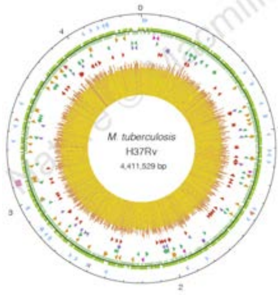
Current and Emerging Technologies for the Diagnosis of Microbial Infections

Edited by
Andrew Sails and Yi-Wei Tang

42

Illumina HiSeq 2500

PacBio RSII



- Optimized WGS analysis pipeline
- Illumina WGS vs de novo PacBio WGS of MTB references
- 0 false-pos., max. 3 % false-neg. SNPs genome-wide
- Validated for *S. aureus* (Roisin et al., CMI, 2016)

GenoScreen

APPLIED MATHS

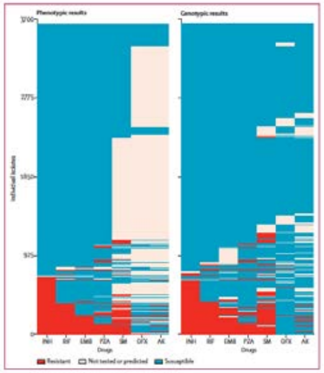
PACIFIC BIOSCIENCES

WGS for prediction of drug resistance: *M. tuberculosis*

THE LANCET Infectious Diseases

Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study

Timothy M Walker, Thomas A Kohl*, Shaheed V Omar*, Jessica Hedge*, Carlos Del Ojo Elias, Phelim Bradley, Zamin Iqbal, Silke Feuerriegel, Katherine E Niehaus, Daniel J Wilson, David A Clifton, Georgia Kapatai, Camilla L C Ip, Rory Bowden, Francis A Drobniewski, Caroline Allix-Béguec, Cyril Gaudin, Julian Parkhill, Roland Diel, Philip Supply, Derrick W Crook, E Grace Smith, A Sarah Walker, Nazir Ismail†, Stefan Niemann†, Tim E A Peto†, and the Modernizing Medical Microbiology (MMM) Informatics Group‡*



- 3,600 genomes
- 89 % predicted phenotypes with sensitivity of 92 % and specificity of > 98%
- Superior to current molecular assays

THE LANCET Respiratory Medicine

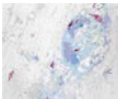
Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study

Louise J Pankhurst, Carlos del Ojo Elias*, Antonina A Votintseva*, Timothy M Walker*, Kevin Cole, Jim Davies, Jilles M Fermont, Deborah M Gascoyne-Binzi, Thomas A Kohl, Clare Kong, Nadine Lemaitre, Stefan Niemann, John Paul, Thomas R Rogers, Emma Roycroft, E Grace Smith, Philip Supply, Patrick Tang, Mark H Wilcox, Sarah Wordsworth, David Wyllie, Li Xu, Derrick W Crook, for the COMPASS-TB Study Group†*

- WGS on newly positive primary cultures in 8 EU and Canada labs
- Full diagnostics 15-21 days faster than classical drug testing
- Cost 7% less annually than current workflows
- Still needs primary culture

Genoscreen Deeplex-MycTB®

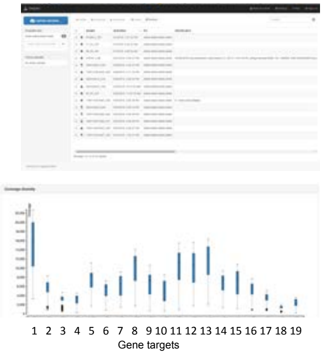
Clinical sample

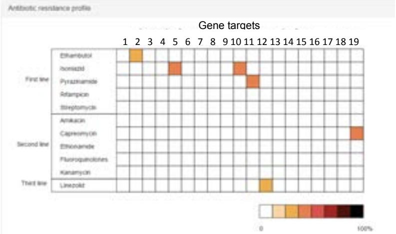


Multiplex PCR
ID, resistance and genotyping targets

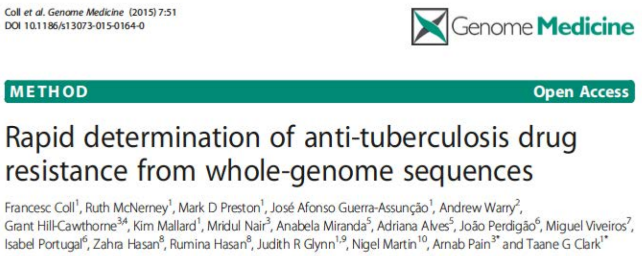
Deep sequencing of multiplexed samples

Cloud-based fast and easy NGS data interpretation





WGS for prediction of drug resistance: *M. tuberculosis* and *S. aureus*



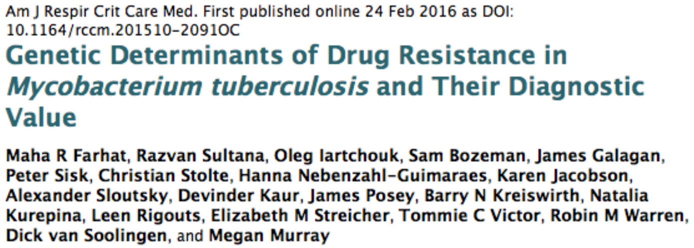
Coll *et al. Genome Medicine* (2015) 7:51
DOI 10.1186/s13073-015-0164-0

Genome **Medicine**

METHOD **Open Access**

Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences

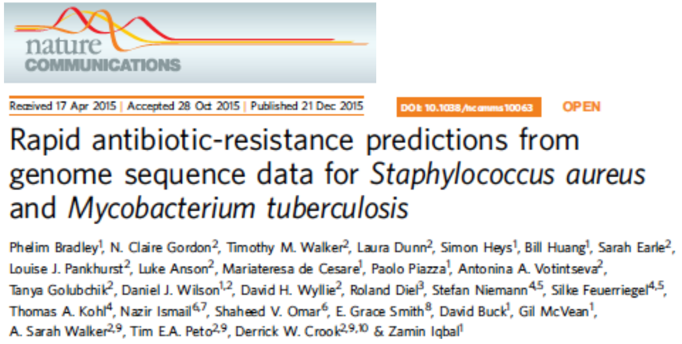
Francesc Coll[1], Ruth McNerney[1], Mark D Preston[1], José Afonso Guerra-Assunção[1], Andrew Warry[2], Grant Hill-Cawthorne[3,4], Kim Mallard[1], Mridul Nair[3], Anabela Miranda[5], Adriana Alves[5], João Perdigão[6], Miguel Viveiros[7], Isabel Portugal[6], Zahra Hasan[8], Rumina Hasan[8], Judith R Glynn[1,9], Nigel Martin[10], Arnab Pain[3*] and Taane G Clark[1*]



Am J Respir Crit Care Med. First published online 24 Feb 2016 as DOI: 10.1164/rccm.201510-2091OC

Genetic Determinants of Drug Resistance in *Mycobacterium tuberculosis* and Their Diagnostic Value

Maha R Farhat, Razvan Sultana, Oleg Iartchouk, Sam Bozeman, James Galagan, Peter Sisk, Christian Stolte, Hanna Nebenzahl-Guimaraes, Karen Jacobson, Alexander Sloutsky, Devinder Kaur, James Posey, Barry N Kreiswirth, Natalia Kurepina, Leen Rigouts, Elizabeth M Streicher, Tommie C Victor, Robin M Warren, Dick van Soolingen, and **Megan Murray**



nature COMMUNICATIONS

Received 17 Apr 2015 | Accepted 28 Oct 2015 | Published 21 Dec 2015 DOI: 10.1038/ncomms10063 OPEN

Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*

Phelim Bradley[1], N. Claire Gordon[2], Timothy M. Walker[2], Laura Dunn[2], Simon Heys[1], Bill Huang[1], Sarah Earle[2], Louise J. Pankhurst[2], Luke Anson[2], Mariateresa de Cesare[1], Paolo Piazza[1], Antonina A. Votintseva[2], Tanya Golubchik[2], Daniel J. Wilson[1,2], David H. Wyllie[2], Roland Diel[3], Stefan Niemann[4,5], Silke Feuerriegel[4,5], Thomas A. Kohl[4], Nazir Ismail[6,7], Shaheed V. Omar[6], E. Grace Smith[8], David Buck[1], Gil McVean[1], A. Sarah Walker[2,9], Tim E.A. Peto[2,9], Derrick W. Crook[2,9,10] & Zamin Iqbal[1]

JCM
Journals.ASM.org

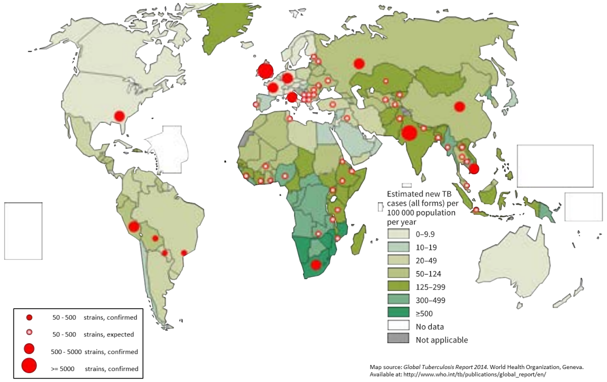
Prediction of *Staphylococcus aureus* Antimicrobial Resistance by Whole-Genome Sequencing

N. C. Gordon,[a] J. R. Price,[b] K. Cole,[b] R. Everitt,[c] M. Morgan,[d] J. Finney,[a] A. M. Kearns,[a] B. Pichon,[a] B. Young,[a] D. J. Wilson,[a] M. J. Llewelyn,[b] J. Paul,[f] T. E. A. Peto,[a] D. W. Crook,[a] A. S. Walker,[a] T. Golubchik[a]

- Multiple, rapidly expanding databases
- Diverse on-line tools for WGS analysis
- Need to/for:
  - Standardize data formats, nomenclatures,…
  - Increase validation of causative mutation candidates
  - Analyze genetic background/epigenetic effects
  - Machine learning algorithms
  - Expert curation
  - Match with quantitative phenotypic resistance levels
  - Link with accessible metadata
  - Synchronize/centralize databases,…

Comprehensive Resistance Prediction for Tuberculosis, an International Consortium (CRyPTIC):

- Univ. of Oxford-led (D. Crook), Wellcome Trust-funded
- 90,000 *M. tuberculosis* complex genomes from worldwide
- 32 collaborators and partners, incl. WHO, US CDC, Chinese CDC, India, South Africa, PHE, etc



Map source: *Global Tuberculosis Report 2014*. World Health Organization, Geneva. Available at: http://www.who.int/tb/publications/global_report/en/

Funding and collaborations

GenoScreen

Cyril Gaudin
Caroline Allix-Béguec
Mathilde Mairey
Yannick Laurent
Stéphanie Duthoy

cnrs advancing the frontiers

Institut Pasteur de Lille

Université Lille Nord de France
Pôle de Recherche et d'Enseignement Supérieur

Inserm
Institut national de la santé et de la recherche médicale

Centre d'Infection & d'Immunité de Lille

Céline Mille
Raffaella Iantomasi

Centre Hospitalier Régional Universitaire de Lille

Nadine Lemaitre
Guy Delcroix

European Commission

SEVENTH FRAMEWORK PROGRAMME

PATHoNGenTrace

THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION
HORIZON 2020

EDCTP

UNIVERSITY OF OXFORD

Timothy Walker
Louise Pankhurst
Tim Peto
Derrick Crook

APPLIED MATHS

Hannes pouseele
Katrien De Bruyne
Bruno Pot

Research Center Borstel
Leibniz-Center for Medicine and Biosciences

Stefan Niemann

PACIFIC BIOSCIENCES

Primo Baybayan
John Harting
Jonas Korlach