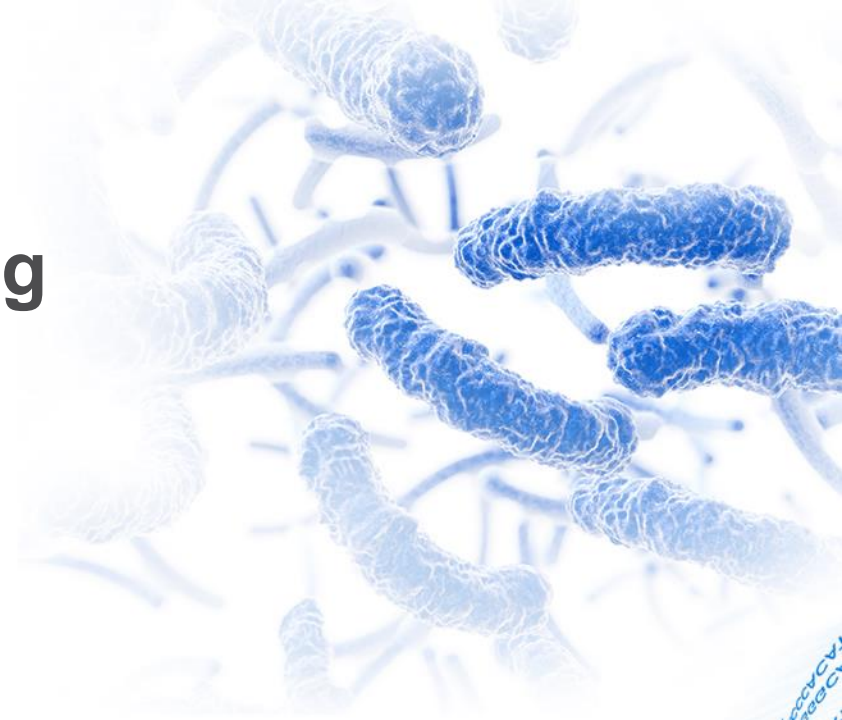


Emerging Opportunities for Next Generation Sequencing in Public Health and Clinical Microbiology



*Susan Knowles
Illumina Inc.
June 2, 2014*

Outline

- ▶ Infectious Disease
- ▶ Illumina Portfolio
- ▶ Publications – Use Cases
 - Public Health
 - Clinical Microbiology



Infectious Disease

A Top Priority for Public Health Policy

Dr. Tom Frieden, Director of US CDC

\$40M Requested for *Advanced Molecular Detection and Response to Infectious Disease Outbreaks*.

Sequencing tools, information technologies and bioinformatics experts to enable faster and more effective infectious disease prevention and control.



Dame Sally Claire Davies, CMO, England

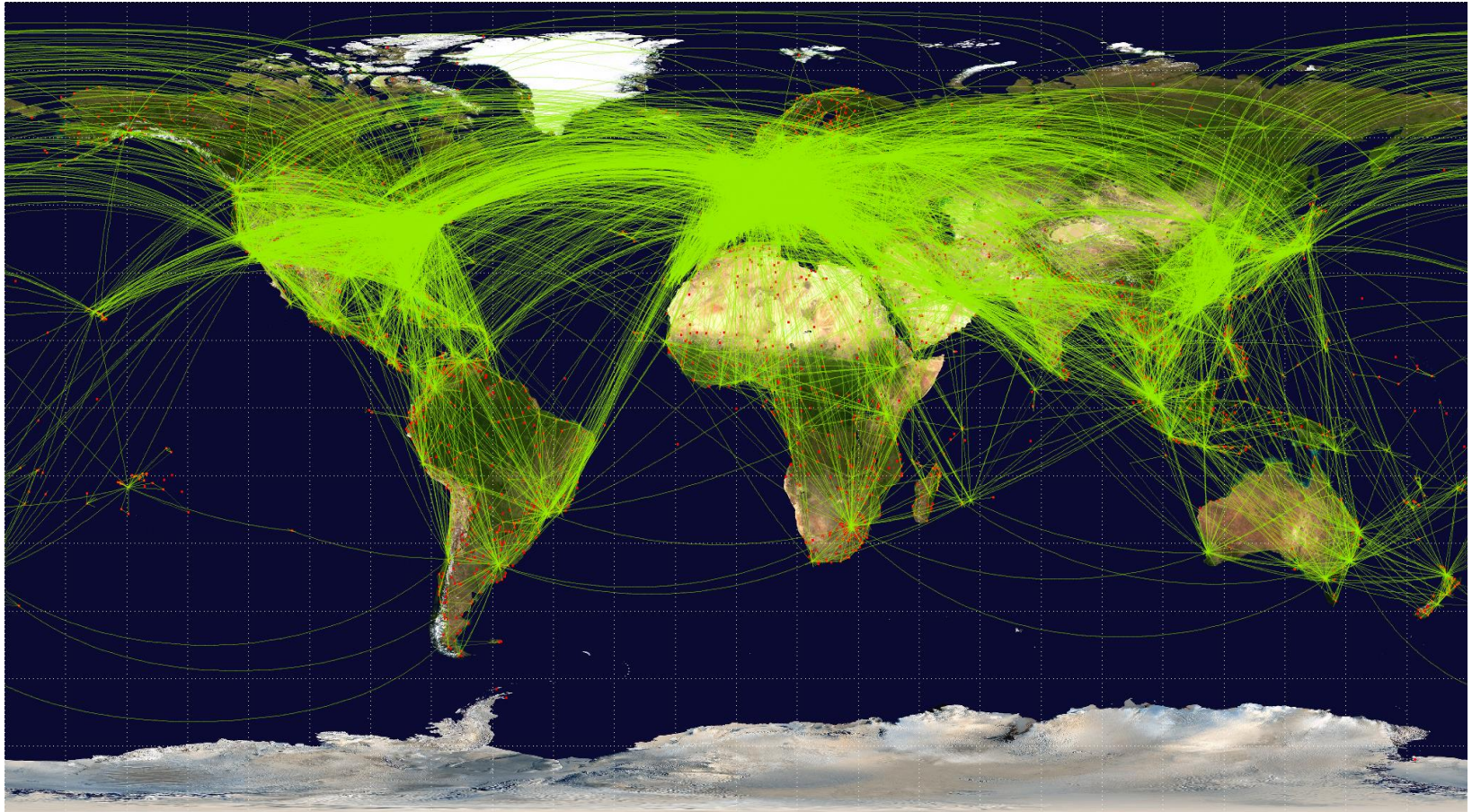
First in-depth report focused on infectious diseases:

- Globally, this group of diseases represents the greatest cause of death and burden of disease.
- New infectious diseases are emerging every year;
- Older diseases are re-emerging as they become resistant to our antimicrobial drugs.



Globalization

“An outbreak anywhere is a risk everywhere”¹

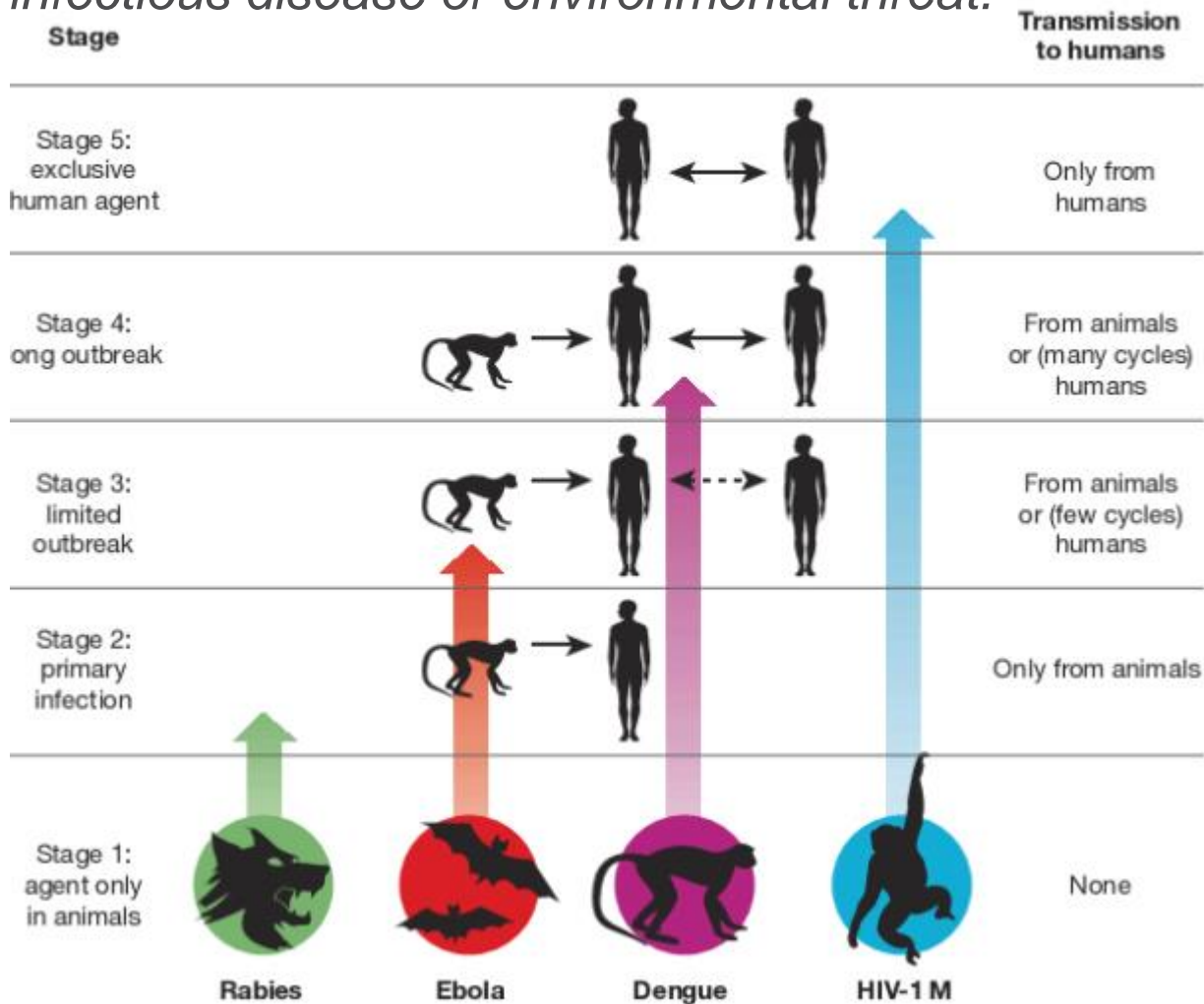


¹Source: Dr. Frieden speech, National Press Club, Sept. 2013.



Emerging Infectious Disease

Daily, the US CDC initiates an investigation of at least one new infectious disease or environmental threat.¹

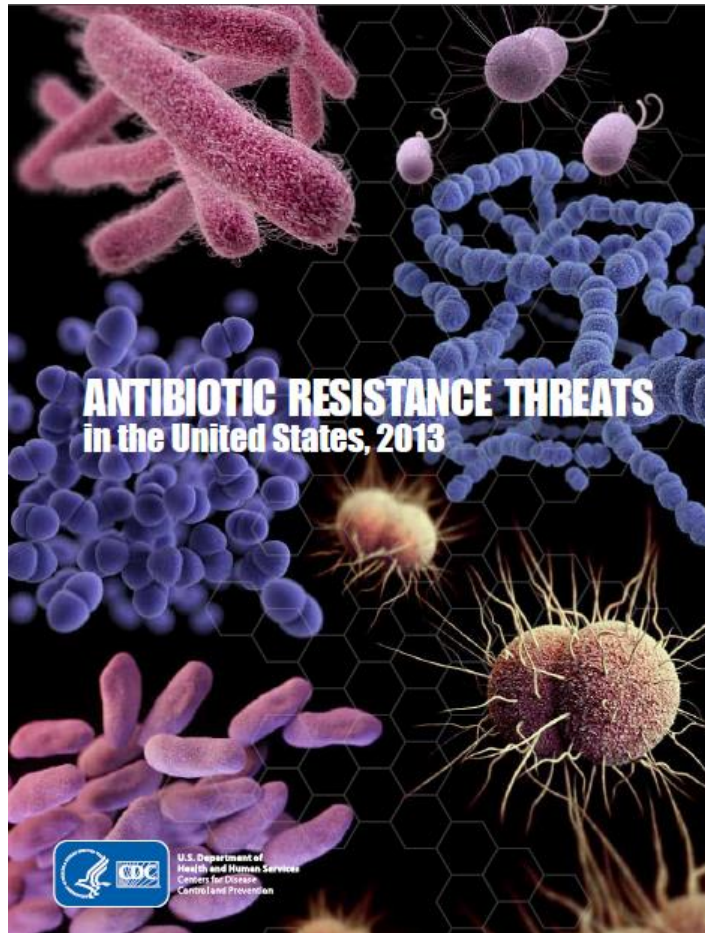


¹Source: Dr. Frieden speech, National Press Club, Sept. 2013.



Antibiotic Resistance

High economic and social costs



US Statistics

- ▶ 2M – number of people are sickened every year with antibiotic-resistant infections,
- ▶ 23,000+ deaths per year
- ▶ \$20 billion in excess direct healthcare costs
- ▶ \$35 billion a year in additional costs to society for lost productivity



Next Generation Sequencing (NGS) & Infectious Disease

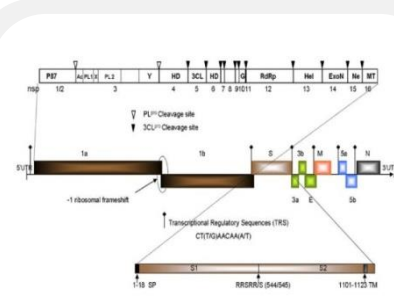
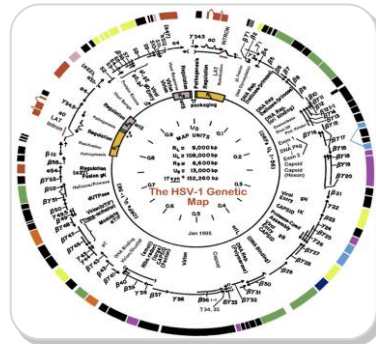
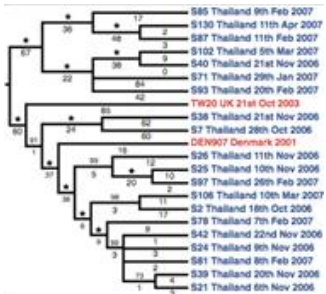
High resolution genomic information enables a range of research

Discovery

Characterization

Rearrangements & Evolution

Host Pathogen Interaction



Next Generation Sequencing (NGS) & Infectious Disease

Applications development

Public Health

- Surveillance
- Transmission



Molecular Dx

- Development of diagnostic tests



Therapeutics

- Vaccine development
- Antibiotic and antiviral development



Illumina, Who We Are

- ▶ Founded in 1998
- ▶ Headquarters in San Diego, CA
- ▶ >2,700 employees
- ▶ Global commercial operations, facilities in 7 countries.
- ▶ IP portfolio of 159 issued patents and 171 pending applications
- ▶ 90% of the worlds DNA sequencing data is from Illumina platforms
- ▶ **\$1000 Genome achieved and enabled for the masses - 2014**



Illumina Vision

Innovating for the Future of Genetic Analysis

From Genome Wide Discovery...



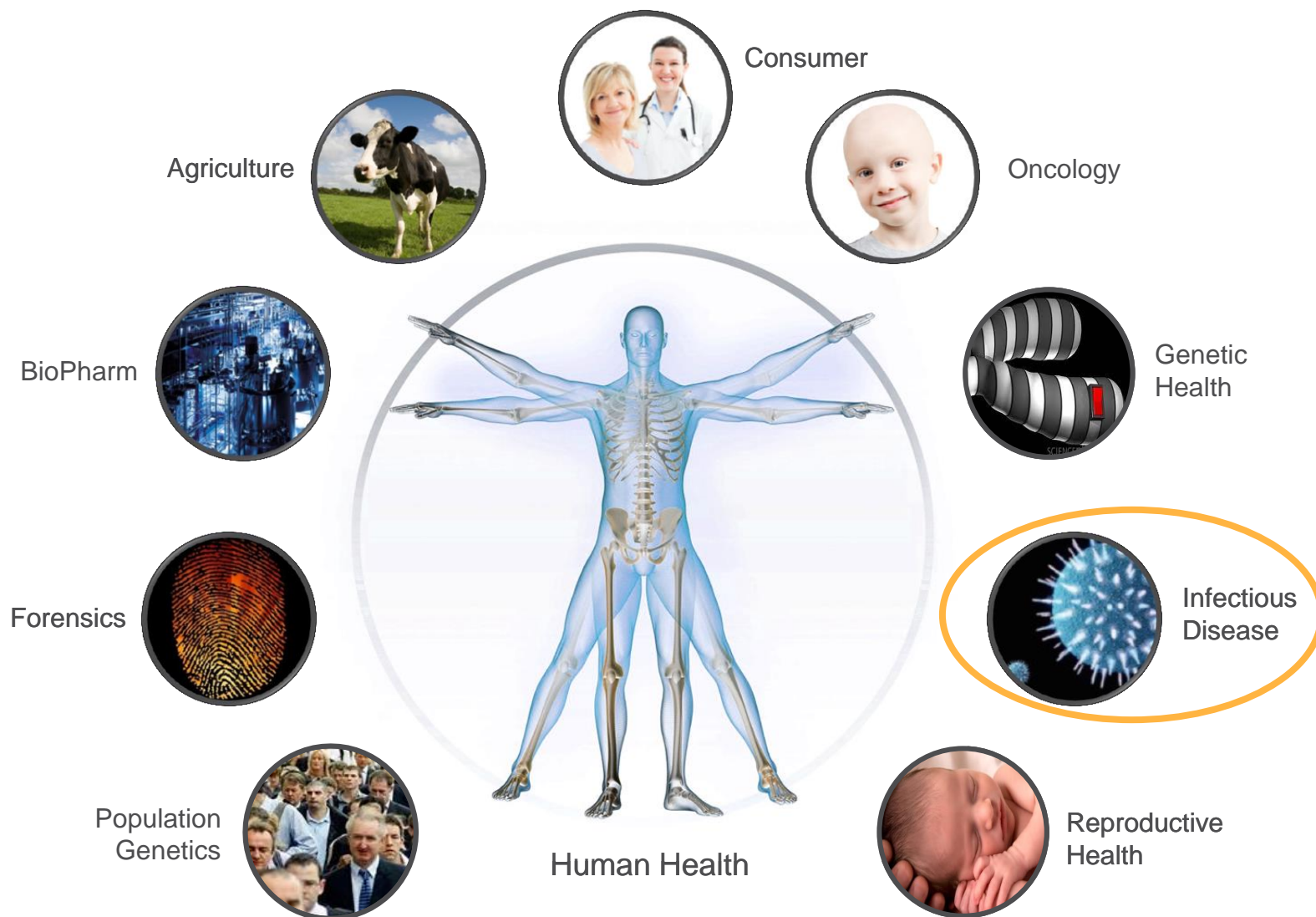
To Targeted Validation and Beyond...

To advance human health by unlocking the power of the genome



Illumina Next Generation Sequencing

Impacting Human Health



The New Illumina Portfolio

Sequencing Power for Every Scale

Regulated Power

Focused Power

Flexible Power

Production Power

Population Power



MiSeqDx

The world's first CE-IVD and FDA cleared NGS platform.



MiSeq

Speed and simplicity for targeted and small-genome sequencing.



NextSeq 500

Speed and simplicity for whole-genome, exome, and transcriptome sequencing.



HiSeq 2500

Power and efficiency for large-scale genomics.



HiSeq X Ten

\$1,000 human genome and extreme throughput for population-scale sequencing.



Introducing NeoPrep



Library prep reimaged.
Unrivaled simplicity.

NeoPrep Library Prep System
Coming summer 2014

With the NeoPrep System, Illumina's market-leading TruSeq and Nextera library prep workflows are about to get even easier.



Throughput to Match Microbiology Applications



High
Throughput



Low
Throughput

Shotgun metagenomics

- ▶ Human microbiome, microbial diversity
- ▶ Gene content and discovery

rRNA Metagenomics

- ▶ Relative abundance of microbial diversity
 - 16S for bacteria and archaea
 - 18S for eukaryotes

Microbial genomics

- ▶ Detection
- ▶ Identification
- ▶ Antibiotic sensitivity testing
- ▶ Molecular epidemiology



Sample to Answer Workflow Solutions

BaseSpace®

MICROBIOLOGY SOLUTIONS

AMPLICON
SEQUENCING

16S rRNA
SEQUENCING

MICROBIAL
WHOLE GENOME

TRANSCRIPT-
OMICS

SHOTGUN
METAGENOMICS

Sample Prep

Sequence

Answer



Sample to Answer Workflow Solutions

BaseSpace®



16S
Metagenomics



De Novo
Assembly



Resequencing



Generate
FASTQ



SPAdes
Genome
Assembler



DNASTar
SeqMan Ngen
Assembler



SRA
Submission



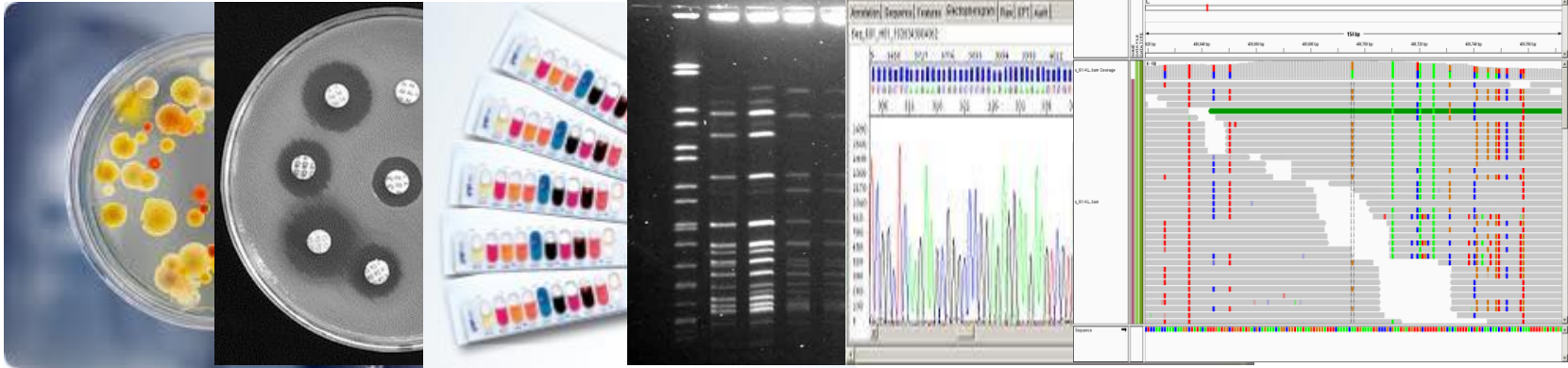
Broad IGV
Genome
Visualization



PathGen DX,
PathSeq Virome

Characterizing Infectious Disease in the Genomics Era

Transitioning to advanced genomic methods



Traditional

- ▶ Culture
- ▶ Serotyping
- ▶ Antibiotic susceptibility
- ▶ Multi-locus Enzyme Electrophoresis
- ▶ Bacteriophage typing

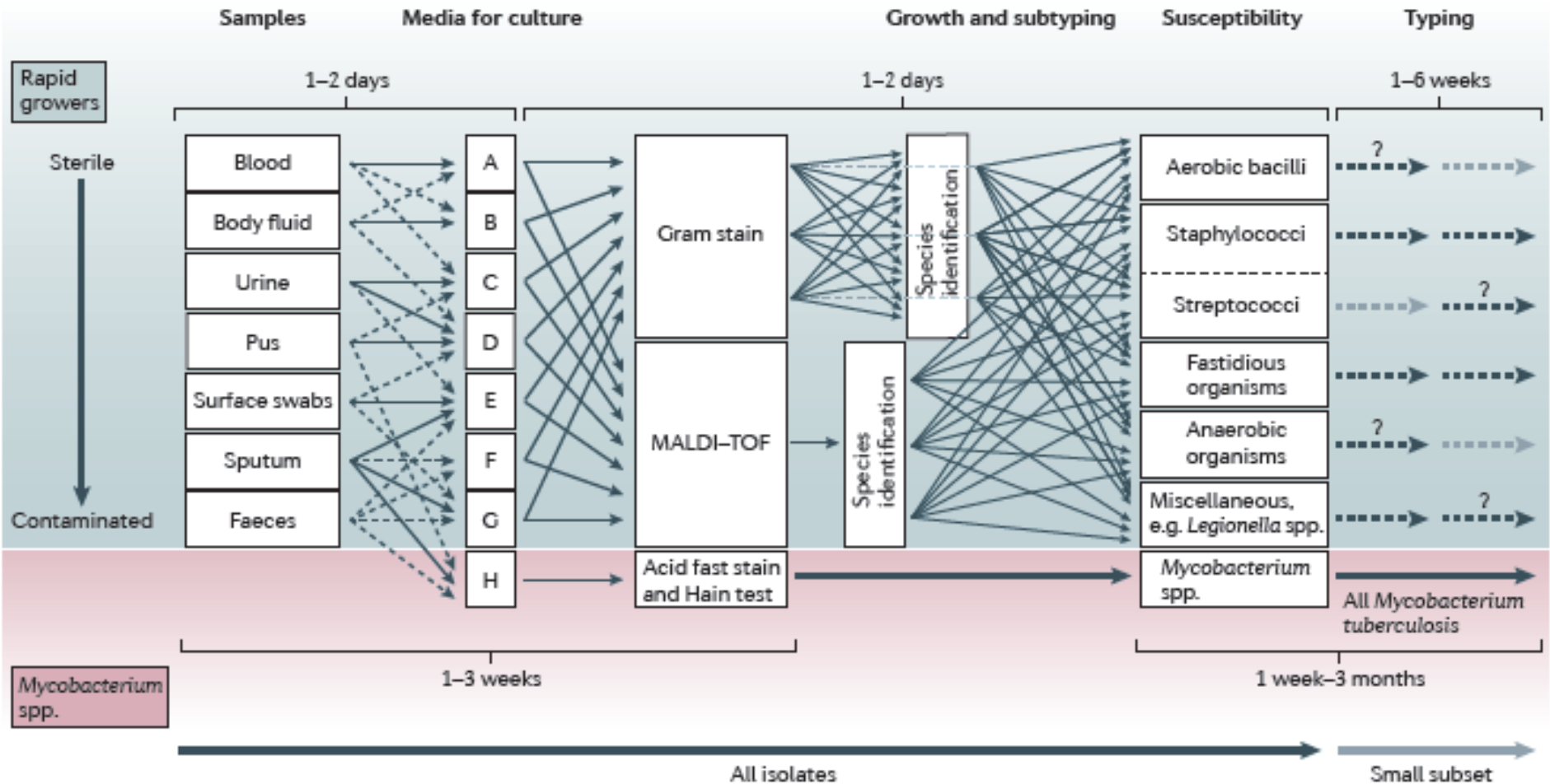
Genotyping

- ▶ Pulse Field Gel Electrophoresis (PFGE)
- ▶ PCR
- ▶ Microarrays
- ▶ Sanger Sequencing
- ▶ Next Generation Sequencing



Today's Market: Clinical Microbiology

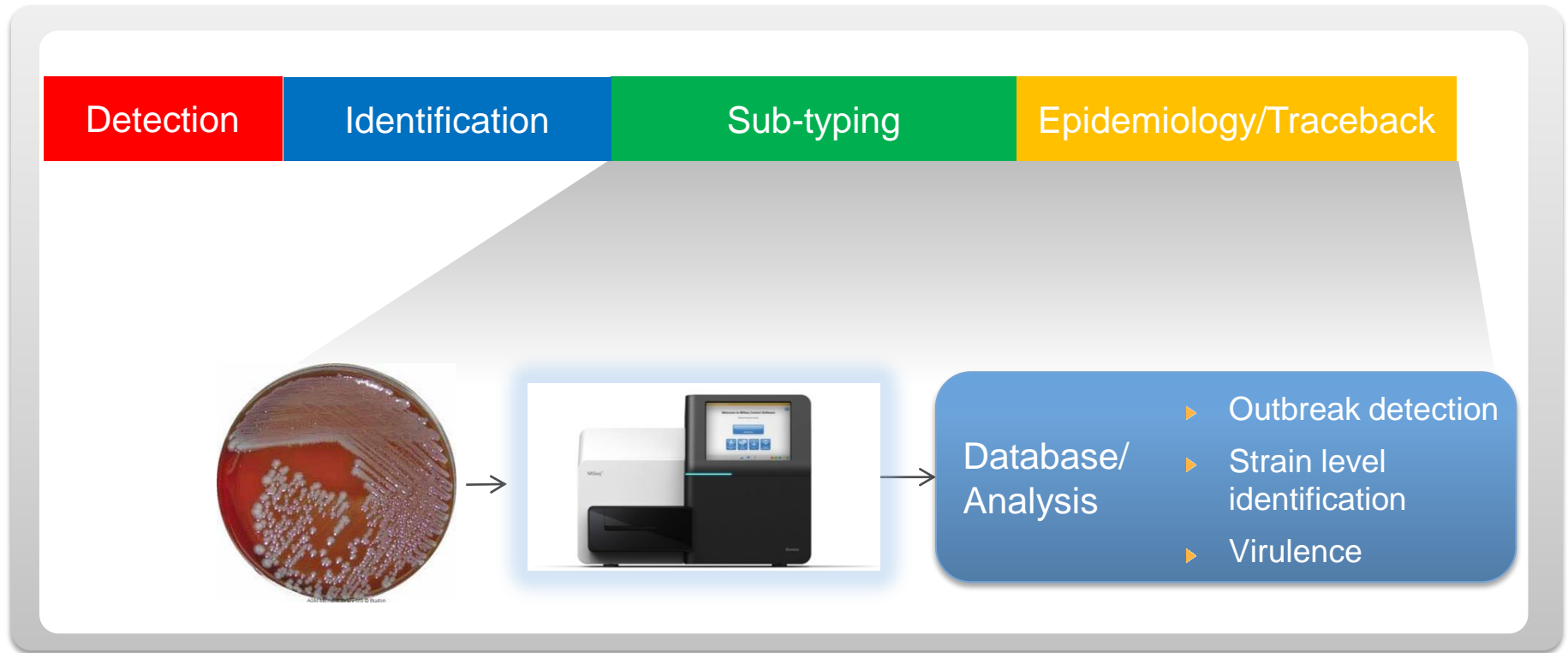
No universal method answers all questions



Pathogen Analysis – Where Does NGS Fit Today?

Pathogen sub-typing and epidemiology

- ▶ Surveillance and outbreak detection – single lab or network of labs
- ▶ Sub-typing and characterization
- ▶ Epidemiology and traceback – e.g., food recalls



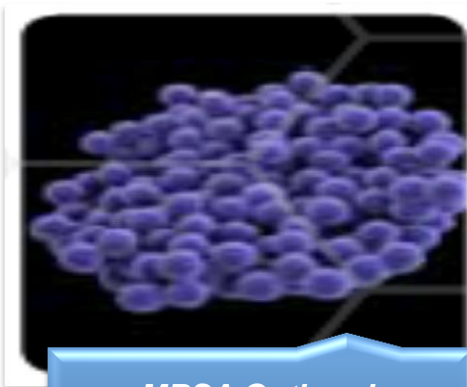
Case Studies



Let's see some real life stories...



*Micobacterium
tuberculosis*
UK 2013



*MRSA Outbreak
Transmission*



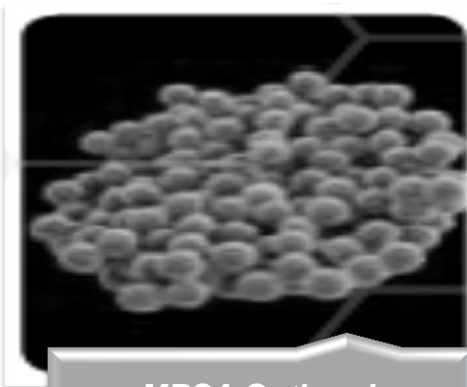
Salmonella enterica
FDA Genome Trakr Network



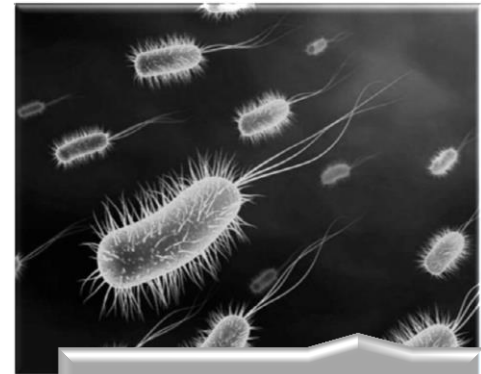
Let's see some real life stories...



*Micobacterium
tuberculosis*
UK 2013



*MRSA Outbreak
Transmission*



Salmonella enterica
FDA Genome Trakr Network



M. tuberculosis outbreak investigations

Calibration & Validation of Genomic Epidemiology

Articles

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

Timothy M Walker*, Camilla L Clift¹, Ruth H Harrell², Jason T Evans, Georgia Kapoti, Martin Dedicoat, David W Eyre, David J Wilson, Peter M Hawkey, Derrick W Crook, Julian Parkhill, David Harris, A Sarah Walker, Roy Bowden, Philip Monk, E Graze Smith, Tim EA Pebody

Summary

Background Tuberculosis incidence in the UK has risen in the past decade. Disease control depends on epidemiological data, which can be difficult to obtain. Whole-genome sequencing can detect microevolution within *Mycobacterium tuberculosis* strains. We aimed to estimate the genetic diversity of related *M tuberculosis* strains in the UK Midlands and to investigate how this measurement might be used to investigate community outbreaks.

Methods In a retrospective observational study, we used Illumina technology to sequence *M tuberculosis* genomes from an archive of frozen cultures. We characterised isolates into four groups: cross-sectional, longitudinal, household, and community. We measured pairwise nucleotide differences within hosts and between hosts in household outbreaks and estimated the rate of change in DNA sequences. We used the findings to interpret network diagrams constructed from 11 community clusters derived from mycobacterial interspersed repetitive-unit-variable-number tandem-repeat data.

Findings We sequenced 390 separate isolates from 254 patients, including representatives from all five major lineages of *M tuberculosis*. The estimated rate of change in DNA sequences was 0.5 single nucleotide polymorphisms (SNPs) per genome per year (95% CI 0.3–0.7) in longitudinal isolates from 30 individuals and 25 families. Divergence is rarely higher than five SNPs in 3 years. 109 (96%) of 114 paired isolates from individuals and households differed by five or fewer SNPs. More than five SNPs separated isolates from none of 69 epidemiologically linked patients, two (15%) of 13 possibly linked patients, and 13 (17%) of 75 epidemiologically unlinked patients (three-way comparison exact $p < 0.0001$). Genetic trees and clinical and epidemiological data suggest that super-spreaders were present in two community clusters.

Interpretation Whole-genome sequencing can delineate outbreaks of tuberculosis and allows inference about direction of transmission between cases. The technique could identify super-spreaders and predict the existence of undiagnosed cases, potentially leading to early treatment of infectious patients and their contacts.

Funding Medical Research Council, Wellcome Trust, National Institute for Health Research, and the Health Protection Agency.

Introduction

Control of *Mycobacterium tuberculosis* can be challenging even in high-income countries. Between 2001 and 2011, incidence of tuberculosis in the UK rose from 11.6 to 14.4 cases per 100 000 people per year,¹ with active disease developing in individuals born outside the UK accounting for the increase.² Detection of tuberculosis outbreaks is guided by mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) genotyping.³ Although transmission between individuals infected with different genotypes can be excluded with this approach, epidemiological data are needed to confirm outbreaks when genotypes match.^{3,4} Collection of such data is difficult if patients are unwilling or unable to volunteer information, as is commonly the case in some of the social groups most at risk of tuberculosis.^{5,6} Even when genotyping does lead to outbreak detection, it offers no insights into the underlying patterns of transmission.

Whole-genome sequencing is an increasingly accessible and affordable alternative to MIRU-VNTR genotyping that can detect microevolution within *M tuberculosis* lineages as

they are transmitted between hosts.^{7–9} Because backwards mutations are rare,² the pattern of accumulated mutations can theoretically suggest direction of transmission during an outbreak. Although whole-genome sequencing has a greater resolution than does MIRU-VNTR genotyping (as established in one specific outbreak),¹⁰ its full public health potential remains to be investigated.

In this study, our main aim was to estimate the genetic diversity of related strains of *M tuberculosis* in the Midlands region of the UK and to investigate where and how our measure of genetic diversity might be used to assess community outbreaks in detail. The region includes the cities of Birmingham and Leicester, where all five clades (lineages) of *M tuberculosis* are found in its ethnically diverse population.^{11,12} Annual incidence of tuberculosis in these cities is up to 50–70 cases per 100 000 individuals.¹

Methods

Study design

We sequenced isolates of *M tuberculosis* from an archive of more than 13 000 frozen cultures obtained between

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See Comment page 301

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- ▶ Retrospective study
- ▶ Comprehensive WGS study of tuberculosis transmission in the Midlands region, UK

Study Aim:

- ▶ How much genetic variation is there in TB isolates obtained at the same time but from different sites in an individual?
- ▶ How much variation accrues in a patient during the course of an infection?
- ▶ In household transmission, when the source of infection is known, how much do isolates vary between patients?
- ▶ Can patients be ruled in or ruled out as part of community outbreaks with thresholds of variation and how often do patterns suggest super-spreading?



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



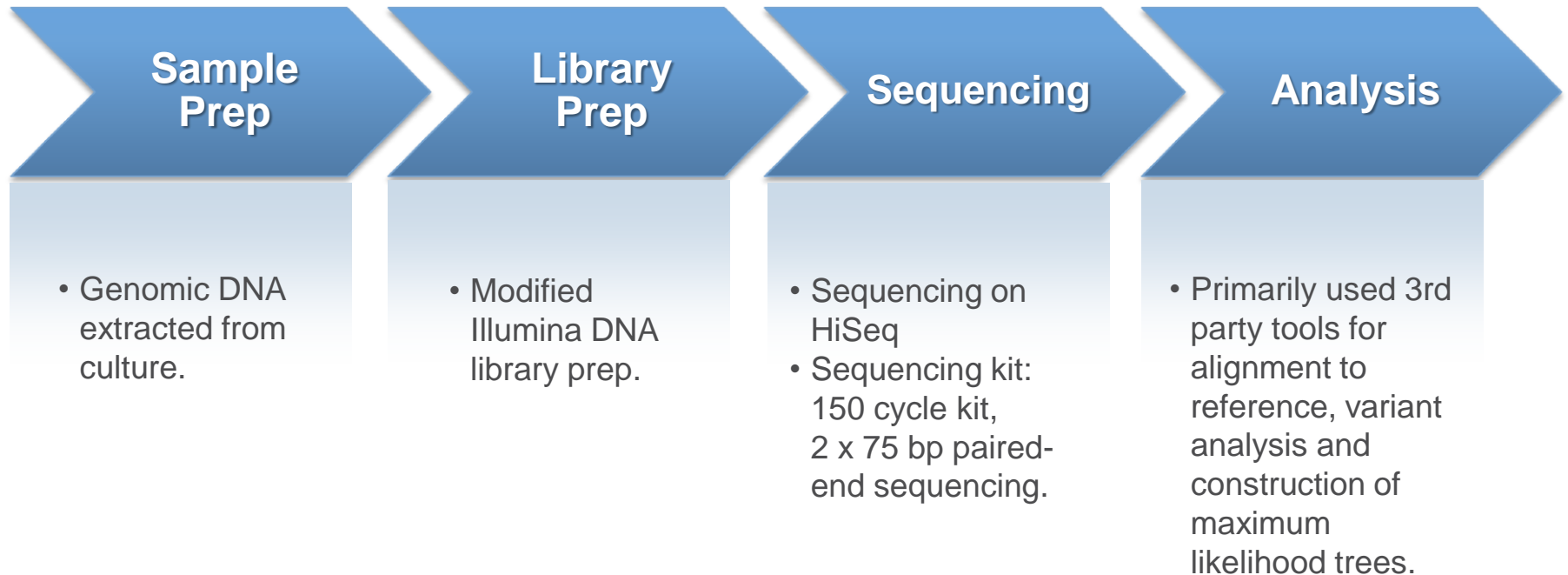
Timothy M Walker*, Camilla L Clp*, Ruth H Harrell*, Jason T Evans, Georgia Kapatai, Martin J Dedicoat, David W Eyre, Daniel J Wilson, Peter M Hawkey, Derrick W Crook, Julian Parkhill, David Harris, A Sarah Walker, Rory Bowden, Philip Monk†, E Grace Smith†, Tim E A Petof

- ▶ Characterized isolates into four groups
 - Cross-sectional
 - Longitudinal
 - Household
 - Community

- ▶ Sequenced 390 separate isolates from 254 patients, including representatives from all five major lineages of *M. tuberculosis*, from an archive of frozen cultures

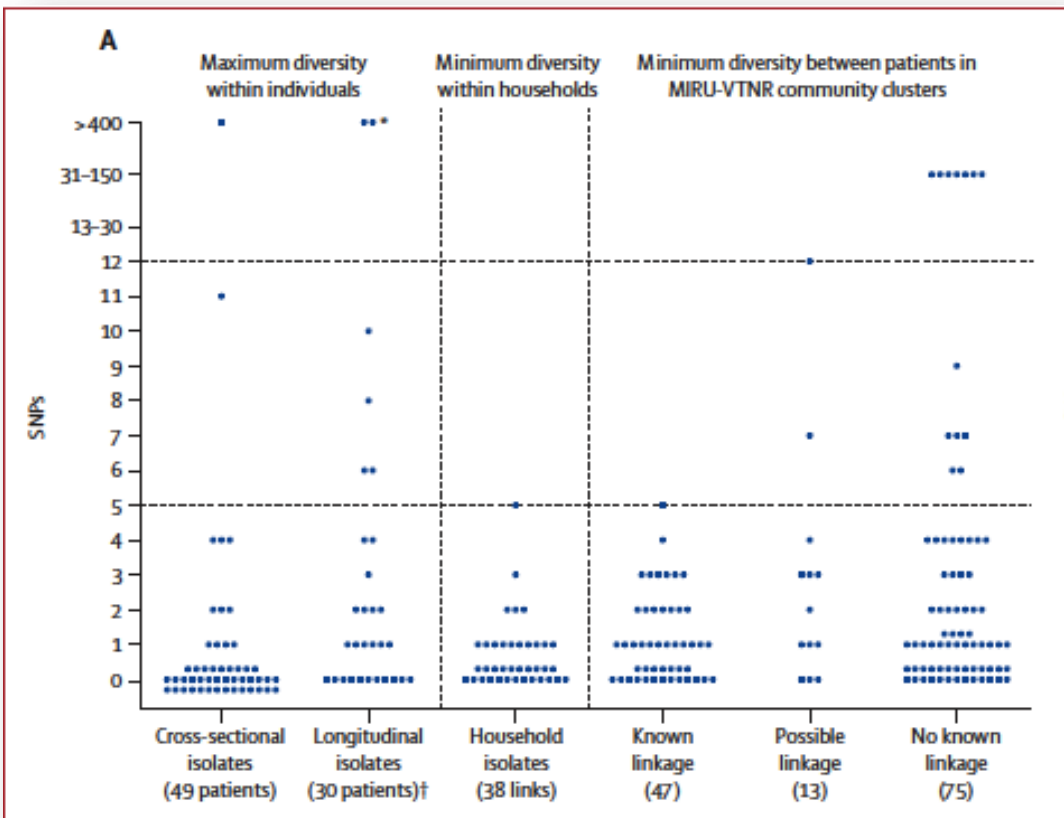
WGS *M. tuberculosis* Sequencing

Workflow



Genetic diversity of related isolates

M. tuberculosis



- ▶ Within-host diversity
 - <5 SNPs/patient (both cross sectional and longitudinal)
- ▶ Threshold for isolates within a household belonging to a specific outbreak cluster: ≤ 5 SNPs
- ▶ Threshold for MIRU-VNTR-based communities differed by ≤ 5 SNPs and not more than 12 SNPs



Genetic distances within 11 community clusters

MIRU-VNTR-based

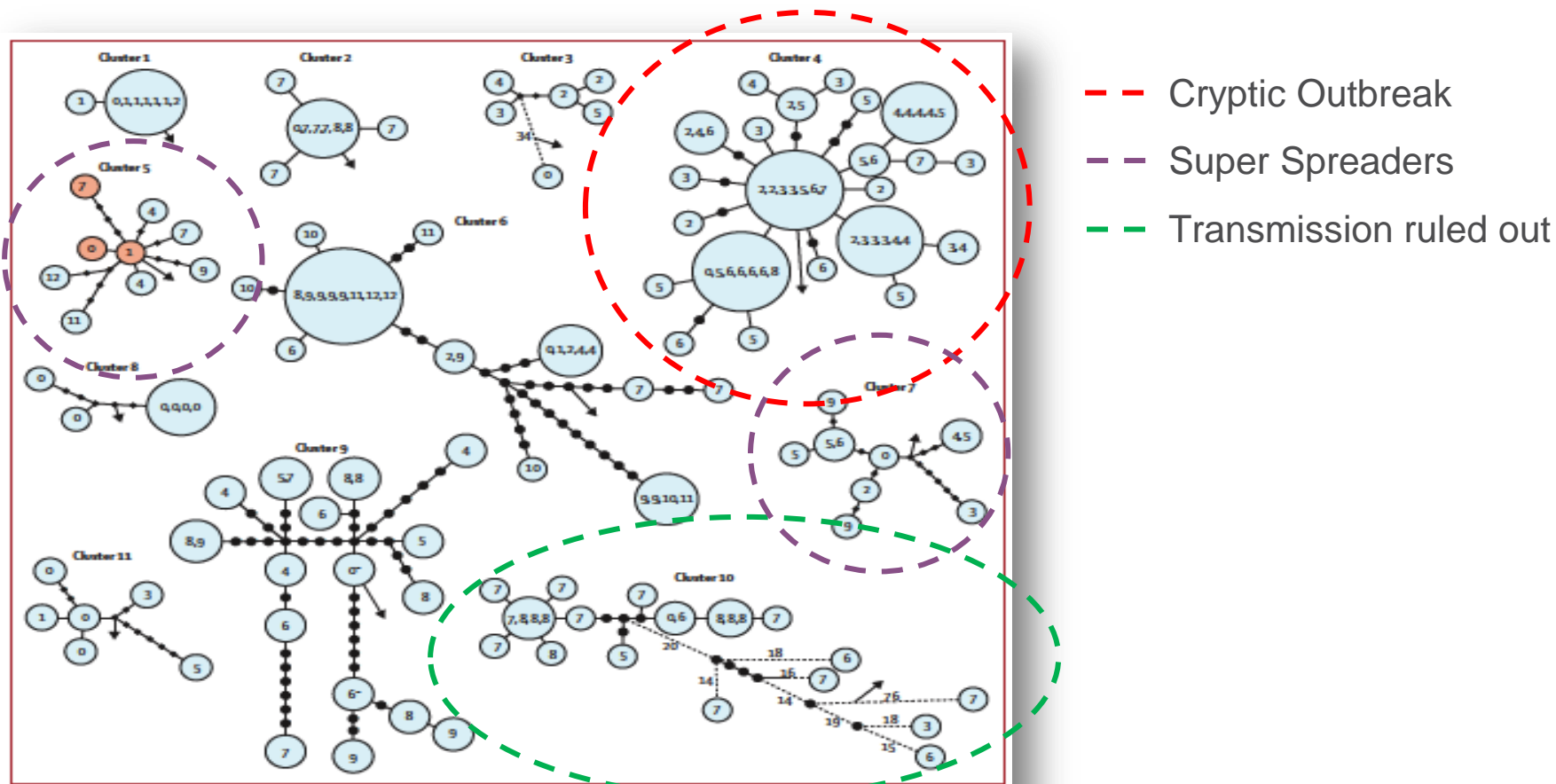


Figure 4: Genetic distances within 11 community clusters

Genetic distances estimated with maximum likelihood. Each blue circle represents a node of people who were infected with isolates separated by no SNPs. Each number within a circle is one patient, the number indicates at which year during the outbreak they were diagnosed (the first infected is represented by 0). For patients with several isolates, the closest in SNPs to the next patient is included. Black circles are added when patients within blue circles are separated by more than one SNP; one black circle represents a difference of one SNP. Dashed lines in clusters three and ten show larger SNP distances (not to scale), with numbers representing the SNP difference. Arrows indicate the next closest isolate in the sequenced collection. Cluster five has three red nodes that were sequenced after the blue nodes; the existence of the central red node was suggested by the constellation of surrounding blue nodes. SNP= single nucleotide polymorphism. *Two isolates from one patient.

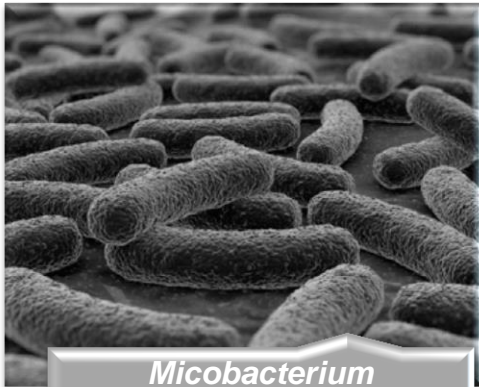


Conclusions

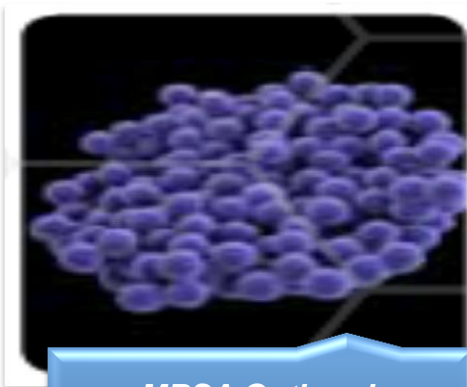
- ▶ Whole genome sequencing of TB using next generation sequencing provides high resolution data for investigating tuberculosis outbreaks and transmission pathways.
- ▶ Within VNTR-defined clusters, WGS offers sufficient resolution to identify or discount outbreaks.
- ▶ Declining costs and time to result of NGS platforms make NGS a valuable tool for complex community outbreaks when epidemiological data is limited.



Let's see some real life stories...



*Micobacterium
tuberculosis*
UK 2013



*MRSA Outbreak
Transmission*



Salmonella enterica
FDA Genome Trakr Network



WGS of a Hospital Outbreak of MRSA

MiSeq Halts Superbug Spread

Background:

- ▶ Hospital infection teams identify 12 infants colonized with MRSA over 6 months (2011).
- ▶ Link suspected, but a persistent outbreak could not be confirmed with conventional methods.

Study Aim:

- ▶ Can WGS help understand transmission and distinguish between MRSA strains.

Results:

- ▶ Identified a carrier that allowed the outbreak to persist.
- ▶ Identified a new MRSA strain not previously seen in the hospital.
- ▶ Showed previously unsuspected transmission between the hospital and community.

Articles

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

Simon Elliott*, Edward J Cartwright*, M Eslee Tink, Matthew T Holden, Nicholas Brown, Anmol, Ogby Stuart, Matthew Elliotts, Michael A Quill, Stephen D Bentley, Oliver Parkhill, Sharon Pebody†

Summary
Background The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) that can persist in the community and replace existing hospital-adapted lineages of MRSA means that it is necessary to understand transmission dynamics in terms of hospitals and the community as one entity. We assessed the use of whole-genome sequencing to enhance detection of MRSA transmission between these settings.

Methods We studied a putative MRSA outbreak on a special care baby unit (SCBU) at a National Health Service Foundation Trust in Cambridge, UK. We used whole-genome sequencing to validate and expand findings from an infection-control team who assessed the outbreak through conventional analysis of epidemiological data and antibiogram profiles. We sequenced isolates from all colonised patients in the SCBU, and sequenced MRSA isolates from patients in the hospital or community with the same antibiotic susceptibility profile as the outbreak strain.

Findings The hospital infection-control team identified 12 infants colonised with MRSA in a 6 month period in 2011, who were suspected of being linked, but a persistent outbreak could not be confirmed with conventional methods. With whole genome sequencing, we identified 26 related cases of MRSA carriage, and showed transmission occurred within the SCBU, from mothers on a postnatal ward, and in the community. The outbreak MRSA type was a new sequence type (ST) 2371, which is closely related to ST22, but contains genes encoding Panton-Valentine leucocidin. Whole genome sequencing data were used to propose and confirm that MRSA carriage by a staff member had allowed the outbreak to persist during periods without known infection on the SCBU and after a deep clean.

Interpretation Whole-genome sequencing holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

Conclusions UK Clinical Research Collaboration Translational Infection Research Initiative, Wellcome Trust, Health Protection Agency, and the National Institute for Health Research Cambridge Biomedical Research Centre.

Introduction
Successful prevention of health-care-associated methicillin-resistant *Staphylococcus aureus* (MRSA) depends on effective programmes of infection control, including detection of transmission events and outbreaks and effective strategies for their containment and prevention.^{1,2} Until recently, the predominant focus of control efforts took place in health-care facilities, where most MRSA infections were caused by a small number of health-care-associated MRSA lineages that were poorly adapted for persistence in the community.^{3,4} This situation has undergone a fundamental shift, with the emergence of lineages of community-associated MRSA such as USA300 in the USA that can be carried for long periods by healthy people.^{5,6} Furthermore, community-associated MRSA is now displacing previously dominant health-care-associated MRSA lineages,⁴ a process that is driven at least in part by repeated admission and discharge of some groups of patients. One implication of the blurring between health-care-associated and community-associated MRSA is that for the purpose of understanding the transmission dynamics of MRSA, hospitals and the community should no longer be regarded as separate entities. This distinction is problematic, because effective linkage between the two settings is challenging for present strategies used for hospital-based infection control.⁷

One approach to tracking of MRSA transmission pathways between health-care facilities and the community could be to obtain and genotype MRSA isolates, and draw inferences on transmission on the basis of their genetic relatedness. Present typing methods are not fit for purpose because they are not sufficiently discriminatory, but this obstacle is set to change with the introduction of whole-genome sequencing.⁸ This technique provides the best discrimination between closely related bacterial isolates, and the rapidly decreasing cost and turnaround time of the technology means that it could become viable in diagnostic laboratories in the near future. Whole-genome sequencing has sufficient discriminatory power to reconstruct intercontinental and local transmission of MRSA lineages.⁹ Furthermore, bench-top, rapid turnaround whole-genome sequencing was able to distinguish between MRSA isolates that were associated with a putative outbreak in a neonatal intensive care unit

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Correspondence: Prof Sharon Pebody, Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK (s.p2@medsch.cam.ac.uk)



Investigating MRSA Transmission

MRSA screen of all infants entering a Special Care Baby Unit (SCBU) and weekly thereafter.

- ▶ 3 infants were carriers
- ▶ Identical antibiotic resistance pattern

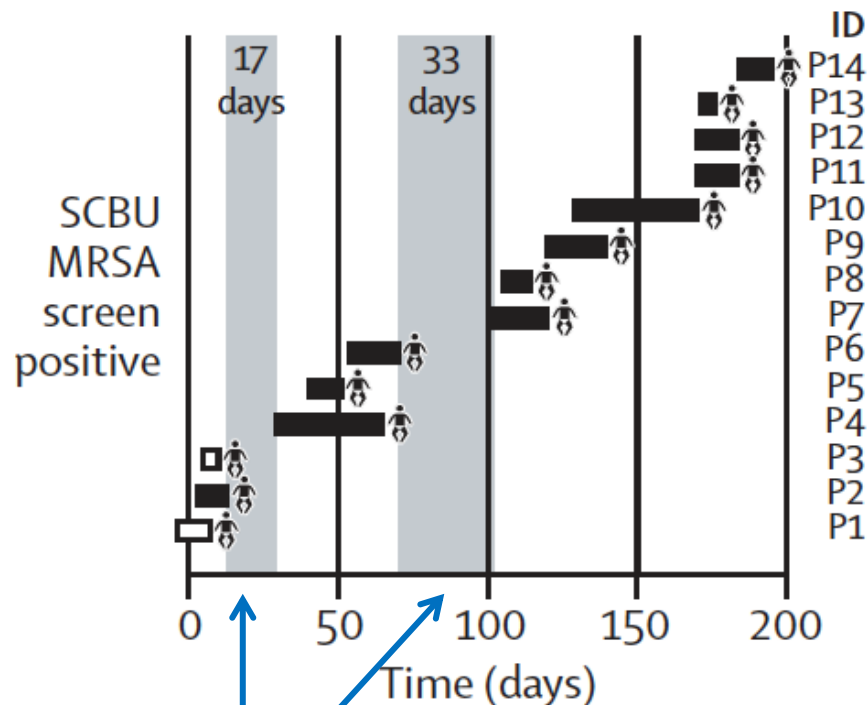
Outbreak?

Investigation triggered:

- ▶ Reviewed 14 MRSA isolates from SCBU during preceding 6 months
 - 14 were a new strain, not seen before in that hospital
 - 3 cases were most common strain in the hospital

Multiple infants putatively linked to an outbreak

Epidemiological Map of SCBU Cases



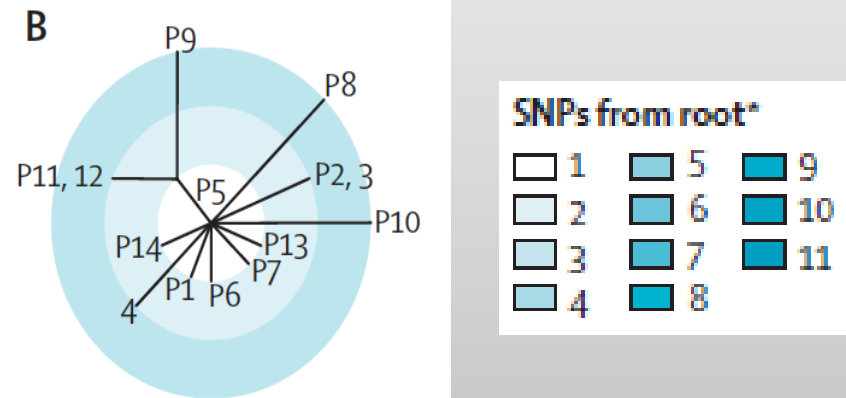
Time gaps, no carriers overlapped. Source?



Investigating MRSA Transmission

- ▶ Identify 10 additional non-SCBU cases over the past 3 month period.
- ▶ Epidemiological link to the SCBU cases?
- ▶ Another MRSA carrier admitted to the SCBU - 64 days after the last positive MRSA case left the SCBU.
- ▶ Sequencing revealed that this case differed from the new strain by 4 SNPs.

Sequenced-based phylogenetic tree, cases 1-14, new strain.



What to do?

Investigating MRSA Transmission

Next steps

- ▶ Now 15 infants are putatively linked to an outbreak
- ▶ Staff member(s) now suspected to be carriers
- ▶ Screened 154 SCBU staff members for MRSA

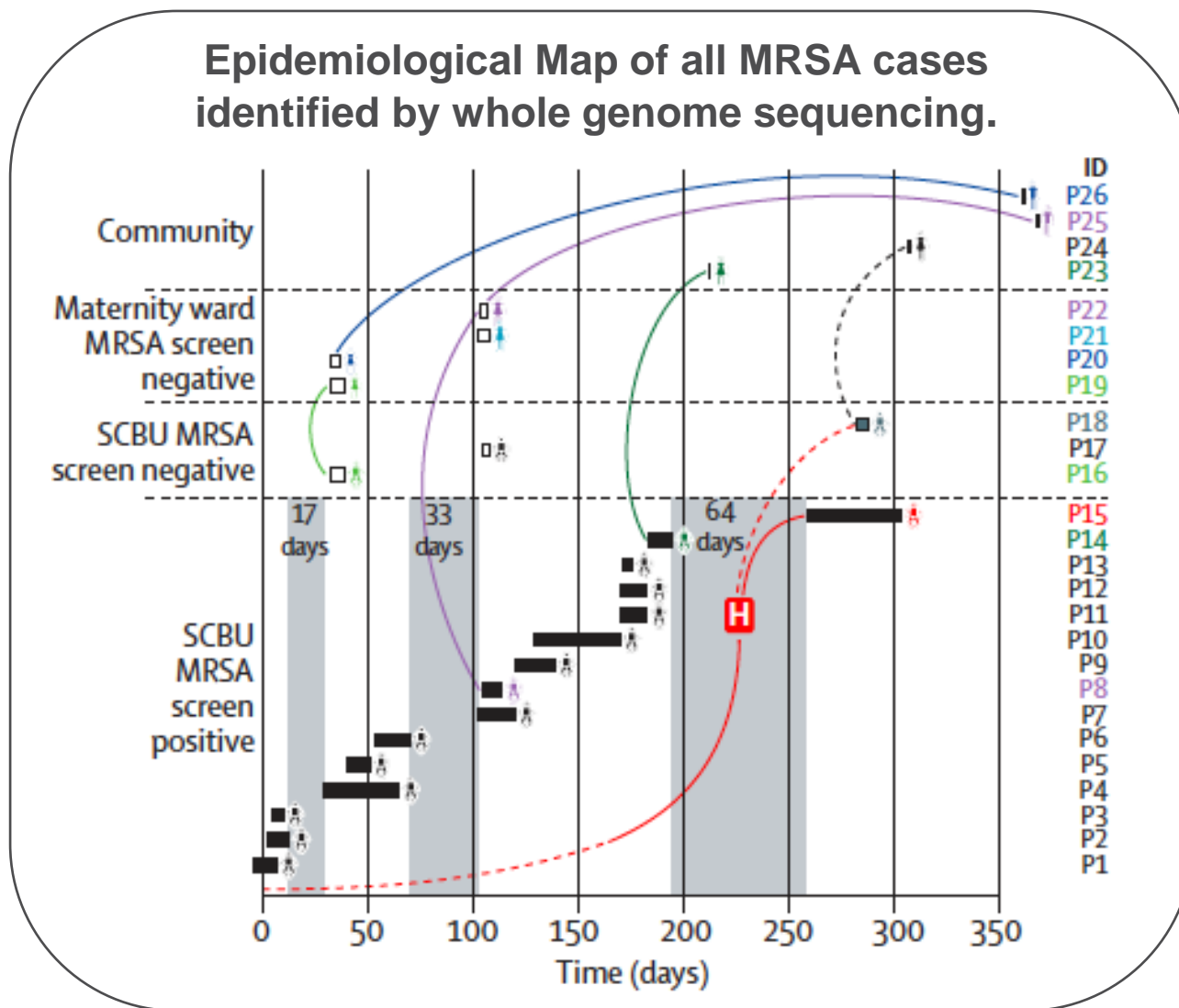
One member was positive

- ▶ Sequencing confirmed that isolate is closely linked to outbreak strains
- ▶ Staff member was relieved of clinical duties and successfully decolonized
- ▶ **Outbreak was halted**



How long was the staff member colonized?

A range of 251 days before to 164 days after the first case

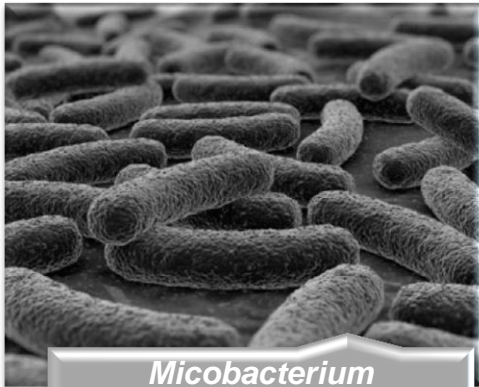


Conclusions

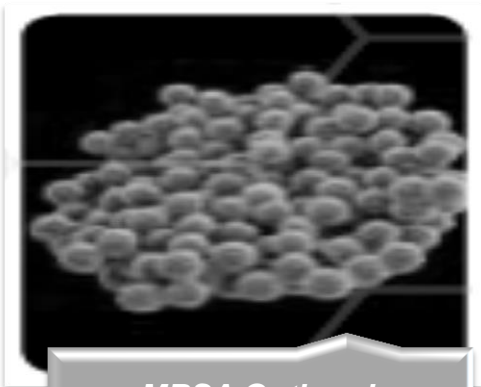
- ▶ NGS enable more precise identification of patients involved in an outbreak of MRSA compared to standard infection control techniques.
- ▶ WGS data and epidemiology mapping had a direct impact on infection control activities.
- ▶ Cost benefit – while there is no detailed analysis, the paper points out that the cost of containing the outbreak was about £10,000. Compared to the cost of sequencing £97/sample.
- ▶ Future needs:
 - A central database for comparison of sequence data with previous local and national isolates.
 - A system for automated interpretation and linking of genome sequence data.



Let's see some real life stories...



Micobacterium tuberculosis
UK 2013



MRSA Outbreak Transmission



Salmonella enterica
FDA Genome Trakr Network

FDA Selects MiSeq for Next Generation Sequencing to Identify Foodborne Pathogens

Food Safety News

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FDA Spends \$17 Million to Go After Pathogens Faster

BY NEWS DESK | SEPTEMBER 19, 2012

The U.S. Food and Drug Administration (FDA) is spending \$17 million on technology it hopes will be fast enough to catch fresh produce with pathogen contamination.

FDA has awarded a five-year contract to Illumina Inc, a San Diego-based technology company involved in accelerating genetic research. Illumina will provide FDA with its MiSeq sequencing systems and reagents for conducting whole genome analysis on produce and produce-related environmental Salmonella and shigatoxigenic E. coli.



**7 State health departments
and 10 FDA-ORA labs**

Genome Trakr Network¹

- ▶ A pilot network and coordinated effort across state and federal labs.
- ▶ Sequencing pathogens collected from foodborne outbreaks, contaminated food products and environmental sources.
- ▶ Genome Trakr - genomic reference database at NCBI where sequencing data is archived.
- ▶ Genome Trakr is open-access to enable analysis in real time, speeding up investigations and contamination control.

Source:

<http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ucm363134.htm>



FDA Protocol

Application: Bacterial whole genome sequencing from culture



- Grow culture

Nextera Library Prepc

- Lyse cells from cultured isolate
- Genomic DNA extraction

Nextera Library Prepc

- Nextera XT
- ~12 samples per run
- Sequencing kit: 500 cycle kit, 2 x 250 bp paired-end sequencing.
- 20x-40x coverage

MiSeq & Primary Analysis

- MiSeq Reporter workflow: Generate FASTQ



- Data sent to FDA or BaseSpace for storage and sharing with FDA and upload to NCBI SRA database and analysis.



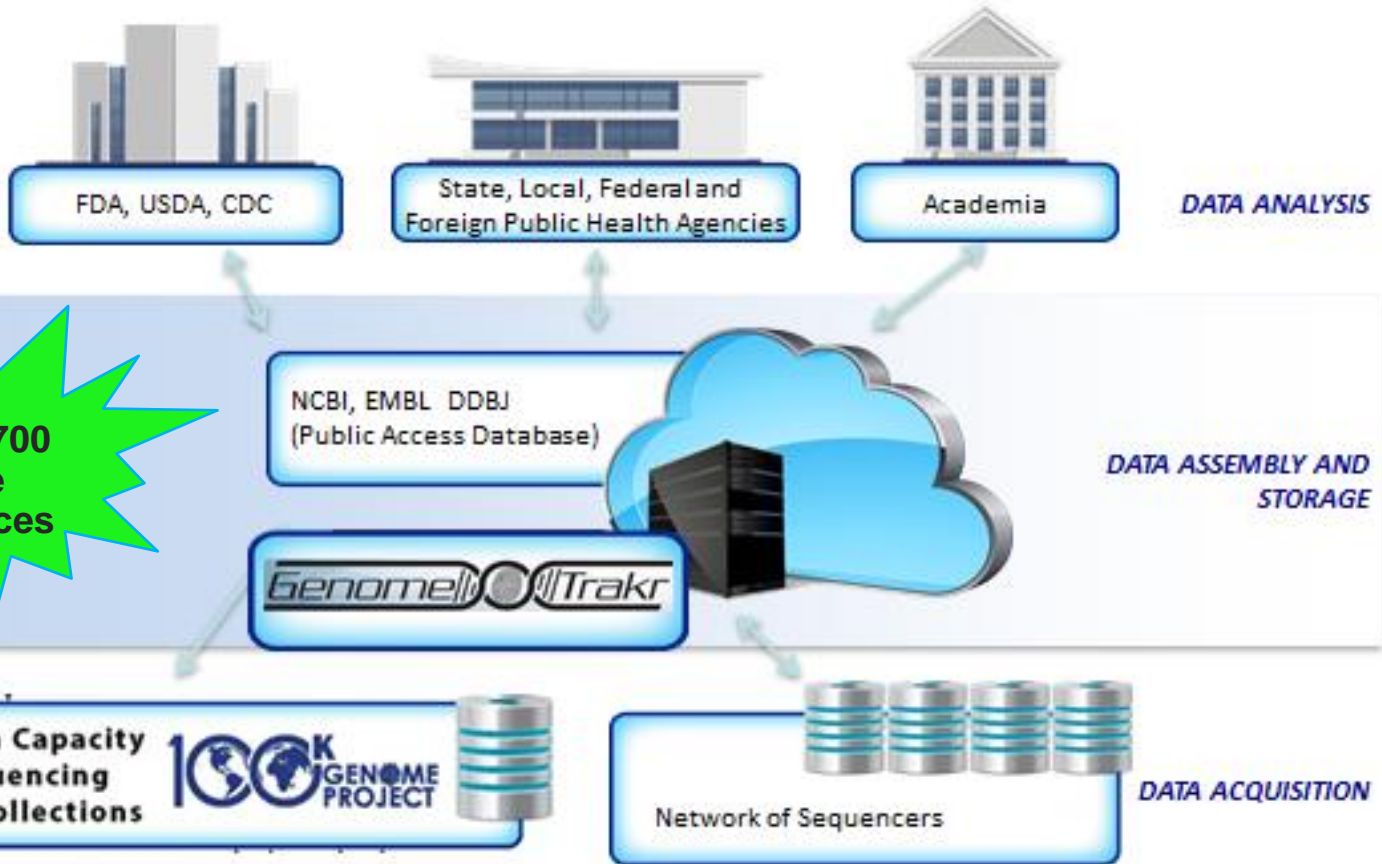
FDA CFSAN Network Model



U.S. Food and Drug Administration
Protecting and Promoting Public Health

www.fda.gov

Our Current Model



Over 4,700 genome sequences



Impact: NGS Used to Assess Food Pathogen Outbreak in Food and Clinical Samples

By RYAN JASLOW / CBS NEWS / March 12, 2014, 12:07 PM

FDA shuts down Roos Foods cheese plant over listeria outbreak



Compared with pulsed-field gel electrophoresis (PFGE), WGS provides clearer distinction between cases and foods that are likely part of a given outbreak and those that are not.



Whole-genome sequences of the *Listeria* strains isolated from Roos Foods cheese products were available after the recall and were found to be highly related to sequences of the *Listeria* strains isolated from the patients.



ILLUMINA Continuously Strives to Improve

Innovating to meet customer needs

NGS Impact

- FDA website: Compared with PFGE, WGS provides clearer distinction between cases and foods that are likely part of a given outbreak and those that are not.

Accessible Methods

- MiSeq delivers most integrated bench top instrument.
- Sample prep products with minimal hands-on steps.
- Automated data analysis available with MiSeq Reporter and on BaseSpace.

Illumina is the NGS Leader

- Great than 85% of all NGS bench top data is generated on the MiSeq.
- Illumina technology was selected by FDA over other bench top platforms for quality and ease of use.

Product Innovation

- Expanding read lengths, 2x300 bp in V3 chemistry
- Expanding BaseSpace applications for microbiology
- Delivering automated solutions for sample prep



Thank You

