Moving Public Health, and Specifically the Battle against Antibiotic Resistance, Into the Era of Next Generation Sequencing

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Associate Director for Science
Division of Healthcare Quality Promotion
Whole Genome Sequencing Conference, Austin, TX
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Disclosures

- No relevant financial disclosures
- For a complete list and description of CDC Foundation-funded projects in which DHQP participates, see http://www.cdcfoundation.org/what/programs/list
- *The findings and conclusions in this presentation are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.*
Major Uses of Infectious Agent Genetic Characterization in Public Health

- Phylogenetic differentiation
  - Distinct epidemiology: environmental source and persistence, host and body site tropism, route of transmission
  - Distinct virulence: morbidity and mortality, disease risk
  - Antibiotic and biocide resistance, vaccine composition and escape
  - Evolutionary ‘slow time-clock’ differentiation-MLST, wgMLST

- Strain/clone differentiation
  - Understanding transmission, outbreak detection
  - Evolutionary ‘fast time-clock’ differentiation-whole/core genome SNP

- Mechanistic understanding and detection of key determinants
  - Antibiotic resistance, epidemiologic, and virulence determinants
  - ‘Genetic sequence of public health importance’
Outbreak detection: Pulsenet

PulseNet connects the dots to detect foodborne outbreaks and prevent over 270,000 illnesses from Salmonella, E. coli and Listeria every year.

Every year PulseNet saves at least half a billion dollars in medical costs and lost productivity.

$1 spent = $70 saved!
Pulsenet future

2017

» Whole genome sequencing will be used for routine surveillance of *Salmonella* in states that have the capacity to conduct sequencing.

» PulseNet will be able to analyze *Vibrio* and *Shigella* whole genome sequencing data.

2018

» PulseNet will be able to analyze *Yersinia* and *Cronobacter* whole genome sequencing data.

» All 50 state public health laboratories will be using whole genome sequencing for routine surveillance.

» Whole genome sequencing will become the new PulseNet gold standard for subtyping pathogens that cause foodborne illness.

2020 and Beyond

PulseNet will use highly sophisticated approaches designed to identify and subtype foodborne pathogens directly from complex clinical samples, without bacterial cultures. The favored approach, known as metagenomics, has the potential to allow extraction of pathogen-specific DNA sequence information directly from complex samples such as stool.
Tuberculosis

- Advancing genotyping
  - Current combination of spoligotyping, IS6110-based RFLP, and MIRU
  - Whole genomic sequencing on a national scale
    - Improving informatics pipelines
    - Increasing state and regional public health laboratory capacity
- Molecular detection of antibiotic resistance
  - Direct NAAT-detection of TB and rifampin resistance (Xpert MTB/RIF assay)
- Future
  - Direct sequence available from the sputum
  - Metagenomics solution for typing and antibiotic resistance
Influenza

- Tracking/surveillance for vaccine effectiveness and disease burden
  - Moving to ‘sequence first’ at CDC and pushing this capability out to state public health laboratories
  - Cloud-based sequence database
  - Hemagglutinin and neuraminidase from the sequence data
- Detection of outbreaks
  - Detection of influenza along with other respiratory viruses in metagenomes from unexplained respiratory disease outbreaks
- Improving vaccine production
  - Detecting gene variants associated with better influenza strain growth in eggs
ANTIBIOTIC RESISTANCE THREATS
in the United States, 2013

Estimated minimum number of illnesses and deaths caused annually by antibiotic resistance*:
At least

2,049,442 illnesses
23,000 deaths

*bacteria and fungus included in this report
Combating AR requires comprehensive, aggressive action across the U.S. gov’t and around the globe.

New Drugs Alone Aren’t Enough to Protect Americans
CDC’s Approach to Combat Antibiotic Resistance Includes Innovation

Connecting the dots to address current and future gaps and opportunities

- Ongoing innovation for new strategies for prevention:
  - Patient-level interventions
  - Healthcare facility interventions
  - Regional interventions
Impact of Next Generation Sequencing

- Challenges
  - Data overload
  - Interpretation
Carbapenem-Resistant *Klebsiella pneumoniae* Producing New Delhi Metallo-/β-Lactamase at an Acute Care Hospital, Colorado, 2012

Carbapenem-Resistant *Klebsiella pneumoniae* Producing New Delhi Metallo-β-Lactamase at an Acute Care Hospital, Colorado, 2012

Whole Genome Sequencing

Carbapenem-Resistant *Klebsiella pneumoniae* Producing New Delhi Metallo-β-Lactamase at an Acute Care Hospital, Colorado, 2012

- Identified 3 areas where improvements in infection control were needed

Burkholderia cenocepacia outbreak, PA

Tom de Man
Analysis To Date

- Clean raw sequencing reads
- *de novo* assemble clean reads into contigs
- Perform in silico MLST
- Determine the antimicrobial resistance (AMR) genotype
- Determine core genome size
- Map clean reads to a genome assembly reference
- Call single nucleotide polymorphisms (SNPs)

***DRAFT Data are preliminary and subject to change***
RESULTS: Species confirmation, MLST, AMR

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Species by Kraken</th>
<th>MLST*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-18-01</td>
<td>Blood</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-02</td>
<td>Blood</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-04</td>
<td>Catheter tip</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-05</td>
<td>Drain in wall - S18</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-06</td>
<td>Drain in wall - S14</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-07</td>
<td>Water of preflush</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-08</td>
<td>Water of preflush</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
<tr>
<td>2017-18-09</td>
<td>RO tubing - S18</td>
<td>Burkholderia cenocepacia</td>
<td>1116</td>
</tr>
</tbody>
</table>

gyrB closest match: 640
141C → 502A
188C → 549T
338T → 699C

* Only four matching MLST alleles with ST1116

No acquired AMR genes identified

***DRAFT Data are preliminary and subject to change
DRAFT****
Example: Core Genome and SNP Calling

Five genomes
Whole genome alignment using parSNP
Identify common regions
Core genome

The core contains important housekeeping genes
Core genome

Regions where we call core SNPs
Accessory genome

The accessory regions contain phage, mobile elements, plasmids etc.
Mauve numbers for all 8 BC isolates

- Cluster coverage in 2017-18-01: 98.37%
- Cluster coverage in 2017-18-02: 99.38%
- Cluster coverage in 2017-18-04: 99.33%
- Cluster coverage in 2017-18-05: 99.35%
- Cluster coverage in 2017-18-06: 99.27%
- Cluster coverage in 2017-18-07: 99.31%
- Cluster coverage in 2017-18-08: 99.28%
- Cluster coverage in 2017-18-09: 99.35%
- Total coverage among all sequences: 99.20% (core genome)

***DRAFT Data are preliminary and subject to change
DRAFT****
Core SNP tree for 8 outbreak isolates

<table>
<thead>
<tr>
<th>Isolate</th>
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</tr>
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<tbody>
<tr>
<td>2017-18-01</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>2017-18-06</td>
<td>Drain in wall - S14</td>
</tr>
<tr>
<td>2017-18-07</td>
<td>Water of preflush</td>
</tr>
<tr>
<td>2017-18-08</td>
<td>Water of preflush</td>
</tr>
<tr>
<td>2017-18-09</td>
<td>RO tubing - S18</td>
</tr>
</tbody>
</table>

***DRAFT Data are preliminary and subject to change DRAFT***
Core SNP tree for 8 outbreak isolates

Transmission events

- Catheter tip <-> Patient

Core genome size: 98.37%

<table>
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<td>RO tubing - S18</td>
</tr>
</tbody>
</table>

***DRAFT Data are preliminary and subject to change DRAFT***
Core SNP table for all 8 outbreak isolates
(Data input for tree on previous slides)

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-18-01</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2017-18-02</td>
<td>5</td>
<td>-</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2017-18-04</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2017-18-05</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

***DRAFT Data are preliminary and subject to change
DRAFT****
Genomic Analysis of the Emergence and Rapid Global Dissemination of the Clonal Group 258 *Klebsiella pneumoniae* Pandemic

**Fig 1.** Genetic diversity of healthcare-associated *K. pneumoniae*.


http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0133727
Genomic Analysis of the Emergence and Rapid Global Dissemination of the Clonal Group 258 *Klebsiella pneumoniae* Pandemic

**Fig 3. Projecting the evolutionary history of ST258.**


http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0133727
New fundamental understanding of *Clostridium difficile* epidemiology through whole genomic sequencing or other ‘fast clock’ typing

- Eyre et al. 2013
  - Enzyme immunoassay for CDI diagnosis
  - Only 38% of new cases linked to a symptomatic (CDI) source

- Curry et al.
  - Cell cytotoxin neutralization assay for diagnosis
  - At least 29% of new cases linked to an asymptomatic source

- Eyre et al. 2017
  - Symptomatic PCR+/toxin- patients transmit as frequently as symptomatic PCR+/toxin+ patients

As better infection control contains transmission from symptomatic (CDI) source, asymptomatic (and mildly symptomatic) patients play a larger role in transmission

Eyre DW et al.  *Clin Infect Dis* 2017;65(3):433–41
SSTAR, a Stand-Alone Easy-To-Use Antimicrobial Resistance Gene Predictor

Tom J. B. de Man, Brandi M. Limbago
Centers for Disease Control and Prevention, Division of Healthcare Quality Promotion, Atlanta, Georgia, USA

ABSTRACT We present the easy-to-use Sequence Search Tool for Antimicrobial Resistance, SSTAR. It combines a locally executed BLASTN search against a customizable database with an intuitive graphical user interface for identifying antimicrobial resistance (AR) genes from genomic data. Although the database is initially populated from a public repository of acquired resistance determinants (i.e., ARG-ANNOT), it can be customized for particular pathogen groups and resistance mechanisms. For
Thinking Beyond The Resistant Bacteria Themselves: Plasmid and Gene Transfer

## Detect & Respond: Creating AR Regional Labs

<table>
<thead>
<tr>
<th>Healthcare Labs</th>
<th>State/Local Labs</th>
<th>Regional Labs</th>
<th>CDC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WEST</strong>&lt;br&gt;Washington State Public Health Laboratories&lt;br&gt;- Core Testing&lt;br&gt;- Candida&lt;br&gt;- N. gonorrhoeae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CENTRAL</strong>&lt;br&gt;Minnesota Department of Health Public Health Laboratory&lt;br&gt;- Core Testing&lt;br&gt;- Candida&lt;br&gt;- C. difficile&lt;br&gt;- Reflex Culture pilot&lt;br&gt;- S. pneumaticae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MOUNTAIN</strong>&lt;br&gt;Texas Department of State Health Services Laboratory&lt;br&gt;- Core Testing&lt;br&gt;- N. gonorrhoeae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MIDWEST</strong>&lt;br&gt;Wisconsin State Laboratory of Hygiene&lt;br&gt;- Core Testing&lt;br&gt;- Reflex Culture pilot&lt;br&gt;- S. pneumaticae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NORTHWEST</strong>&lt;br&gt;Wadsworth Center Bacteriology Laboratory&lt;br&gt;- Core Testing&lt;br&gt;- Candida</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>MID-ATLANTIC</strong>&lt;br&gt;Maryland Public Health Laboratory&lt;br&gt;- Core Testing&lt;br&gt;- N. gonorrhoeae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SOUTHEAST</strong>&lt;br&gt;Tennessee State Public Health Laboratory&lt;br&gt;- Core Testing&lt;br&gt;- Candida&lt;br&gt;- N. gonorrhoeae&lt;br&gt;- Reflex Culture pilot</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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[Map of the United States showing regional labs and their respective services]
CDC & FDA AR Isolate Bank: Sharing Bug Data to Support Drug, Diagnostic Development

CDC gathers resistant bacteria through surveillance/outbreak programs. CDC analyzes the bacteria’s resistance & shares with researchers. Currently includes 496 isolates (on 14 panels).

Since July 2015, CDC has processed 516 orders (more than 46k isolates). New diagnostic tests & antibiotic drugs are developed using the bacteria & data.

Helping healthcare providers know that the tests they use and drugs they prescribe will protect patients.

www.cdc.gov/DrugResistance/Resistance-Bank
CDC’s Approach to Combat Antibiotic Resistance Includes Innovation

Discovering, investigating, and implementing new solutions to protect Americans

**Academic & Healthcare Partners**
- 28 collaboratives discovering new ways to protect patients and scale up effective interventions across health systems
- 11 Prevention Epicenters identifying new prevention strategies to guide clinical practice and maximize public health impact
- 14 studies exploring the gut-drug relationship and the patient’s microbiome
- 260 white papers submitted to CDC’s recent Broad Agency Agreement solicitation

**Industry Partners**
- With the CDC-FDA AR Isolate Bank, supporting development of new drugs and diagnostics with 46,000 isolates and more than 500 orders
- Making public CDC’s sequencing data from AR pathogens to spur innovation, research
Normal microbial flora

- Bacterial cells outnumber human cells 10:1

- **Mutualistic association**

- We provide
  - Nutrients
  - Stable environment

- They provide
  - Nutrients (vitamins)
  - Keep out pathogens
  - Stimulate immune system

Intestinal Microbiota

- $10^{14}$ microorganisms overall
- $10^{4-5}$ species
- Only $\sim$20% ever cultured *in vitro*
- Increasing density
  - $10^1$ stomach
  - $10^3$ duodenum
  - $10^4$ jejunum
  - $10^7$ ileum
  - $10^{12}$ colon
- $10^{12}$ per gram of stool

http://www.wright.edu/~oleg.paliy/research.html
Certain epidemiologic factors in common

- Direct and indirect contact transmission between patients
- Colonized patients far exceed those infected
- Colonization precedes infection by days to weeks and may last weeks to months and even years after

Colonization occurs at:

- Pathologic biofilms
- Body sites normally inhabited by a complex and diverse human microbiota
Key Premise

- The intact human microbiome is a primary host defense for preventing colonization, dominance, transmission, and infection with opportunists or pathobionts
  - Clostridium difficile
  - Multidrug-resistant organisms (VRE, CRE, ESBLs, others)
  - Salmonella, Shigella, and Campylobacter spp.

Why ‘Pathobiont’?
- Commensal: low virulence
- Opportunistic–pathobiont: emphasizes necessity of microbiome disruption in virulence
- Pathogen: high virulence
Antibiotics disrupt your microbiome, wiping out both good and bad bacteria. Resistant bacteria—like MRSA, CRE, and *C. difficile*—can take advantage of this disruption and multiply.

A healthy microbiome helps protect you from infection. Improved antibiotic use and a healthy microbiome can keep us and our communities well.

With this overgrowth, your body is primed for infection. Once colonized, you can easily spread the resistant bacteria with others.

Resistant bacteria—like MRSA, CRE, and *C. difficile*—can take advantage of this disruption and multiply.
Colonization Resistance

Human microbiota

- From birth, environment and host factors impact a person’s microbiota
- Microbial flora acts as an ecosystem, with major and minor players
- To maintain stability
  - Species functional types
  - Redundancy
- Multifactorial
  - Age
  - Vaccination
  - Diet
  - Medications
  - Disease status (e.g., URI, diarrhea)
Long-Term Acute Care Hospital (LTACH)

- Specialty-care hospital
- Patients with complex medical problems
  - Often transferred from ICU
- More individualized, intense treatment
  - Respirator weaning
- Extended stay (20-30 days)
- Antibiotic use extremely high\(^1\)

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1. Gould et al., 2006. ICHE
Question: Can you pick out the two healthy donors?
Question: Can you pick out the two healthy donors?
Microbial Community Composition: Protective species

F. prausnitzi
Microbial Community Composition: Protective species

**Barnesiella**

[Bar chart showing relative abundance of various bacterial species in healthy donors and patients.]

- Others
- Proteobacteria:Enterobacteriaceae
- Proteobacteria:Pseudomonadaceae
- Firmicutes:Ruminococcaceae
- Firmicutes:Erysipelotrichaceae
- Firmicutes:Alcaligenaceae
- Firmicutes:Enterococcaceae
- Firmicutes:Lactobacillaceae
- Firmicutes:Lachnospiraceae
- Bacteroidetes:Rikenellaceae
- Bacteroidetes:Bacteroidaceae
Microbial Community Composition

- **Barnesiella**
- **E. faecium**

Graph showing relative abundance of various microbial communities in healthy donors and patients.
Discussion

- Early description of distal gut microbial communities
  - LTACH patients
- Correlation between antibiotics in the previous 30 days and diversity
  - Not perfect
- Other factors are not easily obtained in a clinical setting
  - Microbiome characterization/diagnosis
Antibiotic Resistance Threat Quantified by Microbiome Indices (MI)

- What is the usual MI seen with antimicrobial X?
- What is the MI permissive for colonization?
- What is the MI that promotes dominance?
- What is the cumulative MI that leads to transmission?

Normal microbiome: Resistant to colonization
Disrupted microbiome: Susceptible to colonization
Colonization MDRO colonizes the gut
Overgrowth & Dominance MDRO overgrows & dominates the gut

Infection & Transmission MDRO infection & potential for transmission
What is the natural history of the microbiome, antibiotic impact?
Example: Reducing Resistance in Hematopoietic Stem Cell Transplant Patients through FMT

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Negative rectal swab at 1 week</th>
<th>Decolonization at 1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Antibiotics (-)</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDM1+</td>
<td>8/14</td>
<td>57</td>
</tr>
<tr>
<td>ESBL+</td>
<td>2/3</td>
<td>67</td>
</tr>
<tr>
<td>Other, carbapenem-resistant</td>
<td>1/2</td>
<td>50</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESBL+</td>
<td>11/11</td>
<td>100</td>
</tr>
<tr>
<td>OXA-48+</td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBL+</td>
<td>2/2</td>
<td>100</td>
</tr>
<tr>
<td>Other, carbapenem-resistant</td>
<td>1/2</td>
<td>50</td>
</tr>
<tr>
<td>Carbapenem-resistant <em>Enterobacter cloacae</em></td>
<td>1/2</td>
<td>50</td>
</tr>
<tr>
<td>Vancomycin-resistant enterococci (VRE)</td>
<td>2/2</td>
<td>100</td>
</tr>
<tr>
<td><em>Acinetobacter ursingii MBL+</em></td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>1/1</td>
<td>100</td>
</tr>
</tbody>
</table>


Belinski et al. Clin Infect Dis 2017
Questions?

Email: ljm3@cdc.gov

For more information, contact CDC
1-800-CDC-INFO (232-4636)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.