Clinical and Public Health Microbiology: A Tsunami of Change

John Besser
CDC, Enteric Diseases Laboratory Branch
Clinical and Public Health Microbiology: A Tsunami of Change

- Clinical diagnostic trends
- Public health challenges
- Public health opportunities
Pasteur était un chimiste et biologiste français qui a prouvé la théorie des germes de la maladie et a inventé le procédé de pasteurisation. Louis Pasteur est né le 27 Décembre 1822 à Dole dans le Jura de la France. Son père était tanneur. En 1847, il a obtenu un doctorat de l’École Normale à Paris.
Rapid Tests and Single-analyte CID DTs
Connection between environmental and human isolates

Courtesy of Dr. Gayle Langley, CDC
Outbreaks

Legionnaires’ outbreak widens to 12 dead in New York

11 August 2015 | US & Canada

Legionnaires’ disease among residents of a long-term care facility: The sentinel event in a community outbreak

Outbreak of Legionnaires’ disease among cruise ship passengers exposed to a contaminated whirlpool spa

The Importance of Clinical Surveillance in Detecting Legionnaires’ Disease Outbreaks: A Large Outbreak in a Hospital With a Legionella Disinfection System—Pennsylvania, 2011–2012

Courtesy of Dr. Gayle Langley, CDC
STEC Incidence Rates by Various Case Definitions, 2013
Syndromic CIDT Panels: GI Disease

- BD Max
- Cepheid GeneXpert
- BioFire FilmArray
- Nanosphere's Verigene Enteric Pathogens (EP) Test
- GenMark Dx eSensor XT-8
- Applied Biocode
- Luminex xTag GPP
- Prodesse ProGastro™ SSCS
Syndromic CIDT Panels: Respiratory

- BD Max
- Cepheid GeneXpert
- BioFire FilmArray
- GenMark Dx eSensor XT-8
- Nanosphere's Verigene Respiratory panels (3)
- Luminex xTag GPP

Martha Iwamoto, MD¹, Jennifer Y. Huang, MPH¹, Alicia B. Cronquist, MPH², Carlota Medus, PhD³, Sharon Hurd, MPH⁴, Shelley Zansky, PhD⁵, John Dunn, DVM⁶, Amy M. Woron, PhD⁶, Nadine Oosmanally, MSPH⁷, Patricia M. Griffin, MD¹, John Besser, PhD¹, Olga L. Henao, PhD¹

(Author affiliations at end of text)

The increased availability and rapid adoption of culture-independent diagnostic tests (CIDTs) is moving clinical detection of bacterial enteric infections away from culture-based methods. These new tests do not yield isolates that are currently needed for further tests to distinguish among strains or subtypes of Salmonella, Campylobacter, Shiga toxin–producing improve patient care by allowing rapid diagnosis, improving sensitivity and simplicity, lowering costs, and by detection of a wider range of pathogens. However, current culture-independent diagnostic methods do not have subtyping ability that enables determination of antimicrobial resistance, detection of clusters of illness, and monitoring of trends. Currently, the

March 12, 2015
New Syndrome-based Culture-Independent Test Panels: Advantages

- Fast
- Efficient workflow
- Good performance
- Wider range of pathogens
Now you can test for 15 key bacteria, viruses, and parasites — all in under 5 hours.
Traditional Culture and Susceptibility Testing
Methods are Slow to Produce Results

Specimen Taken

Hours

24 48 72 96 (4 days!!!)

Culture
Prelim report
Susceptibility test
Susceptibility test (fastidious)
Mixed infections report to doctor

Medical Value

Slide courtesy of Dr. Fred Tenover
Molecular Methods Positively Impact Patient Outcomes

Specimen Taken

PCR testing

Early results optimize antibiotic therapy

PCR report

Medical Value

Final report to doctor for mixed infection

Susceptibility test (fastidious)

Susceptibility test

Culture

0 1-3

24 Hours

Slide courtesy of Dr. Fred Tenover
The “Cloud” of Stool Testing Methods

Send outs:
- Noro PCR
- O & P
- Bacterial isolates to State PHL

C. difficile toxin PCR

Giardia/Crypto EIA

Specimen arrives in Lab

Order entry and correction

Primary Set-Up
- Enteric culture inoculation
- Specimen aliquoting

Virology shell culture
- Rotavirus EIA
- DFA confirmatory Giardia/Crypto

Culture desk (Salmonella, Shigella, Campylobacter, STEC, Yersinia, Vibrio)

Adapted from K. Chapin, 2013
Syndrome-based Stool Testing Panels

Send outs:
- O & P
- Bacterial isolates to State PHL

Specimen arrives in Lab

Order entry and correction

PCR multi-agent panel(s)

Adapted from K. Chapin, 2013
Demise of GC Culture

- Fast, accurate
- Urine specimen, vs urethral swab
- Includes *Chlamydia trachomatis*
- No susceptibility data
- Specimen incompatible with culture
- Empiric treatment, reliance on guidelines
Direction of Clinical Diagnostic Testing for Infections of Public Health Importance

Federal laboratories ➔ State/local Public Health Laboratories ➔ Clinical Laboratories ➔ Point-of-care

Rare infections

- TB
- Diphtheria
- Rabies

Exotic infections

Common infections

Salmonella infection

- Symptoms: fever, abdominal pain, diarrhea, headache, nausea
- Treatment: antibiotics, supportive care

TB (tuberculosis)

- Symptoms: cough, weight loss, fatigue, night sweats
- Diagnosis: sputum smears, chest X-ray

Rabies

- Symptoms: fever, headache, muscle spasms
- Prevention: post-exposure prophylaxis

State/local Public Health Laboratories

Point-of-care

Clinical Laboratories
World's Most Portable Molecular Diagnostics System Unveiled at AACC

GeneXpert Omni to Further Decentralize Critical TB, Virology and Ebola Tests

SUNNYVALE, Calif. and GENEVA, July 28, 2015 /PRNewswire/ -- Cepheid (Nasdaq: CPHD) and FIND today unveiled the GeneXpert® Omni, the world's most portable molecular diagnostics system enabling unprecedented access to accurate, fast and potentially life-saving diagnosis for patients suspected of TB, HIV and Ebola in even the most remote areas of the world.
Direction of Clinical Diagnostic Testing for Infections of Public Health Importance

Federal laboratories

- Exotic infections

State/local Public Health Laboratories
- Home Testing
- Rare infections
  - TB
  - Diphtheria
  - Rabies

Clinical Laboratories

Point-of-care

Common infections

State/local Public Health Laboratories

Home Testing

- Rare infections

Point-of-care

- TB
- Diphtheria
- Rabies

Exotic infections
Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing


The field of diagnostic microbiology is undergoing its most radical transformation since Pasteur.
CIDT Challenges

- Patient care
- Accurate case counting
- Maintaining isolate-based surveillance
CIDT Challenges

- Patient care
  - Interpretation
  - High specificity*
- Accurate case counting
- Maintaining isolate-based surveillance

* detects only what you’re looking for
A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests

T Ripa (torvald.ripa@lthalland.se), P Nilsson

Department of Clinical Microbiology & Infection Control Halland

A new variant of *Chlamydia trachomatis* with a deletion was detected in Sweden, following an unexpected 25% decrease was noted between November 2005 and August 2006 in Halland. The number of patients tested during this period was similar in the corresponding period one year earlier: 9055 compared with 87
# Multicenter Campylobacter Test Evaluation

<table>
<thead>
<tr>
<th>CIDT Test</th>
<th>CIDT positive at clinic and cultured</th>
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<tbody>
<tr>
<td></td>
<td>Total (n)</td>
<td>% Culture positive</td>
<td>% Culture negative</td>
</tr>
<tr>
<td>Product A</td>
<td>238</td>
<td>34%</td>
<td>66%</td>
</tr>
<tr>
<td>Product B</td>
<td>18</td>
<td>89%</td>
<td>11%</td>
</tr>
<tr>
<td>Product C</td>
<td>1</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Unknown test type</td>
<td>369</td>
<td>38%</td>
<td>62%</td>
</tr>
<tr>
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CIDT Challenges

- Patient care
  - Interpretation
  - High specificity*
- Accurate case counting
- Maintaining isolate-based surveillance

* detects only what you’re looking for
Foodborne Illness Acquired in the United States—Major Pathogens
Shalini Sodhioka, Pratibha Shriramkumar, Frederick J. Angulo, Robert V. Tauxe, Marus Amin-Wilhemsen, Sharon J. Reay, Jeffrey R. Jones, and Patricia W. Goffin

Foodborne Illness Acquired in the United States—Unspecified Agents
Shalini Sodhioka, Pratibha Shriramkumar, Frederick J. Angulo, Robert V. Tauxe, and Robert M. Kolterman

How Safe Is Our Food?
J. Glenn Norris, Jr.

Incidence of STEC infections in FoodNet, 2008–2011

Hierarchical scheme for categorizing foods into commodities

Stability in diagnostic test performance
2.36. SALMONELLOSIS (Salmonella spp. other than Salmonella typhi and Salmonella paratyphi)

**Laboratory Criteria**

Isolation of *Salmonella* (other than *Salmonella typhi* and *Salmonella paratyphi*) from stool, urine, body site (e.g. infected wound) or any normally sterile body fluids and tissues (e.g. blood, CSF, bone, synovial fluid, etc.)

**Laboratory Criteria**

At least one of the following four:

- Isolation of an *Escherichia coli* strain that produces Shigatoxin (Stx) or harbours stx1 or stx2 gene(s)
- Isolation of non-sorbitol-fermenting (NSF) *Escherichia coli* O157 (without Stx or stx gene testing)
- Direct detection of *stx1* or *stx2* gene(s) nucleic acid (without strain isolation)
- Direct detection of free Stx in faeces (without strain isolation)
Public Health CIDT Challenges

- Patient care
- **Accurate case counting**
- **Maintaining isolate-based surveillance**
  - Strain tracking (including WGS)
  - Susceptibility surveillance
  - Virulence, vaccine efficacy

*Detects only what you’re looking for*
How Culture-Independent Diagnostics Threaten Public Surveillance

BY LYDIA ZURAW | NOVEMBER 22, 2014

Traditional methods for diagnosing foodborne illness infections such as Salmonella, Campylobacter and E. coli involve cultivating patient samples in an artificial nutrient medium. But tests that don’t require isolates from pure culture are becoming increasingly popular.

There are different kinds of culture-independent diagnostic tests (CIDTs), but they all take a broad look at the DNA in samples, screening for the general types of pathogens that are present. The type of CIDT public health folks think will really overtake culture tests are syndrome-based panels that can test for multiple agents at once. There are five such tests currently licensed for gastrointestinal illnesses, with more expected to follow in coming years.

These CIDTs are particularly attractive to clinicians because, in addition to testing for many different pathogens, they can be faster than traditional methods and can detect bugs that would otherwise be difficult to find. They also don’t need as much equipment or highly trained technicians, so they can save labs money.
CIDT A Non-Issue for “Event-Driven” Outbreaks

• Best way to discover new PATHOGENS
White City, Jackson Co, 1982
Nationwide reporting began in 1912
Reported *Salmonella* infections in the United States, 1920-2006

![Graph showing incidence of Typhoid Fever and Non-typhoid Salmonellosis from 1920 to 2000.](image)

- **Typhoid Fever**
- **Non-typhoid Salmonellosis**

**National salmonella serotype surveillance**

CDC, National surveillance data
Listeriosis Surveillance System

LM case

State/Local Health Agency

CDC

Interview Cases

Isolates

Case / Food questionnaire

Isolates

EMBL (Europe)

NCBI (GenBank)

DDBJ (Japan)

International Nucleotide Sequence Database Collaboration

GenomeTrakR

WGS

PFGE

WGS

PFGE

analysis upload

PulseNet

\textbullet\ Food / animal, environment sampling

FDA USDA

\textbullet\ WGS

\textbullet\ Case-Case Studies

\textbullet\ NCBI (GenBank)

\textbullet\ DDBJ (Japan)

\textbullet\ EMBL (Europe)
Next era?
Listeria Cluster Metrics Before and After WGS

- **No. of clusters detected**
  - Pre-WGS (Sept 2012–Aug 2013): 14
  - WGS Year 1 (Sept 2013–Aug 2014): 19
  - WGS Year 2 (Sept 2014–Aug 2015): 21

- **No. of clusters detected sooner or only by WGS**
  - Pre-WGS (Sept 2012–Aug 2013): N/A
  - WGS Year 1 (Sept 2013–Aug 2014): 6
  - WGS Year 2 (Sept 2014–Aug 2015): 6

- **No. of outbreaks solved (food source identified)**
  - Pre-WGS (Sept 2012–Aug 2013): 1
  - WGS Year 1 (Sept 2013–Aug 2014): 4
  - WGS Year 2 (Sept 2014–Aug 2015): 9

- **Median no. of cases per cluster**
  - Pre-WGS (Sept 2012–Aug 2013): 6
  - WGS Year 1 (Sept 2013–Aug 2014): 4
  - WGS Year 2 (Sept 2014–Aug 2015): 3

- **No. of cases linked to food source**
  - Pre-WGS (Sept 2012–Aug 2013): 6
  - WGS Year 1 (Sept 2013–Aug 2014): 16
  - WGS Year 2 (Sept 2014–Aug 2015): 93

Note that cluster 1508MLGX6-1WGS counted as solved with 24 cases.
Listeria and Caramel Apples

- 35 cases
- 12 states
- 34 hospitalizations
- 7 deaths
Blue Bell Recalls All Products After Listeria Outbreak

By AUSTIN RAMZY  APRIL 21, 2015

Blue Bell Creameries, which distributes frozen desserts to about half of the United States, said that it was voluntarily recalling all of its products after the bacteria listeria was found in two cartons of ice cream.

CDC using new technology to track listeria illnesses

WASHINGTON – The government is as a bit of luck - to track an outbreak of ice cream products.

Texas-based Blue Bell Creameries reported listeria was found in a variety of the company's frozen treats. The massive recall followed several smaller recalls as health officials across the country have rapidly worked to track the outbreak, which is so far linked to 11 listeria illnesses, including three deaths. The problems have raised questions about the future of the 108-year-old, family-owned creamery.

Genome mapping helps track Blue Bell recall

New sequencing tech shows listeria problems can be traced to 2010.

Brenham-based Blue Bell Creameries recalled all its products this week as listeria was found in a variety of the company’s frozen treats. The massive recall followed several smaller recalls as health officials across the country have rapidly worked to track the outbreak, which is so far linked to 11 listeria illnesses, including three deaths. The problems have raised questions about the future of the 108-year-old, family-owned creamery.
Public Health WGS Workflow

Nomenclature server
Calculation engine
Trimming, mapping, de novo assembly, SNP detection, allele detection

PH databases

Users at CDC and in the States

Sequencer
Raw sequences
External storage: NCBI, ENA, BaseSpace

Calculation engine
Trimming, mapping, de novo assembly, SNP detection, allele detection

LIMS

PH databases

Nomenclature server

Allele databases

Genus/species
Serotype
Pathotype
Resistance
7-gene MLST
rMLST
cMLST
wgMLST

Data pathway
Proposed data pathway
Analysis request

Users at CDC and in the States

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**Point-and-Click Epidemiology**

*case-case study still in development*
### Subtyping Methods: Isolate Dependency

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<th>Method</th>
<th>Isolates Required?</th>
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<tr>
<td>PFGE</td>
<td>Yes</td>
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<tr>
<td>MLVA</td>
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<tr>
<td>WGS</td>
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Nationwide reporting began in 1912
Reported Salmonella infections in the United States, 1920-2006

CDC, National surveillance data
It is ironic that new tests which are likely better for patient care could result in many thousands of additional cases per year if appropriate measures are not taken.
Clinical and Public Health Microbiology: A Tsunami of Change

- Clinical diagnostic trends
- Public health challenges
- Public health opportunities
Multi-analyte DNA-based Panels

- Polymicrobial infections
- New tracking possibilities

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Luminex xTag GPP panel
### Multi-analyte DNA-based Panels

- Multiple agents
- New tracking possibilities

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Luminex xTag GPP panel
Multi-analyte DNA-based Panels

- Multiple agents
- New tracking possibilities
- Increased case reporting?

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Luminex xTag GPP panel
Strategy to Address Loss of Isolates for Public Health Activities

1. Preserve cultures
   - Surveillance by current methods (serotyping, AST, PFGE, MLVA etc.)
   - Device industry consult
   - Regulatory / legal
   - Reimbursement
   - New isolate recovery methods
   - Sentinel surveillance

2. Prepare for the future working on pure cultures
   - Surveillance by whole genome sequencing (WGS)
     - Sequence-based infrastructure
     - Large genome databases

3. Metagenomics No cultures
   - Amplicon sequencing (short-term)
   - Shotgun metagenomics (longer-term)

Surveillance and diagnostics by metagenomics
Sexually Transmitted Diseases (STDs)

Gonococcal Isolate Surveillance Project

The Gonococcal Isolate Surveillance Project (GISP) was established in 1986 to monitor trends in antibiotic susceptibilities of strains of N. gonorrhoeae in the United States. GISP works to improve case detection, reporting, and antibiotic susceptibility data collection among state health departments, CDC, and other national surveillance programs. The project's primary aim is to monitor the spread of antibiotic-resistant gonorrhea and guide public health strategies to control gonorrhea.

Figure 6. Age distribution of GISP participants and nationally reported gonorrhea cases in men, 2006

View at CDC.gov/STD
TN Burden

- 6/15-9/15

Isolates 81%
Other 19%

EIA + broth 67%
Syndrome CIDT 21%
HD / o/b 12%

Courtesy of Dr. Amy Woron, TN Dept of Health
TN Burden

- **6/15-9/15**
  - Isolates 81%
  - Other 19%

- **7/1 – 1/31**
  - 70 from + EIA
  - 304 Syndrome CIDT
    - 293 Diatherix
    - 11 Luminex

Courtesy of Dr. Amy Woron, TN Dept of Health
Multi-State Isolate Recovery Project

Goal:

Provide data for isolate recovery recommendations (by APHL Food Safety CIDT Workgroup)
Projects:
- Media study
- Specimen study (to study process and biostability)
- Prospective study
Strategy to Address Loss of Isolates for Public Health Activities

1. **Preserve cultures**
   - Surveillance by current methods (serotyping, AST, PFGE, MLVA etc.)
   - Device industry consult
   - Regulatory / legal
   - Reimbursement
   - New isolate recovery methods
   - Sentinel surveillance

2. **Prepare for the future working on pure cultures**
   - Surveillance by whole genome sequencing (WGS)
   - Sequence-based infrastructure
   - Large genome databases

3. **Metagenomics (No cultures)**
   - Amplicon sequencing (short-term)
   - Shotgun metagenomics (longer-term)

Surveillance and diagnostics by metagenomics
CDC: Flu shot less effective this year because current virus has mutated

By Debra Goldschmidt and Jen Christensen, CNN

Updated 11:11 AM ET, Wed December 10, 2014

New tool could help prevent the flu 02:15
AMD and Influenza Surveillance

Expansion of 3C.2a in US and Bangladesh

Global expansion of 3C.3a

Tree Courtesy of U of Cambridge, UK

Adapted from slide provided by John Barnes, CDC
Starting Material

Sputum  Dx Culture  Subculture

From Dr. James Posey, ICEID 2015
Patient Eats Contaminated Food

Stool Sample Collected

Public Health Laboratory Receives Sample

1 - 3 days

Contact with health care system: 1 - 5 days

Patient Becomes Ill

Diagnosis: 1 - 3 days

Shipping: 0 - 7 days

Sero typing & DNA fingerprinting: 2 - 10 days

Salmonella Identified

Case Confirmed as Part of Outbreak

Foodborne Bacterial Outbreak Detection: Potential Improvements in Timeline
Human Feces

- Microbial genomes
  - Bacteria
  - Viruses
  - Parasites
  - Fungi
- Other genomes
  - Human
  - Food Animals
  - Plants

Science. 336:8 1246-1247
Public Health “Metagenomics”: From Specimen To Answer

- Amplicon sequencing
- “Shotgun” metagenomics
- Single-cell sorting and sequencing

$ MB $ $ $$$

TB
CaliciNet: Culture-Independent Since 1968

CaliciNet

National Norovirus Outbreak Network
Amplicon Sequencing

<table>
<thead>
<tr>
<th>Pathogen Marker</th>
<th>Virulence Determinants</th>
<th>AR Markers</th>
<th>STRAIN TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bifidobacterium spp.</td>
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<td></td>
<td></td>
<td></td>
<td>Bacteroides spp.</td>
</tr>
<tr>
<td>□</td>
<td>■</td>
<td>■</td>
<td>STEC (STRAIN 1)</td>
</tr>
<tr>
<td>□</td>
<td>■</td>
<td>■</td>
<td>STEC (STRAIN 2)</td>
</tr>
<tr>
<td>□</td>
<td>■</td>
<td>■</td>
<td>Non-pathogenic E. coli</td>
</tr>
<tr>
<td>□</td>
<td>■</td>
<td>■</td>
<td>Enterobacter spp.</td>
</tr>
</tbody>
</table>
Amplicon Sequencing
O157:H7 Intact Shiga-toxin Converting Phages

Phages are genetically similar overall with some conserved flanking regions.

Lori Gladney

Stx1 Phages w/Stx1A,1B

Stx2 Phages w/Stx2A,2B
Core gene alignments (no gaps).

Choose a pseudorandom subset (40).

Create uncorrected pairwise distance matrix.
  # diffs / alignment length

Cluster by furthest neighbor (complete linkage).

Repeat (100x)

Compare cluster assignments to all core gene classification via Adjusted Wallace Coefficient.

The Wallace coefficient quantifies the likelihood that an isolate appears in the same cluster by two different methods.

Provides directional information (i.e. can detect when one method is more discriminatory than the other).

Adjusted version takes into account that the agreement between two methods could be due to chance alone.

From Dr. Jo Williams
- Subset of 40 core genes with perfect bidirectional cluster agreement
- Alignment length 35,785 bp (end gaps stripped)
- Furthest neighbor (complete linkage) clustering
  - 33 clusters at uncorrected distance of 0.001 (36 SNPs)
  - Clusters of > 1 genome highlighted by line at bottom
- Bootstrap support from 100 replicates

**Note:**
- Relative relationships of clusters differ
- Bootstrap values generally lower
Amplicon Sequencing: Phasing Solutions

- Identify pathogen-specific PCR targets. Sources of variation:
  - Flanked by heterogeneous regions
  - Presence/absence

- Quantitative marker linkage

- Sequence non-specific markers (e.g. 16S, 18S…amplified metagenomics)
Public Health Metagenomics: From Specimen To Answer

- Amplicon sequencing
- “Shotgun” metagenomics
- Single-cell sorting and sequencing
Metagenomics Enteric Public Health Applications

- In situ pathogen characterization
- Pathogen discovery
- Food identification (in and outside the body)
- Root cause analysis
- Population biology (e.g. dysbiosis)
- Host factors
**In-situ** Pathogen Characterization

A Culture-Independent Sequence-Based Metagenomics Approach to the Investigation of an Outbreak of Shiga-Toxigenic *Escherichia coli* O104:H4

Nicholas J. Loman, MBBS, PhD  
Chrystala Constantinidou, PhD  
Martin Christner, MD  
Holger Rohde, MD  
Jacqueline Z.-M. Chan, PhD  
Joshua Quick, BSc  
Jacqueline C. Weir, MSc  
Christopher Quince, PhD  
Geoffrey P. Smith, PhD  
Jason R. Betley, PhD  
Martin Aenfelbacher, MD

**Importance** Identification of the bacterium responsible for an outbreak can aid in disease management. However, traditional culture-based diagnosis can be difficult, particularly if no specific diagnostic test is available for an outbreak strain.

**Objective** To explore the potential of metagenomics, which is the direct sequencing of DNA extracted from microbiologically complex samples, as an open-ended clinical discovery platform capable of identifying and characterizing bacterial strains from an outbreak without laboratory culture.

**Design, Setting, and Patients** In a retrospective investigation, 45 samples were selected from fecal specimens obtained from patients with diarrhea during the 2011 outbreak of Shiga-toxigenic *Escherichia coli* (STEC) O104:H4 in Germany. Samples were subjected to high-throughput sequencing (August-September 2012), followed by a 3-phase analysis (November 2012-February 2013). In phase 1, a de novo assembly approach was developed to obtain a draft genome of the outbreak strain. In phase 2, the depth of coverage of the outbreak strain genome was determined in each sample.
Shotgun Metagenomics

SIGNAL TO NOISE

PHASING
Human Feces

- Microbial genomes
  - Bacteria
  - Viruses
  - Parasites
  - Fungi

- Other genomes
  - Human
  - Food Animals
  - Plants

Science. 336:8 1246-1247
Metagenomics: Signal-to-Noise
(just considering bacteria)

8x10^9 reads (~1Tbase)

1x10^{11} organisms/mL

For a positive stool specimen containing 1x10^5 CFU/mL STEC, you might expect (at best) <0.15X genome coverage from a full HiSeq* run.

*For reference purposes only; does not imply endorsement.

“Clutter Mitigation” Strategies

NUCLEIC ACID EXTRACTION

DNA RNA TNA

POST-EXTRACTION AND LIBRARY CONSTRUCTION

LIBRARY

SEQUENCING

Differential cell lysis
Filtering, Concentration
Separation/Pulldown
Direct amplification
Laser capture, microfluidics

Nucleases (RNAse/DNAse)
cDNA conversion
rRNA depletion
Bind/degrade CpG methylated DNA
Preferential separation (mass, seq, chem)
Genome/transcriptome amplification
Sequencing platform selection
Library method and parameters
Size selection

New platforms/approaches
Multiplexing and pooling
Bioinformatic strategies

Many variables impact sequence yield, quality and bias.
New Long-read Sequencing Technology
“Hi-C” Crosslinking Technology

Figure 1. Overview of Hi-C technology
A) Hi-C detects chromatin interaction both within and between chromosomes by covalently crosslinking protein/DNA complexes with formaldehyde. B) The chromatin is digested with...
<table>
<thead>
<tr>
<th>Factor</th>
<th>Likelihood of being resolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>High</td>
</tr>
<tr>
<td>Sequencing read length (and error rate)</td>
<td>High</td>
</tr>
<tr>
<td>Metagenomic-specific software, pipelines</td>
<td>High</td>
</tr>
<tr>
<td>Computing processing power, bandwidth</td>
<td>High</td>
</tr>
<tr>
<td>Signal to noise</td>
<td>High</td>
</tr>
</tbody>
</table>
Salmonella Heidelberg Outbreaks, 2013

- Very short incubation
- High morbidity/mortality
Questions

- Can we detect Salmonella signal in a metagenomic sample?
- Can we distinguish the two outbreaks using metagenomic shotgun sequencing?
- Is another unrecognized pathogen involved?
Phylogeny of isolates and metagenomes

(Across 2.1 M sites out of 4.4 M)
Dear Whoever you are—
I can’t believe what I just had to do and I damn sure can’t believe what you have to do—I thought I was dedicated to my job but this is something else—I really feel for you—
The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.