Whole Genome Sequencing in Foodborne Outbreak Detection:
How Food Safety Gets Better, But Companies Can Feel Worse

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CDC provides the vital link between illness in people & the food safety systems of government agencies & food producers.
More Disclaimers / Disclosures

1. Thank you for invitation & lunch

2. Speaker reserves the right to say something stupid, wrong or incredibly obvious.

3. Findings & conclusions in this presentation are those of the author & do not represent the views of the Centers for Disease Control & Prevention
How Food Safety Gets Better…

- ↓↓↓ Mortality since 1900
- Foodborne diseases are still very common
- Since 1990s, ↓ Campylobacter, Listeria, E coli O157 & Yersinia infections
- Little change in Salmonella. Vibrio increasing
- ↓↓ Hepatitis A is disappearing since 1950
- Norovirus *in vitro* culture developed
...But Companies Can Feel Worse

- Emerging Diseases (e.g., food allergy, E coli O157, cyclosporiasis)
- Genomics (e.g., DNA sequencing) & Information Technology:
  
  Accelerating pace of change

- The world is changing both public & private sectors.

We need to talk more going forward!
Accelerating pace of change...

- 1854 Era of Classical Epidemiology & Microbiology
  1920’s serotyping, 1940’s phage typing


- 2014 PulseNet Genome Sequencing Era
Trends of “Fingerprinting” for Bacteria

- Serotyping
- Phage typing
- Bacteriocin typing
- Ribotyping
- Plasmid profiles
- PFGE (1984)
- MLST
- MLVA
- AFLP
- RAPD
- MBMS*
- WGS**

* Microarray-based multi-target sequencing
** Whole Genome Sequencing

Source: Efrain M. Ribot, CDC
Listeria Outbreaks & Incidence, 1983-2013

<table>
<thead>
<tr>
<th>Era</th>
<th>Outbreaks per year</th>
<th>Median cases per outbreak</th>
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No. outbreaks

Incidence (per million pop)
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**Listeria Outbreaks & Incidence, 1983-2014**

- **Incidence (per million pop)**
- **No. outbreaks**

- **Outbreak**
- **Incidence**
Listeriosis Outbreaks and Incidence*, 1983-2016

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<td>69</td>
<td>11</td>
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*2016 incidence rate preliminary data from FoodNet
WGS detects more outbreaks than by PFGE

Number of Salmonella outbreaks detected with laboratory data
Public Health Agency of Canada

Year
0 20 40 60 80 100 120
2012 2013 2014 2015 2016 2017
Enteritidis
Heidelberg
Typhimurium

WGS

Courtesy C. Nadon, Public Health Agency of Canada
How Food Safety Gets Better...

Infectious Disease Mortality, United States, 1900-1996

Armstrong et al, JAMA 1999
46% of deaths in 1910 were from infectious diseases, compared to 3% in 2010.
How Food Safety Gets Better…

The fall of *Salmonella* Typhi, USA, 1920-2010

Preliminary Data : National Notifiable Diseases & FoodNet
Incidence of Hepatitis A, United States, 1952-2015

2016 data: 2,007 reported cases
as of Dec 15, 2018, reported cases = 10,582

Source: National Notifiable Disease Surveillance System, CDC
Crude Pathogen rates per 100,000
FoodNet 1996-2017

Since 1996-1998

↓ E. coli O157
↓ Campylobacter
↓ Listeria
↓ Yersinia

± Salmonella

↑ Vibrio

http://www.cdc.gov/foodnet/index.html
Since 1996-1998

↓ *E. coli* O157
↓ *Listeria*
↓ *Yersinia*
↑ *Vibrio*

Crude Pathogen rates per 100,000
FoodNet 1996-2016

HACCP Rule, 1996

E. coli

Yersinia

Listeria

Vibrio

Cyclospora

http://www.cdc.gov/foodnet/index.html
Foodborne Disease: 1900-39

- Amoebiasis
- Botulism
- Brucellosis
- Cholera
- Hepatitis
- Salmonellosis
- Scarlet fever (streptococcus)
- Septic sore throat (Strep zooepidemicus)
- Staphylococcal food poisoning
- Tapeworms
- Trichinosis
- Tuberculosis, bovine
- Typhoid fever
“Prediction is very difficult, esp. about the future.”
According to Yogi Berra, or Niels Bohr, or Albert Einstein, or Mark Twain, or Somebody

*It is “time to close the book” on the problem of infectious diseases.* (1969)
Jesse Steinfeld, MD, U.S. Surgeon General, 1969-73

“The future of infectious diseases will be very dull.” (1972)
Macfarlane Burnet, 1960 Nobel Prize Winner In Physiology/Medicine

*Told students that there were “no new diseases to be discovered.”* (1976)
Lewis Thomas, Dean Yale Medical School
Microbes appear 3.5 Billion years ago
Man appears ~130,000 years ago in Africa

https://biomimicry.net/earths-calendar-year-4-5-billion-years-compressed-into-12-months/
...But Companies Can Feel Worse

The rise of reported other *Salmonella* infections, USA, 1920-2010

![Graph showing the incidence per 100,000 population of non-typhoid salmonellosis from 1920 to 2010.](Preliminary Data: National Notifiable Diseases & FoodNet)
Foodborne Disease, 21st Century

1. Norovirus (1972)
2. Salmonella
3. Clostridium perfringens
4. Campylobacter (1972)
5. Staphylococcal
6. Shigella
7. E Coli O157 etc (1980s)
8. Yersinia enterocolitica (1976)

Listeria (1982)
Cyclospora (1996)
vCJD/BSE (1996)

* Scallan et al, 2011
Emerging Peanut Allergy


Quartz | qz.com
Data: Journal of Allergy and Clinical Immunology

J Allergy Clin Immunol 2010;125:1322-6
Why are food allergies increasing?

- Genetic
- Exposure in Utero or during lactation
- Medications (antibiotic, antacids)
- Microbiome (Gut microflora)/Hygiene Hypothesis
- Processed Foods. GMO
- Cross-reactive antigens
- Non-oral exposure
- Timing of food introduction to infants

American Academy of Pediatrics (AAP)

• 2000, AAP recommends delay:
  – cow's milk until age 1 year;
  – egg until age 2 years; &
  – peanuts, tree nuts & fish until age 3 years.

• 2008, AAP withdraws; no evidence for 2000 recommendations

• 2015, AAP recommends:
  – highest risk infants introduced to peanut as early as 4-6 mos of age
  – infants not at high risk, suggest that peanut be introduced “freely” into the diet

Era of Classical Epidemiology & Microbiology

• How do we know it’s food? Outbreak investigation

• “Church Picnic” or “sore thumb”

Large number of cases in one jurisdiction
  – Detected by affected group
  – Local investigation
  – Local food handling error (s)
  – Local solution
January 12
A pediatric gastroenterologist notified the Washington State Dept of Health of increase in emergency dept visits for bloody diarrhea & the hospitalization of 3 children with hemolytic uremic syndrome.
E. Coli 0157:H7 WA State 1993 by date of exposure*

*cases who ate a JIB hamburger on a single day

US and primary culture-confirmed cases = 333

T J Barrett, CDC retired

Improved cooking temps

Public alert

First report

January

Number
PulseNet Era: circa 1996 - present

Pulsed-field gel electrophoresis (PFGE) makes “invisible” outbreaks visible
PulseNet Era: circa 1996 - present

Pulsed-field gel electrophoresis (PFGE) makes “invisible” outbreaks visible.

- Small numbers of cases in many jurisdictions
- Detected by lab-based subtype surveillance
- Multistate / Country Multi-disciplinary investigation
- More challenging to investigate
- Higher stakes?
- Identifying “new” foods/ingredients
Shiga-toxin producing *E. Coli* in 12 states

- In February, 14 lab isolates with uncommon PFGE
  - 12 states
  - Age range = 13 - 70 years, median = 20 y.o.
  - 79% female ♀ ♂
  - 1 beef isolate in PulseNet library in 2015
    - broccoli & romaine lettuce from interviews

- Asked the states to re-interview
“Focus group” Interviews: Flour Hypothesis

- 10 /10 reported they or household member baked
- 8 /10 specifically remembered baking something homemade in week before illness began (5 definite, 3 maybe)
- Of the 5 who definitely baked:
  - 4 / 5 ate or tasted the raw dough or batter
  - 3 / 4 used Brand A flour; the 4th used either Brand A or another brand
  - 2 still had the bags of Brand A flour used before illness
    - Both bags produced in same plant within one day
    - Both people reported eating raw cookie dough
## Selected Exposures

**E coli Cases vs Patients with other Illness.**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Cases</th>
<th>Other Patients</th>
<th>odds ratio (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Used <em>Brand A</em> flour</td>
<td>19/30 (63)</td>
<td>7/78 (9)</td>
<td>6.87 (1.23–38.35)</td>
</tr>
<tr>
<td>Ate any peanut butter</td>
<td>23/29 (79)</td>
<td>35/77 (45)</td>
<td>0.83 (0.14–5.11)</td>
</tr>
</tbody>
</table>

Included questions about exposure to other flour:
- Brand *B*
- Brand *C*
- Brand *D*
- Brand *E*
- Brand *F*
FDA Traceback Data

- Detailed product information from 3 ill people with leftover packages of *Brand A* flour
- Records collected from restaurants linked to ill people
  - In early May 2016, identified 3 young children exposed to raw dough at restaurants in different states
  - All played with the raw dough & some ate it
- *E. coli* from ill people closely related genetically to outbreak strain
- Identified that flour was produced in the same week in November 2015 at the *Company A facility in Kansas City*, Mo
On May 31, following a conference call among FDA & CDC, Company A conducted a voluntary recall of flour.

June 10, FDA isolated *E coli* from leftover flour samples from Arizona, Colorado, & Oklahoma.

- Flour isolates closely related genetically by WGS to clinical isolates.

In July, FDA conducted WGS on an different strain of *E coli* isolate provided by Company A.

- Flour isolate closely related genetically to a patient’s isolate.
- This ill person subsequently included in the investigation.
People infected with the outbreak strains of *E. coli* O121 or *E. coli* O26, by state of residence, Dec 2015 - Sep 28, 2016 (n=63)
Genome Sequencing Era

In 1995, the Hubble Space Telescope found distant galaxies & star clusters never seen before.
DNA extraction
DNA shearing
Library prep
Sequencing
Whole Genome Sequencing

1 BACTERIAL GENOME ~3M “LETTERs” (base pairs)

Reading whole genome 1 letter pair per second = ~35 days

For NGS (next generation sequencing) we start with a whole genome book, shred it into thousands to millions of pieces (sequence reads), & then try to tape it back together using computer algorithms.
Building “Family Tree” – more than one way to draw a tree

- Many different ways to display trees
- Branches that connect to the terminal node are the important branch lengths to indicate relatedness
Seeing things we haven’t seen before

- “New” food/vehicles
- Recall trigger outbreak investigation
  - Instead of vice versa
- “Old foods” in challenging communication scenarios
- Long-tail, “never-ending” outbreak
  - “Outbreaks” are a “continuous variable”
- Definition of an outbreak changing?
  "Future foodborne outbreaks more likely to be:
  More dispersed & smaller: ‘low & slow’ "
### 35 new vehicles identified in multistate outbreaks 2006 – April 2018

1. Bagged spinach  
2. Carrot juice  
3. Peanut butter  
4. Broccoli powder on a snack food  
5. Dog food  
6. **Pot pies/frozen meals**  
7. Canned hot dog chili sauce  
8. Fresh hot chili peppers  
9. Black pepper  
10. Tahini sesame paste  
11. **Raw cookie dough**  
12. Aquatic water frogs  
13. Fresh papaya  
14. Frozen mamay fruit pulp  
15. Bologna  
16. In-shell hazelnuts  
17. Pine nuts  
18. Par-cooked, broiled chicken livers  
19. Scraped tuna  
20. Cashew cheese  
21. Bearded dragons  
22. Sugar cane juice  
23. Sprouted chia seeds  
24. Almond butter  
25. Caramel apples  
26. Sprouted nut butters  
27. Dried mushrooms (in truffle oil puree)  
28. Crested geckos  
29. Pistachios  
30. **Wheat flour**  
31. Powdered meal supplements  
32. Soy nut butter  
33. Frozen packaged vegetables  
34. Fresh Frozen & Dried Coconut  
35. Kratom powder
Washington cases, Jack in the Box outbreak, 1992–1993

- 639 ill
- 172 hospitalized
- 45 with HUS
- 3 died
- Recall prevented an estimated 800 primary cases
People infected with the outbreak strains of *E. coli* O121 or *E. coli* O26, by date of illness onset, Dec 21, 2015 - Sep 5, 2016 (n=56)

Multistate Outbreak of Listeriosis Linked to Blue Bell Creameries Products

Month of Illness Onset
Outbreak of Listeriosis Linked to Recalled Stone Fruit

- July 2014 recall receives extensive media coverage
- Many inquiries to CDC FDA & health depts from concerned clinicians & public
- Many of whom had received automated telephone calls informing them that they had purchased recalled fruit.
- During July 19–31, the CDC Listeria website received >500,000 page views
- Stone fruit isolates obtained from company
  - 4 human isolates in 2014 with PFGE match
  - Patient 1 ate recalled nectarines & peaches
  - Patient 2 ate peaches, possibly recalled ones
  - Patient 3 did not eat recalled fruits
  - Patient 4; no exposure information available
Stone Fruit WGS: Epi-Lab Concordance

- #1 & #2 patient isolates highly-related to stone fruit isolates
  - MA patient & MN patient ate nectarines & peaches

- #3 & #4 patient isolates not highly-related to stone fruit isolates
  - SC patient did not eat recalled fruits & IL patient had no exposure information available
Salmonella Enteritidis (SE) & eggs from a small farm – Tennessee, 2016

Regulatory action for SE in eggs is focused on farms with \( \geq 3000 \) hens

- DOH began sequencing SE prospectively in 2016,

**1st outbreak**
- 6 cases from Restaurant A: Cohort study - Steak with Béarnaise sauce (made with eggs)
- Eggs from “small” local Farm X (<3000 hens)
- Environment cultures on Farm X negative for SE

A month later, **2nd outbreak** (9 cases);
- mayo with raw eggs at Restaurant B;
  - WGS, within 3 SNPs of first outbreak
  - Eggs also from Farm X
  - Reinvestigation of Farm X: Found SE in chicken litter
  - Restaurant B changed egg suppliers
- All receiving eggs educated not to use them raw

Salmonella Enteritidis (SE)
& frozen stuffed breaded raw chicken products, Minnesota, 2015

Minnesota DOH began sequencing SE

**Cluster #1**: 5 illnesses

- Ate *Brand X* of frozen stuffed breaded raw chicken entrée
- Same WGS strain found in product
- Product distributed to many states
- 2.4 M pounds recalled

**Cluster #2**: 15 illnesses (including 7 in other states)

- Ate *Brand Y* frozen stuffed breaded chicken products
- Same WGS strain found in frozen product
- Product distributed to many states
- 1.7 M pounds recalled

- Most patients knew product was raw, & followed cooking instructions
- Some even checked the internal temperature
- USDA now considering further standards for products like this

[www.cdc.gov/salmonella/outbreaks/](http://www.cdc.gov/salmonella/outbreaks/)
Change #2: The Look – Investigation Notices

- When will this be used?
  - Investigations lacking specific advice to consumers, but there is still a need to communicate
  - May be used for enteric zoonotic outbreaks
  - Likely to be used less frequently than Food Safety Alerts, but gives us flexibility
Change #2: The Look – Food Safety Alerts

- **When will this be used?**
  - Investigations with specific advice to consumers (often a product recall)

- **Main differences from Investigation Notices:**
  - Use of the orange alert symbol
  - Advice is the first section at the top of the page
% of illnesses due to **recognized outbreaks**, FoodNet, 1996-2017

- **Campylobacter**: <1%
- **Salmonella**: 6%
- **STEC (e.g., E. coli O157)**: 11%

Reference: [https://wwwn.cdc.gov/foodnetfast/](https://wwwn.cdc.gov/foodnetfast/)
Thank you

Food Microbiology

“...Fasten your seatbelts, its going to be a bumpy night!”

- Margo Channing (Bette Davis) *All About Eve*, 1950
Factors influencing development of Infectious Diseases

Environment

Agent

- longevity & infectivity outside host
- host distribution, abundance, infection
- e.g. cholera, hantaviral disease, hookworm, schistosomiasis

Host

- tissue tropisms, pathogenicity, immune response, host specificity
- e.g. rabies, Lyme disease, malaria, cryptosporidiosis

- nutrition
- hygiene
- treatment
- housing
- e.g. TB, HIV/AIDS, diarrheal diseases, acute respiratory infections
Other concerns with WGS

• WGS turnaround time issues
  • Still long (~ 7 work days)

• Cost

• Cluster triage
  • Not resources to investigate all outbreaks
  • Which should be investigated?

• Culture-independent diagnostic testing (CIDT)
  • We are losing the isolates!
The stool microbiome

- **Microbes**
  - Bacteria
  - Viruses
  - Parasites
  - Fungi
- **Other cells**
  - Human
  - Food Animals
  - Plants

Science. 336:8 1246-1247