



From the lab bench to the Internet: Experience with PulseNet

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What is PulseNet?

- PulseNet is the National molecular subtyping network for foodborne disease surveillance established by the Centers for Disease Control and Prevention in 1996
- Pulsed-field Gel Electrophoresis (PFGE) is used to subtype pathogens
- PFGE images normalized using customized software
- PFGE patterns are electronically submitted to National database located at CDC in Atlanta, GA



DEPARTMENT OF HEALTH & HUMAN SERVICES



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Laboratory Investigation of a Multistate Food-Borne Outbreak of *Escherichia coli* O157:H7 by Using Pulsed-Field Gel Electrophoresis and Phage Typing

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Two hundred thirty-three isolates of *Escherichia coli* O157:H7 were analyzed by both pulsed-field gel electrophoresis (PFGE) and bacteriophage typing. All 26 isolates from persons whose illness was associated with a recent multistate outbreak of *E. coli* O157:H7 infections linked to the consumption of undercooked hamburgers and all 27 isolates from incriminated lots of hamburger meat had the same phage type and the same PFGE pattern. Twenty-five of 74 *E. coli* O157:H7 isolates from Washington State and 10 of 27 isolates from other states obtained during the 6 months before the outbreak had the same phage type as the outbreak strain, but only 1 isolate had the same PFGE pattern. PFGE thus appeared to be a more sensitive method than bacteriophage typing for distinguishing outbreak and non-outbreak-related strains. The PFGE patterns of seven preoutbreak sporadic isolates and five sporadic isolates from the outbreak period differed from that of the outbreak strain by a single band, making it difficult to identify these isolates as outbreak or non-outbreak related. Phage typing and PFGE with additional enzymes were helpful in resolving this problem. While not as sensitive as PFGE, phage typing was helpful in interpreting PFGE data and could have been used as a simple, rapid screen to eliminate the need for performing PFGE on unrelated isolates.

The genesis of PulseNet

- In 1996, 4 public health laboratories in 4 States and an USDA laboratory submitted *E. coli* O157:H7 PFGE patterns to CDC
- In 1997, WASPHL developed 1-day PFGE protocol
- In 1997-98, PulseNet 1-day standardized PFGE protocol
 - Harmonization of CDC and WASPHL

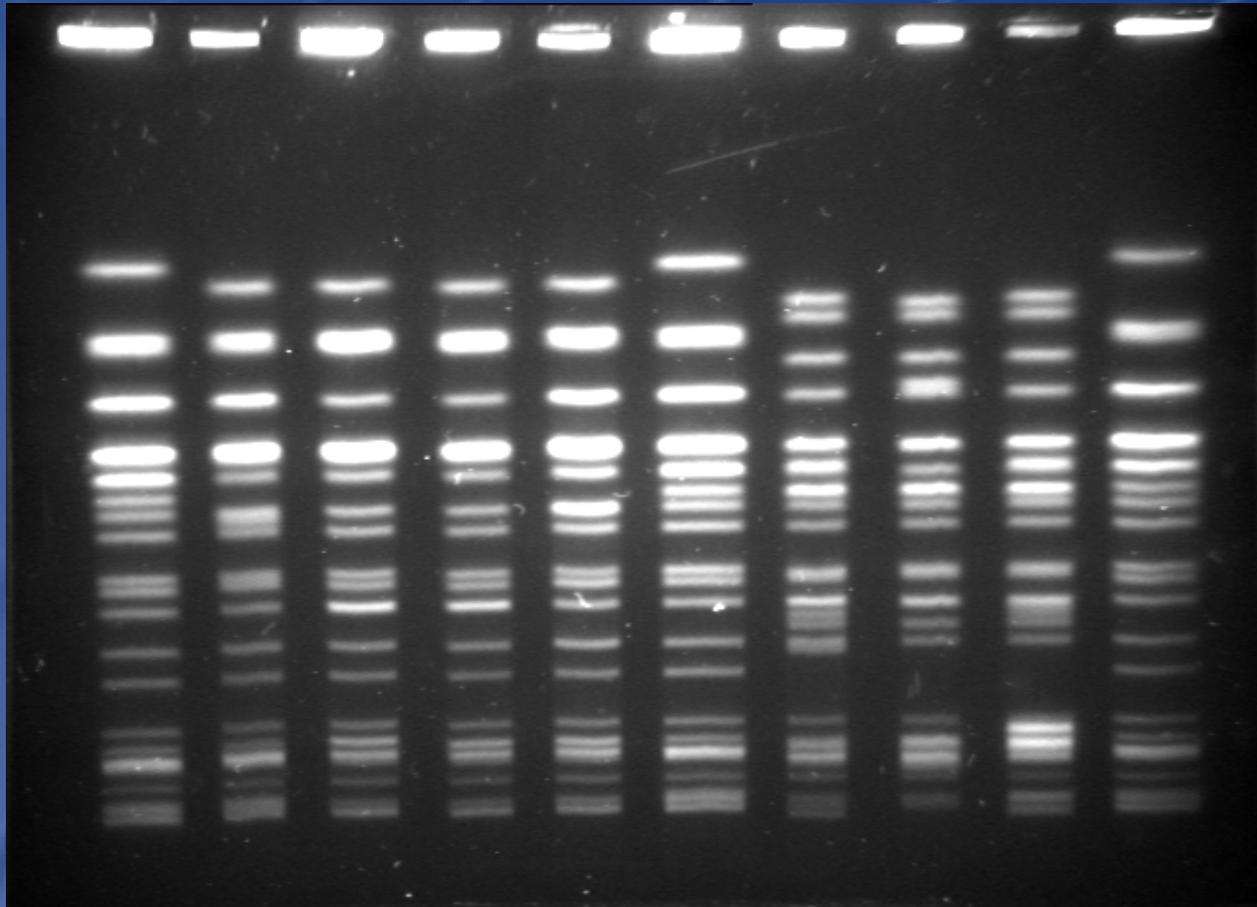
Components of PulseNet

- Laboratory
- Data analysis
- Communication network

Laboratory

- Standardization of rapid one-day (24 hr) PFGE protocol
 - *E. coli* O157:H7, *Salmonella*, *Shigella*, *Listeria monocytogenes*
- Development of new protocols
 - *Campylobacter*, *Clostridium perfringens*, and *Vibrio cholerae*
- Certification and proficiency testing
- Training workshops

E. coli O157:H7 PFGE gel



Data analysis

- PFGE images analyzed using Molecular Analyst Fingerprinting Plus (Bio-Rad) software
 - Gels normalized using same molecular size standard
- Training workshops
- Certification and proficiency testing
- In 2001, the PulseNet *E. coli* database was converted to a new on-line database system
 - Public health laboratories submit PFGE patterns via the internet
 - *Listeria monocytogenes* and *Salmonella* databases will be on-line by 2002

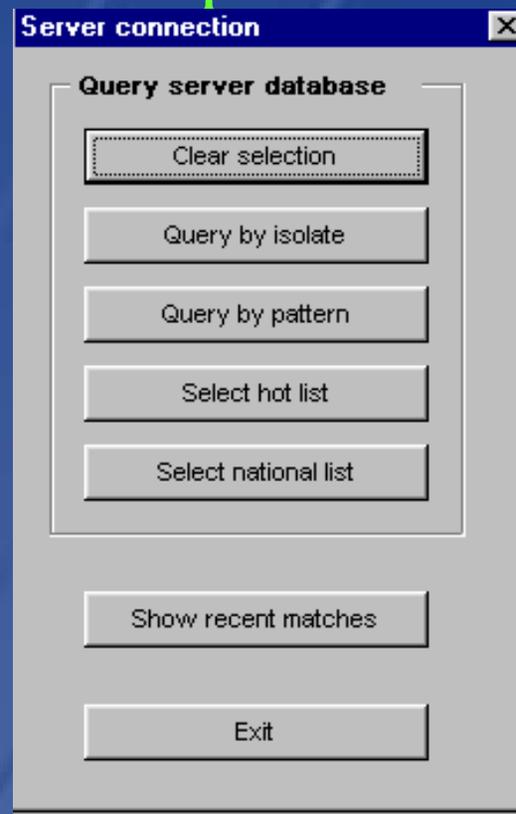
BioNumerics Server



- Upload & download of information
- Internet based

PulseNet “Client” BioNumerics software

- On-line system allows public health labs to actively submit and view PFGE patterns from the National database.



Information fields

Entry properties [X]

Source

City: []

County: []

State: GA []

Country: USA []

Outbreak: []

Source Type: Human []

Source Site: Unknown []

Toxin: []

Phage Type: []

SeroType: E. Coli O157:H7 []

Isolate status: []

NARMS-EB

FoodNet isolate

Patterns

Xbal	Elnl
Kk100298,2	Kk100298,7

Isolat. Date: [] Thursday, January 10, 2002 []

Receiv. Date: [] Thursday, January 10, 2002 []

Upload date:

Antibiotics... []

Submit... []

OK []

Cancel []

Additional lab data

BioNumerics

File Edit Database Experiments Comparison Identification Scripts

Key	LabID	SourceCountry	SourceState	S	1	2	3	4
1	01P0151	USA	RI	Pi	●	-	-	-
2	2001-G0001116	USA	OR	U	●	-	-	-
3	2001G-0001096	USA	OR	U	●	-	-	-
4	2001G-0001130	USA	OR	U	●	-	-	-
5	2001G-0001131	USA	OR	U	●	-	-	-
6	2001G-0001140	USA	OR	U	●	-	-	-
7	5733	USA	GA		●	●	-	-
8	6039	USA	TX		●	●	-	-
9	6039/#1	USA	TX		-	●	-	-
10	6141	USA	GA		●	●	-	-
11	6142	USA	GA		●	●	-	-
12	6153	USA	TN		-	●	-	-
13	6154	USA	TX		-	●	-	-
14	6157	USA	TX		-	●	-	-
15	f5716	USA	KY		●	-	-	-
16	F5717	USA	FL		●	-	-	-
17	F5730	USA	GA		●	-	-	-
18	F5731	USA	GA		●	-	-	-
19	F5733	USA	GA		●	-	-	-
20	F5739	USA	GA		●	-	-	-
21	F5816	USA	IL		●	-	-	-
22	STANDARD-ecoli				●	●	●	-

Experiments

Fingerprint types

- 1 ↓ PFGE-Xbal
- 2 ↓ PFGE-BlnI
- 3 ↓ PFGE-Spel

Character types

- 4 antibio

Files

- ↓ STD-Ecoli-Xbal
- Kk100298
- RI01057
- OR00111
- mf010926,Scott

Comparisons

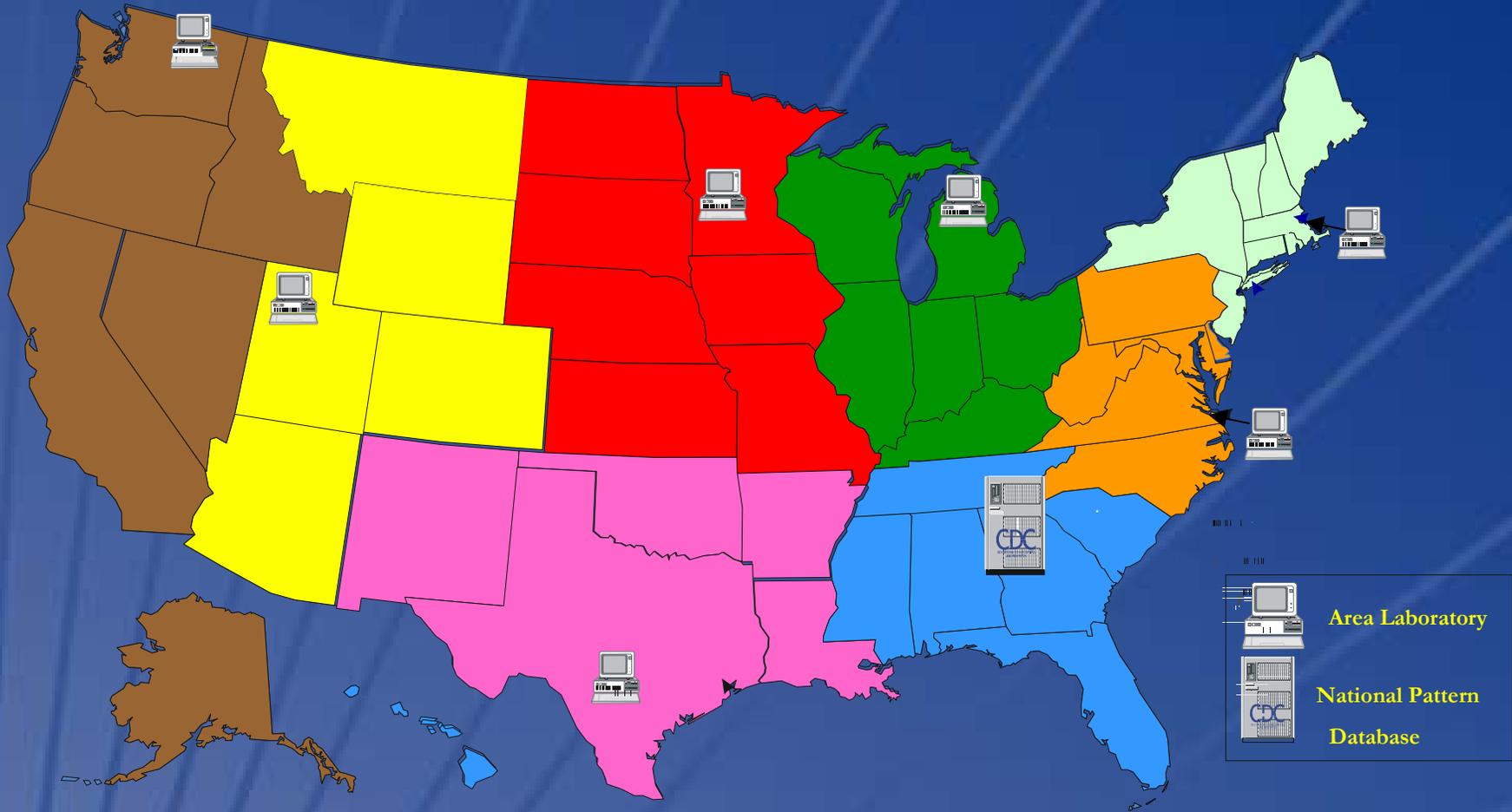
- 092701 comp

Libraries

Communication network

- Listserv postings
 - Cluster detection
 - Outbreak investigations
 - Technical support
- “PulseNet News” Newsletter
 - Quarterly publication
- Annual meeting
 - April 8-10, 2002

The National PulseNet Network - 2002





PulseNet North

PulseNet North



Partnership with
Canada has been
particularly
rewarding

PulseNet has revolutionized foodborne disease surveillance

- Real-time laboratory data available to epidemiologists
 - Cluster detection
 - Outbreak investigation
 - Scope of outbreak
 - Identification of source of outbreak
 - Effectiveness of prevention measures

PulseNet at Work

- Hudson beef outbreak, 1997
 - CO SPHL and USDA-FSIS performed PFGE typing of clinical and hamburger isolates
 - Patterns transmitted electronically to CDC
 - Found to be indistinguishable from each other within minutes of receiving the hamburger pattern
 - Determined that the scope of the outbreak was limited within 48 hours

PulseNet Activity, 1996-2001

	1996	2000	2001
No. of pathogens	1	4	7
No. of U.S. participating laboratories	16	18	64
<i>Escherichia coli, Salmonella, Shigella, Listeria, Campylobacter, Clostridium perfringens, Vibrio cholerae</i>			

PFGE patterns submitted to PulseNet Databases



PulseNet Activity 2001: *E. coli* O157:H7

TIFF files received	704
No. labs submitting TIFF	44
PFGE patterns received	3788
Clusters/outbreaks investigated	44
No. of laboratories certified	25
No. of laboratories on-line	19

Expanding the Net: Beyond Foodborne Pathogens

■ 2000

- Division of Bacterial and Mycotic Diseases / MSPB
 - *N. meningitidis* and *B. pertussis*
- Division of Health Quality Promotion
 - MRSA
- Division of Viral and Rickettsial Diseases
 - Calicinet

2001

- Division of Bacterial and Mycotic Diseases / RDB
 - *S. pneumoniae*
 - *Legionella sp.*

**CPHL will coordinate the establishment of
PulseNet Europe in 2002**

