

MLVA Interpretation for Epis



Ashley Sabol, MS

Research Microbiologist

PulseNet USA

Enteric Diseases Laboratory Branch

Centers for Disease Control and Prevention

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Objectives

- ❑ **Describe the science behind MLVA**
- ❑ **Explain when to include MLVA in an outbreak case-definition**
- ❑ **Discuss the utility of PFGE vs. MLVA**
- ❑ **Discuss how to interpret MLVA results**
- ❑ **To provide outbreak examples on how MLVA was used to formulate or change case definitions in these outbreaks**

MLVA OVERVIEW

Multiple-Locus VNTR Analysis (MLVA)

VNTR = Variable-Number Tandem Repeat

Locus 1

Strain A: VNTR array 4x3 = allele 3

atgggtaatccgctcgACgCACgCACgCgccaatcgatacgat

Strain B: VNTR array 4x5 = allele 5

atgggtaatccgctcgACgCACgCACgCACgCACgCgccc

Locus 2

Strain A: VNTR array 3x4 = allele 4

ggtaccggtaaagcgcACCACCACCACCTtgacactgccggttg

Strain B: VNTR array 3x6 = allele 6

ggtaccggtaaagcgcACCACCACCACCACCACCTtgacac

Possible MLVA subtypes = $(n_1) \times (n_2) \times (n_3) \dots (n_x)$

**Bacterial
genome**

Locus 2

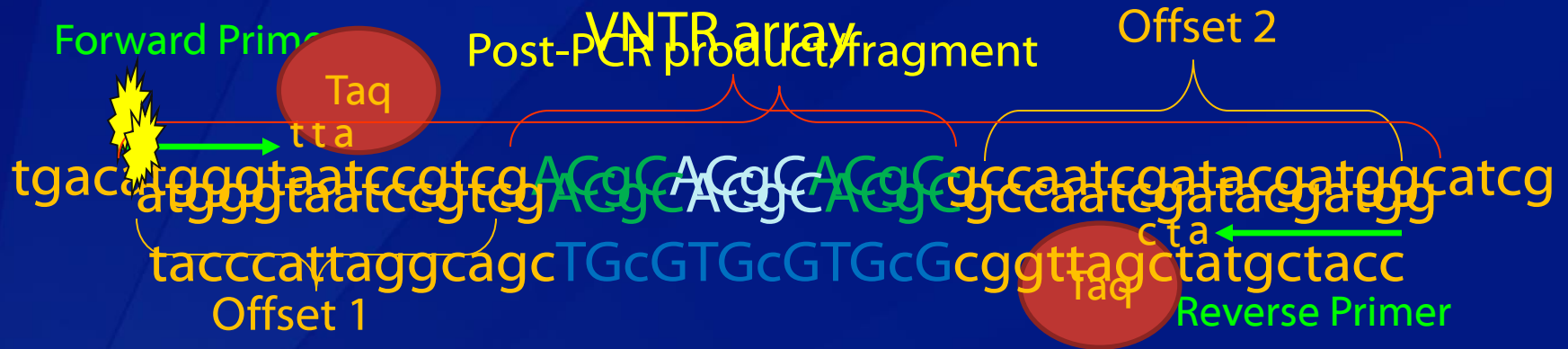
Locus 4

Locus 1

Locus 3



Generating Fragments of Target Sequences



MLVA Process

(Multiple Locus Variable-Number Tandem Repeat Analysis)

Bacterial Culture



Boil bacteria and release DNA

- 1 Scientists take bacterial cells from an agar plate and boil the cells to release DNA.



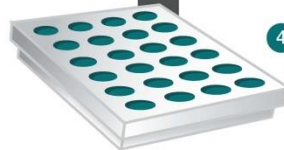
PCR amplification

- 2 Scientists have to detect the DNA region needed for this type of fingerprinting, called the variable-number tandem repeat arrays (VNTR). To do this, they use polymerase chain reaction (PCR), which combines the DNA with chemicals to amplify the VNTR.

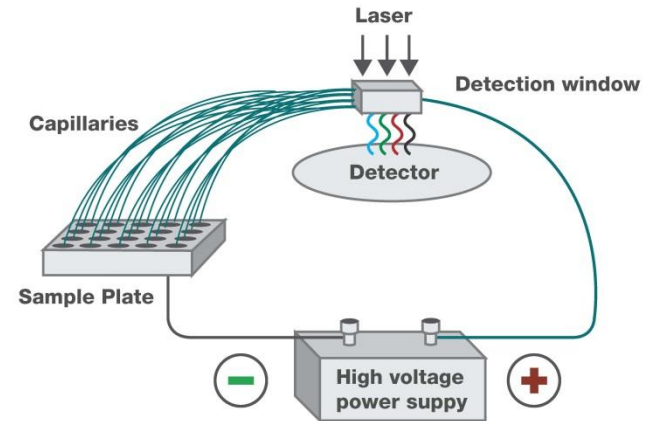
PCR product analysis



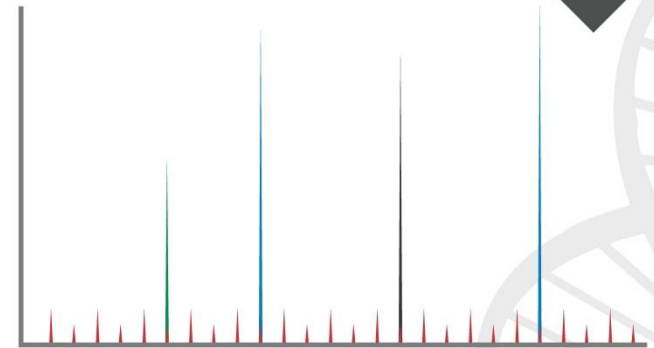
- 3 After PCR, scientists must determine the size of the PCR products. The different sizes will tell scientists how related the bacterial strains are to each other.



- 4 Scientists load the PCR products into a sample analysis plate and mix them with chemicals that help them determine the size of the product.



- 5 Using capillary electrophoresis, the fragment analysis solution is run through a gel matrix in an electric field to determine the sizes of the DNA fragments.



- 6 The data output of the MLVA process is called an electropherogram. It shows the DNA standards of known size in red, and the sizes of the PCR products in blue, green, and black. The PCR products sizes are converted into allele types using special software, which lets scientists determine how closely they are related.

DATA INTERPRETATION

Why PFGE and MLVA can yield different results?

- **PFGE**

- Variation in infrequent restriction sites due to:
 - Insertions
 - Deletions
 - Recombination

- **MLVA**

- Variation in VNTRs due to:
 - Slipped strand mispairing (SSM)
 - i.e. DNA polymerase slippage
 - Primary mechanism
 - Recombination
 - Rare in VNTRs; results in large copy number differences

VNTRs and infrequent restriction sites evolve at different rates among different strains.

Significance of a Single Repeat Difference

- **Each assay consists of a mixture of highly to moderately variable and conserved loci**
 - The difference between “highly variable” and “conserved” loci generally relates to repeat length
 - In general, the **shorter the repeat unit** the more variation is seen in terms of copy numbers
 - Conversely, **longer repeat units** are typically more stable in outbreak situations

Eight VNTR loci included in the PulseNet MLVA protocol for STEC O157

VNTR	Genetic region	Repeat size (bp)	No. of repeats		No. of alleles/Null alleles	Diversity
			Min	Max		
O157-3	Hypothetical protein	6	2	26	25/Yes	90.9
O157-9	Not ORF	6	1	25	25/Yes	91.0
O157-17	Hypothetical protein	6	2	18	14/No	78.3
O157-19	Hypothetical protein	6	2	13	12/No	73.9
O157-25	Not ORF	6	1	13	11/No	58.9
O157-34	YigL	18	4	12	9/No	71.9
O157-36	Not ORF ¹	7	3	19	18/Yes	86.9
O157-37	Hypothetical protein ¹	6	3	22	19/Yes	74.5

¹On pO157 plasmid

PulseNet MLVA protocols

– Existing protocols

- STEC O157 – 8 loci
- *Salmonella* serotype Typhimurium (Group B) – 7 loci
- *Salmonella* serotype Enteritidis (Group D) – 7 loci

– In-house protocols

- *Salmonella* serotype Newport – 6 loci
- *V. cholerae* – 6 loci

– Development abandoned

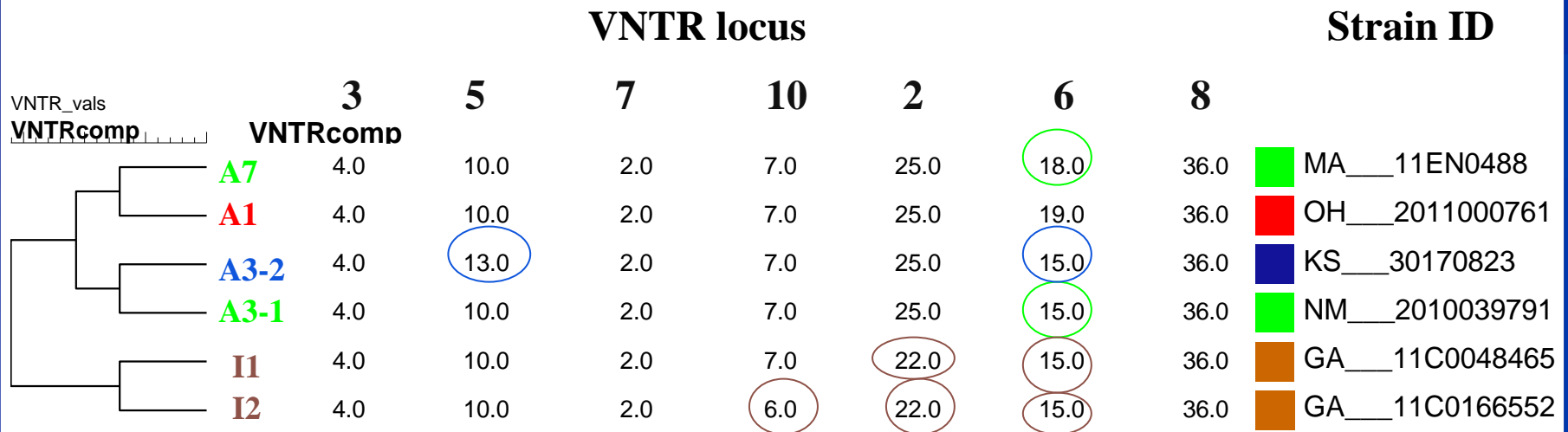
- *Listeria monocytogenes* – 11 loci
- *S. sonnei*
- Non-O157 STEC

Interpretation criteria for MLVA patterns during an outbreak

- **STEC O157**
 - Up to three repeats at a single locus or one repeat at two loci
 - No variation at locus 34
- ***Salmonella* serotype Typhimurium**
 - Up to four repeats at a single locus (loci 5, 6, and 10)
 - No variation at loci 2, 3, 7, and 8
- ***Salmonella* serotype Enteritidis**
 - One repeat (locus 2) or two repeats (locus 5) at a single locus
 - No variation at loci 1, 3, 6, 8 and 9
- ***Salmonella* serotype Newport**
 - One repeat at a single locus (loci 5 and 6)
 - No variation at loci A, B, L and M

Quantifying the degree of relatedness by MLVA in *Salmonella* Typhimurium

(Laboratory exposure to strain ATCC 14028 – Fall 2010 - 2012)



- Main outbreak pattern A1
- Patterns highly related to the main outbreak pattern: A3-1, A7
- A pattern closely related to pattern A3-1: A3-2
- Patterns not closely related to A-series: I1, I2

Implementation of MLVA in PulseNet USA

- Used as a complementary technique to PFGE for:
 - Selected emerging multi-state clusters prioritized by CDC PulseNet Lab
 - Special investigations by CDC, USDA, FDA, and local & state epidemiologists

Uses of MLVA

- Separate background cases from outbreak related cases
 - Important for strains with high background rates (common PFGE patterns)
- Assess relatedness of closely related PFGE patterns
 - Temporarily related clusters
 - Help with decisions when assessing the extent of the outbreak (e.g. variant *Blnl*-patterns)

Isolate selection for MLVA typing

- Representative isolates
 - Large multi-state PFGE defined clusters
 - Wide geographical and temporal distribution
 - States with multiple PFGE matches
 - Food or animal isolates
 - Isolates from patients with food exposure data
- All isolates
 - MLVA used as part of the case definition to define the scope of the outbreak

Variation in MLVA patterns among isolates of the same PFGE defined strain

- The more common the PFGE pattern, the more variation can be expected
 - Applies in many cases, particularly in STEC O157 and *Salmonella* ser. Typhimurium, but exceptions have been seen both ways
 - Some strains are clonal no matter which subtyping method is used
- The usefulness of MLVA for a certain PFGE defined strain needs to be established empirically

Parts of outbreak investigation where additional subtyping plays role

Additional differentiation and stringent agent definitions (e.g. “indistinguishable”)

- Cluster detection (and linking clusters)
- Hypothesis generation and testing
- Informational tracebacks
- Testing of food/water/environment
- Determination of outbreak scope

Expanding the case definition with multiple PFGE types

STEC O157 cluster 1310MNEXH-1

VNTR loci

3 34 9 25 17 19 36 37

Strain ID

XbaI pattern

BlnI pattern

	3	34	9	25	17	19	36	37	Strain ID	XbaI pattern	BlnI pattern
	10.0	9.0	9.0	3.0	7.0	4.0	9.0	7.0	CT__337438001	EXHX01.0074	EXHA26.0569
	10.0	9.0	7.0	3.0	7.0	6.0	9.0	7.0	USDA_210050700	EXHX01.0074	EXHA26.0569
	15.0	9.0	5.0	4.0	8.0	6.0	9.0	7.0	PA__M13016133001A	EXHX01.0074	EXHA26.0569
	13.0	9.0	15.0	4.0	7.0	6.0	10.0	10.0	MN__E2013017527-1	EXHX01.0074	EXHA26.0569
	13.0	9.0	15.0	4.0	7.0	6.0	10.0	10.0	MN__I2013017320-2	EXHX01.0074	EXHA26.0569
	20.0	9.0	19.0	4.0	7.0	6.0	11.0	9.0	CA__M13X05015	EXHX01.0074	EXHA26.0569
	7.0	9.0	11.0	4.0	7.0	5.0	10.0	8.0	MD__MDA13165523	EXHX01.0074	EXHA26.0569
	11.0	10.0	5.0	4.0	8.0	6.0	10.0	8.0	IL__C13EN001082	EXHX01.0074	EXHA26.0569
	11.0	10.0	5.0	4.0	8.0	6.0	10.0	8.0	MN__E2013020390		
	11.0	10.0	5.0	4.0	8.0	6.0	10.0	8.0	MN__E2013020391		
	11.0	10.0	5.0	4.0	8.0	6.0	10.0	8.0	MN__I2013019298-1	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013022933	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013022944	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013023037	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013023162	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013023168	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013023290	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013023383	EXHX01.0074	EXHA26.0569
	10.0	8.0	16.0	3.0	8.0	6.0	10.0	8.0	CO__HUM-2013023384	EXHX01.0074	EXHA26.0569
	10.0	10.0	5.0	5.0	8.0	6.0	7.0	9.0	IN__14ENT0419	EXHX01.0074	EXHA26.0569
	13.0	8.0	14.0	4.0	8.0	4.0	11.0	7.0	OR__13100200594	EXHX01.0074	EXHA26.0569
	12.0	9.0	16.0	4.0	6.0	4.0	11.0	7.0	WA__18943	EXHX01.1401	EXHA26.0569
	11.0	10.0	5.0	4.0	6.0	5.0	11.0	10.0	WY__13013546	EXHX01.0074	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00009645	EXHX01.0074	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00009901	EXHX01.0074	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00009963	EXHX01.0077	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00010059	EXHX01.0074	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00010258	EXHX01.0074	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00010259	EXHX01.0074	EXHA26.0569

← Ground beef isolates

STEC O157 cluster 1309MLEXH-3 (1309PAEXH-1 combined)

VNTR loci								Strain ID	XbaI pattern	BlnI pattern	
3	34	9	25	17	19	36	37				
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	CT__339676001	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	CT__339960001	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	DE__B521900	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	MA__13EN6862	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	NH__1309040080	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	NY__IDR1300029856-1P	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042738	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042795	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042796	EXHX01.0077	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042797	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042799	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042817	EXHX01.0077	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042914	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	OH__2013042949	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015016001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015077001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015162001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015163001A	EXHX01.0077	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015311001A	EXHX01.0077	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015406001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015407001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015408001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015503001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015551001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015646001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13015968001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13016134001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13016135001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13016136001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13016635001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13016954001A	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	WA__18680	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	WA__18929	EXHX01.0008	EXHA26.0569
	10.0	9.0	10.0	3.0	7.0	4.0	9.0	7.0	PA__M13016229001A	EXHX01.0077	EXHA26.0569
	10.0	9.0	9.0	3.0	7.0	4.0	8.0	7.0	CAOC_SB132480203	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	ARS3_RM16686	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	NH__1307110005	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	OR__13090901987	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	OR__13091203543	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	WA__18874	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	WA__18902	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	WA__18903	EXHX01.0008	EXHA26.0569
	9.0	9.0	16.0	4.0	7.0	4.0	8.0	14.0	WA__18915	EXHX01.0008	EXHA26.0569
	8.0	9.0	12.0	5.0	7.0	4.0	5.0	13.0	AZ__AZ00009963	EXHX01.0077	EXHA26.0569
	9.0	9.0	14.0	3.0	9.0	6.0	13.0	8.0	PA__M13014524001A	EXHX01.0008	EXHA26.0569

A1

A2

Conclusions

- **Benefits of MLVA**

- Useful in outbreaks with common PFGE patterns, common exposures and multiple PFGE patterns
- Can be used to both restrict and expand the case definition
- The degree of relatedness of patterns can be quantified
- Technically simple, high throughput method

- **Challenges with MLVA**

- Highly variable VNTR targets tend to evolve during outbreaks
- Development of data interpretation guidelines requires large databases and solid epidemiological data
- Protocols highly serotype-specific

Future of MLVA at the Beginning of the Genomics Era

- Effective immediately, CDC PulseNet Central lab cutting capacity for MLVA by 75%
- Area labs highly encouraged to get certified
- Prioritizing clusters where MLVA is implemented

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Quaerskioons?

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.