

# WGS Analysis of *Salmonella montevideo* Isolates from Multiple Outbreaks at a Restaurant Between 1997 and 2012



Julia Wolfe, MS  
Orange County Public Health Lab  
Supervising PH Microbiologist  
Molecular Testing



# The Outbreak That Just Kept Reoccurring

*S. montevideo* outbreak in a chicken fast food restaurant started in 1997, reappeared in 2000, then again in 2012.

1997 (n=6): Catered event, 5 people became ill

1 employee tested positive

All environmental samples were negative

2000 (n=10): 4 sporadic community cases

6 employees tested positive

All environmental samples were negative

2012 (n=48): 8 sporadic community cases & 11 patrons

18 employees tested positive

11 environmental samples were positive

# 2012: Finally, the Smoking Gun was Found

First round of environmental samples (n=30):

All negative



Second round of intense environmental investigation: OC Team

(Epi, EH, & Lab) + CDPH

+ private environmental group:

*S. montevideo* was found!



# Not Your Routine Environmental Samples

Chicken cutting board  
was clean

A film underneath the chicken  
cutting board had *S. montevideo*



# Not Your Routine Environmental Samples

An oily film on the floor next to the chicken grilling station grew *S. montevideo*



# Not Your Routine Environmental Samples

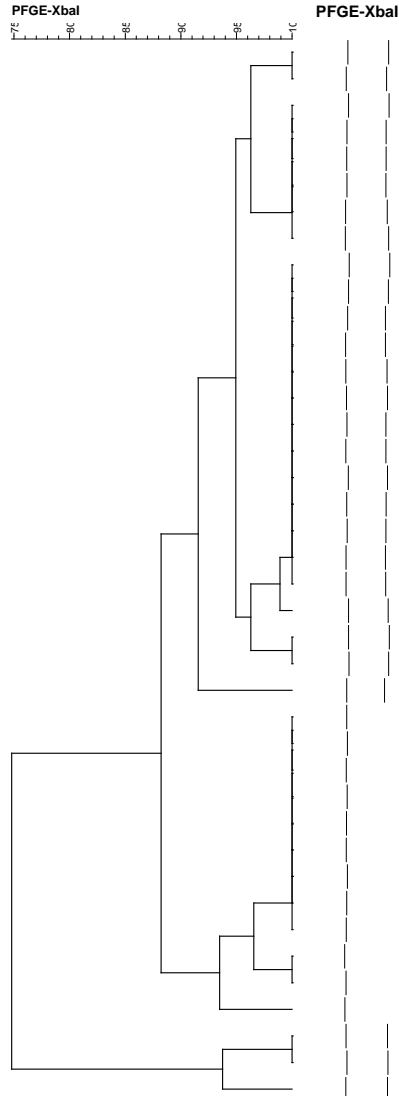
A film inside a container  
which holds cooking tools  
grew *S. montevideo*



The soil from an outside rock garden grew *S. montevideo*!!



# PFGE results using XbaI restriction enzyme (n=40)



WGS_id	WGS Clade No	IsolatDate	PFGE-XbaI-pattern	SourceType
PNUSAS032090	1	2000-08-31	JIXX01.0037	Human
PNUSAS032494	4	2012-07-27	JIXX01.0964	Environmental
PNUSAS032086	1	2000-08-12	JIXX01.0964	Human
PNUSAS032096	3	2012-05-26	JIXX01.0964	Human
PNUSAS032099	4	2012-05-28	JIXX01.0964	Human
PNUSAS032100	4	2012-05-28	JIXX01.0964	Human
PNUSAS032490	4	2012-07-26	JIXX01.0964	Human
PNUSAS032496	3	2012-07-03	JIXX01.0964	Environmental
PNUSAS032085	1	2000-08-10	JIXX01.0966	Human
PNUSAS032089	1	2000-08-21	JIXX01.0966	Human
PNUSAS032092		2012-03-16	JIXX01.0966	Human
PNUSAS032093	3	2012-04-10	JIXX01.0966	Human
PNUSAS032094	4	2012-05-03	JIXX01.0966	Human
PNUSAS032097		2012-05-26	JIXX01.0966	Human
PNUSAS032482	4	2012-05-28	JIXX01.0966	Human
PNUSAS032483	4	2012-05-28	JIXX01.0966	Human
PNUSAS032485		2012-05-30	JIXX01.0966	Human
PNUSAS032487	4	2012-07-03	JIXX01.0964	Environmental
PNUSAS032488	4	2012-07-03	JIXX01.0966	Environmental
PNUSAS032493	4	2012-07-27	JIXX01.0966	Human
PNUSAS032497	4	2012-07-09	JIXX01.0966	Environmental
PNUSAS032091			JIXX01.0019	Human
PNUSAS032087	1	2000-08-10	JIXX01.0034	Human
PNUSAS032088	1	2000-08-11	JIXX01.0034	Human
PNUSAS032486		2012-06-18	JIXX01.0040	Human
PNUSAS032098	2	2012-05-27	JIXX01.0097	Human
PNUSAS032484	2	2012-05-28	JIXX01.0097	Human
PNUSAS032489	2	2012-07-26	JIXX01.0097	Human
PNUSAS032492	4	2012-07-27	JIXX01.0097	Human
PNUSAS032495	2	2012-08-03	JIXX01.0097	Environmental
PNUSAS014342		2012-08-03	JIXX01.0097	Environmental
PNUSAS014344		2012-08-15	JIXX01.0097	Environmental
PNUSAS014345		2012-09-24	JIXX01.0097	Environmental
PNUSAS014341		2012-08-03	JIXX01.0097	Environmental
PNUSAS032095	2	2012-05-07	JIXX01.0097	Human
PNUSAS032491	2	2012-07-26	JIXX01.1139	Human
PNUSAS014343		2012-08-03	JIXX01.1077	Environmental
PNUSAS034861		2012-06-14	JIXX01.1294	Human
PNUSAS035119		2012-07-25	JIXX01.1294	Human
PNUSAS034862		2012-07-15	JIXX01.1250	Human

← 1997 isolate

# 2012 PFGE Results (pre WGS era)

PFGE was performed on 29 staff/patrons and 11 environmental isolates.

- Eight PFGE patterns with less than 4 band differences between them were identified.
- The isolate from the first outbreak in 1997 was not part of these three clusters.
- Two of the three clusters contained isolates from 2000 and 2012.
- Each of the 3 clusters contained patron, employee, and environmental isolates with fingerprint patterns that were less than 2 band differences.

Conclusion: PFGE data suggests that the isolates from 2000 and 2012 were related to the 1997 isolate because they had fingerprints that differed by less than 4 bands and we had strong Epi links.



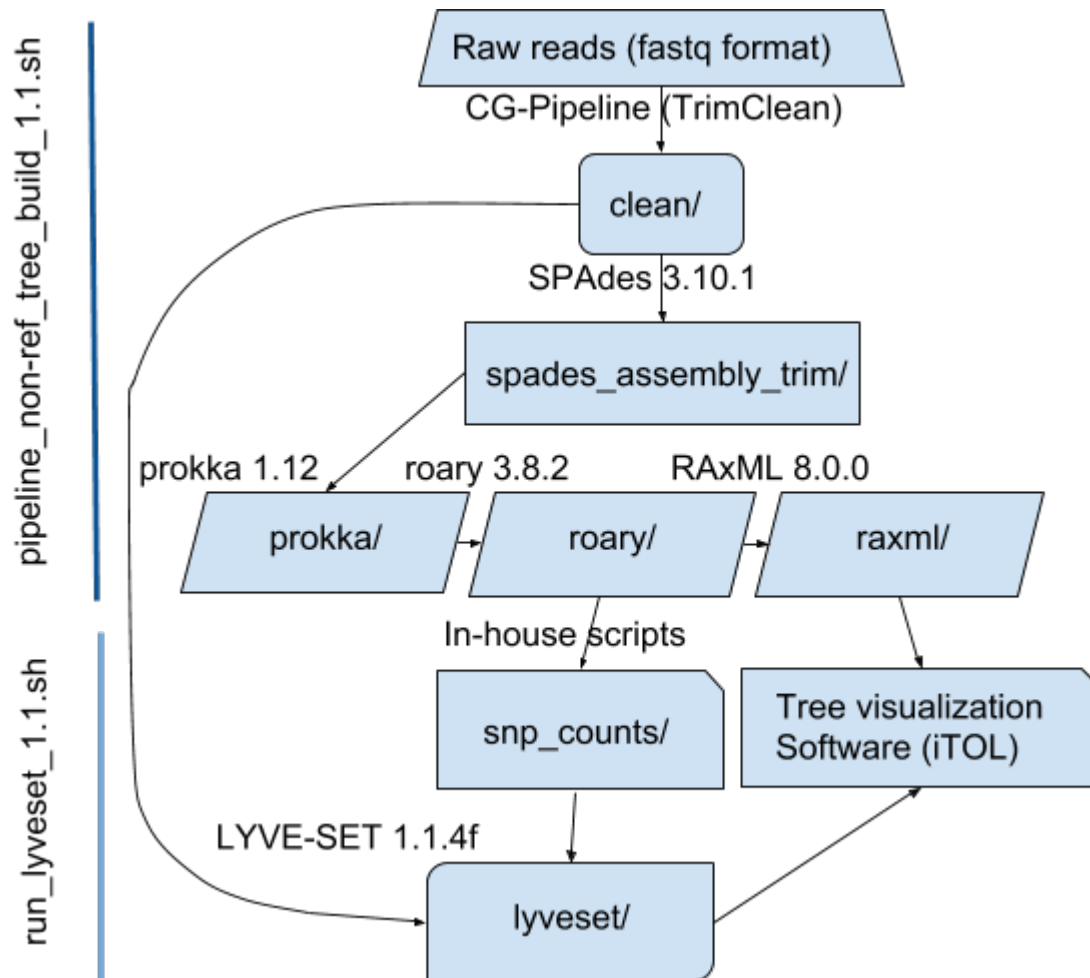
# 2018 OC Performed Retrospective WGS testing

- OCPHL started WGS wet lab in the fall 2016.
- Our Epis requested a retrospective WGS testing of the outbreak.
- We decided to test 40 isolates from the outbreak.
- We were confident in the wet lab portion but we reached out to Colorado PHL for help with WGS analysis

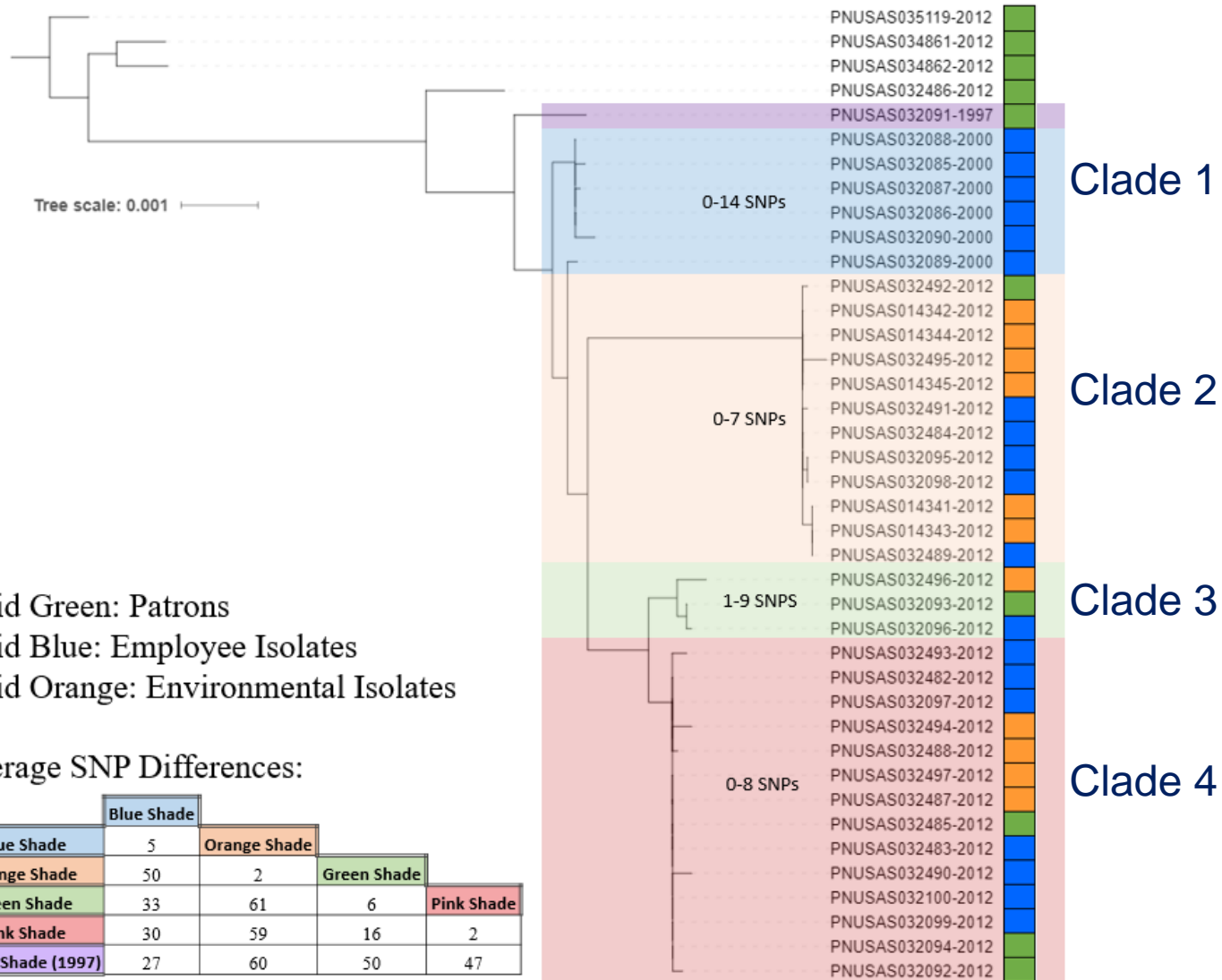


**COLORADO**  
Department of Public  
Health & Environment

# Non-reference LYVE\_SET Flow Diagram



# Lyve-SET hqSNP analysis (n=40)



# SNP matrix table (N=40)

	PNUSAS03119	PNUSAS03460	PNUSAS03482	PNUSAS03246	PNUSAS03091	PNUSAS03098	PNUSAS03095	PNUSAS03087	PNUSAS03246	PNUSAS03090	PNUSAS03089	PNUSAS03242	PNUSAS03142	PNUSAS03144	PNUSAS03249	PNUSAS03145	PNUSAS03095	PNUSAS03098	PNUSAS03141	PNUSAS03143	PNUSAS03248	PNUSAS03096	PNUSAS03099	PNUSAS03242	PNUSAS03097	PNUSAS03146	PNUSAS03248	PNUSAS03147	PNUSAS03149	PNUSAS03100	PNUSAS03098	PNUSAS03104	PNUSAS03095								
PNUSAS035119		30	31	91	108	101	104	100	106	104	113	119	111	117	136	140	140	110	140	140	99	139	128	125	126	126	124	124	124	126	120	120	124	127							
PNUSAS034861	30		20	95	111	106	109	107	105	109	107	139	124	135	141	142	142	143	143	141	144	107	145	133	128	129	130	127	127	131	127	127	126	126	126	130	124	124	124	127	
PNUSAS034862	31	20		97	113	109	112	110	107	112	110	140	125	137	143	145	144	147	147	143	147	109	147	136	131	132	133	130	129	134	130	130	129	129	133	125	127	127	131		
PNUSAS032486	91	95	97		50	44	48	47	43	48	47	80	72	75	79	82	81	83	83	80	83	61	83	73	68	69	69	66	65	70	67	66	66	66	66	70	63	64	63	68	
PNUSAS032091	108	111	113	50		25	28	27	25	28	27	60	57	58	66	62	62	62	62	63	47	64	53	48	49	49	46	46	50	47	46	46	46	46	50	45	45	45	48		
PNUSAS032088	101	106	109	44	25		2	1	0	4	9	49	47	48	54	50	51	51	51	50	50	39	51	36	31	32	31	28	28	28	28	28	28	28	32	27	27	27	30		
PNUSAS032085	101	109	112	48	28	2		3	2	6	12	52	49	50	56	53	53	54	54	53	53	40	54	38	33	35	35	32	32	32	32	31	31	36	30	30	30	33			
PNUSAS032087	104	107	110	47	27	1	3		1	5	11	50	48	49	56	52	52	52	51	52	52	40	53	37	32	33	33	30	30	34	31	30	30	30	34	29	29	29	32		
PNUSAS032086	100	105	107	43	25	0	2	1		0	10	49	47	47	50	50	50	50	49	50	50	39	51	36	31	32	32	29	29	29	29	29	33	28	28	28	28	30			
PNUSAS032090	106	109	112	48	28	4	6	5	6		14	53	51	52	58	55	54	56	56	55	55	40	56	40	35	36	36	33	33	33	33	36	34	33	33	33	32	32	35		
PNUSAS032089	104	107	110	47	27	9	12	11	10	14		47	43	45	52	48	47	49	49	48	48	36	48	30	25	27	27	24	24	24	24	24	24	23	23	28	22	21	21	25	
PNUSAS032492	139	139	146	80	60	49	52	50	49	53	47		1	1	6	1	2	1	2	2	3	3	3	66	60	59	62	59	58	63	60	59	59	59	59	59	63	59	58	58	60
PNUSAS014342	119	126	125	72	57	47	49	48	47	51	43		4	4	0	1	0	1	1	2	2	2	63	57	57	58	56	56	60	57	56	56	56	56	56	56	56	56	57		
PNUSAS014344	131	126	137	75	58	48	50	49	47	51	45		1	0	4	0	1	0	1	1	2	2	63	58	58	60	57	57	60	58	57	57	57	57	61	57	57	56	59		
PNUSAS032495	137	141	143	79	66	54	56	56	50	58	52	6	4	4		4	6	5	6	6	7	5	7	70	64	64	64	66	63	63	63	63	63	63	63	63	63	63	64		
PNUSAS014345	137	142	145	82	62	50	53	52	50	55	48		1	0	0	4	1	1	1	2	2	2	66	60	60	62	59	59	63	60	59	59	59	59	63	59	58	58	58	60	
PNUSAS032491	136	142	144	81	62	51	53	52	50	54	47	2	1	1	6	1		1	2	2	3	2	66	60	60	62	59	59	63	60	59	59	59	63	59	58	58	58	60		
PNUSAS032484	140	143	147	83	62	51	54	52	50	56	49	1	0	0	5	0		1	1	2	2	2	67	60	61	63	60	60	64	61	60	60	60	64	60	59	58	58	62		
PNUSAS032095	140	143	147	83	62	51	54	51	49	56	49	2	1	1	6	1	2	1		0	3	3	65	59	61	62	60	59	64	60	59	59	59	59	63	58	58	57	61		
PNUSAS032098	135	141	143	80	62	50	53	52	50	55	48	2	1	1	6	1	2	1	0		3	3	65	60	60	62	59	59	63	60	59	59	59	63	59	59	59	58	60		
PNUSAS014341	140	144	147	83	63	50	53	52	50	55	48	3	2	2	7	2	3	2	3	2	3	3	66	60	60	62	59	59	63	60	59	59	59	63	59	59	59	58	61		
PNUSAS014343	99	107	109	61	47	39	40	39	40	36	3	2	2	2	5	2	2	2	3	3	0		0	53	47	47	48	46	46	49	47	46	46	46	46	46	46	46	47		
PNUSAS032489	139	145	147	83	64	51	54	53	51	56	48	3	2	2	7	2	3	2	3	2	3	0		67	61	61	63	60	60	64	61	60	60	64	60	60	60	59	63		
PNUSAS032093	128	133	136	73	53	36	38	37	36	40	30	66	63	63	70	66	66	67	65	65	66	53	67		7	9	20	17	17	21	18	17	17	17	21	17	17	17	19		
PNUSAS032094	125	128	131	68	48	31	33	32	31	35	25	60	57	58	64	60	60	60	59	60	60	47	61	7		7	1	16	13	13	17	14	13	13	13	13	13	13	15		
PNUSAS032096	126	129	132	69	49	32	35	33	32	36	27	59	57	58	64	60	60	61	61	60	60	47	61	9		17	14	14	18	15	14	14	14	14	14	14	14	14	14	16	
PNUSAS032493	128	130	133	69	49	31	35	33	32	36	27	62	58	60	66	62	62	63	62	62	62	48	63	20	16	17		3	3	7	4	3	3	3	3	7	3	3	5		
PNUSAS032482	125	127	130	66	46	28	32	30	29	33	24	59	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		4	1	0	0	0	0	0	0	0	0	2		
PNUSAS032097	124	127	129	65	46	28	32	30	29	33	24	58	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		4	1	0	0	0	0	0	0	0	0	2		
PNUSAS032494	128	131	134	70	50	32	36	34	33	36	28	63	60	60	64	60	63	63	63	63	49	64	21	17	18	7	4		4	1	0	0	0	0	0	0	0	0	0		
PNUSAS032488	124	127	130	67	47	29	31	31	30	34	24	60	57	58	64	60	60	61	60	60	60	47	61	18	14	15	4	1		1	1	1	1	1	1	1	1	1	3		
PNUSAS032497	125	127	130	66	46	28	32	30	29	33	24	58	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		0	0	0	0	0	0	0	0	0	0	0	2	
PNUSAS032487	124	126	129	66	46	28	32	30	29	33	24	58	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		0	0	0	0	0	0	0	0	0	0	0	2	
PNUSAS032485	123	126	129	66	46	28	31	30	29	33	23	59	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		0	0	0	0	0	0	0	0	0	0	0	2	
PNUSAS032483	122	126	129	66	46	28	31	30	29	33	23	59	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		0	0	0	0	0	0	0	0	0	0	0	2	
PNUSAS032490	128	130	133	70	50	32	36	34	33	36	28	63	60	61	67	63	63	64	63	63	63	50	64	21	17	18	7	4	4	8	5	4	4	4	4	4	4	4	5		
PNUSAS032100	118	124	125	63	45	27	30	29	28	32	22	59	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		0	0	0	0	0	0	0	0	0	0	0	1	
PNUSAS032099	120	124	127	64	45	27	30	29	28	32	21	58	56	57	63	59	59	60	60	59	59	46	60	17	13	14	3		0	0	0	0	0	0	0	0	0	0	0	1	
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PNUSAS032092	124	127	131	68	48	30	33	32	30	35	25	60	57	59	64	60	60	62	61	60	61	47	61	19	15	16	5	2		2	6	3	2	2	2	2	5	1	1	1	

# Retrospective WGS study

- The phylogenetic tree from Iyve-SET hgSNP analysis identified 4 clades
- One clade contained isolates from 2000, the other three clades were from 2012 isolates. SNP differences between clades ranged from 13 to 70 SNPs.
- The 1997 isolate differed from the 2000 clade by a minimum of 25 SNPs
- The 1997 isolate differed from the 2012 clades by a minimum of 45 SNPs.
- The 2000 clade differed from 2012 isolates by a minimum of 21 SNPs.

# Summary

- PFGE and WGS identified three out of four clusters which contained patron, employee, and environmental isolates.
- WGS analysis of the case isolates alone may have suggested that the 2012 clades were unrelated (13 – 70 SNPs difference), but the strong epidemiological data (employee, patrons, and environmental isolates) clearly tied these clades together.
- The environmental isolates were unique in that they suggested that by 2012, *S. montevideo* organisms were surviving in several biofilms within and outside of the restaurant.
- These results suggest that the Salmonella strains from 2000 and 2012 were residing in the restaurant since 1997 and ultimately caused sporadic outbreaks over 15 years.



# Thank you

## Acknowledgements:

**CDPHE: Joel Sevinsky & Logan Fink**

**OC Microbiologists: Rina Tjiptahadi, Angelica Torres, & Karen Nguyen**

**OC Epidemiologists**

