

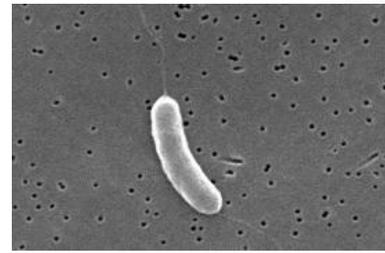
Molecular characterization of *Vibrio vulnificus* isolated from Eastern oysters (*Crassostrea virginica*) from the northeastern coast of the US and comparison with clinical strains.

Steven Pitchford, Yann Reynaud, Sophie De Decker,  
Gary Wikfors, Christopher Brown

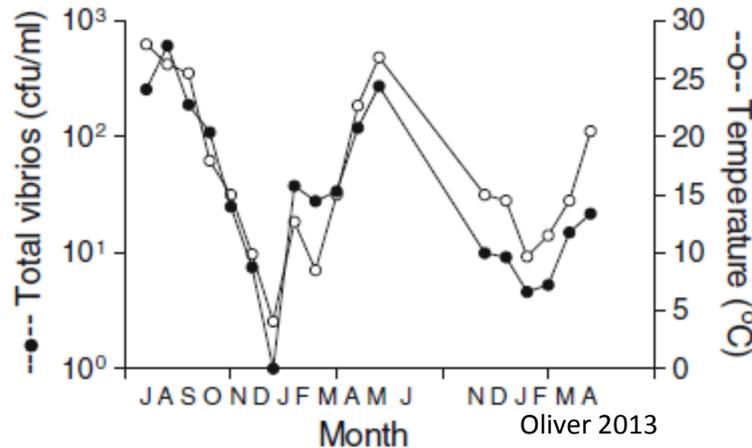
NOAA, NEFSC, 212 Rogers Avenue, Milford 06460, CT, USA



# Vibrio vulnificus



- Gram negative, halophilic , curved rods
- Distribution worldwide-occurring naturally in coastal and estuarine water, sediments; also seafood including oysters, shrimp and fish
- CFU/ml strongly correlated with water temperature

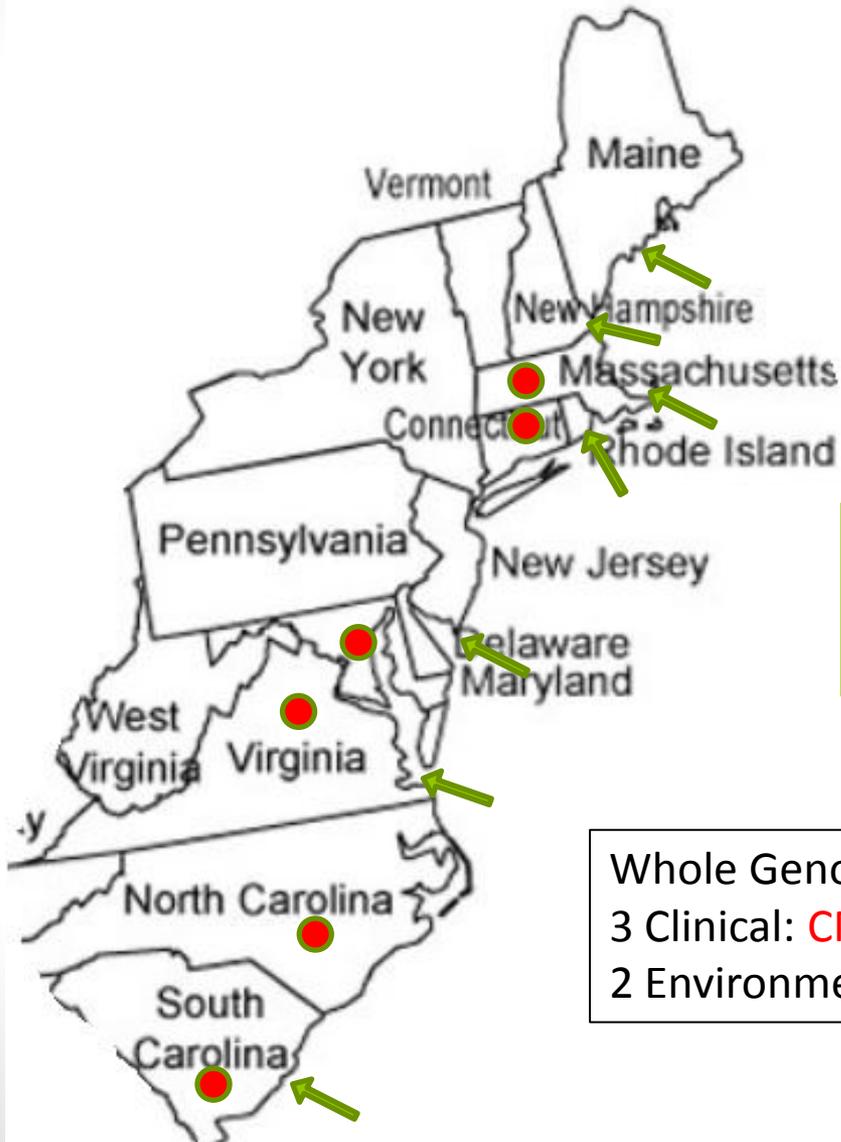


- In the US, most infections occur from May-October
- A large majority of these are in 5 states bordering on the GOM:  
AL. FL. LA. MS. TX.
- Sources of infection:
  - Consumption of raw or undercooked shellfish esp. oysters
  - Open wounds.





# Source of strains used in study



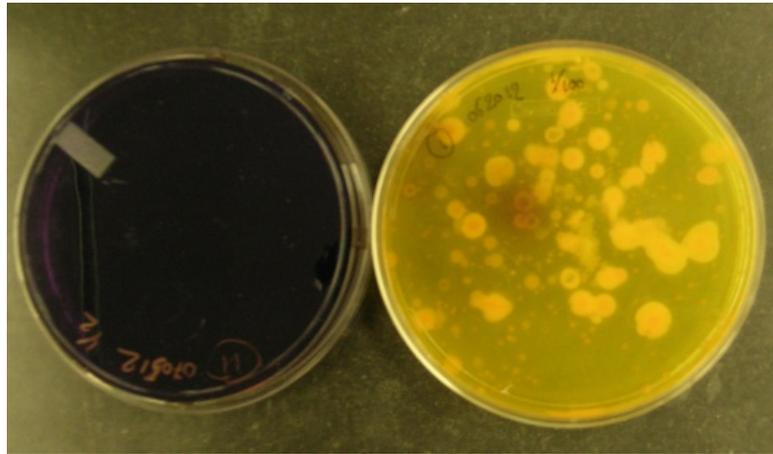
● **Clinical:** 18 strains provided by the CDC from 6 states, collected 2003-2010. Isolated from blood, wound, stool and gall bladder

← **Environmental:** June-Oct 2012  
Oysters sampled from 13 farms and sites in 7 states. Water temp. > 20C

Whole Genome Sequence (WGS) strains-*in silico*  
3 Clinical: **CMCP6, YJ016, MO-24/O,**  
2 Environmental from oysters **JY1701 and JY1305**

# Oyster Screening

Oyster tissues were processed under BSL2 conditions and plated on CPC+ agar (Cellobiose-polymyxin B-colistin plus (Warner and Oliver 2007))

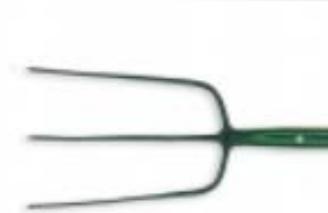


Yellow colonies were confirmed as Vv by PCR for the cytolysin gene (vvhA)

60 strains of *V.vulnificus* were isolated from oysters



# Polyphasic Molecular Typing



## 1. Genotype

**Biotype:** based on biochemical, serological, and genetic analysis

- **Biotype 1:** Most common-cause majority of human infections.
- **Biotype 2:** Primarily eel pathogens, some cases of human infection.
- **Biotype 3:** First outbreak Israel-Handling tilapia led to human infections

For this study-multiplex PCR was used (Sanjuan, et. al. 2007)

**CPS Operon (Capsular Polysaccharide)** (Chatzidaki-Livanis et al., 2006)

- **Allele 1:** 87% Clinical strains
- **Allele 2:** 87% Environmental strains

**vcg (virulence correlated gene)** (Warner et al. 2008)

- **C type:** 90% of clinical strains
- **E type:** 87 % of environmental strains

**16sRNA** (Aznar et al. 1994 ; Nillson et al. 2003)

- **16sRNA Type A:** 94% of environmental strains
- **16sRNA Type B:** 71% of clinical strains

## 2. MLST Multilocus Sequence Typing

4 house keeping genes

2 common  
virulence genes

Gene	Sequence length bp	Polymorphic sites nt	Alleles number
16S rRNA	387	15	4
glp	591	56	41
gyrB	633	64	41
rpoD	930	59	39
pilF	528	63	41
vvhA	761	75	43
House keeping	2541	194	73
Virulence	1289	138	69
All	3830	332	81

## 3. Virulence

- Whole genome comparisons of E and C strains (Morrison 2012, Gulig 2010)
- Genes specific to clinically pathogenic strains and absent from environmental strains.

- Aryl A (arylsulfatase A found on genomic island XII)
- MtlABC (PTS system mannitol-specific transporter subunit II B)
- NanA (N-acetylneuraminatase lyase-involved in sialic acid catabolism)

# Genotype Results

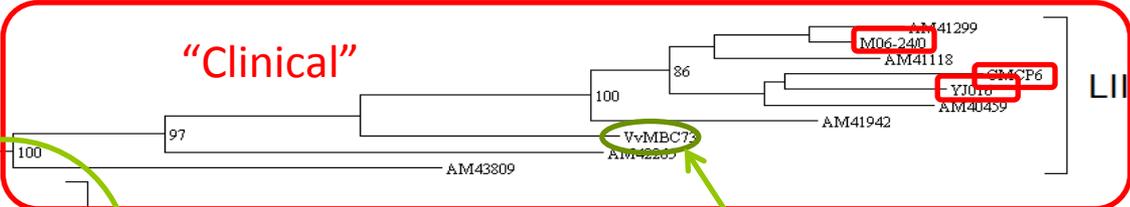
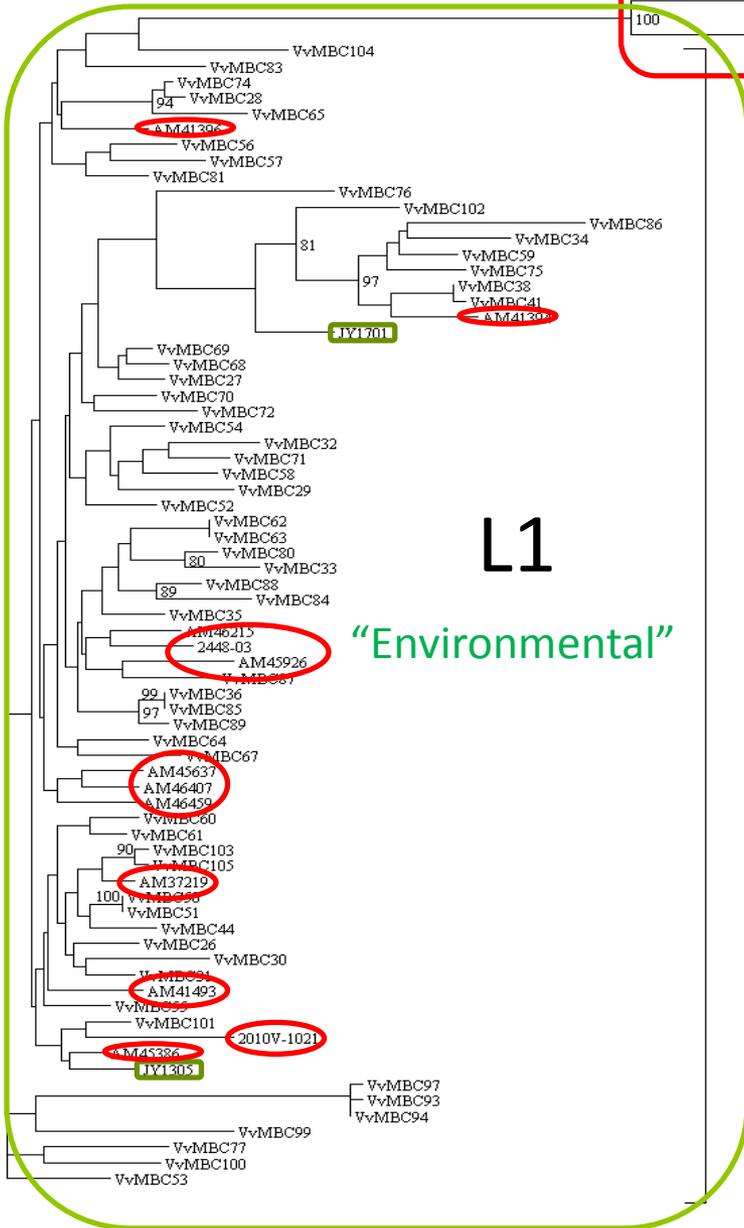
All 83 strains-Biotype 1

“Environmental”  
profile: AE2

“Clinical”  
Profile: BC1

Loci	Clinical (CDC/WGS) n=21	Environmental (Oysters/WGS) n=62
	Genotype distribution (%)	
CPS allele		
1	23.8 (87%)	16.1
2	71.4	59.7
0 (absent)	4.1	24.2
vcg		
C	52.4 (90%)	3.2
E	47.6	96.8
16s RNA Type		
A	61.9	100
B	38.1 (94%)	0

# MLST Results



9/21 clinical strains

1/62 Oyster strains

“Clinical Profile” BC1  
 CPS 1= 5/9  
 16s B= 9/9  
 vcg C= 9/9

61/62 oyster strains

12/21 clinical strains

“Environmental Profile:” AE2  
 CPS 2= 12/12  
 16s A= 12/12  
 vcg E= 10/12

3830 bp, 6 genes 16Sr DNA, *rpoD*, *gyrB*, *glp*, *pilF* and *vvhA* 83 *V. vulnificus* strains. NJ, Kimura’s 2, 1000 bootstraps replicates

# Genes linked to virulence

	Clinical	Clinical-LII	Clinical-LI	Environ.	Clin-wgs	Env-wgs
	n=21	n=9	n=12	n=62	n=3	n=2
Arylsulfatase A	71.4%	100%	50%	16.1%	3/3	0/2
MtIABC	90.5%	100%	83.3%	61.3%	3/3	0/2
NanA	95.2%	100%	91.6%	37.1%	3/3	0/2

First polyphasic typing study of Vv strains from the NE US

**Genes linked to virulence in clinical strains of *V. vulnificus* were found in many of the strains isolated from oysters. These strains have the potential ability to trigger infection in humans**



**However, GENOTYPE DOES NOT PREDICT VIRULENCE !**



**The only way to confirm pathogenicity is to perform the iron dextran treated mouse assay. VvMBC73 has been sent to Paul Gulig / University of Florida for testing-awaiting results.**

## So- just why was this study done?

After all, compared to the GOM, Vv infections are very rare in the NE

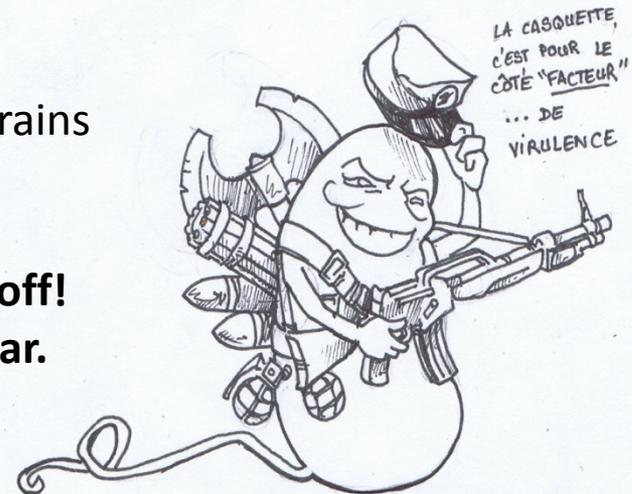
When the FDA was considering a ban on harvest and sale of raw oysters from GOM during warm temperatures, a question was raised –if Vv is so wide spread and can be found in virtually all oysters above 20C, why aren't MORE people getting sick from NE oysters?

That was the original impetus for this study : to compare southern and northern Vv strains and determine if there are genetic differences in virulence.

However, s..t happens and the project morphed.

Virulence factors are indeed present in northern Vv strains and just waiting for the right **trigger**.

**Don't piss this guy off!  
Or make him "hot" under the collar.**



# Molecular Typing of Environmental and Clinical Strains of *Vibrio vulnificus* Isolated in the Northeastern USA

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