Characterization of *Vibrio* spp. on CPC+, CHROMagar Vibrio, and TCBS, and Proposed Cross-Plating Method for Isolation of *Vibrio vulnificus* from Environmental Samples

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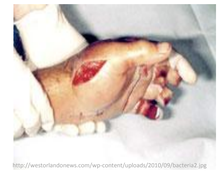
Vibrio vulnificus

- Found in estuaries and brackish waters worldwide
- Contaminates raw seafood and shellfish
- Can cause severe wound infections and septicemia
- Responsible for 95% of seafood related deaths in US
- Rates of infection are increasing compared to other food borne pathogens



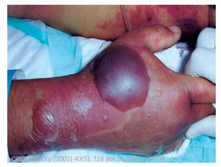
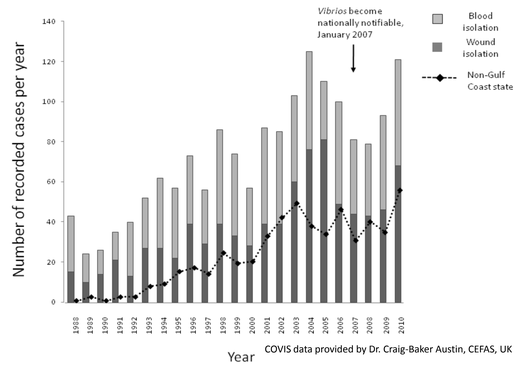


Figure 1. *Vibrio vulnificus* cases reported in the USA, 1988-2010



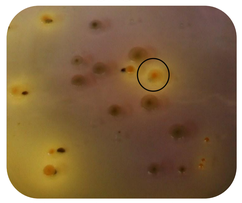
COVIS data provided by Dr. Craig-Baker Austin, CEFAS, UK

Monitoring the presence of this bacterium in estuarine waters and shellfish is of medical and economic importance

Current Methods of Detection

Vibrio vulnificus can be grown on selective an differential media
(circles indicate one colony of *V. vulnificus*)

CPC+

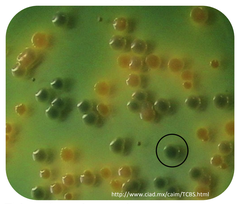


Cellobiose polymyxin B colistin agar

CHV

CHROMagar Vibrio

TCBS



Thiosulfate citrate bile salts sucrose agar

Problem with this method

Other *Vibrio* species can look like *V. vulnificus* on these media
This often occurs when *V. vulnificus* populations are at low levels

Identification of False Positive Isolates

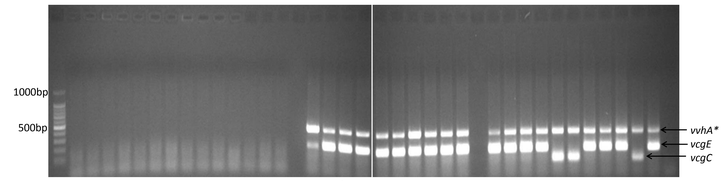
To determine which *Vibrio* species can appear like *V. vulnificus* on these media we grew 17 *Vibrio* species on each medium

Species that can grow like *V. vulnificus* on each medium		
CPC+	**CHV**	**TCBS**
V. parahaemolyticus	*V. cholerae*	*V. parahaemolyticus*
V. alginolyticus	*V. hollisae*	*V. harveyi*
V. harveyi	*V. mimicus*	*V. anguillarum*
	V. fluvialis	*V. mimicus*
	V. aestruianus	*V. hollisae*
		V. metschnikovii
		V. nigripulchritudo
		V. proteolyticus
		V. pelagius
		V. leiognathi

Several *Vibrio* species can grow like *V. vulnificus* on the three different media however, of those tested **none of them grew like *V. vulnificus* on all three**

Confirmatory Method of Detection

Each *V. vulnificus* presumptive colony must be confirmed by genetic testing



(**vvhA* used to identify V. vulnificus, *vcgC/E* used to identify the two genotypes of this species)

Our lab uses multiplex PCR to confirm *V. vulnificus* presumptive isolates[1]

Cost Analysis of the Current Methods

Presumptive *V. vulnificus* isolated from North Carolina oysters[2]			
	Isolated using CPC+		Isolated using CHV
Years	2005-2006	2007-2010	2010
Number of Isolates Tested	367	3623	456
# Confirmed by Genetic Testing	149	25	18
Estimated Cost Per Sample Set	$287	$2,826	$356
Estimated Man-Hours Consumed	11 hrs	113 hrs	14 hrs

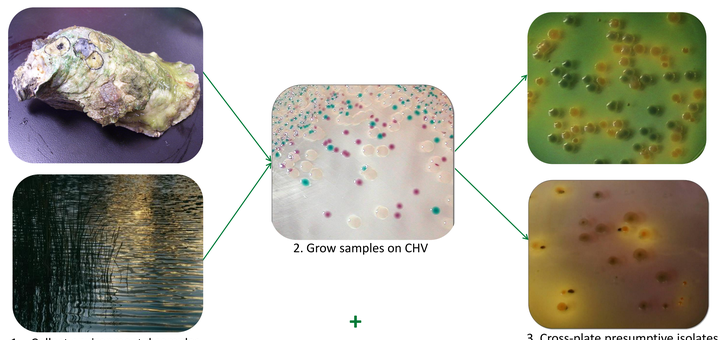
PCR confirmation of *V. vulnificus* presumptive isolates is expensive, requires an experienced technician, and is time consuming.

Many countries may not have access to these resources, therefore an inexpensive, efficient, non-molecular based technique would be economically valuable

Developing a New Method of Detection

Hypothesis: Using a new media-based cross-plating method, we can increase the ability to accurately detect *V. vulnificus* from environmental samples, thereby eliminating or reducing the need for genetic testing

Workflow:



1. Collect environmental samples of interest

3. Cross-plate presumptive isolates onto CPC+ and TCBS

Isolates that appear like *V. vulnificus* on all three media are referred to as **triple positives** and are predicted to be *V. vulnificus*

References and Acknowledgements

1. Warner, E. and J. D. Oliver (2008). "Multiplex PCR assay for detection and simultaneous differentiation of genotypes of *Vibrio vulnificus* biotype 1." *Foodborne Pathogens and Disease* 5(5): 691-693.
2. Froelich, B. A., T. C. Williams, et al. (2012). "Apparent Loss of *Vibrio vulnificus* from North Carolina Oysters Coincides with a Drought-Induced Increase in Salinity." *Appl Environ Microbiol* 78(11): 3885-3889.

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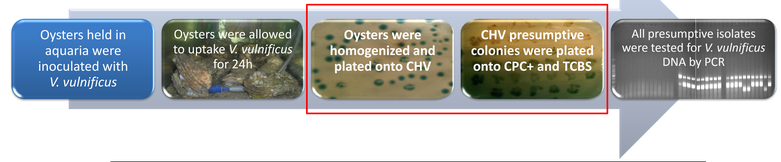
Oyster Uptake Experiment (Proof of Concept)

Methods:



Results:

Ability to Accurately Detect *V. vulnificus* in Infected Oysters		
	Current Method	New Method
# of original isolates	92	92
# of *V. vulnificus* presumptive isolates	92	40
# confirmed by genetic testing	40	40
% Correctly identified	43.5%	100%

Using the new cross-plating method resulted in 100% accuracy in detecting *V. vulnificus* from artificially infected oysters

This method reduced the amount of genetic testing needed by 56.5% and increased the efficiency of detecting *V. vulnificus* by 130%

Is the new method effective for environmental isolation and detection of *V. vulnificus*?

Methods: The new cross-plating method was performed on a variety of environmental samples and the costs of each method were compared

Results:

Ability to Accurately Detect *V. vulnificus* in Environmental Samples		
	Current Method	New Method
# of original isolates	152	152
# of *V. vulnificus* presumptive isolates	152	49
# confirmed by genetic testing	54	46
% Correctly identified	35.5%	92.8%
Cost Analysis		
Estimated cost of testing (with PCR)	$119	$44
Estimated cost of testing (without PCR)	Not feasible (too inaccurate)	**$3 without genetic testing**

For this set of 152 environmental isolates, the new cross-plating method **resulted in a 161% increase in the efficiency of detecting *V. vulnificus***

We were able to successfully detect *V. vulnificus* with 92.8% accuracy, **without the need for genetic testing, reducing the cost by 97.5%**

Conclusions and Implications

We suggest that this new and simple cross-plating technique will provide a method for the isolation and detection of *V. vulnificus* that is **more accurate, more time efficient, and more cost effective**, particularly when molecular methods are not available.

SUMMARY OF BENEFITS:

Increases accuracy in detecting *V. vulnificus*

Reduces and/or eliminates the need for genetic testing

Reduces time and money spent on isolation and detection methods