Characterization of *Enterobacteriaceae* and *Vibrio spp.* Occurrence in Aquatic Systems

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Introduction

Vibrio spp:

- Naturally occurring bacteria in aquatic environments[1]
- Pathogenic if ingested or enter an open wound, causing Vibriosis[2]
- Form symbiotic biofilms with other bacteria in the environment[3]
- Examples: *Vibrio cholerae, Vibrio vulnificus, Vibrio parahaemolyticus*[1,2]

Figure 1: *Vibrio cholerae*[4]

Vibriosis is Increasing with Climate Change

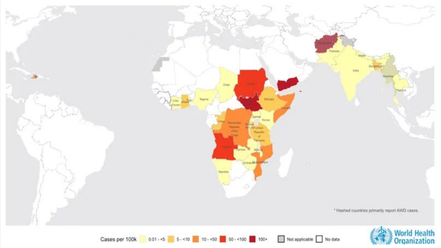

Figure 2: Vibriosis disproportionately Impacts Low and Middle Income Countries[5]

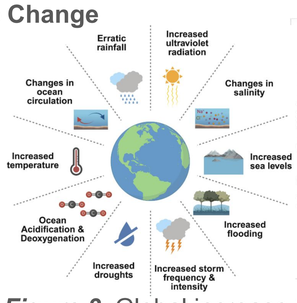

Figure 3: Global increase in Vibriosis is associated with environmental factors[1,7]

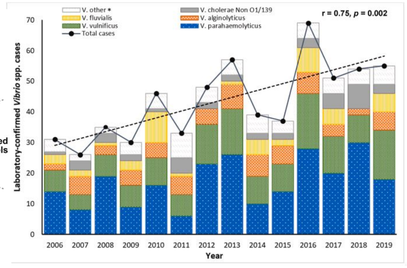

Figure 4: Vibriosis cases are increasing in Maryland[6]

Enterobacteriaceae Co-occurence:

- Enterobacteriaceae (Enterobacteria) are bacteria that can live in animal digestive tracks, such as *E. coli*, *Salmonella*, and *Camplyobacter*. Coliforms are a class of enterobacteria[8]
- *Vibrio* may be negatively correlated with other enterobacteria in the environment, but this relationship has not been extensively studied[9]

Objectives:

1. Statistically explore co-occurrence relationships between enterobacteria and *Vibrio spp.*
2. Determine if the presence of other enterobacteria can be used as a proxy for *Vibrio*, in addition to climate change
3. Provide further evidence that *Vibrio spp.* are native to the environment

Methods

Data Sources:

1. CDC: Bacteria, Enterics, Amoebae, and Mycotics (BEAM) dashboard[10]
2. Maryland Department of Health: Selected Notifiable Conditions Reported in Maryland (2015-2024)[11]

Case Data Analysis:

1. Combined incidence rates for each county
2. Calculated Spearman's correlation coefficient and p-value, adjusted using False Discovery Rate (FDR)[12]

$$P_j = \frac{\binom{N_1}{j} \times \binom{N-N_1}{N_2-j}}{\binom{N}{N_2}}$$

Figure 4: Adapted Hypergeometric Test[13]
N: Total number of states
$N_{1/2}$: Number of states pathogen 1/2 is present
j: Co-occurring states

Presence-Absence Analysis

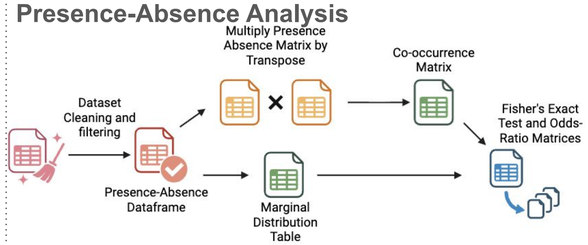

Figure 5: Presence Absence Data Workflow
BEAM report isolate data converted into a state-level presence-absence Dataframe[14]. Marginal totals derived from total state counts, and co-occurrence matrix constructed by transpose matrix multiplication[13, 14, 15]. Fisher's Exact Test and odds ratio calculated for each pathogen pair, and adjusted using False Discovery Rate (FDR) correction[13, 14]

Results and Discussion

Figure 6: Distribution of Enteric Pathogens across 10 U.S. HHS Regions

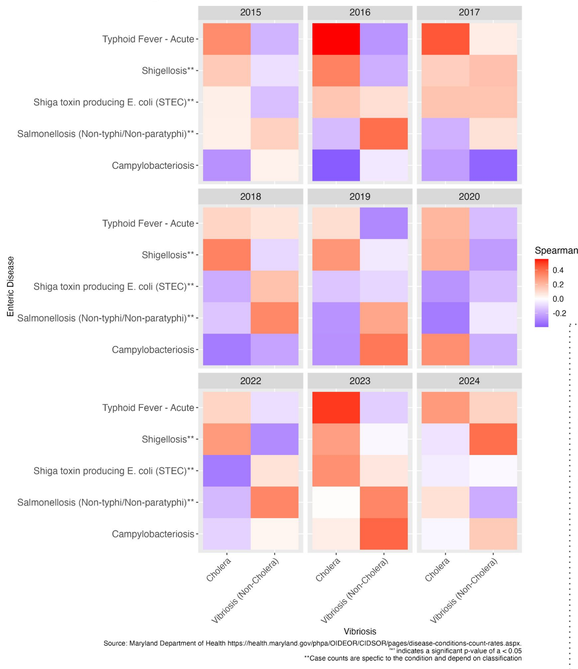

Figure 8: Spearman Correlation of Enteric Disease Case Numbers for all Maryland Counties

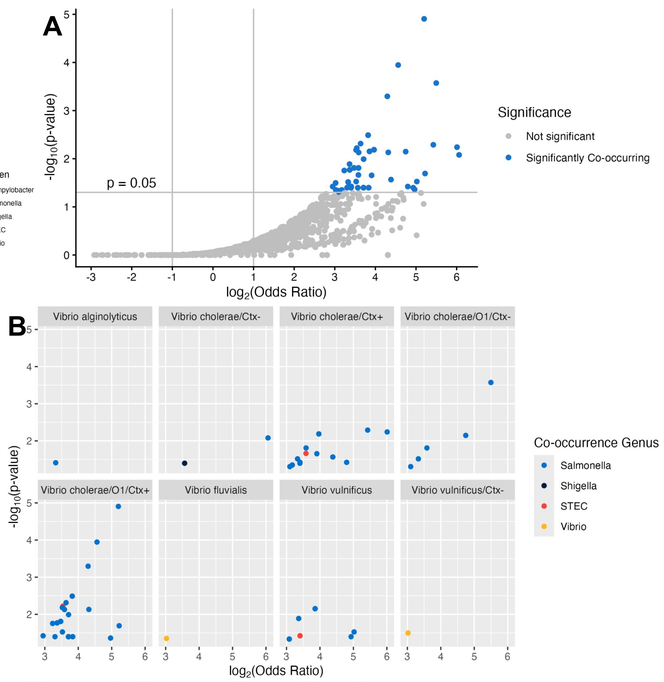

Figure 7: Fisher's Exact Test Enrichment of Presence-Absence Co-Ocurence Matrix
A) Volcano Plot of all co-occurrence pairs and **B)** Significant co-occurrence pairs faceted by Vibrio species.

Discussion:

1. *Vibrio* environmental isolates are consistently low when other sampled enterobacteria counts are high (Figure 6)
2. *Vibrio* may not be significantly positively associated with the presence of other environmental enteric pathogens (Figure 7A)
3. Most of the few significant pairs were *Vibrio cholerae* Ctx+, which pass virulence factors to other aquatic bacteria (Figure 7B)
4. There may be no significant correlation between infectious coliforms and Vibrio, suggesting that coliforms may obtain pathogenicity from non-environmental sources (Figure 8)

Conclusions:

The preliminary results suggest a lack of correlation of the incidence of Enteric bacteria and *Vibrio spp.*, which strongly supports the habitat of Vibrio spp. being the natural environment

Future Directions

- Perform Coliform and *Vibrio* water testing at 4 sites along the Potomac River
- Characterize the flora of these sites using metagenomics
- Calculate co-occurrence significance at these sites by using Fisher's Exact Test

References and Acknolegements

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