**Research Project Title:** Monitoring of emergent microbial pathogens in recreational beaches at Gulf of Mexico marine beaches.

**Project Overview:**

Problem:

Very little environmental information is available about the relationship between fecal indicator bacteria, microbial source tracking markers and waterborne pathogens in beaches impacted by urban runoff at the Gulf of Mexico. The list includes nosocomial and opportunistic pathogens such as *Stenotrophomonas sp*, *Elizabethkingia sp*, and *Pseudomonas aeruginosa*, and *Vibrio* sp. A common characteristic among these microbial opportunistic pathogens is their multidrug resistance. Their distribution in the environment, fate and transport, and relationship to more widely distributed fecal indicators and pathogens is unknown.

Objectives:

* Monitor the distribution of opportunistic pathogens in beaches located at the Gulf coast in Florida, Mississippi, and Texas.
* Compare presence of pathogens as a function of watershed land uses (i.e., hospital presence, CSOs, frequency of SSOs).
* Establish relationship to EPA recommended indicators and MST markers and identify whether established indicators can be used as a surrogate for the presence of the selected waterborne pathogens.

Research Approach:

Marine recreational waters were collected at each of three locations: Picnic Island (PI) FL, Courthouse Road Beach (CH) MS, and Sylvan Park Beach (SB) Tx. Samples were collected by contractor laboratories three days per week (Monday, Tuesday, and Thursday). Collection was performed from June thru December of 2022, following a standardized protocol. Water samples were transported to the U.S. Environmental Protection Agency research facility (Cincinnati, OH) on ice in sterilized containers with shipping temperature maintained at ≤4 ◦C. Water filtration for qPCR testing and enrichment cultures was conducted immediately upon arrival to the laboratory. Additional details about sample collection and DNA extraction can be found in Kelleher et al 2025.

Alkaline peptone broth was used to produce enrichment cultures. DNA was extracted using 500 uL of the enriched samples and filter membranes from unenriched samples following the GeneRite DNA-EZ kit standard protocol. Quantitative PCR (qPCR) was performed with primers to discern amount of *Vibrio cholerae* in this system. Additionally, DNA metabarcoding was conducted targeting the V4 region of the 16S rRNA gene. Both analytical methods used previously published amplification primers. Library preparation and sequencing was conducted using dual indexing 2x300 paired-end Illumina MiSeq sequencing in alignment with standard protocols.

Scope and Significance:

* Understanding the fate and transport of emergent waterborne pathogens and potential exposure to recreators
* Children are particularly susceptible to the emergent pathogens of interest, the information on the relationship to traditionally identified pathogens and indicators will be useful to evaluate how QMRA can be used to address risk to children from swimming exposure.
* Relationship to MST markers will be used to classify polluted sites and/or hot spots based on human waste levels and the presence of pathogens and the influence of non-point pollution sources in mixed-use watersheds (with emphasis on the presence of hospitals, landfills, brownfields, etc.).

References:

Julie Kelleher, Mike Cyterski, Brian R. McMinn, Stephanie Dean, Adin C. Pemberton, Jessica R. Willis, Adam Diedrich, Seth McWhorter, Richard A. Haugland, Orin C. Shanks, Asja Korajkic. 2025. Cultured and molecular measures of fecal indicator microbes in Gulf of Mexico recreational waters, Science of The Total Environment, Volume 966, 178741, ISSN 0048-9697,

https://doi.org/10.1016/j.scitotenv.2025.178741.