

Microbial Source Tracking 2025

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Conceptual Model

- Metagenomic

- Culture/library-dependent



Water sample



Concentrating pipette



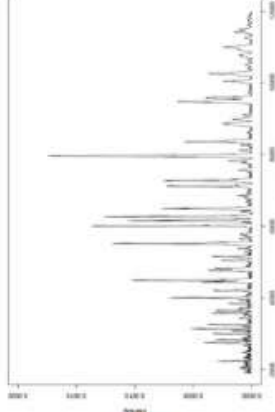
dPCR



IDEXX Enterolet

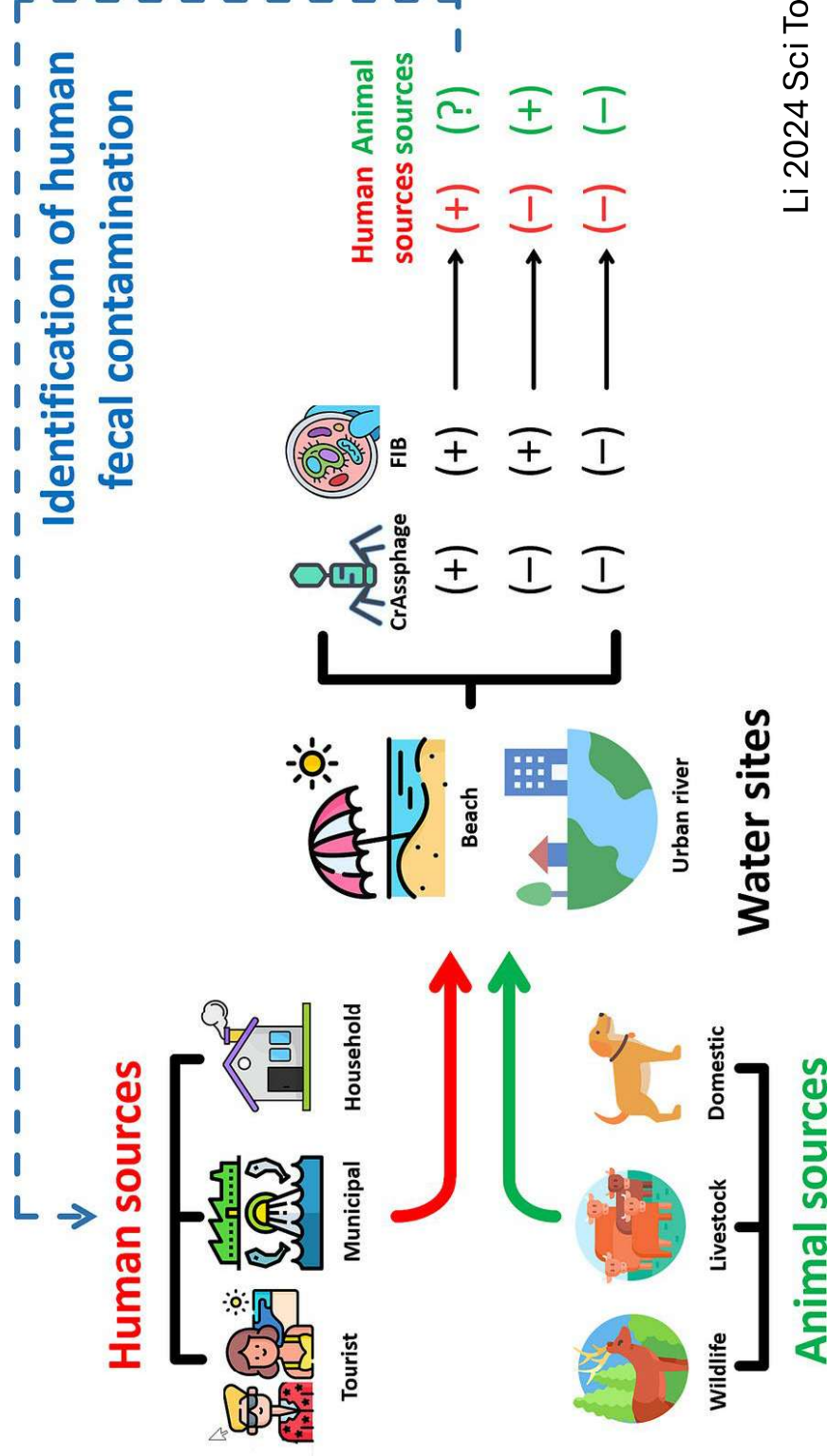


Differential and selective media



Mass spectra

Assessment of crAssphage as a human fecal source tracking marker in the lower Great Lakes



A loop-mediated isothermal amplification assay for the rapid and quantitative tracking of fecal contamination sources in water

- LAMP primers target region used in qPCR assays for crAssphages
- Adaptable to point-of-sampling protocol
- Quantifiable with portable qPCR system



<https://doi.org/10.1016/j.envres.2025.121162>

Colorimetric LAMP

- Tubes held at 65 C for 15 min
- pH sensitive dye reports production of nucleic acid
- Requires minimal equipment



SOP Development

- Routine application of LAMP and other nucleic acid amplification protocols to detect phages will require method standardization
- Filtration of acidified samples is laborious
- Concentrating pipettors show promise but clog easily with estuarine samples
- Current research focuses on evaluating prefiltration with 5 micron eDNA filter



Culture-Dependent: Microbial Source Tracking Database (MSTbase)

- Human waste samples were collected from wastewater treatment plants (WWTPs) and onsite wastewater treatment systems (OWTS).
- Animal waste samples were sourced from dogs, harbor seals, cattle, gull droppings, laboratory rats, and guano from a bat colony.
- Water and sediment samples were collected from Texas A&M University Galveston.
- Libraries were generated by Enterolert kit (IDEXX, Westbrook, ME) and subsequently plated on *Enterococcus* ChromoSelect
- Mass spectra generated by MALDI-TOF

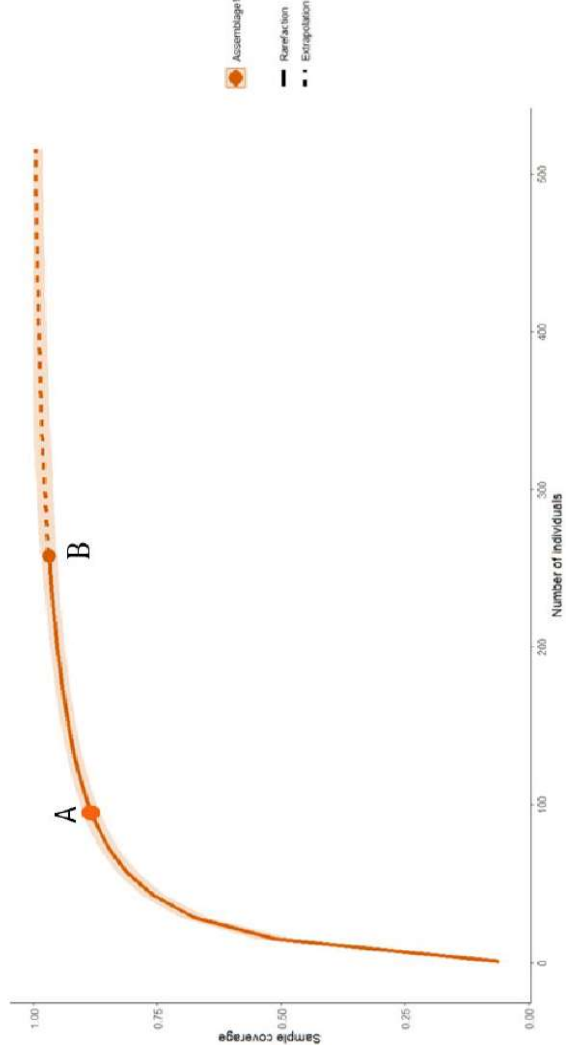


Table 1. *Enterococcus* species identified by MALDI-TOF MS in a Six-Month Period

Species	Total	Jan-25	Dec-24	Nov-24	Oct-24	Sep-24	Aug-24
<i>Enterococcus faecium</i>	2,985	2,300	161	43			481
<i>Enterococcus faecalis</i>	3,264	1,640	70	339	41	24	1,150
<i>E. casseliflavus</i>	1,261	979	33	224	25		
<i>Enterococcus mundtii</i>	821	581	113	111	8	8	
<i>Enterococcus hirae</i>	625	536		41		24	24
<i>E. thailandicus</i>	132	132					
<i>Enterococcus gallinarum</i>	124	123		1			
<i>Enterococcus avium</i>	127	104	8	4			11
<i>Enterococcus COW09</i>	0	49	32	63			
<i>Enterococcus COW12</i>	0	18	4	24			
<i>Enterococcus COW41</i>	0			1			
<i>other Enterococci</i>	19			19			
Sum	9,358	6,462	421	870	74	56	1,666

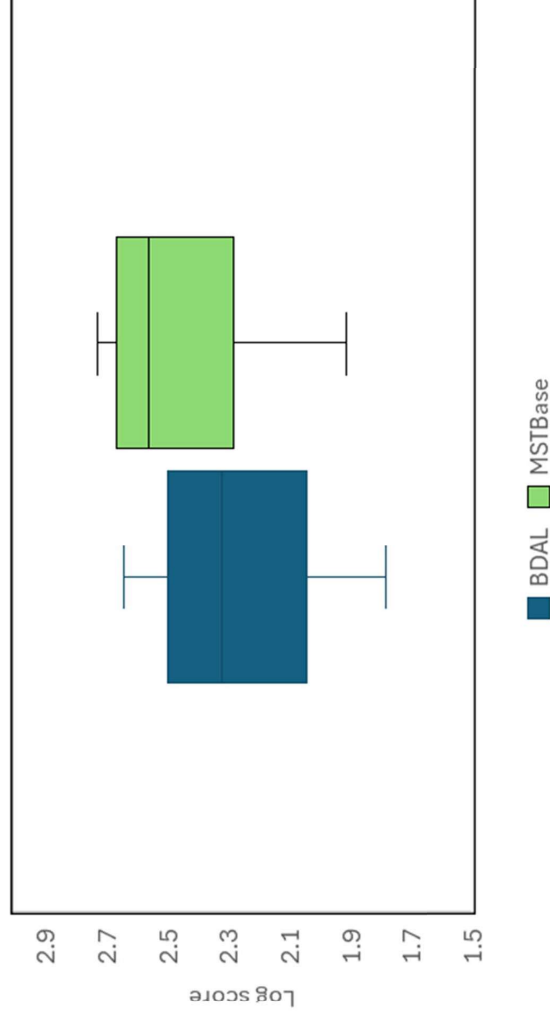
Sample coverage

- A. First 100 isolates covers ~87% of the *Enterococcus spp.* in library.
- B. At ~200 isolates coverage reaches ~98% coverage.

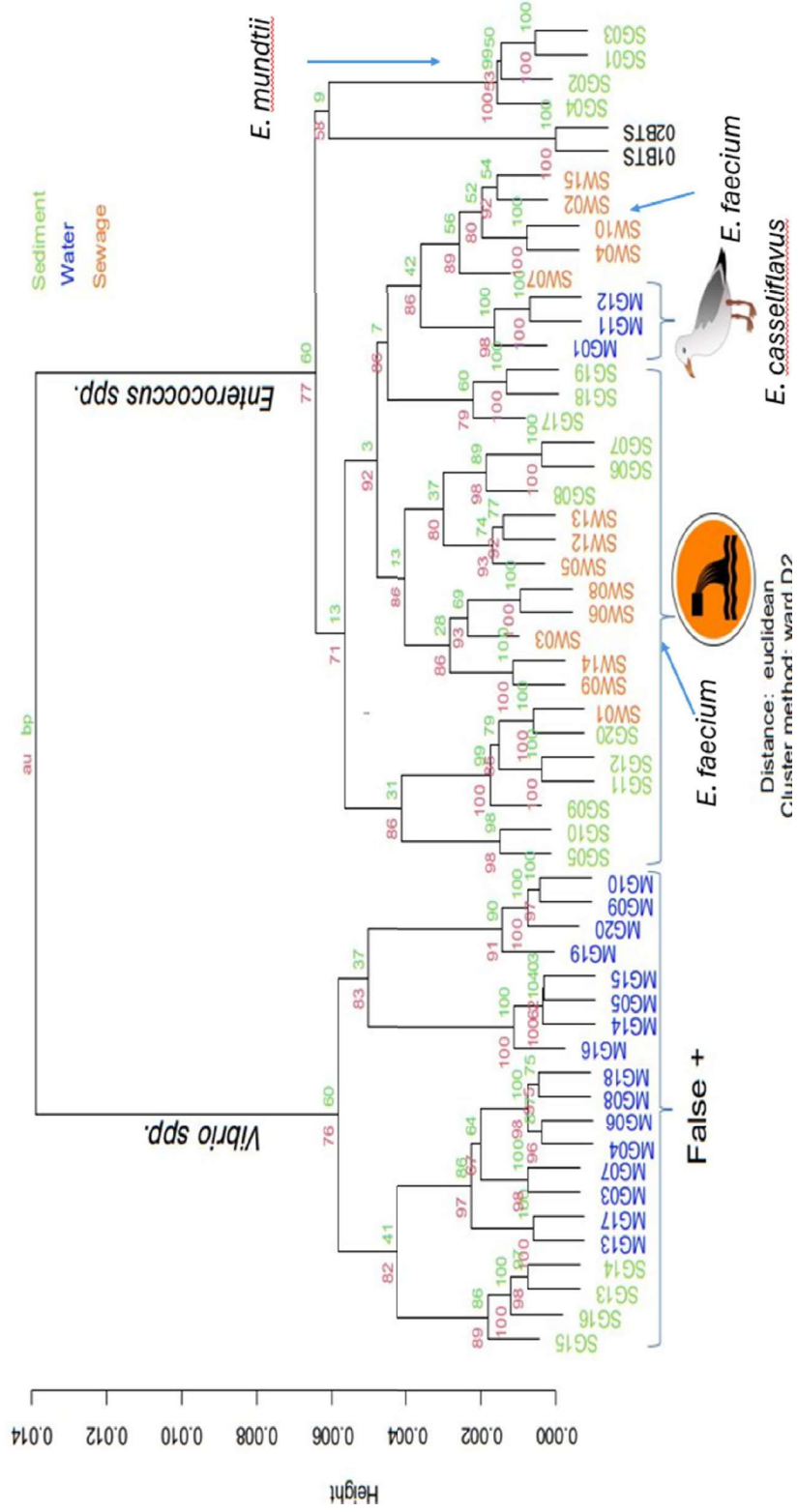


MSTdatabase

- MALDI-TOF systems use commercial database of mass spectra “fingerprints” for microbial identification
- Database contains mostly clinical isolates
- 208 Main Spectra Profile (MSPs) were constructed using FlexAnalysis v3.4 and Compass Explorer
- *Enterococcus spp.*, collected from bats, were re-identified using MSTbase
- Percent change of MSTbase over the BDAL database was ~8.3% indicates MSTbase significantly increased identification over the BDAL database



Cluster dendrogram with p-values (%)



Conclusions

- qLAMP for crAssphages protocol validated
- MALDI-TOF MS shows promise for identifying the source of FIB
- *Vibrio* species may give false positives with IDEXX kit

Acknowledgements

- We'd like to say thank you to Yvonne Mendoza, Julia York, Maddie Joseph, Madison Prince, and all the other undergraduates, for their assistance with this project.
- This project received support from
 - Texas Commission of Environmental Quality
 - National Science Foundation
 - Environmental Protection Agency