Geospatial Distribution of Antimicrobial Resistance Genes in US Rivers and Streams

Jay L. Garland, Ph.D.
Nichole Brinkman, Ph.D.
Scott Keely, Ph.D.
Michael Jahne, Ph.D.
U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Systems Exposure Division

United States Environmental Protection Agency

- ***Overall mission***: To protect human health and the environment

- **Office of Water**: ensures drinking water is safe, and restores and maintains oceans, watersheds, and their aquatic ecosystems.
  - ***Office of Science and Technology***: develops recommended safe water quality levels for toxics, nutrients, and pathogens to help ensure our nation's waters can be used for fishing, swimming, and drinking water
  - ***Office of Wastewater Management***: supports the Clean Water Act by promoting effective wastewater treatment, disposal and management
  - ***Office of Wetlands, Oceans, and Watersheds***: works to protect our freshwater, estuarine, coastal and ocean ecosystems.

- ***Office of Research & Development***: Provides scientific foundation for credible decision-making to safeguard human health and ecosystems from environmental pollutants.

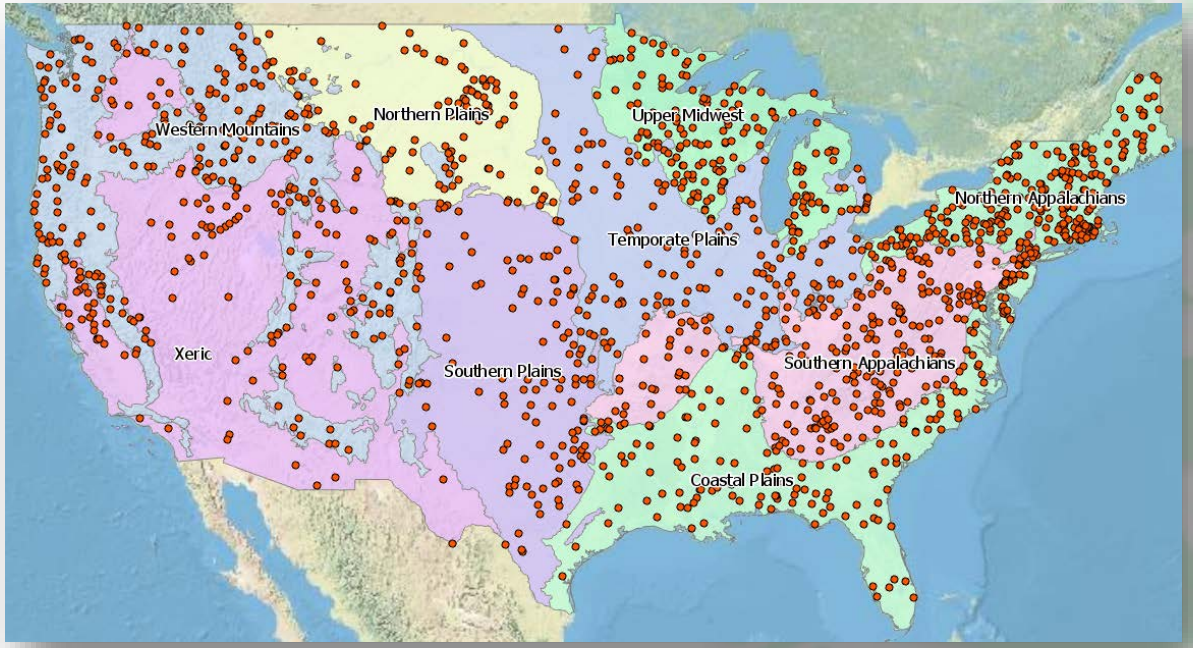
National Watershed Work Opportunity to Monitor ARGs

- The National Aquatic Resource Surveys (NARS) are collaborative programs between the EPA, states, and tribes to assess the quality of the nation's coastal waters, lakes and reservoirs, rivers and streams, and wetlands using a statistical survey design
  - National Rivers and Streams Assessment (NRSA)
  - National Coastal Condition Assessment (NCCA)
  - National Lakes Assessment (NLA)
  - National Wetland Condition Assessment (NWCA)

- Surveys are conducted annually; 5 year survey cycle

National Aquatic Resource Surveys (sampling sites)

National Rivers and Streams Assessment (NRSA)



National Coastal Condition Assessment (NCCA)

(Colors show different ecoregions)

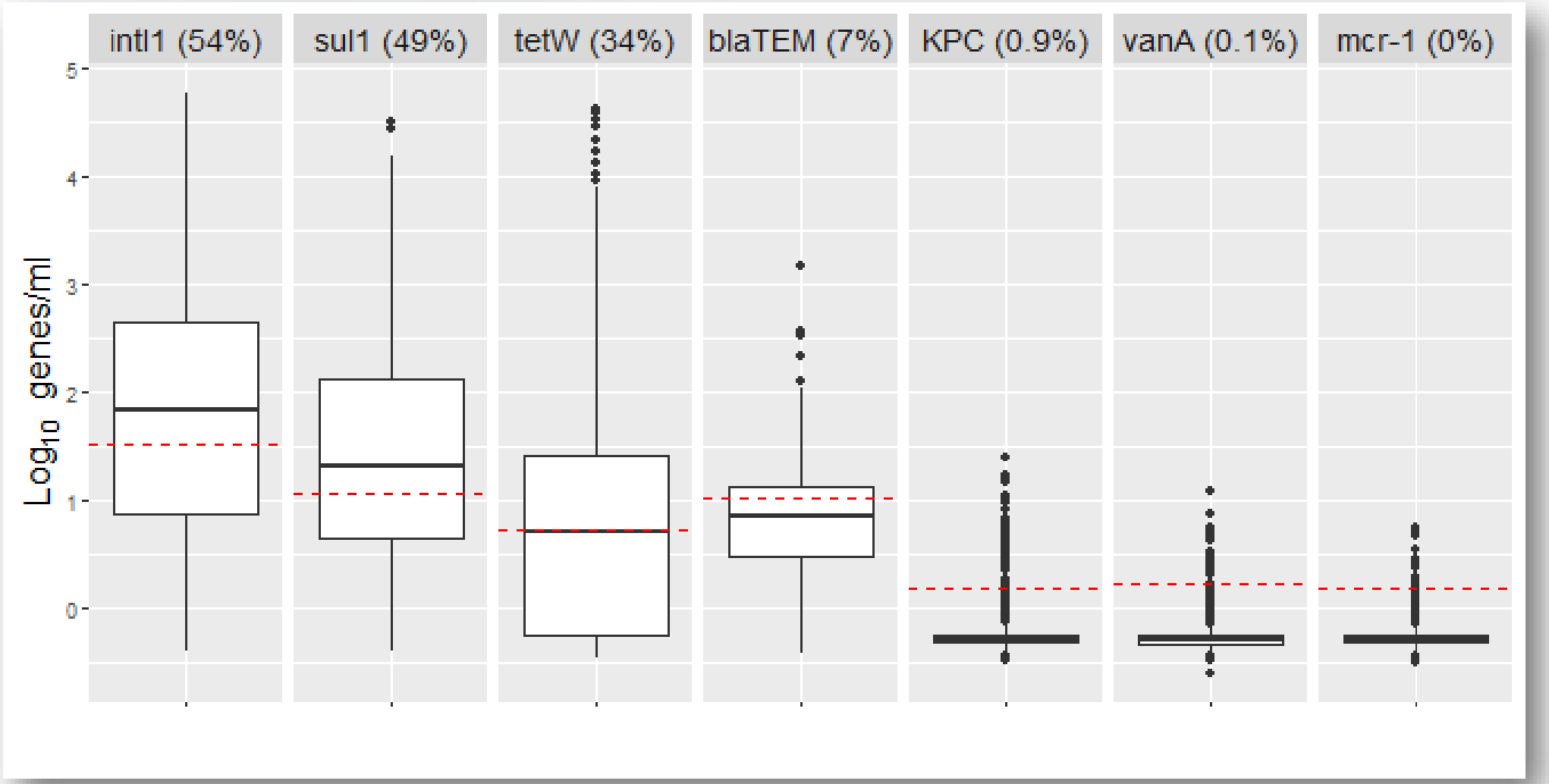
Approach for ARGs

- Enterococcus US EPA Method 1609 was used for sample processing
  - QAQC (holding time standards)
  - Filtered 50 mL water samples collected as part of the NRSA
  - Bead beating was used to disrupt cells (1609)
  - Salmon sperm DNA recovery control
- DNA was extracted using GeneRite DNA-EZ kit
  - Stored at -80C
- Droplet digital PCR (ddPCR, BioRad) was used to quantify specific gene targets
  - Each ddPCR well represents 0.83 mL of stream water
  - A well contains thousands of droplets (nanoliter endpoint PCR reactions)
  - Bayesian statistics was used to estimate final concentrations and credible intervals
- Values greater than the LLOQ and survey weights together were used to calculate the occurrence of ARGs in river kilometers

Selected AMR Genes

Gene	Antimicrobial Target	Mechanism of Action	Abundance in screened samples
bla_{TEM}	Beta-lactam	Cell wall synthesis	Medium
KPC	carbapenem	Cell wall synthesis	Low
NDM	carbapenem	Cell wall synthesis	Low
OXA-48	carbapenem	Cell wall synthesis	Low
VIM	carbapenem	Cell wall synthesis	Low
vanA	vancomycin	Cell wall synthesis	Low
mecA	methicillin	Cell wall synthesis	Low
qnrA	quinolone	DNA replication	Low
tetB	tetracycline	Protein synthesis	Low
tetM	tetracycline	Protein synthesis	Medium
tetW	tetracycline	Protein synthesis	Medium
mcr1	colistin	Cell membrane	Low
sul1	sulfonamide	Anti-metabolism	High
intI1	Not applicable	Captures gene cassettes	High
intI2	Not applicable	Captures gene cassettes	low

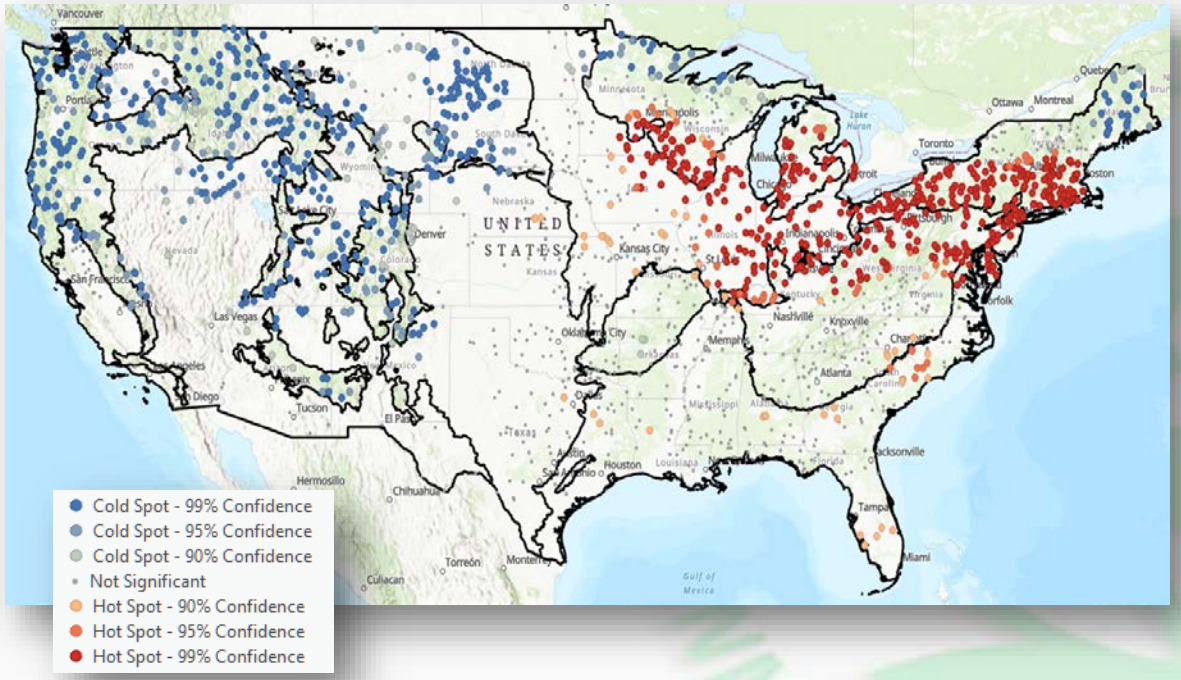
Median Concentration of Targeted Genes



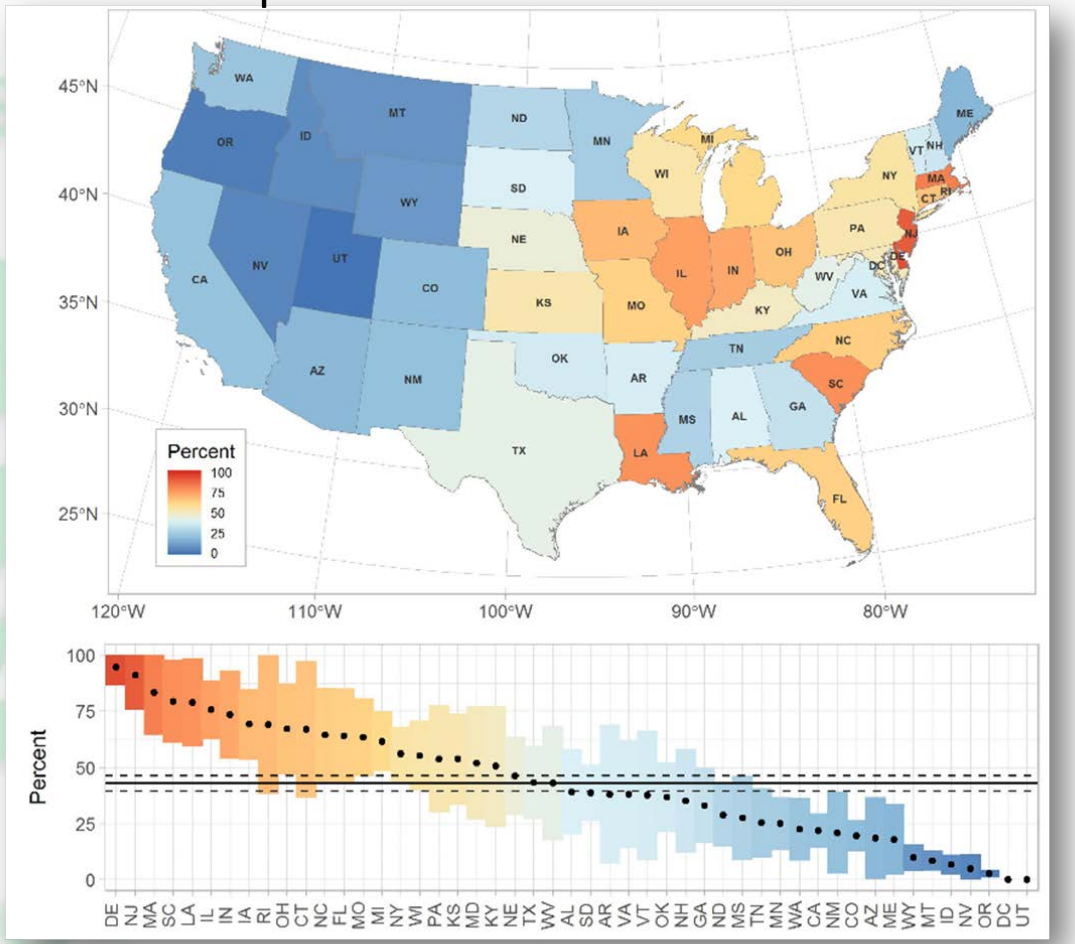
n=1937 samples

Geospatial Distribution intl1

Hotspot Analysis of *intl1* (occurrence =54%)

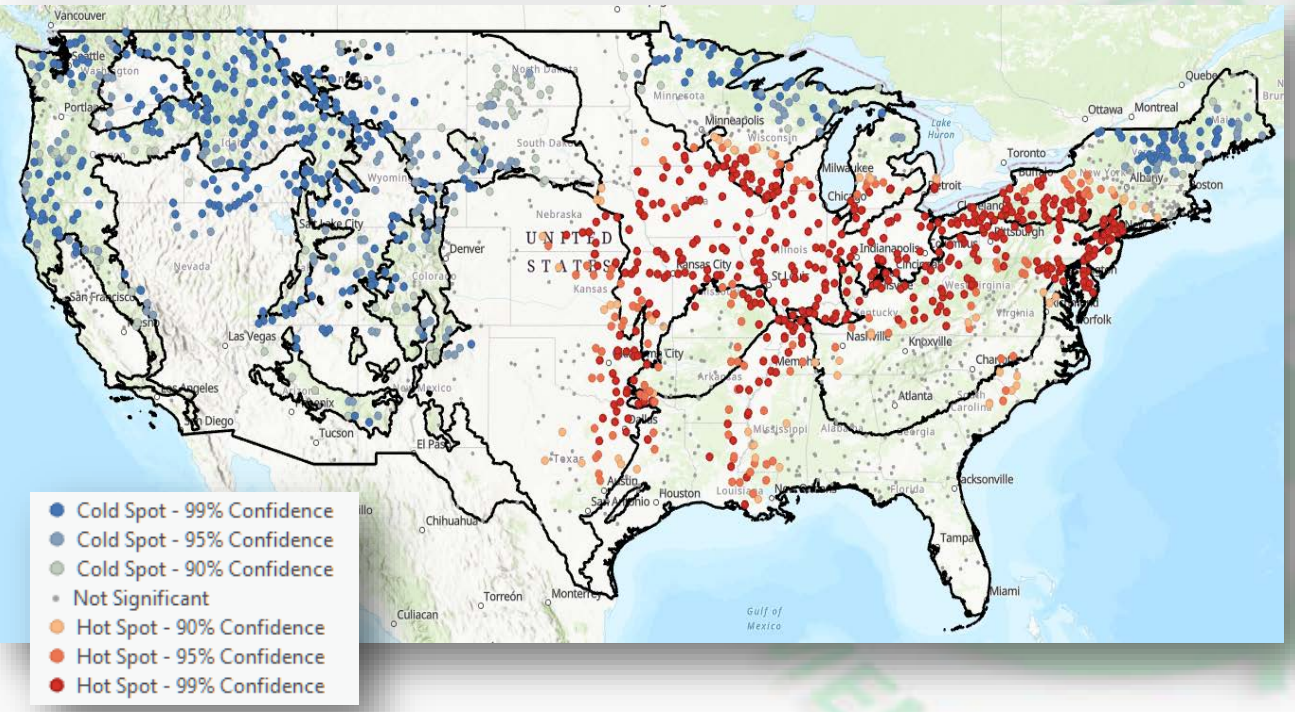


Percent of river km above lower limit of quantification

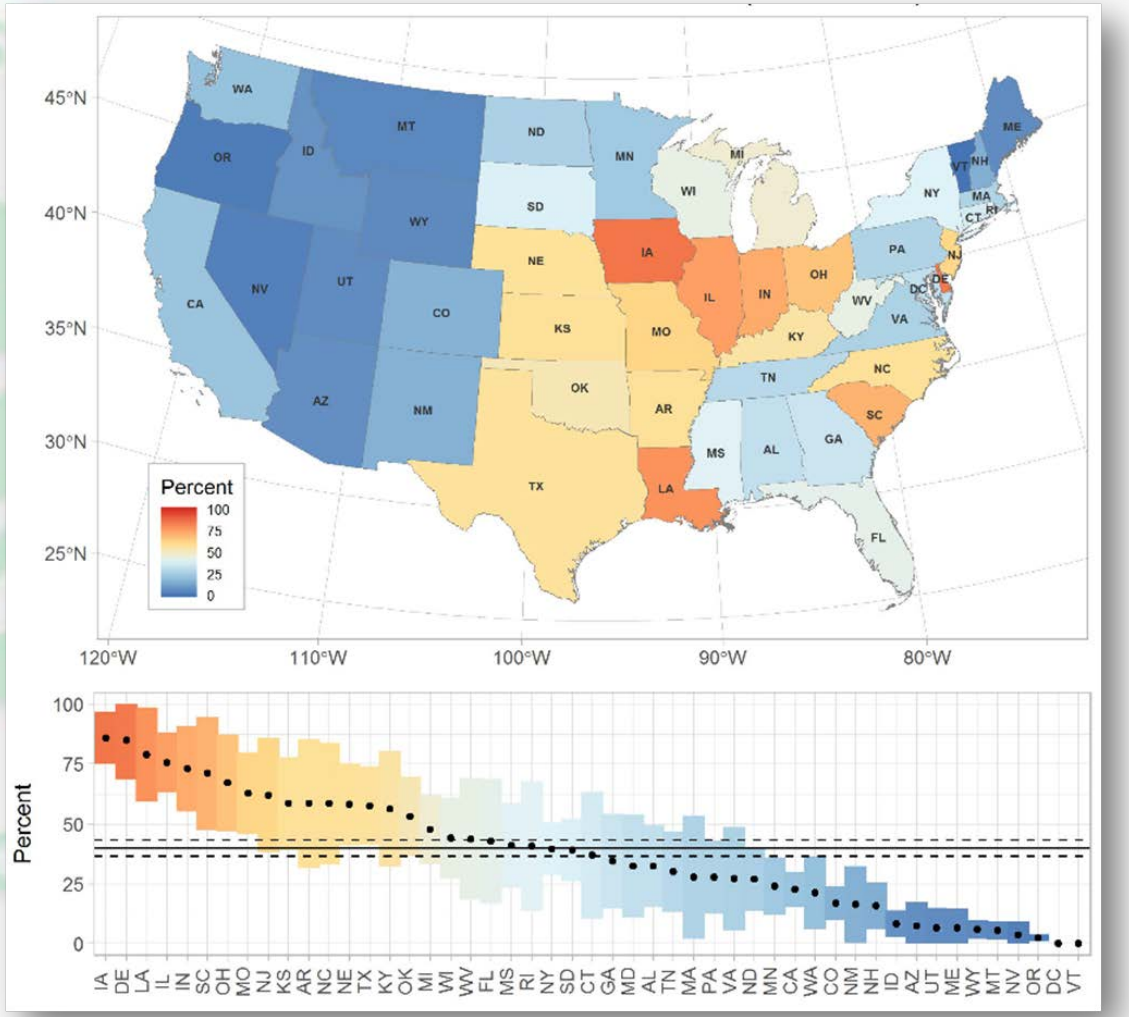


Geospatial Distribution sul1

Hotspot Analysis (occurrence =49%)



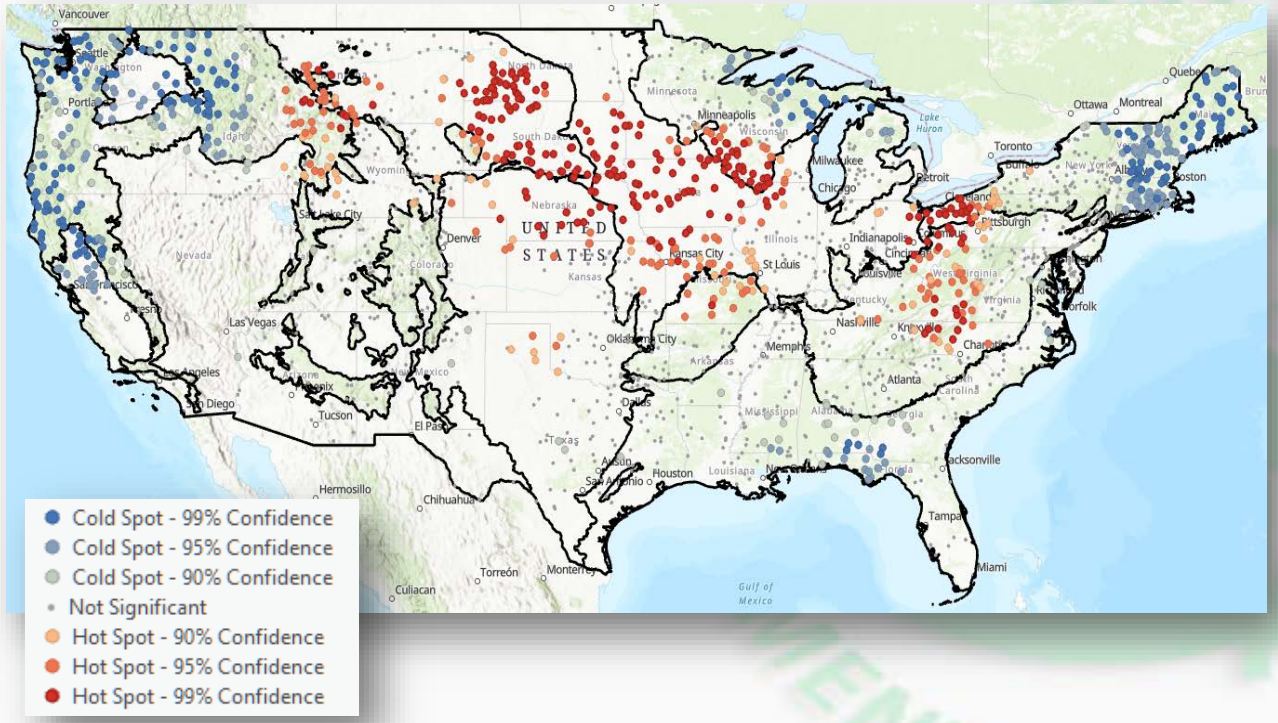
Percent of river km above lower limit of quantification



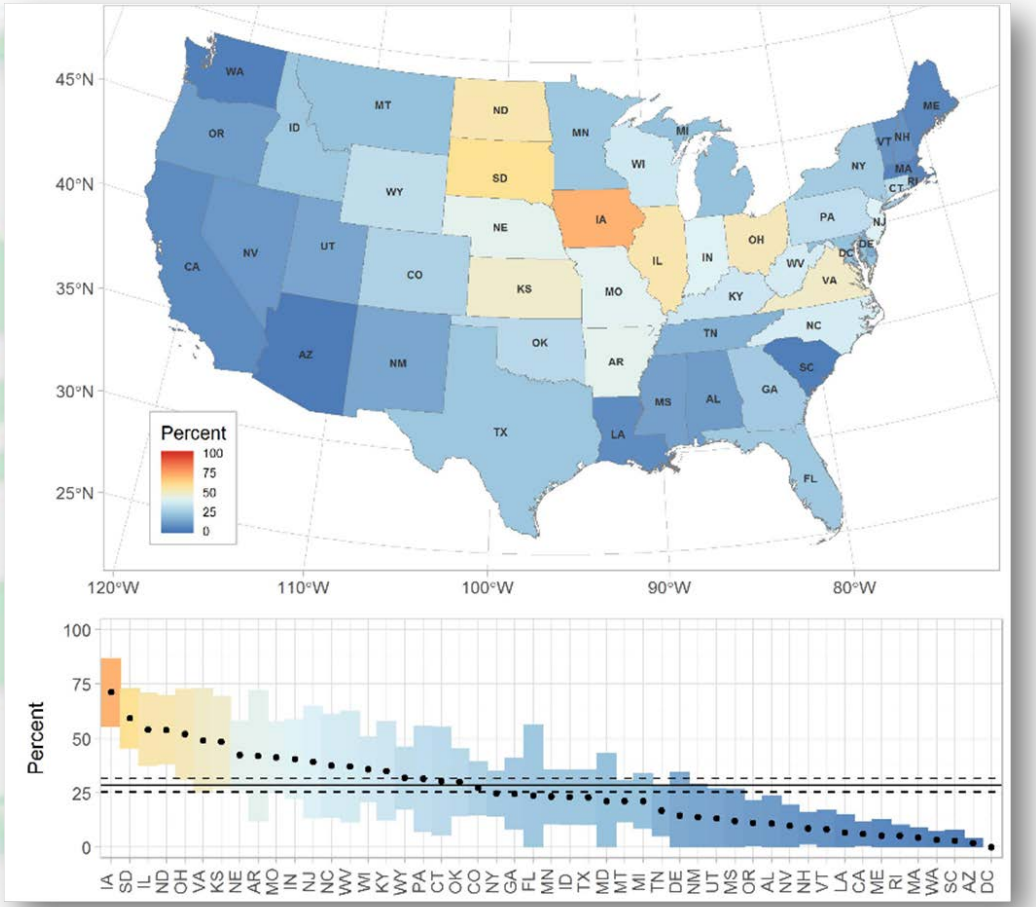
Geospatial Distribution

tetw

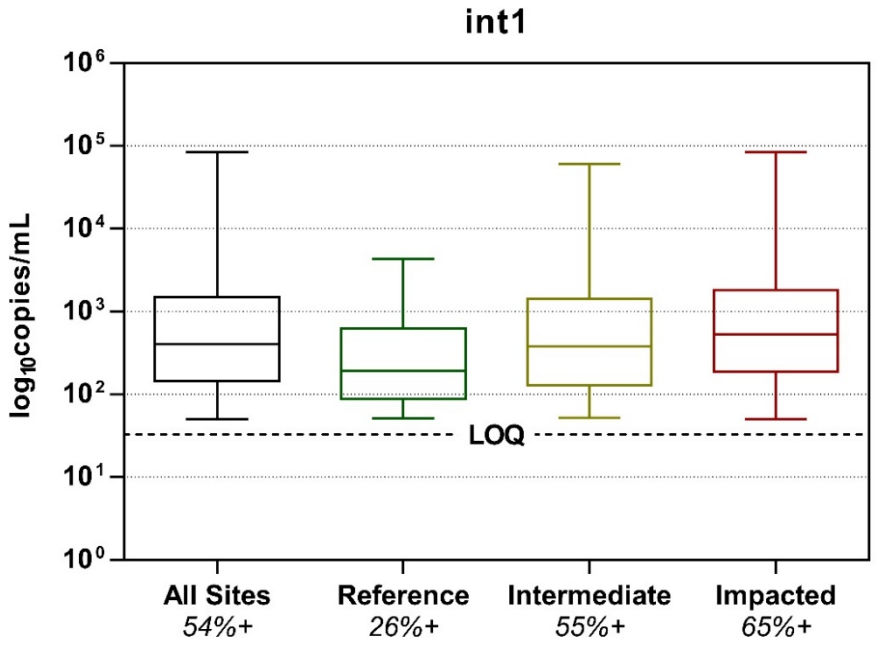
Hotspot Analysis (Occurrence = 34%)

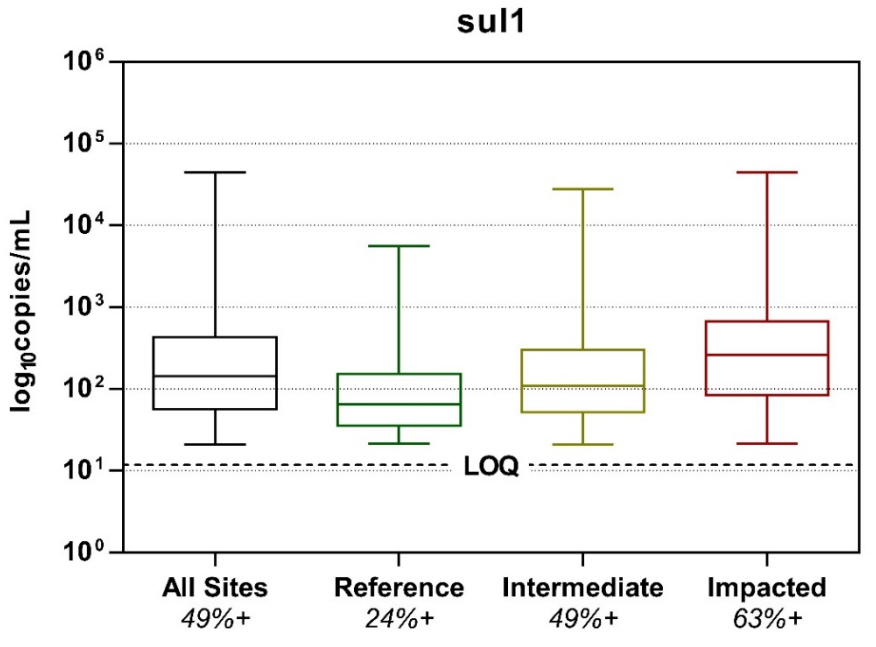


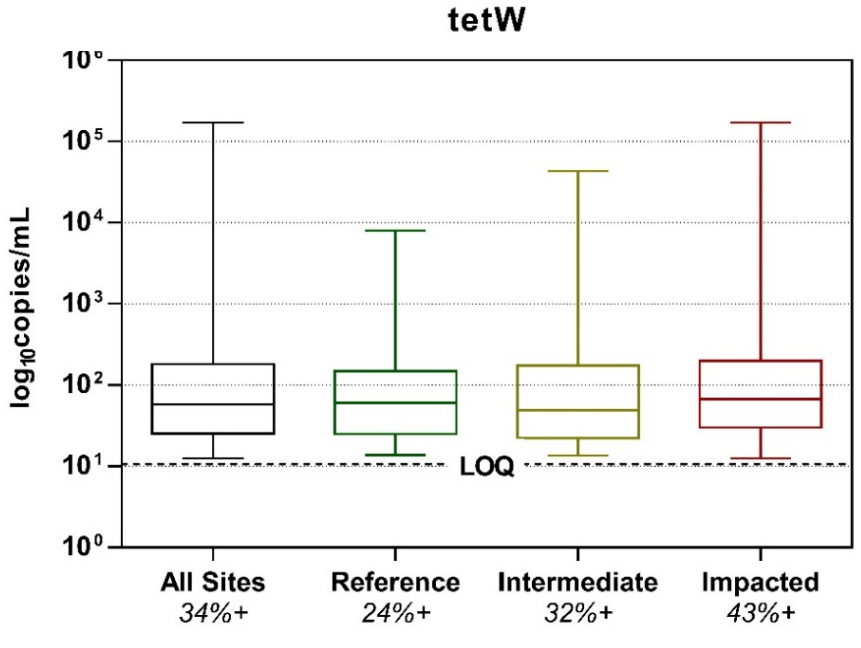
Percent of river km above lower limit of quantification



Impacted vs Reference Sites





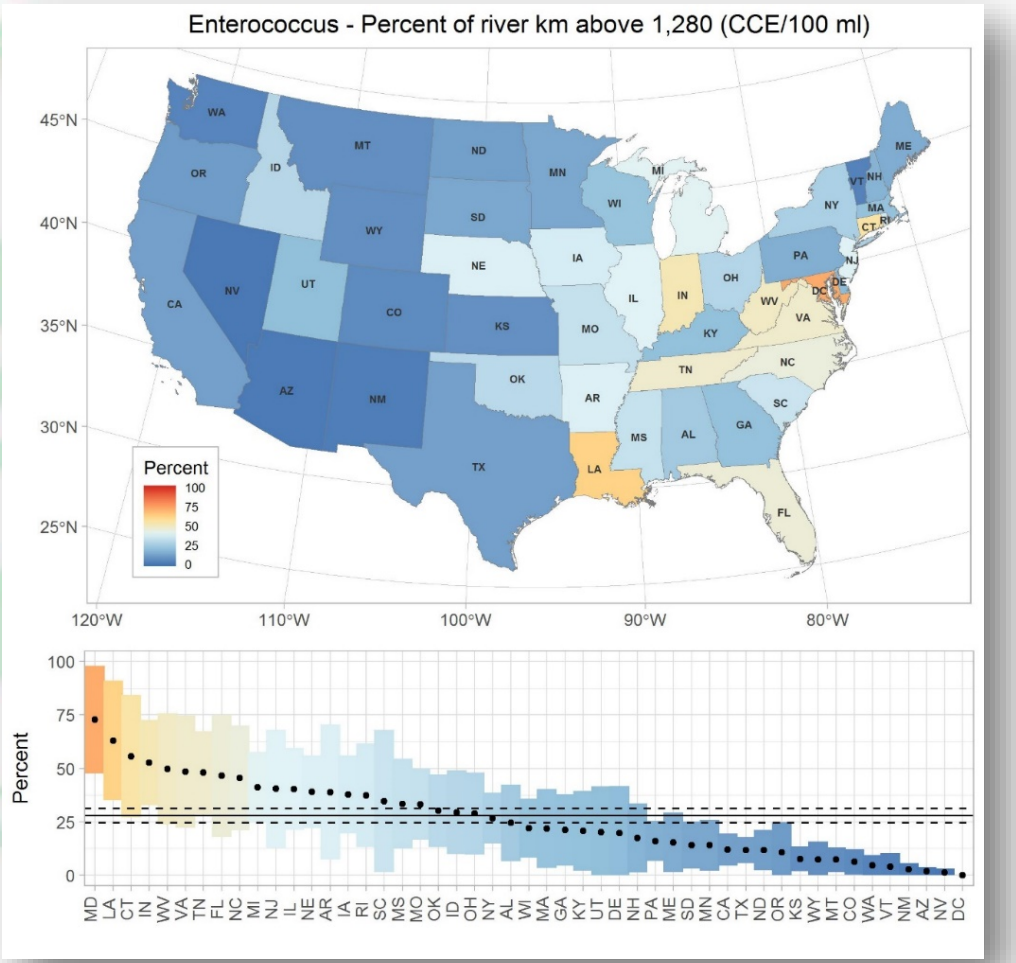


Geospatial Distribution

Enterococcus (fecal indicator)

Hotspot Analysis (Occurrence = 34%)

Percent of river km above lower limit of quantification



Next Steps

- Continue NARS analysis
  - Analyze 2015 Coastal Condition Assessment Samples
  - Analyze the 2018-2019 NRSA samples
  - Develop predictive models of occurrence (drivers)
- Human exposure modeling
  - Use NRSA ARG predictive models to estimate recreational exposure risk
    - AMR complexities challenge traditional QMRA approach
  - Map geospatial distribution of potential risk by activity type and age group to estimate state- and national-level exposure burdens
  - More targeted analysis within recreational water studies
    - To include culture based analysis of pathogens
- Wastewater analysis
  - Focus on high strength streams from hospitals & other health care facilities

Acknowledgments

- Office Research and Development, US EPA
  - Emily Wheaton, MS
  - Richard Haugland, PhD
  - Roy Martin, PhD
  - Scott Leibowitz, PhD
  - Ryan Hill, PhD
- Office of Water (OST)
  - Sharon Nappier, PhD
- USEPA funded and collaborated in the research described herein.
- Richard Mitchell, PhD, Karen Blocksom, PhD Tony Olsen, PhD of USEPA provided assistance in data acquisition