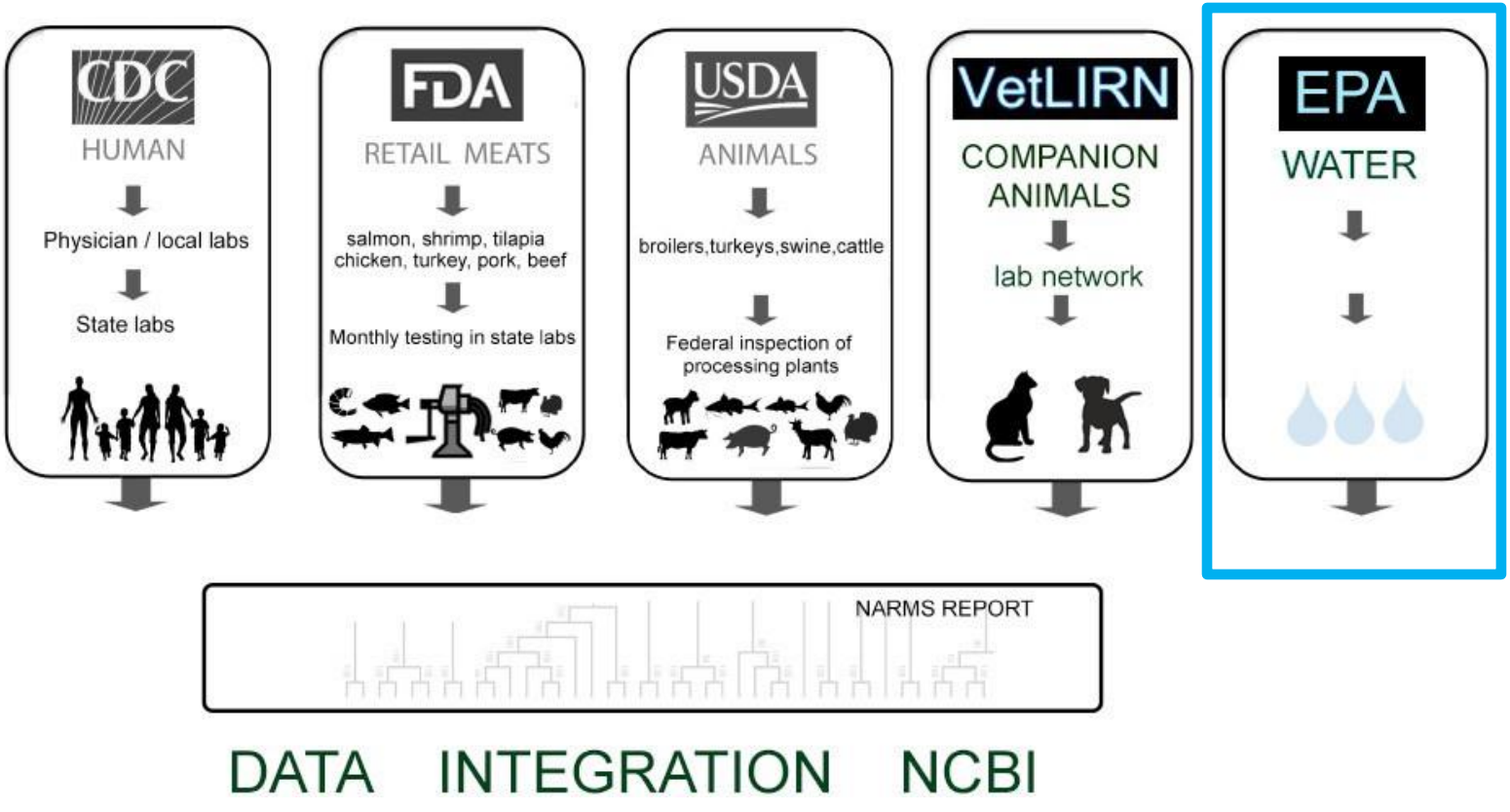
Cross-Agency Collaboration: NARMS and EPA AMR in the Environment

Andrea Ottesen PhD

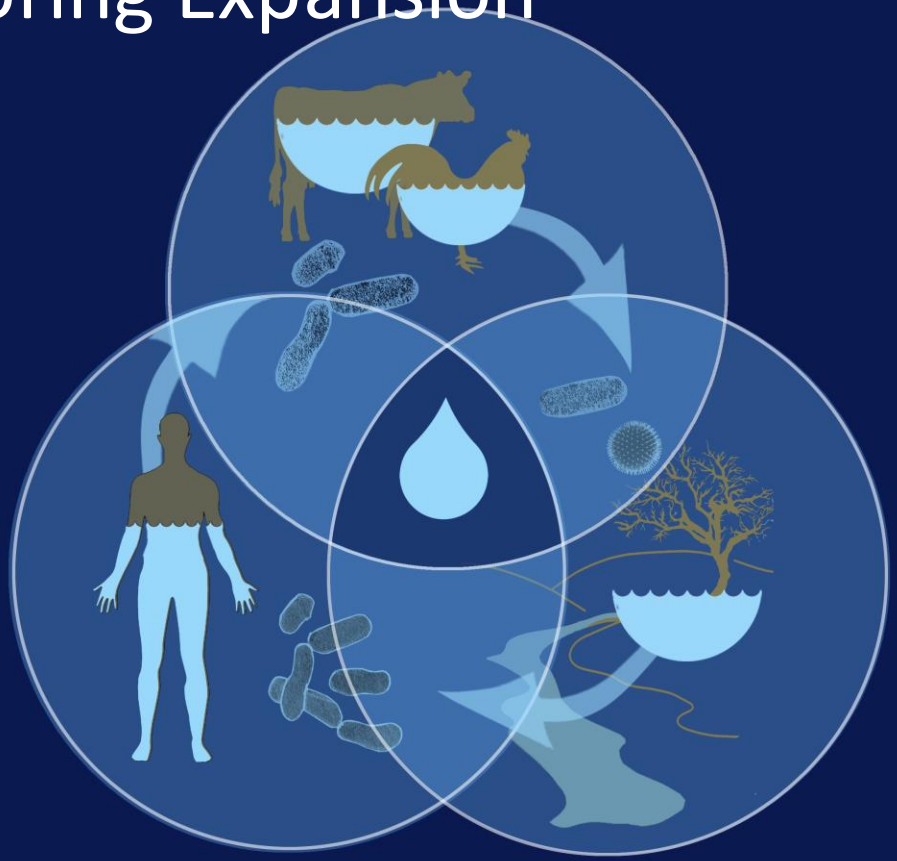
Center for Veterinary Medicine
Office of Veterinary Product Surveillance and Compliance
AMR Research Branch
Andrea.Ottesen@fda.hhs.gov

NARMS INTEGRATED SURVEILLANCE



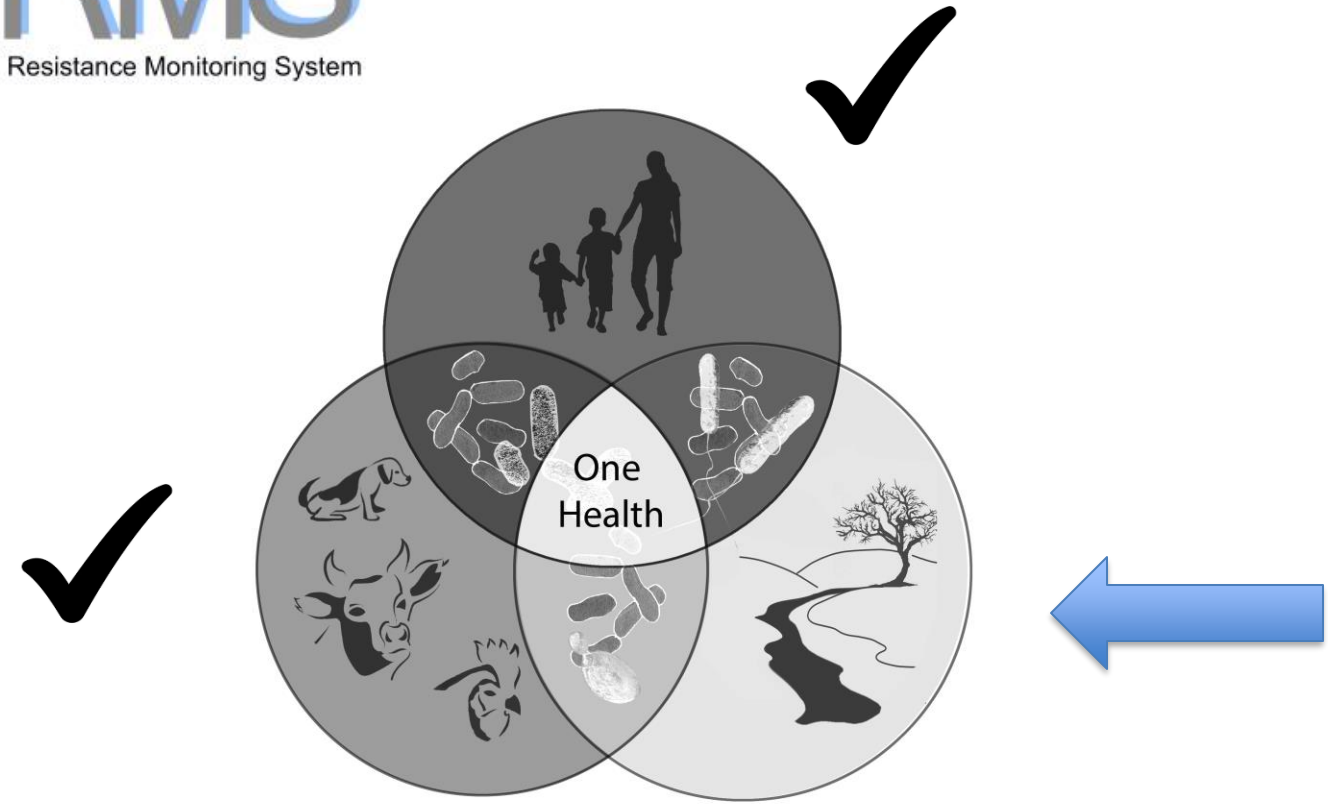
Veterinary Laboratory Investigation and Response Network, Environmental Protection Agency

AMR Monitoring Expansion



NARMS
National Antimicrobial Resistance Monitoring System

FDA




Alison Franklin


Jay Garland


Ben Davis


Mike Jahne

NRSA



Two Foci: NRSA sites and Little Miami River in Ohio

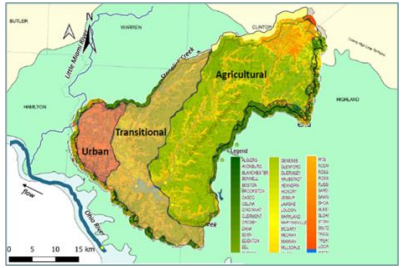
1. National Rivers and Streams Assessment (NRSA) study with 2000 sites across the country, culturing key pathogens and metagenomic evaluation

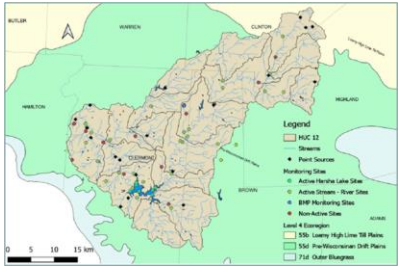
2. High resolution watershed study in Ohio

Isolates: *Salmonella, ESBL E. coli, Enterococcus*

Metagenomes: CI and Enriched

Watershed Study: East Fork of Little Miami River (Ohio)













FDA

Metagenomic

Synechococcaceae	25.87129
Synechococcus	7.411881
Cyanobium	4.619358
Polynucleobacter	3.624273
Methylophilaceae	2.642325
Burkholderiales	1.965825
Microcystis	1.536707
Flavobacterium	1.337165
Erythrobacteraceae	1.278177
Microcystis aeruginosa	0.807889
Aphanizomenonaceae	0.744664
Aeromonas veronii	0.677083
Raphidiopsis brookii	0.434181

Other 47%

BPW

Aeromonas veronii	19.61416
Aeromonas	13.11536
Pseudomonas guangdongensis	7.808017
Aeromonas caviae	6.448325
Clostridium bifermentans	4.966663
Aeromonadaceae	4.775516
Cetobacterium somerae	3.591674
Kurthia	3.277574
Pseudomonas otitidis	3.219884
Comamonas aquatica	2.113339
Vibrio fischeri	1.759849
Vibrio vulnificus	1.514175
Vibrio	0.680173

Other 27%

RV

Salmonella enterica	43.65412
Escherichia coli	22.09408
Salmonella diarizonae	18.13907
Salmonella	9.064401
Escherichia	1.621242
Cronobacter malonaticus	0.83324
Cronobacter	0.337717
Aeromonas veronii	0.307909
Enterobacter hormaechei	0.280082
Aeromonas caviae	0.267738
Citrobacter freundii	0.263912
Enterobacter cloacae	0.261563
Escherichia CL4	0.137805

Other 2%

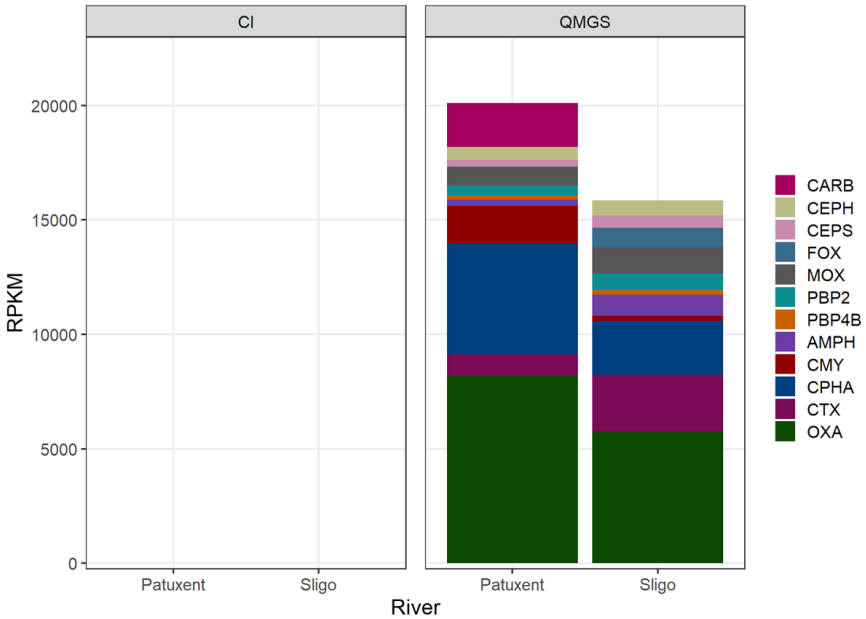
TT

Proteus mirabilis	34.06761
Enterobacter cloacae	12.61134
Morganellaceae	9.658205
Proteus	9.01444
Salmonella enterica	8.381177
Enterobacter kobei	4.058408
Klebsiella pneumoniae	2.61847
Citrobacter sedlakii	2.361597
Providencia stuartii	1.894028
Providencia rettgeri	1.616131
Salmonella diarizonae	1.060506
Shewanella haliotis	0.968642

Other 11%

expediting source tracking, illuminating unseen serovar diversity in outbreaks, lab contamination, emerging pathogens, AMR transmission

What can this approach do for AMR ?



PLOS WATER

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Advancing antimicrobial resistance monitoring in surface waters with metagenomic and quasimetagenomic methods

Andrea Ottesen, Brandon Kocurek, Padmini Ramachandran, Elizabeth Reed, Seth Commichaux, Gunnar Engelbach, Mark Mammel, Sanchez Saint Fleurant, Shaohua Zhao, Claudine Kabera, Amy Merrill, Nathalie Bonin, Hannah Worley, [...], Errol Strain [view all]

Published: December 14, 2022 • https://doi.org/10.1371/journal.pwat.0000067

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Enrichment Procedure

(Modified Standard Method 9260.B2)

- Water sample (~1L) filtered with diatomaceous earth & 3uM filter
- Filter with diatomaceous earth cake added to BPW:
  - 18-24H @ 37°C
- BPW enrichment (1ml) added to Gram Negative Broth (9mL) and TT Broth (9ml):
  - GN: 24H @ 37°C
  - TT: 48H @ 37°C
- GN & TT enrichments (100ul) added to RV broth (9.9mL):
  - 24H @ 37°C

‣ Appl Environ Microbiol. 2022 May 9;88(10):e00393-22. doi: 10.1128/aem.00393-22

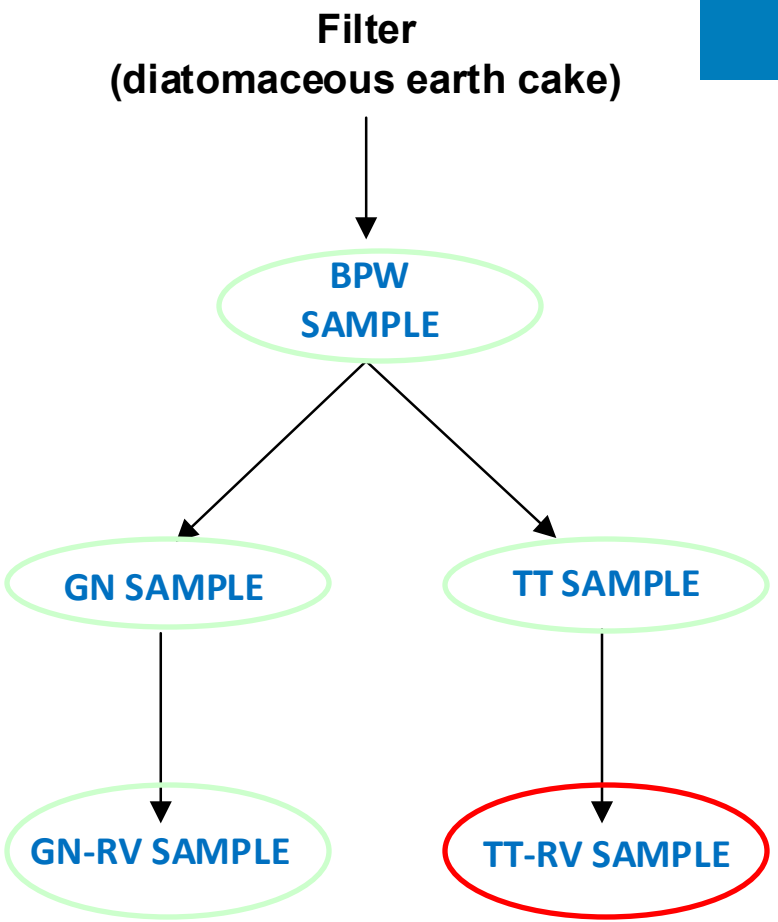
Analysis of *Salmonella enterica* Isolated from a Mixed-Use Watershed in Georgia, USA: Antimicrobial Resistance, Serotype Diversity, and Genetic Relatedness to Human Isolates

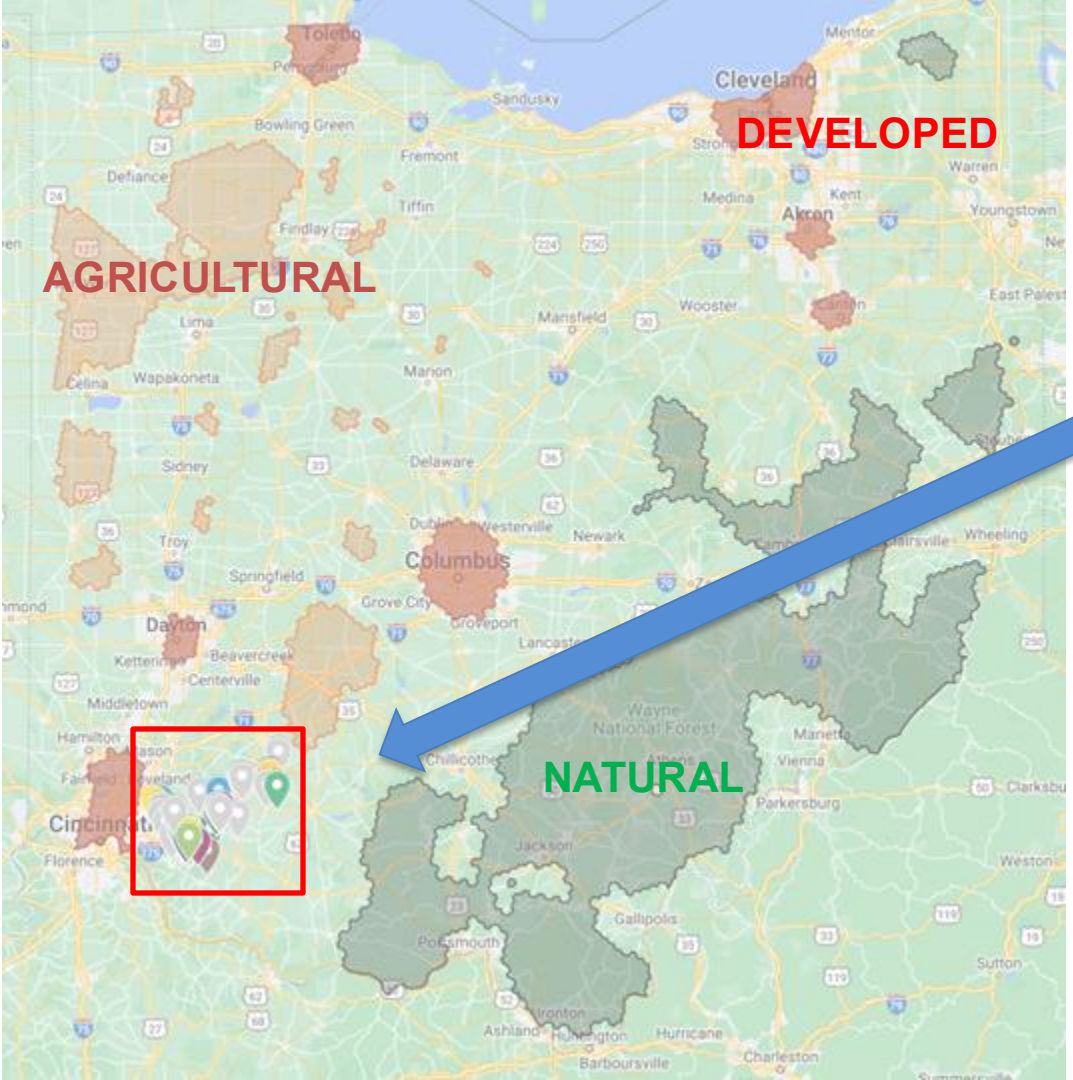
Sohyun Cho [a,b], Lari M Hiott [a], Sandra L House [a], Tiffanie A Woodley [a], Elizabeth A McMillan [a], Poonam Sharma [a,*], John B Barrett [a], Eric S Adams [c], Joshua M Brandenburg [b,d], Kelley B Hise [d], Jacob M Bateman McDonald [e], Elizabeth A Ottesen [f], Erin K Lipp [g], Charlene R Jackson [a], Jonathan G Frye [a,✉]

Editor: Christopher A Elkins[h]

‣ Author information ‣ Article notes ‣ Copyright and License information

PMCID: PMC9128517 PMID: 35532233

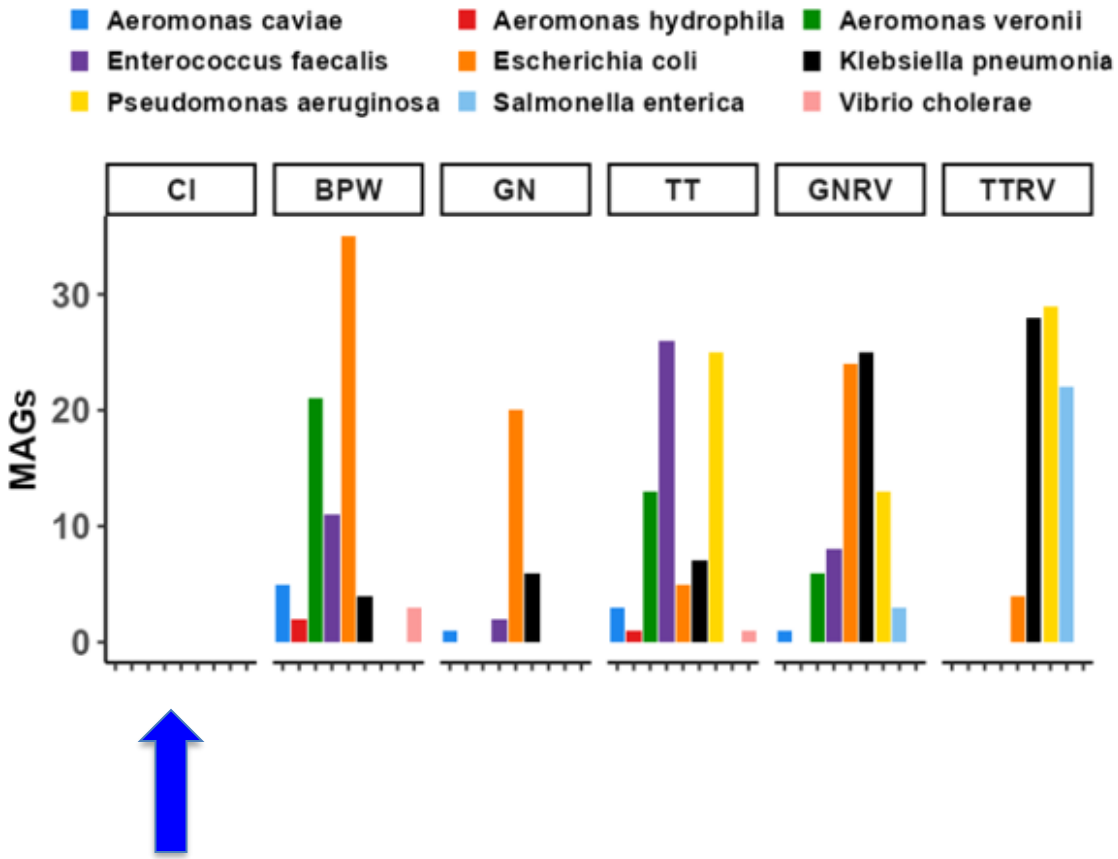




Ohio Watershed

- 36 sites across the East Fork Little Miami River (EFLMR) watershed (Ohio)
  - Mixed Use Watershed
- Culture Independent and Enrichments:
  - BPW, GN, GN/RV, TT, TT/RV

More support for the NGS enrichment (quasimetagenomic) approach for detection of AMR



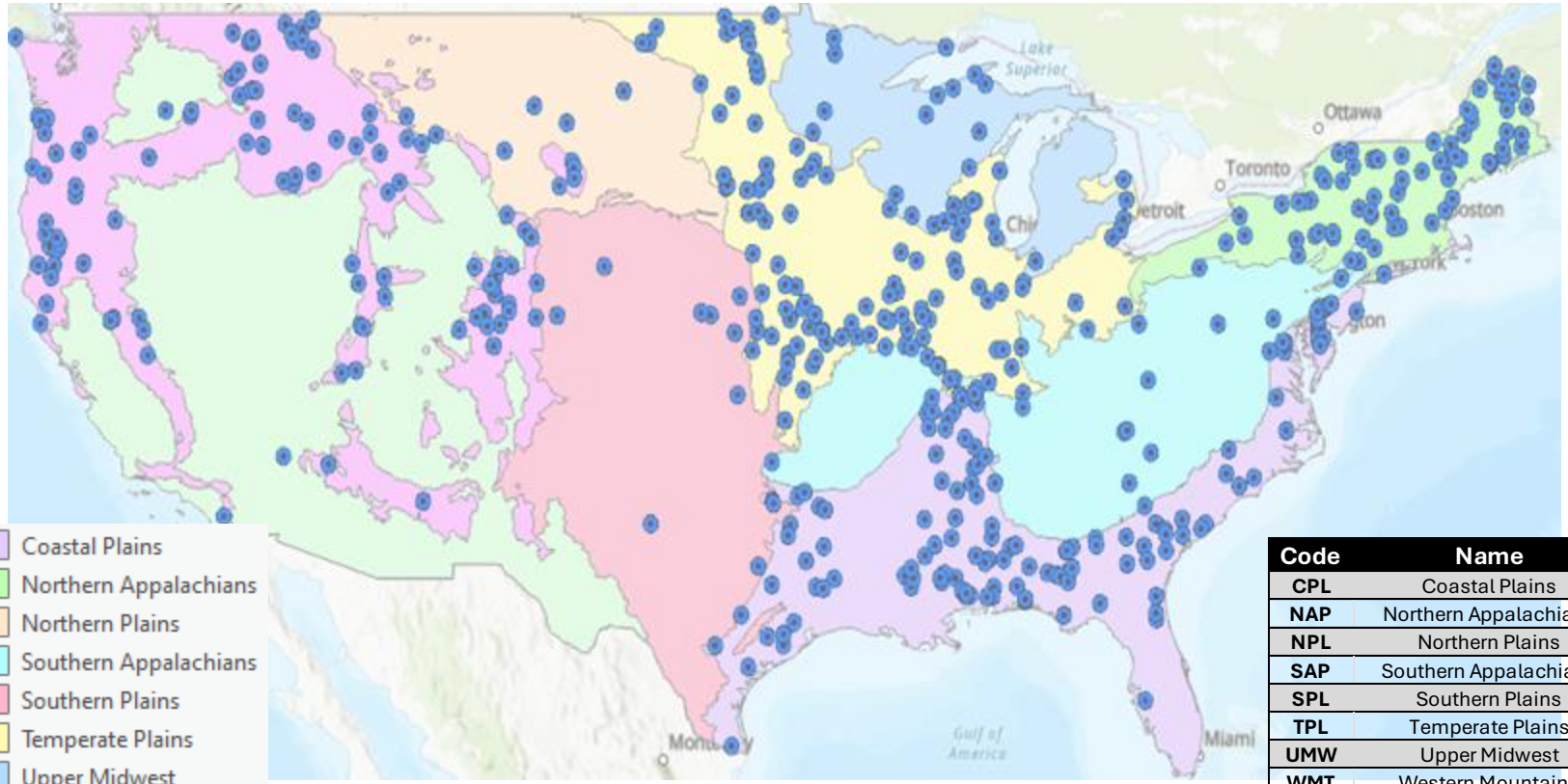
National Rivers and Streams Assessment NRSA

- 513 **TT-RV** samples
  - 42 US States (n=508) & Guam (n=5)

- 9 NRSA EcoRegions (Level III)
- 16 USGS Hydrologic Basins
- 353 USGS Level 8 Hydrologic Unit Regions
- Culture Results (*Salmonella*):
  - Positive (+) → n= 215
  - Negative (-) → n= 298

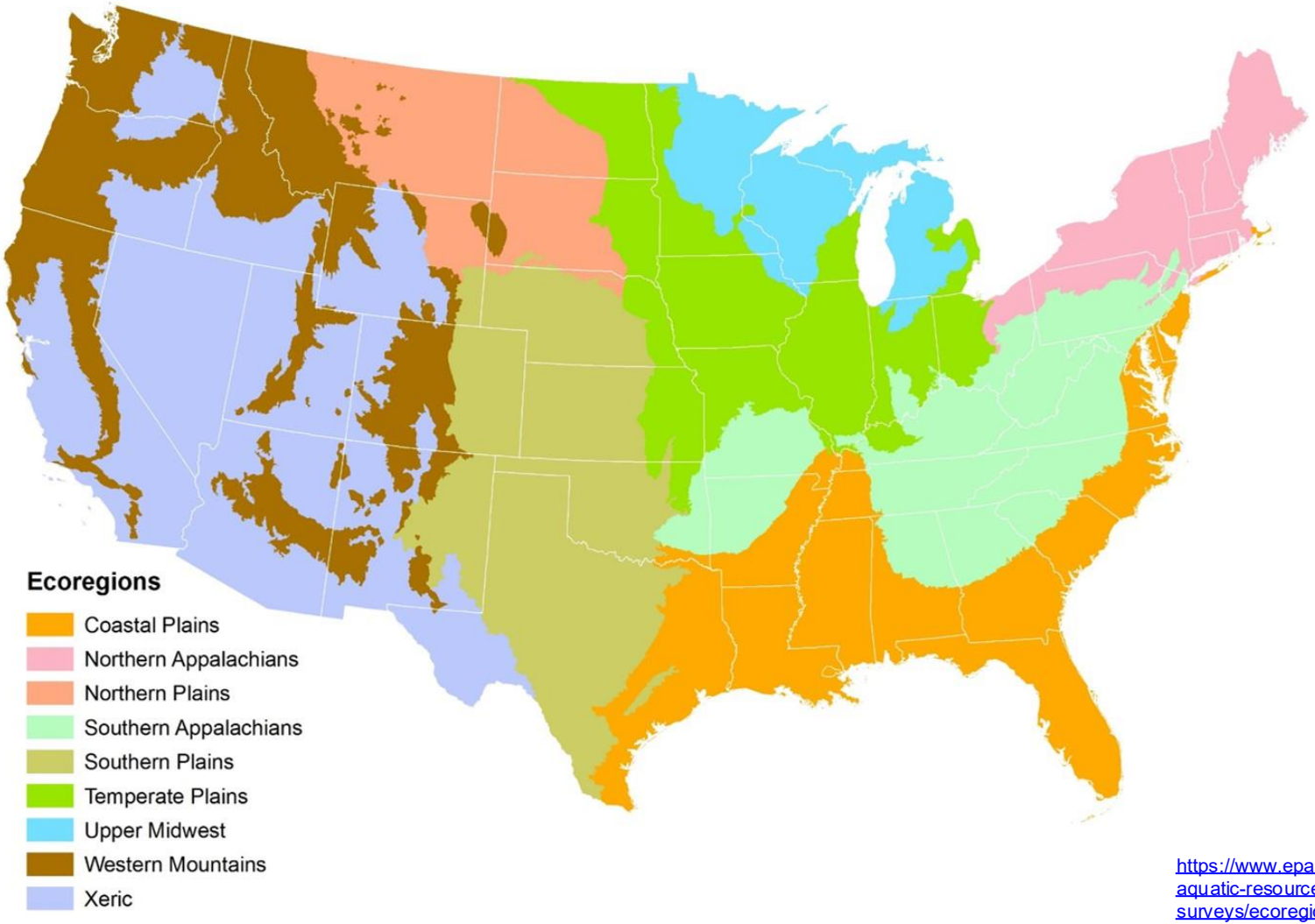


National Rivers and Streams Assessment: NRSA



- Coastal Plains
- Northern Appalachians
- Northern Plains
- Southern Appalachians
- Southern Plains
- Temperate Plains
- Upper Midwest
- Western Mountains
- Xeric

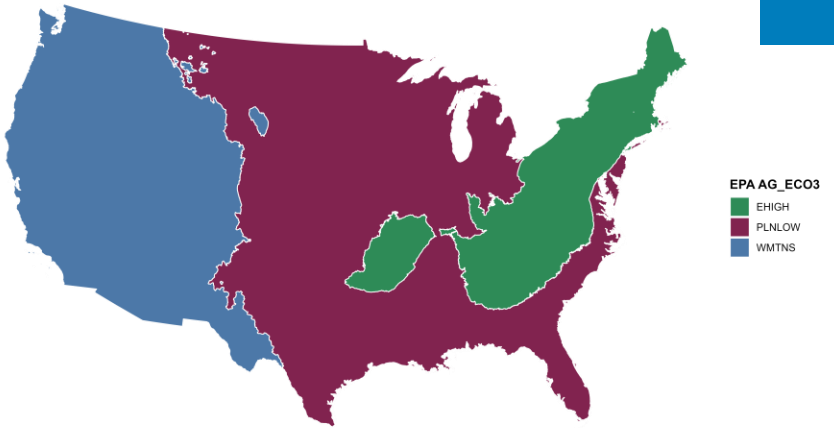
Code	Name	n=
CPL	Coastal Plains	139
NAP	Northern Appalachians	82
NPL	Northern Plains	6
SAP	Southern Appalachians	19
SPL	Southern Plains	10
TPL	Temperate Plains	128
UMW	Upper Midwest	14
WMT	Western Mountains	104
XER	Xeric	6

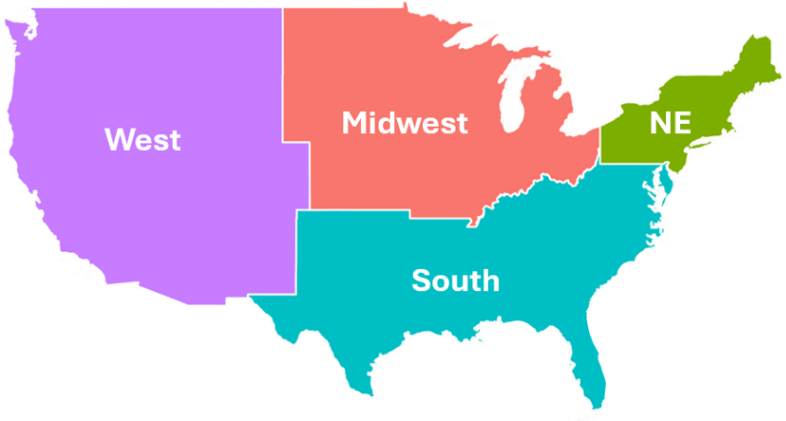


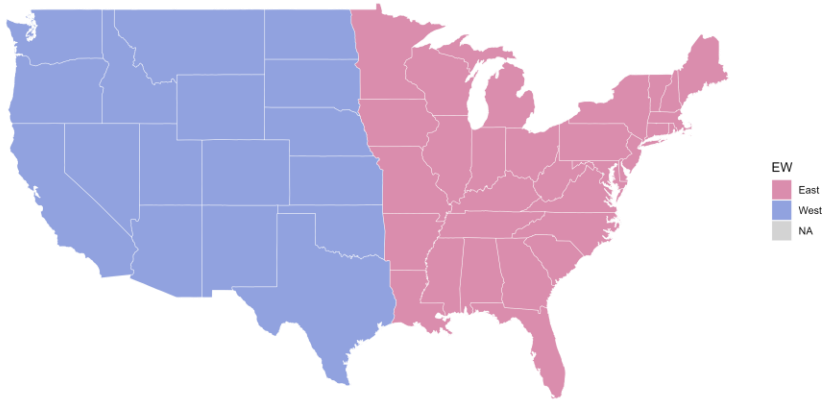
https://www.epa.gov/national-aquatic-resource-surveys/ecoregions-used-national-aquatic-resource-surveys

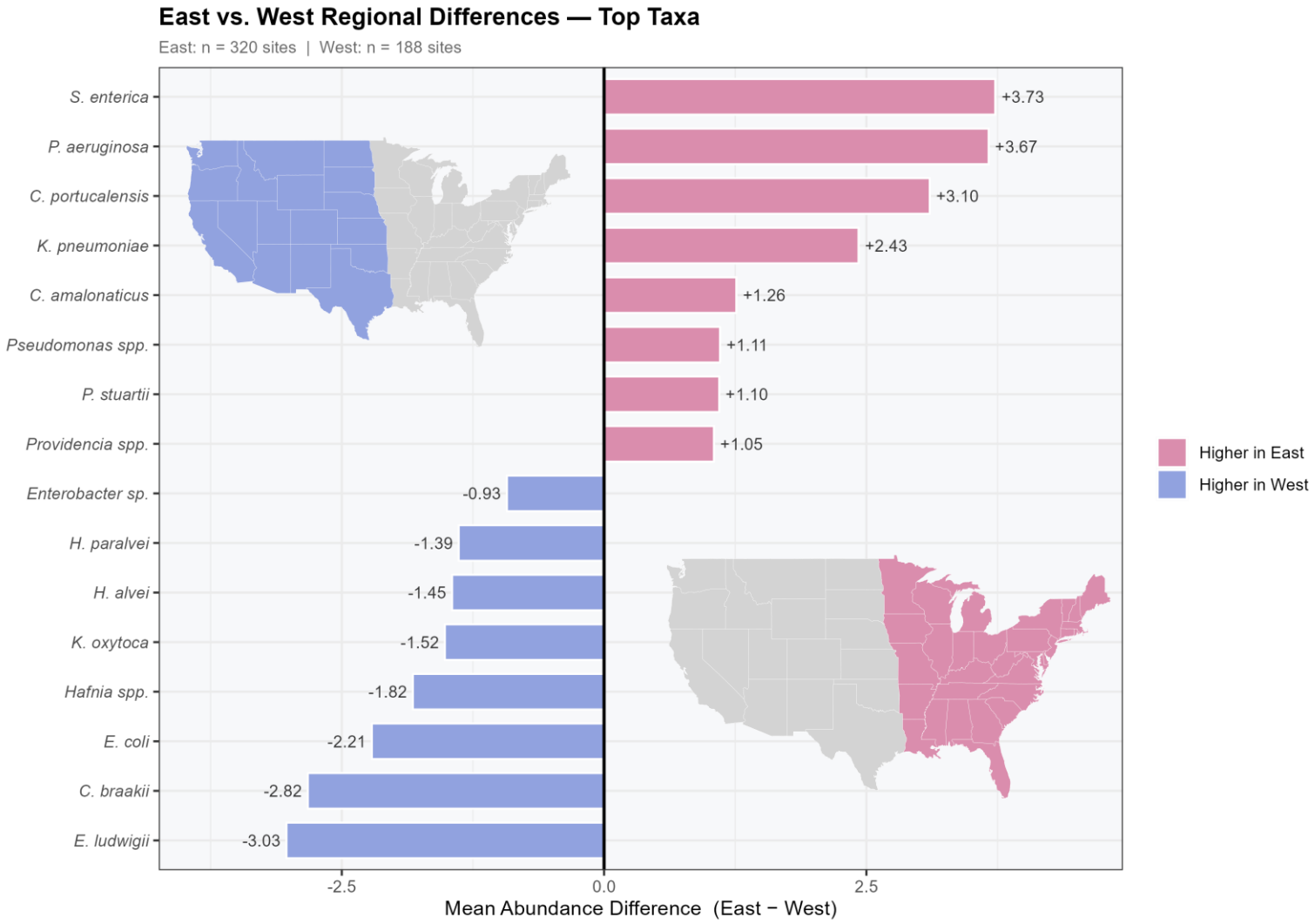
Eco Regions, HUC regions, Census regions, East-West

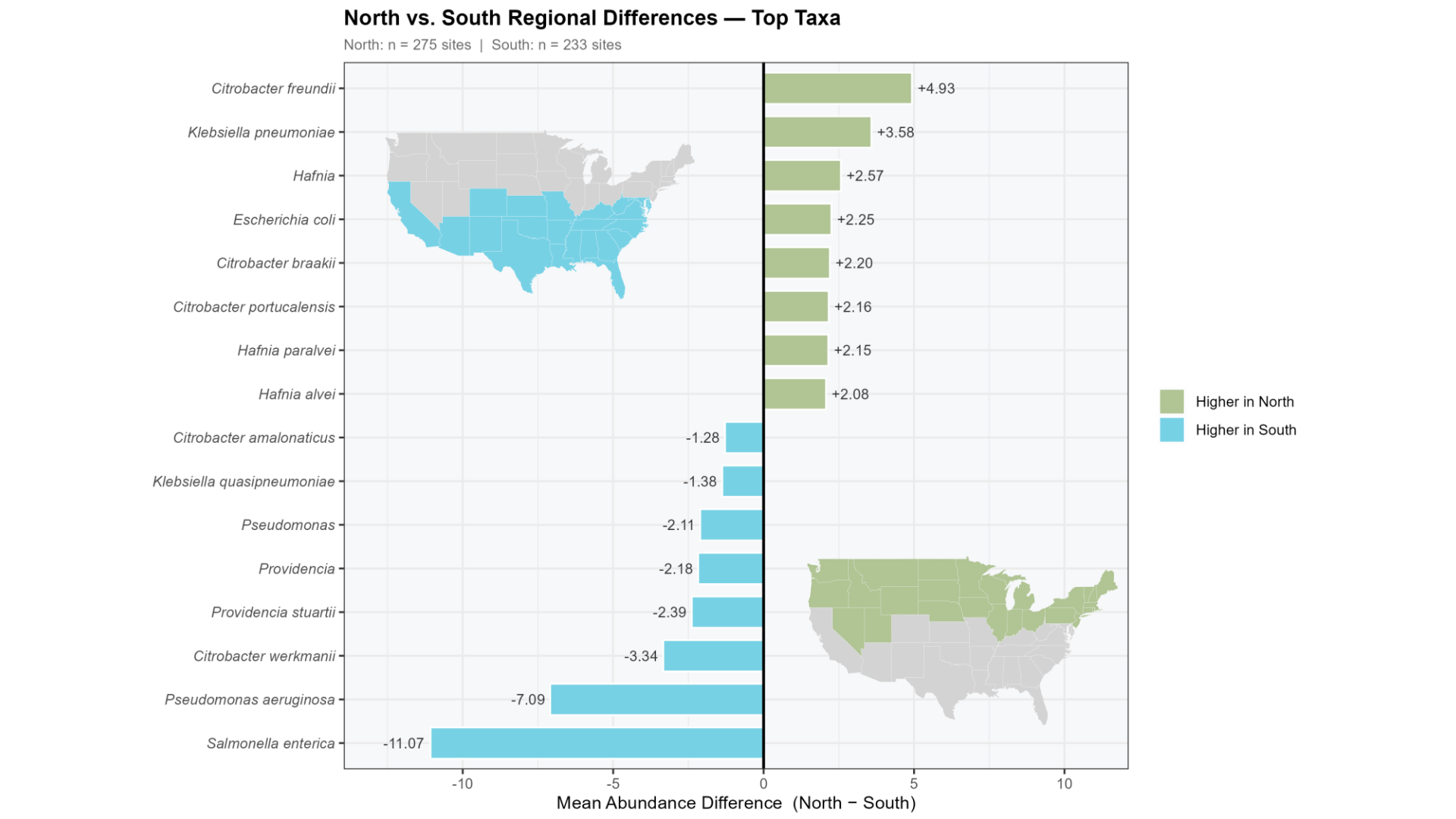









East vs. West Regional Differences — Top Taxa
East: n = 320 sites | West: n = 188 sites
S. enterica
P. aeruginosa
C. portucalensis
K. pneumoniae
C. amalonaticus
Pseudomonas spp.
P. stuartii
Providencia spp.
Enterobacter sp.
H. paralvei
H. alvei
K. oxytoca
Hafnia spp.
E. coli
C. braakii
E. ludwigii
+3.73
+3.67
+3.10
+2.43
+1.26
+1.11
+1.10
+1.05
-0.93
-1.39
-1.45
-1.52
-1.82
-2.21
-2.82
-3.03
-2.5
0.0
2.5
Mean Abundance Difference (East − West)
Higher in East
Higher in West


North vs. South Regional Differences — Top Taxa
North: n = 275 sites | South: n = 233 sites
Citrobacter freundii
Klebsiella pneumoniae
Hafnia
Escherichia coli
Citrobacter braakii
Citrobacter portucalensis
Hafnia paralvei
Hafnia alvei
Citrobacter amalonaticus
Klebsiella quasipneumoniae
Pseudomonas
Providencia
Providencia stuartii
Citrobacter werkmanii
Pseudomonas aeruginosa
Salmonella enterica
+4.93
+3.58
+2.57
+2.25
+2.20
+2.16
+2.15
+2.08
-1.28
-1.38
-2.11
-2.18
-2.39
-3.34
-7.09
-11.07
-10
-5
0
5
10
Mean Abundance Difference (North − South)
Higher in North
Higher in South

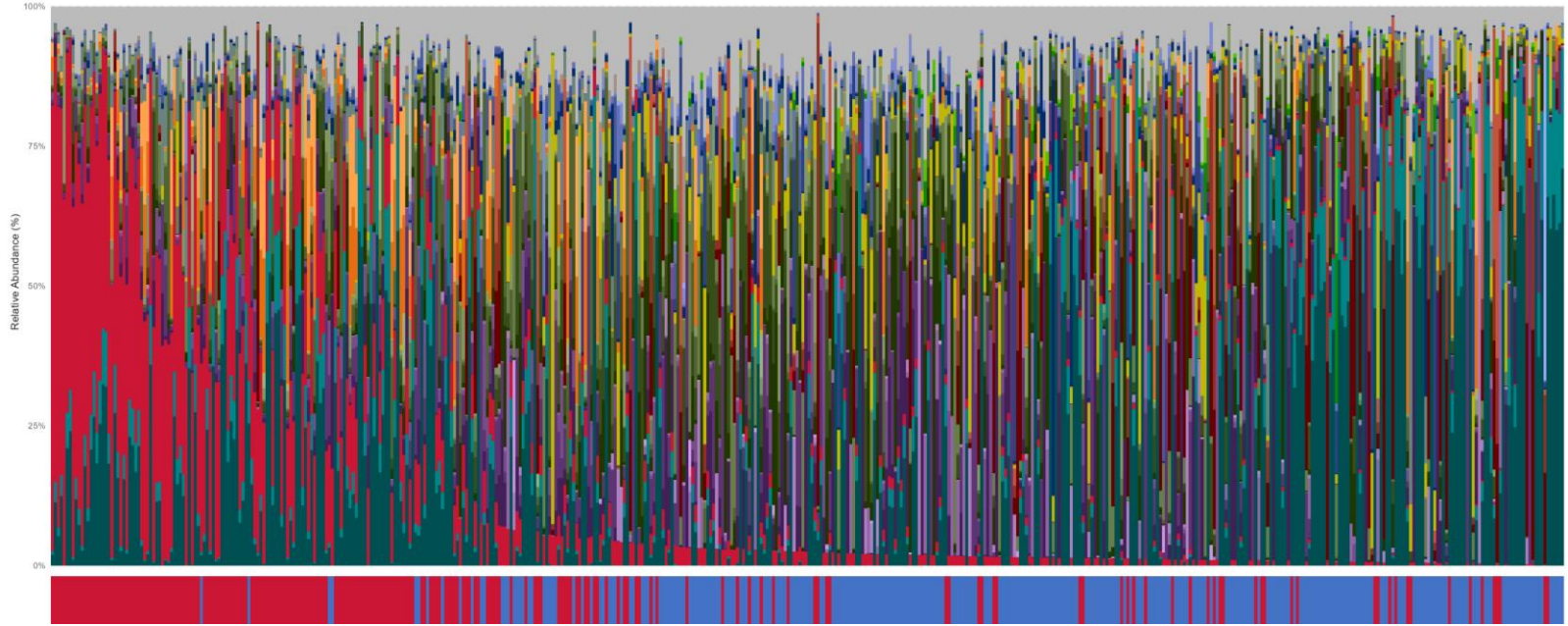
Salmonella detection rate by Census Region



Region	% Sites *Salmonella* +
R3. South	**79.1%** ⚠
R2. Midwest	37.6%
R4. West	21.3%
R1. Northeast	15.0%

The Southern region has a markedly higher *Salmonella* detection rate, consistent with warmer temperatures, higher agricultural runoff, and greater *Salmonella* carriage in livestock and wildlife populations in that region.

Bacterial Community Composition

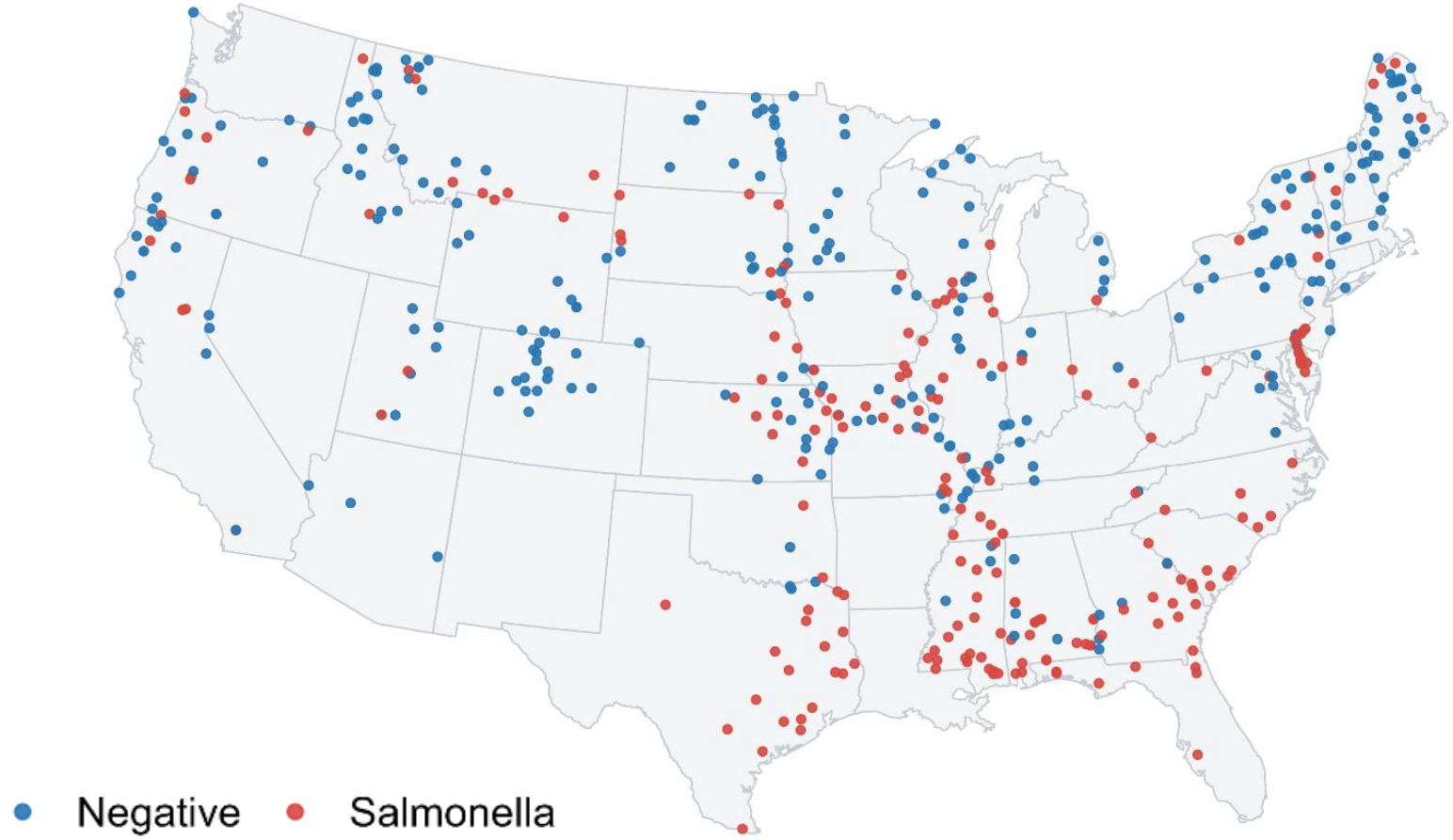


Individual Water Samples (sorted by *S. enterica* abundance, high → low)

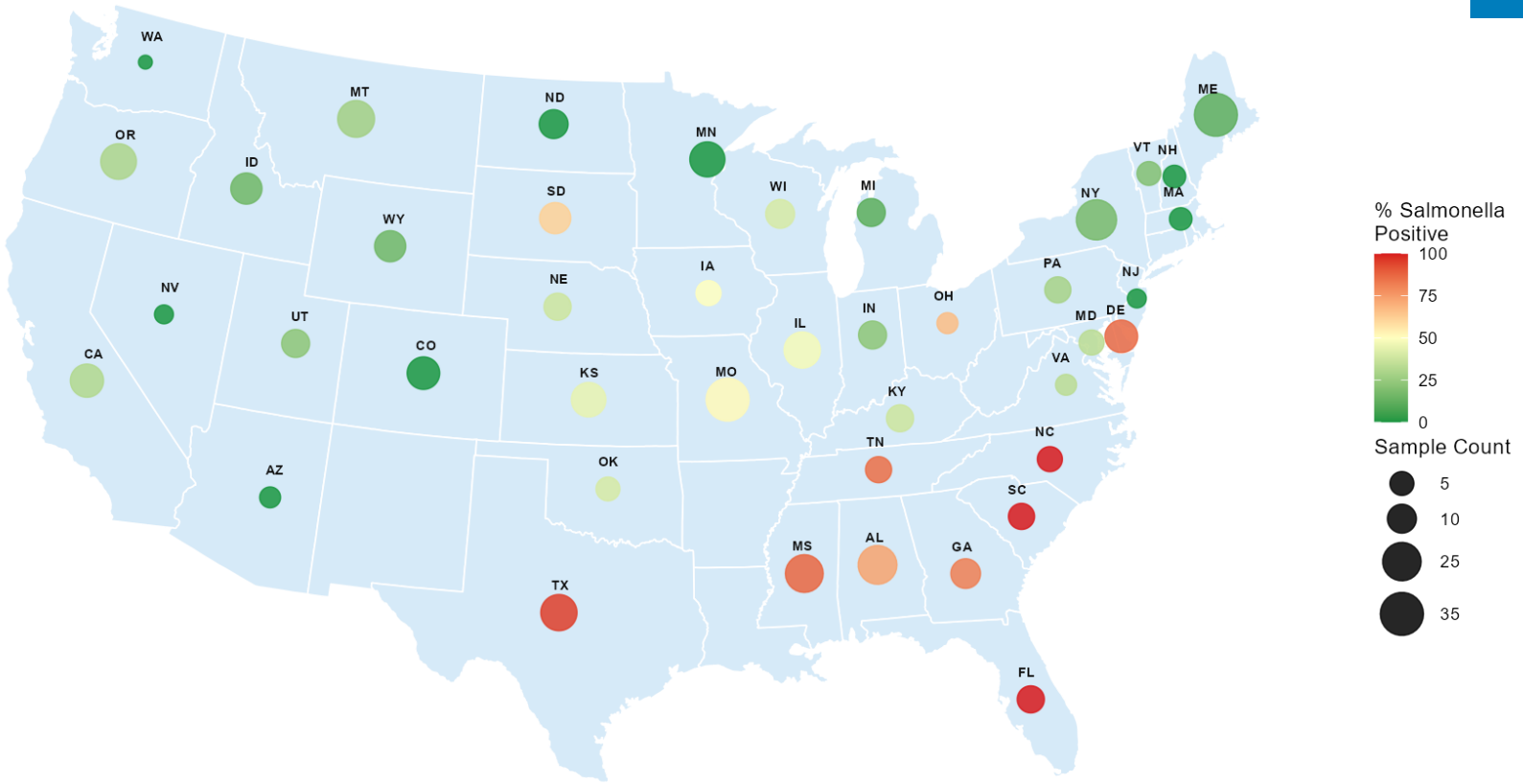
Status

- Negative
- Salmonella Positive

- Other genera < 20%
- *Alcaligenes faecalis*
- *Serratia ureilytica*
- *Serratia*
- *Enterobacter cancerogenus*
- *Enterobacter kobei*
- *Enterobacter* sp.
- *Enterobacter hormaechei*
- *Enterobacter ludwigii*
- *Enterobacter cloacae*
- *Proteus mirabilis*
- *Providencia stuartii*
- *Providencia*
- *Escherichia coli*
- *Hafnia alvei*
- *Hafnia paralvei*
- *Hafnia*
- *Citrobacter rodentium*
- *Citrobacter amalonaticus*
- *Citrobacter braakii*
- *Citrobacter werkmanii*
- *Citrobacter portucalensis*
- *Citrobacter freundii*
- *Klebsiella michiganensis*
- *Klebsiella oxytoca*
- *Klebsiella variicola*
- *Klebsiella quasipneumoniae*
- *Klebsiella aerogenes*
- *Klebsiella pneumoniae*
- *Salmonella enterica*
- *Pseudomonas*
- *Pseudomonas aeruginosa*


Negative
Salmonella

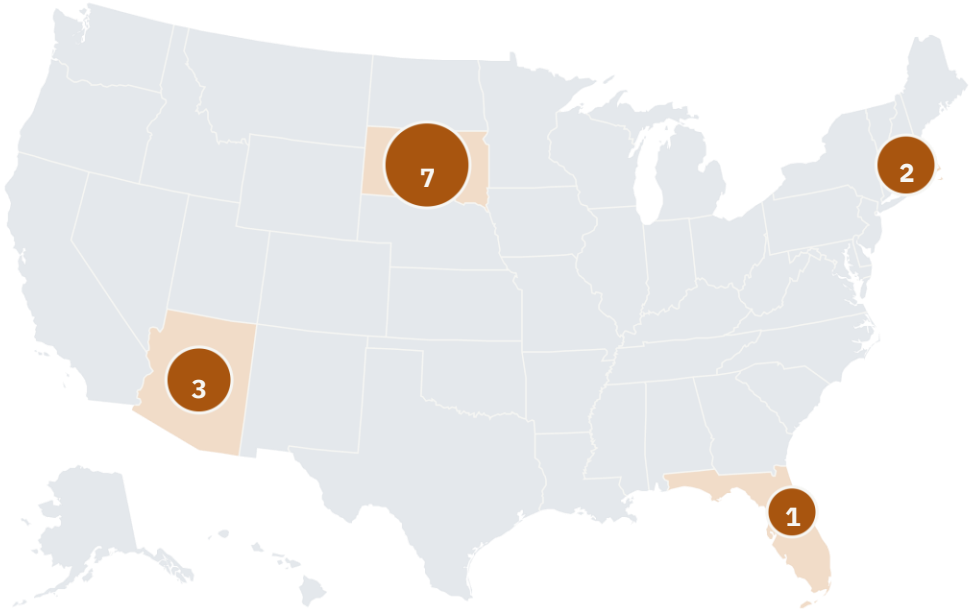
Salmonella Culture Results



Sequenced by Genometrakr Labs

IDENTICAL TO A CLINICAL ISOLATE · 0 SNPS

13 river isolates identical to human cases

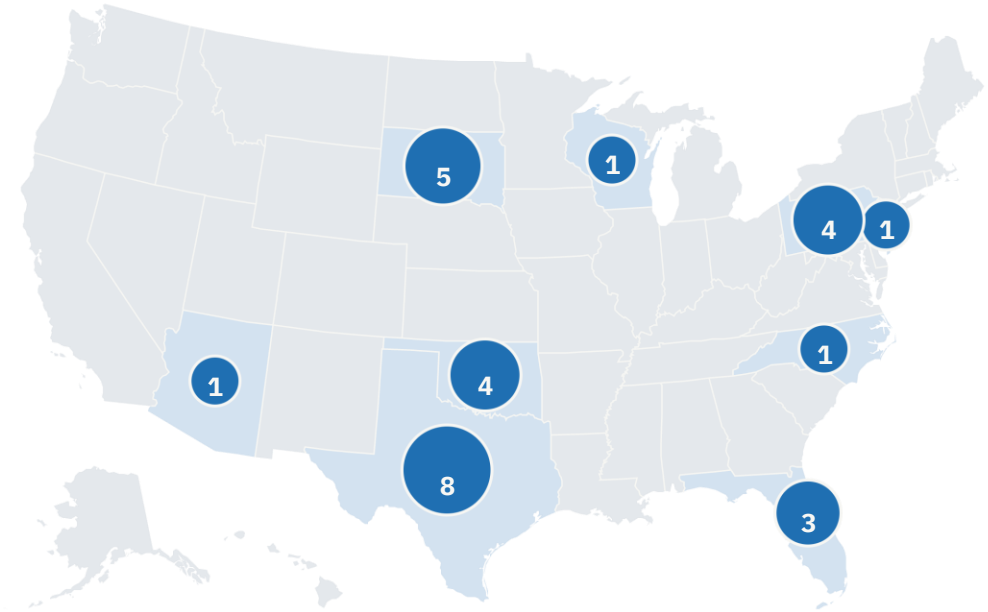


State	No.	Serotype	SNP cluster
SD	7	Bredeney	PDS000002230
AZ	3	Typhimurium	PDS000013845
MA	2	Braenderup	PDS000076604
FL	1	Poona	PDS000009710

Location is the sampling state recorded in NCBI; site-level coordinates are not in the export. Source: NCBI Pathogen Detection, PRJNA1186127.

ONE SNP FROM A CLINICAL ISOLATE

28 river isolates one SNP from human cases



State	No.	Serotype	SNP cluster
TX	8	Newport	PDS000063294
SD	2	Bredeney	PDS000002230
SD	2	Oranienburg	PDS000004062
SD	1	Newport	PDS000063294
OK	4	Braenderup	PDS000076604
PA	4	Typhimurium	PDS000239240
FL	3	Newport	PDS000063294
AZ	1	Typhimurium	PDS000013845
NC	1	Newport	PDS000063294
NJ	1	Newport	PDS000063294
WI	1	Newport	PDS000063294

9 states, 5 serotypes. Circle size and number = river isolates sampled in that state. Cluster IDs shown without version suffix.

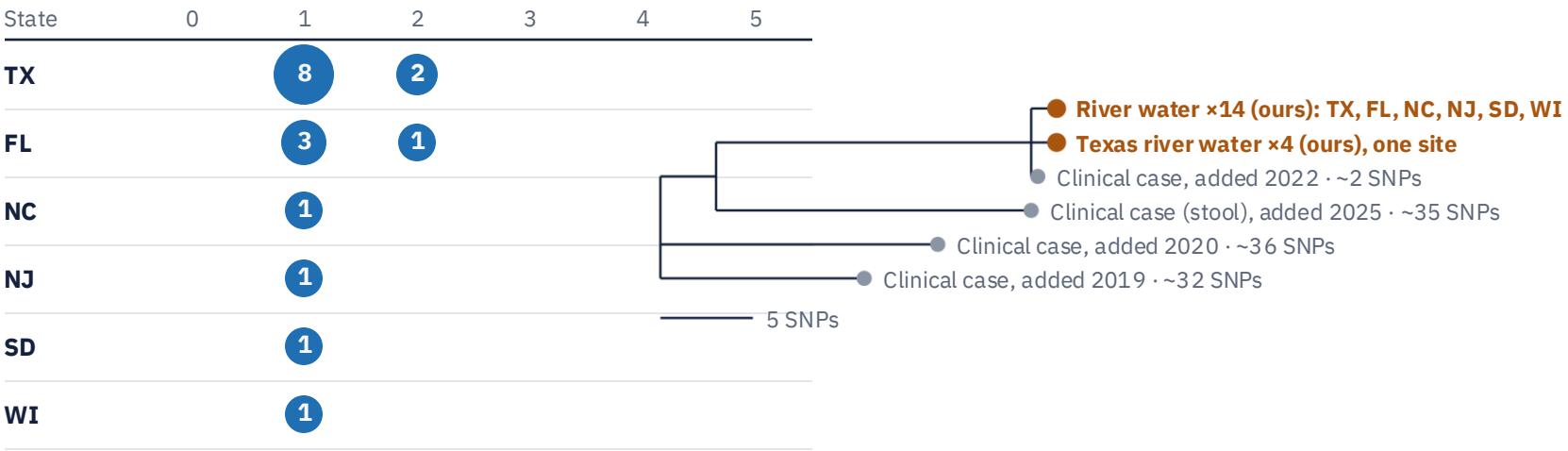
Location is the sampling state recorded in NCBI; site-level coordinates are not in the export. Source: NCBI Pathogen Detection, PRJNA1186127.

SALMONELLA NEWPORT Cluster · PDS000063294

Same Newport strain in six states

18 isolates · 1–2 SNPs from clinical · collected Aug 8–10, 2023 · no acquired AMR genes

River isolates by SNP distance to nearest clinical isolate

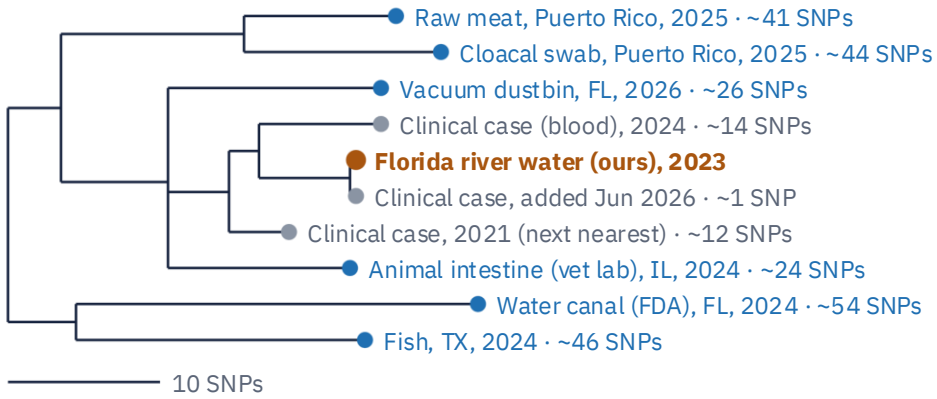


Number in circle = river isolates. SNP tree not yet available (see final steps).

Cluster membership: NCBI Pathogen Detection export of PDS000063294 (22 isolates: 18 river, 4 clinical).

SALMONELLA POONA · CONDENSED SNP TREE · PDS000009710

Florida isolate matches a 2026 case



Pruned from the NCBI SNP tree of PDS000009710 (1,316 tips): every non-human source kept (one of two canal isolates shown). Tree distances run 1–2 SNPs above NCBI's pairwise counts.

SALMONELLA ORANIENBURG Cluster· PDS000027137 + 2 RELATED

Iowa river isolates from 2023 in a growing lineage

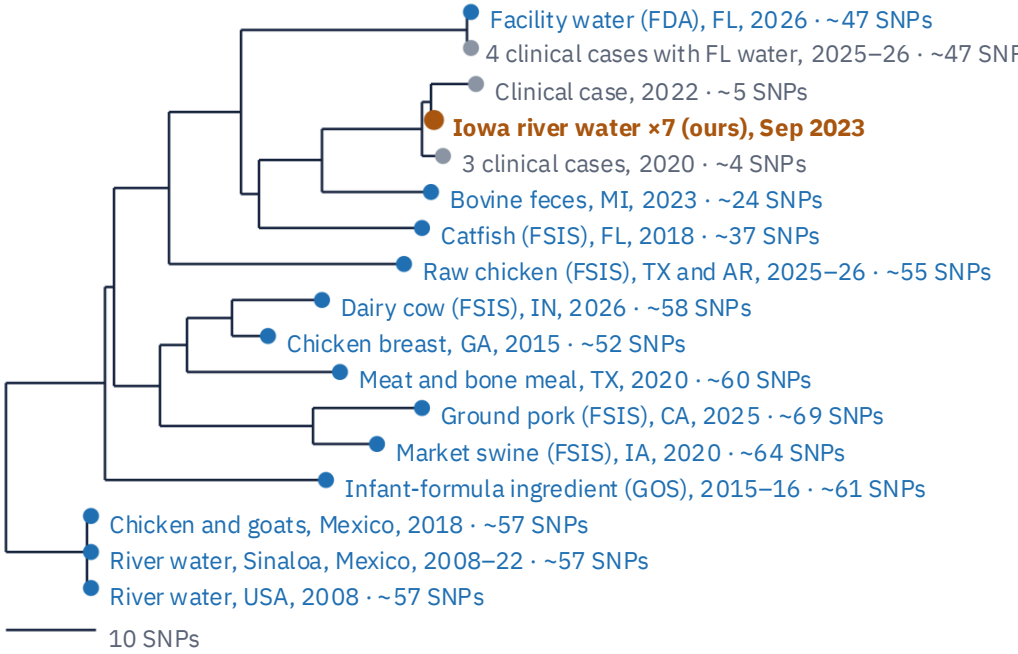
12 isolates in 3 clusters · 1–5 SNPs from clinical · collected Jun–Sep 2023 · no acquired AMR genes

River isolates by SNP distance to nearest clinical isolate

Site	0	1	2	3	4	5
IA 9/23				6	1	
IA 6/23					2	1
SD		2				

IA 9/23 = PDS000027137 · IA 6/23 = PDS000105180 · SD (7/23) = PDS000004062. Rows labeled by collection month. Number in circle = river isolates.

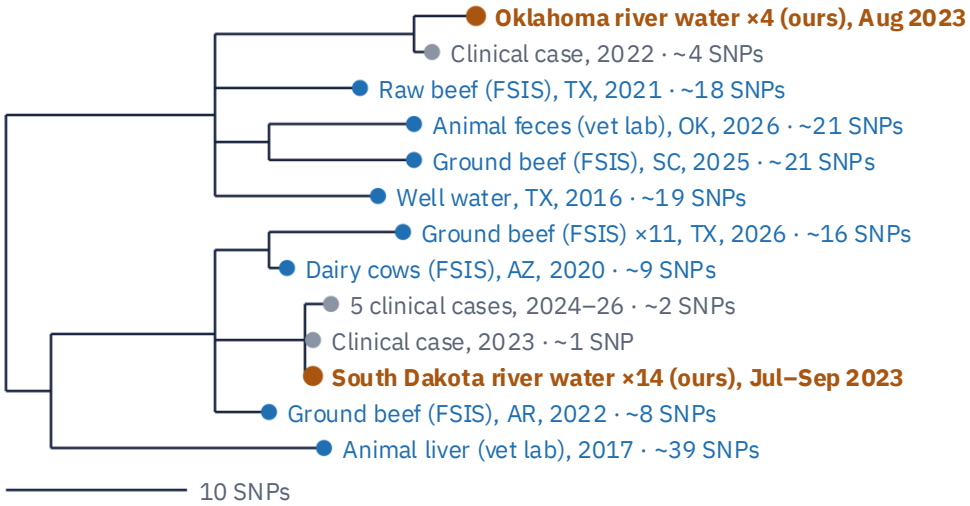
SD cluster: North Dakota cattle isolates (2021, 2025) are 1 SNP from clinical.



Cluster membership: NCBI Pathogen Detection export. Outbreak #1387 link is a candidate, not confirmed. Source: FDA CORE.

SALMONELLA BREDENEY Cluster· CONDENSED SNP TREE · PDS000002230

A persistent Bredeney reservoir in South Dakota

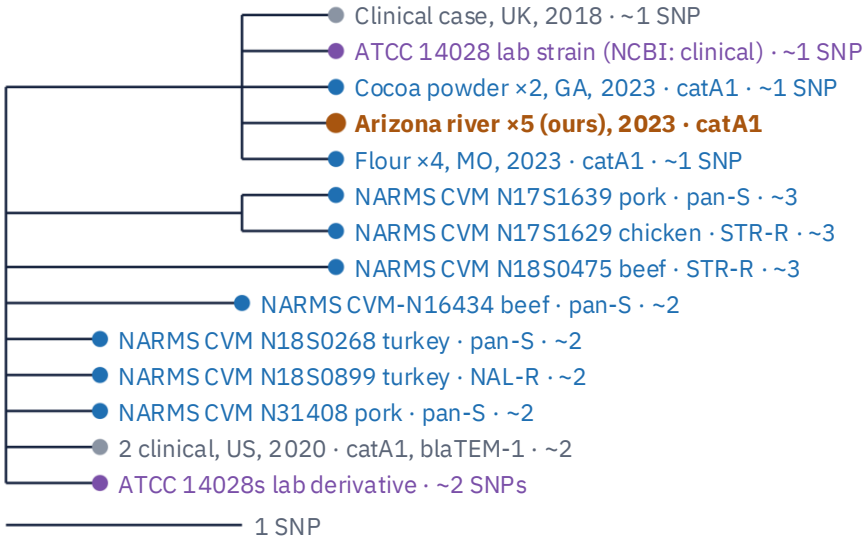


South Dakota isolates sit on the beef and cattle branch

Pruned from the NCBI SNP tree of PDS000002230; distances to our nearer river group. Tree distances run 1–2 SNPs above NCBI's pairwise counts.

SALMONELLA TYPHIMURIUM Cluster· CONDENSED SNP TREE · PDS000013845

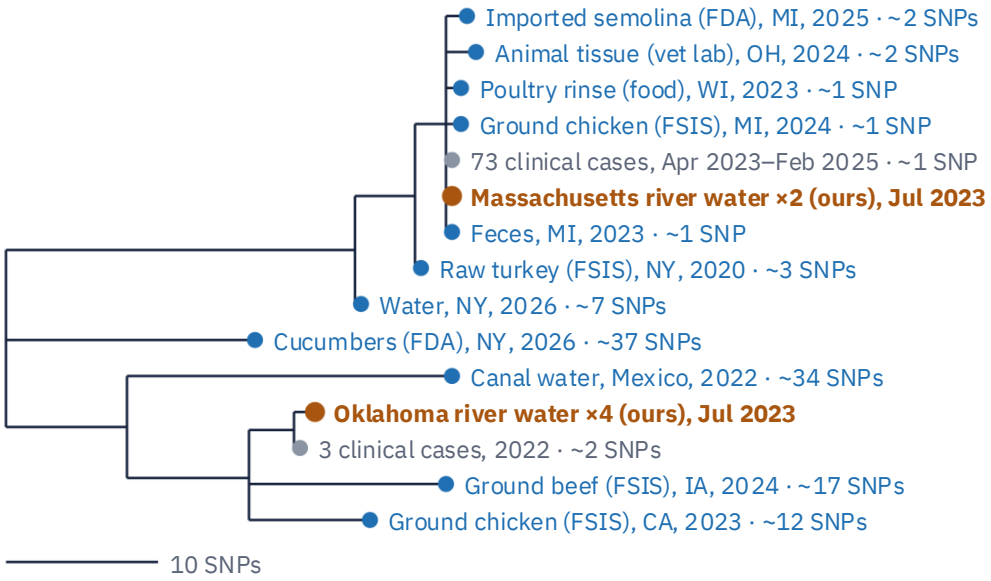
Arizona isolates match a lab control strain more closely than isolates



Pruned from the NCBI SNP tree of PDS000013845 (1,397 isolates). NAL = nalidixic acid, STR = streptomycin, pan-S = no resistance on the NARMS panel.

***SALMONELLA* BRAENDERUP Cluster· CONDENSED SNP TREE · PDS000076604**

Massachusetts isolates sit in a 2023 poultry-linked branch



WHAT THE TREE SHOWS

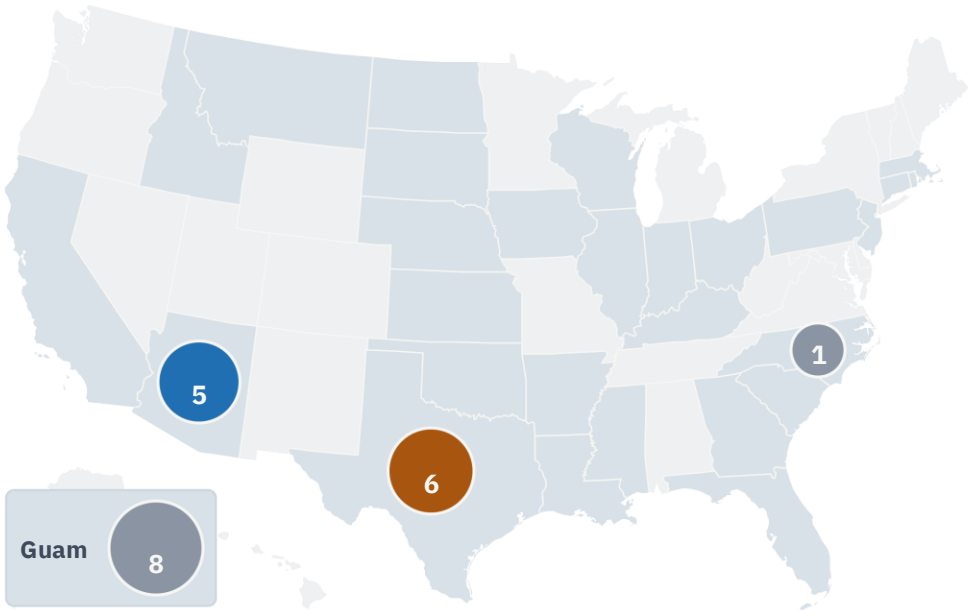
MA is within ~1 SNP of 73 cases added Apr 2023–Feb 2025, most in summer 2023, when the river was sampled. OK sits on its own branch near three 2022 cases.

FOOD LINK ?

WI poultry rinse (2023) and MI ground chicken (2024) are ~1 SNP from MA. Cucumber and canal isolates are 34+ SNPs from both.

Pruned from the NCBI SNP tree of PDS000076604 (7,114 isolates); distances to our nearer river group. Tree distances run 1–2 SNPs above NCBI's pairwise counts.

ANTIMICROBIAL RESISTANCE in ISOLATES

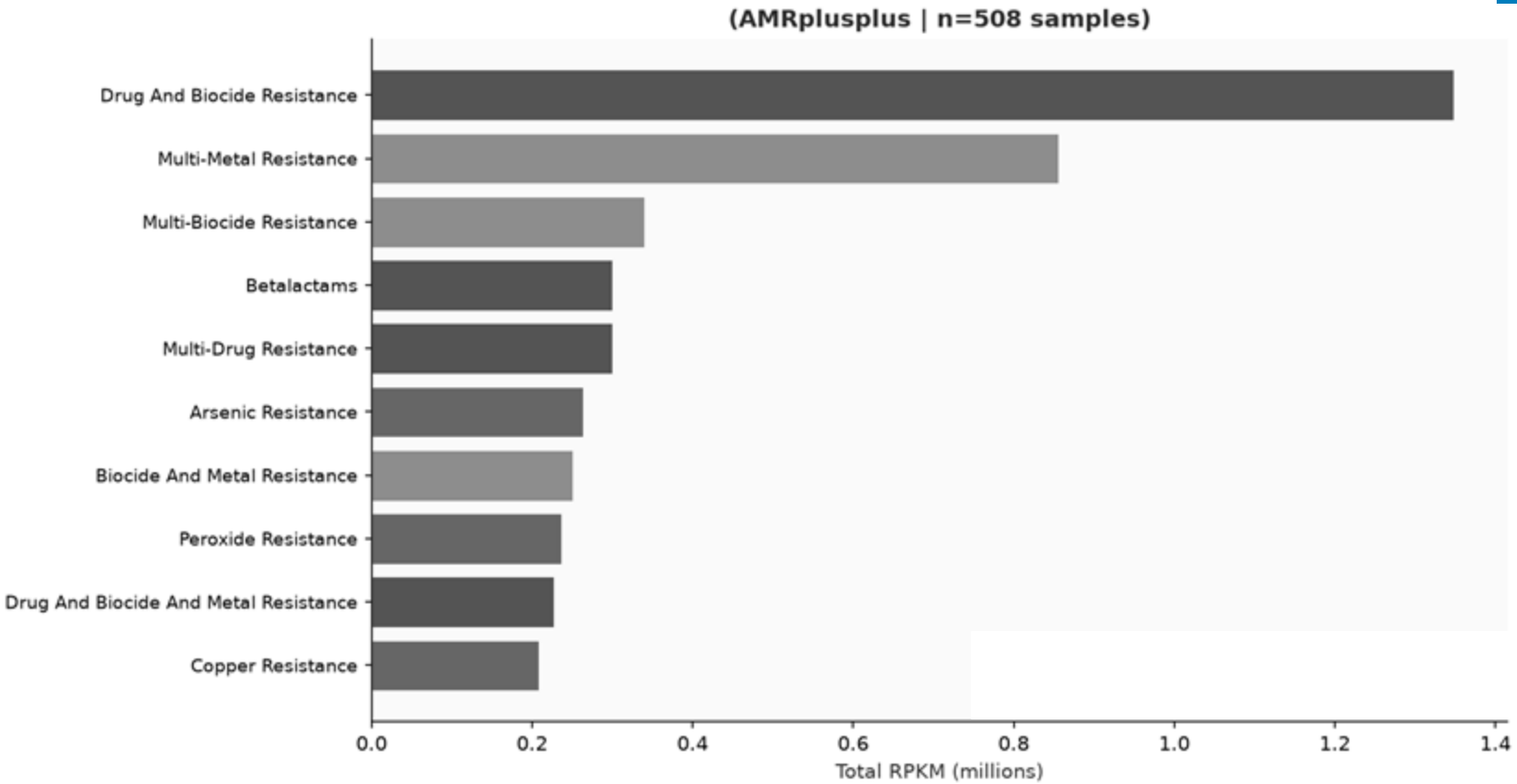


Multidrug · TX · 6
Anatum: ampicillin, phenicols, sulfonamides, tetracycline, streptomycin. 6 SNPs from clinical.

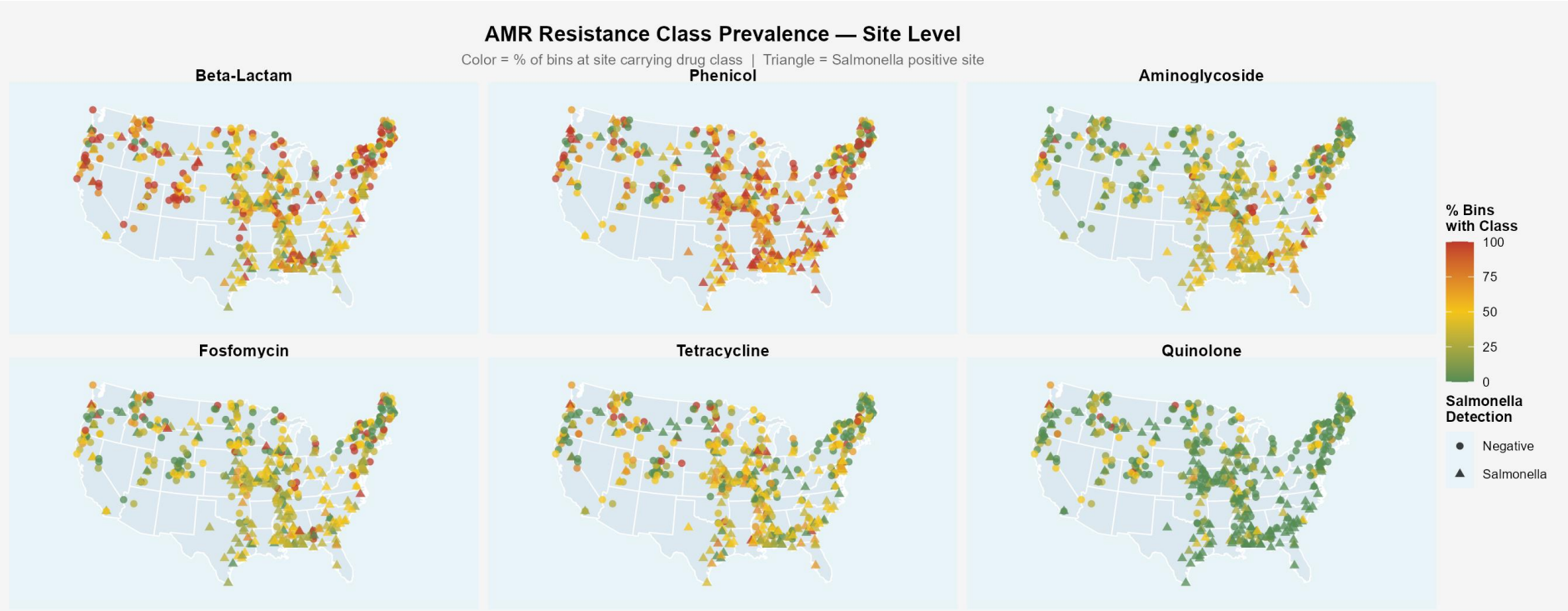
Single class · AZ · 5
Typhimurium: catA1, chloramphenicol. Possible lab control strain.

Low-level or uncertain · Guam 8, NC 1
Guam Orientalis and Lansing: fosA7 variants. NC Manhattan: partial blaEC.

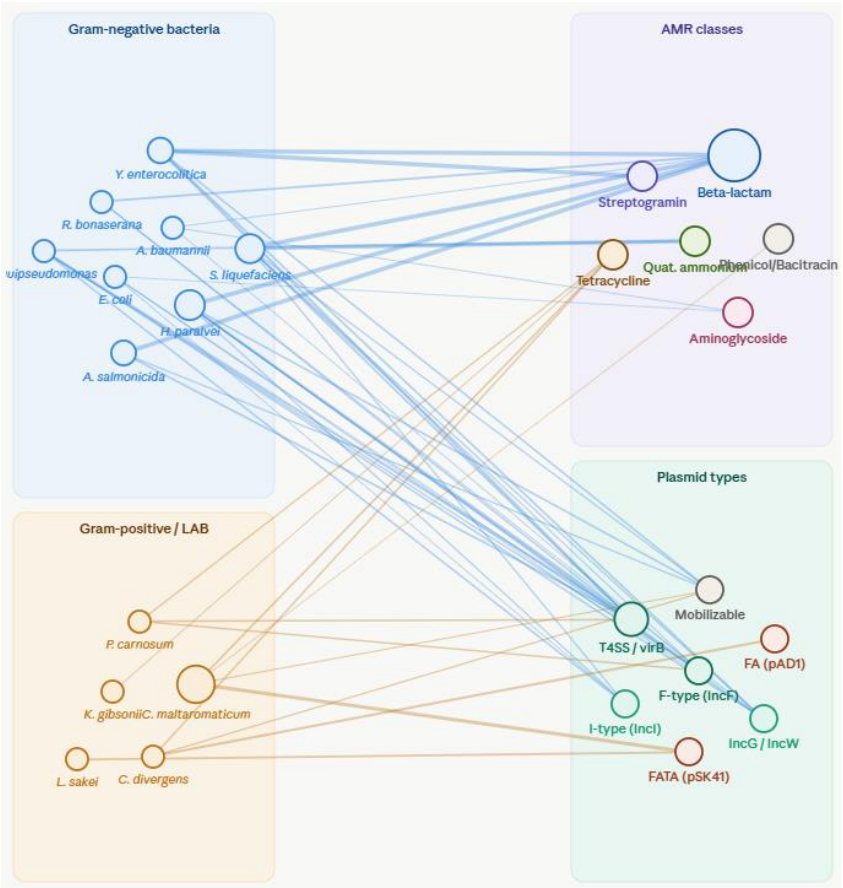
AMR Class Burden by Total RPKM

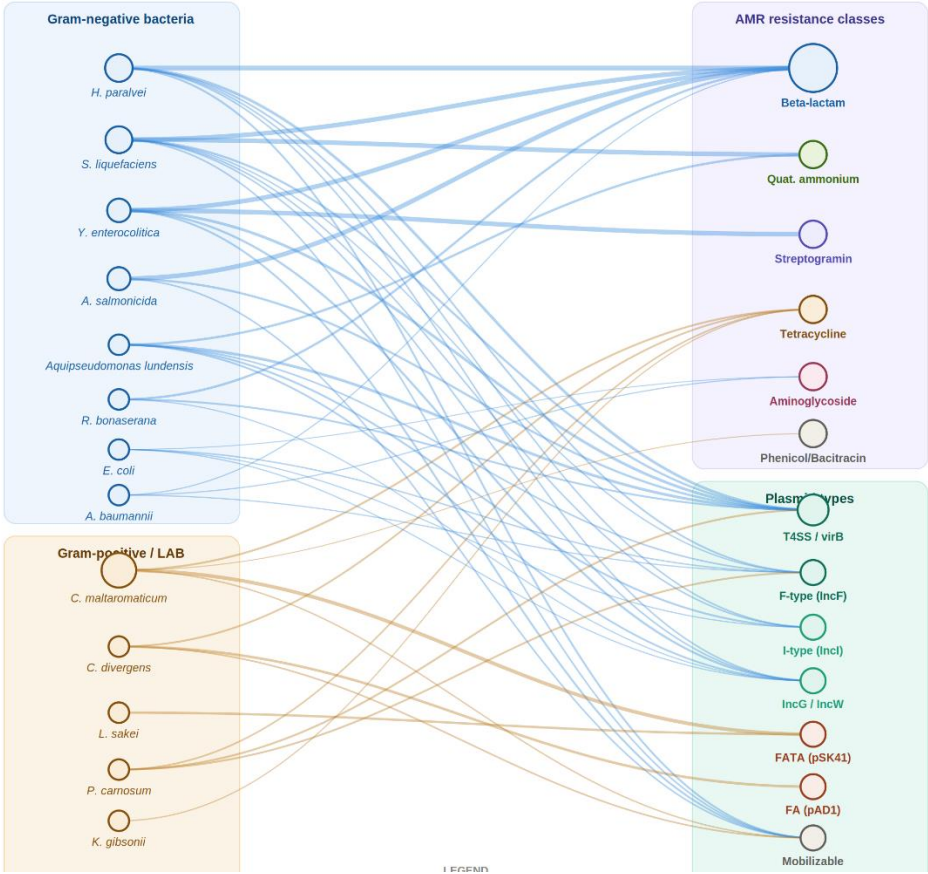


AMR class distribution across sites



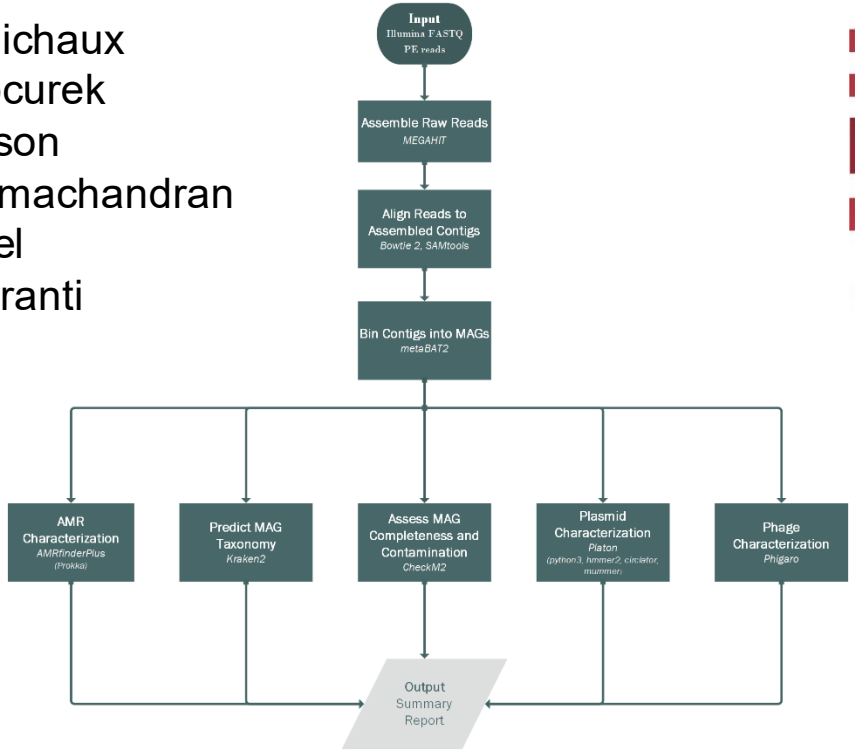
Linking AMR genes, and plasmids to specific taxa with metagenomic data

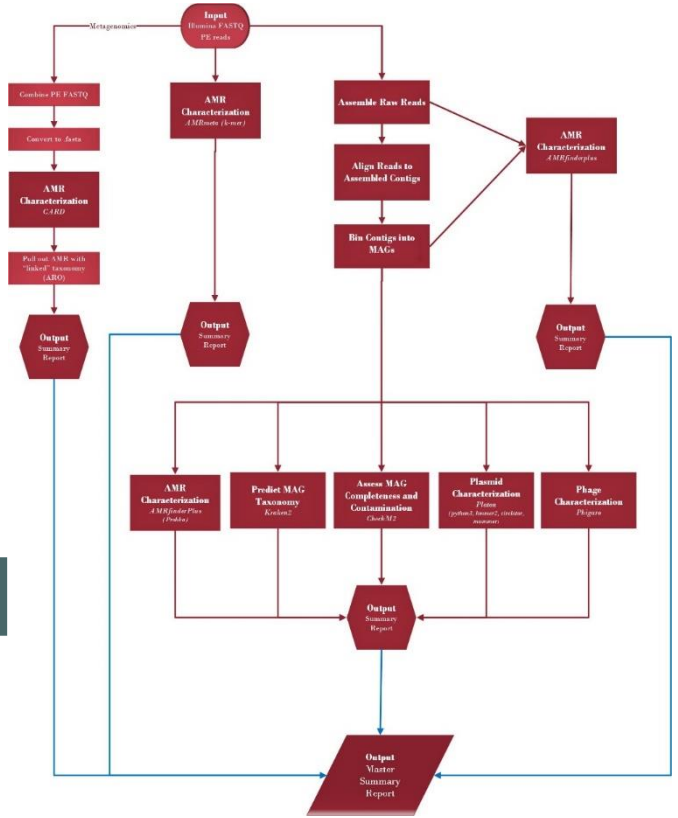




Bioinformatic Pipelines – how can we take advantage of what metagenomic and quasismetagenomic data can provide and assign confidence to the linkage of AMR and associated hosts

Seth Commichaux
Brandon Kocurek
David Erickson
Padmini Ramachandran
Filipp Rondel
Kranti Kongranti





MAGGIC

Metagenomically **A**ssembled **G**enome **G**eneration with **I**ntegrated **C**lassification

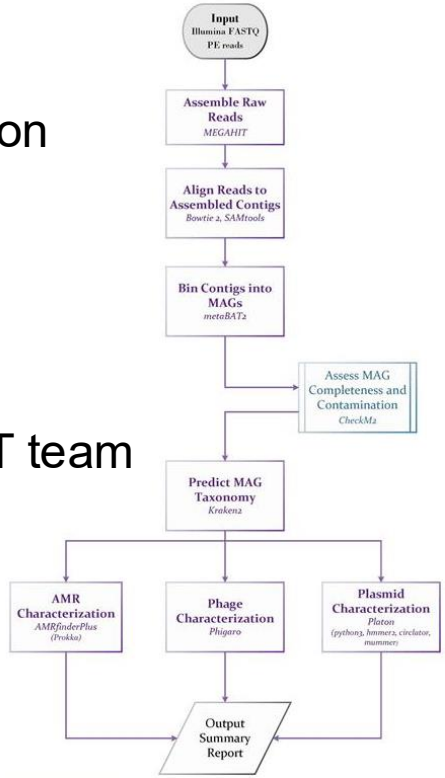


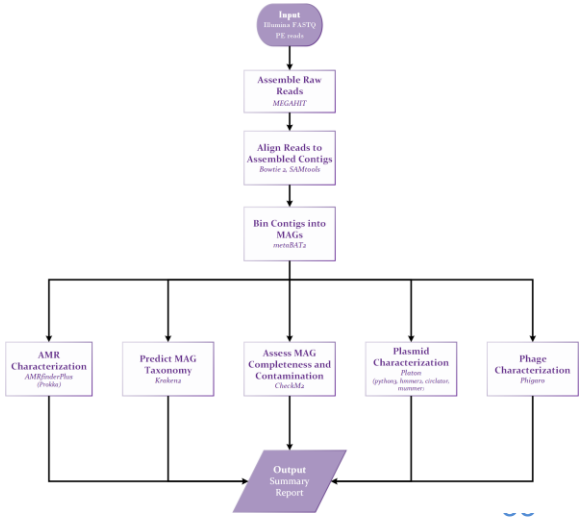
Chief Scientist Grant
Dr. Filipp Rondel, and Dr. David Erickson
Dr. Seth Commichaux

Dr. Kranti Konganti

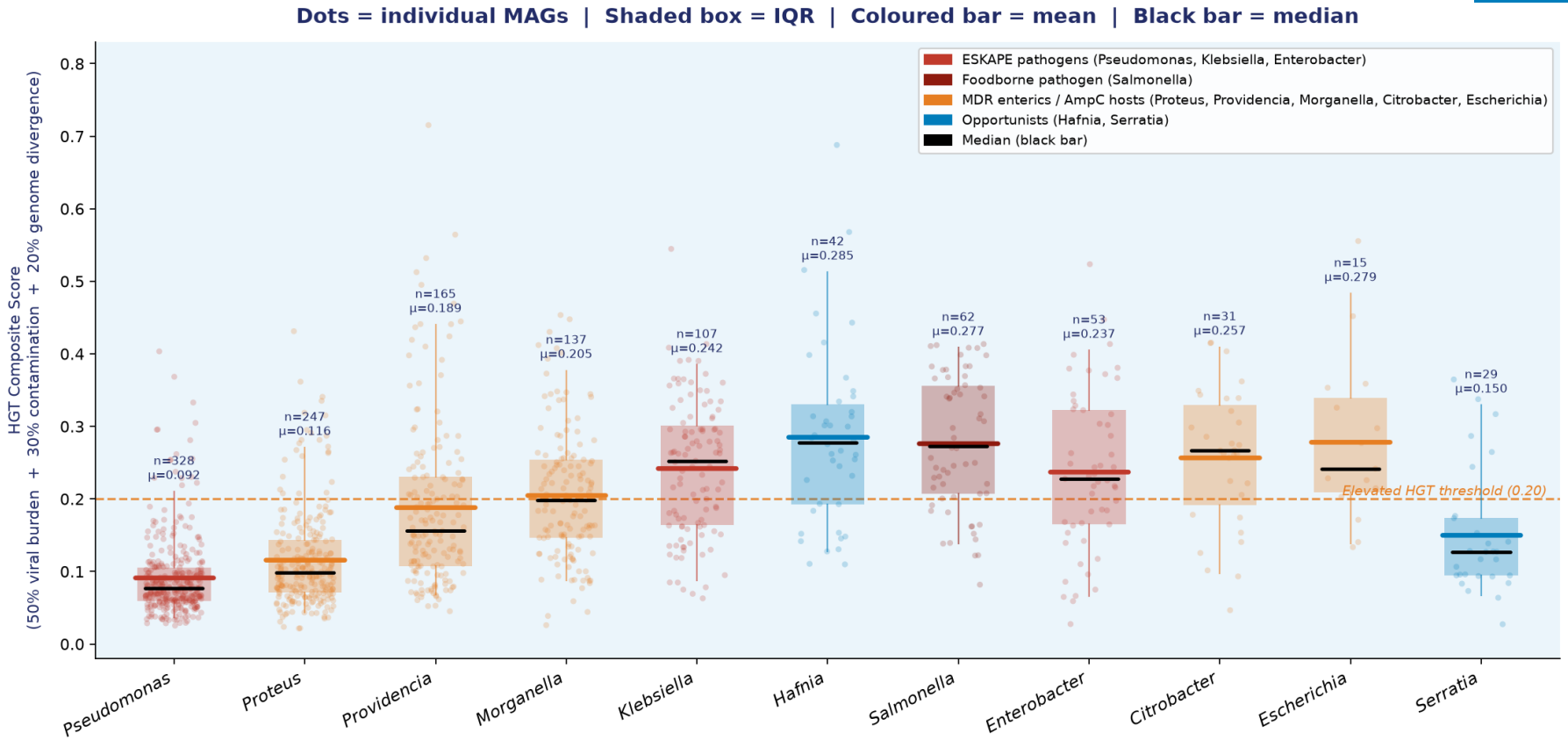
Dr. Padmini Ramachandran and OAMT team

Brandon Kocurek

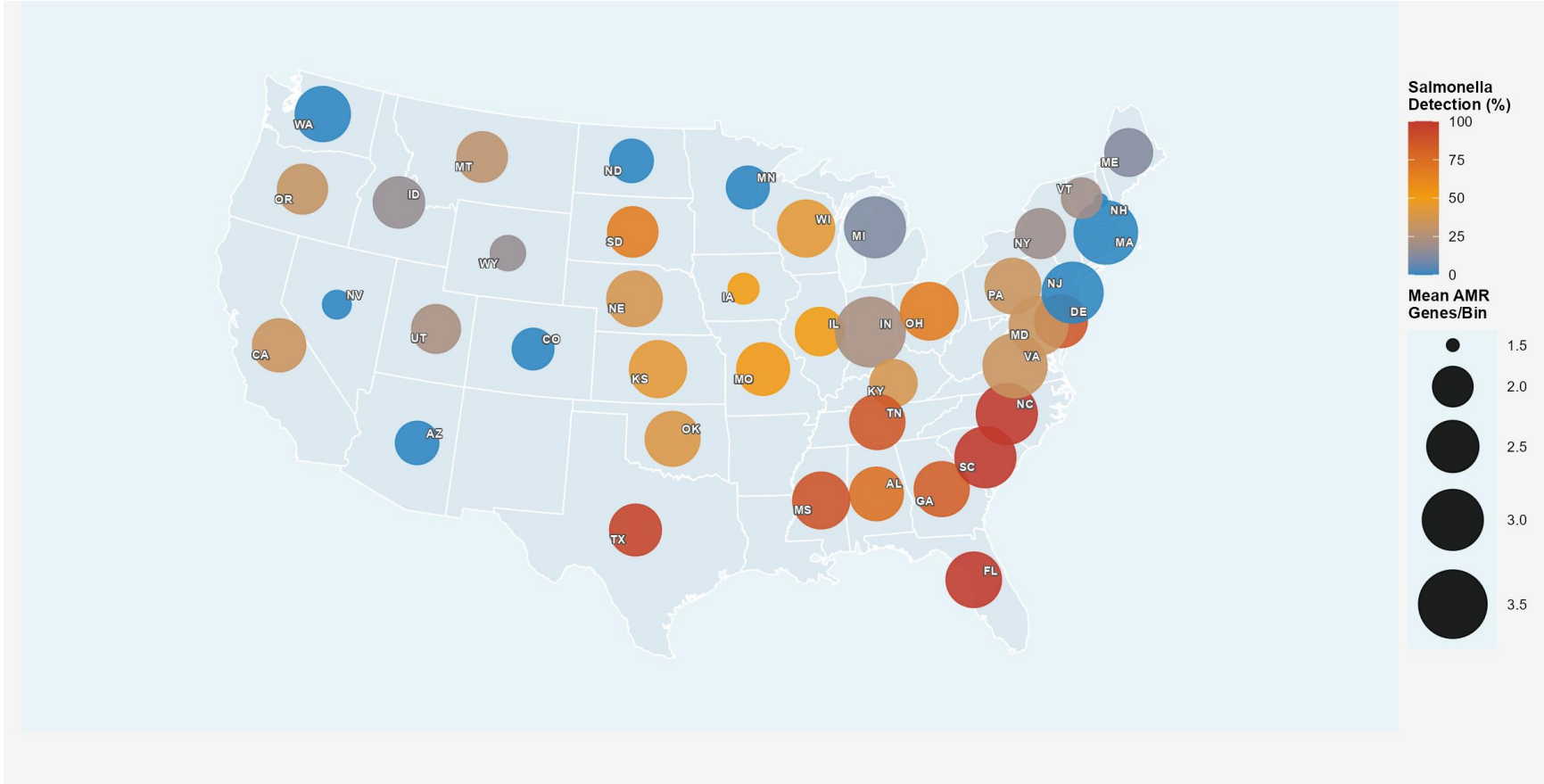




‘HGT Risk Score’ across significant genera

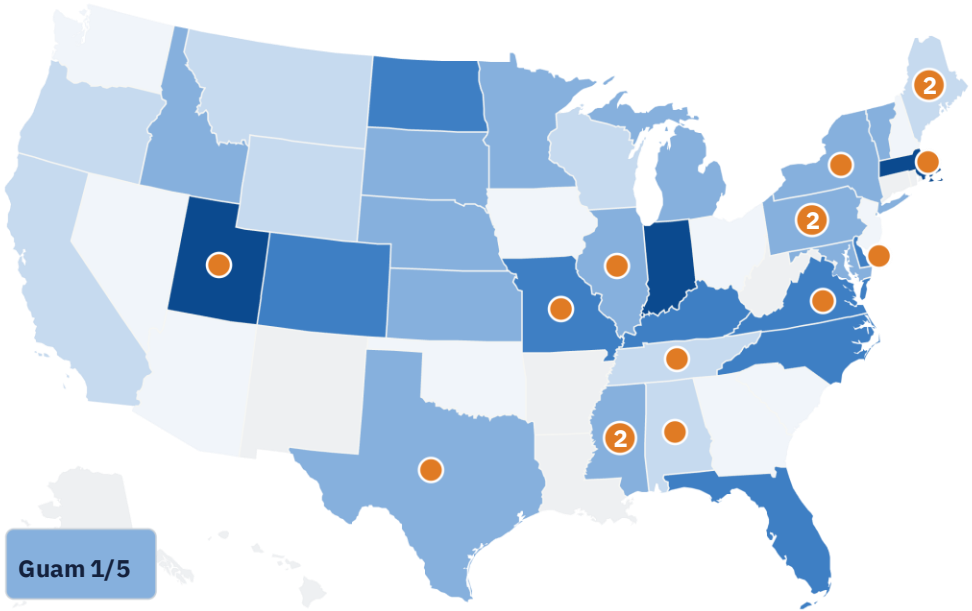


Mean AMR gene # per *Salmonella* bin



MOBILE ANTIBIOTIC RESISTANCE · 513 RIVER METAGENOMES

Mobile resistance is patchy; colistin genes span the East



Samples with any mobile marker

- None
- 1–14%
- 15–29%
- 30–44%
- 45%+
- mcr-9/-10 sample (2 = two)

OVERALL

109 of 513 samples (21%) carry at least one, in 33 of 43 states and territories.

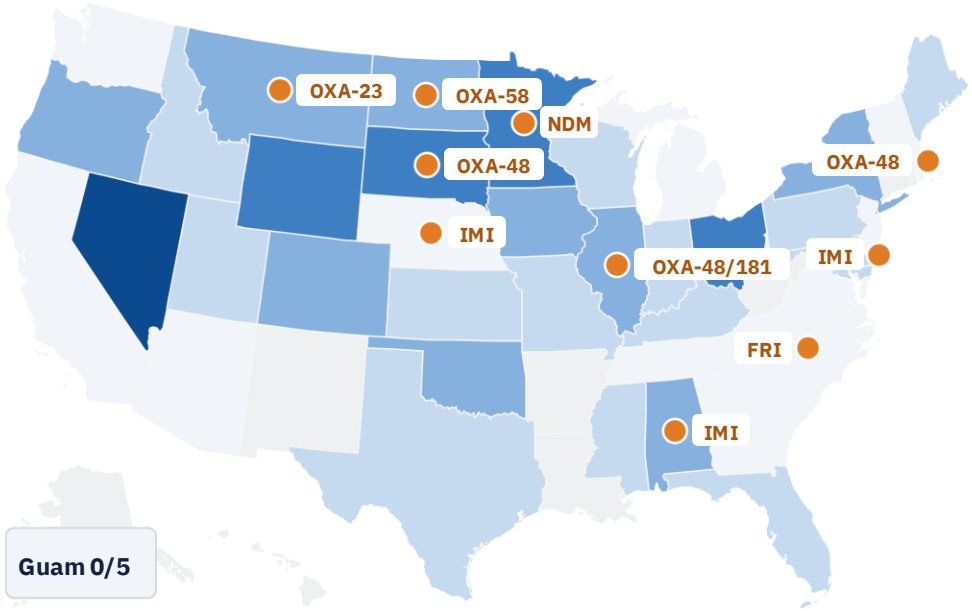
COLISTIN

16 samples carry mcr-9 or mcr-10, in 13 states; all also carry copper and silver operons.

Markers: sul1, sul2, qacEΔ1, blaTEM, tet(A), floR, aph(3″)-Ib/aph(6)-Id, mcr, catA1 (AMRFinderPlus on reads). State level; no site coordinates.

CARBAPENEMASE GENES · 513 RIVER METAGENOMES

Mostly intrinsic *Aeromonas* genes; acquired ones are single reads



Samples with intrinsic genes (cphA, TRU, POM)

- None
- 1–14%
- 15–29%
- 30–44%
- 45%+
- Acquired carbapenemase read hit

INTRINSIC

70 of 513 samples (14%), almost all chromosomal cphA and TRU in Aeromonas.

ACQUIRED

10 samples, 1–10 reads each; no KPC, VIM or IMP, and none in Salmonella.

Shading: AMRFinderPlus and CARD read hits. Markers: CARD read hits (IMI in AL also AMRFinderPlus and an Enterobacter MAG). Needs confirmation.

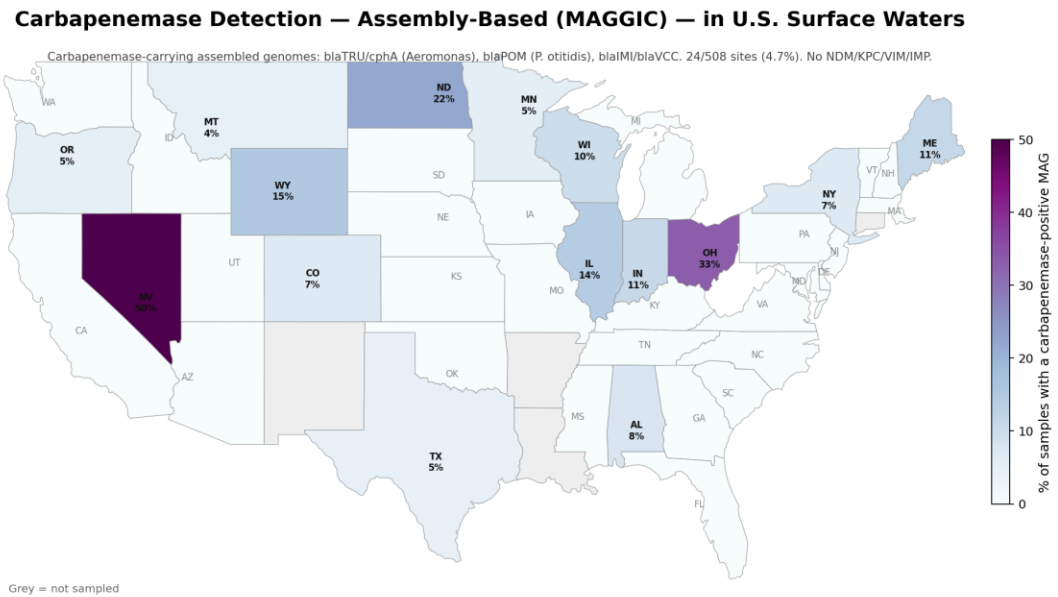
Carbapenemase Summary

Mobile carbapenem resistance was uncommon (~5% of sites).

Most carbapenemase observed was environmentally derived and dominated by intrinsic chromosomal enzymes of aquatic *Aeromonas* and *Pseudomonas*.

Clinically significant carbapenemase genes were observed in 2 sites but disagreement between databases preventing reporting at this time.

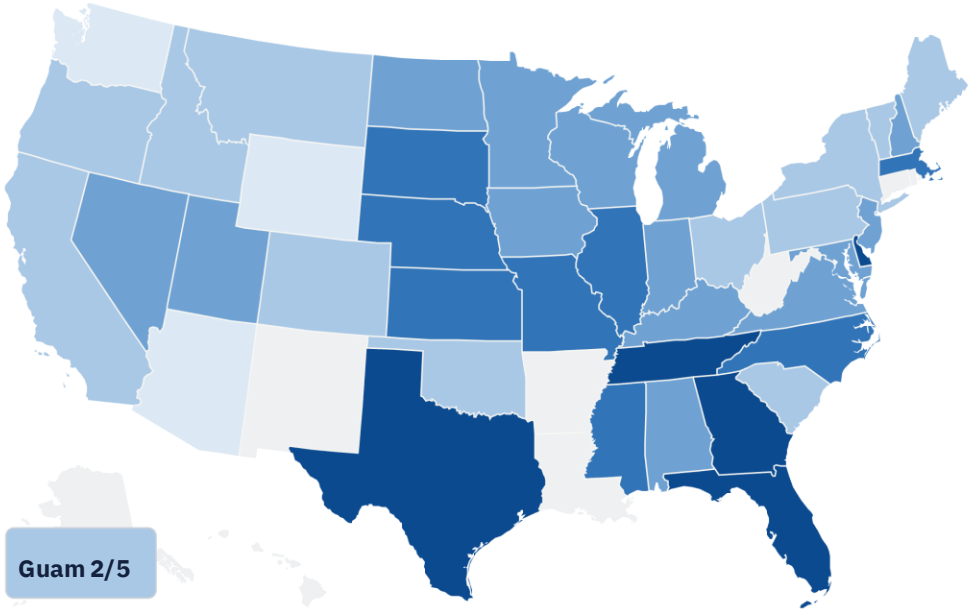
Clinically important carbapenemases were essentially absent from the NRSA samples.



Host organism	Carrier MAGs	Carbapenemase Genes	Nature
Aeromonas enteropelogenes	**11**	blaTRU / cphA	Intrinsic chromosomal MBL
Aeromonas veronii	**10**	cphA / blaTRU / blaVCC	Intrinsic chromosomal MBL
Metapseudomonas* (*Pseudomonas*) *otitidis	**3**	blaPOM / blaPOM-1	Intrinsic chromosomal MBL
***Enterobacter* sp. (Kobei)**	**1**	blaIMI	Class A; potentially mobilizable
Unclassified	**1**	cphA	—

HEAVY METALS · TELLURIUM RESISTANCE (TER GENES)

Tellurium resistance is most common in the Southeast



Share of samples with ter genes

- 0–24%
- 25–49%
- 50–69%
- 70–84%
- 85–100%
- Not sampled

OVERALL

302 of 513 metagenomes (59%) carry ter genes, in 41 of 43 states and territories.

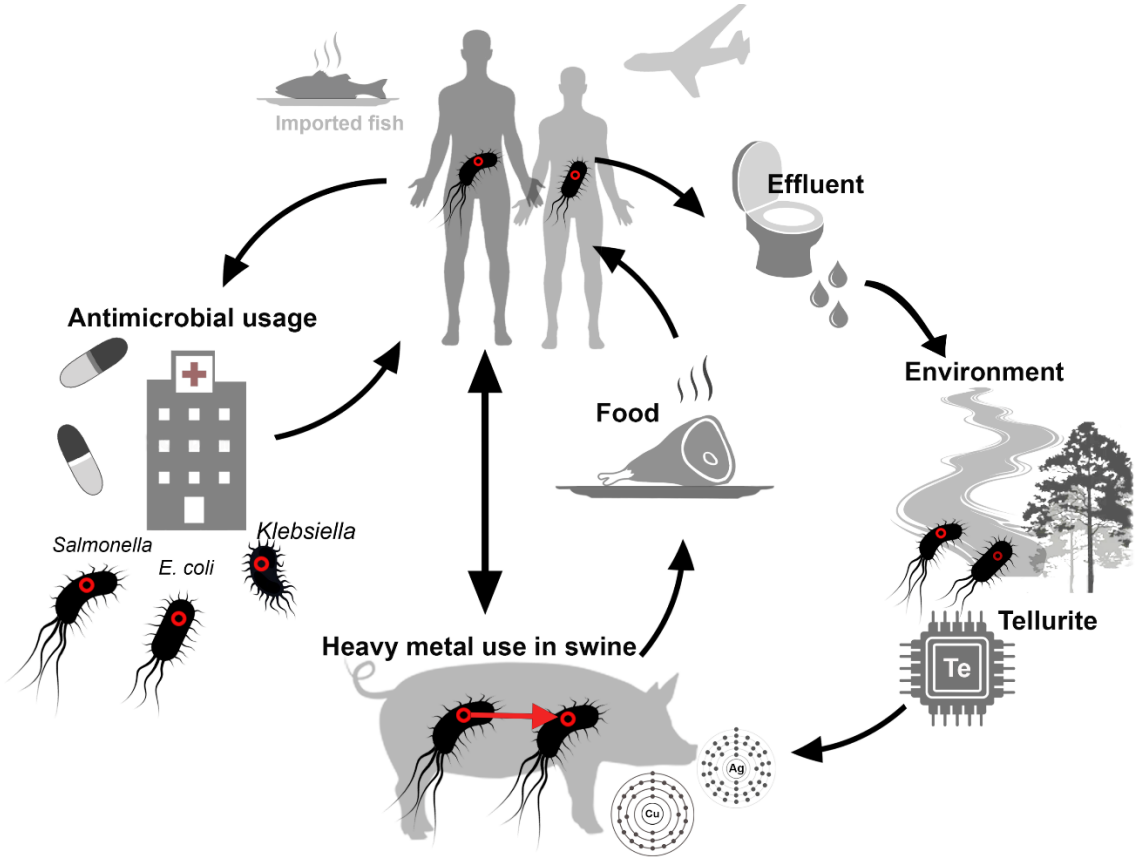
HIGHEST

DE 15/15, **GA** 10/11, **TX** 19/21, **FL** 7/8, **MS** 20/24: mostly Coastal Plains.

LOWEST

Mountain West and Maine: WY, CO, ME and OR at 23–35%.

ter genes (terB, terC, terD, terE, terW, terZ) by AMRFinderPlus on reads.


Imported fish
Effluent
Antimicrobial usage
Environment
Food
Salmonella
E. coli
Klebsiella
Heavy metal use in swine
Tellurite
Te
Ag
Cu



Cong Li et al

AMR Summaries

- **Near-Universal Presence of AmpC β-Lactamases (blaCMY) 89.2% of Sites**
  - AMRfinderplus (453/508 samples), CARD (CMY-74, -82, -83, -93, -101 variants each in >88% of samples), AMRplusplus (Beta-lactam class ranks #4 by total RPKM)
- **Quinolone Resistance Is Ubiquitous and Multi-Mechanistic >90% of Sites**
  - AMRfinderplus (oqxA: 93.3%, oqxB: 87.9%), CARD (QnrB variants in 83–92% of sites depending on *Salmonella* status), AMRplusplus (quinolone-class hits)
- **Fosfomycin Resistance (fosA) in >97% of All Sites**
  - AMRfinderplus (494/508 samples, the single most prevalent gene in the dataset), AMRplusplus (Fosfomycin class detected), Bactikmer (dominant hosts *K. pneumoniae*, *Pseudomonas* known carriers)
- **Heavy Metal & Biocide Co-selection Is a Major Environmental AMR Driver**
  - AMRfinderplus (arsenic resistance *arsABCDR* cluster: 96–97% prevalence, #3–#5 genes by total count), AMRplusplus (Multi-Metal Resistance: #2 class by RPKM at 856K; Biocide resistance: #3 and #9), MAGGIC (metal resistance genes found across nearly all high-quality MAGs)
- **AMR Burden Is Independent of *Salmonella* Detection Status**
  - AMRfinderplus and CARD prevalence values for blaCMY (85.8% vs. 92.9%), QnrB (83.4% vs. 91.6%), and efflux genes are comparably high or even higher in *Salmonella*-negative sites

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ML/AI/BI, Computer programming



Katherine Huebner
AMU



Brandon Kocurek
Data mgmt., Scientific computing



Cong Li
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Lucas Harrison
Molecular genetics, AI



Ryan McDonald
Molecular microbiology



Sampa Mukherjee
Genetics, cloning



Andrea Ottesen
MGS for environmental monitoring, diagnostics



Daniel Tadesse
Microbiome of food and animals



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Procurement



Sanchez Jn Charles
Phenotyping



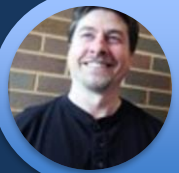
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Genomics



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Data management



Shawn McDermott
Isolate management, logistics



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Laboratory Manager

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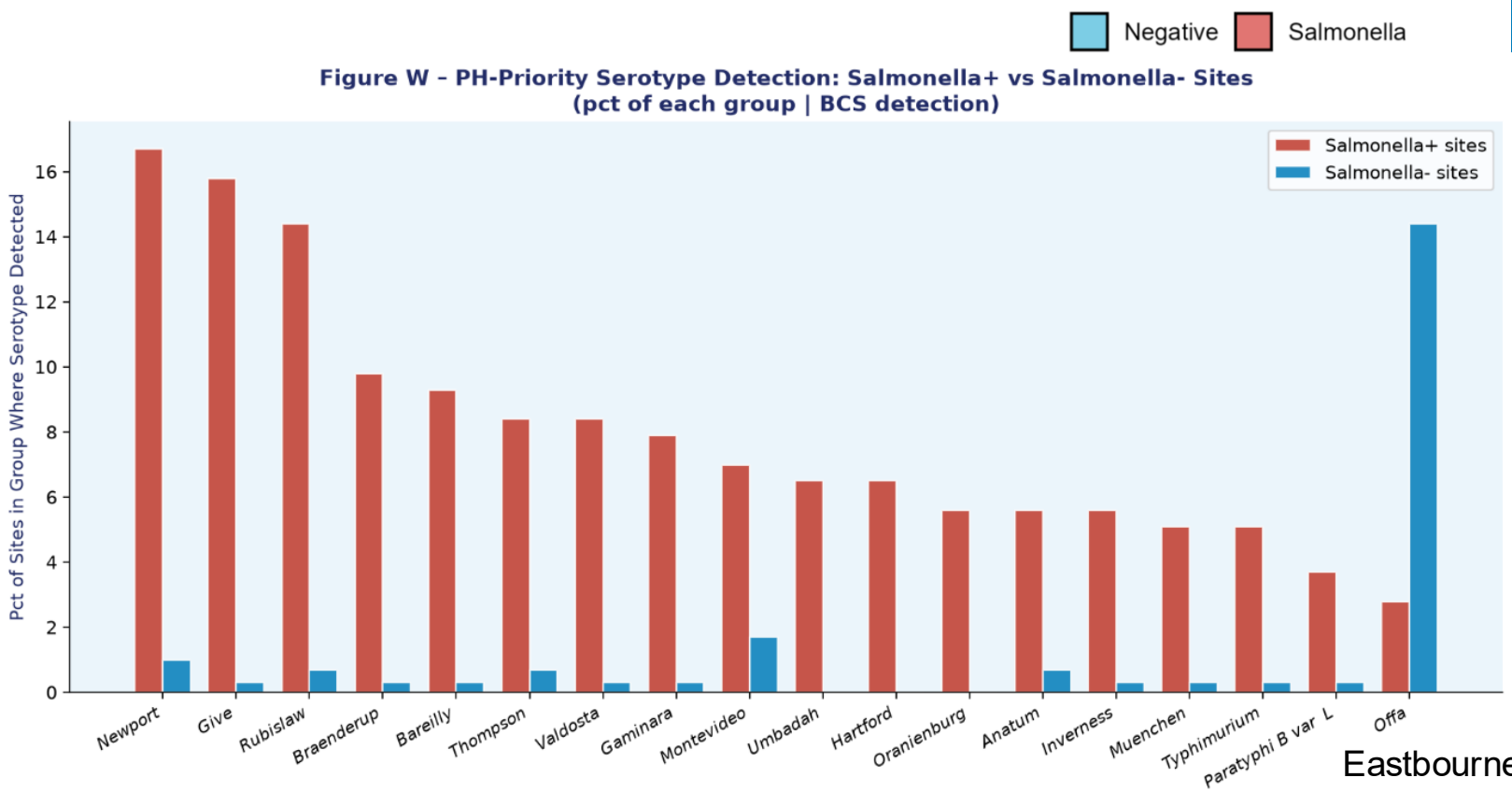
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