EPA Office of Research & Development Initiatives to Monitor Environmental AMR and Model Risk

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NARMS
National Antimicrobial Resistance Monitoring System

- **FDA**
  - Pat McDermott, Errol Strain, Claudine Kabera, Andrea Ottesen, Daniel Tadesse, Heather Harbottle, Christopher Grimm, Heather Tate, Wesley Hunter, Jie Zheng
- **USDA**
  - Kim Cook, Jim Wells, Clinton Williams, Manan Sharma, Mark Ibekwe, Lisa Durso, Johnathan Frye
- **CDC**
  - Amy Kirby, Dan Weller, Sue Gerber, Jason Folster, Andrew Huang,

Outline

- Interagency collaboration on developing a surface water pilot as part of NARMS
- Upcoming request for proposals for evaluating fate and transport of AMR through municipal wastewater treatment & relative impacts on the environment
- Developing of quantitative microbial risk assessment models for relevant exposures
  - Current work on waterborne exposures
  - Upcoming work on QMRA models for crop use of antimicrobials

Larson et al. 2018. ***Critical Knowledge Gaps and Research Needs Related to the Environmental Dimensions of Antibiotic Resistance.***
Environment International 117, 132-138

Relative Contributions of Different Sources

Role of Environment on Evolution of Resistance

Human/Animal Health Impacts from Environmental Exposures

Efficacy and Feasibility of Interventions

National-Scale Surface Water Efforts

Initiatives for Addressing Antibiotic Resistance in the Environment*: Current Situation and Challenges*
https://wellcome.org/sites/default/files/antimicrobial-resistance-environment-report.pd (2018)

- **Environmental waters one of the areas in the report**
  - Geospatial distribution of resistance to inform risk
  - Sources & selective pressures for amplification/transmission
  - Define & standardize sampling/analysis methods

"Following the NARMS Review Subcommittee recommendations to incorporate the three major domains of the One Health model (humans, animals, environment), an important theme of this strategic plan is the expansion of testing to examine resistance in animal pathogens and the environment. For environmental monitoring, what constitutes the best sampling points will be refined over time. Surface waters as confluence points of ecosystems differentially affected by built environments is a starting point."

NARMS Strategic Plan 2020-2025

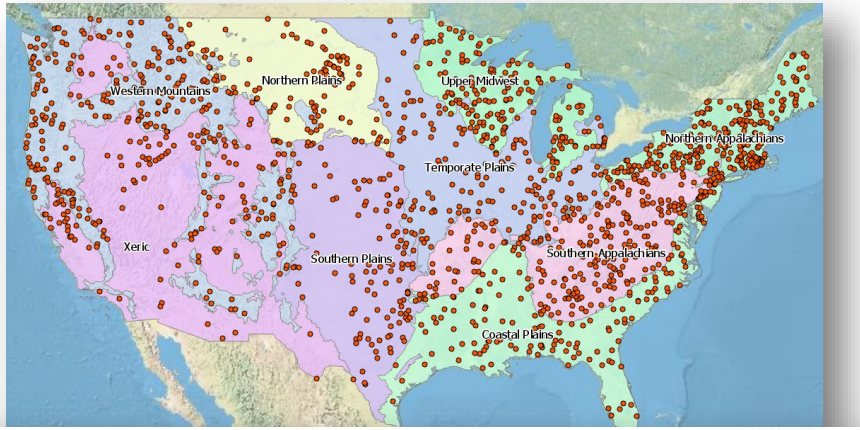
Surface Water AMR Monitoring (SWAM) Objectives

- A pilot environmental effort within a One Health focused NARMS

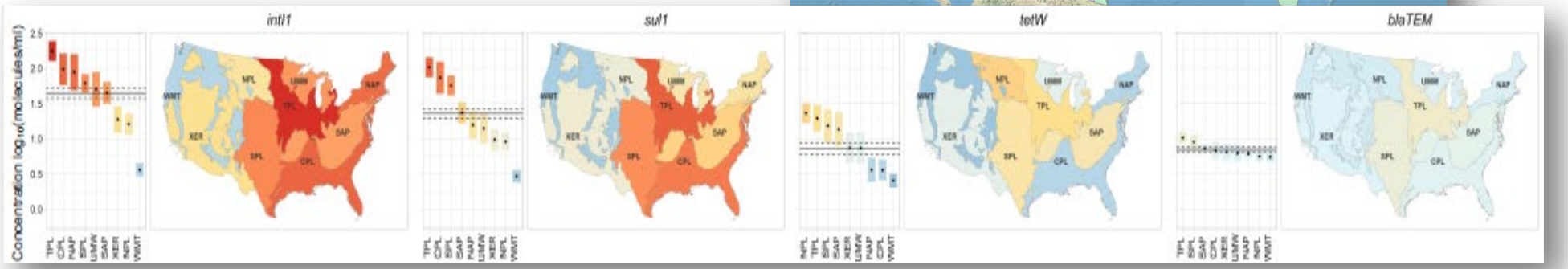
- Develop a national-scale, quantitative assessment of AMR within surface water:
  A. Standardized measure (and library of samples) to monitor trends as part of NARMS
  B. Input to models of AMR risks for various end uses of water (recreational, drinking, agricultural, water reuse)
  C. Help quantify drivers of occurrence and selective pressures for potential amplification
  D. Identify critical control points and assess current and new mitigation strategies

Designing the Study

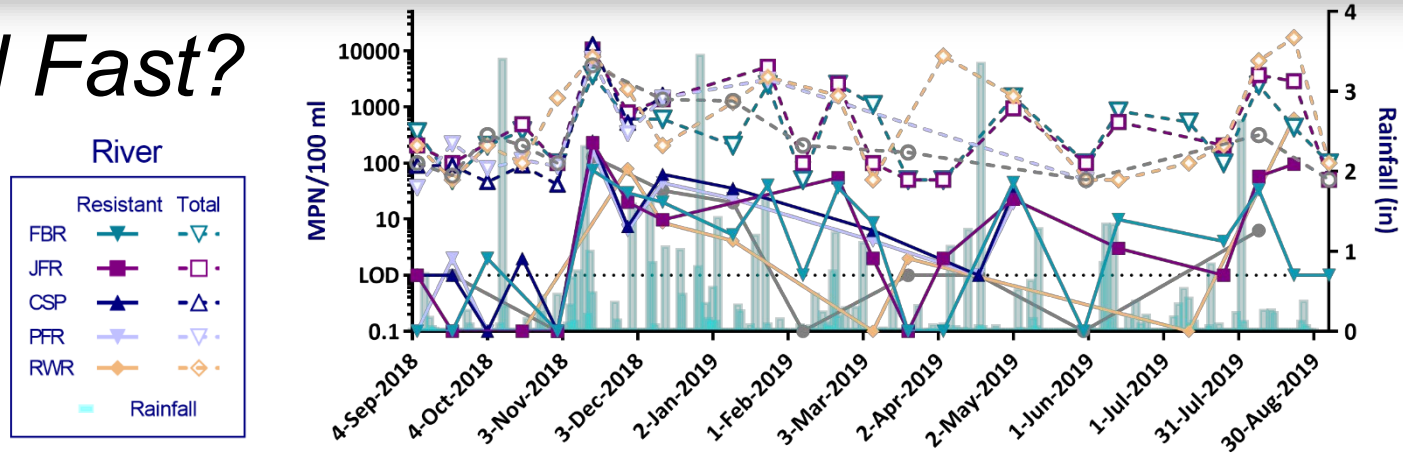
Go Big and Slow?



EPA National Rivers and Streams Assessment
5 year, probabilistic survey of aquatic resource



Or Small and Fast?



CDC Preliminary Surface Water Study in Chattahoochee River

NARMS
National Antimicrobial Resistance Monitoring System

Phased design for SWAM

Phase 1	SWAM Pilot	Statistical Design Subgroup discussions	Initial testing of methodologies	FY21-1st half FY22
Phase 2			Watershed based assessment to evaluate methodologies before national sampling and serve as a demonstration project for future watershed studies	Spring FY22-Spring FY23
Phase 3			Probabilistic national survey to provide statistically valid estimates of AMR status and trends in surface water, using methods tested in the other phases	Summers 2023-24
Phase 4			Continued probabilistic national monitoring together with expanding number of (partner-led) intensive watershed studies across the country	2024+

Analytical Targets

- **Culture**
  - *Enterococci, E.coli:* Links to existing water quality methods
    - Will quantify and determine resistance to specific antibiotics
  - *Salmonella:* Links to food cycle & NARMS
    - Presence/absence

- **Targeted Gene Analysis**
  - Defined panel of antibiotic resistance genes important to human, animal, and environmental health, including fecal source trackers (~90-100 genes)

- **Metagenomics**
  - Define environmental resistome in surface waters
  - Determine new genes to quantify via targeted gene analysis

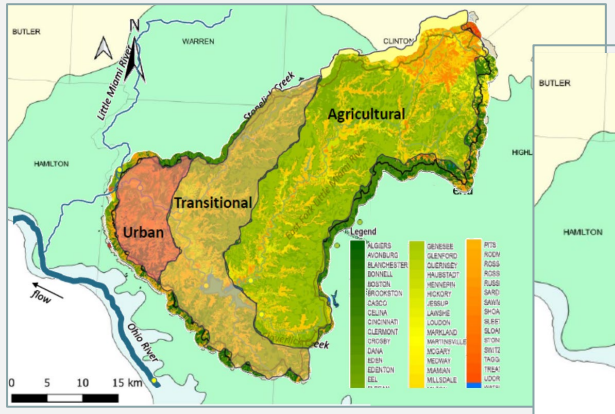
Additional Culture Method Details

- **E. coli** – Modified mTEC method (Modification of EPA Standard Method 1603)
  - Cefotaxime resistance
  - Enumeration of both resistant and susceptible types
  - Whole genome sequencing of as many isolates as possible (FDA-CVM)

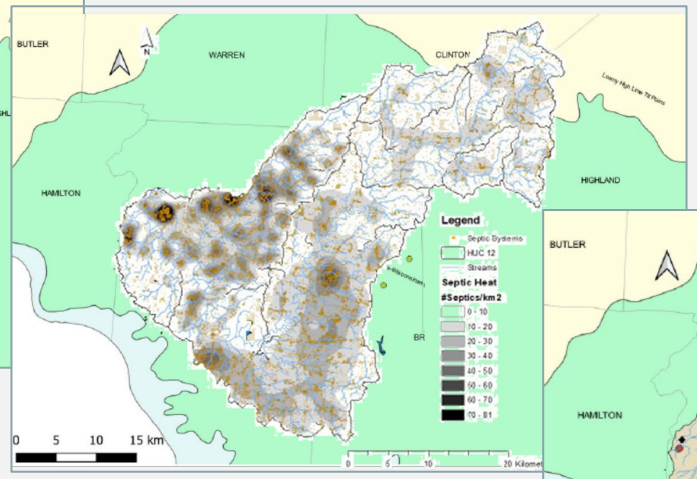
- ***Enterococcus spp.*** – Modified mEI method (Modification of EPA Standard Method 1600)
  - Vancomycin resistance
  - Enumeration of both resistant and susceptible types

- ***Salmonella*** – modified EPA Standard Method 0260.B2
  - Glass wool and cellulose powder filtration followed by enrichment
  - Presence/absence only
  - Whole genome sequencing of all isolates (FDA-CVM)

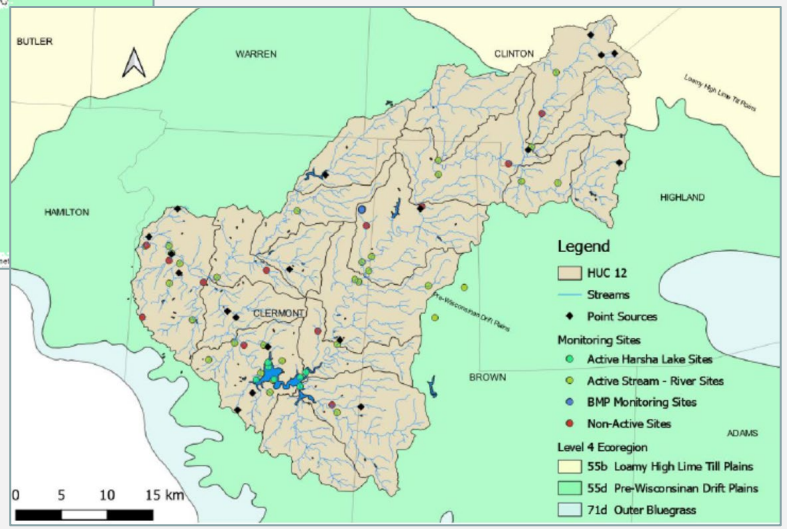
East Fork Little Miami Watershed AMR Pilot



Urban- Ag transition



12K septic systems mapped



Point sources and rec waters in relation to sample sites

Will determine minimum reporting and data quality objectives for comparisons to NRSA and future watershed studies

Additional watershed studies needed:

- High livestock inputs
- Highly urbanized systems
- Regional variation

EPA United States Environmental Protection Agency

Watershed studies complement NRSA design

Is there temporal/seasonal variation in antimicrobial resistant bacteria and genes?

Are there environmental reservoirs of AMR?

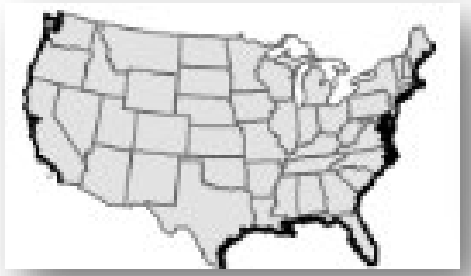
What are the relative contributions of different AMR sources (e.g., septic, WWTP, livestock, wildlife)

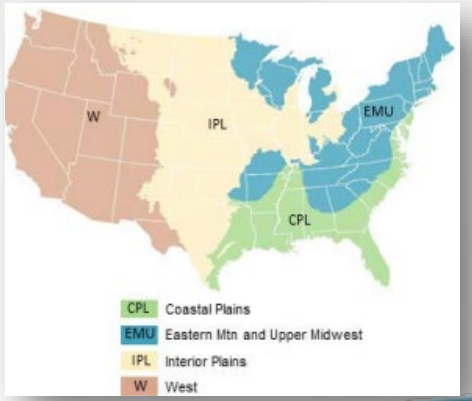
What are the watershed-scale drivers and attenuators of AMR?

How can we mitigate AMR at local scales?

National Water Resources: Opportunity to Monitor AR

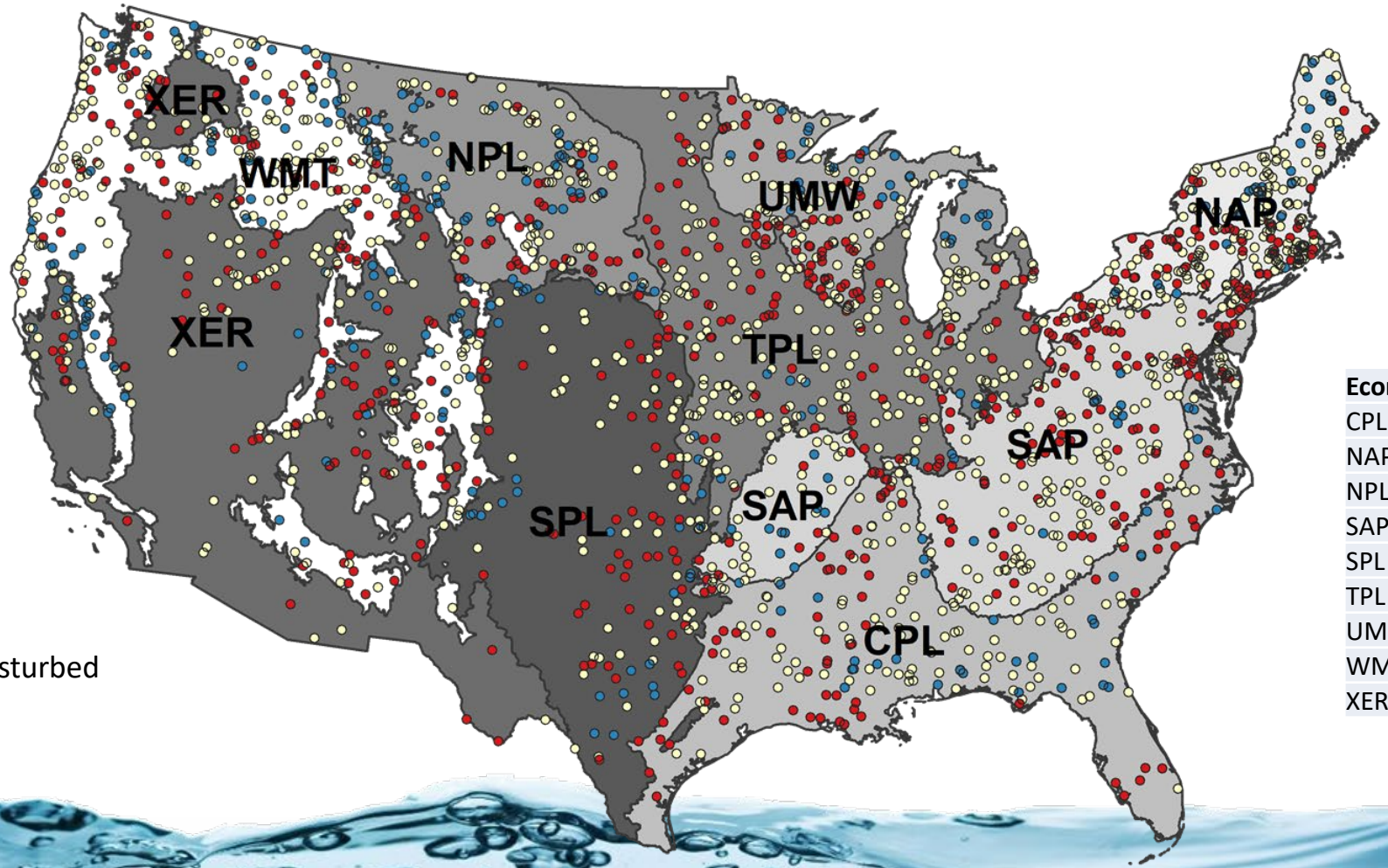
- The National Aquatic Resource Surveys (NARS) are collaborative programs between the EPA, states, and tribes to assess the quality of the nation's waters
  - National Rivers and Streams Assessment (NRSA)
  - National Coastal Condition Assessment (NCCA)
  - National Lakes Assessment (NLA)
  - National Wetland Condition Assessment (NWCA)
- Surveys are conducted annually
  - 5-year survey cycles





Approach – National Rivers and Streams Assessment (NRSA) Survey

Ecoregion Abbreviations:
Coastal Plains (CPL)
Northern Appalachians (NAP)
Northern Plains (NPL)
Southern Appalachians (SAP)
Southern Plains (SPL)
Temperate Plains (TPL)
Upper Midwest (UMW)
Western Mountains (WMT)
Xeric (XER)

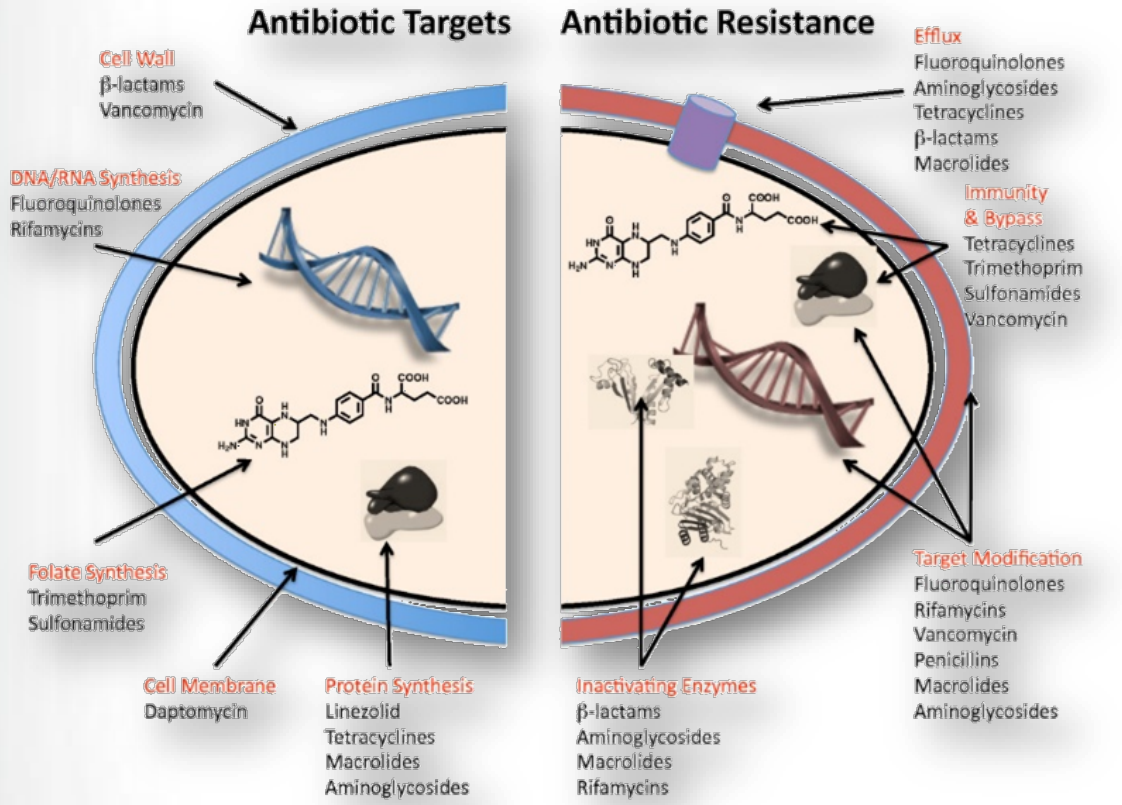


Study years: 2013-2014
N= 1853
~1.2 million kilometers

blue is Least Disturbed
yellow is Intermediate Disturbed
red is Most Disturbed

Ecoregion	total km
CPL	198824
NAP	138082
NPL	27108
SAP	289341
SPL	38818
TPL	185850
UMW	101648
WMT	186538
XER	44017

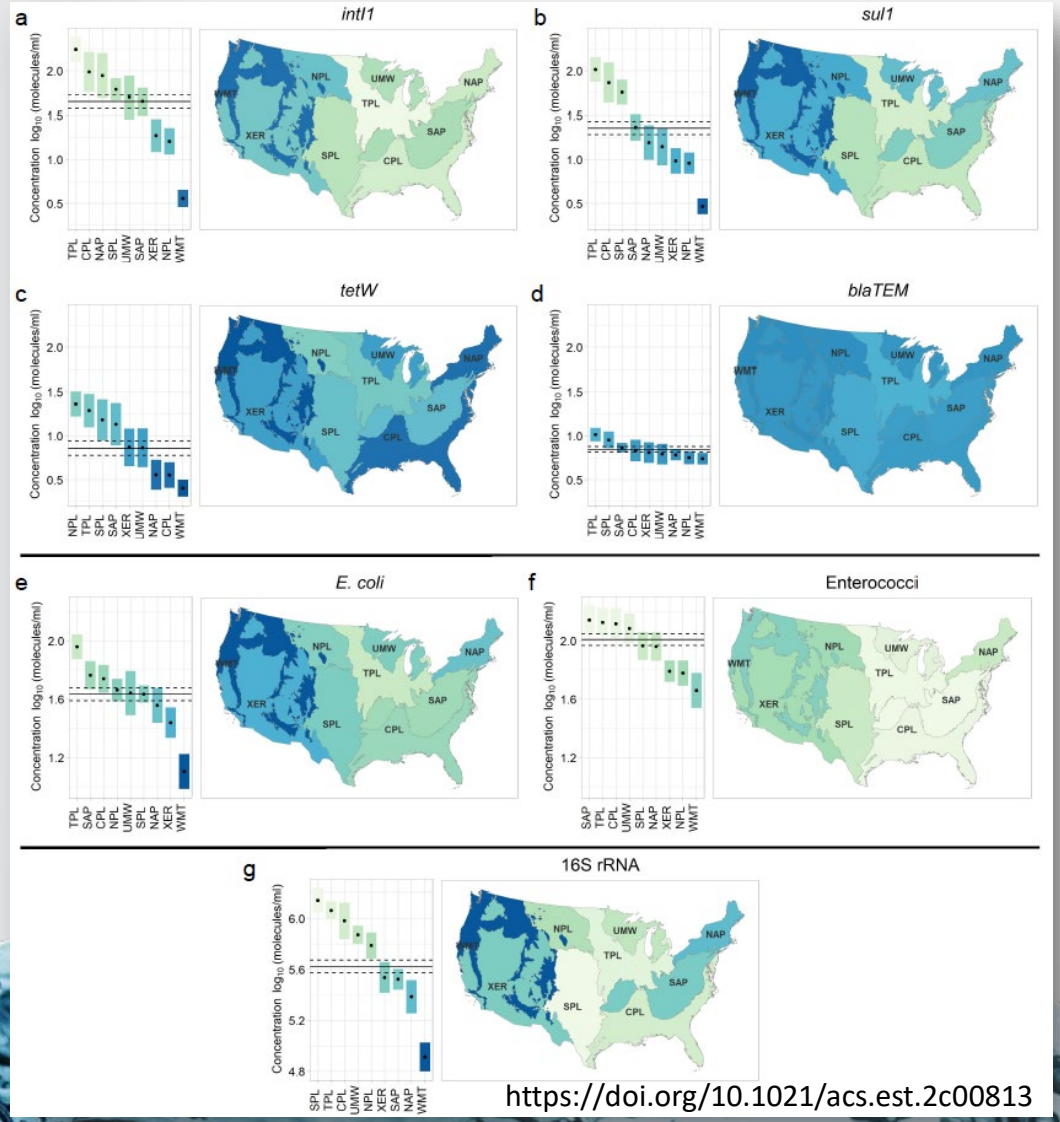
Genes Included in the Pre-Pilot



Wright, G. D. (2010)

- class 1 integron-integrase (*intI1*)
- sulfonamide resistance (*sul1*)
- tetracycline resistance (*tetW*)
- beta-lactam resistance (*blaTEM*)
- *Klebsiella pneumoniae* carbapenemase (*KPC*)
- vancomycin resistance (*vanA*)
- colistin resistance (*mcr-1*)
- 16S and 23S rRNA for total and fecal indicator bacteria (enterococci and *E. coli*)

Results: Geospatial Distribution of ARG



https://doi.org/10.1021/acs.est.2c00813

Observations:

Both *intI1* and *sul1* were high in the Plains (except for NPL) and Appalachians and low in NPL, XER, and WMT

tetW was high in the Plains (except for CPL) and SAP and low in the NAP, CPL, and WMT

blaTEM was high in the TPL and SPL and low everywhere else.

E. coli was high in the TPL, SAP CPL and low in the NAP, XER and WMT

Enterococcus was high in the SAP, TPL, CPL and low in the XER, NPL and WMT

16S rRNA gene was high in the SPL, TPL, CPL and low in the SAP, NAP and WMT

KPC, *vanA* and *mcr-1* were too low for analysis

Baseline Analysis

Published March 1, 2016
J. Environ. Qual. 45:420–431 (2016) doi:10.2134/jeq2015.06.0327
Journal of Environmental Quality SPECIAL SECTION
ANTIBIOTICS IN AGROECOSYSTEMS: STATE OF THE SCIENCE

How Should We Be Determining Background and Baseline Antibiotic Resistance Levels in Agroecosystem Research?

Michael J. Rothrock, Jr.,* Patricia L. Keen, Kimberly L. Cook, Lisa M. Durso, Alison M. Franklin, and Robert S. Dungan

Hypothesis: ARGs are associated with environmental impairment

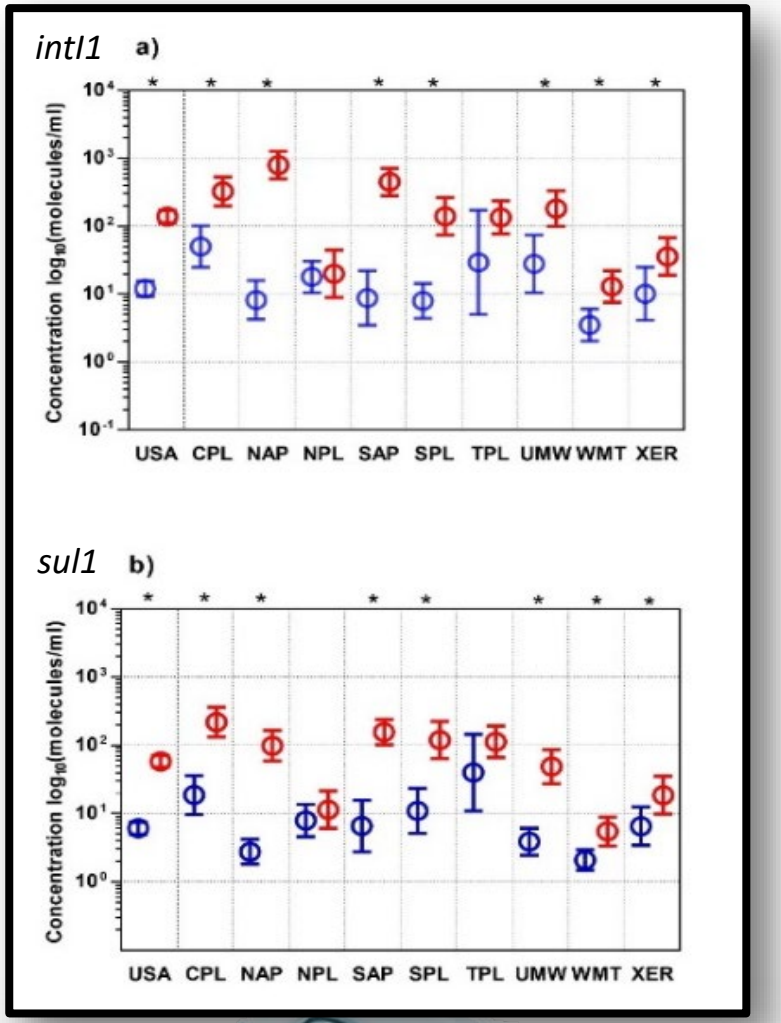
- Good condition (Least Disturbed Sites) associates with low gene concentrations
- Poor condition (Most Disturbed Sites) associates with high gene concentrations

https://www.epa.gov/national-aquatic-resource-surveys/national-rivers-and-streams-assessment-2013-2014-report

Least Disturbed Sites (LDS)	Ranges	
Total P (µg/L)	≤20	≤150
Total N (µg/L)	≤750	≤4500
Cl^- (µeq/L)	≤200	≤2000
$SO4^{2-}$ (µeq/L)	≤200	≤400
ANC (µeq/L)+ DOC (mg/L)	≥50 + ≥5	≥50 + ≥5
Turbidity (NTU)	≤5	≤50
Riparian Disturbance Index	≤0.5	≤2
% fine substrate	≤15	≤90

Most Disturbed Sites (MDS)	Ranges	
Total P (µg/L)	>100	>500
Total N (µg/L)	>1500	>15000
Cl^- (µeq/L)	>1000	>10000
$SO4^{2-}$ (µeq/L)	>1000	>4000
ANC (µeq/L) + DOC (mg/L)	<0 <5	<0 <5
Turbidity (NTU)	>10	>100
Riparian Disturbance Index	>3	>4
% fine substrate	>50	>100

Baseline Results: LDS versus MDS



Least Disturbed Sites

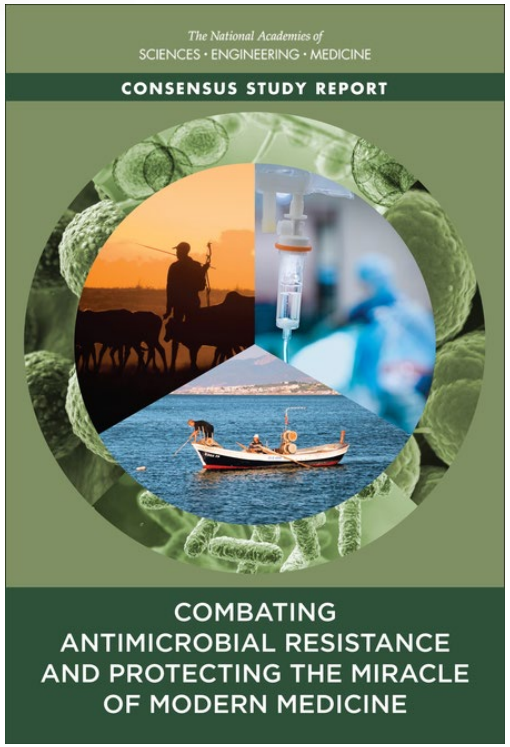
Most Disturbed Sites

* Credible differences

Conclusions

- ARGs showed significant geospatial patterns at national scale
- Good quality rivers/streams had lower ARG concentrations than poor quality ones
- These data suggest *intI1* can be used as an *operational* ecological condition indicator, but more research is needed
- Baseline analysis findings:
  - Urbanization and poor watershed integrity were significantly associated with high concentrations of *intI1* and *sul1*
  - Poor watershed integrity, but not urbanization, was associated with high concentrations of *tetW*
  - Urbanization and poor watershed integrity were not associated with *blaTEM*
- 2023-24 NRSA cycle: same statistical design but expanded analytical targets, larger volumes

2021 National Academy of Sciences Report



The challenge for environmental monitoring is to determine what factors amplify resistance in the environment and what factors encourage their transmission

Water treatment plants are…. not equipped to eliminate resistance traits or drug residues….an important bridge between human made contamination and the natural environment

Strengthening - Combating Antimicrobial Resistance and Protecting the MiracSurveillancele of Modern Medicine - NCBI Bookshelf (nih.gov)

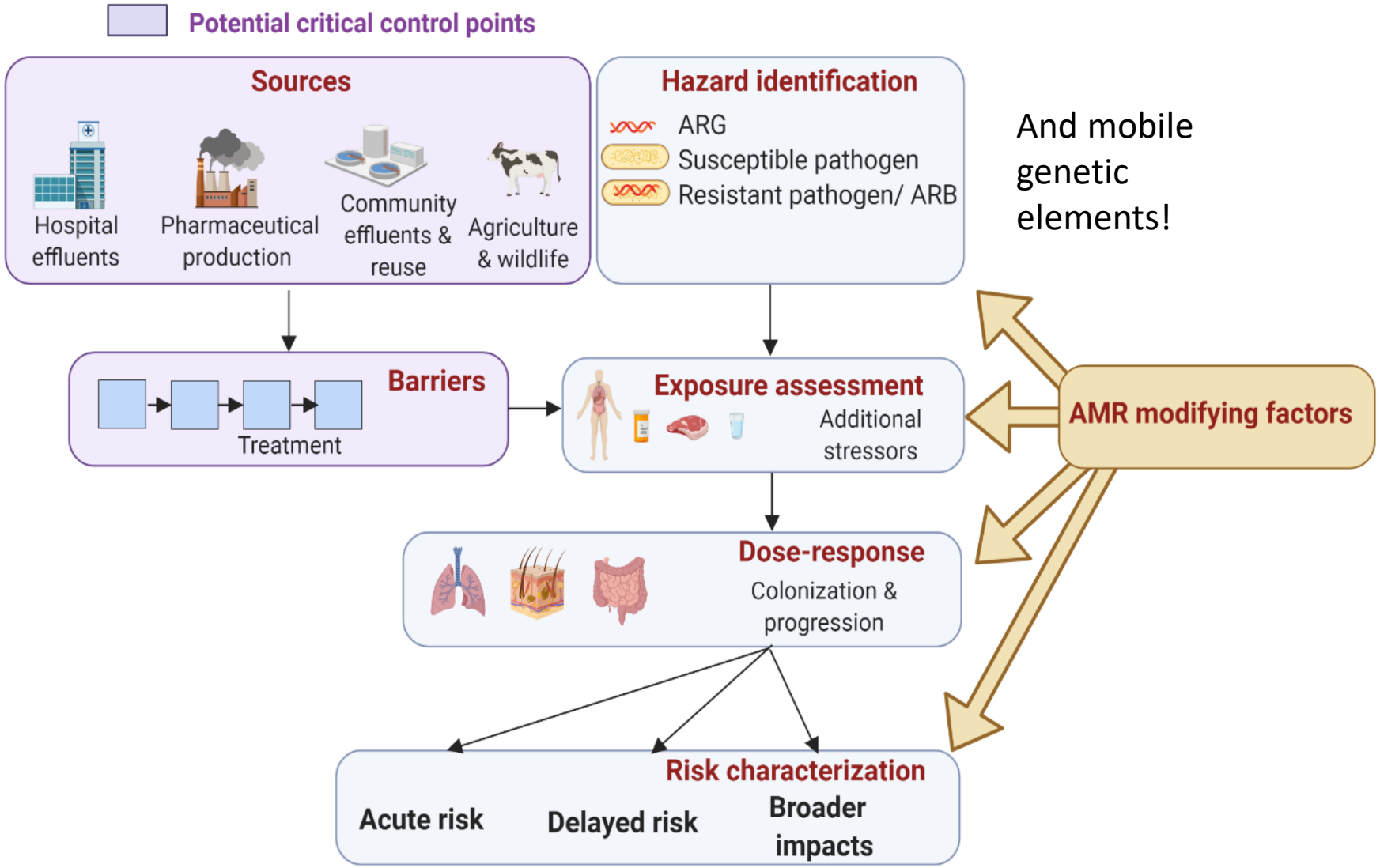
Recommendation 4.2 The EPA should provide guidance and resources to states for testing point source discharges at wastewater treatment plants for antimicrobial resistance traits and integrating these data with other surveillance networks”

National Priorities: Evaluation of Antimicrobial Resistance in Wastewater and Sewage Sludge Treatment and Its Impact to the Environment

This RFA will solicit research on selection and removal efficiency of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in wastewater treatment plants. It will also request research on the relative significance of wastewater effluent as a source of ARB and ARGs in receiving waters…to be released spring 2023

What factors do we need for “risk assessment +”?

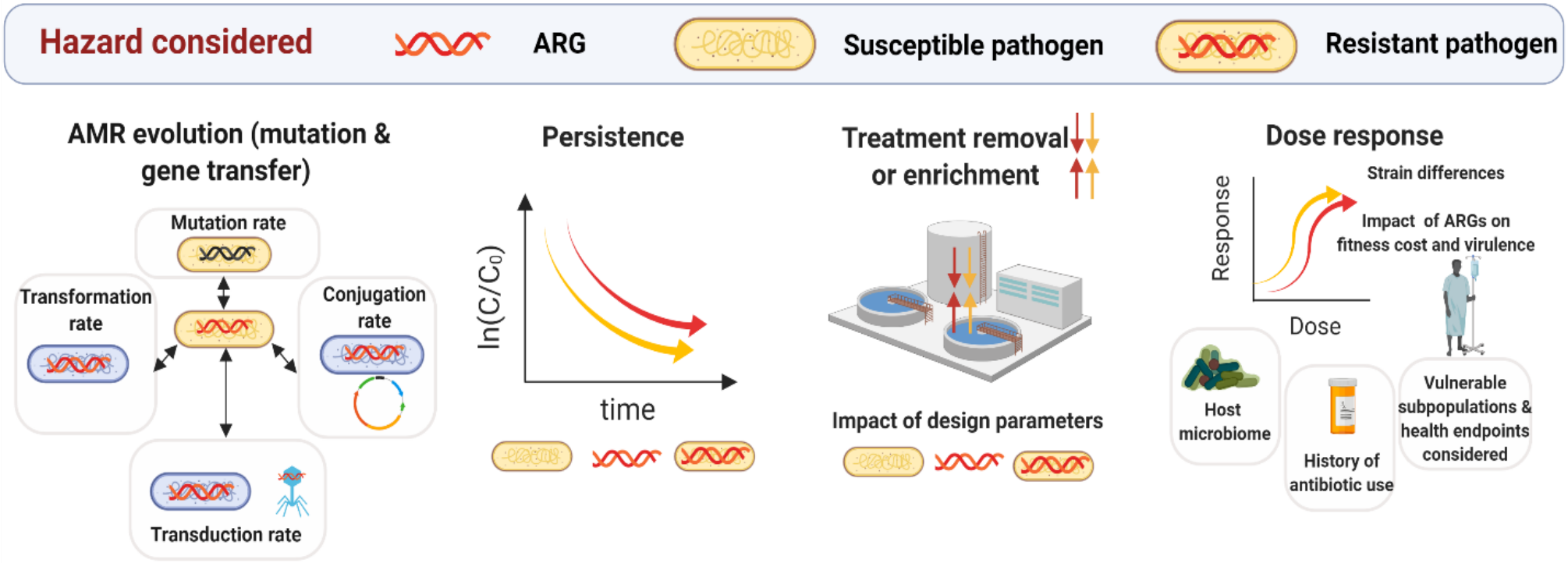
Systematic literature review, stakeholder focus group (40+), advocate/activist interviews (n=6)



And mobile genetic elements!

Hamilton et al. ES&T in revision

What are the key "modifying factors"?



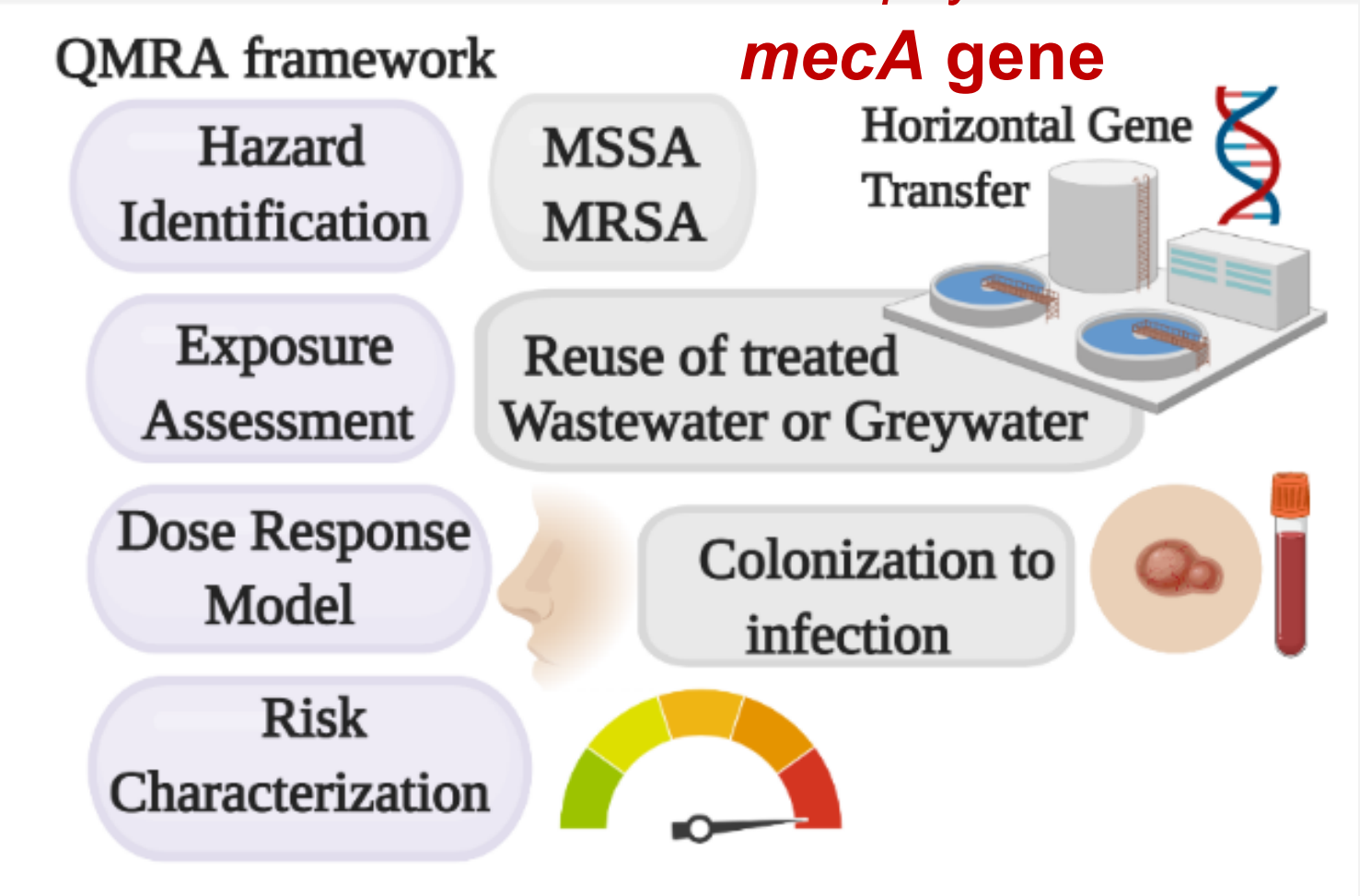
Modifying factors are a function of:

ARG and vector ARB identity
Antibiotic concentrations
Heavy metals
Other biological stressors
Water quality parameters
Microbial community
…and many more

Hamilton et al. ES&T in revision

An example incorporating a gene transfer parameter

MSSA= Methicillin-susceptible *Staphylococcus aureus*
MRSA= Methicillin-resistant *Staphylococcus aureus*



***mecA* gene**

Mary Schoen, Soller Environmental

"Log increase value"

$$LIV_{HGT.MRSA} = \log_{10}\left(1 + \frac{HGT*(1-F_r)}{F_r*(1+HGT)}\right)$$

Where *HGT* = ratio of transconjugant/recipient cells and F_r = fraction of bacteria resistant at start of treatment process

Schoen, M.E., Jahne, M.A., Garland, J., Ramirez, L., Lopatkin, A.J. and Hamilton, K.A., 2021. Quantitative microbial risk assessment of antimicrobial resistant and susceptible Staphylococcus aureus in reclaimed wastewaters. *Environmental Science & Technology*, *55*(22), pp.15246-15255.