

**'CROWD'-SOURCING ENVIRONMENTAL PATHOGENOMICS:  
Enteric Pathogens and Resistance Plasmids  
Isolated from Stream Sediments in the  
Shenandoah Valley of Virginia**

**JAMES B. HERRICK, PH.D.**

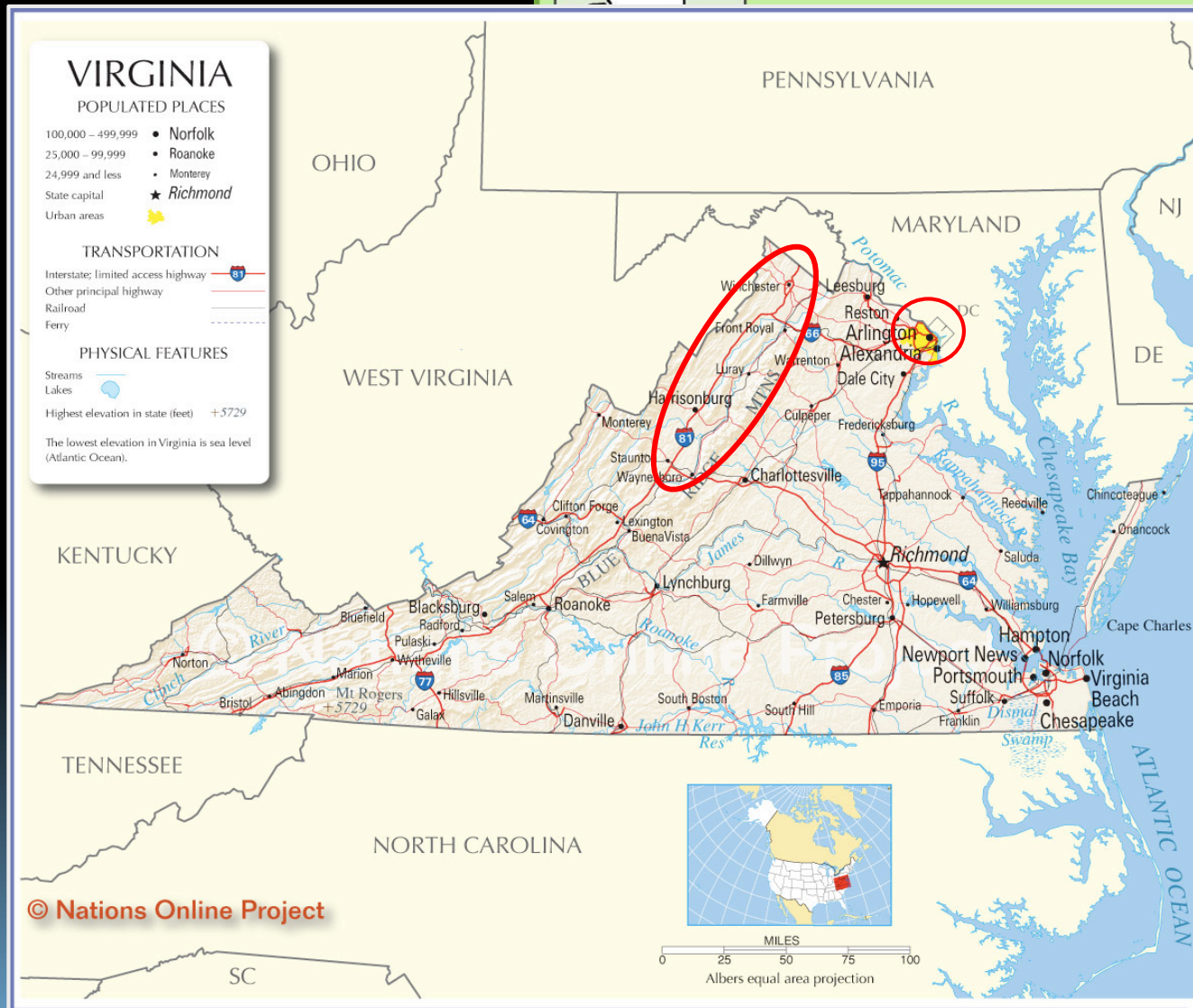
*James Madison University*

Harrisonburg, Virginia USA

*"The future of humanity and microbes likely will unfold as episodes of a suspense thriller that could be titled 'Our Wits Versus Their Genes'."*

-- Joshua Lederberg

# THE SHENANDOAH VALLEY OF VIRGINIA









# MANURE PRODUCTION IN THE SHENANDOAH VALLEY

**Table A. 2012 Estimated Phosphorus Output from Poultry and Cows, Augusta, Page, Rockingham, and Shenandoah Counties**

Type	Number of Animals	Manure Output	Phosphorus (pounds)
<b>Chickens</b>			
Broilers	157,380,630	196,726 tons	4,485,348
Pullets	1,286,348	32,101 tons	251,545
Layers	939,008	32,560 tons	250,715
<b>Turkeys</b>			
	16,534,511	148,811 tons	3,258,961
<b>Cows</b>			
	528,943	1.28 billion gallons	5,056,038
<b>Total</b>	<b>176,669,440 animals</b>	<b>410,198 tons and 1.28 billion gallons</b>	<b>13,302,607</b>

*Note: Manure generation and phosphorus content shown on a recoverable or as-is basis. Sources: USDA 2012 Agricultural Census, Virginia Department of Conservation and Recreation 2014 Nutrient Management Standards and Criteria, and the Chesapeake Bay Program. See Appendix A for methods.*



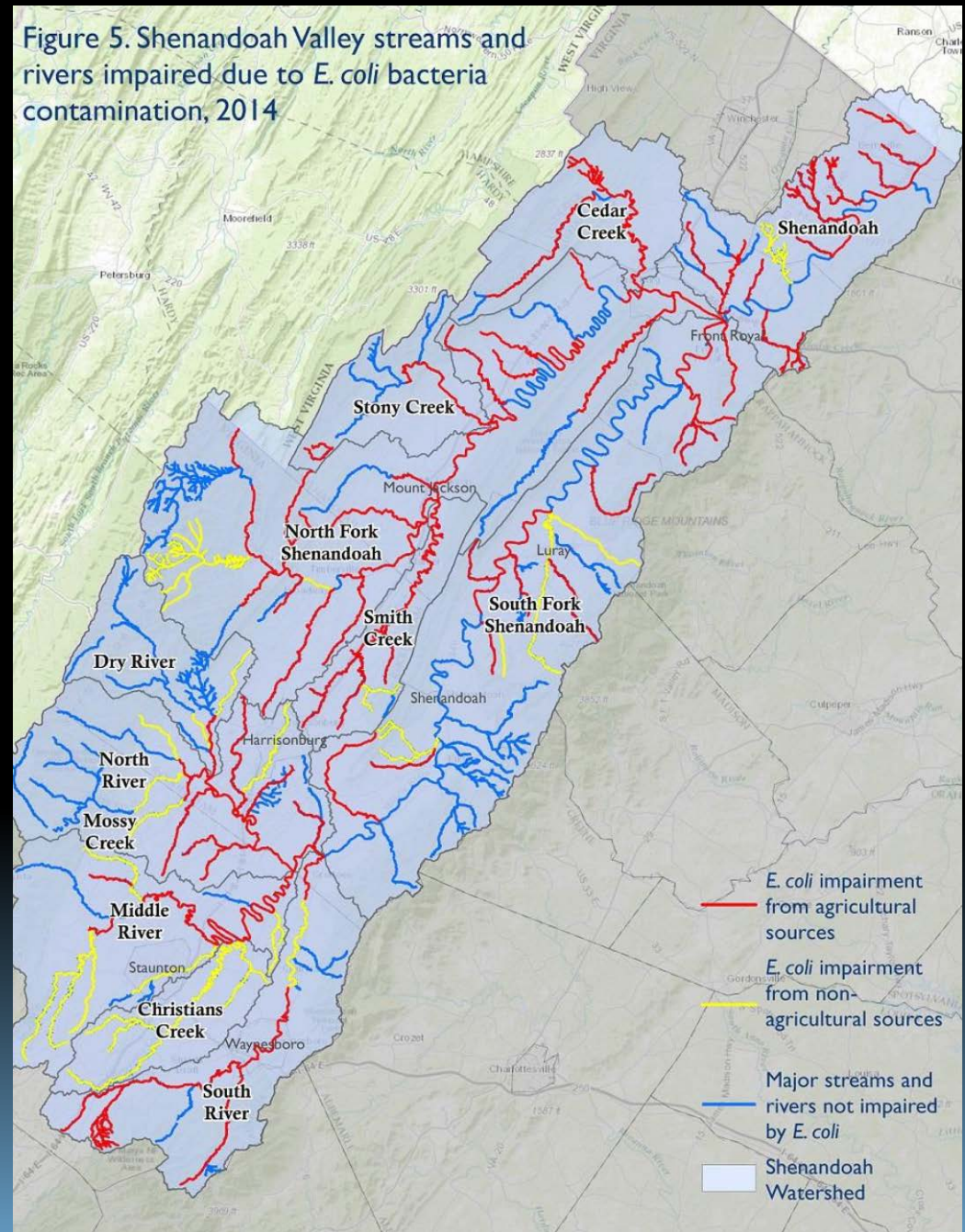




Field application of poultry litter

# CONTAMINATED WATERWAYS

Figure 5. Shenandoah Valley streams and rivers impaired due to *E. coli* bacteria contamination, 2014





Details | Basemap

Share Print ▾ | Measure



### Legend

#### Monitoring Locations

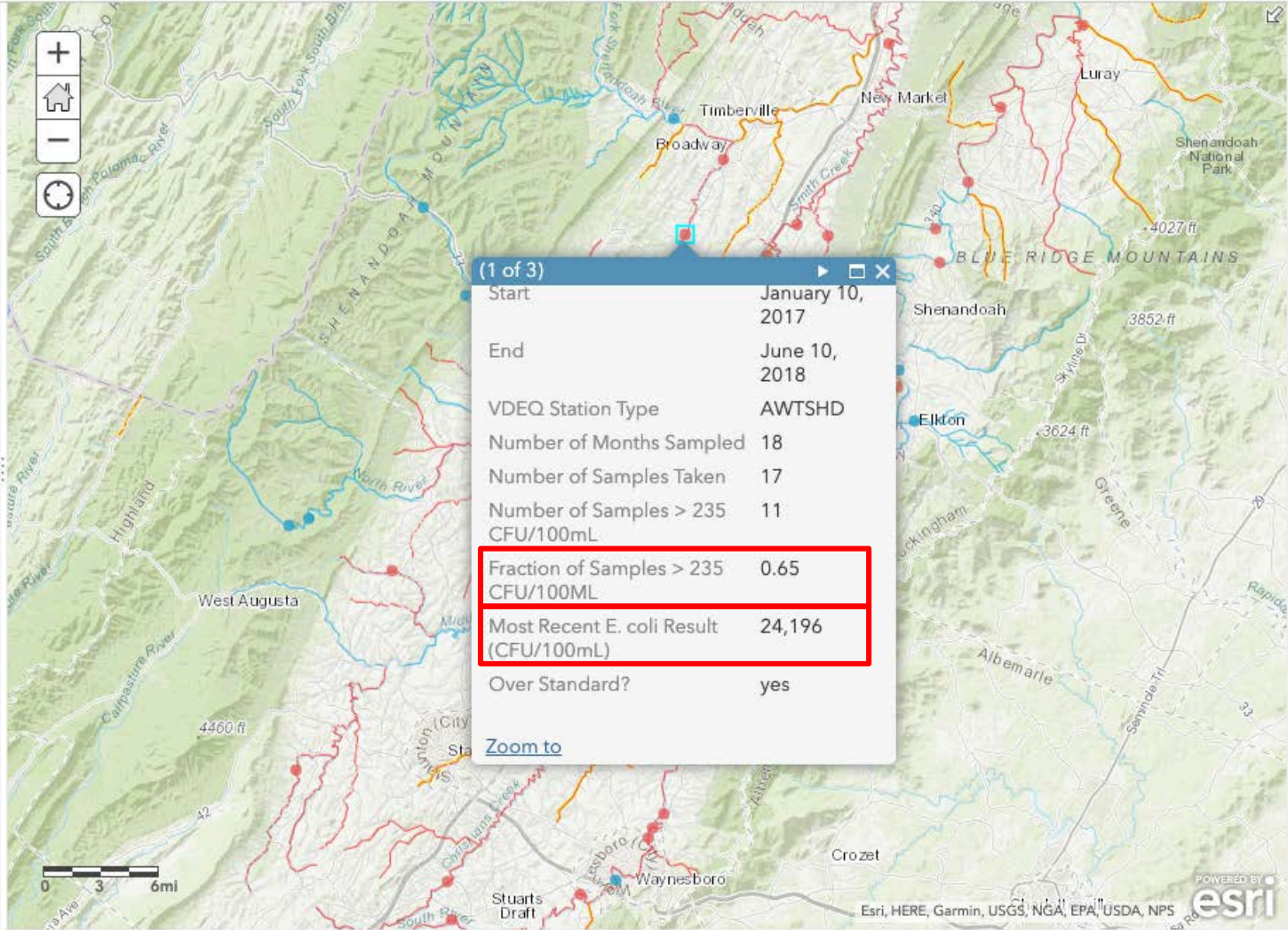
Unsafe for swimming?

- yes
- no

#### Recreational Use Status

- Not Supporting
- Fully Supporting
- Not Assessed
- Insufficient Information

#### Needs Clean Up Plan (TMDL)



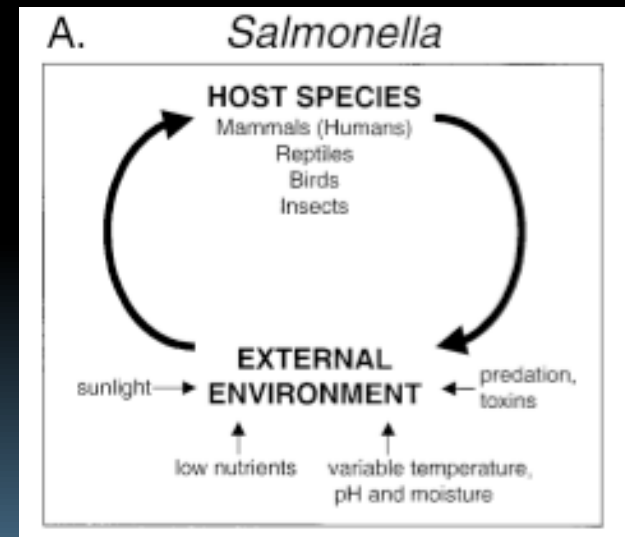
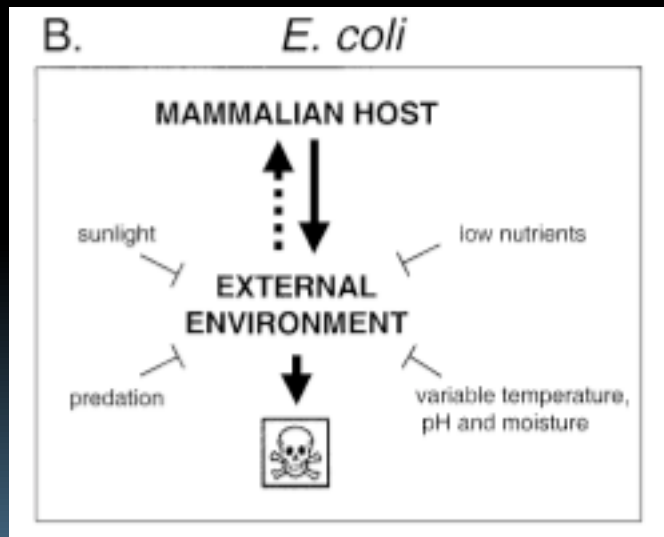


Are there fecal pathogens in these impacted  
waterways?

# CONTRASTING LIFESTYLES OF *SALMONELLA* AND *E. COLI* IN AQUATIC, SOIL, AND AGRICULTURAL ENVIRONMENTS

*E. coli* → negative growth rates in most secondary habitats

*Salmonella* → uses secondary habitats to facilitate passage to new hosts



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2003, p. 3687-3694  
0099-2240/03/\$08.00+0 DOI: 10.1128/AEM.69.7.3687-3694.2003  
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Vol. 69, No. 7

## MINIREVIEW

Role of Nonhost Environments in the Lifestyles of *Salmonella*  
and *Escherichia coli*

Mollie D. Winfield and Eduardo A. Groisman\*



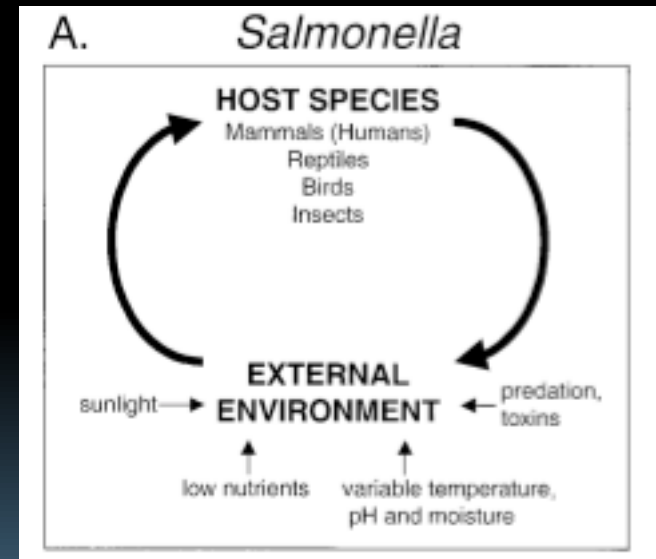
# CONTRASTING LIFESTYLES OF *SALMONELLA* AND *E. COLI* IN AQUATIC, SOIL, AND AGRICULTURAL ENVIRONMENTS

*E. coli* → negative growth rates in most secondary habitats

*Salmonella* → uses secondary habitats to facilitate passage to new hosts

## *Salmonella*:

- Has high survival rates in aquatic systems
- Is more resistant than *E. coli* to environmental fluctuations
- Can persist in a poultry house for > 1 year
- Can survive and grow in soil for at least a year
- Exhibits four-fold greater adherence to sediment particles than *E. coli*



Annals and Environmental Microbiology, July 2003, p. 3667-3684  
0092-2465/03/070367-18. DOI: 10.1128/AEM.73.7.3667-3684.2003  
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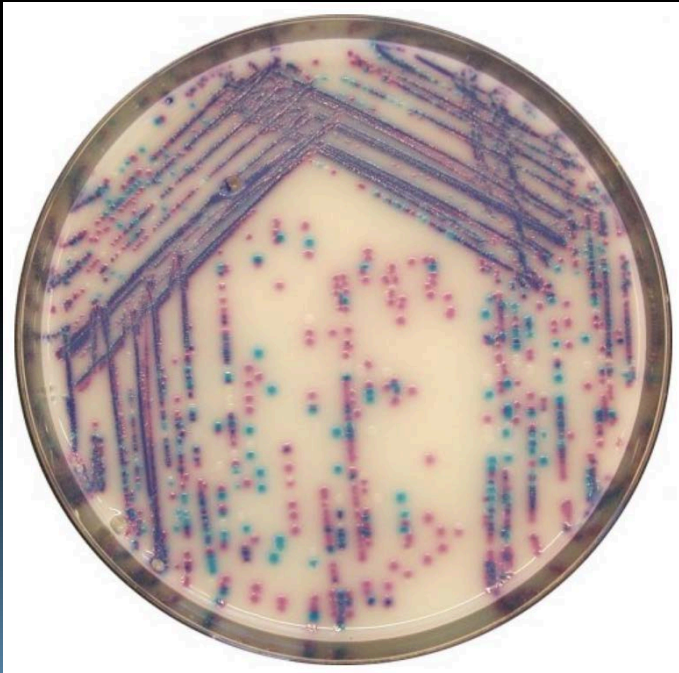
Can we recover them?



# WHY SAMPLE SEDIMENTS?

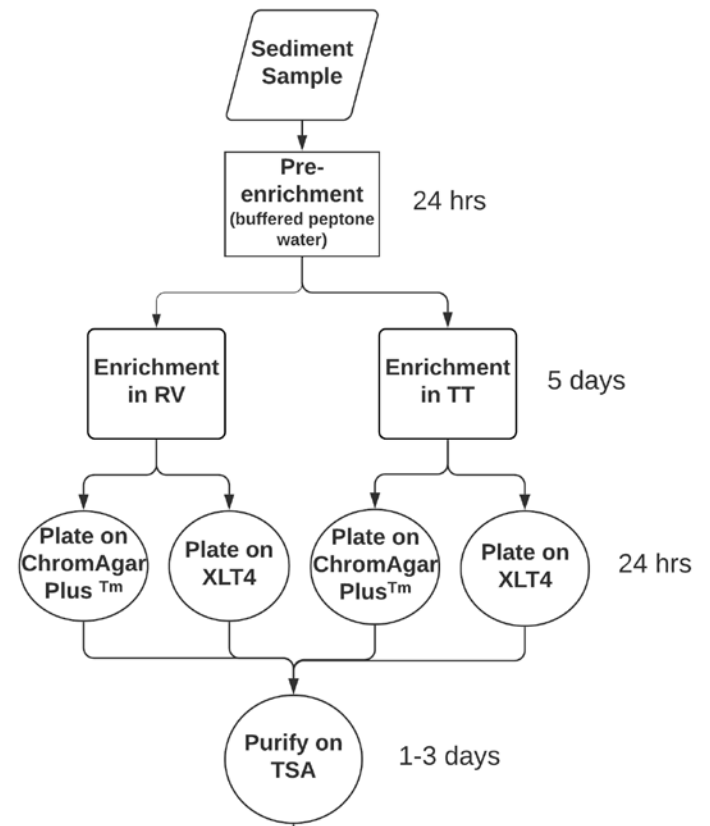
- Total bacterial counts in sediments typically much higher than in the water column
- *Salmonella* and other fecal bacteria can persist in sediments
- Sediment populations may represent a more long-term reservoir

# SALMONELLA ISOLATION AND IDENTIFICATION WORKFLOW

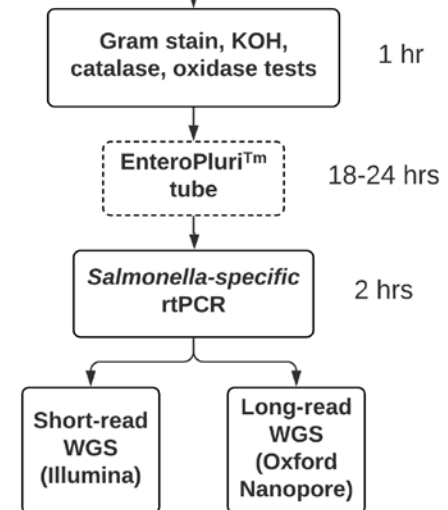


ChromAgar Plus

## Isolation



## Identification









































# 'Crowd'-sourcing isolation, ID, and basic genomics



# SAMPLING & METADATA (EPICOLLECT5)

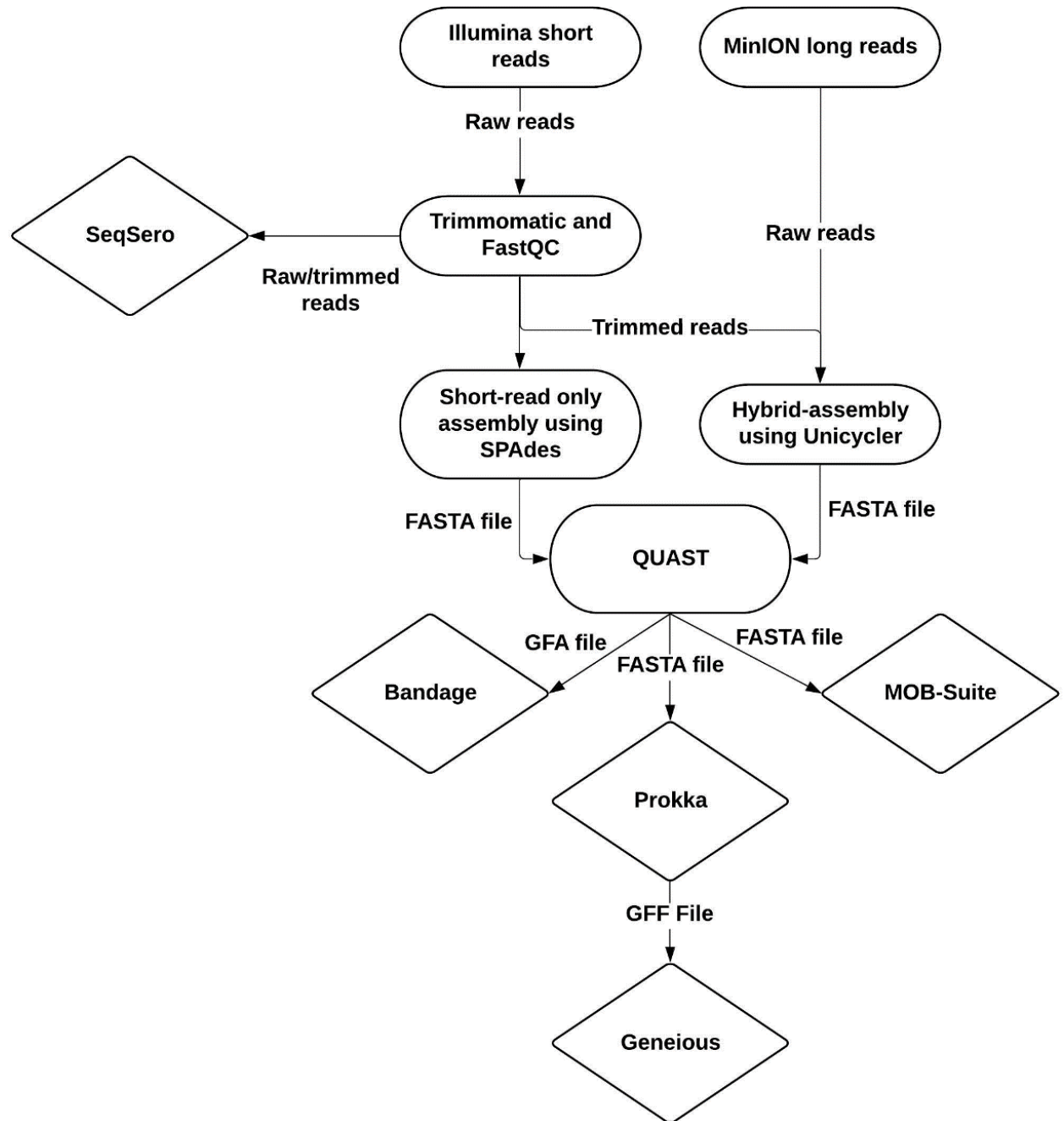
Filter by title

FROM: 22 JAN, 18 TO: 02 SEP, 19 NEWEST ▾ X

View	Delete	Edit	Title	Created At	Enter latitude and longitude (re...	Who collected this sample?	Photo of sampling site	Water Temperature (T)	Salinity
			02/09/2019 Muddy Cr...	2nd Sep, 2019	38.466871, -78.975172	Guilliams		22.9	.15
			02/09/2019 Muddy Cr...	2nd Sep, 2019	38.466927, -78.97457	Emily		22.3	.26
			02/09/2019 Muddy Cr...	2nd Sep, 2019	38.466983, -78.975062	May		22.3	0.26
			02/09/2019 Muddy Cr...	2nd Sep, 2019	38.46693, -78.974626	Brian Swinehart		22.3	.26
			02/09/2019 Cooks Cr...	2nd Sep, 2019	38.419992, -78.939783	Bryan		20.5	.27
			02/09/2019 Cooks Cr...	2nd Sep, 2019	38.419909, -78.939513	Devan		21.6	0.33
			02/09/2019 Other (ent...	2nd Sep, 2019	38.419926, -78.939629	Guilliams		21.6	.33
			02/09/2019 Cooks Cr...	2nd Sep, 2019	38.419972, -78.939768	Brian Swinehart		21.4	.33
			02/09/2019 Cooks Cr...	2nd Sep, 2019	38.372947, -78.934662	May Heivilin		23.4	0.28



# BIOINFORMATICS WORKFLOW



**Tools** ☆ ↑

search tools

**Get Data**

NGS TOOLBOX

- NGS: QC and manipulation
- NGS: Assembly
- NGS: Screening and Prediction
- NGS: Mapping
- NGS: Annotations
- NGS: CFSAN SNP Pipeline (beta)
- NGS: Phylogenetics
- NGS: Megablast

METAGENOMICS TOOLBOX

- Metagenomics: Metaphlan
- Metagenomics: Diamond
- Metagenomics: Kraken
- Metagenomics: Mitokmer
- Metagenomics: Lefse
- Metagenomics: Graphlan
- Metagenomics: Functional Profiling
- Metagenomics: Assembly
- Metagenomics: AMR

## Welcome to GalaxyTrakr: open-source bioinformatics for public health.

This site is intended for use by GenomeTrakr laboratories and their collaborators to assist in the analysis of genomic data for foodborne pathogens. This instance of Galaxy is hosted in a public environment and no personally identifiable (PII) or commercial confidential information should be uploaded.

### --!!--Information and Announcements--!!--

Please re-import the skesamlst workflow that was updated a few days ago. Previous versions are no longer working and are causing errors when running. Thank you.

Access CFSAN SNP Pipeline workflows in the shared workflows screen.

Post in the official Galaxy GenomeTrakr board on the Redmine Site: [Click here](#)

[Click here](#) to access the GalaxyTrakr User Guide

Forgot Password? [Email GalaxyTrakr Support Team](#)

Take an interactive tour: [Galaxy UI](#) [History](#) [Scratchbook](#)

Galaxy is an open platform for supporting data intensive research. Galaxy is developed by The Galaxy Team with the support of many contributors.

**History** ↻ + 🗑️ ⚙️

search datasets

**HJ1 Sample**

42 shown, 21 deleted

1.11 GB

- 63: SeqSero Results
- 60: SRR5886281\_2
- 59: HJ01\_SRR5886281\_1
- 47: staramr on data 22: results.xlsx
- 46: staramr on data 22: settings.txt
- 45: staramr on data 22: results.tsv
- 44: staramr on data 22: summary.tsv
- 43: staramr on data 22: hits a list
- 42: Quast: Report (PDF)
- 41: Quast: Report (HTML)
- 40: Quast: Report (tabular)



☰	<a href="#">BLG1a - Navigating and Using GalaxyTrakr</a>	✓	⋮
☰	<a href="#">BLG1b: Salmonella Serotyping</a>	✓	⋮
☰	<a href="#">BLG2a: FastQC &amp; Trimmomatic</a>	✓	⋮
☰	<a href="#">BLG2b-Naming Files</a>	✓	⋮
☰	<a href="#">BLG3a: Genome Assembly</a>	✓	⋮
☰	<a href="#">BLG3b: Assessing Assembly Quality</a>	✓	⋮
☰	<a href="#">BLG3c: Ordering and Viewing Assembled Contigs</a>	✓	⋮
☰	<a href="#">OSF Misc view-only link (RefSeq file)</a>	✓	⋮
☰	<a href="#">RefSeq Salmonella Files</a>	✓	⋮
☰	<a href="#">bacWGSTdb spreadsheet</a>	✓	⋮
☰	<a href="#">BLG4: Gene Annotation</a>	✓	⋮
☰	<a href="#">BLG5 : Resistance Gene Detection</a>	✓	⋮



# WHOLE GENOME SEQUENCING PLATFORMS

## Illumina Sequencing (2<sup>nd</sup> gen)



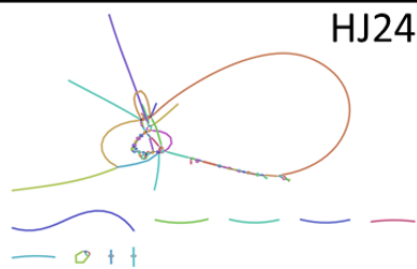
Produces **highly accurate** (>99.9%),  
**short reads** (~50 - 300 bp)

## Oxford Nanopore MinION

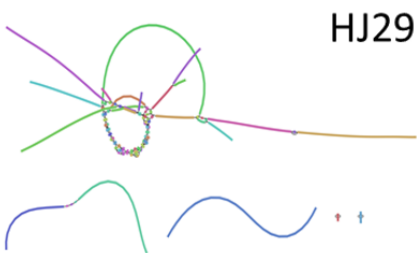


Produces **lower accuracy** (~85-95%),  
**long reads** (> 1 kb)

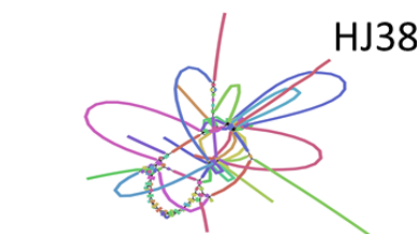
Combine data for hybrid, *de novo* genome assembly



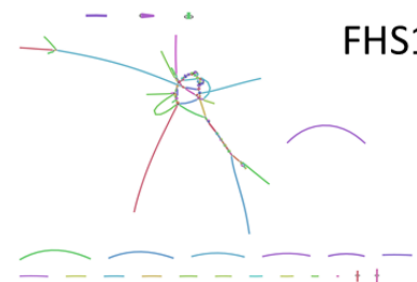
- # contigs: 71
- Largest contig: 1,166,988bp
- Total length: 4,749,189bp
- N50: 253,998



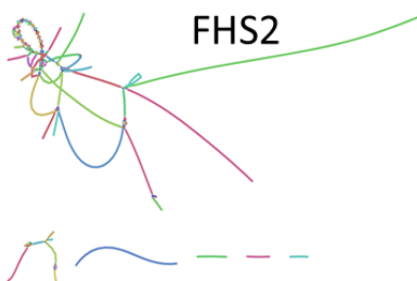
- # contigs: 49
- Largest contig: 639,599
- Total length: 4,791,305bp
- N50: 380,501bp



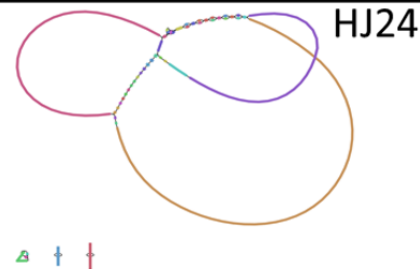
- # contigs: 82
- Largest contig: 465,385bp
- Total length: 4,952,373bp
- N50: 169,481bp



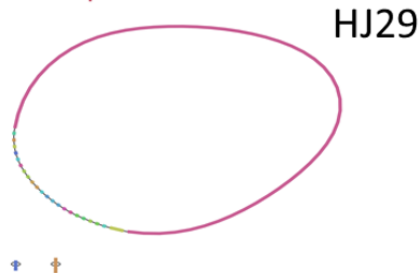
- # contigs: 80
- Largest contig: 457,762bp
- Total length: 4,773,345bp
- N50: 177,457bp



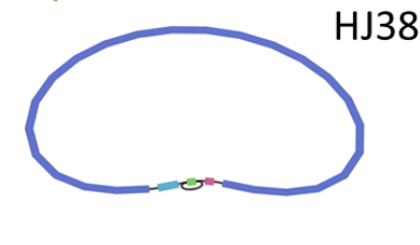
- # contigs: 72
- Largest contig: 1,029,429bp
- Total length: 4,947,084bp
- N50: 245,290bp



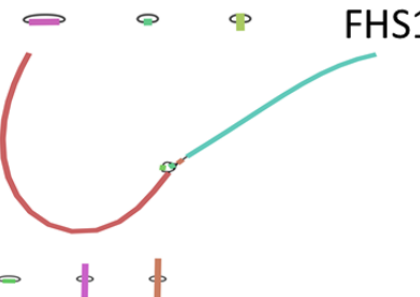
- # contigs: 50
- Largest contig: 2,101,776bp
- Total length: 4,789,900bp
- N50: 1,276,038bp



- # contigs: 25
- Largest contig: 4,744,553bp
- Total length: 4,843,076bp
- N50: 4,744,553bp



- # contigs: 7
- Largest contig: 3,204,242bp
- Total length: 5,044,956bp
- N50: 3,204,242bp

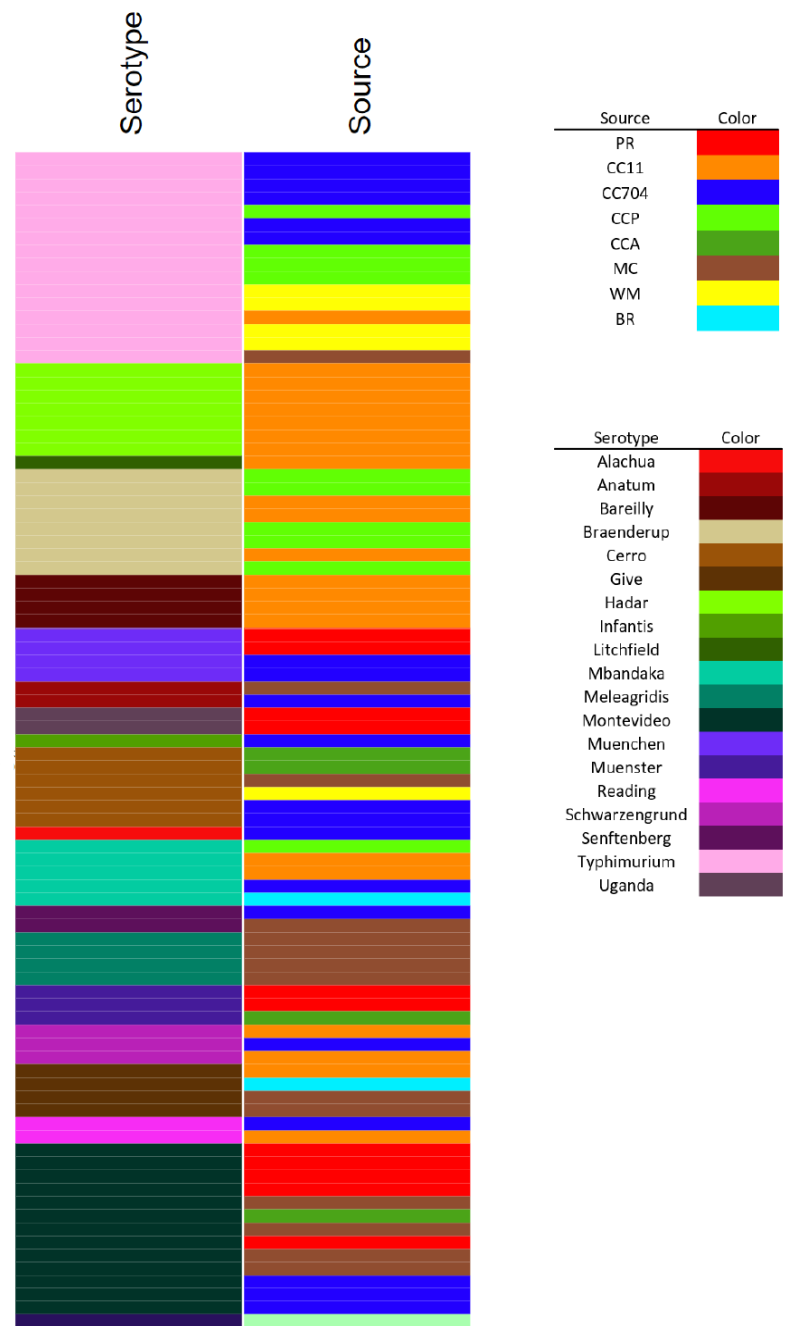


- # contigs: 8
- Largest contig: 2,986,139bp
- Total length: 4,863,594bp
- N50: 2,986,139bp



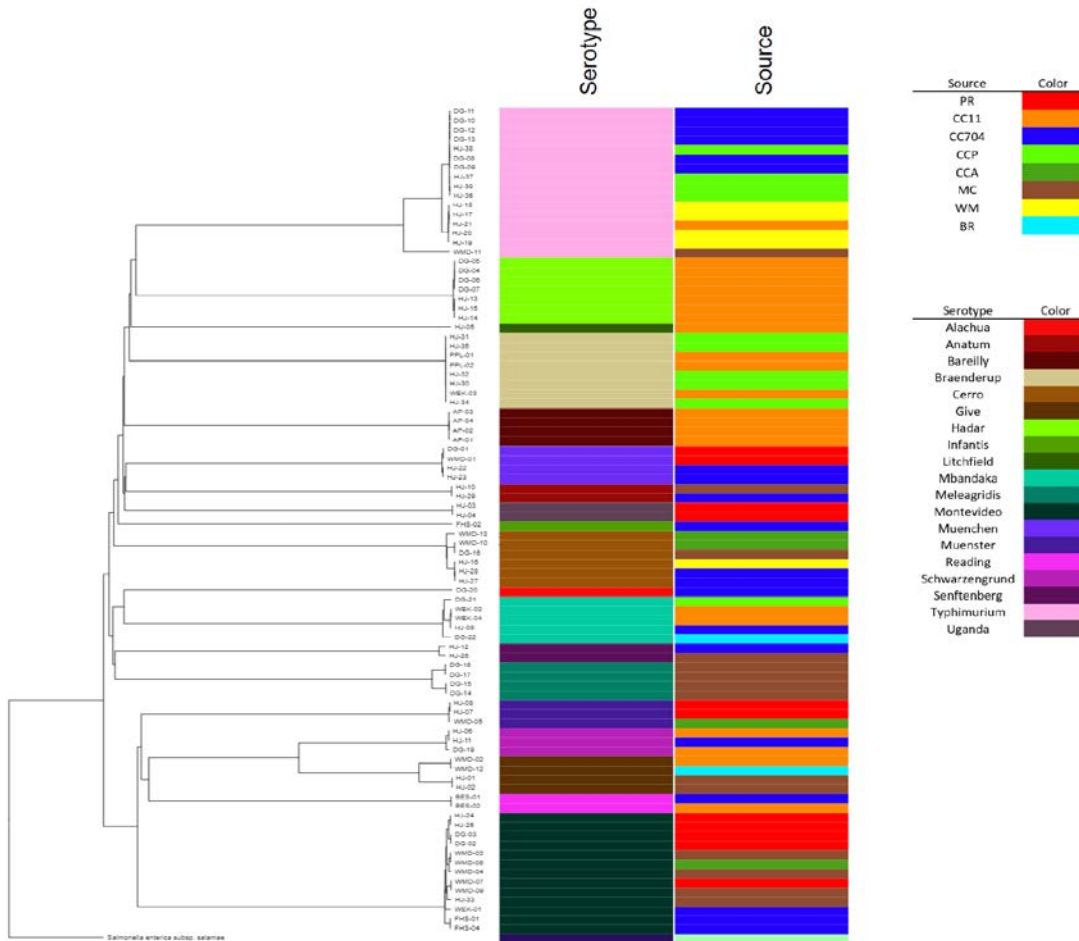
- # contigs: 21
- Largest contig: 4,571,921bp
- Total length: 5,040,259bp
- N50: 4,571,921bp

# Serotype diversity and distribution of *Salmonella enterica* from stream sediments

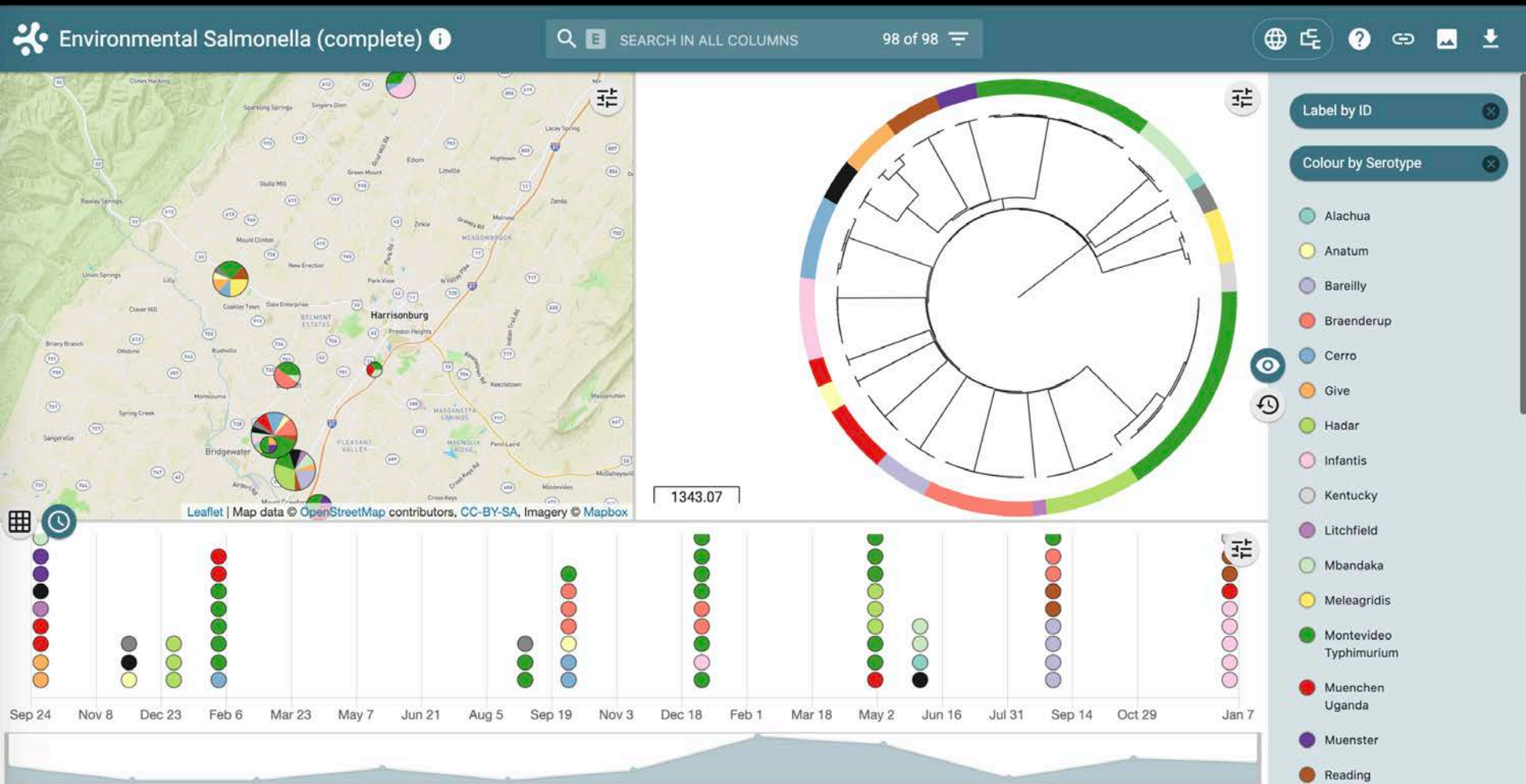




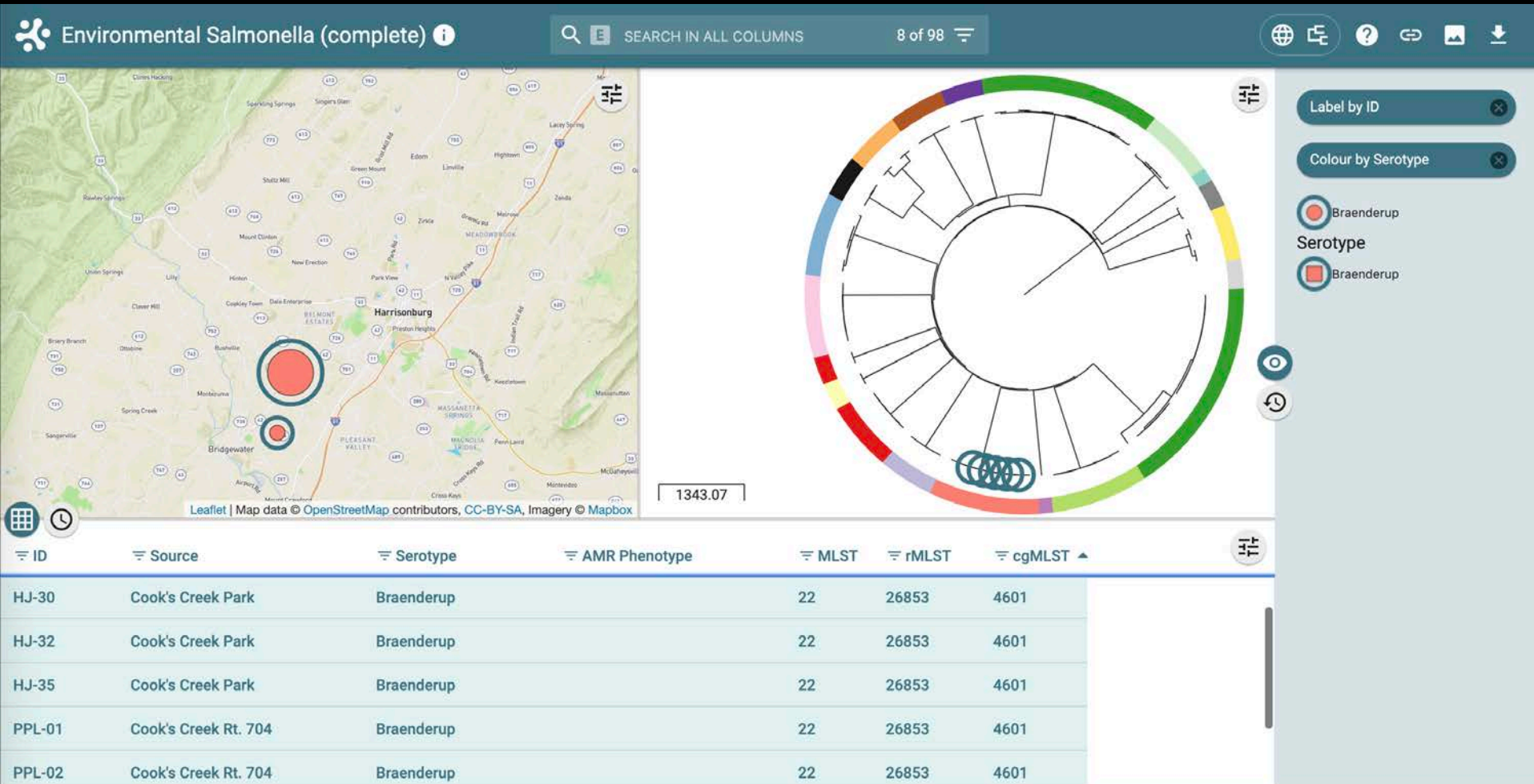
# 48/88 isolates are members of the top 20 human disease-causing serotypes



# VISUALIZATION OF ISOLATE DATA USING MICROREACT



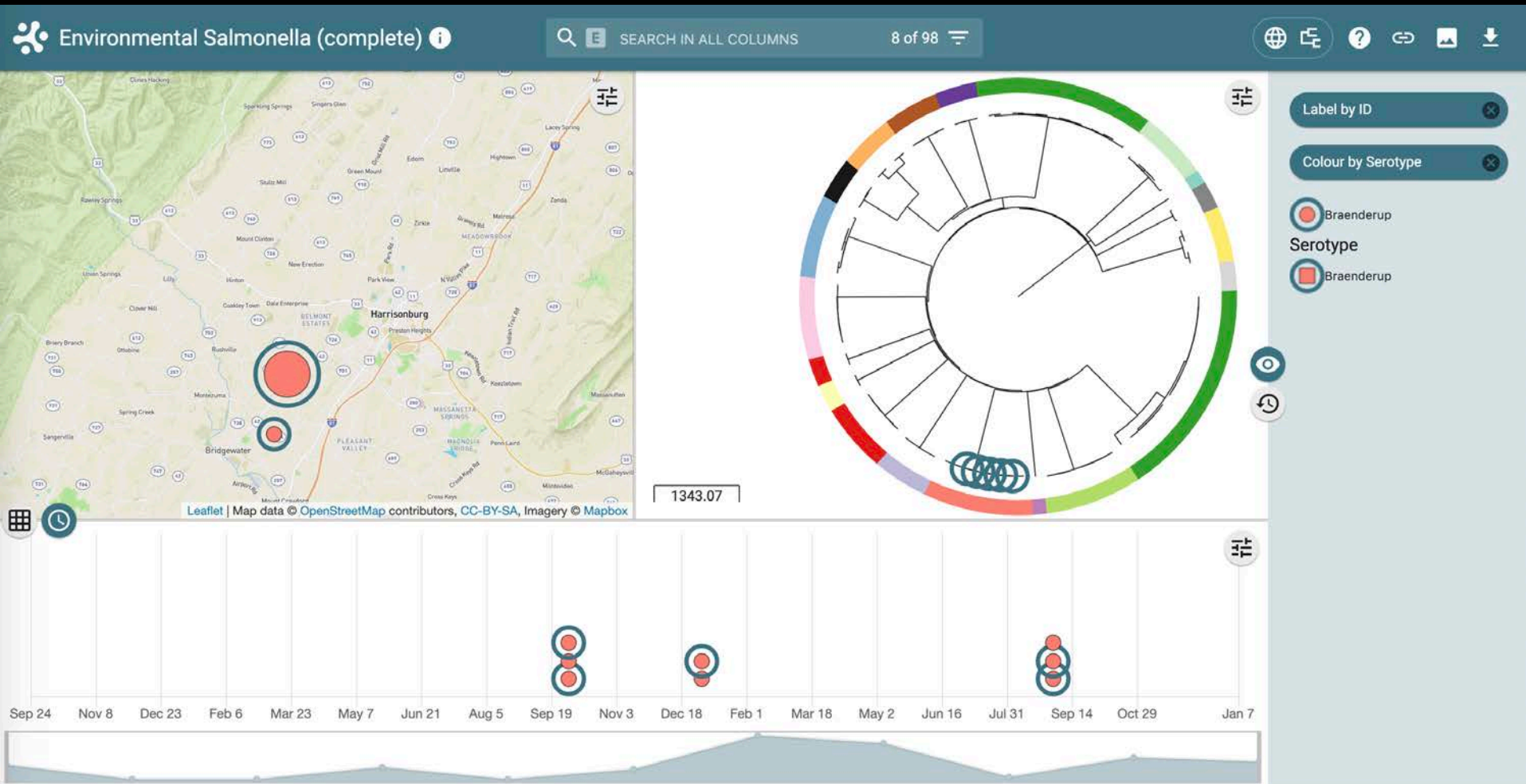
# Identical S. Braenderup found in across multiple locations



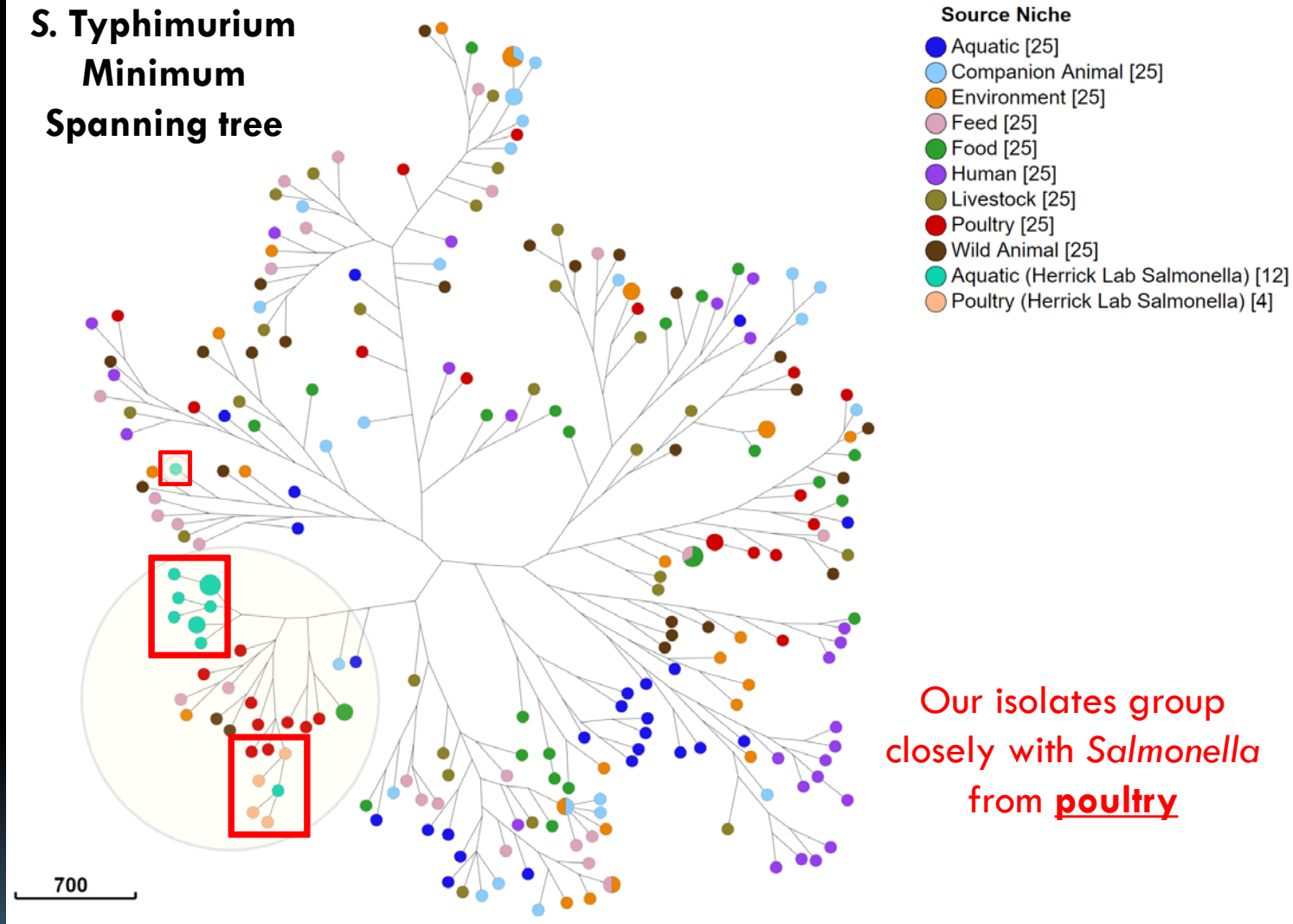
Enterobase: Alikhan NF, Zhou Z, Sergeant MJ, Achtman M (2018) "A genomic overview of the population structure of Salmonella." PLoS Genet 14 (4): e1007261.



# Identical S. Braenderup found in across multiple locations & timepoints



## S. Typhimurium Minimum Spanning tree



# MOBILE GENETIC ELEMENTS (MGEs)



A photograph of a Daily Mail newspaper advertisement. The top part features a yellow banner with "FREE DVD" and "FREE LILY COLLECTION" offers. Below this, a headline reads "Hospital infection soars by 22 per cent in just three months" followed by "1,000 SUPERBUG VICTIMS A WEEK".

**THE WORLD AT WAR** **FREE DVD** **FREE LILY COLLECTION**  
GET YOUR SECOND SPECIAL BONUS DISC FREE TODAY WORTH £25 FOR EVERY READER

Pick up from WHSmith or we can post you the whole set

Hospital infection soars by 22 per cent in just three months

**1,000 SUPERBUG VICTIMS A WEEK**

A microscopic image showing numerous small, rod-shaped bacteria, likely antibiotic-resistant staphylococci, scattered across a dark background.

**Drug-Proof Superbug Turns Deadly**

Antibiotic-resistant staph kills more Americans than AIDS

Oct 16, 2007 11:28 PM CDT

# “SUPER- PLASMIDS”

## Drug-resistant superbug spreading in Europe's hospitals

By James Gallagher  
Health and science correspondent, BBC News

© 29 July 2019



Superbugs resistant to emergency antibiotics are spreading in hospitals, a Europe-wide study shows.



Volume 73, Issue 12

December 2018

## Carriage of *bla*<sub>KPC-2</sub> by a virulence plasmid in hypervirulent *Klebsiella pneumoniae*

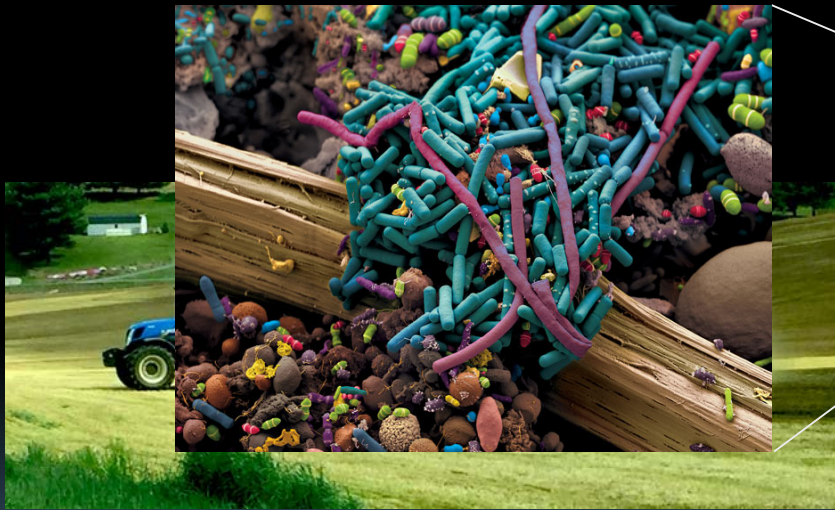
Ning Dong, Dachuan Lin, Rong Zhang, Edward Wai-Chi Chan, Sheng Chen ✉

*Journal of Antimicrobial Chemotherapy*, Volume 73, Issue 12, December 2018, Pages 3317–3321, <https://doi.org/10.1093/jac/dky358>

**Published:** 15 September 2018    **Article history** ▼

# Environmental reservoirs of transmissible antibiotic resistance and virulence?

Beyond the possible health implications of persistent pathogens in streams, what is the effect of having soil and stream bacteria constantly bathed in high numbers of fecal bacteria?

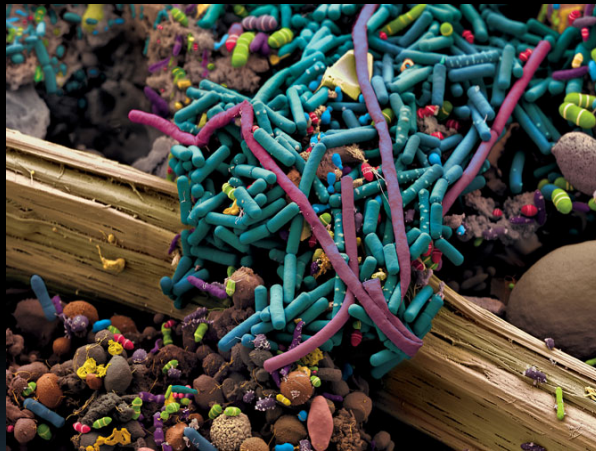


- Introduced bacteria may persist long enough for horizontal gene transfer (HGT), especially in sediments
- Possible “hot spots” for HGT due to:
  - Large total surface area
  - High numbers
  - High nutrient content





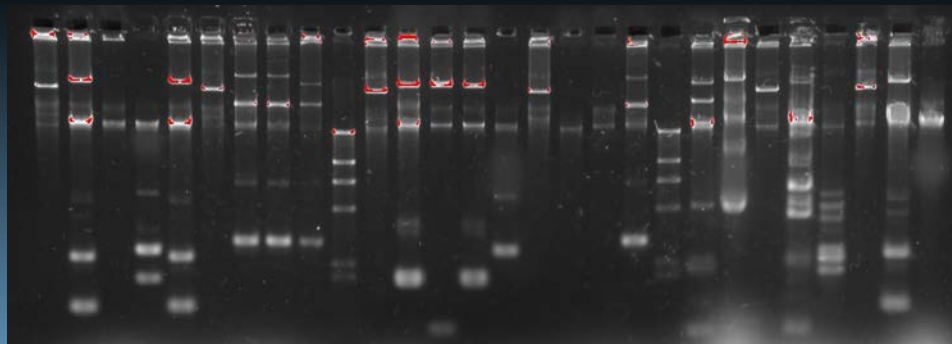
# Potential *in situ* recombination and transmission in stream populations



- Genes, transposons, genomic islands, and other mobile genetic elements may also recombine *in situ* within native and introduced stream populations
- These may then be transmissible to and from pathogens (e.g. via plasmids)

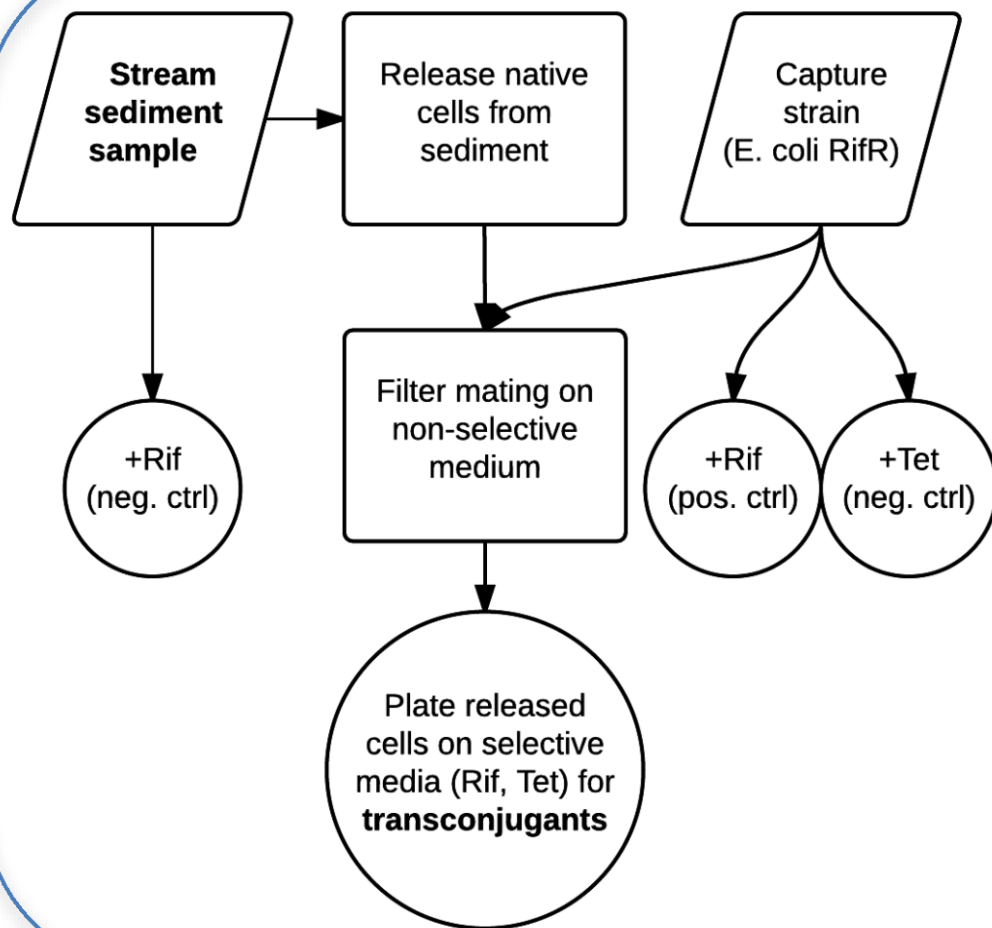
# ANTIMICROBIAL RESISTANCE GENES (ARGs) IN ISOLATED *SALMONELLA* PRIMARILY PLASMID-BORNE

- 84% of the ARGs in isolated *Salmonella* were plasmid-borne\*
- All the *S. Typhimurium* ARGs were plasmid-borne\*
- Some (nearly) identical isolates differed only in whether their ARGs were on plasmids or not



\*Exclusive of *aac6-laa* and *aac6-ly*, which are part of the core genome.

EXOGENOUS CAPTURE OF  
TRANSMISSIBLE PLASMIDS FROM  
STREAMS

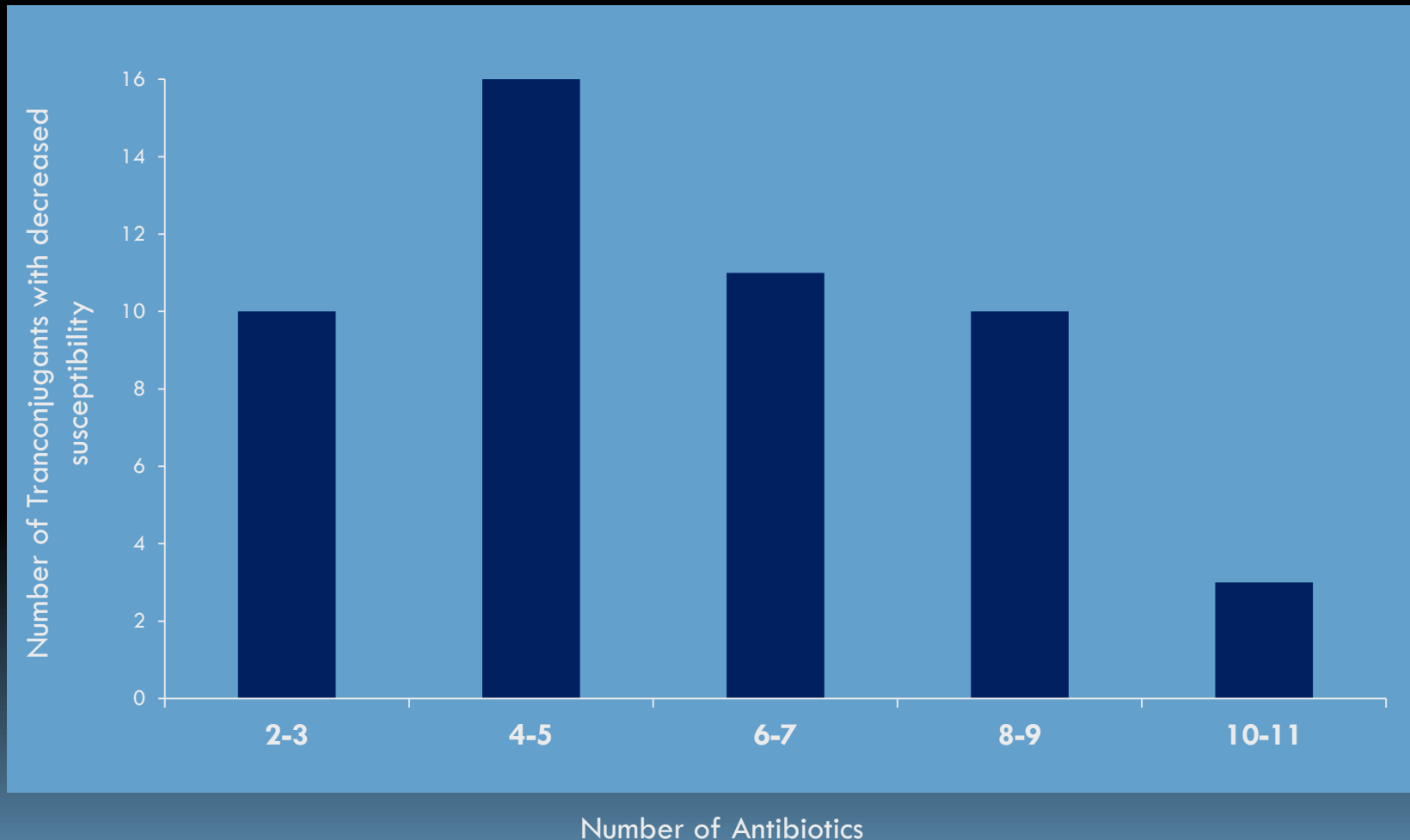


**Figure 1. Exogenous capturing of tetracycline resistance plasmids from sediment.** Plasmids captured from stream sediment samples by releasing native cells from sediment and conjugating with a rifampicin-resistant strain of *E. coli*. Transconjugants selected on tetracycline- and rifampicin-amended medium. Plasmids purified and electroporated into an electrocompetent *E. coli* strain and tested for decreased antibiotic susceptibility to 12 antibiotics relative to un-electroporated strain.

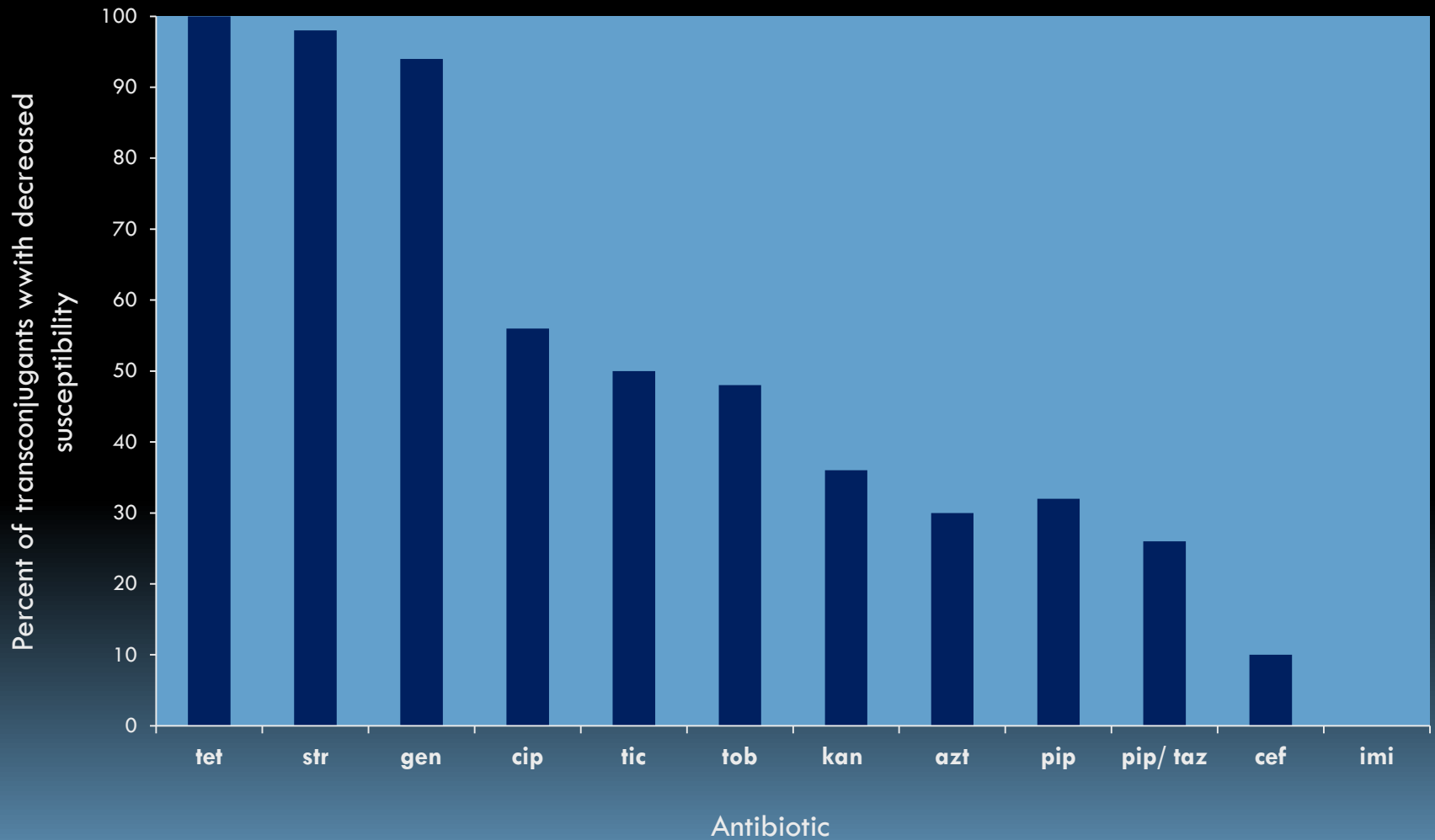


# Plasmids captured by *Pseudomonas* sp. from Muddy Creek sediments conferred decreased susceptibility to multiple antibiotics

(n = 50 plasmids)

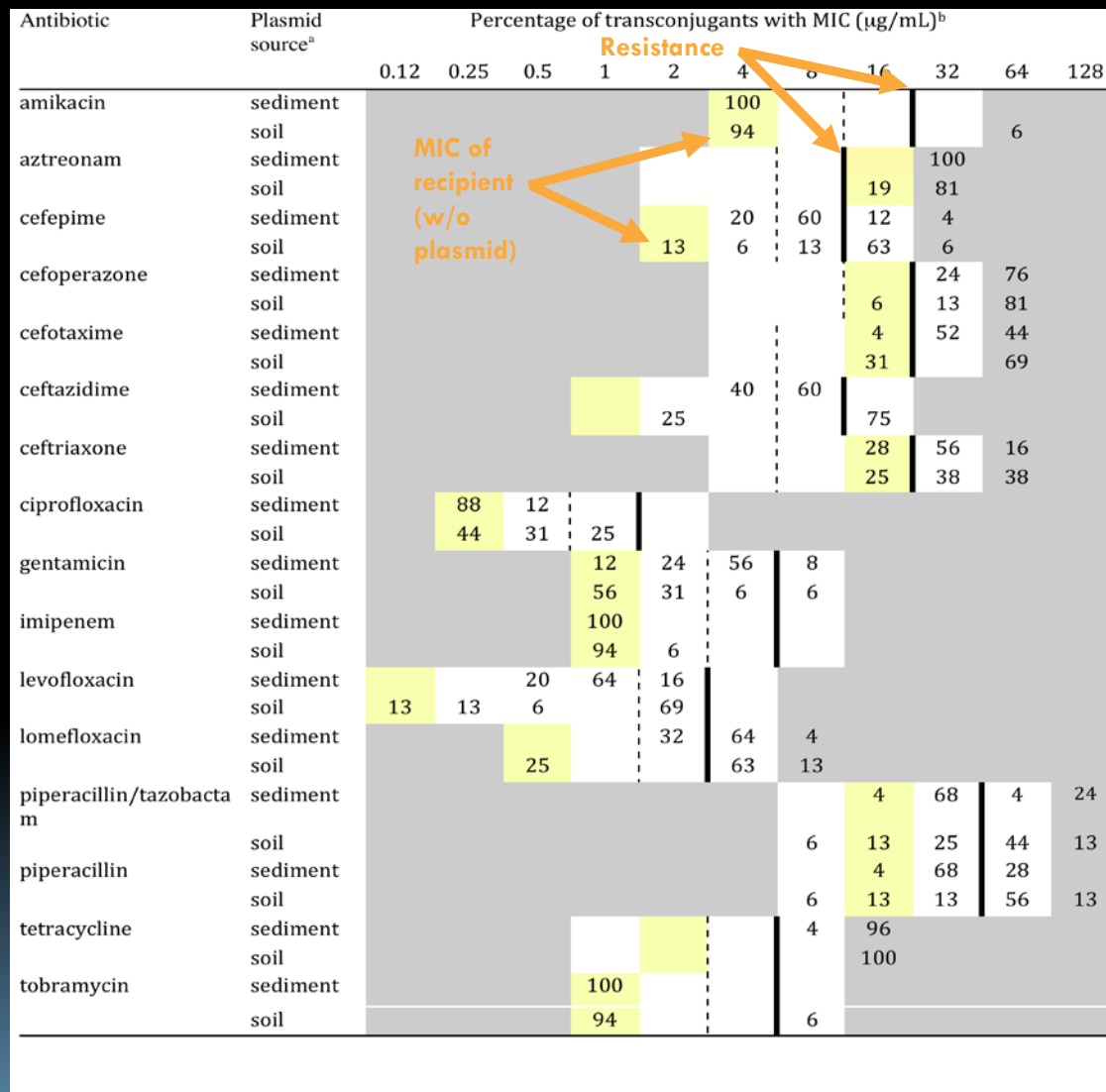


# Percentage of transconjugants encoding decreased susceptibility to 12 antibiotics






# MICs of plasmids captured from litter-amended soil and adjacent stream sediments

- MIC of recipient strain
- Resistance breakpoint for *P. aeruginosa*
- Susceptibility breakpoint for *P. aeruginosa*



## MICs of plasmids captured from litter-amended soil and adjacent stream sediments

**Ceftriaxone – 3<sup>rd</sup> generation**  
**Cefoperazone – 3<sup>rd</sup> gen**  
**Cefotaxime – 3<sup>rd</sup> gen**  
**Ceftazidime – 3<sup>rd</sup> gen**  
**Cefepime – 4<sup>th</sup> gen**

 MIC of recipient strain  
 Resistance breakpoint for *P. aeruginosa*  
 Susceptibility breakpoint for *P. aeruginosa*

Antibiotic	Plasmid source <sup>a</sup>	Percentage of transconjugants with MIC (µg/mL) <sup>b</sup>											
		0.12	0.25	0.5	1	2	4	8	16	32	64	128	
amikacin	sediment						100						
	soil						94						6
aztreonam	sediment									100			
	soil								19	81			
cefepime	sediment						20	60	12	4			
	soil					13	6	13	63	6			
cefoperazone	sediment									24	76		
	soil								6	13	81		
cefotaxime	sediment								4	52	44		
	soil								31	69			
ceftazidime	sediment						40	60					
	soil					25			75				
ceftriaxone	sediment								28	56	16		
	soil								25	38	38		
ciprofloxacin	sediment	88	12										
	soil	44	31	25									
gentamicin	sediment				12	24	56	8					
	soil				56	31	6	6					
imipenem	sediment				100								
	soil				94	6							
levofloxacin	sediment			20	64	16							
	soil	13	13	6		69							
lomefloxacin	sediment					32	64	4					
	soil			25			63	13					
piperacillin/tazobactam	sediment								4	68	4	24	
	soil							6	13	25	44	13	
piperacillin	sediment								4	68	28		
	soil							6	13	13	56	13	
tetracycline	sediment							4	96				
	soil								100				
tobramycin	sediment				100								
	soil				94			6					



# Plasmids captured from streams in the Shenandoah Valley have commonly conferred phenotypic resistance to:

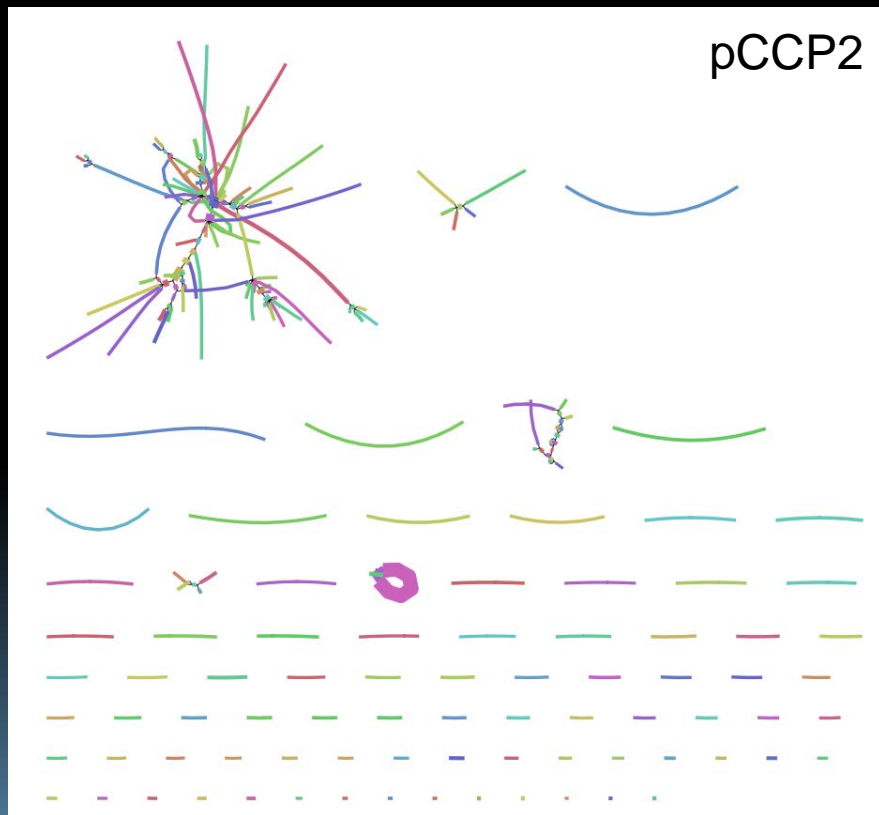
- Tetracycline
- Streptomycin
- Gentamicin\*
- Ciprofloxacin
- Lomefloxacin
- Ticarcillin\*
- Tobromycin\*
- Kanamycin
- Aztreonam\*
- Piperacillin\*
- Piperacillin/Tazobactam\*
- Ceftriaxone\*
- Cefepime\*
- Cefoperazone
- Ceftzadime \*
- Cefotaxime\*

\*Antibiotics generally reserved for human-only use

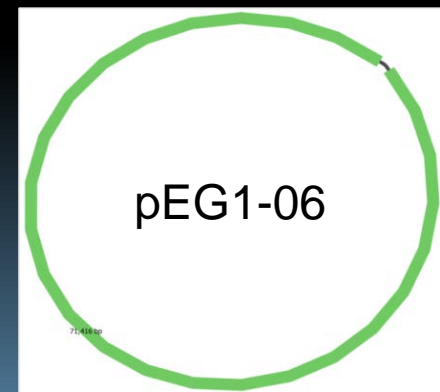
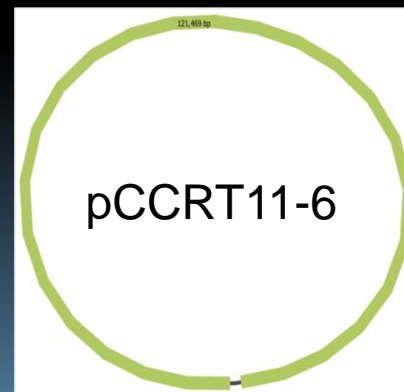
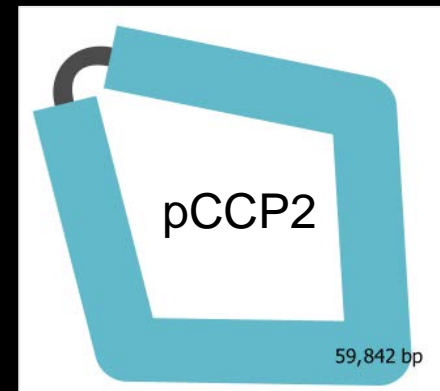
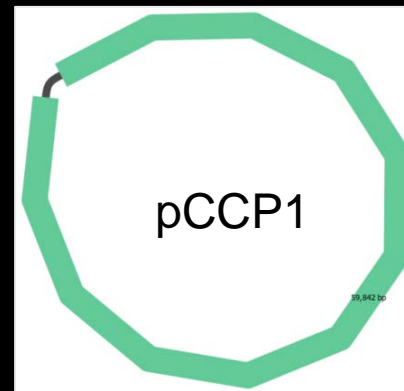
What is the genetic basis for the phenotypic resistance we see in these resistance plasmids?

# BANDAGE GRAPHS OF HYBRID-ASSEMBLED CAPTURED PLASMIDS

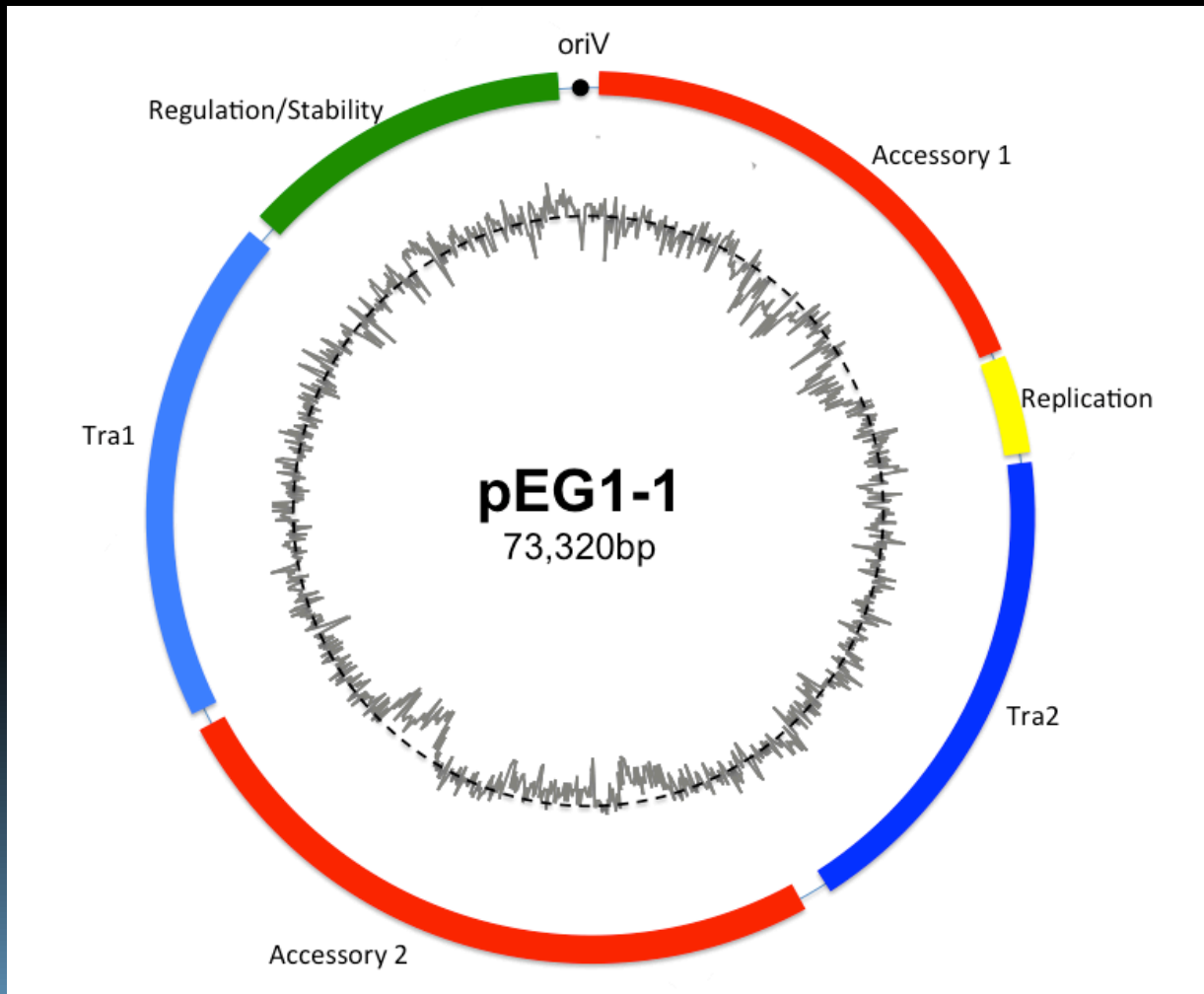
Short-read only



Hybrid short + long read



# pEG1-1: a multiresistance plasmid captured from uncultivated stream sediment bacteria

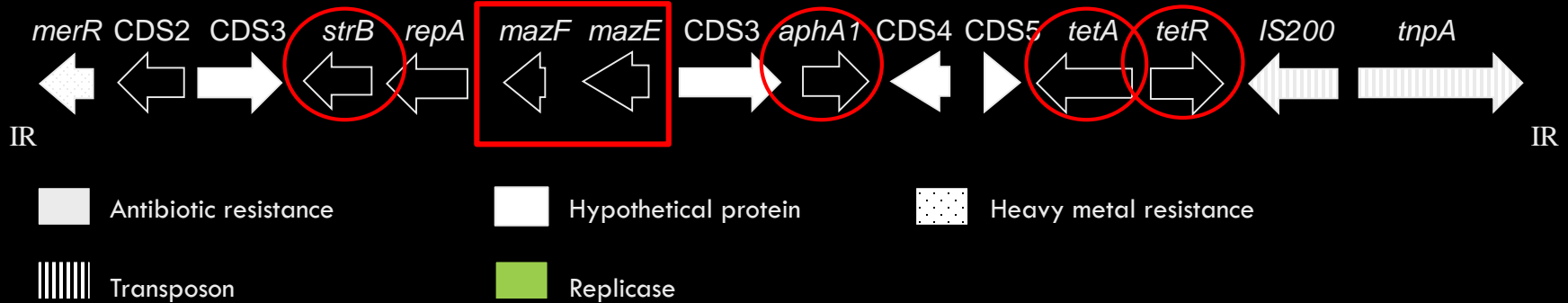


- Captured using *E. coli* host
- IncP-1 $\beta$  Plasmid (very broad host range)
- Encodes phenotypic resistance to tetracycline, kanamycin, piperacillin, ticarcillin, tobramycin, piperacillin/tazobactam, and cefepime
- Two potentially mobile accessory modules



# Accessory Module 1

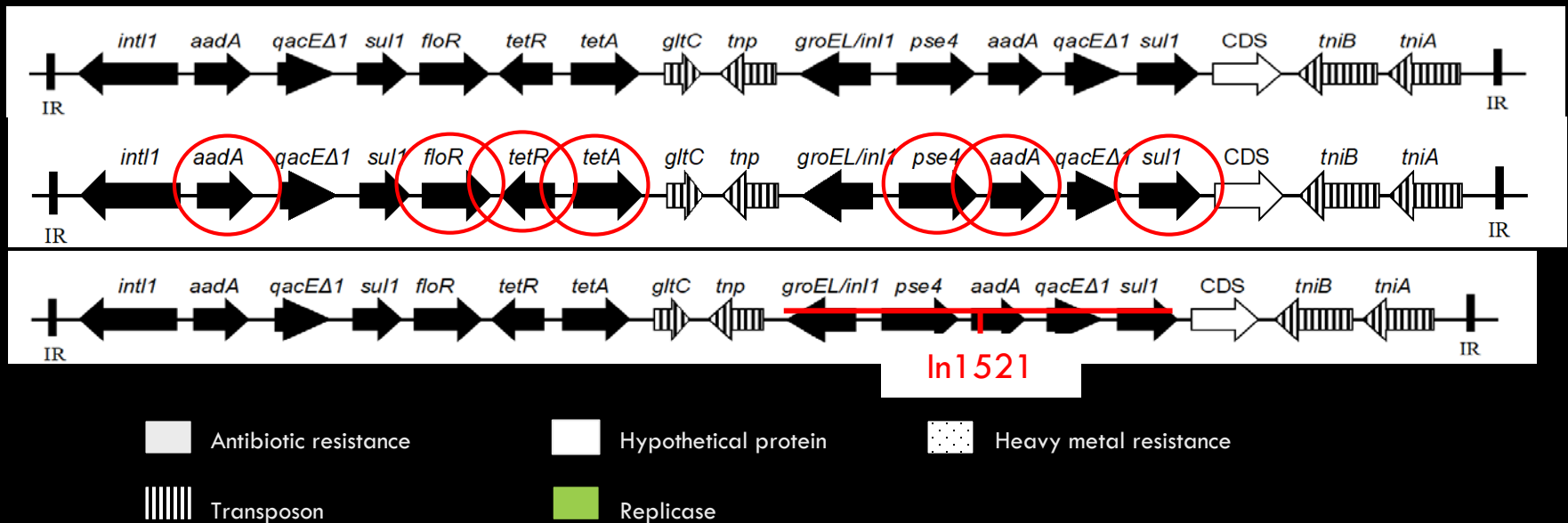
Tn21-related transposon (15,054bp)



- One large Tn21 transposon

- *mazE/F* toxin/antitoxin “plasmid addiction” system

# ACCESSORY MODULE 2

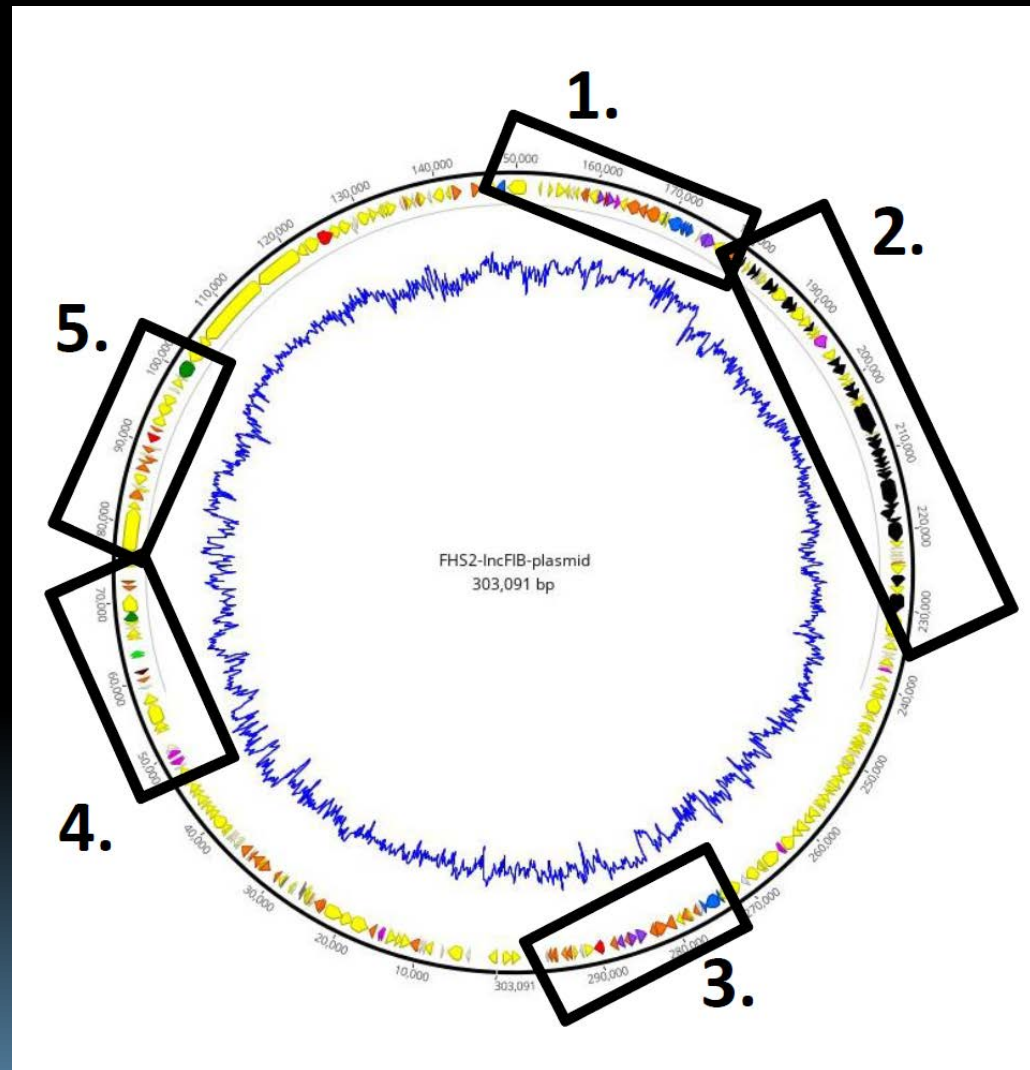


A “complex integron”, typically associated with (chromosomal) *Salmonella* genomic island 1 (SGI1)

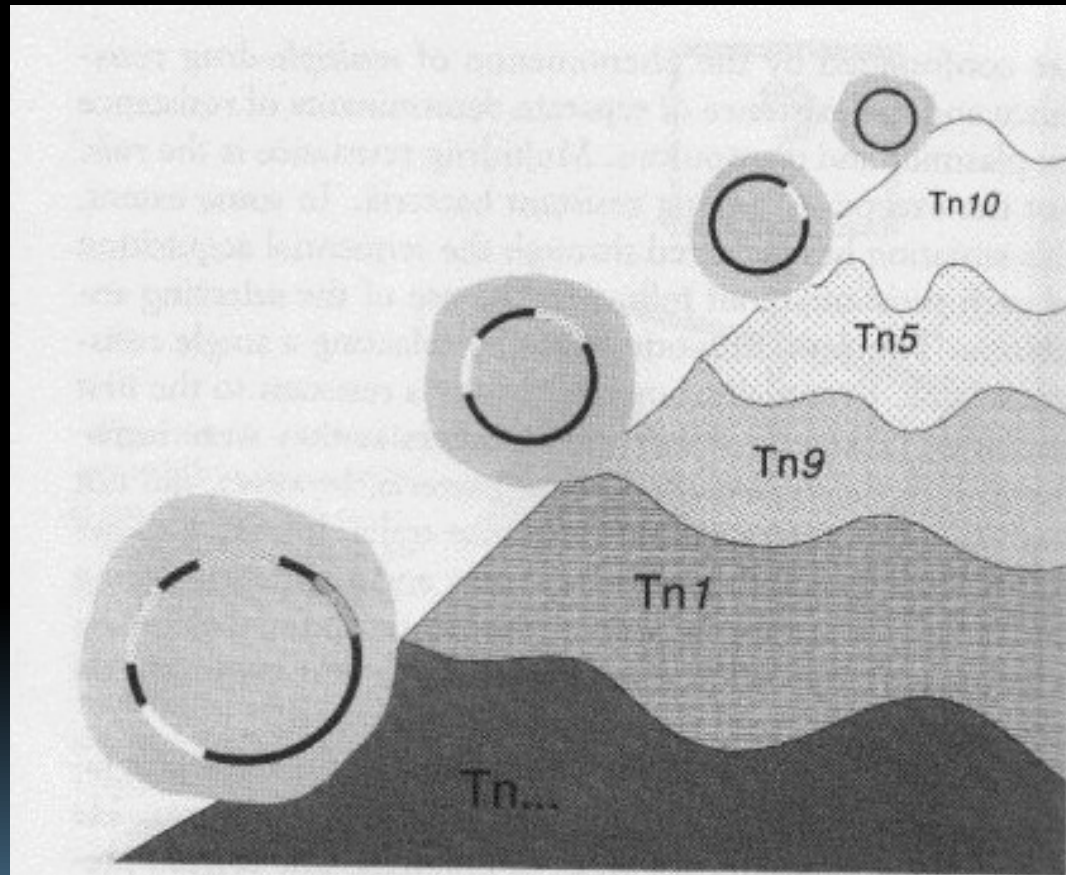
- First identified in multidrug resistant *Salmonella* Typhimurium DT104
- Found on chromosomes or in association with IncA/C plasmids
- In1521 is a new class 1 integron (*in1521*)

# Plasmid pFHS2

- Transmissible 303 kb megaplasmid identified in *Salmonella* Infantis FHS-02
- Isolated from sediment of Cooks Creek, near Dayton Virginia



# Plasmids pick up transposons as they move through the environment



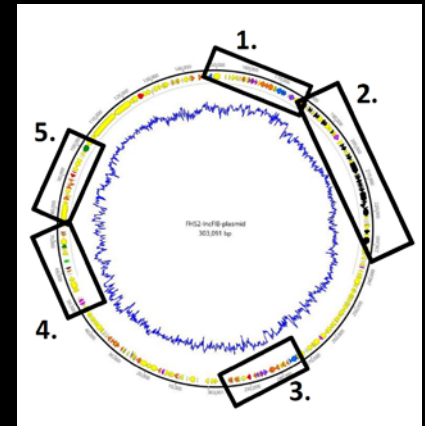
*Figure 4.11. Plasmids pick up resistance genes on transposons as they move through the environment. By retaining previous ones and picking up new ones, they increase in size, like a snowball rolling down a hill (Bonnie Marshall, Tufts University School of Medicine).*

Levy, S. B. 2002. *The Antibiotic Paradox*. Perseus, Cambridge MA.

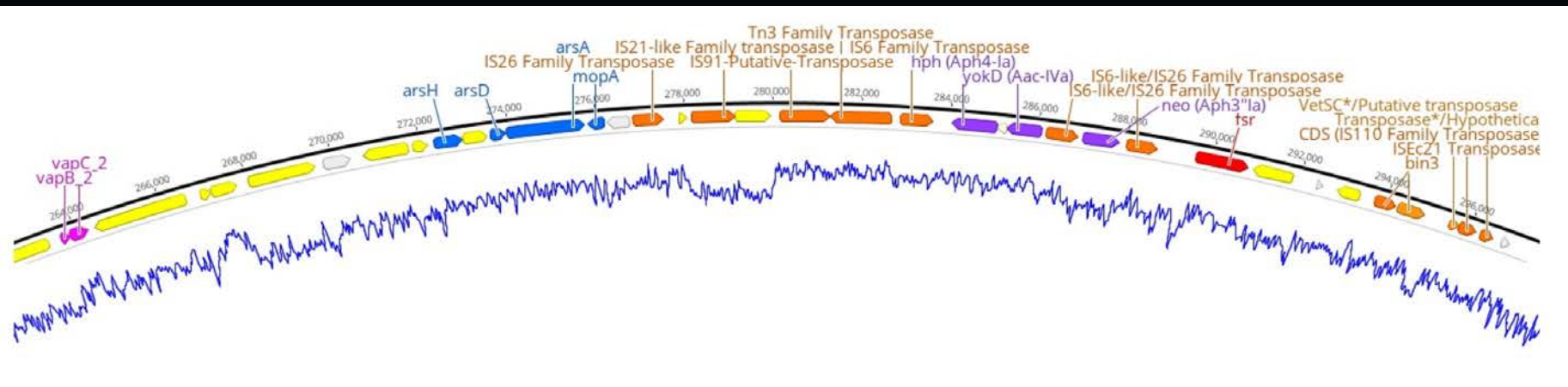


# Plasmid pFHS2

- 11 predicted AR genes
- 11 predicted heavy metal resistance genes (mercury, arsenic, tellurite, molybdenum)
- Large potential for co-selection (genetic hitch-hiking)
- 4 predicted 'plasmid addiction' systems
- Multiple transposons & IS elements
- Phage-associated virulence gene (*gipA*), associated with Peyer's patch colonization and macrophage survival



## Accessory gene region 3



# IMPLICATIONS

- Bacterial communities in stream sediments may harbor **significant reservoirs of pathogens, as well as resistance, virulence & other genes.**
- These genes are often found on **mobile genetic elements.**
- Animal husbandry carried out on a massive scale **may be adding to these reservoirs.**
- The ease of recovery of transmissible MDR plasmids suggests that these reservoirs exist, and that **plasmids and other MGEs may facilitate gene recombination and spread to and from pathogens.**



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