Genetic AMR Predictions and Big Data – Implications for One Health

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Overview

1. Why do we need to predict AMR?
2. Intro to the technology
3. How well does it work?
4. Animal health surveillance
5. Data sharing

1. Why do we need to “predict” antimicrobial resistance (AMR) in animal health?

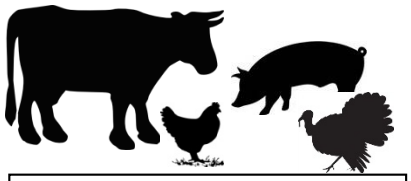
MICROORGANISMS WITH A THREAT LEVEL OF SERIOUS

Foodborne Pathogens

- Multidrug-resistant ***Acinetobacter*** — Rarely resistant in animals
- Drug-resistant ***Campylobacter***
- Fluconazole-resistant ***Candida*** (a fungus) — Rarely resistant in animals
- Extended spectrum β-lactamase producing **Enterobacteriaceae (ESBLs)**
- Vancomycin-resistant ***Enterococcus*** (VRE) — Rarely resistant in animals
- Multidrug-resistant ***Pseudomonas aeruginosa*** — Common; intrinsic resistance
- Drug-resistant **non-typhoidal *Salmonella***
- Drug-resistant ***Salmonella*** Typhi — Specific to humans
- Drug-resistant ***Shigella*** — Specific to humans
- Methicillin-resistant ***Staph*** — Common; originated from humans
- Drug-resistant ***Streptococcus pneumoniae*** — Rarely resistant in animals
- Drug-resistant **tuberculosis** — Specific to humans

NARMS
National Antimicrobial Resistance Monitoring System

Surveillance of Resistance: as of Jan 2018



Animal Population
Slaughterhouse

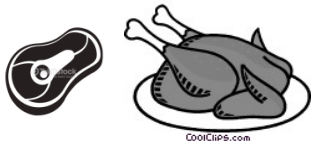
Cecal Sampling (2013)
Non-regulatory

HACCP (1997)
regulatory

USDA

Cattle, Chicken, Turkey

Chicken only



Retail Meats (2002)
Grocery Store
- Chicken
- Ground turkey
- Pork chop
- Ground beef

Imported Foods
Distributors
- Spices
- Seafood
- Produce
- Feed
- Etc.

20 State and university labs

ORA Denver lab

Chicken and Turkey only

FDA

Human Population (1996)
Medical Office

Physician Visit

Local Lab

50 State Labs (Salmonella and Shigella)

CDC

KEY

- *Campylobacter*
- Non-typhoidal *Salmonella*
- *Enterococcus*
- Generic *E. coli*
- Typhoidal *Salmonella*
- *E. coli* O157
- Non-cholera *Vibrio*
- *Shigella*

Heather Tate, FDA

What about animal health?

Puppies spread antibiotic-resistant infections to 118 people, CDC reports

Drugged puppies blamed for spreading diarrhea superbugs in multi-state outbreak

Officials were stunned by the amount of antibiotics the puppies had been given.

Puppies are making people sick — and it's people's fault

Health Sep 22, 2018 2:44 PM EDT

Antibiotic-resistant bacteria that have infected more than 100 people and that have been linked to pet store puppies appear to have spread at least in part because healthy dogs were given antibiotics — a decision that all but surely fostered antibiotic resistance.

Raw pet food sales growing despite health warnings

Consumer interest and sales of raw meat dog and cat food have been growing consistently over the past few years.

TIM WALL

Fueled by interest in ancestral and high-protein, fresh-meat diets, consumer interest in and sales of raw meat dog and cat food have been growing consistently over the past few years, despite scientists' warnings about raw

pet food safety. P
analysts and scier
into the raw pet fo
"If there are ne
industry, of cours
of inquiries we ge
Wuest, director of
at Buhler. "And fr
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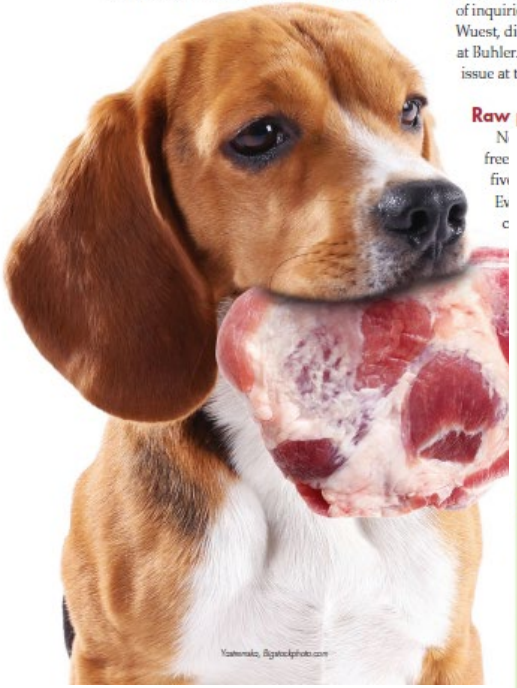


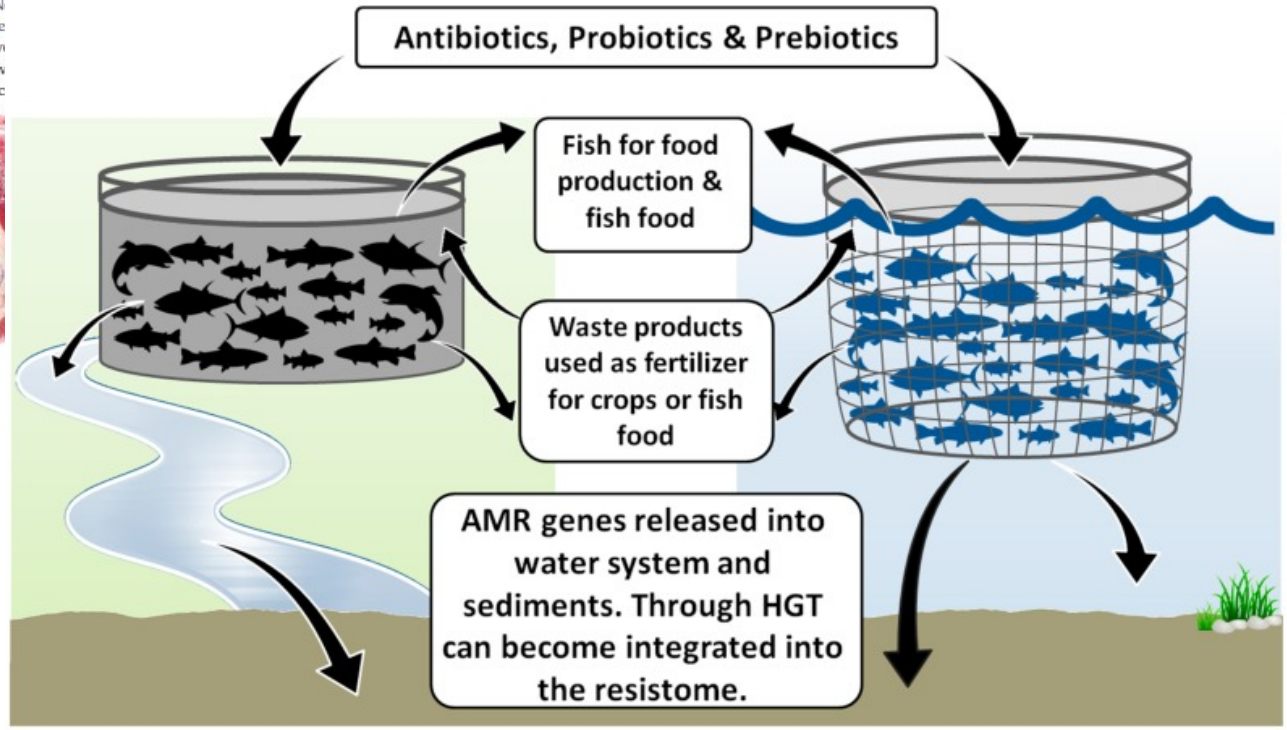
Raw pet food sales grew in 2017

Mar Drugs. 2017 Jun; 15(6): 158.
Published online 2017 Jun 1. doi: 10.3390/md15060158
PMCID: PMC5484108
PMID: 28587172

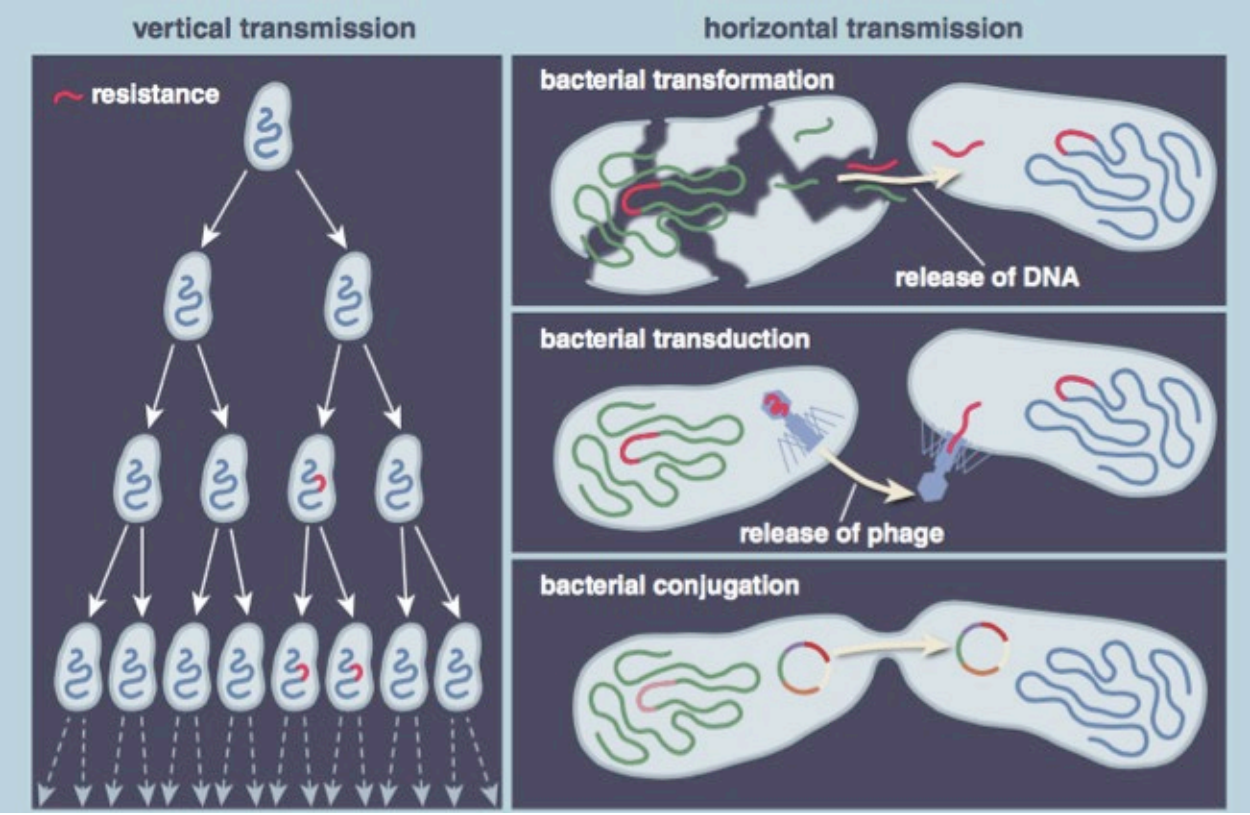
The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions

Joy E. M. Watts,[1,*] Harold J. Schreier,[2] Lauma Lanska,[1] and Michelle S. Hale[3]

Allen Place, Academic Editor, Rosemary Jagus, Academic Editor, and Paul Long, Academic Editor

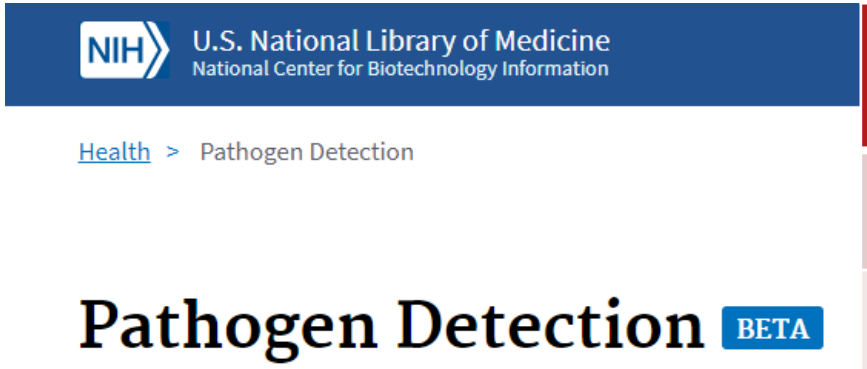


AMR is acquired both vertically and horizontally



Dantas. Am Sci. 2014

National databases for tracking AMR are heavily human-focused

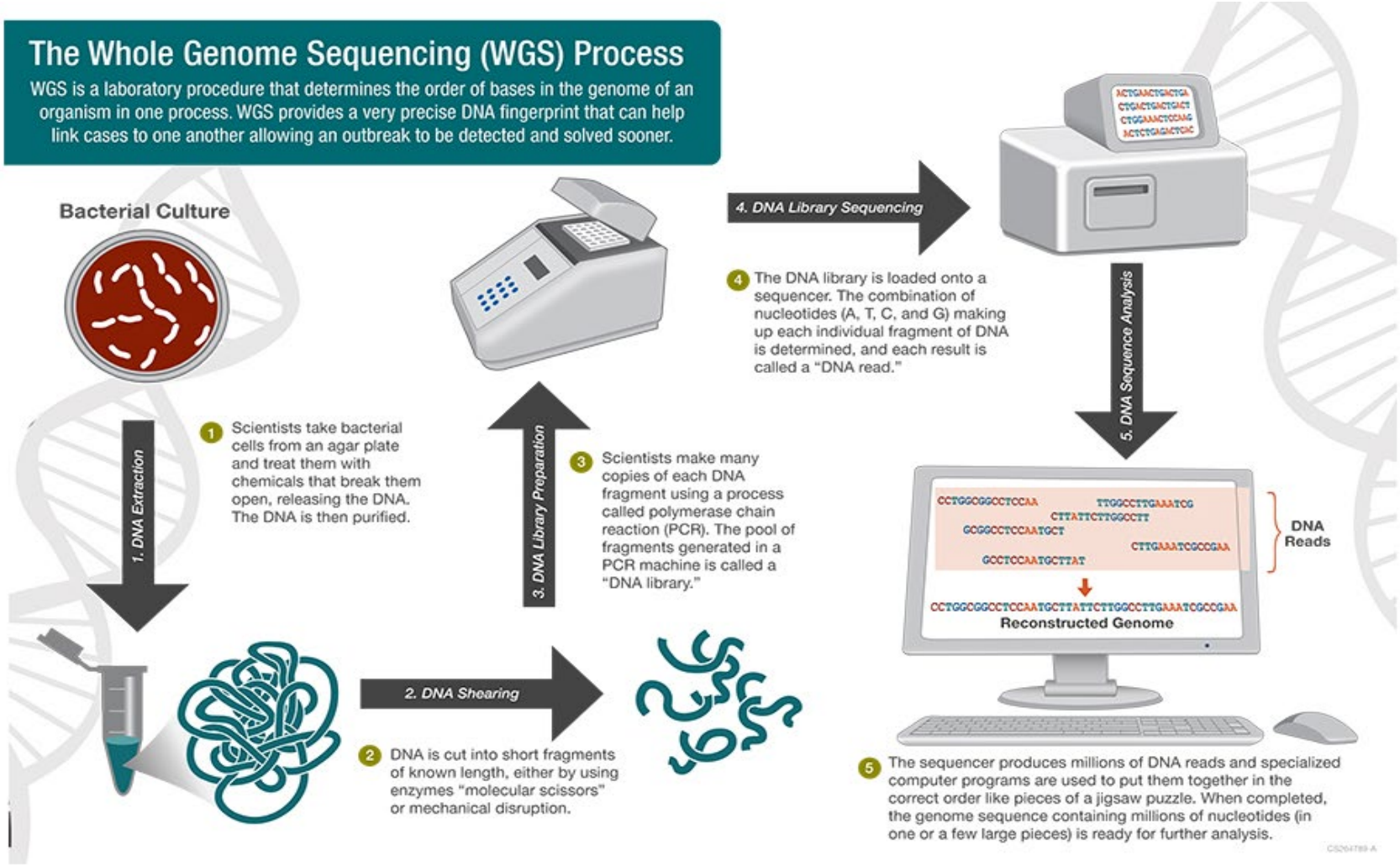


Organism	Total isolates (10/3/18)
Salmonella	153,255
E. Coli	54,679
Listeria monocytogenes	20,879
Campylobacter jejuni	21,838
Klebsiella pneumoniae	7,774

www.ncbi.nlm.nih.gov/pathogens/

2. Intro to the technologies

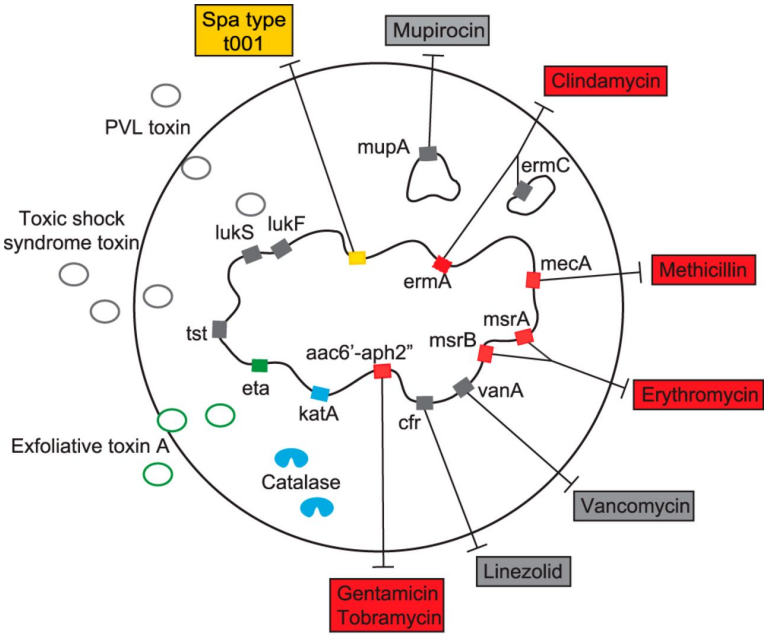
The Whole Genome Sequencing Process



https://www.cdc.gov/pulsenet/pathogens/protocol-images.html#wgs

Bacterial WGS in VDLs

- Performed on cultures (costs ~$50-200)
- Nationally harmonized lab procedures (with FDA/CDC/state health)
  - NCBI integration through GenomeTrakr or PulseNet
- Confirms species, subspecies, isolate relatedness
- Large databases mined to predict features (functional genomics):
  - Serotype
  - Virulence factors
  - AMR



Leopold et al. J. Clin. Microbiol. 2014

WGS procedural overview at AHDC

Pure Culture

DNA Extraction

QC

Optional Submission Point

Library Prep

QC

Optional Submission Point

Normalization and Pooling

Load and Run

QC

Data Transfer by Cloud

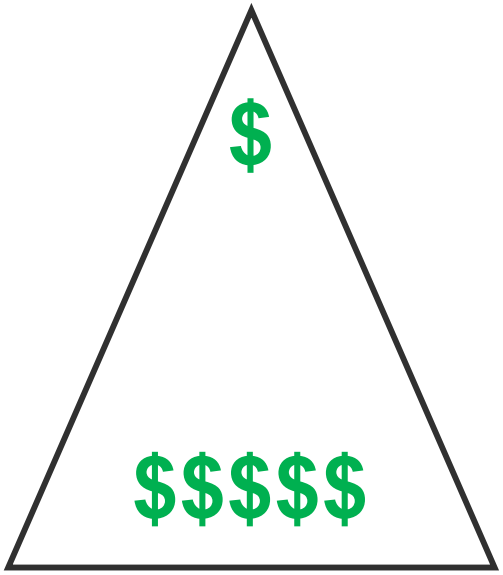
Typical AMR analysis pipeline

- Reads trimmed and assembled
  - Trimmomatic and SPAdes
- Core genome phylogenies constructed
  - Parsnp and FastTree
- Screened for antimicrobial resistance genes (**ARGs**) with ABRicate
  - Multiple databases
  - 90% threshold for identity and coverage

Culture-independent AMR detection methods

Mostly for environmental testing
– some commercial clinical tests for humans

- Targeted amplification (PCR of multiple known resistance genes)
- Targeted metagenomics (sequencing of many known resistance genes)
- Shotgun metagenomics (sequences "all" DNA)
  - captures the known and unknown



3. How well does AMR prediction work?

WGS AMR predictions by gene have good correlation with phenotype

Bacterium	Gen/Phe correlation	Reference
Salmonella enterica	99.7%	Zankari et al. 2013, J Antimicrob Chemother
	99.00%	McDermott et al. 2016, Antim Agents Chemother
Escherichia coli	97.1%	Stoesser et al. 2013, J Antimicrob Chemother
	98.5%	Tyson et al. 2015, J Antimicrob Chemother
Camplylobacter spp.	99.2%	Zhao et al. 2015, J Antimicrob Chemother
Staph. aureus	98.8%	Gordon et al. 2014, J Antimicrob Chemother
Pneumococcus	98%	Metcalf et al. 2016, Clin Microbiol Infect
Enterobacteriaceae (B-lacs)	100%	Shelburne et al. 2017, Clin Infect Dis
Mycobacterium	95.3%	Phelan et al. 2016, Genome Med
	92.3%	Walker et al. 2015, Lancet Infect Dis

Publications from NARMS

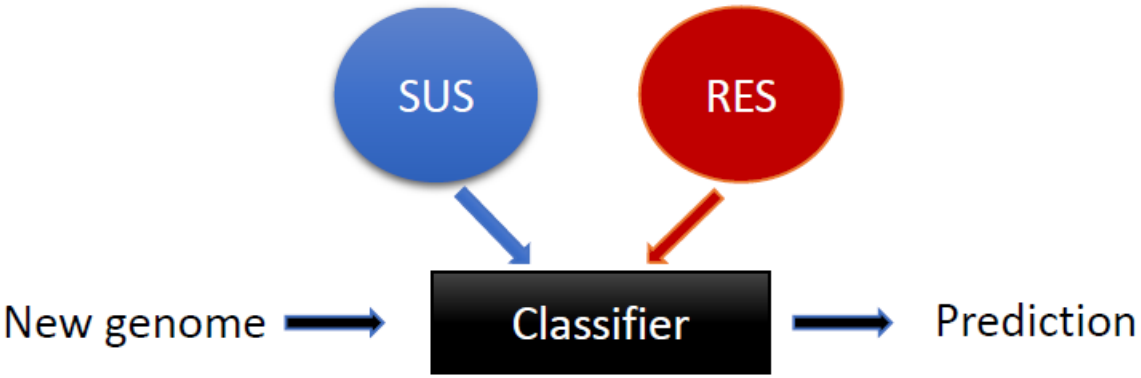
Genotype-Phenotype Concordance for *Salmonella*, all drugs tested

		Phenotype	
		Resistant	Not resistant
Genotype	PR	1478	48
	NPR	127	31443

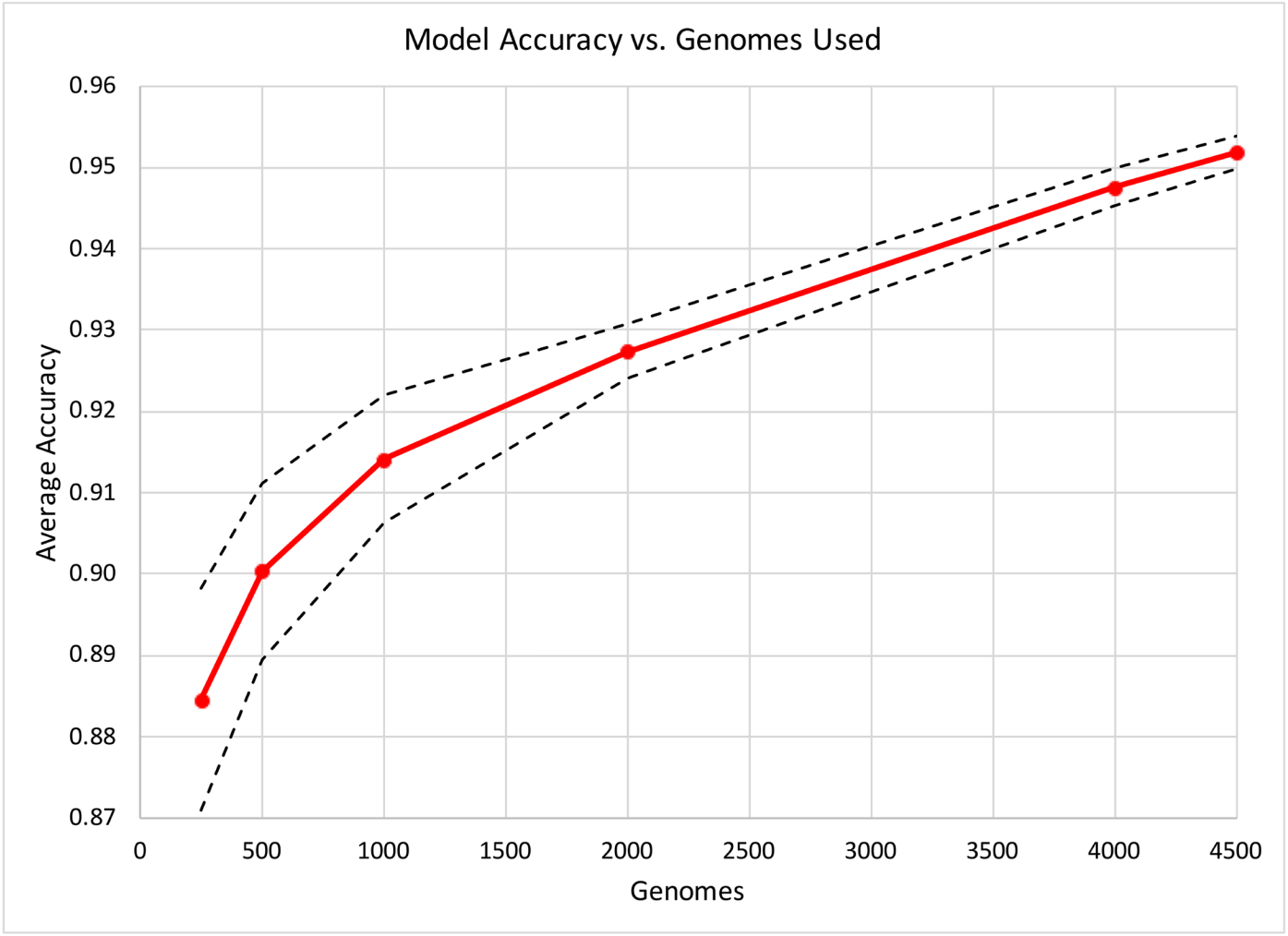
Measure	Value (%)
Sensitivity	92.1
Specificity	99.9
Positive Predictive Value	96.9
Negative Predictive Value	99.6
Kappa coefficient	0.94 (very good)

Jason Folster, CDC

Prediction of MICs by machine learning

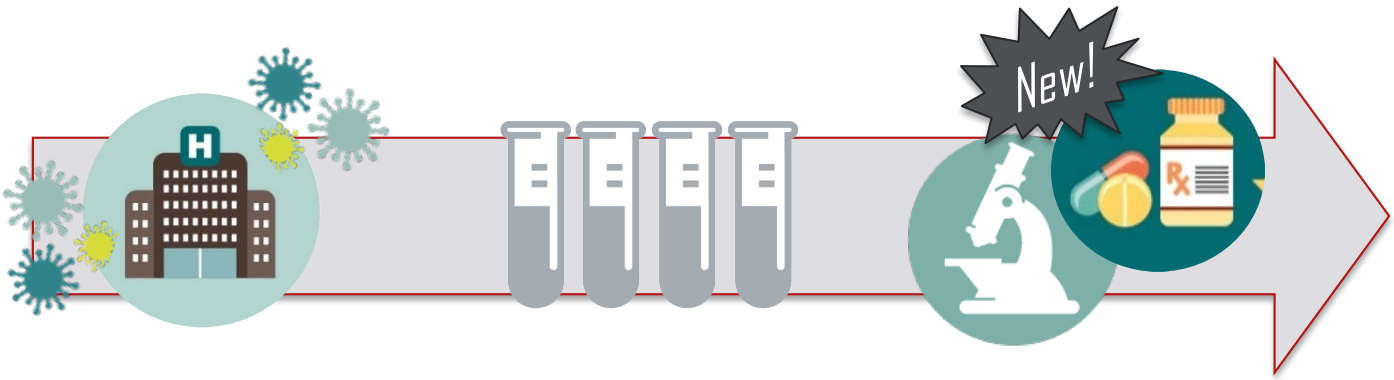


Predicting MICs for *Salmonella*



Nguyen et al., BioRxiv 2018 (Davis Lab)

CDC & FDA AR Isolate Bank:
Sharing Bug Data to Support Drug, Diagnostic Development



CDC gathers resistant bacteria through surveillance/outbreak programs.

CDC analyzes the bacteria's resistance & shares with researchers. The Bank currently includes 228 isolates (on five panels).

New diagnostic tests & antibiotic drugs are developed using the bacteria & data. Since July 2015, CDC has processed 63 orders.

Helping healthcare providers know that the tests they use and drugs they prescribe will protect patients.

Jean Patel, CDC

Antibiotic / Antimicrobial Resistance

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What's this? Submit

CDC & FDA Antibiotic Resistance (AR) Isolate Bank

AR ISOLATE BANK

The CDC and FDA AR Isolate Bank provides information on resistance to support innovation in diagnostics and drug development. CDC provides isolates (bacteria isolated from a specimen, like blood or food) to approved institutions.

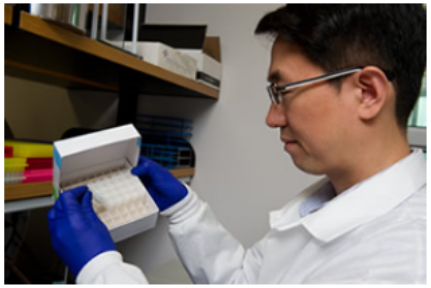
On This Page

> "The isolates helped us challenge our diagnostic tests to ensure they can detect a variety of resistance targets," -Biotechnology company"

Advancing the Fight against Antibiotic Resistance

As of January 2018, the AR Isolate Bank shipped more than 2,000 isolate panels. The AR Isolate Bank helps:

- Strengthen diagnostics by validating lab tests
- Inform research and development to
  - develop drugs like antibiotics and antifungals
  - develop diagnostic devices, tests, or assays
  - satisfy a request or support an application to FDA
- Perform testing to ensure drug effectiveness
- Study biology and pathogenic mechanisms
- Detect new and unusual public health resistance threats

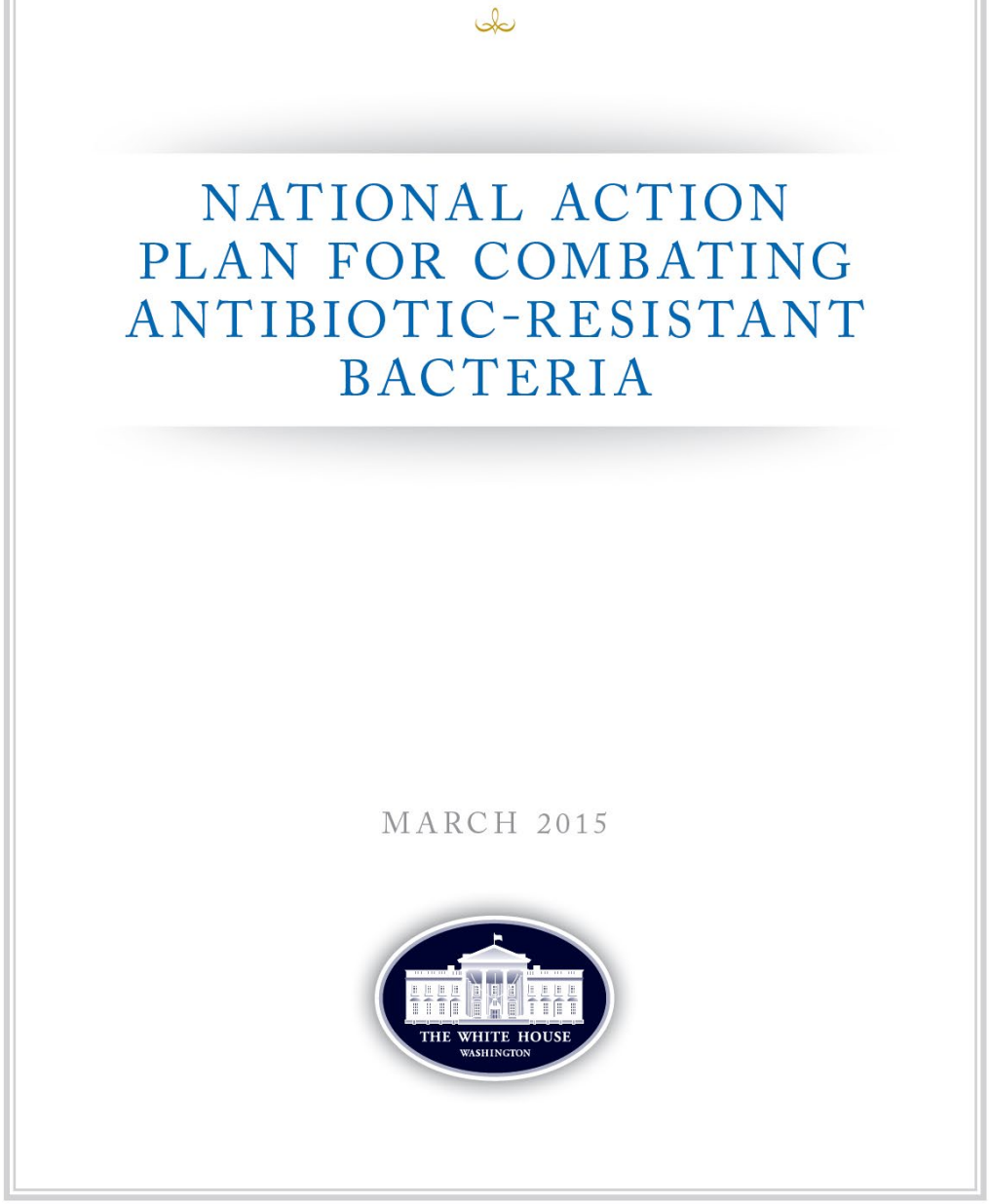


The AR Isolate Bank shipped more than 2,000 isolate panels as of January 2018.

https://www.cdc.gov/drugresistance/resistance-bank/

4. Animal health surveillance

GOAL 2: Strengthen **National One-Health Surveillance Efforts** to Combat Resistance



New national pilot veterinary surveillance efforts (2017-18)

- **USDA National Animal Health Laboratory Network**
  - 19 vet diagnostic labs
  - First year in progress (aiming for 3,000 isolates)
    - *Salmonella* (cattle, swine, poultry, horses, dogs, cats)
    - *E. coli* (same as above)
    - *Mannheimia haemolytica* (cattle)
    - *Staphylococcus intermedius* group (dogs and cats)
  - Including secure (HL7 compliant) messaging

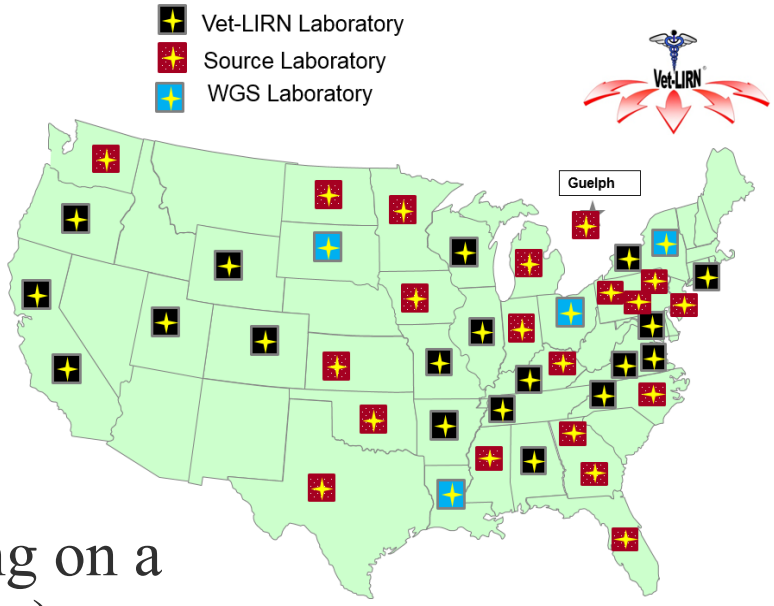
USDA Year 1: Progress – electronic messaging

To address the unique requirements for protecting information as part of the AMR Pilot study, a staged approach has been developed to support electronic results data submission.

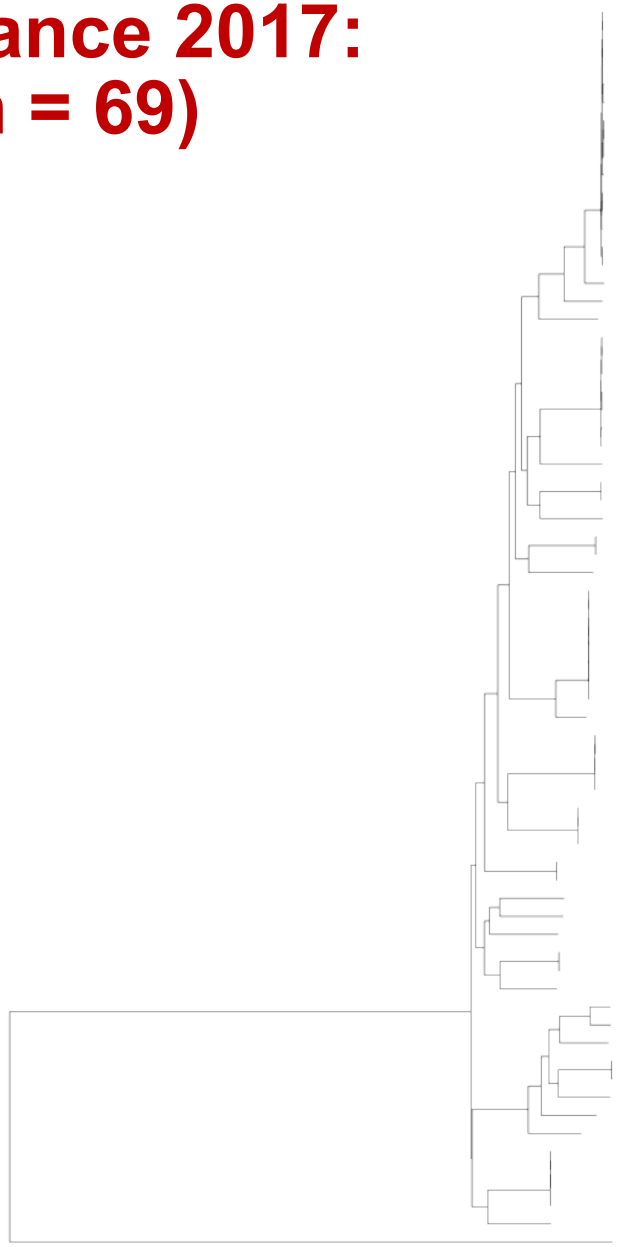
- Step 1 – all laboratories were provided with a spreadsheet with required data entry fields for HL7 messaging of AST data
  - Laboratories submit AST data to NAHLN Program Office on these spreadsheets – data is reviewed and validated prior to uploading to APHIS database.
- Step 2 [*in progress*] – script developed to convert data from spreadsheets to HL7 format, which is then messaged directly to APHIS database
  - Script has been developed, NAHLN program office is beta-testing for errors
  - Once beta testing is complete, script will be provided to laboratories for use
  - Goal – at least 5 laboratories using this method of data reporting by the end of Sep (FY 2018)

New national pilot veterinary surveillance efforts (2017-18)

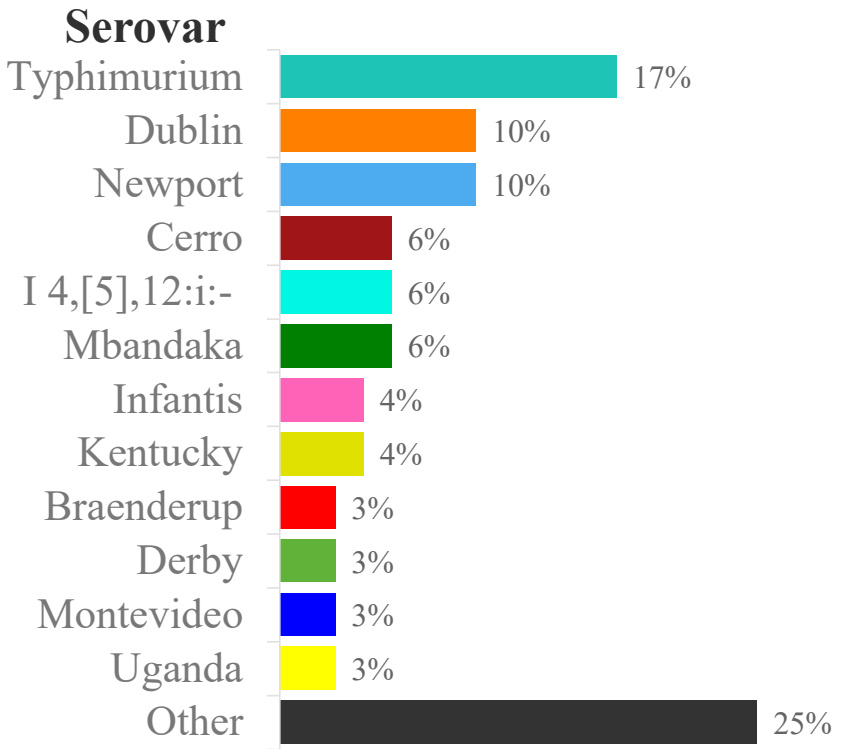
- **FDA Veterinary Laboratory Investigation and Response Network**
  - 20 vet diagnostic labs
  - ~2,000 isolates collected in 2017
    - *Salmonella* (all hosts)
    - *E. coli* (dogs)
    - *S. pseudintermedius* (dogs)
  - Including whole genome sequencing on a subset (done by 4 additional vet labs) uploaded to NCBI in near real-time
  - In 2018 (year 2), adding 5 additional labs and broadening the scope of pathogens

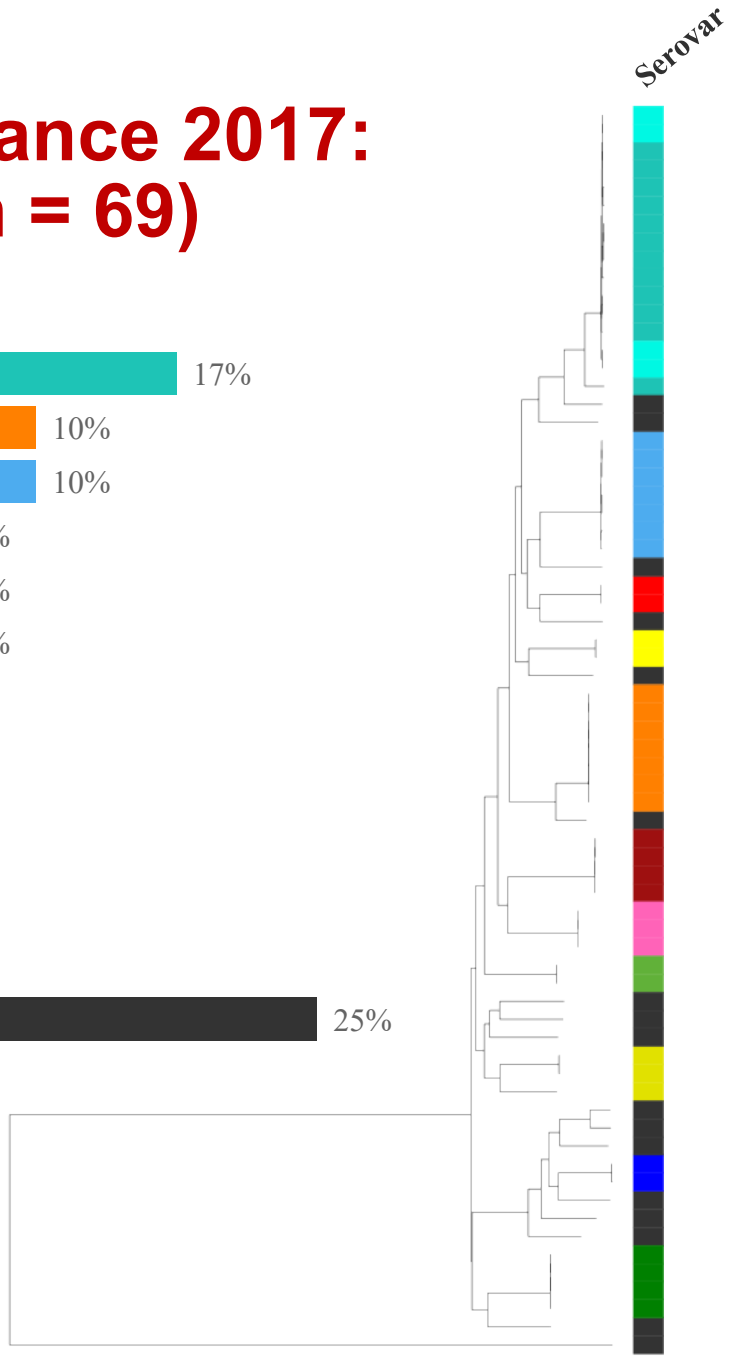


FDA national WGS surveillance 2017: *Salmonella* (n = 69)

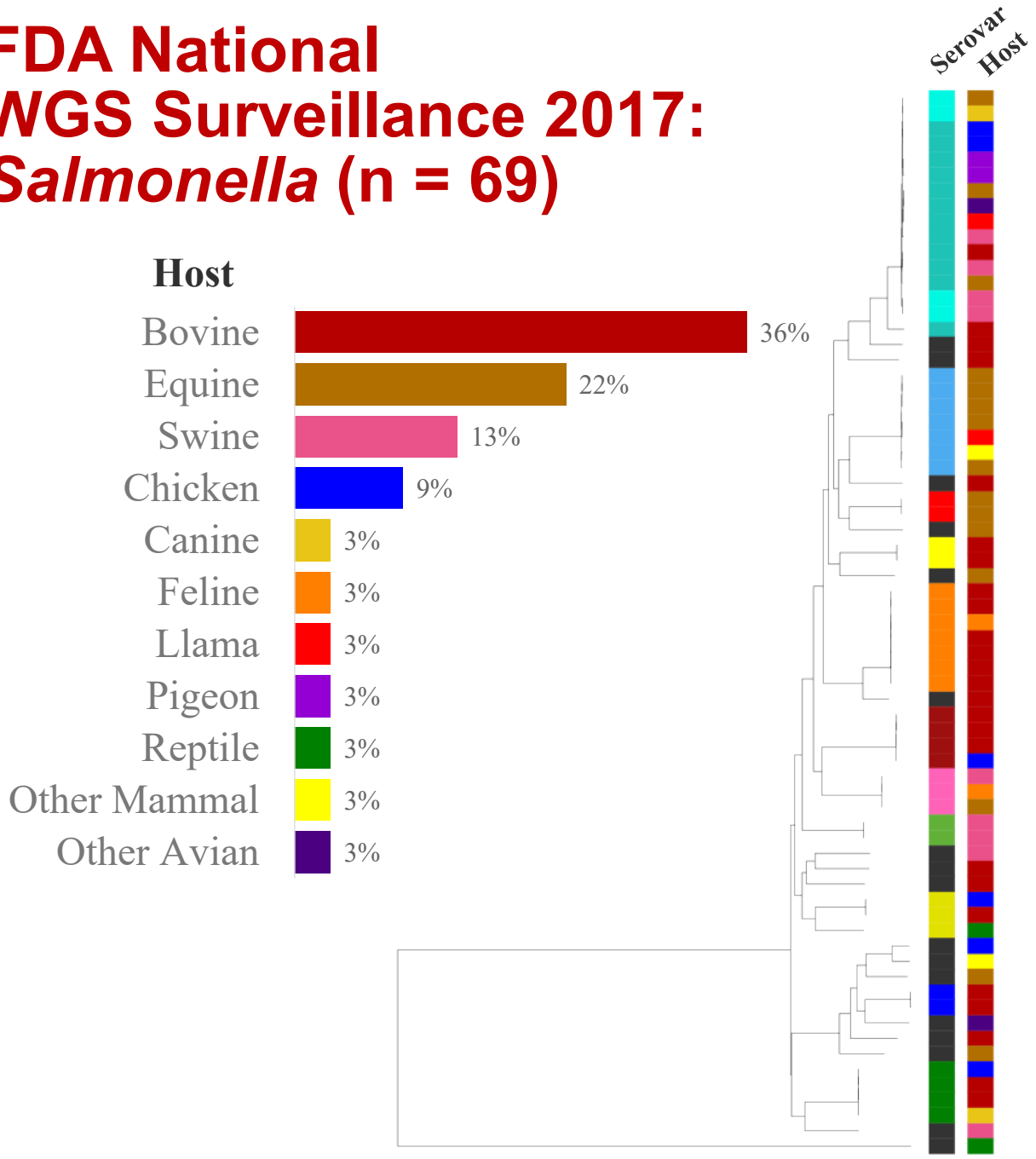


FDA National WGS Surveillance 2017: *Salmonella* (n = 69)





FDA National WGS Surveillance 2017: *Salmonella* (n = 69)



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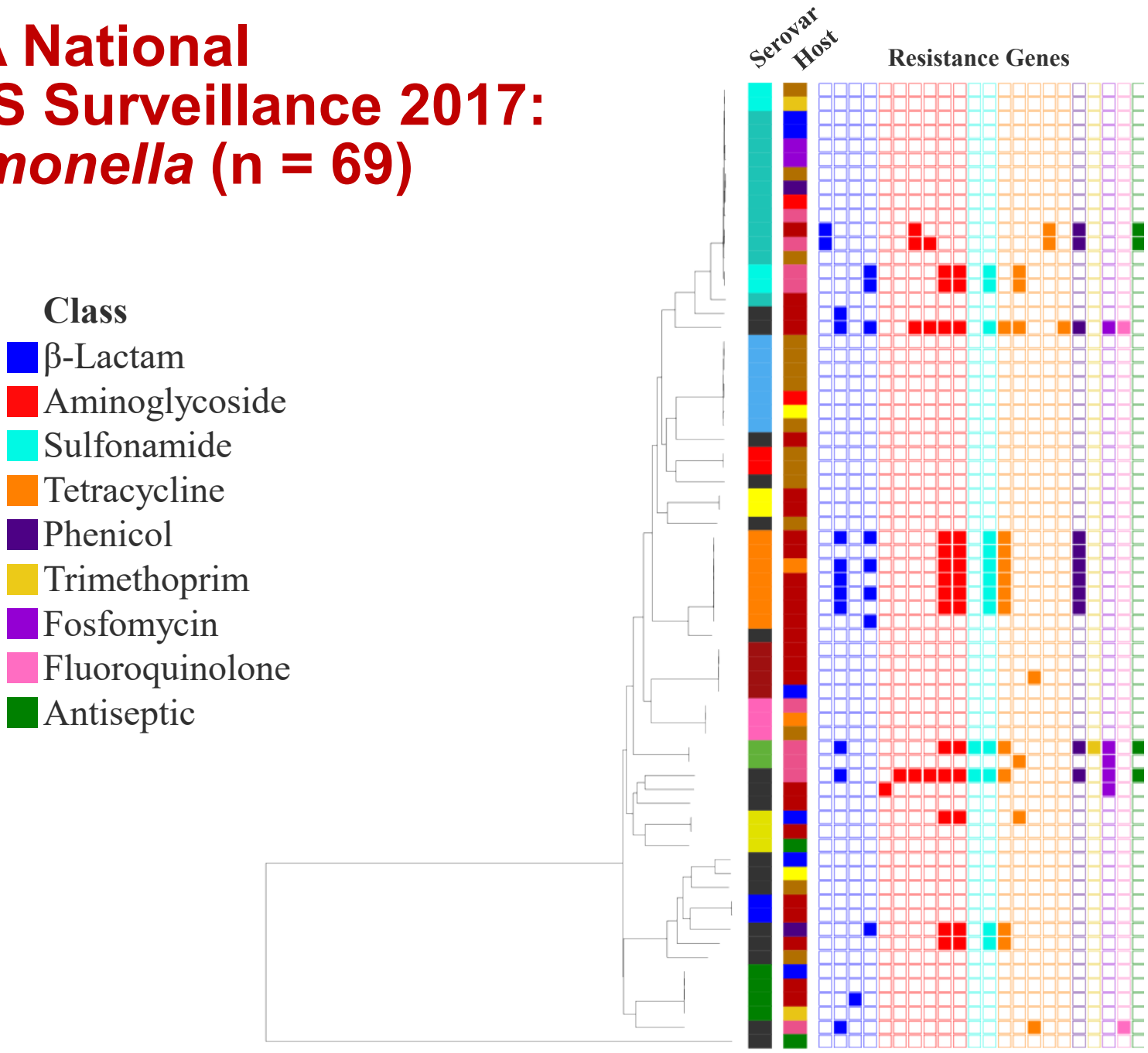
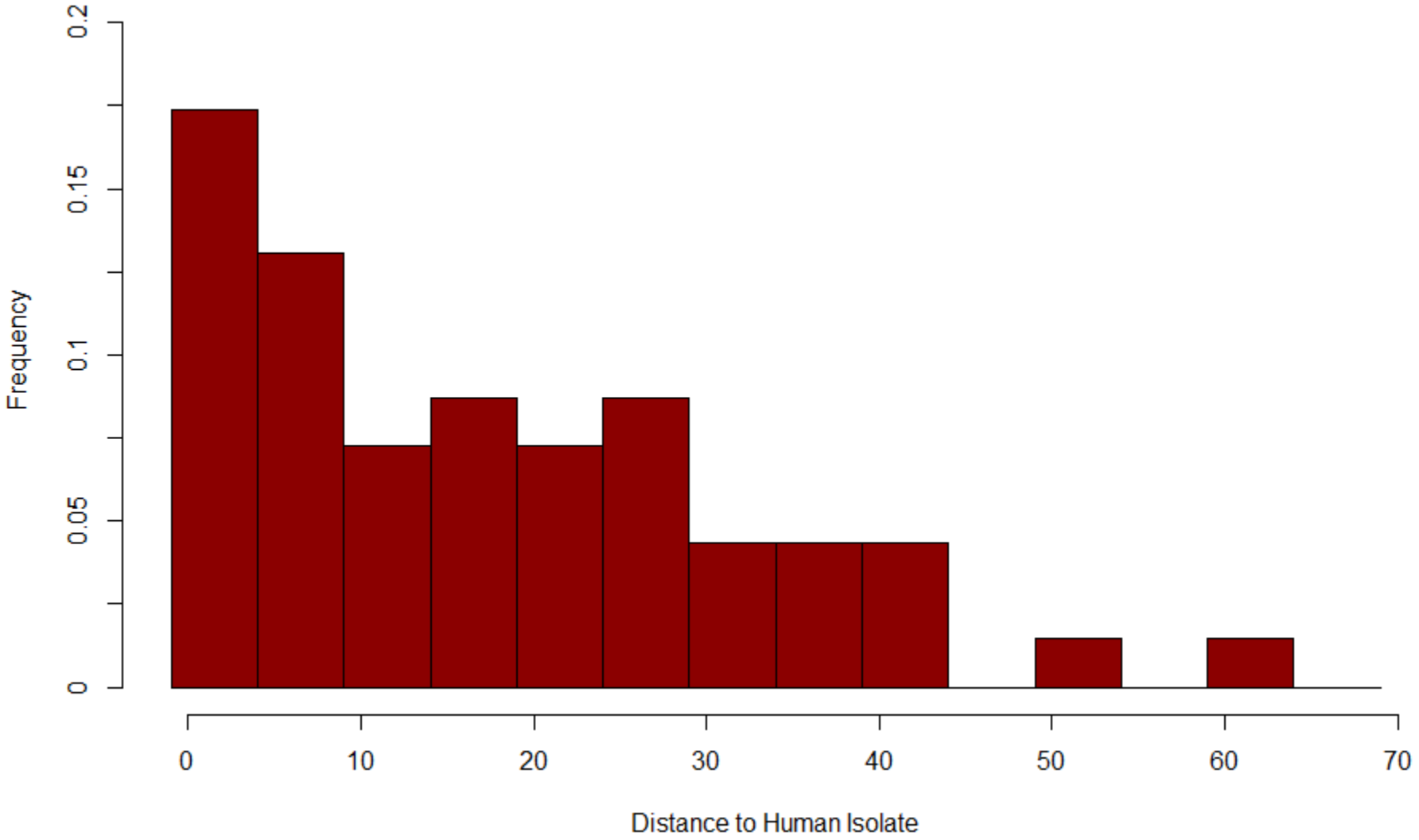
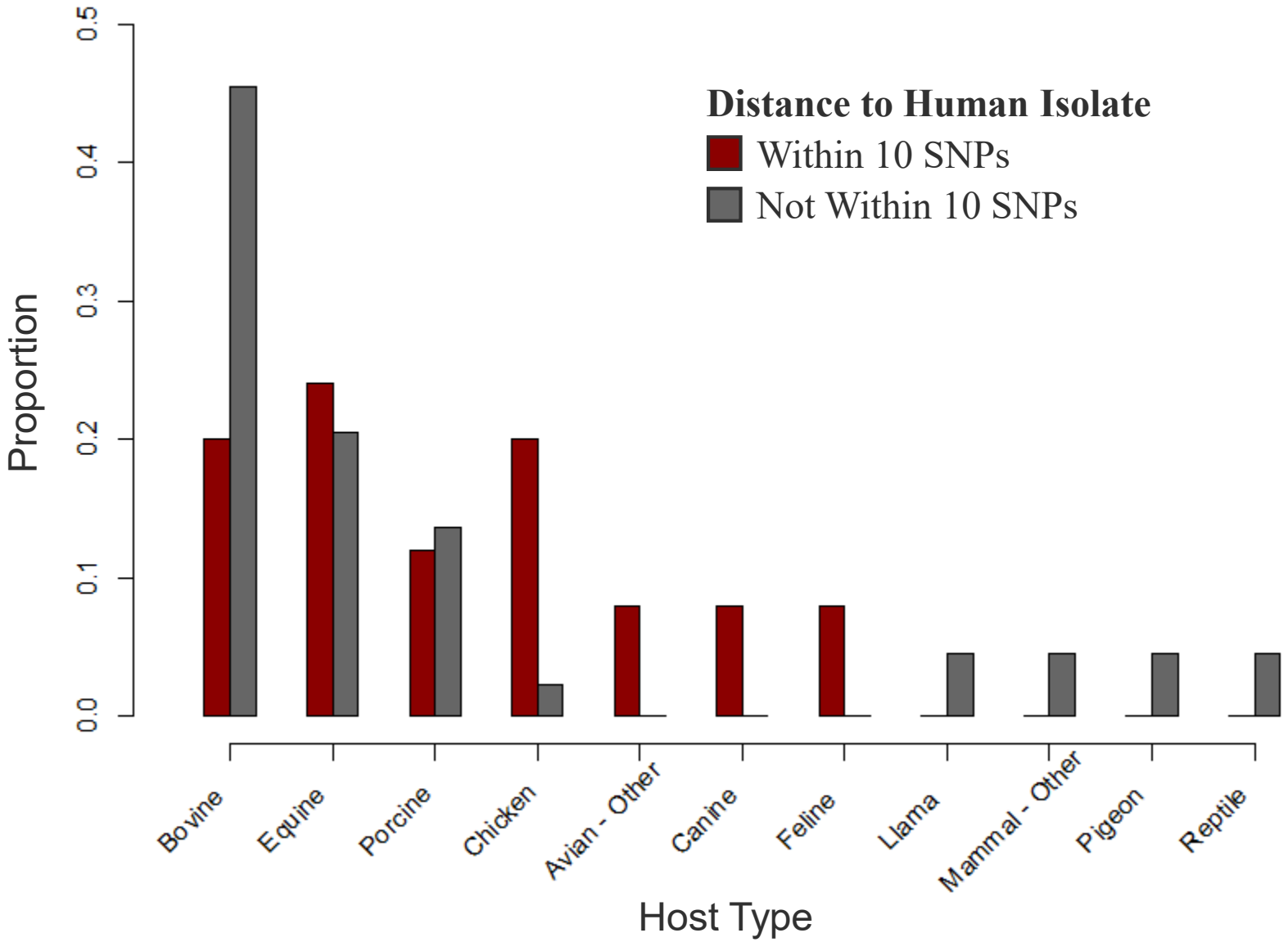
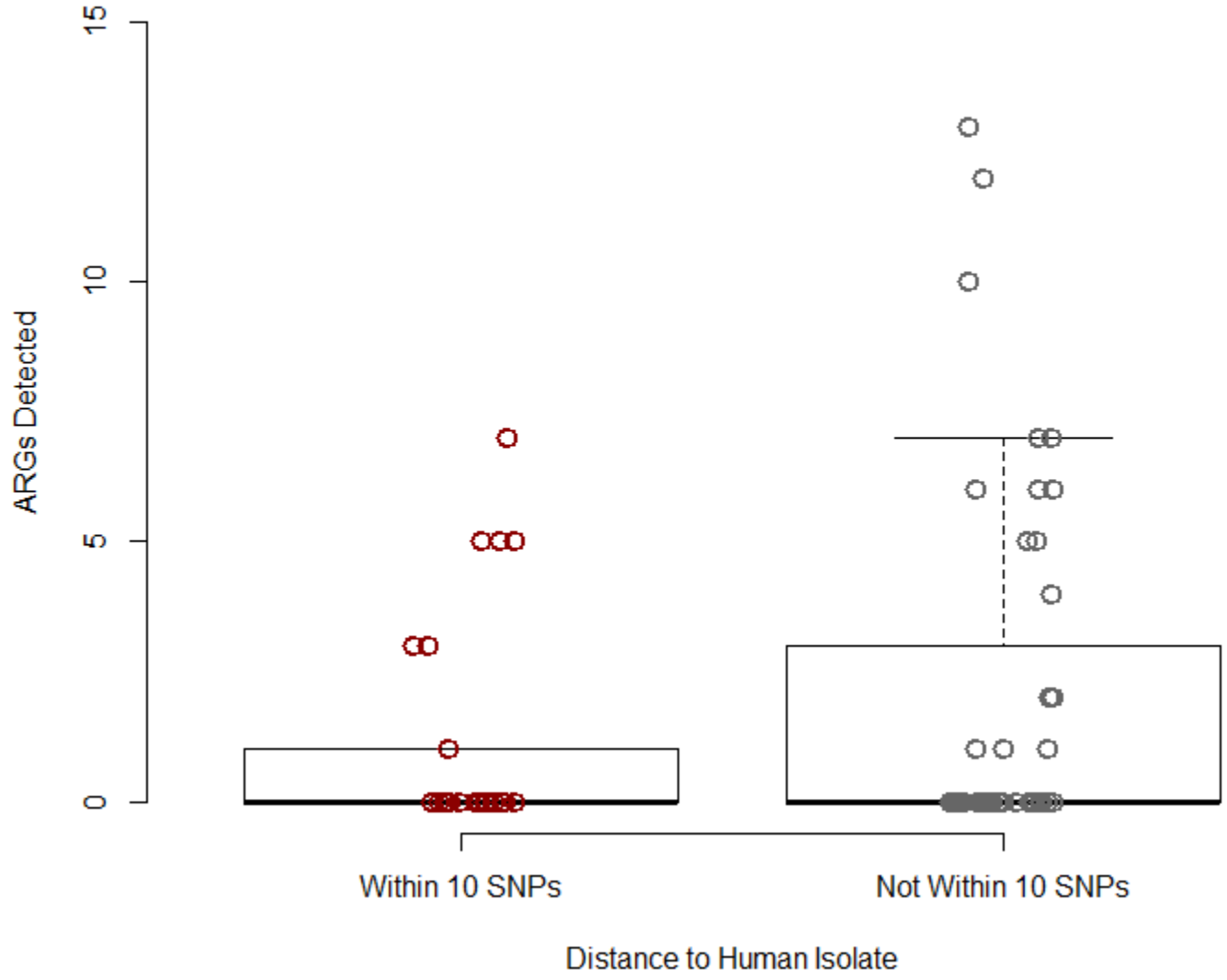


Veterinary *Salmonella* isolates are closely related to human cases (n = 54)

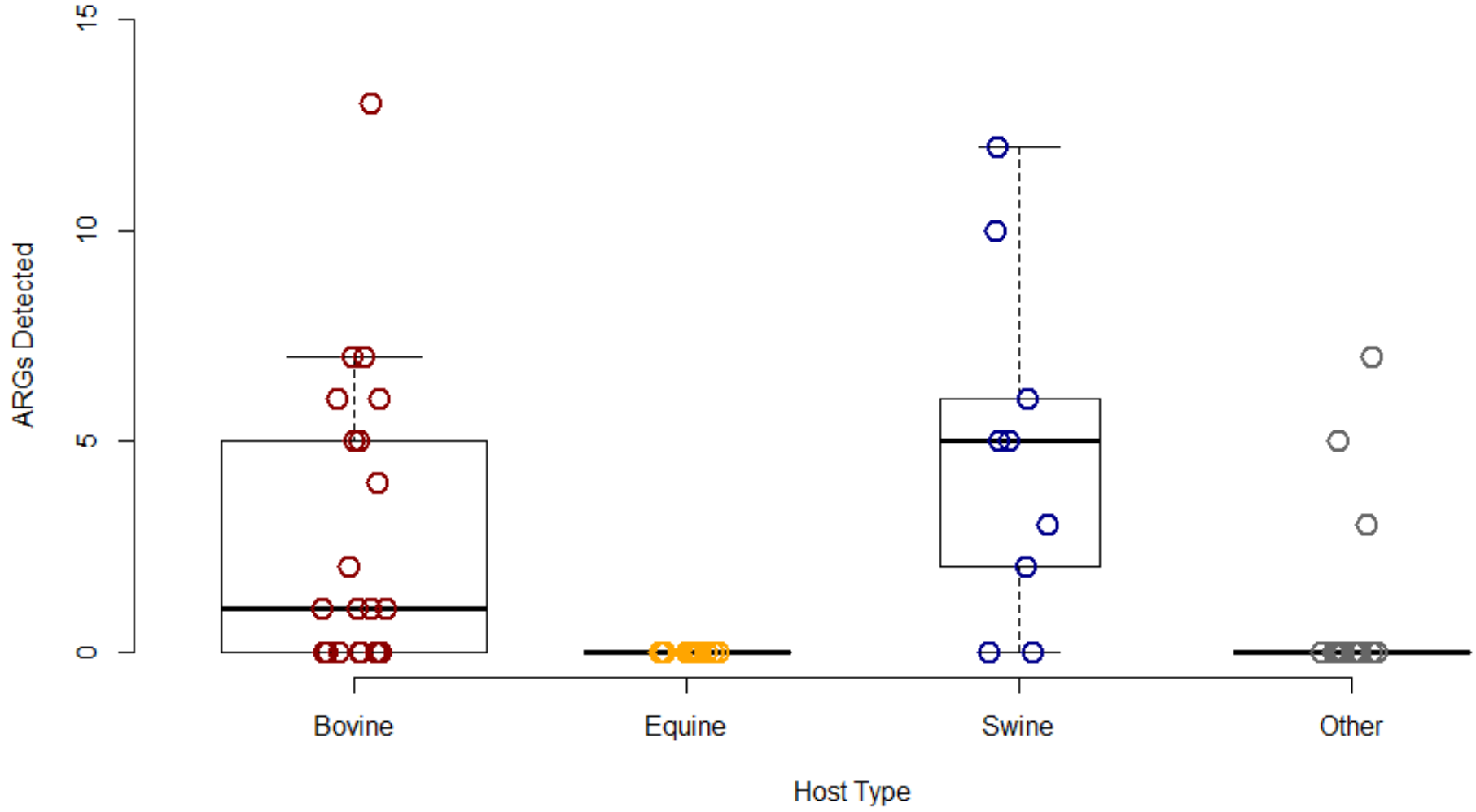



Distance to Human Isolate
Within 10 SNPs
Not Within 10 SNPs
Proportion
0.0
0.1
0.2
0.3
0.4
0.5
Bovine
Equine
Porcine
Chicken
Avian - Other
Canine
Feline
Llama
Mammal - Other
Pigeon
Reptile
Host Type

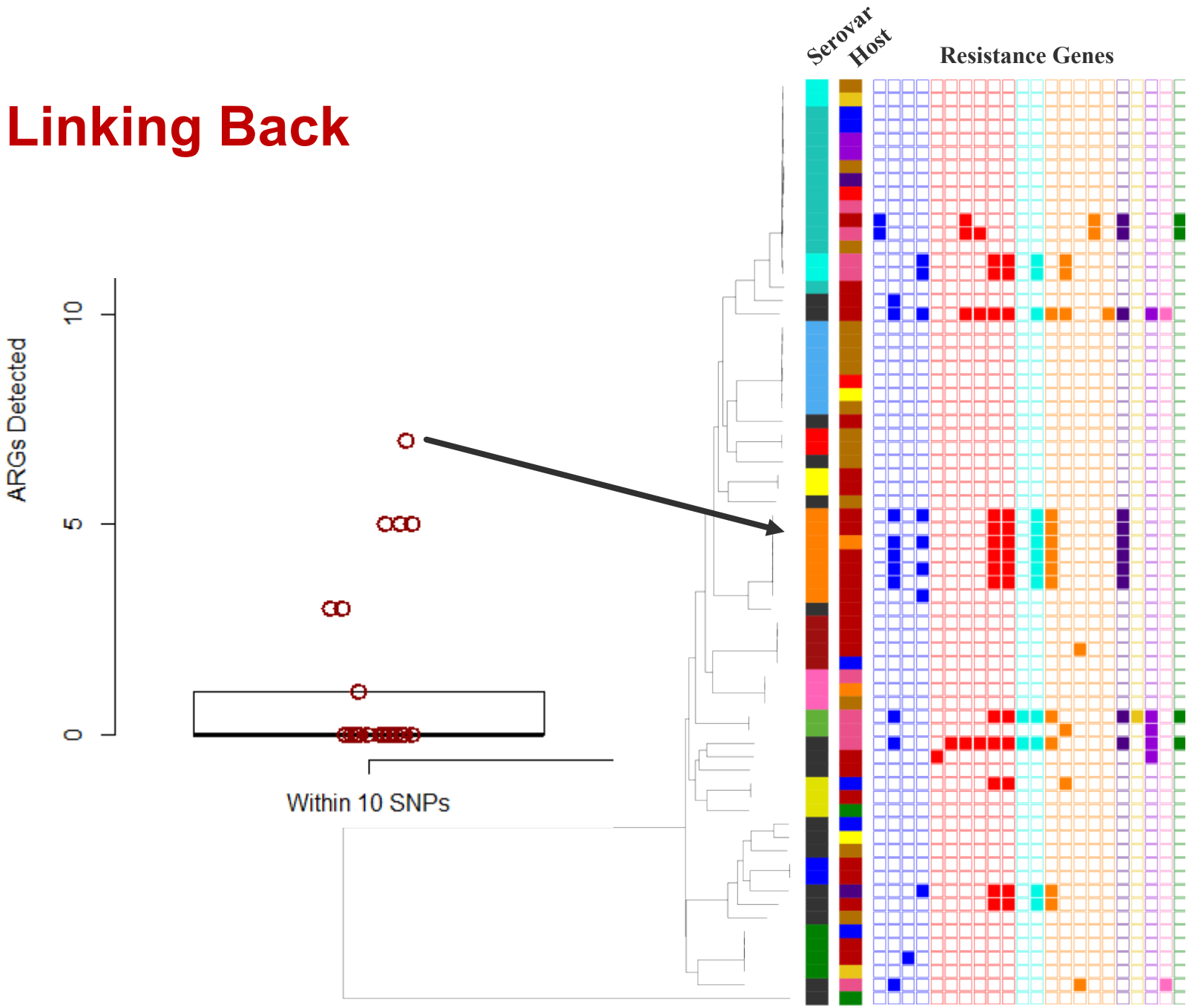
Distribution of ARGs by Human Distance



Distribution of ARGs by Host Type

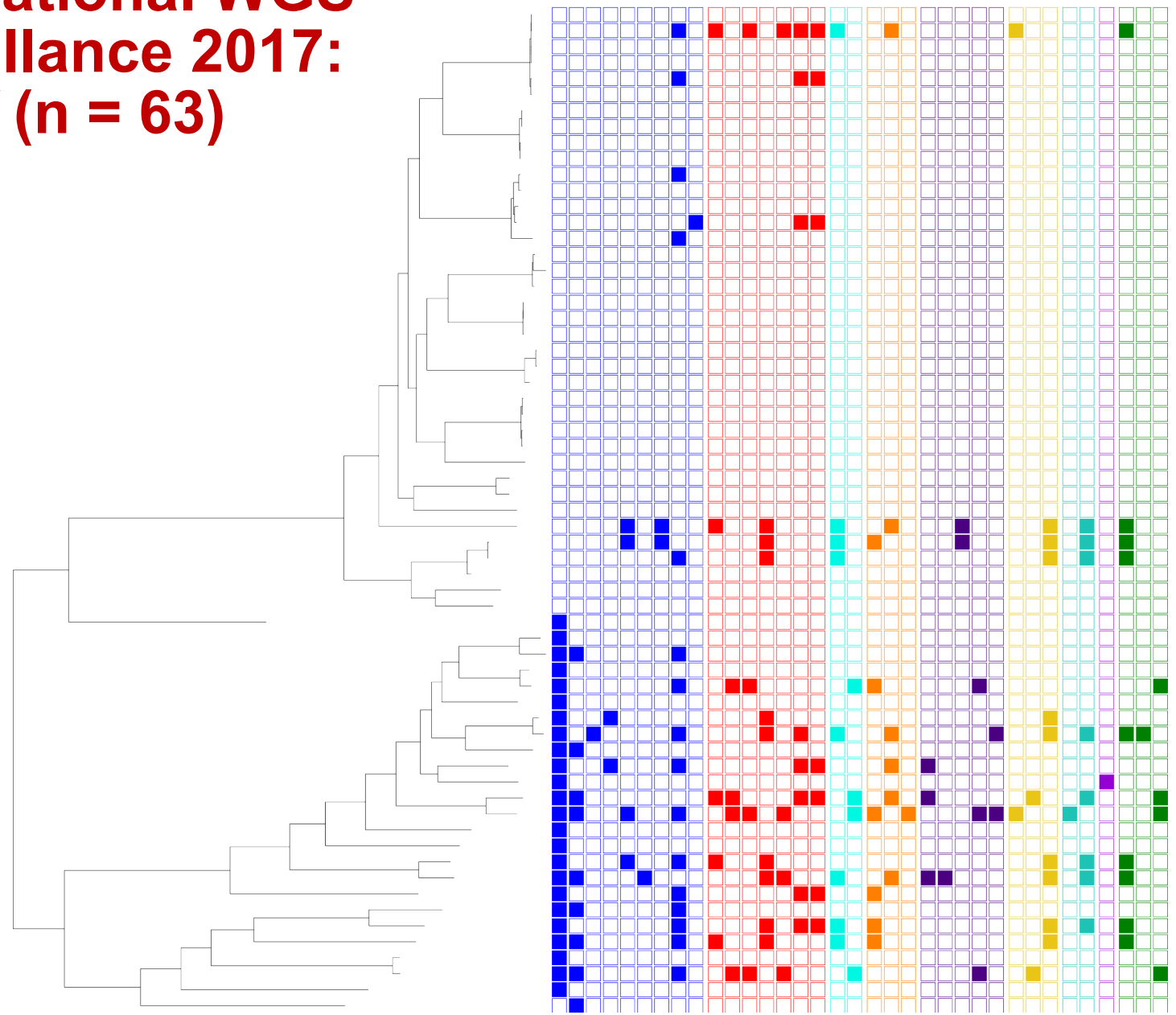


Linking Back

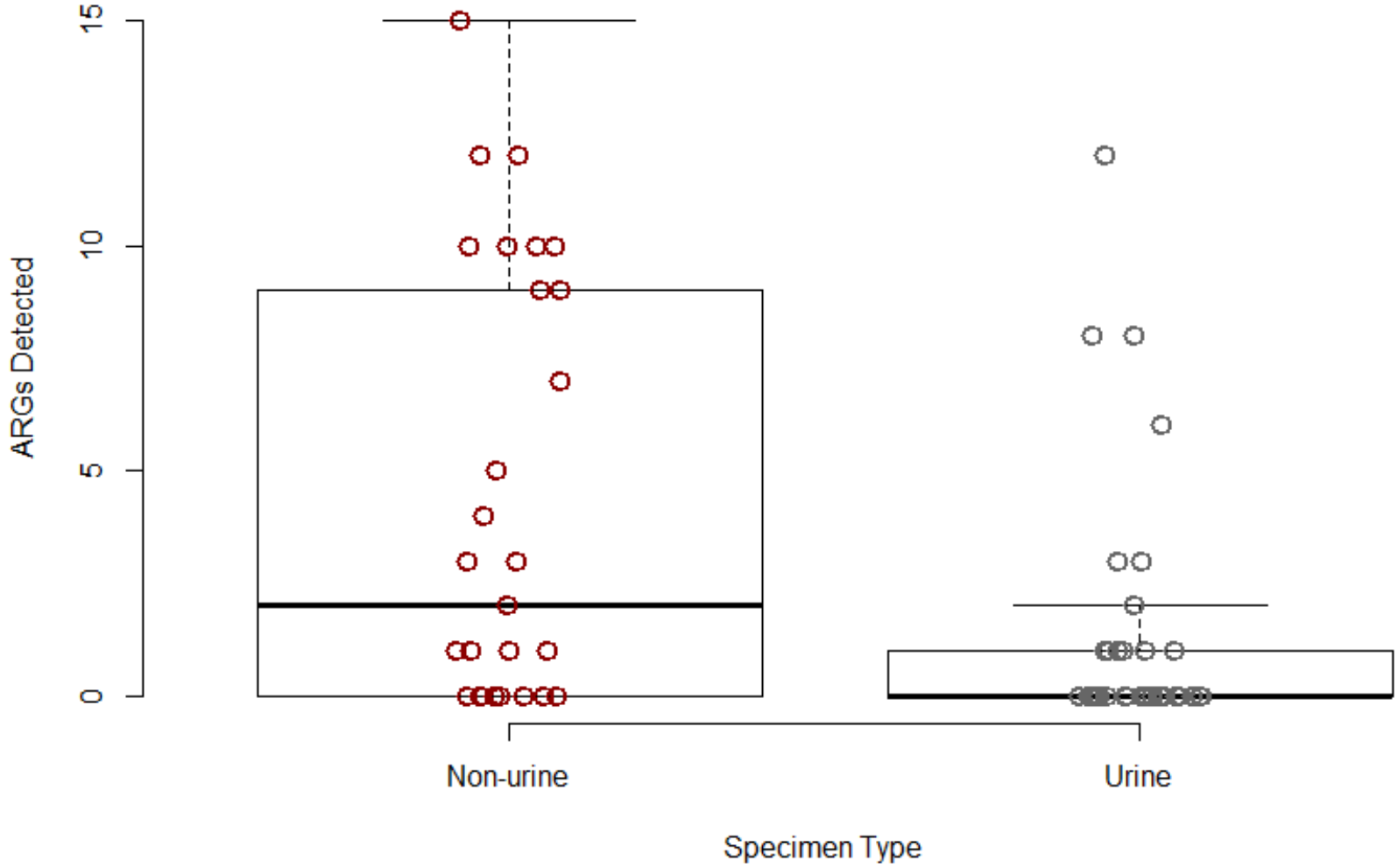


E. coli

FDA National WGS Surveillance 2017: *E. coli* (n = 63)



ARGs by Specimen Type



Relation to Human Isolates?

- Very few in SNP clusters with human isolates, despite predominance of human samples in database
- Capturing a different subset of population
- Why it matters
  - May still be linked to human infections not covered by typical surveillance frameworks
  - Shared genetic repertoire

Most extreme case

Nearly pan-resistant *E. Coli* from canine fecal sample

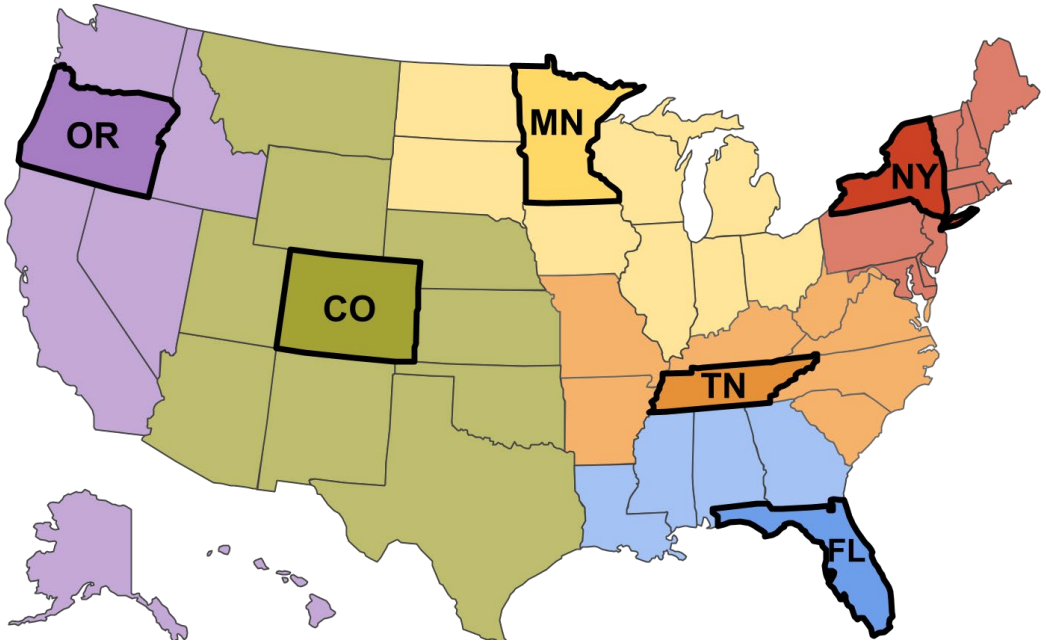
ECOL-17-VL-NY FL-0002	aac(3)-Iid (gentamicin) aadA1 (streptomycin) aph(3")-Ib (streptomycin) aph(3')-Ia (kanamycin) aph(6)-Id (streptomycin) blaCMY-2 (penicillins, amoxi-clav, cephalosporins) blaTEM-1 (penicillins) catA1 (phenicols) dfrA14 (trimethoprim) mph(A) (macrolides) qacL (disinfectants) sul2, sul 3 (sulfonamides) tet(B) (tetracycline) gyrA mutations (fluoroquinolones)

FDA pilot AMR surveillance study:

Dr. Olga Ceric,
Sunday at 10:45am
Chicago C (Epidemiology)

5. Bringing big data from animals and humans together

Integrated Food Safety
Centers of Excellence



Strategies for One Health AMR data sharing

- A meeting for animal and public health laboratories and stakeholders
- Sponsored by the New York Integrated Food Safety Center of Excellence
- Held May 2018 at the Cornell College of Veterinary Medicine, Ithaca, NY

Meeting Highlights

- Critical time to coordinate and standardize!
- NARMS and NCBI emerged as “best practice” common databases for AST, WGS, and metadata
- Reducing granularity of location would allow enhanced metadata to be provided
- A tiered system with a 3rd party protector of identifiable information proposed as safeguard for confidentiality

One Health Data Sharing: next steps

- **State-level pilot projects**
- **Identify 3rd parties for tiered confidentiality**
- **Incorporate animal health data in NARMS** from both public/academic and corporate labs

One Health Data Sharing: next steps

- **Share syndromic, regional antibiograms**
  - Stewardship and continuing education initiatives currently supported by AVMA, USDA, CDC, and state public health agencies.
  - Scientific publication
  - Popular press – improve stakeholder awareness
  - Having veterinarians and the public see the summary data will help incentivize participation

Take-home points

1. People and animals share pathogens and pathogens share genes

2. By monitoring ARGs in animal populations, we can better protect both animal and human health

Acknowledgments 1

- Cornell AHDC
  - **Patrick Mitchell**
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  - Denise Archer
- FDA
  - **Olga Ceric**
  - **Sarah Nemser**
  - Jen Jones
  - Renate Reimschussel
  - Maria Sanchez
  - Ruth Timme
- Vet-LIRN WGS Collaborators
  - OH (Yan Zhang & Melanie Prarat)
  - LSU (Laura Peak)
  - SD (Joy Scaria, Linto Antony)
  - WA (Claire Miller)
- NY Source Labs
  - PA (Donna Kelly)
  - FL (David Simon)
  - MS (Martha Pulido-Landínez)
  - NJ (Sarmila DasGupta)
  - GA (Becky Wilkes)

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Acknowledgments 2

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- Gen Meredith

CDC NCEZID

- **Misha Robyn**
- **Megin Nichols**
- Dawn Sievert
- Chris Braden

NY State Ag & Markets

- Dave Smith

USDA NAHLN

- Beth Harris

NARMS

- Jean Whichard - CDC
- Heather Tate - FDA

Cornell CVM

- François Elvinger
- Lorin Warnick

Thank you!

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