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USAHA Committee on One Health
10/24/18

One Health benefits of using pathogen WGS as a tool for herd management

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Overview

1. Pathogen Whole Genome Sequencing (WGS) strategies
2. Source tracking
3. Pathotyping
4. Antimicrobial resistance

1. How pathogens are sequenced

Clinical Goal: provide **rapid, low-cost, clinically actionable data in lay terms** for veterinarians & producers

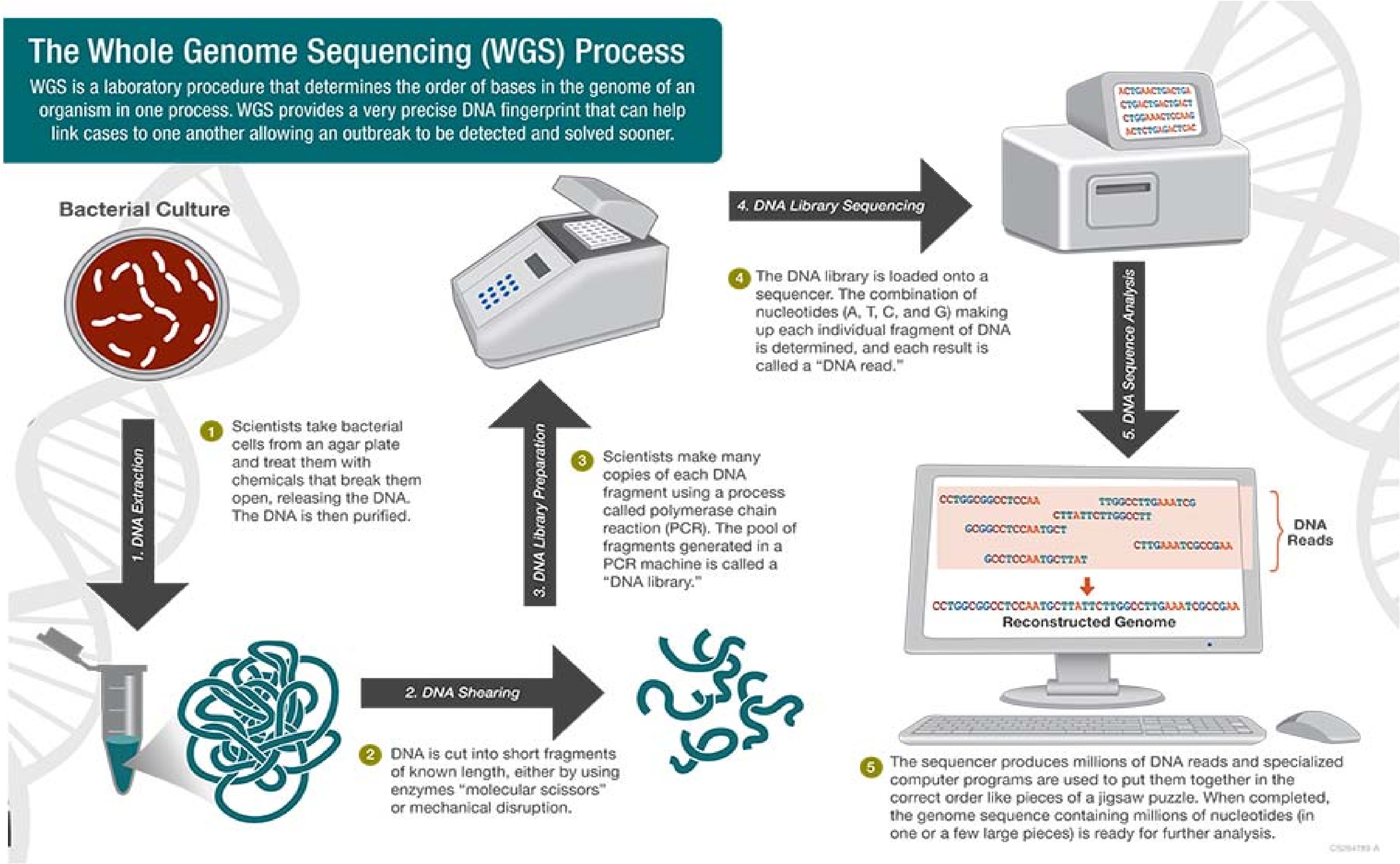
Most common strategies for pathogen WGS

Pure cultures (shotgun)

Amplicons

Selective extraction

The Whole Genome Sequencing Process



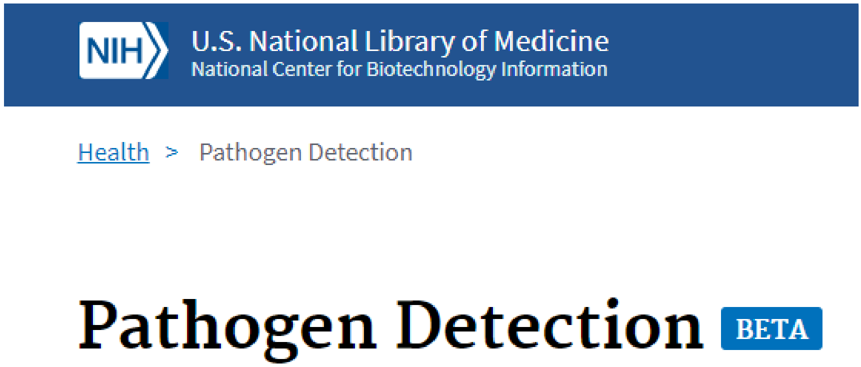
The Whole Genome Sequencing (WGS) Process

WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

1. Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.
2. DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.
3. Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a "DNA library."
4. The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."
5. The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.

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

National pathogen WGS database: heavily human-focused



NIH U.S. National Library of Medicine
National Center for Biotechnology Information

Health > Pathogen Detection

Pathogen Detection BETA

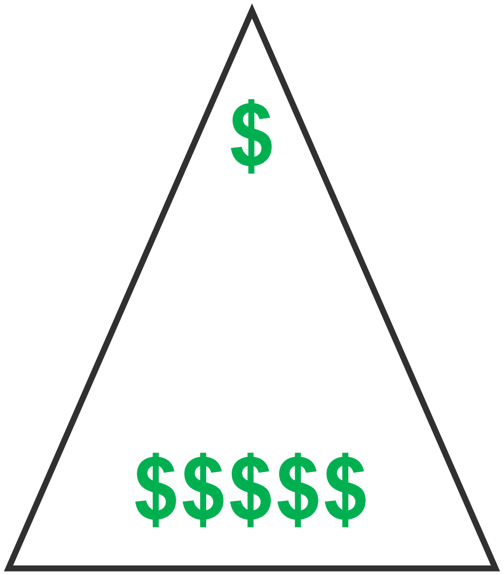
Organism	Total isolates (10/3/18)
Salmonella	153,255
E. Coli	54,679
Listeria monocytogenes	20,879
Campylobacter jejuni	21,838
Klebsiella pneumoniae	7,774
Staphylococcus pseudintermedius	249

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

Culture-independent detection methods

Mostly for environmental testing
– some commercial clinical tests for humans

- Targeted amplification (PCR of multiple genes)
- Targeted metagenomics (sequencing of many genes)
- Shotgun metagenomics (sequences “all” DNA)



News › World › Europe

DNA blunder creates phantom serial killer

Police admit they wasted 15 years hunting for the 'Woman Without a Face'

She was a mysterious serial killer known as the "The Woman Without a Face" and detectives across Europe spent more than 15 years doing their utmost to bring her to justice for at least six brutal murders and a string of break-ins. Yesterday, however, they were forced to admit that she probably didn't exist.

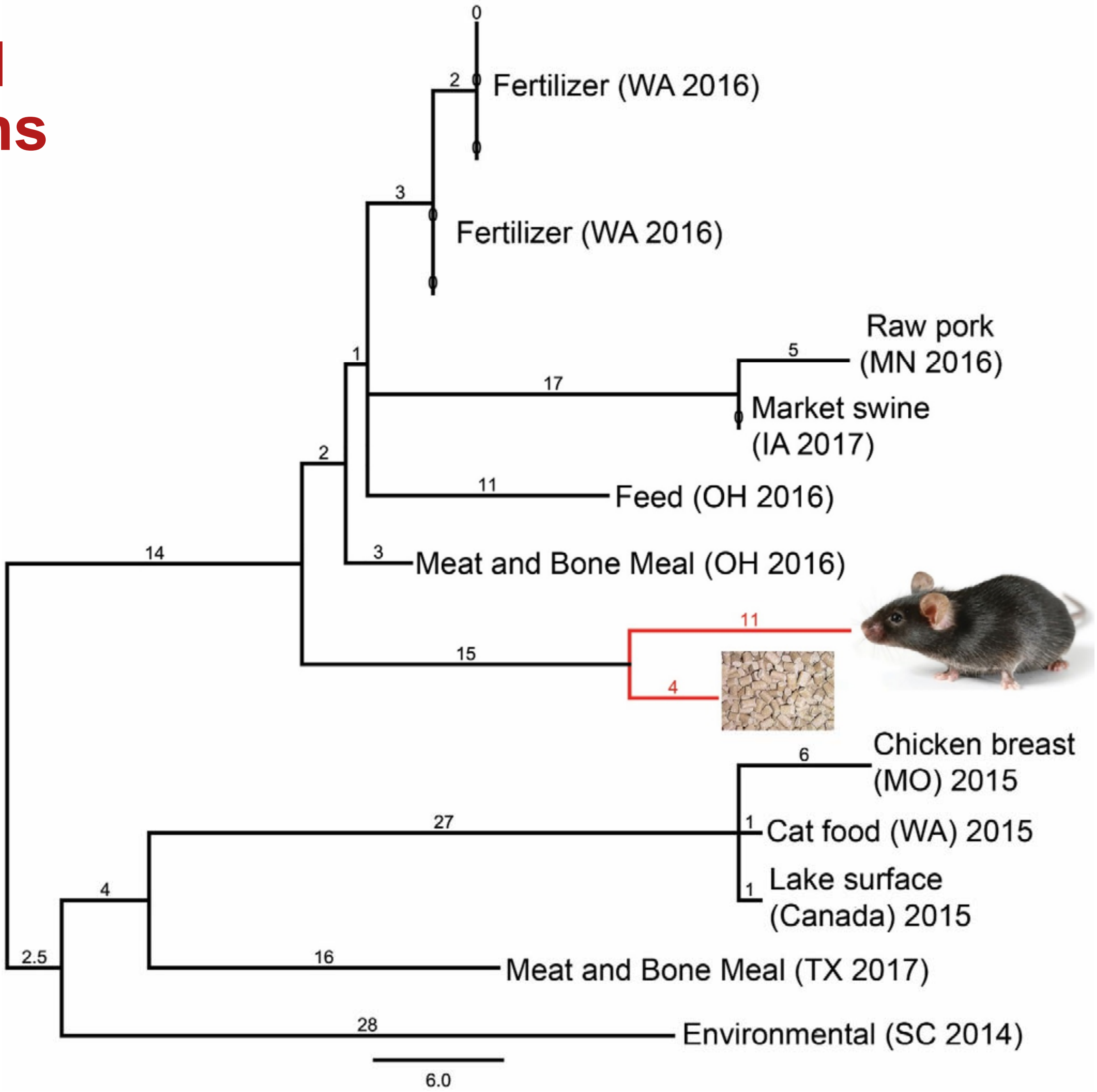
The only clues that "The Woman Without a Face" left behind at 40 different crime scenes were DNA traces. These were collected on cotton swabs, supplied to the police in a number of European countries. Now police investigators have established that in all probability the DNA had not been left by their quarry but by a woman working for the German medical company supplying the swabs, who had inadvertently contaminated them.

German police who had been leading the hunt said they had probably been involved in one of the longest and most perplexing wild goose chases in criminal history. "This is a very embarrassing story," admitted police spokesman Josef Schneider.

2. Source tracking

Clinical Goals: pin point specific common sources of outbreak strains, monitor efficacy of autogenous bacterins/vaccines

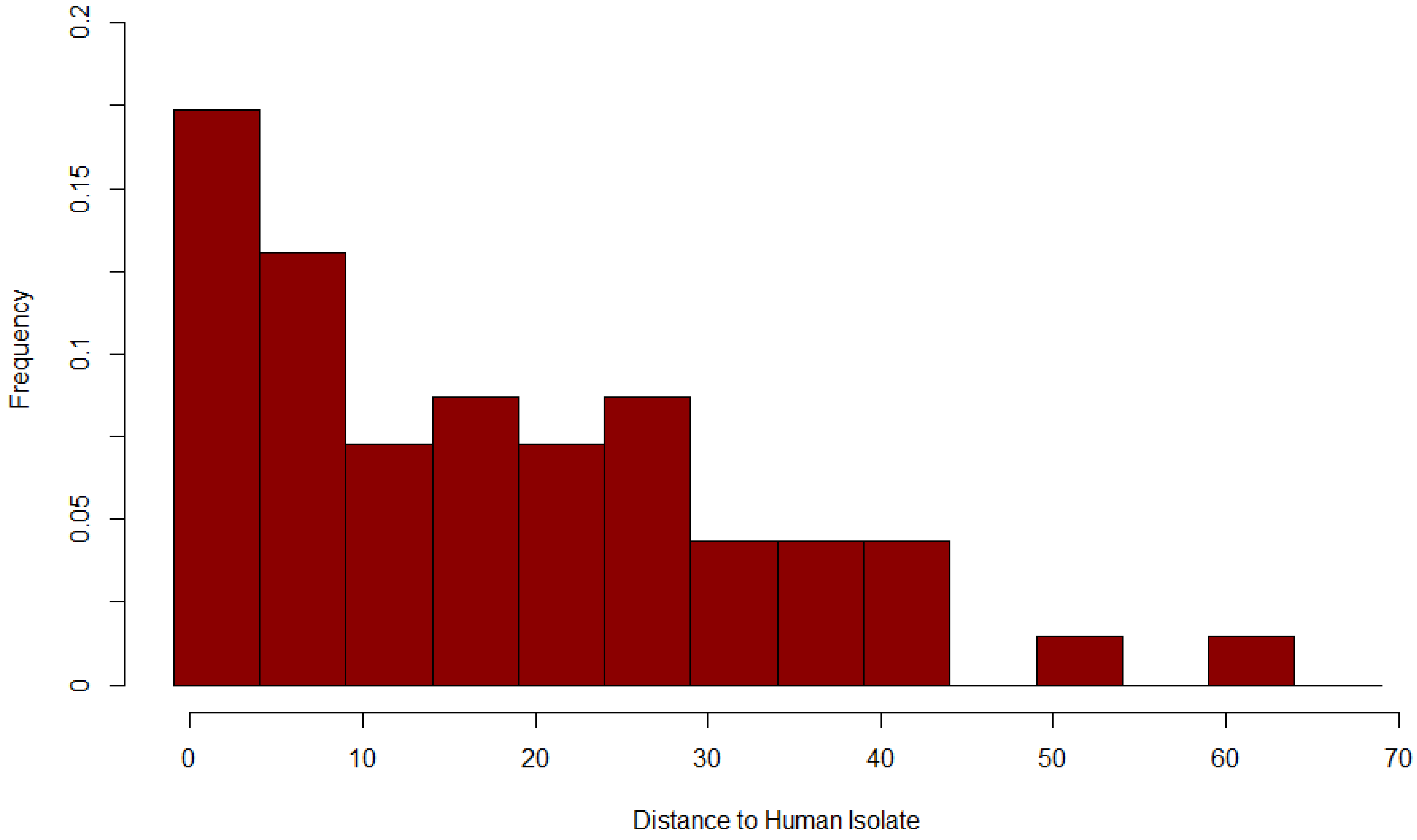
Linking animal and feed strains

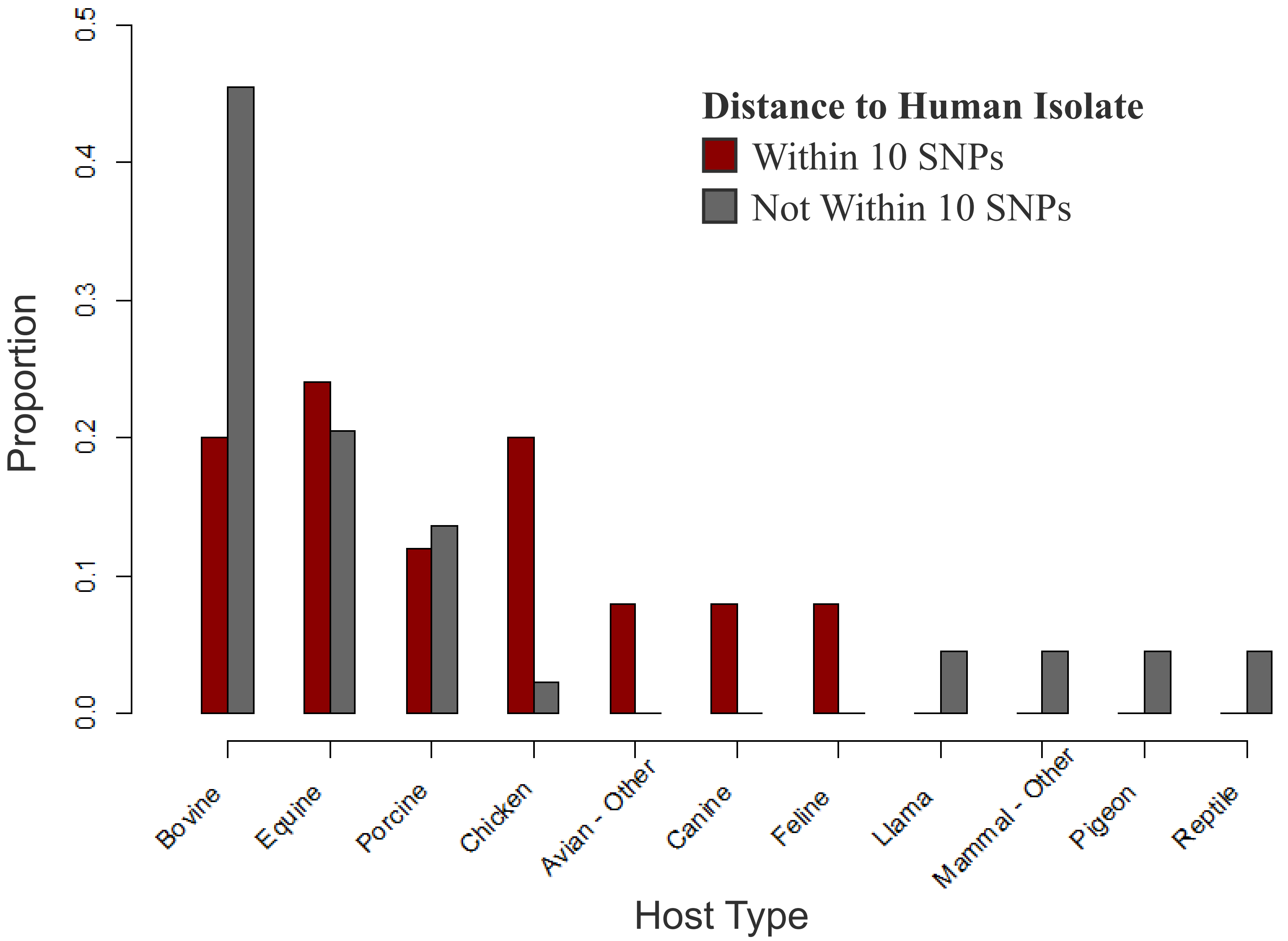


Managing environmental *Salmonella* contamination on dairy farms

Picture credit: www.gea.com

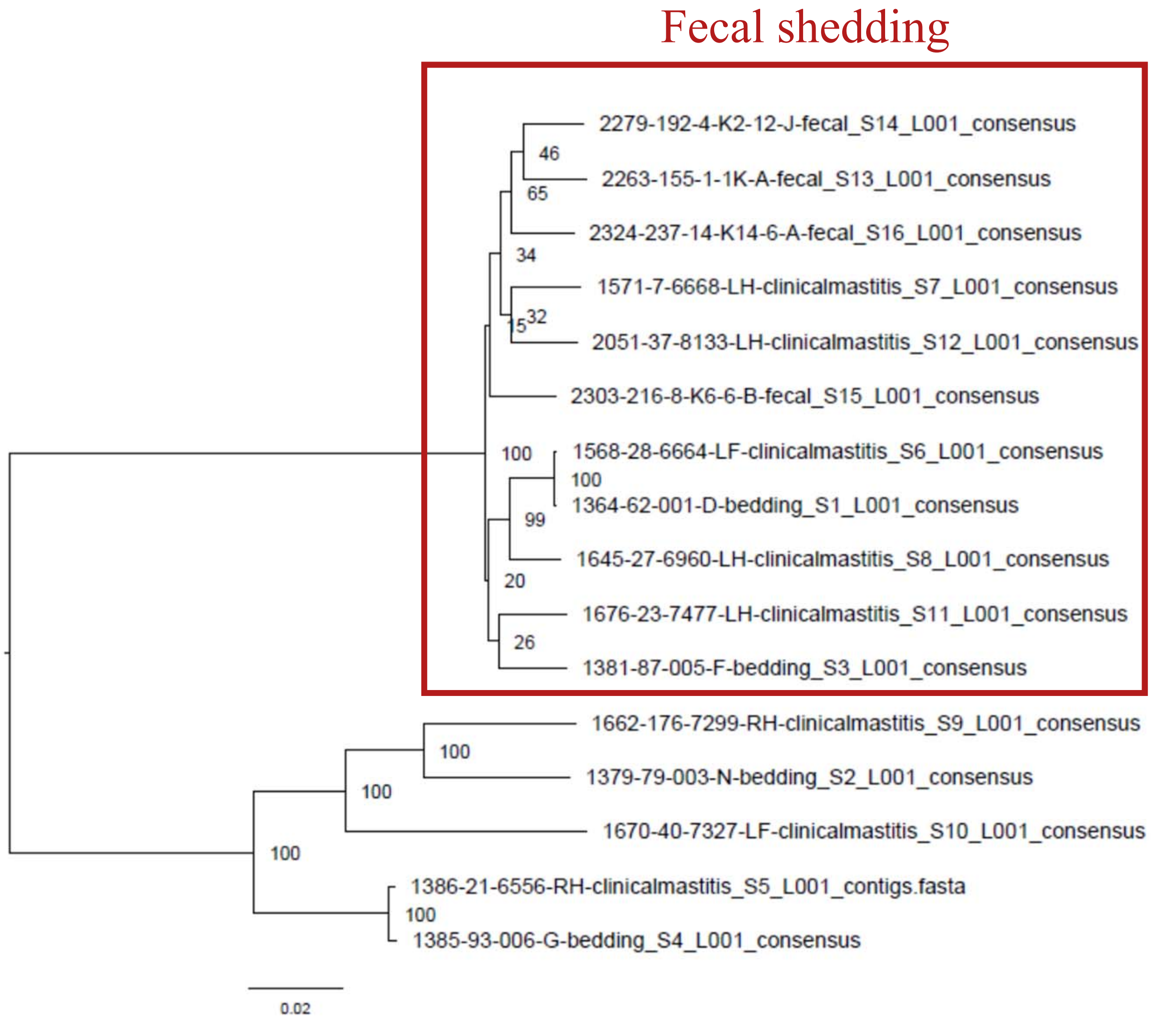
FDA Vet-LIRN *Salmonella* surveillance, 2017 SNP Distance to Human Isolate (n = 54)



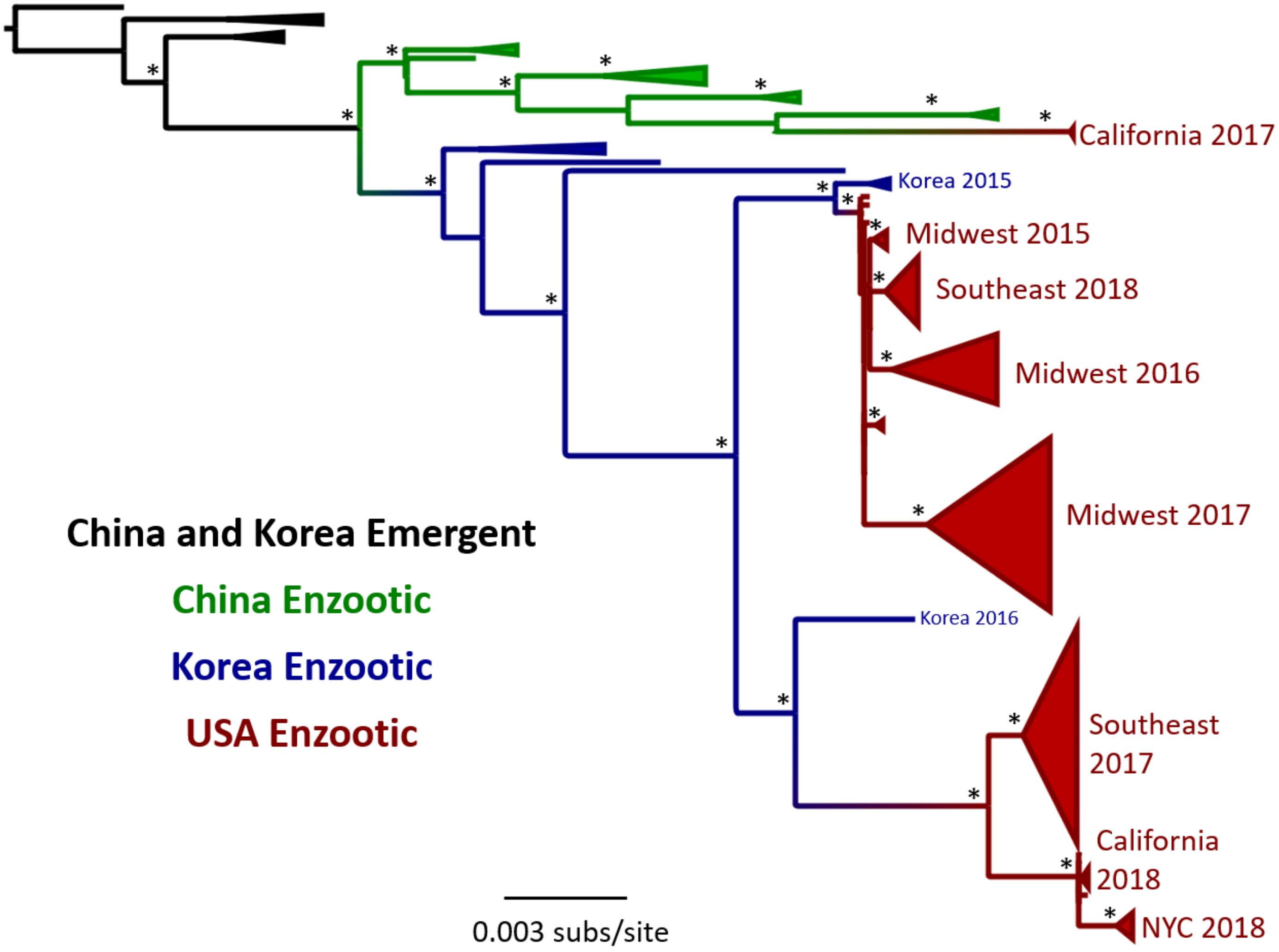

Distance to Human Isolate
Within 10 SNPs
Not Within 10 SNPs
Proportion
0.0
0.1
0.2
0.3
0.4
0.5
Bovine
Equine
Porcine
Chicken
Avian - Other
Canine
Feline
Llama
Mammal - Other
Pigeon
Reptile
Host Type

Managing recurring *Klebsiella* mastitis

- Dairy herd of 940 cows
- Bulk milk SCC = 293,000 / mL
- Compared clinical, fecal, and environmental isolates to assess shedding potential



Monitoring new introductions of canine influenza to the USA

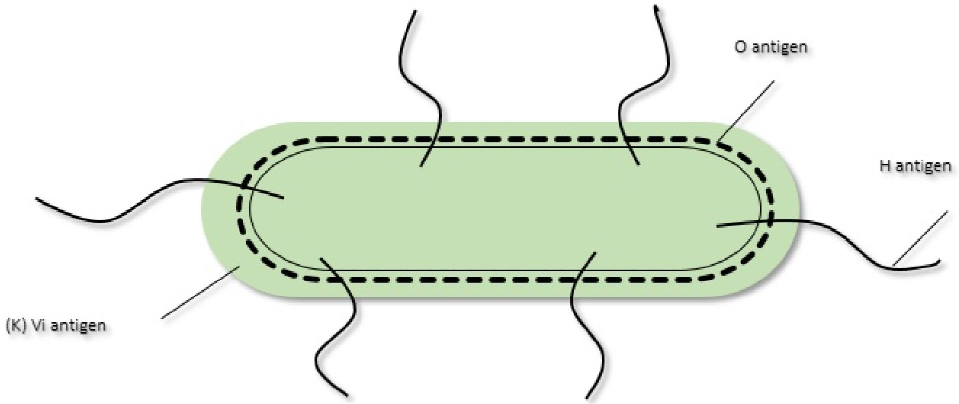


Collaboration with Ian Voorhees and Colin Parish

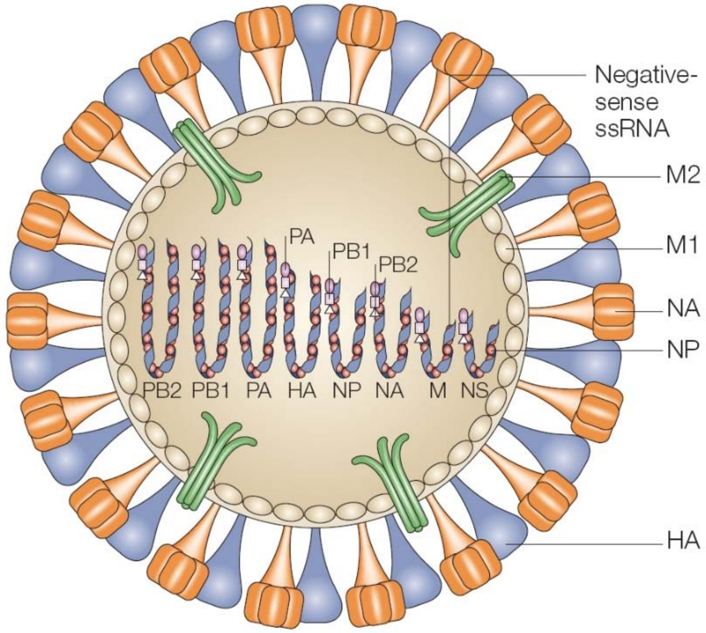
3. Pathotyping and species ID

Clinical Goal: differentiate normal host bacteria from those of clinical significance

Serotype prediction in bacteria and viruses



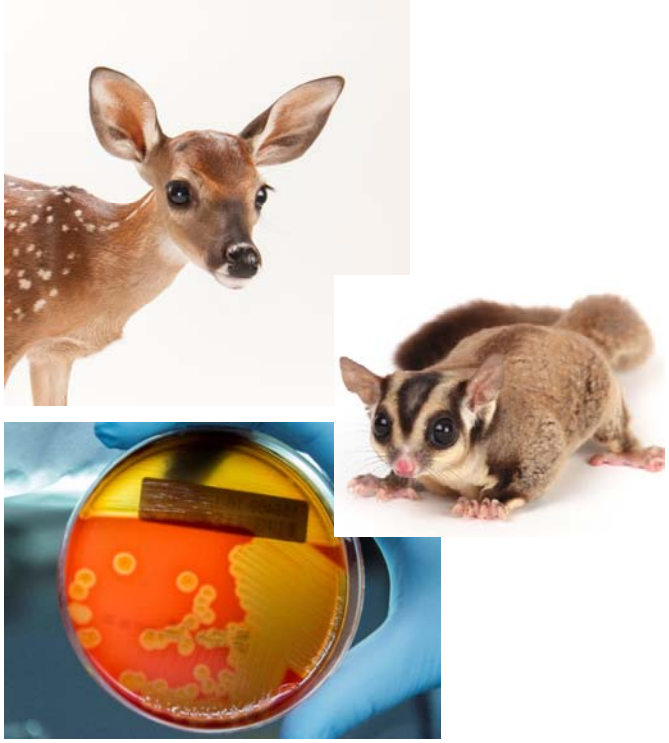
Nmhealth.org



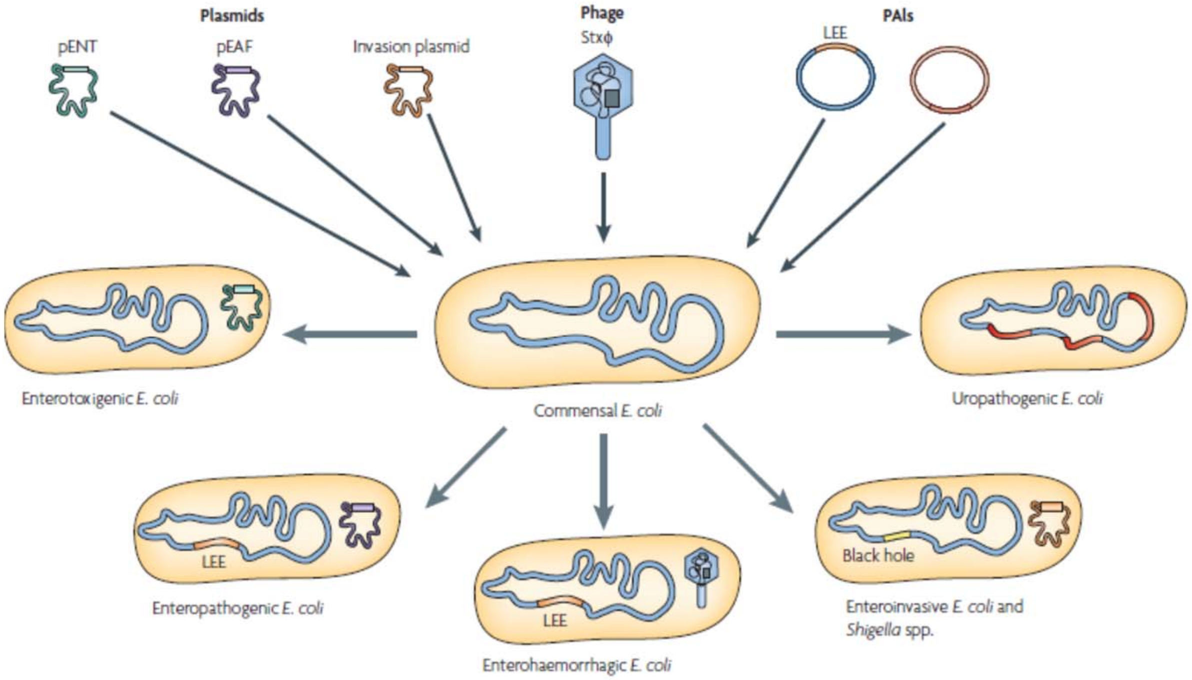
Nature Reviews Microbiology

More precise speciation

- Many bacteria are not well identified by 16S alone (or by phenotypic characteristics of conventional microbiology)
  - Define/predict which biochemical tests are appropriate
- Many viral isolates go unspeciated due to lack of specific tests
  - Complement other tests such as EM, IFA, PCR, cytotoxicity, chloroform resistance



Bacterial toxin gene detection



Nature Reviews Microbiology

Informed wildlife management

- Food production
- Water resources

Image from Kurt Bauschardt, citypages.com

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4. Antimicrobial resistance

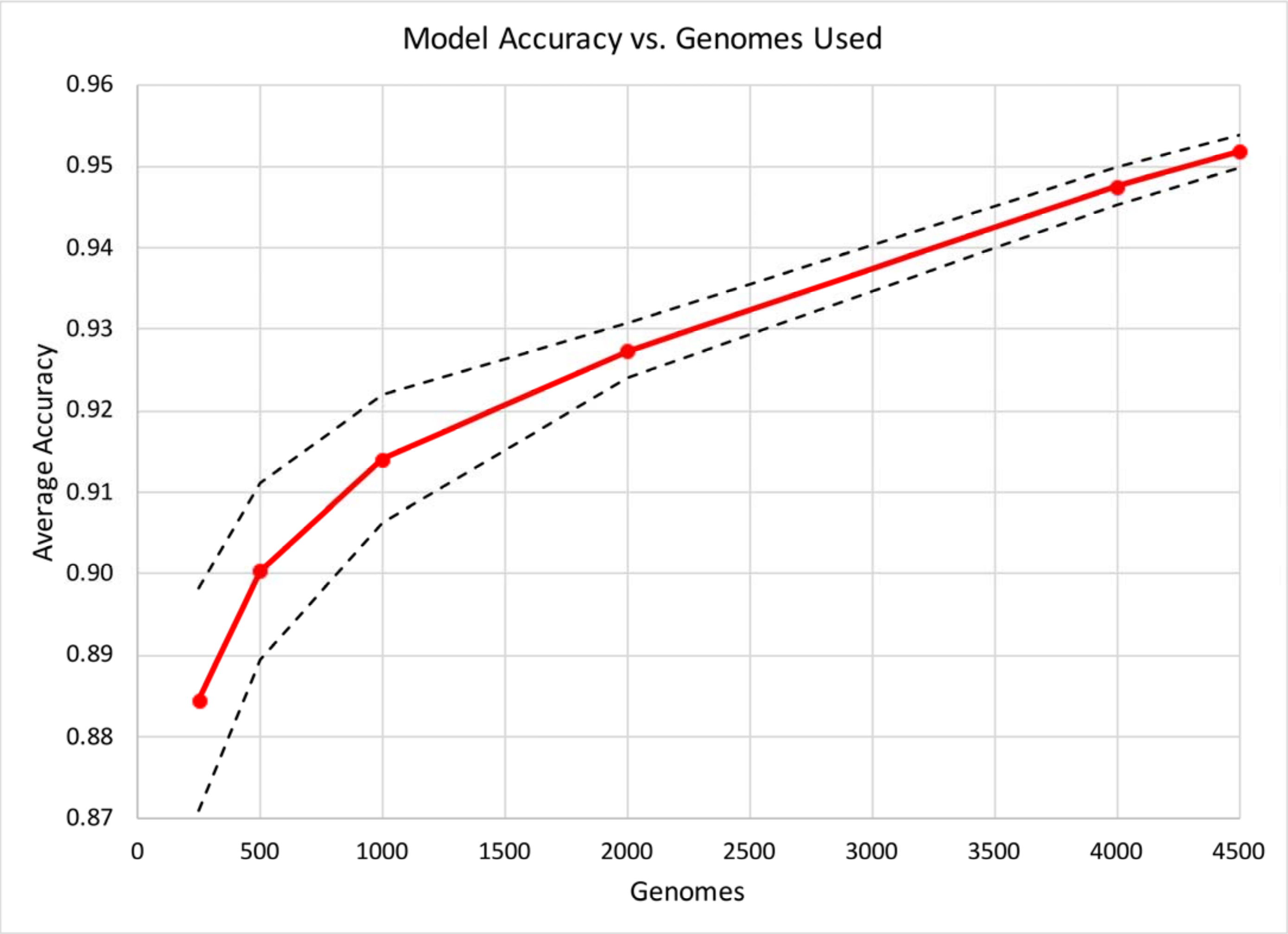
Clinical Goals: detect markers that are not covered by species-specific AST panels, make antibiograms to support judicious use initiatives

WGS AMR predictions by gene have good correlation with sensitivity results

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

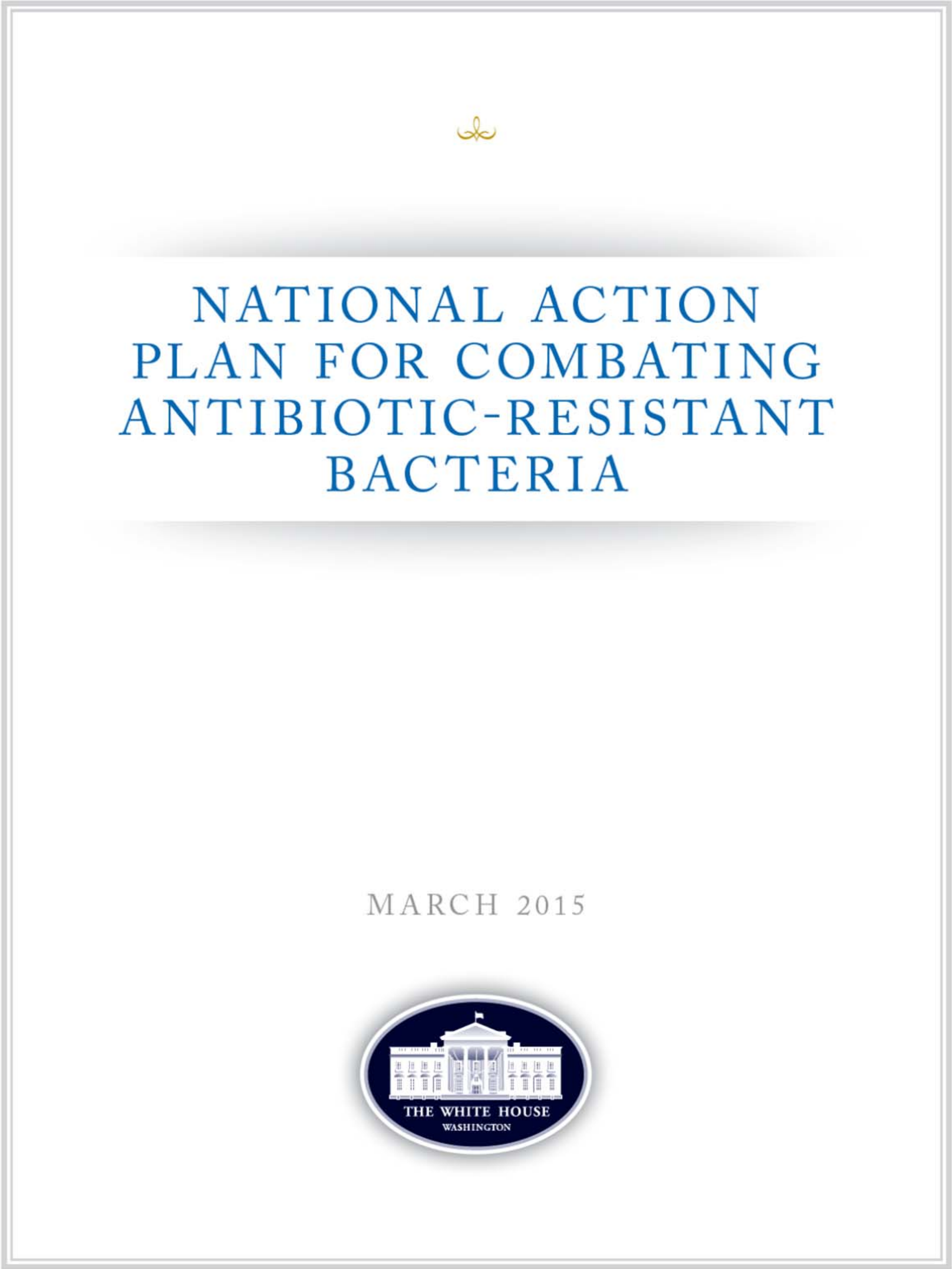
Publications from NARMS

MICs can now be predicted for some bacteria



Nguyen et al., BioRxiv 2018 (Davis Lab)

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

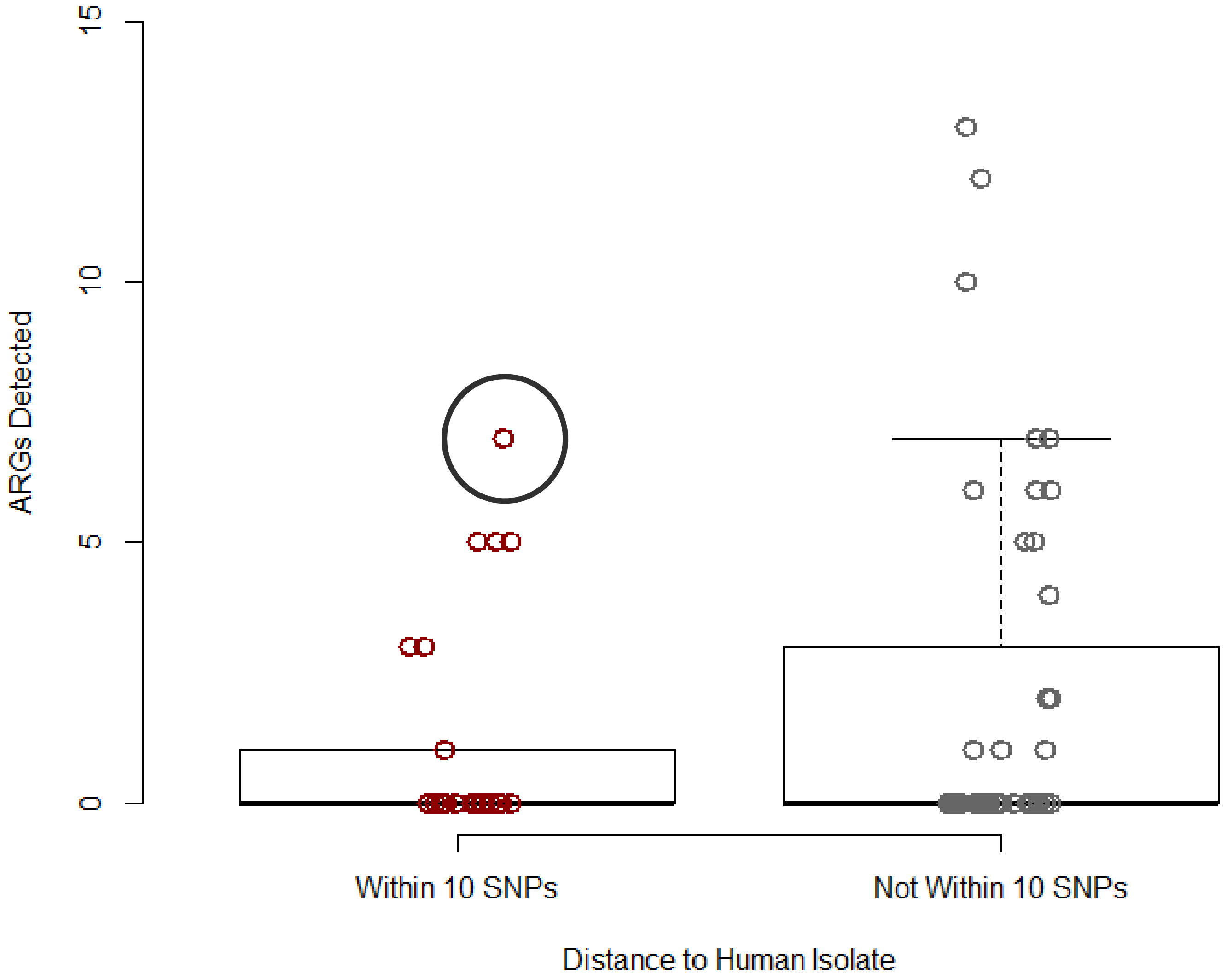


NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

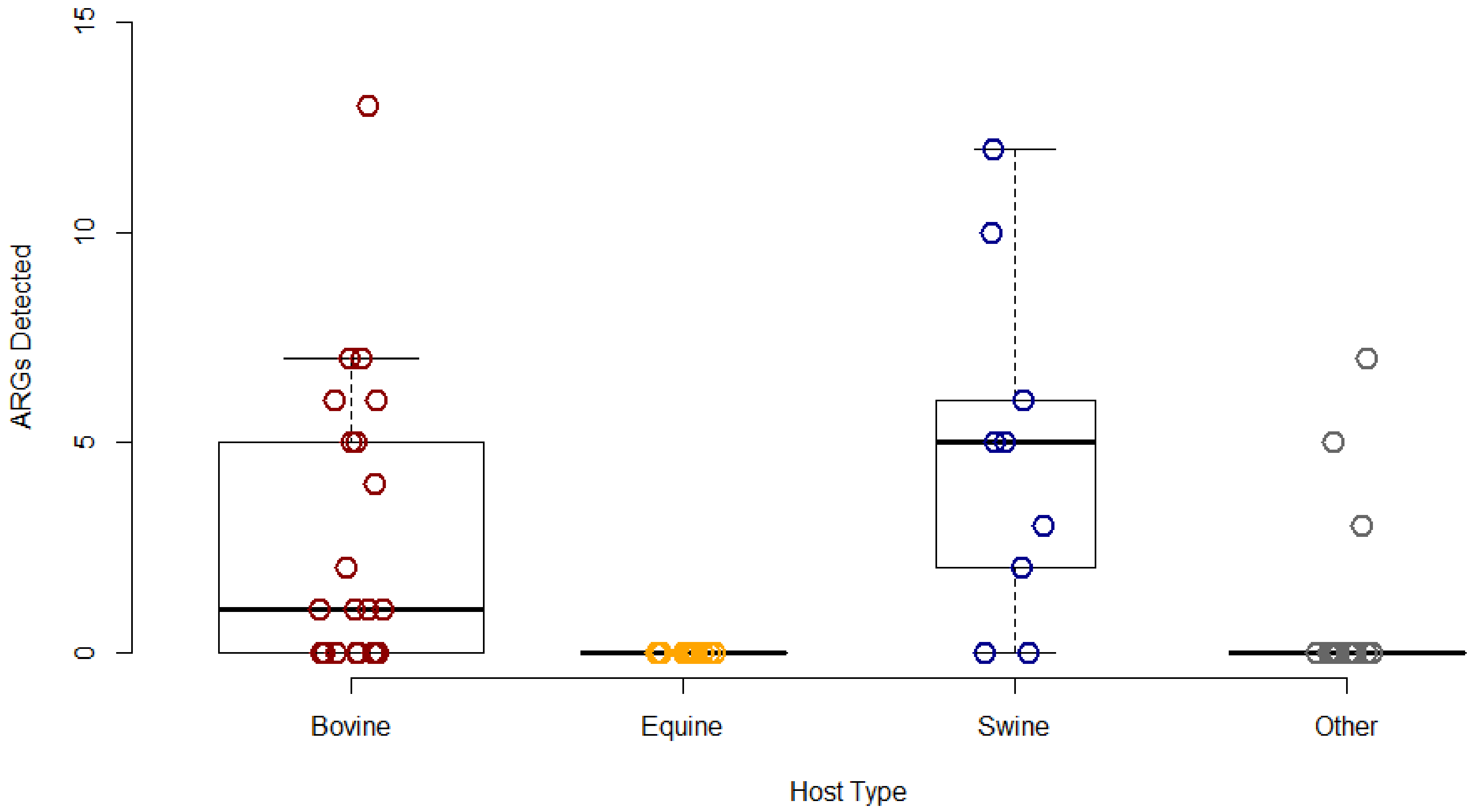
MARCH 2015

THE WHITE HOUSE
WASHINGTON

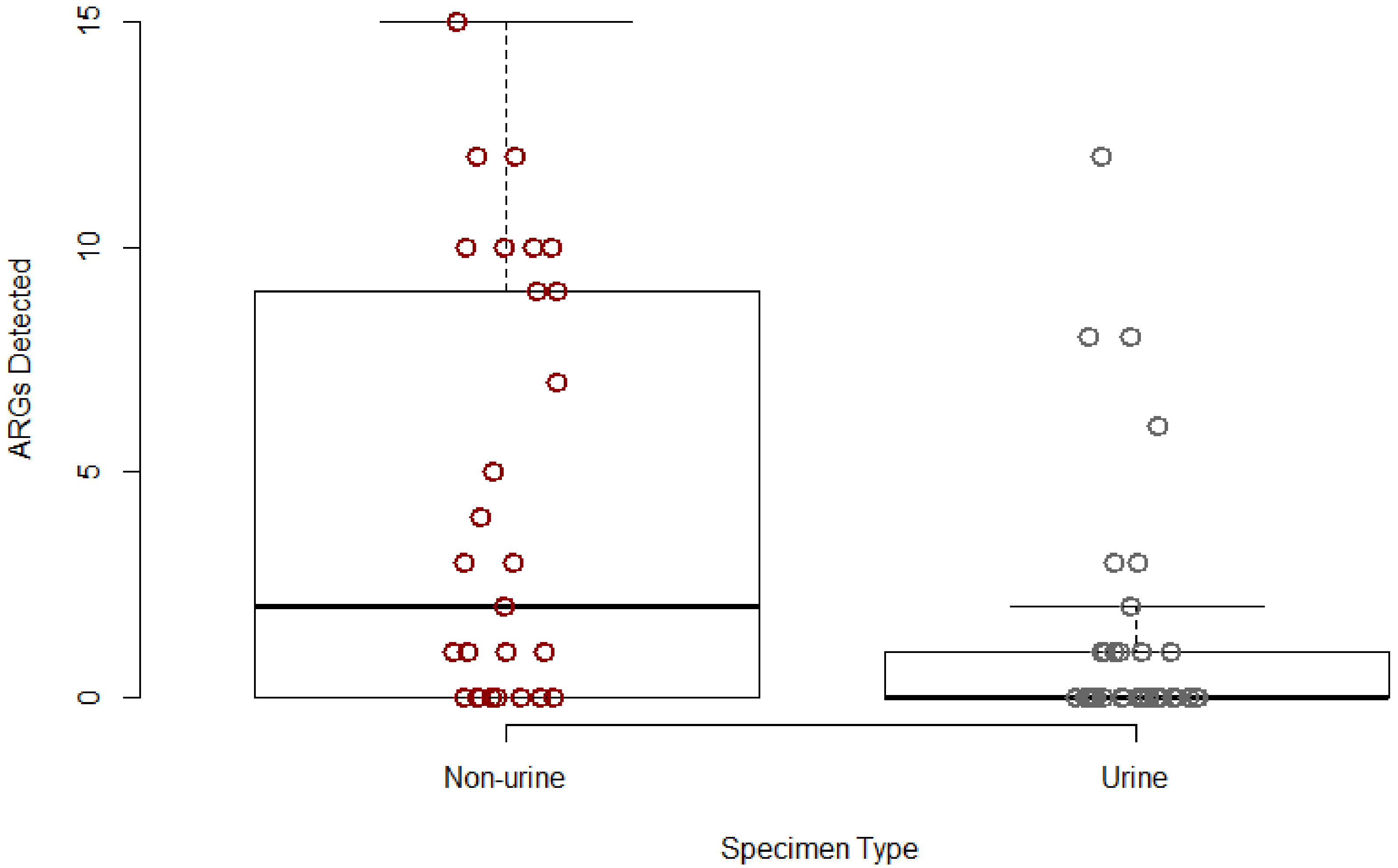
Distribution of Antibiotic Resistance Genes (ARGs) in *Salmonella* by Human Distance



Distribution of *Salmonella* ARGs by Host Type



Canine *E. Coli* ARGs by Specimen Type



Most extreme case – from 2017

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, cephlosporins)
blaTEM-1 (penicillins)
catA1 (phenicols)
dfrA14 (trimethoprim)
mph(A) (macrolides)
qacL (disinfectants)
sul2, sul 3 (sulfonamides)
tet(B) (tetracycline)
gyrA mutations (fluoroquinolones)

In collaboration with FDA Vet-LIRN, Unpublished

One Health AMR Data Sharing

- Meeting sponsored by NY Integrated Food Safety Center of Excellence held May 2018 with stakeholders from different sectors
- Surveillance from WGS AMR data is more rapidly available than traditional methods
  - NARMS monitors NCBI for emerging resistance threats
  - Also publically accessible to researchers and other agencies
- Importance of data integrity, security, and confidentiality
  - NAHLN study emphasizing secure messaging
- A tiered system with a 3rd party protector of identifiable information proposed as safeguard for confidentiality

Take-home points

1. WGS supports preventative controls in herd management

2. Open data is critical for disease surveillance

Acknowledgments

AHDC/NYSVDL Molecular Diagnostics - R&D Team

Laura Goodman
Patrick Mitchell
Renee Anderson
Brittany Chilson
Derek Rothenheber
Ava Jarvis

Cornell University
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NY Integrated Food Safety Center of Excellence

- Martin Wiedmann
- Renato Orsi
- Gen Meredith
- Andie Newman – NYSDOH
- Megin Nichols – CDC NCEZID

FDA Vet-LIRN

- Olga Ceric
- Sarah Nemser
- Renate Reimschuessel

USDA NAHLN

- Beth Harris

Cornell AHDC/NYSVDL

- Anil Thachil
- Rebecca Franklin-Guild
- Paolo Moroni
- Gloria Gioia
- François Elvinger

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