The Role of WGS in Monitoring Antimicrobial Resistance in Foodborne Bacteria

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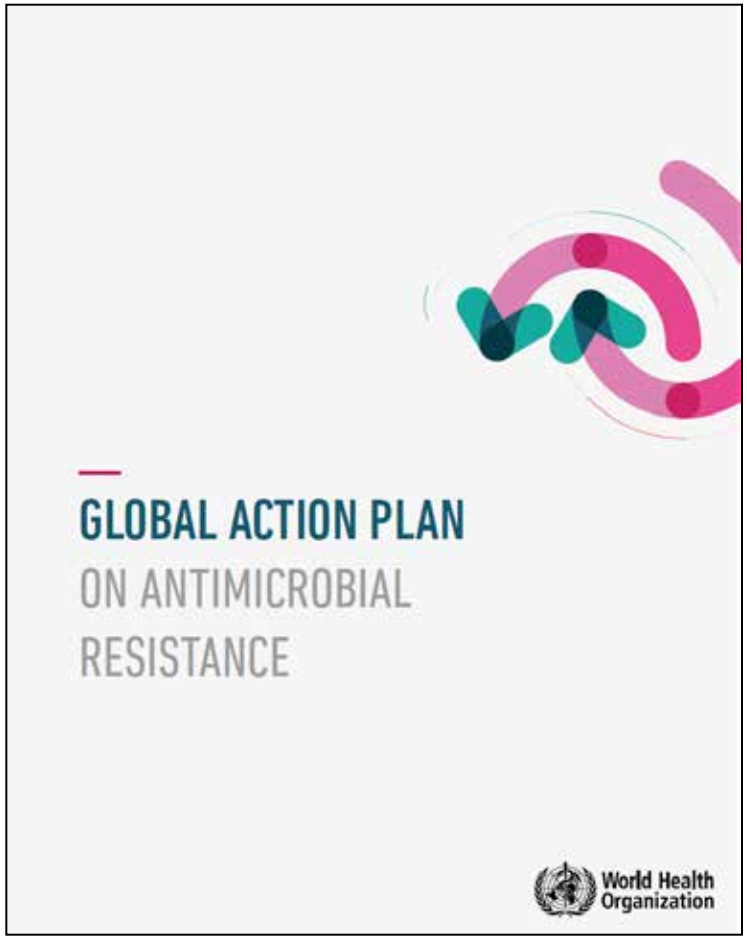
Antibiotic resistance as a global one health challenge

- The development of resistance is linked to how often antibiotics are used.
- Because many antibiotics belong to the same class of medicines, resistance to one specific antibiotic agent can lead to resistance to a whole related class.
- Resistance that develops in one organism or location can also spread rapidly and unpredictably … and can affect antibiotic treatment of a wide range of infections and diseases.
- Drug-resistant bacteria can circulate in populations of human beings and animals, through food, water and the environment, and transmission is influenced by trade, travel and both human and animal migration.
- Resistant bacteria can be found in food animals and food products destined for human consumption.

Antibiotics are societal drugs – S.B. Levy



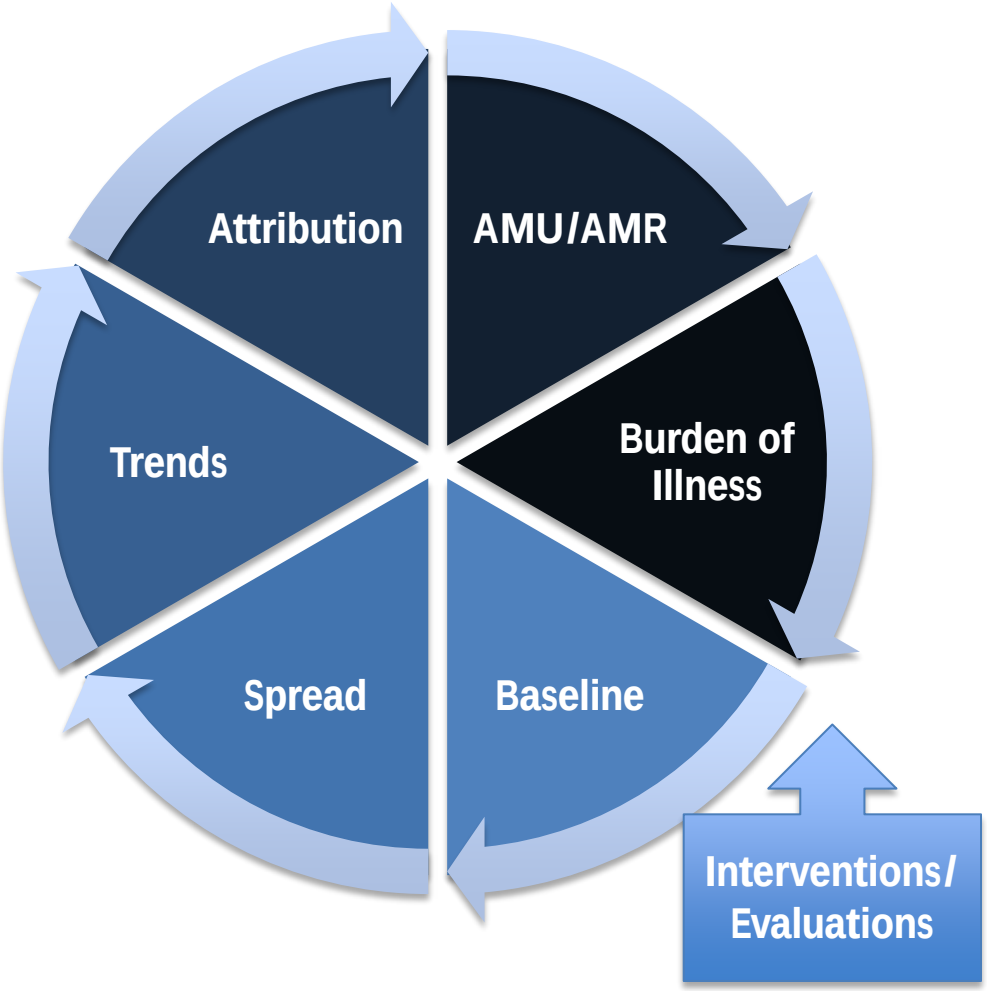
What is integrated surveillance of antimicrobial resistance in foodborne bacteria?

The coordinated sampling and testing of bacteria from food animals, foods, (the environment) and clinically ill humans; and the subsequent evaluation of antimicrobial resistance trends throughout the food production and supply chain using harmonized methods.

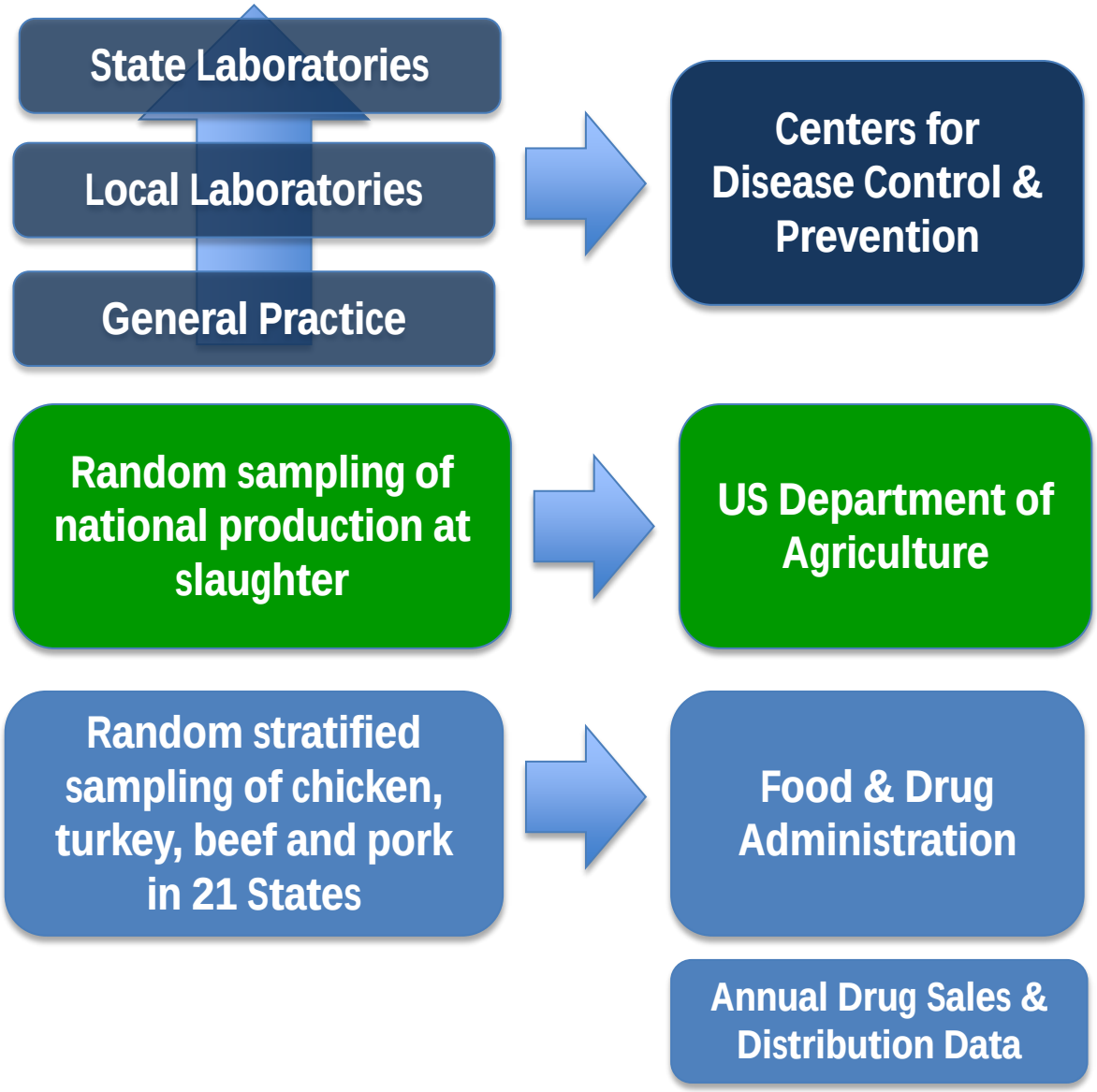
Source: WHO-AGISAR report

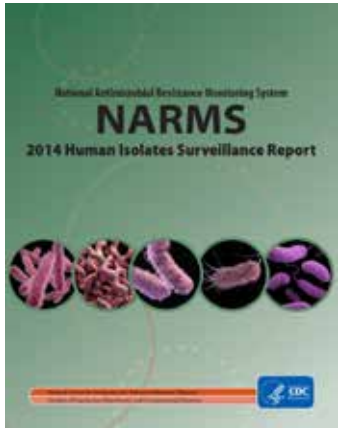
The Purpose of NARMS

1. Monitor resistance trends
2. Disseminate timely information
3. Conduct research
4. Prioritize outbreaks
5. Assist the FDA in making regulatory decisions



NARMS Structure

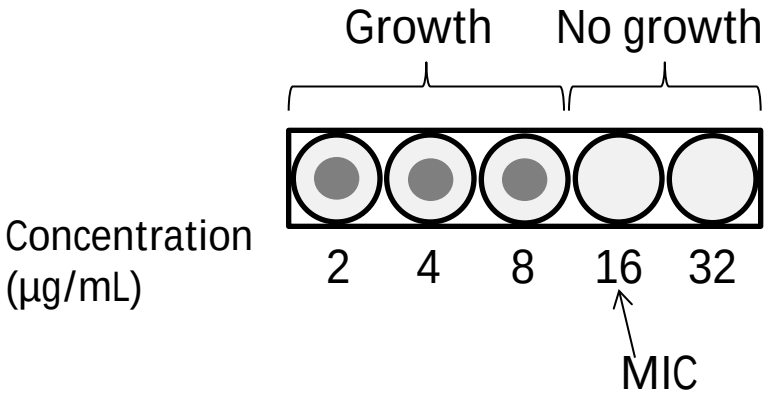




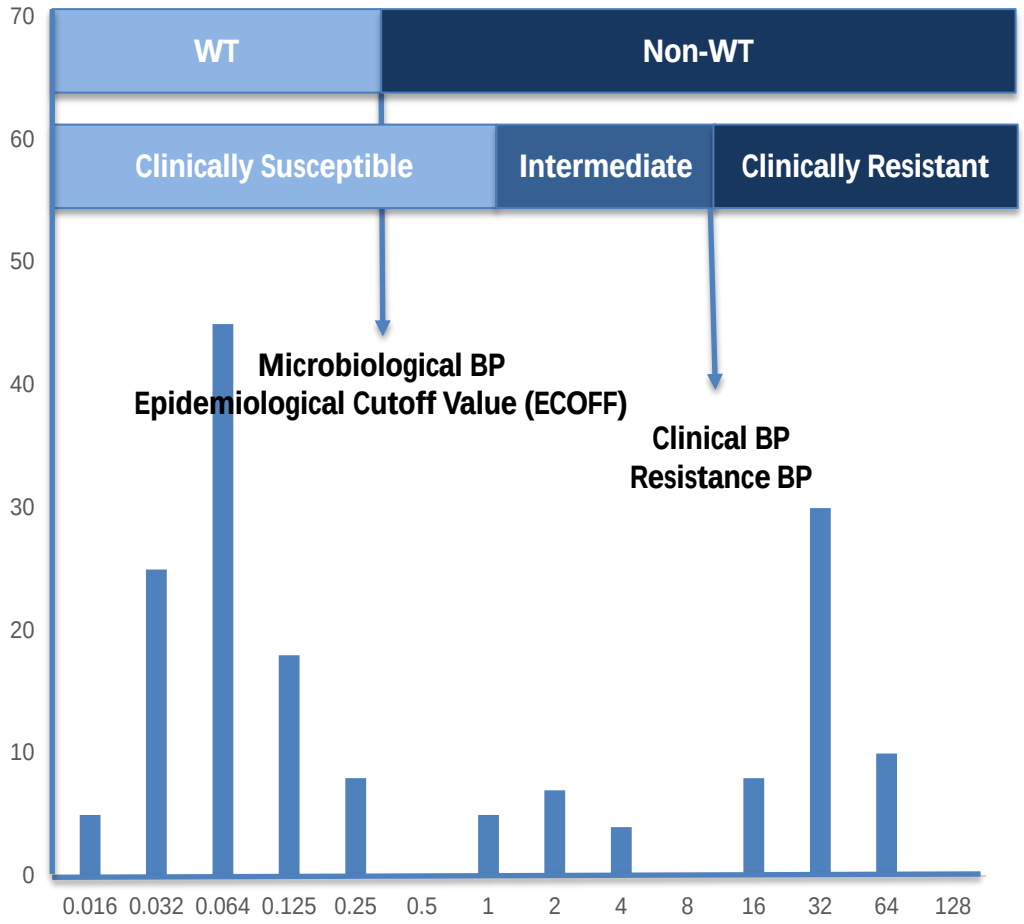
Antimicrobial Susceptibility Testing

- **Antimicrobial susceptibility testing by broth microdilution on 96-well plates**
  - Uses standard methods of the Clinical and Laboratory Standards Institute (CLSI)

- **Susceptibility testing involves detecting minimum inhibitory concentrations (MIC)**
  - High numbers indicate resistance (µg/L)

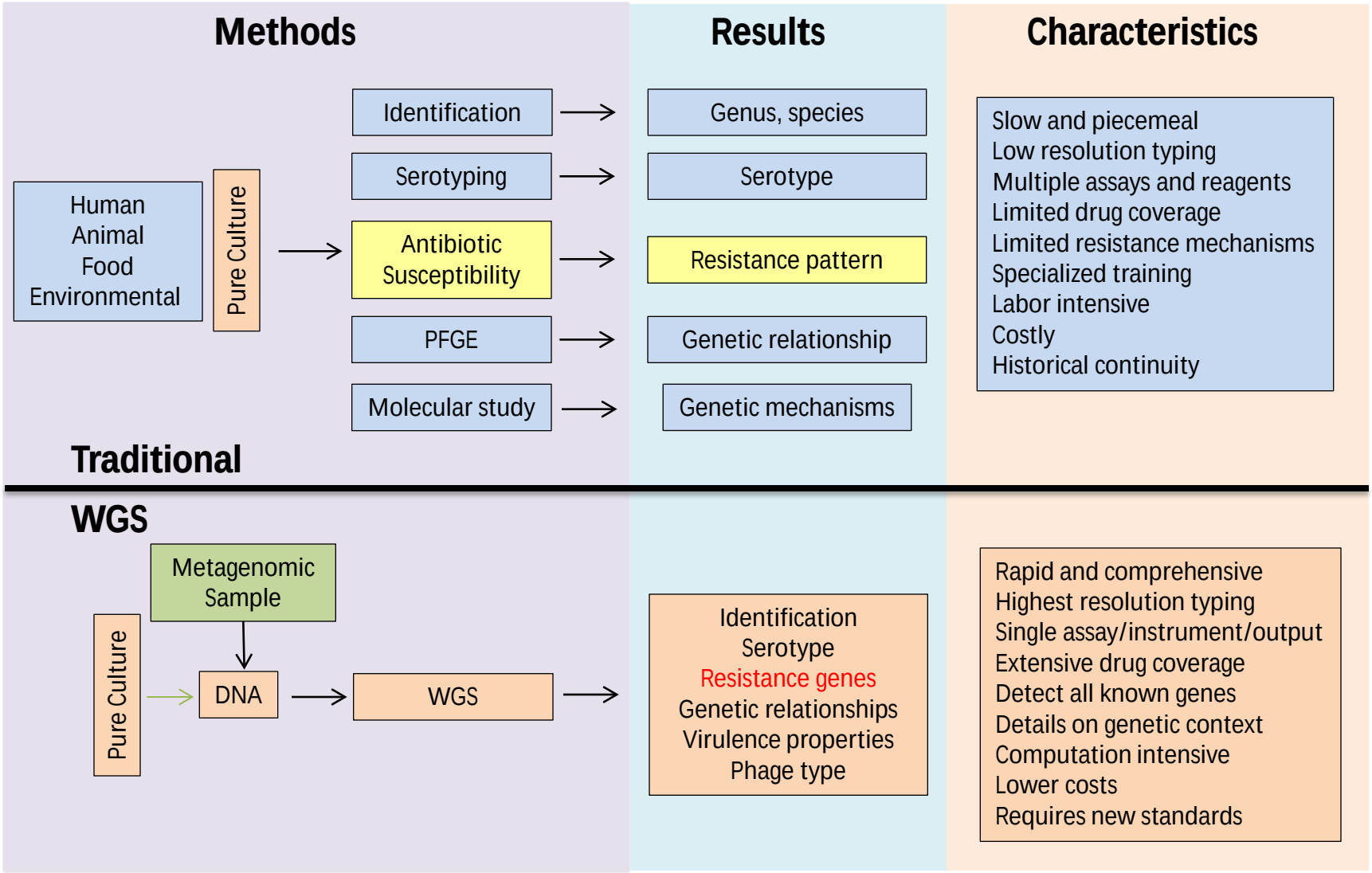


Breakpoints – The Art and Science of Drawing a Line Somewhere



- *"Resistant strains are not inhibited by the usually achievable systemic concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely, and clinical efficacy has not been reliable in treatment studies".* -CLSI

Predicting Resistance: From Genotype to Phenotype



NARMS Genotype-Phenotype Correlations

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

CrossMark

Whole-Genome Sequencing for Detecting Antimicrobial Resistance in Nontyphoidal *Salmonella*

Patrick F. McDermott,[a] Gregory H. Tyson,[a] Claudine Kabera,[a] Yuansha Chen,[a] Cong Li,[a] Jason P. Folster,[b] Sherry L. Ayers,[a] Claudia Lam,[a] Heather P. Tate,[a] Shaohua Zhao[a]

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Whole-Genome Sequencing Analysis Accurately Predicts Antimicrobial Resistance Phenotypes in *Campylobacter* spp.

S. Zhao,[a] G. H. Tyson,[a] Y. Chen,[a] C. Li,[a] S. Mukherjee,[a] S. Young,[a] C. Lam,[a] J. P. Folster,[b] J. M. Whichard,[b] P. F. McDermott[a]

J Antimicrob Chemother 2015; **70**: 2763–2769
doi:10.1093/jac/dkv186 Advance Access publication 3 July 2015

Journal of Antimicrobial Chemotherapy

WGS accurately predicts antimicrobial resistance in *Escherichia coli*

Gregory H. Tyson[1], Patrick F. McDermott[1], Cong Li[1], Yuansha Chen[1], Daniel A. Tadesse[1], Sampa Mukherjee[1], Sonya Bodeis-Jones[1], Claudine Kabera[1], Stuart A. Gaines[1], Guy H. Loneragan[2], Tom S. Edrington[3], Mary Torrence[4], Dayna M. Harhay[5] and Shaohua Zhao[1]*

High consistency between antimicrobial resistance genotypes and phenotypes in NARMS isolates

- **Michael Feldgarden***, Vyacheslav Brover*, Uddai Desay•, Jason P. Folster§, Daniel H. Haft*, Jovita Haro•, William Klimke*, Andre McCullogh§, Patrick McDermott†, Cesar Morales, Arjun B. Prasad*, Mustafa Simmons•, Glenn Tillman•, Gregory Tyson†, Jamie Wasilenko•, Shaohua Zhao†
- *National Center for Biotechnology Information/NLM/NIH; †Center For Veterinary Medicine/FDA; §National Center for Emerging and Zoonotic Infectious Diseases/OID/CDC; •Food Safety and Inspection Service/USDA

A total of 6,008 NARMS *Salmonella enterica*, *Campylobacter* spp., and *E. coli* collected from animals, food, and humans between 2002 – 2015 were tested, for which we have both genomic and phenotypic data.

- A total of 81,431 susceptibility tests were performed, 97% (79,345) were consistent with the resistance genotypes.
- 2,109 isolates (35%) were pan-susceptible.

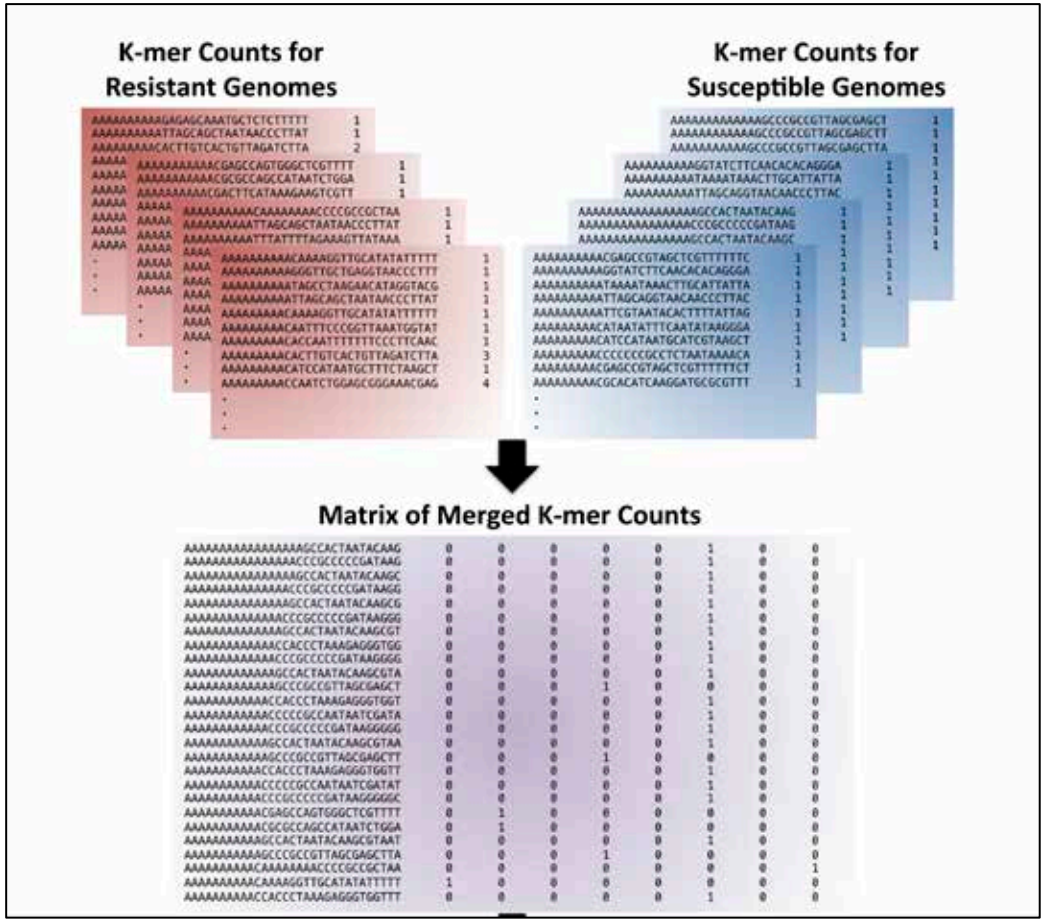
- *Campylobacter*, 93% (6,930) of susceptibility tests were consistent with the resistance genotype
- *S. enterica* 97% (76,821) were consistent with the resistance genotype.
- *E. coli*, 99% (561) of susceptibility tests were consistent with the resistance genotype.

- 7.6% (967) of positive tests for resistance were from isolates that did not have a known acquired AMR gene, representing 48% of total inconsistencies.
- Only 1.4% (1,029) of sensitive isolates possessed a resistance gene that should have conferred resistance.

Machine Learning-Based MIC Prediction:

Collaboration of NARMS and Argonne National Laboratories

- Find non redundant k-mers
- Use counts or presence vs. absence
- Merge to form a matrix
- Based on ± 1 two-fold dilution
- Use an ML method to make the prediction (Current method, AdaBoost)
- Used 4,700 NARMS isolates with phenotype/genotype data



Predicting MICs in *Salmonella*

	Accuracy	95% CI	Samples
Ampicillin	0.97	[0.96-0.97]	4501
Amoxy-Clav	0.97	[0.97-0.98]	4502
Ceftriaxone	0.97	[0.97-0.98]	4502
Azithromycin	0.99	[0.99-1.00]	1641
Chloramphenicol	0.99	[0.99-0.99]	4502
Ciprofloxacin	0.98	[0.98-0.99]	4502
Trimethoprim-Sulfa	0.99	[0.99-0.99]	4501
Sulfisoxazole	0.96	[0.95-0.97]	4154
Cefoxitin	0.96	[0.95-0.97]	4502
Gentamicin	0.93	[0.92-0.94]	4502
Kanamycin	0.98	[0.97-0.99]	924
Nalidixic Acid	0.98	[0.97-0.98]	4502
Streptomycin	0.91	[0.91-0.92]	4502
Tetracycline	0.98	[0.98-0.98]	4502
Ceftiofur	0.99	[0.99-0.99]	4502
Total	0.97	[0.97-0.97]	60741

FDA Announces Implementation of GFI #213, Outlines Continuing Efforts to Address Antimicrobial Resistance

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Update (2/17/17): CVM has completed its audit of affected applications and revised the final number of new animal drug applications that have withdrawn production indications from 22 to 31.

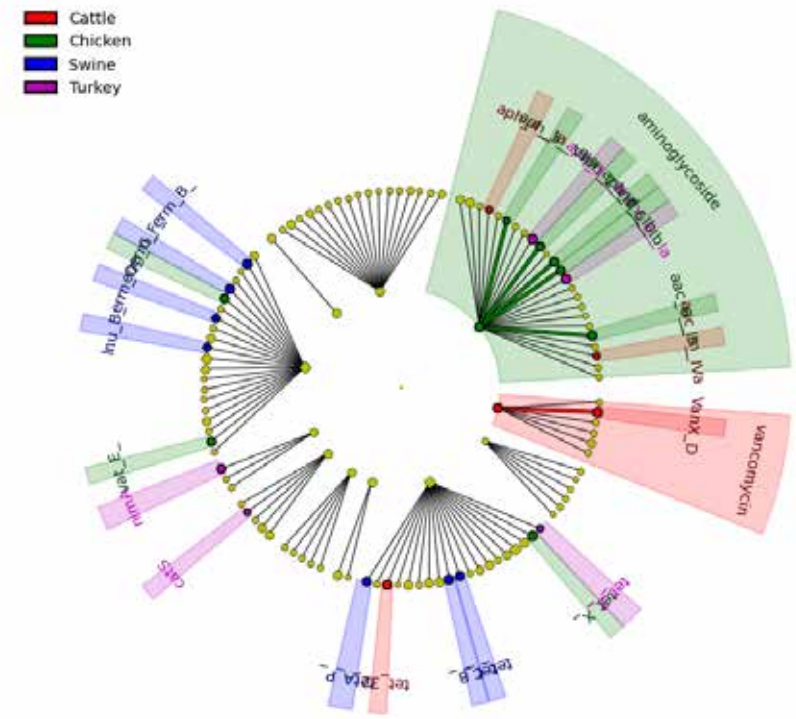
January 3, 2017

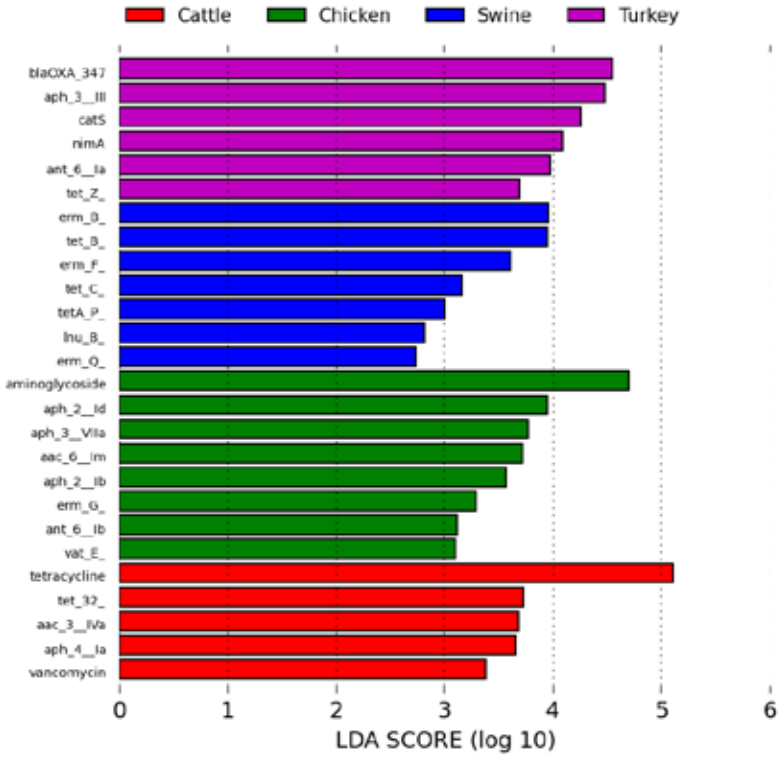
Today, the U.S. Food and Drug Administration announced that it has completed the implementation of Guidance for Industry #213, a process begun in 2013 to transition antimicrobial drugs with importance in human medicine (medically important antimicrobials) that are used in the feed or drinking water of food-producing animals to veterinary oversight and eliminate the use of these products in animals for production (e.g., growth promotion) purposes.

7 Classes Affected

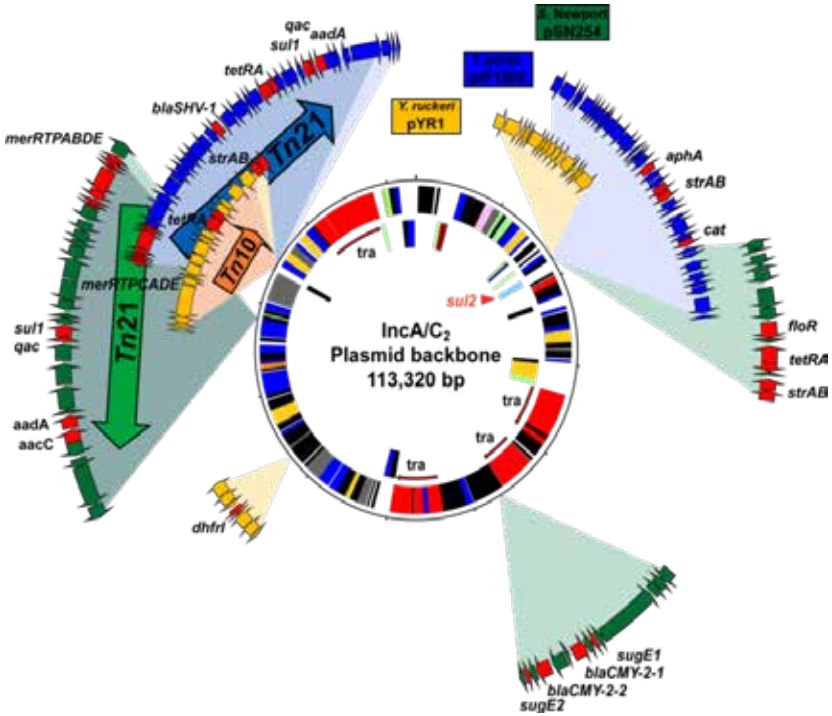
1. Aminoglycosides (streptomycin, spectinomycin)
2. Lincosamides (lincomycin)
3. Macrolides (tylosin, erythromycin)
4. Penicillins (penicillin G procaine)
5. Streptogramins (virginiamycin)
6. Sulfonamides (sulfamethazine)
7. Tetracyclines (chlortetracycline, oxytetracycline)

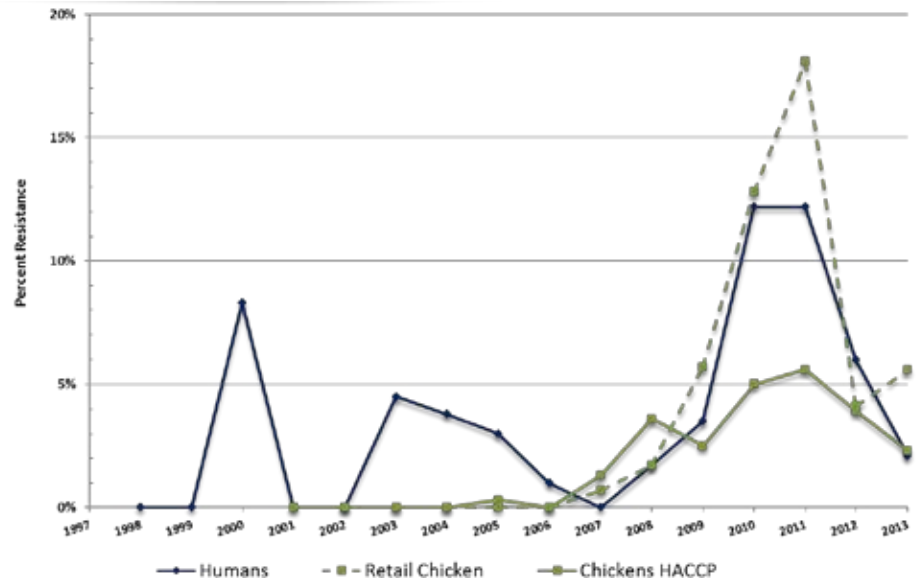
Metagenomic Surveillance
Resistance Genes by Animal Origin





WGS gives new answers to old questions





- Recent descent from a well-preserved plasmid backbone
- Resistance to compounds not tested
- Reveals new possible drivers of AMR

Welch TJ, et al. Multiple antimicrobial resistance in plague: An emerging public health risk. PLoS One. 2007 Mar 21;2(3):e309.

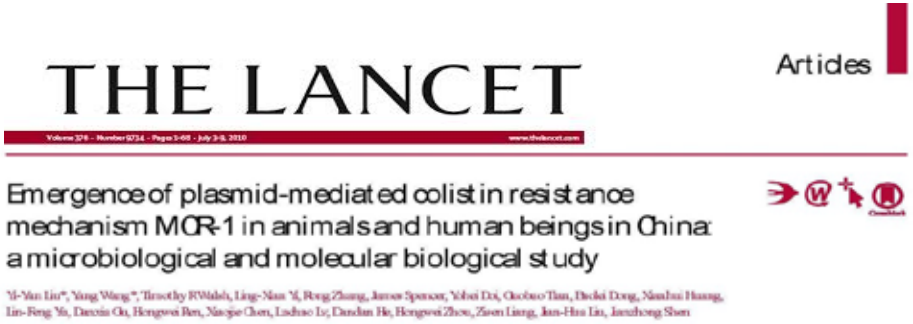
- Eight different Gen^R genes were found.
- Six for the first time in *Campylobacter*

aph(2")-If
aph(2")-I_n3
aph(2")-I_n4
aph(2")-Ib
aph(2")-Ig
aph(2")-Ic
aac(6")-Ie/aph(2")-Ia
aac(6")/aph(2")_n1

Zhao S, et al. Novel gentamicin resistance genes in Campylobacter from humans and retail meats in the USA. J Antimicrob Chemother. 2015 May;70(5):1314-21.

Plasmid-Mediated Colistin Resistance

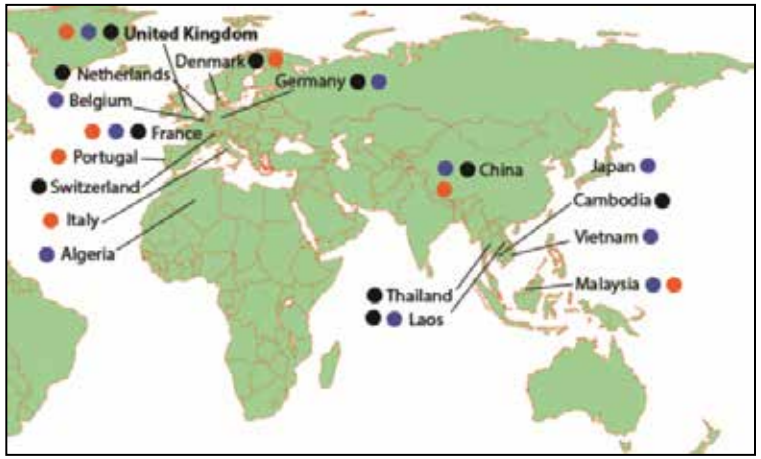
- Colistin is used as a last-resort drug to treat patients with multidrug-resistant infections, including CRE
- The *mcr-1 (*mobile colistin resistance*)* gene was the first plasmid-mediated resistance mechanisms discovered. First reported in China, November 2015
- Without opening a freezer door, we screened over 155,000 bacterial genomes, about 7,000 from NARMS, and none contained the gene
- By selective culture enrichment, our partners at USDA found *mcr-1* in *E. coli* isolates collected from the intestines of two pigs (out of 2,000 samples tested)
- Metagenomic analysis of blinded samples also detected the *mcr-1* gene.


THE LANCET

Articles

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

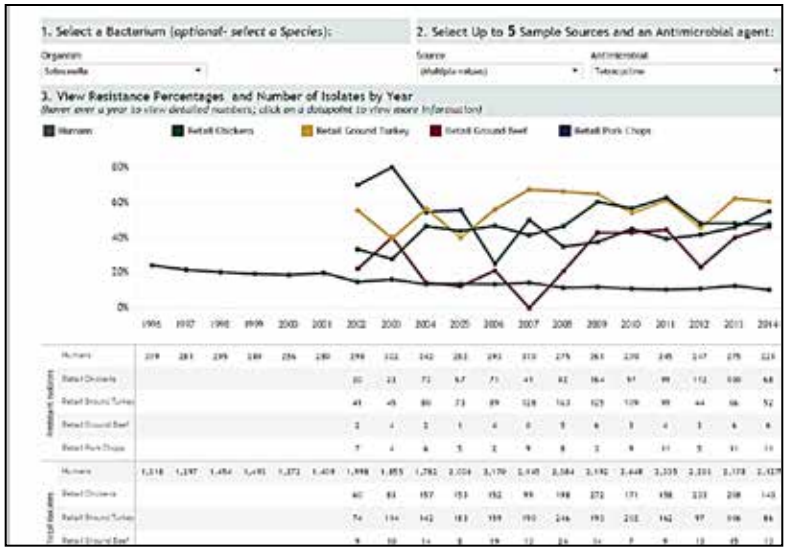
Bacteria that resist last-resort antibiotics were found in China two months ago, now they're everywhere

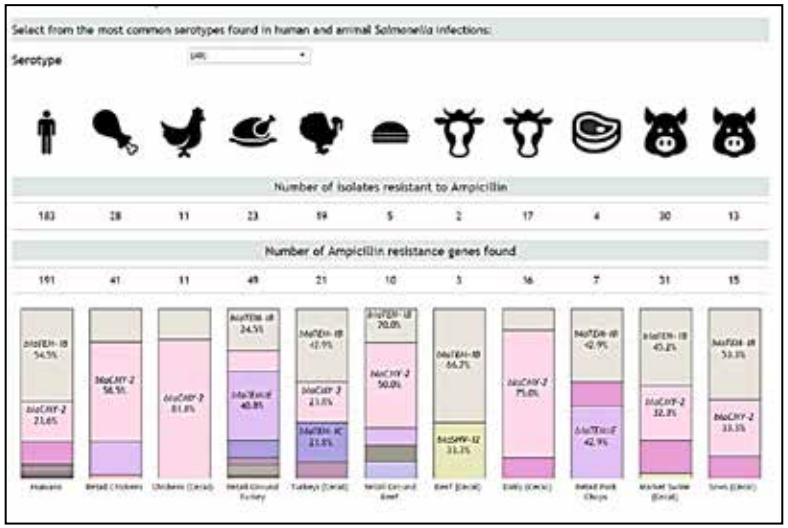


NARMS Resistomics

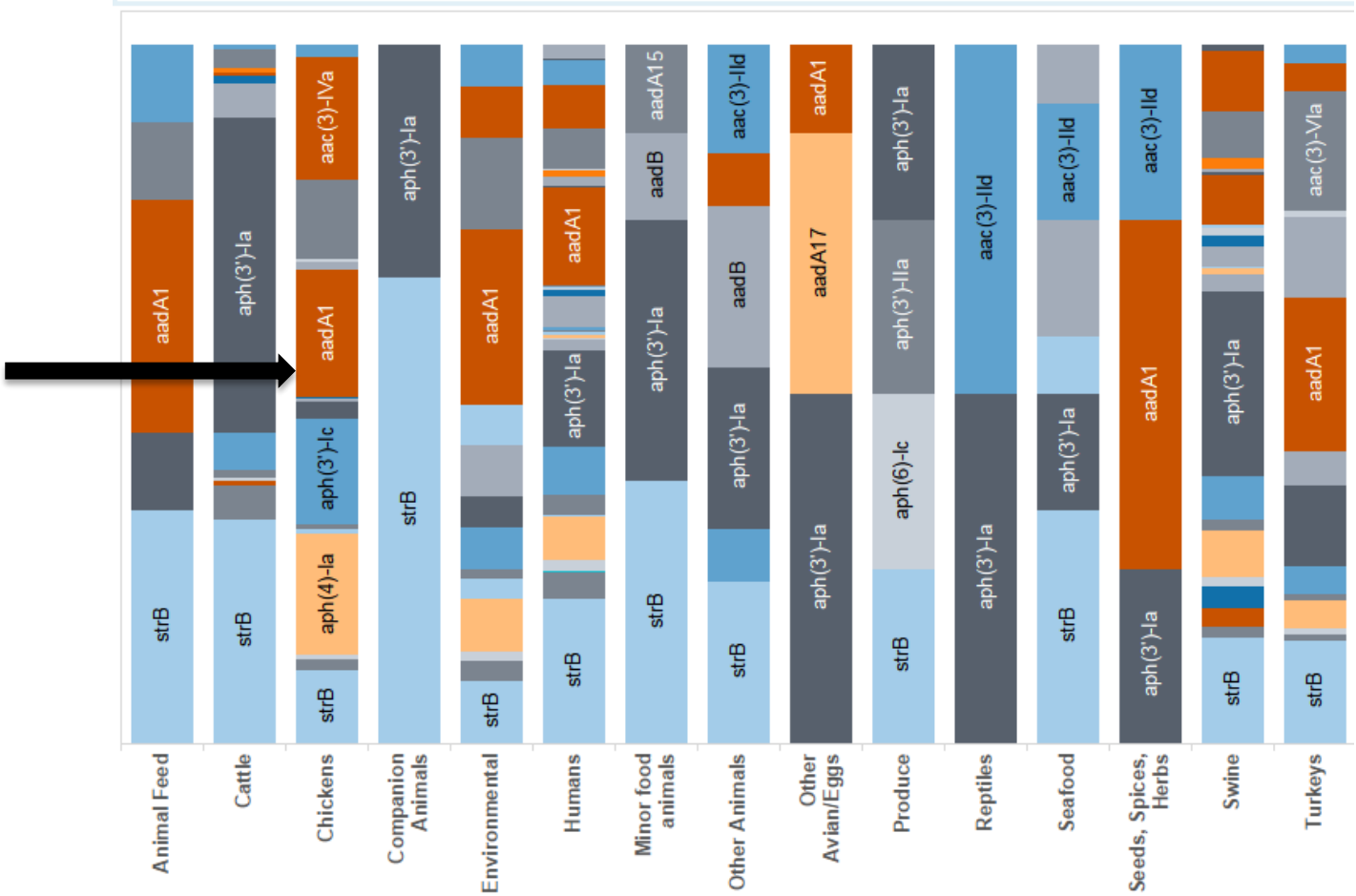
Download NARMS Isolate Level Data

- Human Clinical Cases
- Retail Meats
- Food Producing Animals
  - HACCP 1997-2005
  - HACCP 2006-2013
  - Cecal





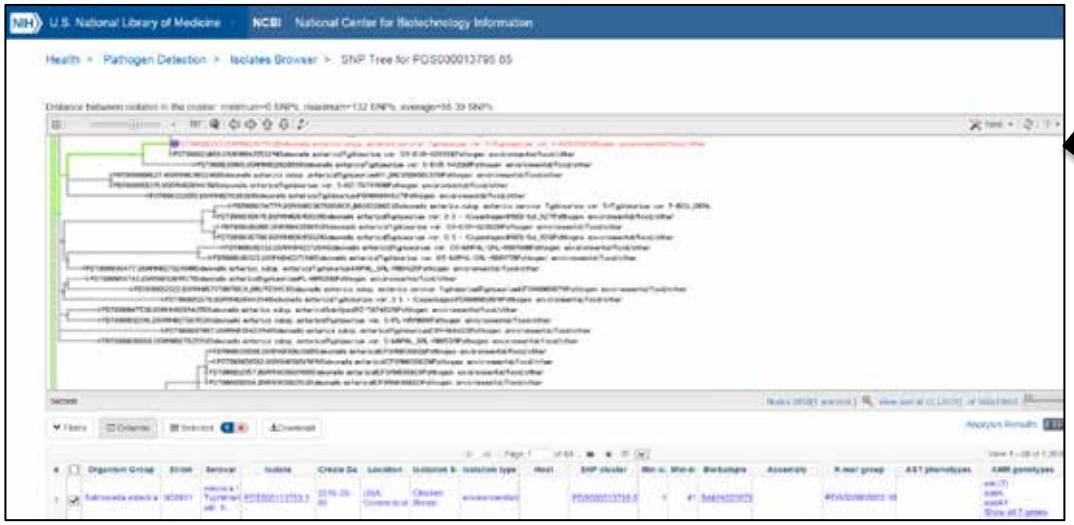
RESISTOME TRACKER
Salmonella



RESISTOME TRACKER
Salmonella

NARMS isolates are marked by green squares. ****Note: Some NARMS isolates do not have corresponding NARMS bioproject IDs and therefore are not marked with green squares. Biosamples may have more than one entry because there is more than one SRA number per Biosample.*

Bio Sample	Genotype	Geographic Location	Serovar	Sources	Year of NCBI Release Date	Click Buttons for Links to NCBI
SAMN02367912	aadA1 aac(3)-VIa sul1 sul2 tet(A)	USA: Connecticut	Typhimurium var. 5-	chicken breast	2016	Click Here
SAMN02367962	aac(3)-VIa strB aadA1 blaTEM-1B blaCMY-2 sul1 sul2 tet(A) tet(B)	USA: New Mexico	Litchfield	chicken breast	2016	Click Here
SAMN02368023	aac(3)-VIa aadA1 strB strA sul1 tet(C) tet(B)	USA: New Mexico	Kentucky	chicken wing	2016	Click Here
SAMN02699266	aadA1 dfrA15 sul1	USA: MN	Kentucky	chicken breast	2016	Click Here



RESISTOME TRACKER
Salmonella

What *Salmonella* resistance genes appear for the first time in the NCBI dataset? Note: list only goes back 6 months

Day of NCBI Release Date	Gene	Class	Geographic Location	Sources	Bio Sample	Click Buttons for Links to NCBI
10/5/2017	mcr5	Polymixin	Australia	feces	SAMN07739130	Click Here
9/30/2017	blaTEM-29	Beta-lactam	United Kingdom: ..	human	SAMN07719053	Click Here
9/29/2017	blaTEM-138	Beta-lactam	United Kingdom: ..	human	SAMN07717931	Click Here
9/28/2017	blaCTX-M-114	Beta-lactam	Unknown	Null	SAMN07718851	Click Here
	blaTEM-77	Beta-lactam	Unknown	Null	SAMN07718910	Click Here
9/27/2017	blaTEM-130	Beta-lactam	USA	missing	SAMN07687877	Click Here
	QnrB17-1	Quinolone	USA:MN	lung (porcine)	SAMN07420427	Click Here
9/26/2017	blaTEM-122	Beta-lactam	United Kingdom: ..	human	SAMN07695381	Click Here
	blaTEM-150	Beta-lactam	United Kingdom: ..	human	SAMN07695269	Click Here
	mcr2	Polymixin	Belgium	pork carcasses	SAMEA104175516	Click Here

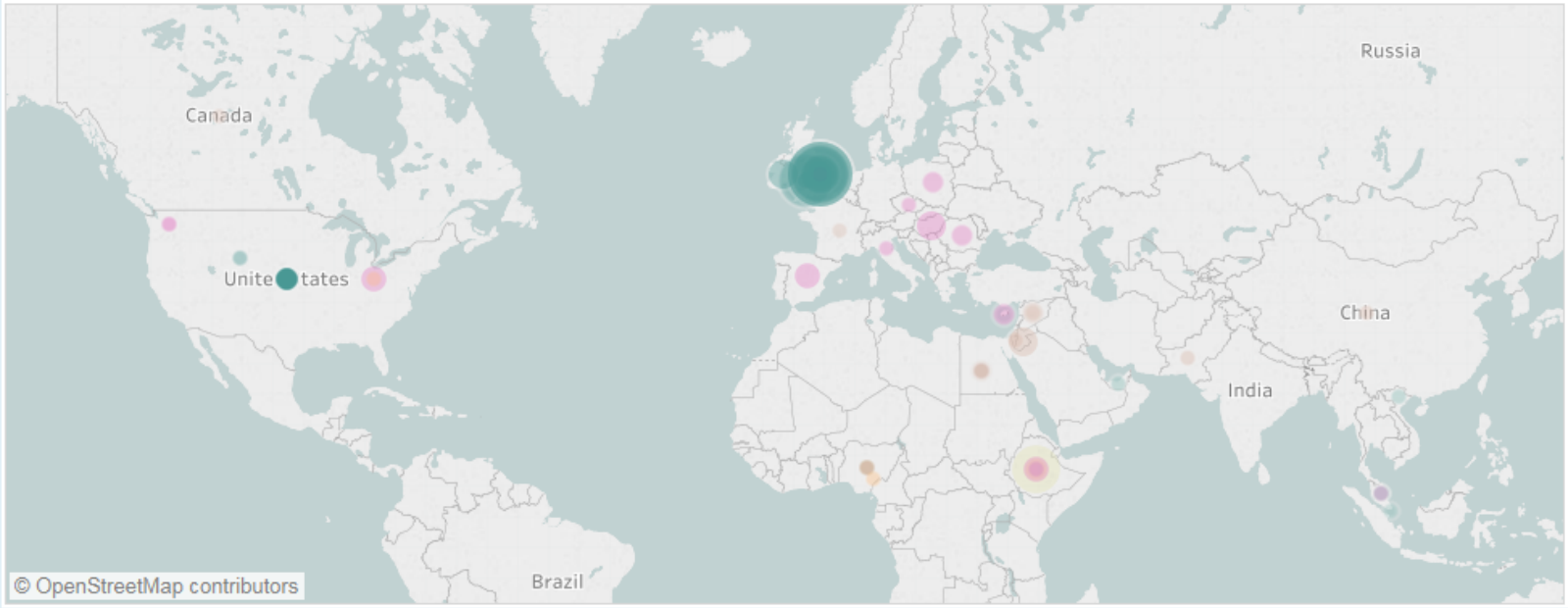
When was the first *Salmonella* isolate with the following resistance gene *collected*?

QnrA1

Gene	Class	Collection Years	Geographic Location	Source	Bio Sample	Click Buttons for Links to NCBI
QnrA1	Quinolone	2003	Malawi	blood	SAMEA1940373	Click Here

RESISTOME TRACKER
Salmonella

Global Distribution of Isolates With aac(3)-Id Genes by Collection Year- 2017



Gene

aac(3)-Id

Sources

- Cattle
- Chickens
- Environmental
- Humans
- Other/Unknown
- Produce
- Reptiles
- Seafood
- Seeds, Spices, Herbs
- Swine

Collection Year

2017

Show history

One Health-One Method

- Easy to lean and use
- Overcomes the limitations of classic microbiology
  - Specialized lab processes (WGS)
  - Need to rely on cultivatable organisms (MGS)
- Permits looking farther with fewer resources and lower costs
  - Sensitivity - detect emerging hazards equal in sensitivity to selective enrichment
  - Reanalysis - retrospective surveillance
  - Discovery
  - One Health and beyond: domestic and wild animal populations, environmental, pets, vectors and transmission pathways.
- Reveals new associations including determinants of zoonotic transmissibility
  - New possibilities to attribute pathogens/resistances to source, more targeted response
  - Richer backdrop of data to evaluate environmental and ecological drivers
- Greater confidence in decision making
- Universality. Ease of sharing and comparing, but need international harmonization and cooperation

Acknowledgements

- FDA (CVM, CFSAN, ORA)
- CDC (NCZEID)
- USDA (FSIS, ARS, APHIS)
- NIH/NCBI
- ANL/Univ Chicago
- State Public Health Labs, Universities

This communication is consistent with 21 CFR 10.85 (k) and constitutes an informal communication that represents my best judgment at this time but does not constitute an advisory opinion, does not necessarily represent the formal position of FDA, and does not bind or otherwise obligate or commit the agency to the views expressed.

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