Antimicrobial Resistance Surveillance Using Genomics

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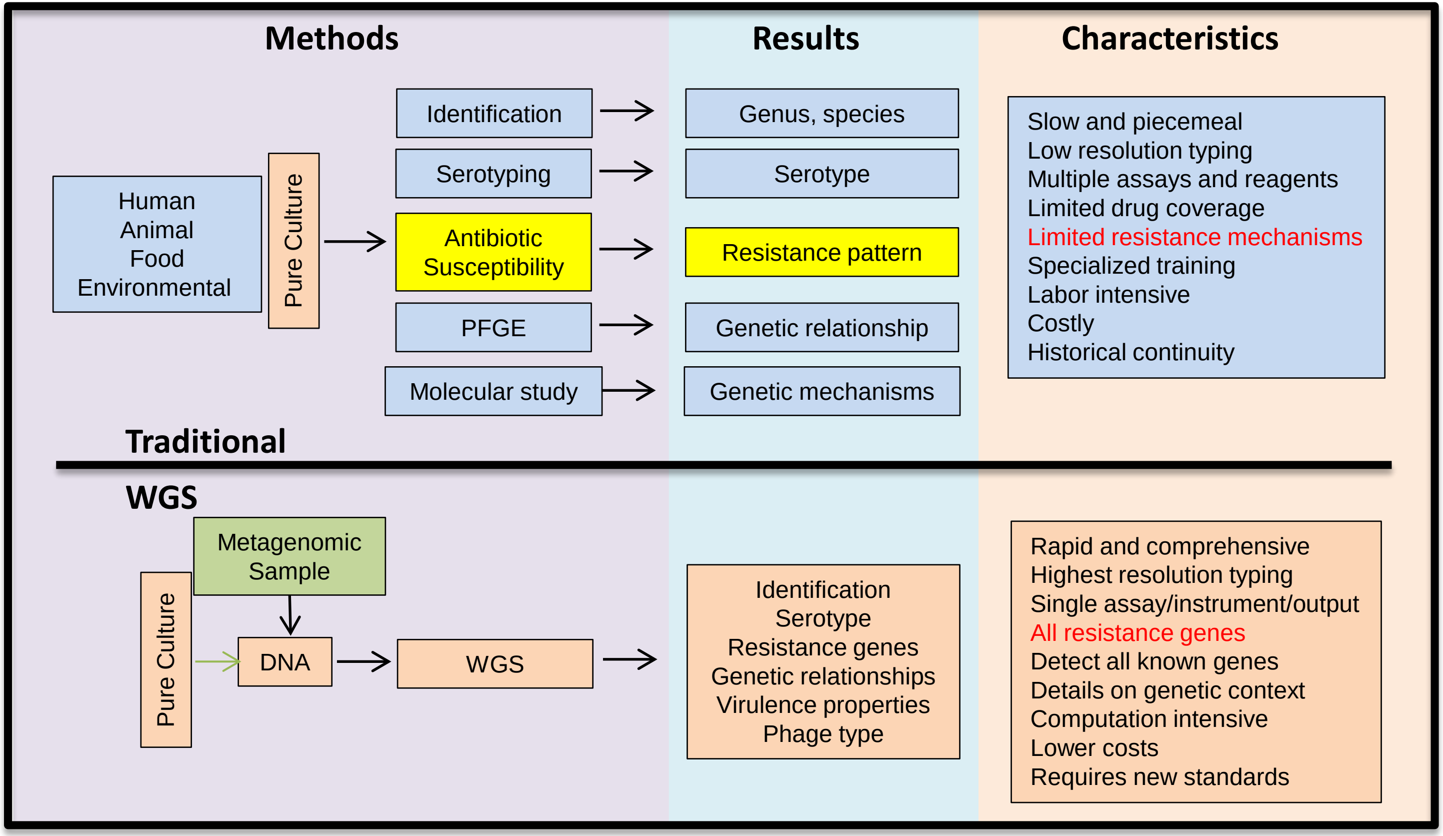
Predicting Resistance: From Genotype to Phenotype



Methods **Results** **Characteristics**

Human
Animal
Food
Environmental

Pure Culture →

- Identification → Genus, species
- Serotyping → Serotype
- Antibiotic Susceptibility → Resistance pattern
- PFGE → Genetic relationship
- Molecular study → Genetic mechanisms

Slow and piecemeal
Low resolution typing
Multiple assays and reagents
Limited drug coverage
Limited resistance mechanisms
Specialized training
Labor intensive
Costly
Historical continuity

Traditional

WGS

Metagenomic Sample → DNA
Pure Culture → DNA → WGS →

Identification
Serotype
Resistance genes
Genetic relationships
Virulence properties
Phage type

Rapid and comprehensive
Highest resolution typing
Single assay/instrument/output
All resistance genes
Detect all known genes
Details on genetic context
Computation intensive
Lower costs
Requires new standards

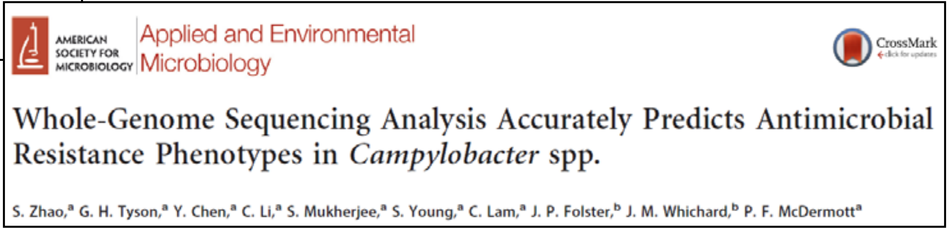
NARMS Genotype-Phenotype Correlations

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

CrossMark click for updates

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]



AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

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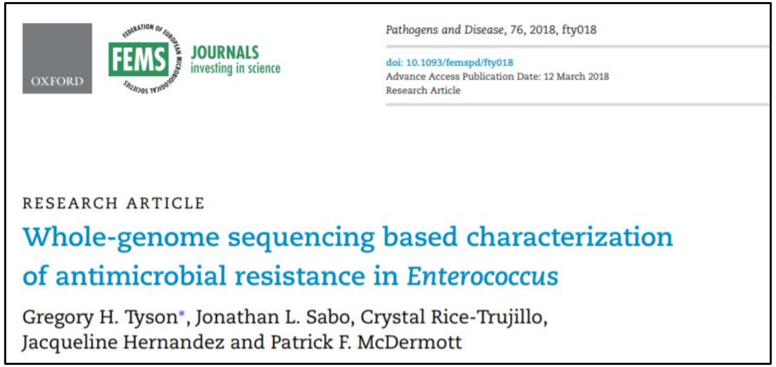
Whole-Genome Sequencing Analysis Accurately Predicts Antimicrobial Resistance Phenotypes in *Campylobacter* spp.

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]



OXFORD | FEMS FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES | JOURNALS investing in science

Pathogens and Disease, 76, 2018, fty018

doi: 10.1093/femspd/fty018
Advance Access Publication Date: 12 March 2018
Research Article

RESEARCH ARTICLE

Whole-genome sequencing based characterization of antimicrobial resistance in *Enterococcus*

Gregory H. Tyson*, Jonathan L. Sabo, Crystal Rice-Trujillo, Jacqueline Hernandez and Patrick F. McDermott

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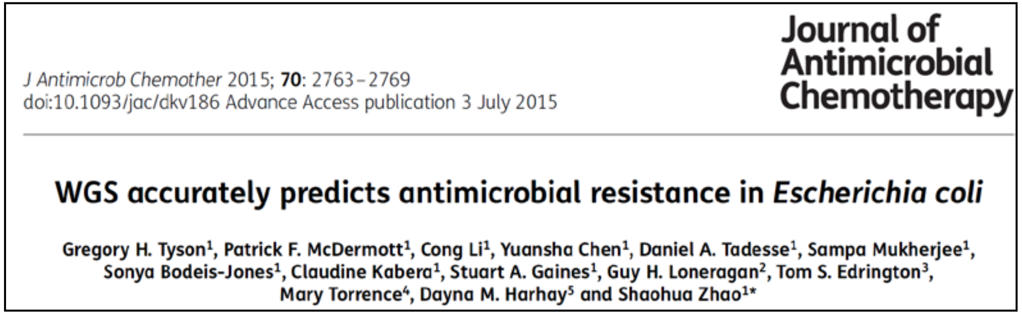


J Antimicrob Chemother 2015; **70**: 2763–2769
doi:10.1093/jac/dkv186 Advance Access publication 3 July 2015

Journal of Antimicrobial Chemotherapy

WGS accurately predicts antimicrobial resistance in *Escherichia coli*

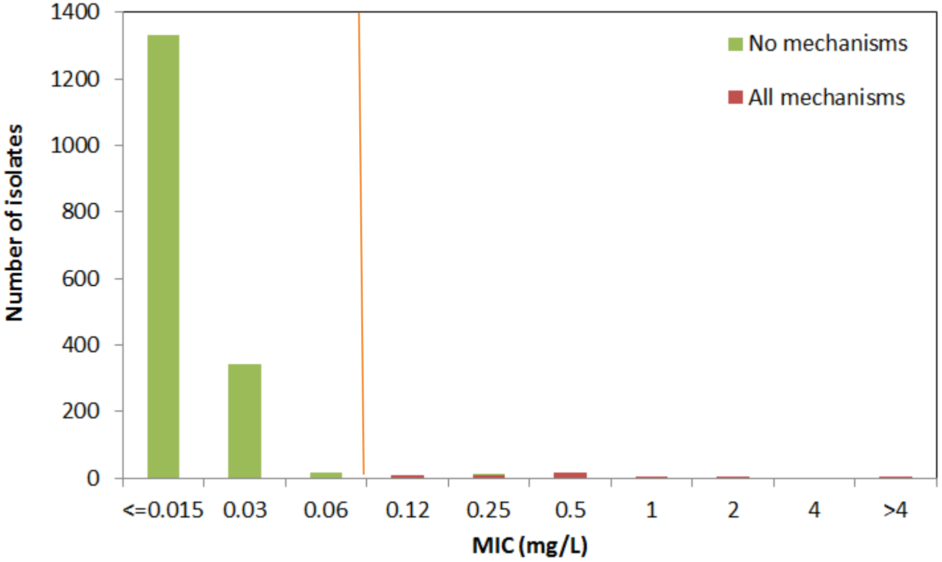
Gregory H. Tyson[1], Patrick F. McDermott[1], Cong Li[1], Yuansha Chen[1], Daniel A. Tadesse[1], Sampa Mukherjee[1], Sonya Bodeis-Jones[1], Claudine Kabera[1], Stuart A. Gaines[1], Guy H. Loneragan[2], Tom S. Edrington[3], Mary Torrence[4], Dayna M. Harhay[5] and Shaohua Zhao[1]*

Predicting Resistance from WGS

Bacterium	Gen/Phe correlation	Reference
Salmonella enterica	**99.70%**	Zankari et al., 2013, J Antimicrob Chemother
	99.00%	McDermott et al., 2016, Antimicrob Agents Chemother
Escherichia coli	**97.10%**	Stoesser et al., 2013, J Antimicrob Chemother
	98.50%	Tyson et al 2015., J Antimicrob Chemother
Campylobacter spp.	**99.20%**	Zhao et al 2015., J Antimicrob Chemother
Staphylococcus aureus	**98.80%**	Gordon et al 2014., J Antimicrob Chemother
Pneumococcus	**98.00%**	Metcalf et al 2016, Clin Microbiol Infect
Enterobacteriaceae (B-lacs)	**100.00%**	Shelburne et al, 2017 Clin Infect Dis
Mycobacterium	**95.30%**	Phelan et al 2016. Genome Med
	92.30%	Walker et al 2015. Lancet Infect Dis

Ciprofloxacin MICs by Mechanism and GCVs

MIC (mg/L)	WT	*qnr* genes	One *gyrA* mutation	Two *gyrA* mutation
≤ 0.015	1333			
0.03	344			
0.06	17			
0.12		1	7	
0.25	1	4	5	
0.5		17	1	
1		4		
2		1		
4				
> 4				3



Tyson GH, Zhao S, Li C, Ayers S, Sabo JL, Lam C, Miller RA, McDermott PF. *Establishing genotypic cutoff values to measure antimicrobial resistance in* Salmonella. Antimicrob Agents Chemother. 2017 Feb 23;61(3).

Summary of GCVs

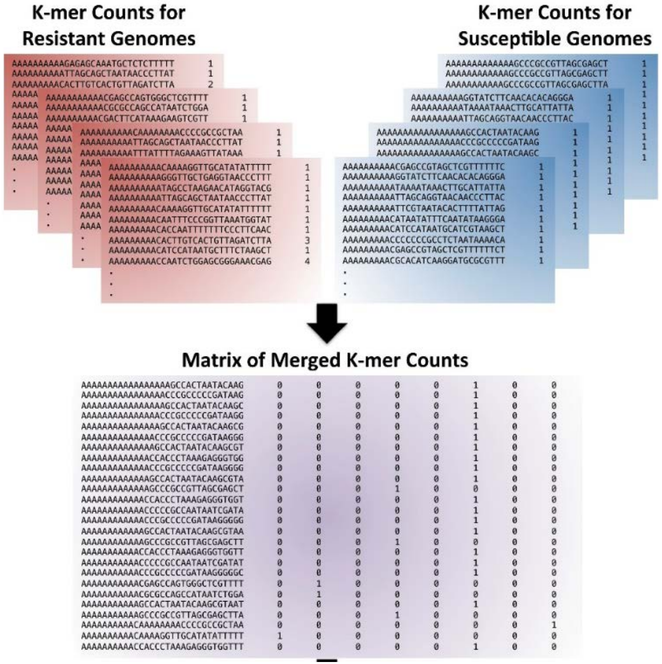
Antimicrobials	CLSI susceptible (S): treatment success likely	EUCAST ECV: wild-type (WT)	GCV: no resistance mechanism (NRM)
Ampicillin	≤ 8	≤ 4	≤ 8
Amoxicillin-clavulanate	≤ 8	None	≤ 2
Cefoxitin	≤ 8	≤ 8	≤ 8
Ceftriaxone	≤ 1	None	≤ 1
Ceftiofur	≤ 2	≤ 2	≤ 2
Gentamicin	≤ 4	≤ 1	≤ 2
Tetracycline	≤ 4	≤ 4	≤ 4
Chloramphenicol	≤ 8	≤ 16	≤ 16
Ciprofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
Nalidixic acid	≤ 16	≤ 16	≤ 8
Azithromycin	None	None	≤ 16
Sulfisoxazole	≤ 256	None	≤ 256
Trimethoprim-sulfamethoxazole	≤ 2	≤ 1	≤ 0.05

Tyson GH, Zhao S, Li C, Ayers S, Sabo JL, Lam C, Miller RA, McDermott PF. *Establishing Genotypic Cutoff Values To Measure Antimicrobial Resistance in* Salmonella. Antimicrob Agents Chemother. 2017 Feb 23;61(3).

Using machine learning to predict antimicrobial minimum inhibitory concentrations and associated genomic features for nontyphoidal *Salmonella*

Marcus Nguyen, S. Wesley Long, Patrick F McDermott, Randall J Olsen, Robert Olson, Rick L Stevens,
Gregory H Tyson, Shaohua Zhao, James J Davis

- Used 5,278 NARMS isolates with phenotype/genotype data
- Susceptibility to 16 antibiotics
- Find non redundant k-mers
- Merge R and S to form a matrix
- Generated XGBoost-based machine learning models to predict MIC ± 1 two-fold dilution



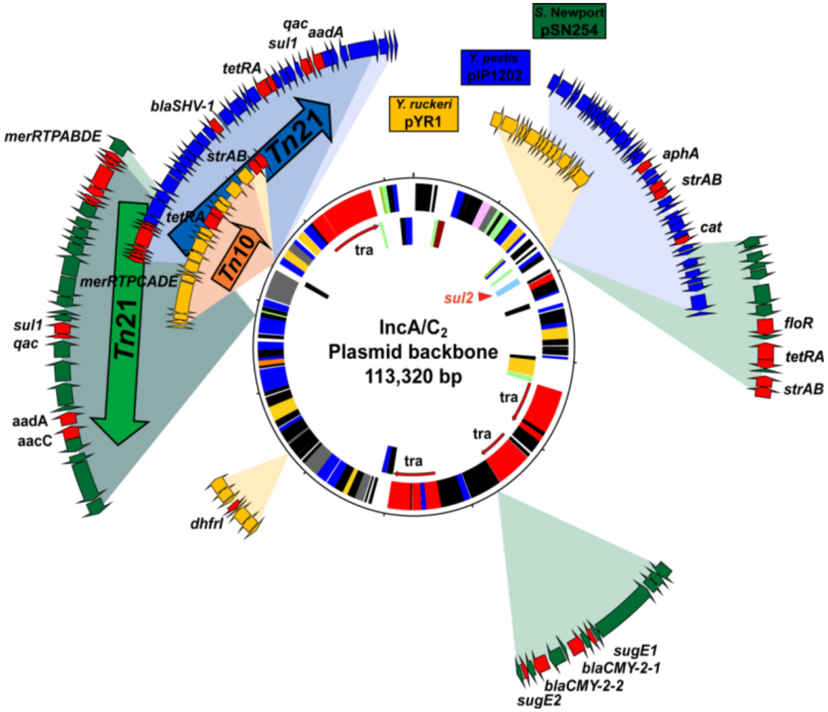
Using machine learning to predict antimicrobial minimum inhibitory concentrations and associated genomic features for nontyphoidal *Salmonella*

Marcus Nguyen, S. Wesley Long, Patrick F McDermott, Randall J Olsen, Robert Olson, Rick L Stevens, Gregory H Tyson, Shaohua Zhao, James J Davis

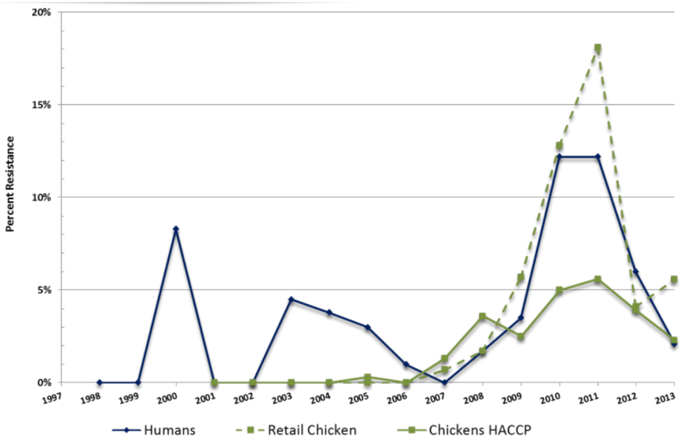
- The MIC prediction models have average accuracies between 95-96% within ± 1 two-fold dilution factor.
- The models are capable of predicting susceptible and resistant MICs with no *a priori* information about the underlying gene content of the genomes.
- By using diverse genomes for training sets, MIC prediction models with accuracies >90% can be generated with fewer than 500 genomes.
- Despite annual fluctuations in AMR gene content in the sampled genomes, this approach for predicting MICs is stable year after year.
- Evaluating loci for including in R-gene screening

	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

WGS gives new answers to old questions



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



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

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

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

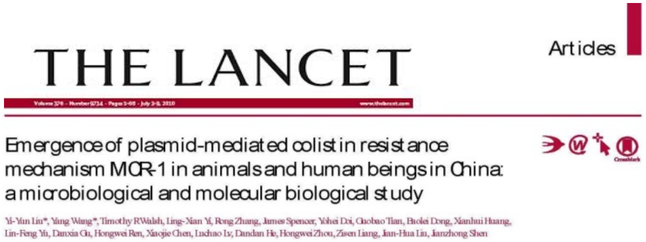
- Colistin is used as a last-resort drug to treat patients with multidrug-resistant infections, including CRE

- 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.



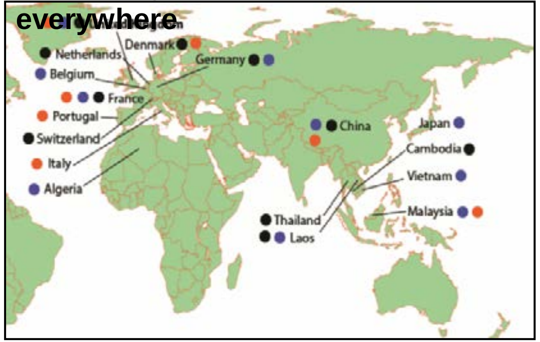
THE LANCET

Articles

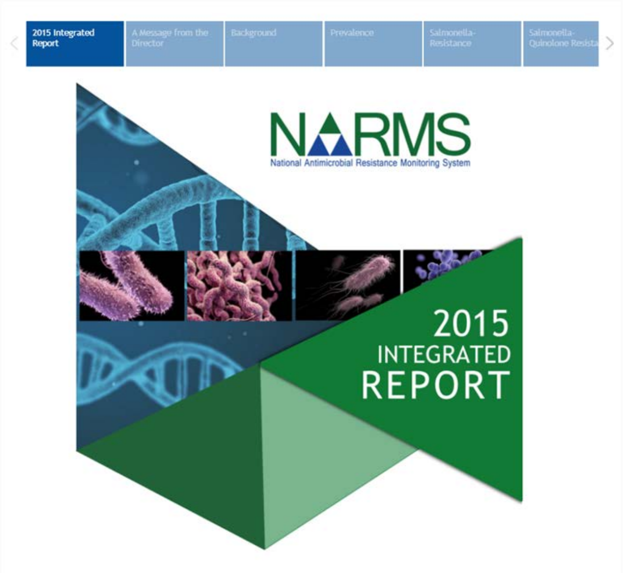
Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen

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



Integrated reporting of genomics: transforming bewildering complexity to intelligible order



Google-> NARMS Integrated Report

NARMS Now: Integrated Data

National Antimicrobial Resistance Monitoring System

Resistance by Species and Serotype

Compare antimicrobial resistance in bacteria from animals and humans. You have the option to choose from four different organisms, 14 animal and meat sources, and several antimicrobials.

Resistance by Sample Source and Place

Compare resistance to a single antimicrobial agent in bacteria from multiple sources and see the geographic distribution of the resistant bacteria.

Resistance Genes in *Salmonella*

Explore the distribution of resistance genes from *Salmonella*, and see distinct and common resistance genes in bacteria from different sample types.

Resistance to Multiple Antimicrobial Agents

Compare any combination of multiple antimicrobial agents for resistance over time, and compare by source.

Multidtrug Resistance

Compare multidrug resistance trends for *Salmonella* and *Campylobacter*. Additionally, compare resistance in *E. coli* and *Enterococcus* from retail meat and food animals over time.

References

For those interested in other NARMS resources, including the isolate level data, go to the reference page.

Adaptations of Food Safety Importance

(1) Thermal tolerance
(2) Dessication resistance
(3) Osmotic/Ionic tolerance
(4) Quat resistance
(5) Chlorine resistance
(6) Biofilm persistence
(7) Surface adherence
(8) Antibiotic resistance
(9) Antimicrobial resistance
(10) Ecological fitness
(11) Heavy metal resistance
(12) Metabolic persistence
(13) Enhanced hydrophobic fitness
(14) Produce invasiveness
(15) Flower invasiveness
(16) Root system invasiveness
(17) Acid resistance
(18) Surface water fitness
(19) In vivo plant migratory fitness
(20) Soil fitness
(21) Capsaicin resistance
(22) Swarming
(23) Trans-ovarian poultry colonization
(24) Fecal persistence (poultry)
(25) Yolk content invasion
(26) Multidrug resistance
(27) External amoeba harborage
(28) Internal amoeba harborage
(29) Acyl-homoserine lactone (AHL)
(30) KatE stationary-phase catalase
(31) In vivo migratory fitness
(32) RDAR phenotype
(33) The ‘Weltevreden’ type
(34) Peristence within the tomato**

Representative genes from the literature that may be linked to resistance, which may affect preventative controls.

ltrC	LMM7_2442	cold stress	Chaturongakul et al., 2008
pdhB	LMM7_1083	oxidative stress	Chaturongakul et al., 2008
rsbV	lmo0893	regulator of sigB activity	Chaturongakul et al., 2008
rsbW	lmo0894	regulator of sigB activity	Chaturongakul et al., 2008
rsbX	BN389_09250	regulator of sigB activity	Chaturongakul et al., 2008
gadC	lmo2362	acid stress	Chaturongakul et al., 2008; Begley and Hill 2015
gadA	lmo0447	acid stress	Chaturongakul et al., 2008;Begley and Hill 2015
opuCA	LMRG_00880	osmotic stress	Fraser et al., 2003
opuCB	LMRG_00879	osmotic stress	Fraser et al., 2003
opuCC	LMRG_00878	osmotic stress	Fraser et al., 2003
opuCD	LMRG_00877	osmotic stress	Fraser et al., 2003
arcA	lmo0043	acid stress	Gahan and Hill, 2014
arcB	LMHCC_0975	acid stress	Gahan and Hill, 2014
arcC	BN389_00490	acid stress	Gahan and Hill, 2014
argB	lmo1589	acid stress	Gahan and Hill, 2014
argC	lmo1591	acid stress	Gahan and Hill, 2014
argD	lmo1588	acid stress	Gahan and Hill, 2014
argG	lmo2090	acid stress	Gahan and Hill, 2014
argH	lmo2091	acid stress	Gahan and Hill, 2014
argR	lmo1637	acid stress	Gahan and Hill, 2014
uspA	lmo0515	general stress response	Gomez et al., 2011
rpoN	LMM7_2503	osmotic stress;cold stress	Okada et al., 2006
sigL	BN389_24240	cold stress	Raimann et al., 2009
betL	LMM7_2191	osmotic stress;cold stress	Tasara and Stephan 2006

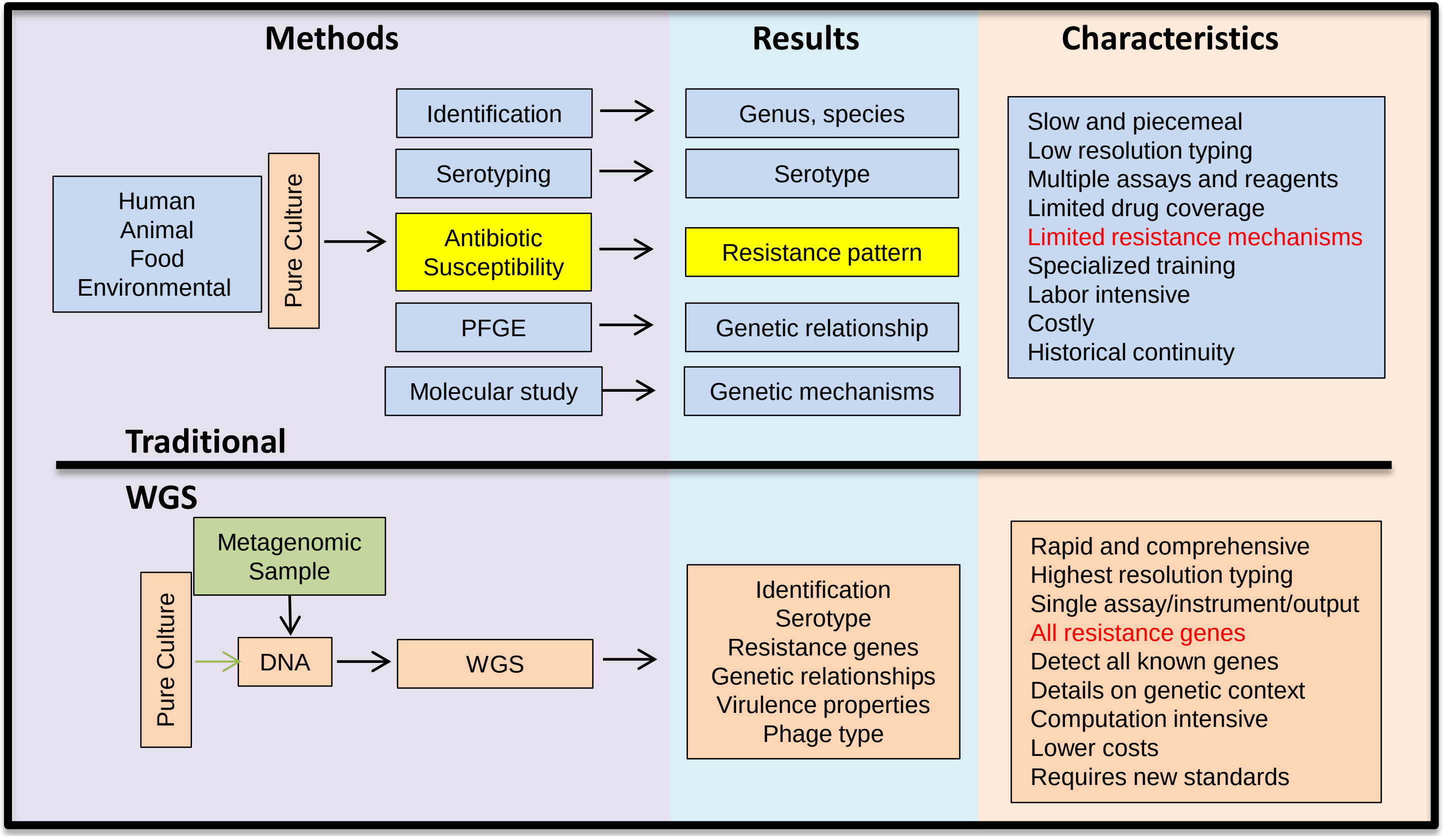
Mine the literature, mine the WGS databases.
Match Genotype with Phenotype

Making Data Accessible: NARMS Now

- Human Clinical Cases – Click the link to be taken to CDC's NARMS Now: Human Data where you can download the human data.
- Retail Meats – Last updated 10/20/2017
- Food Producing Animals
  - HACCP 1997-2005 – Last updated 8/21/2015
  - HACCP 2006-2015 – Last updated 10/20/2017
  - Cecal 2013-2015 – Last updated 10/20/2017
  - Data Dictionary

- Nearly 200,000 entries of isolate-level data for *Salmonella, Campylobacter, E. coli* and *Enterococcus* collected since 1996.
- *Salmonella* from 14 human, food and animals sources
- Extensive metadata including MICs to 16 antibiotics
- WGS data on all food *Salmonella* since 2002
- WGS data on human and animal *Salmonella* since 2014
- 2018-2019 goal is towards publication of real time WGS data to public domain

One Health, One Method



Methods | **Results** | **Characteristics**

Traditional

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Environmental

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PFGE → Genetic relationship

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Historical continuity

WGS

Metagenomic Sample ↓ DNA

Pure Culture → DNA → WGS →

Identification
Serotype
Resistance genes
Genetic relationships
Virulence properties
Phage type

Rapid and comprehensive
Highest resolution typing
Single assay/instrument/output
All resistance genes
Detect all known genes
Details on genetic context
Computation intensive
Lower costs
Requires new standards

Antimicrobial Resistance Surveillance

One Health - One Method

- Highest practical resolution of structural traits in microbial members of an ecosystem
- Accurate prediction of clinical resistance, and perhaps MIC.
- Resistance to compounds not tested such as disinfectants and heavy metals, and other potential drivers of resistance
- Deep surveillance into previously hidden associations (*e.g.*, co-resistance with plasmid type, virulence) including determinants of zoonotic transmissibility of resistance
- Source attribution for more precise intervention
- Retrospective resistance surveillance
- Metagenomics to escape limitations of classical microbiology
- Look farther with few resources: domestic and wild animal populations, animal feed, environmental transmission & exposure pathways.
- Global resistance emergence and spread
- Greater confidence in public health decision making

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Ian Plumb
Dawn Sievert

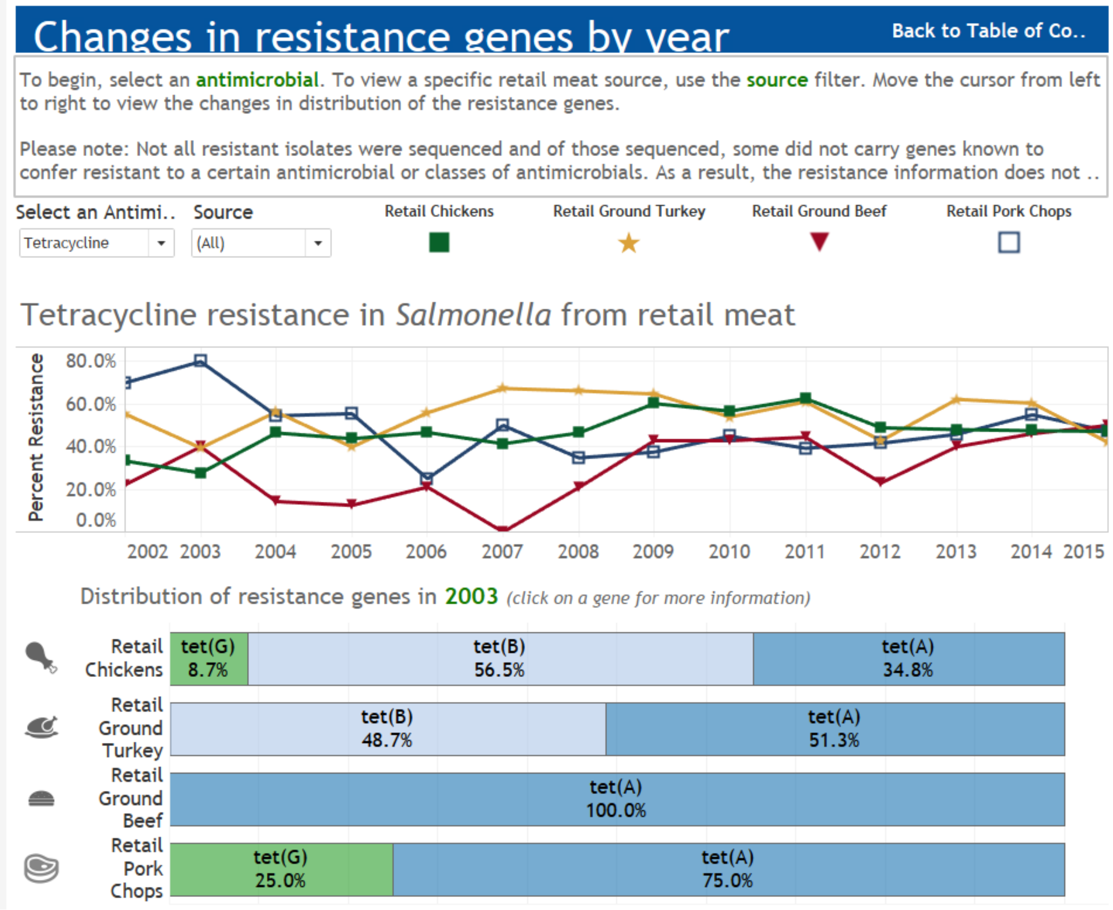
FDA/CFSAN

Mark Allard
Eric Brown
Errol Strain
Ruth Timme

Non-federal partners

- Public health laboratories in all 50 states
- Universities
- Research collaborators

FDA U.S. FOOD & DRUG
ADMINISTRATION



Changes in resistance genes by year

Back to Table of Co..

To begin, select an **antimicrobial**. To view a specific retail meat source, use the **source** filter. Move the cursor from left to right to view the changes in distribution of the resistance genes.

Please note: Not all resistant isolates were sequenced and of those sequenced, some did not carry genes known to confer resistant to a certain antimicrobial or classes of antimicrobials. As a result, the resistance information does not ..

Select an Antimi..: Tetracycline

Source: (All)

Retail Chickens 🟩 Retail Ground Turkey ★ Retail Ground Beef ▼ Retail Pork Chops ☐

Tetracycline resistance in *Salmonella* from retail meat

Distribution of resistance genes in 2003 *(click on a gene for more information)*

Retail Chickens: tet(G) 8.7% | tet(B) 56.5% | tet(A) 34.8%

Retail Ground Turkey: tet(B) 48.7% | tet(A) 51.3%

Retail Ground Beef: tet(A) 100.0%

Retail Pork Chops: tet(G) 25.0% | tet(A) 75.0%

RESISTOME TRACKER

Salmonella

Select an icon or alert below to get started.

https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm570694.htm

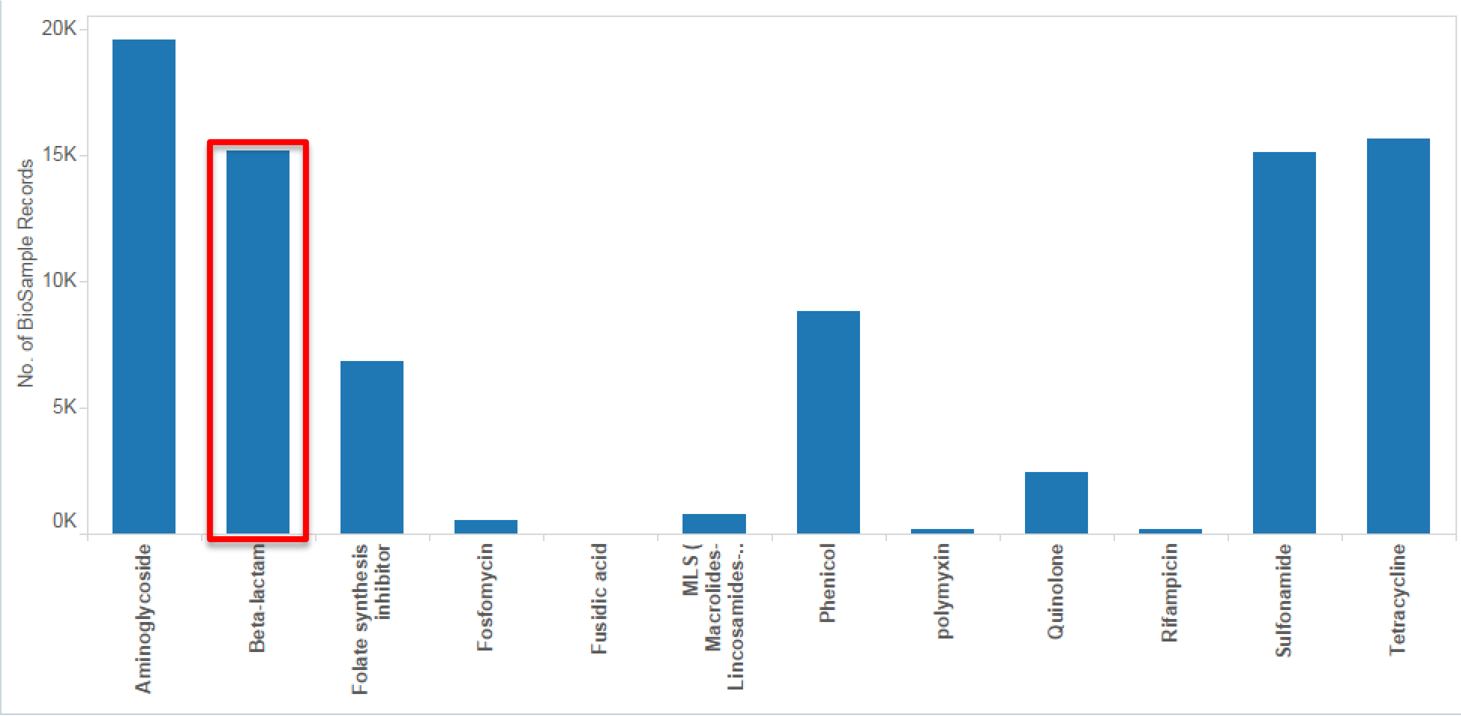
RESISTOME TRACKER

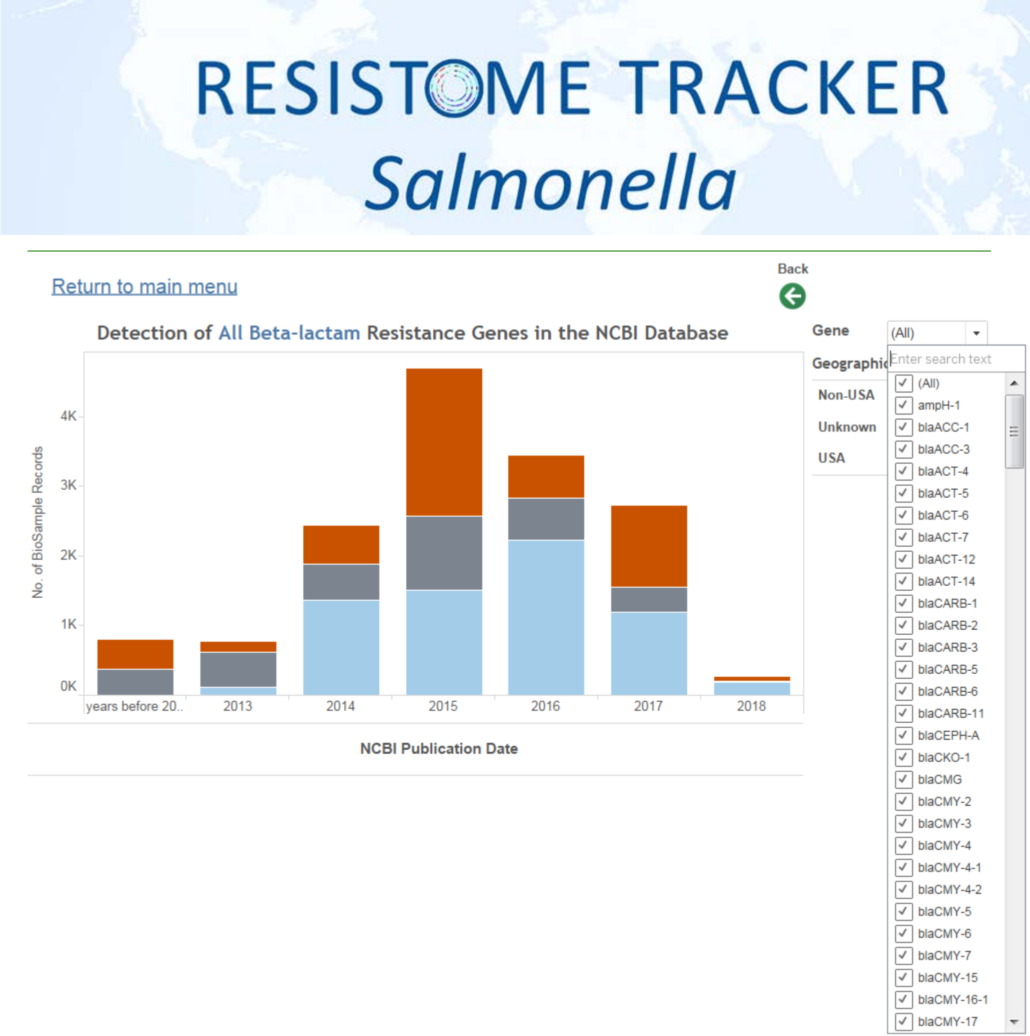
Salmonella

Select an icon or alert below to get started.

https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm570694.htm

RESISTOME TRACKER
Salmonella





RESISTOME TRACKER

Salmonella

Return to main menu

Back

Detection of All Beta-lactam Resistance Genes in the NCBI Database

Gene (All)

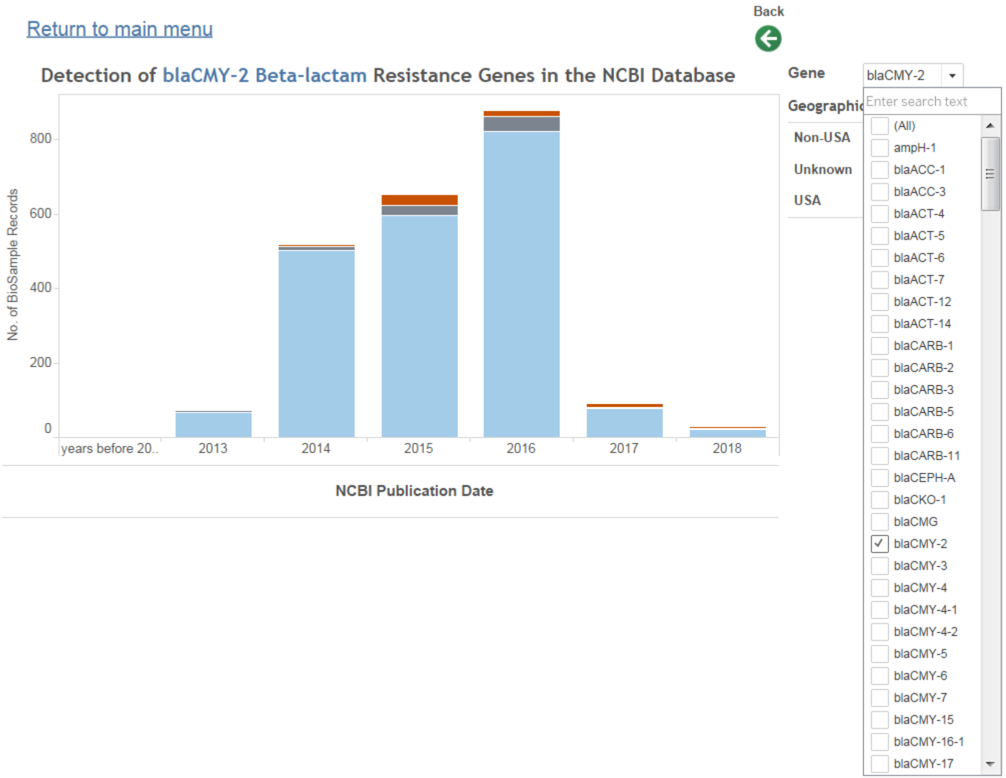
Geographic

- Non-USA
- Unknown
- USA

Enter search text

- ☑ (All)
- ☑ ampH-1
- ☑ blaACC-1
- ☑ blaACC-3
- ☑ blaACT-4
- ☑ blaACT-5
- ☑ blaACT-6
- ☑ blaACT-7
- ☑ blaACT-12
- ☑ blaACT-14
- ☑ blaCARB-1
- ☑ blaCARB-2
- ☑ blaCARB-3
- ☑ blaCARB-5
- ☑ blaCARB-6
- ☑ blaCARB-11
- ☑ blaCEPH-A
- ☑ blaCKO-1
- ☑ blaCMG
- ☑ blaCMY-2
- ☑ blaCMY-3
- ☑ blaCMY-4
- ☑ blaCMY-4-1
- ☑ blaCMY-4-2
- ☑ blaCMY-5
- ☑ blaCMY-6
- ☑ blaCMY-7
- ☑ blaCMY-15
- ☑ blaCMY-16-1
- ☑ blaCMY-17

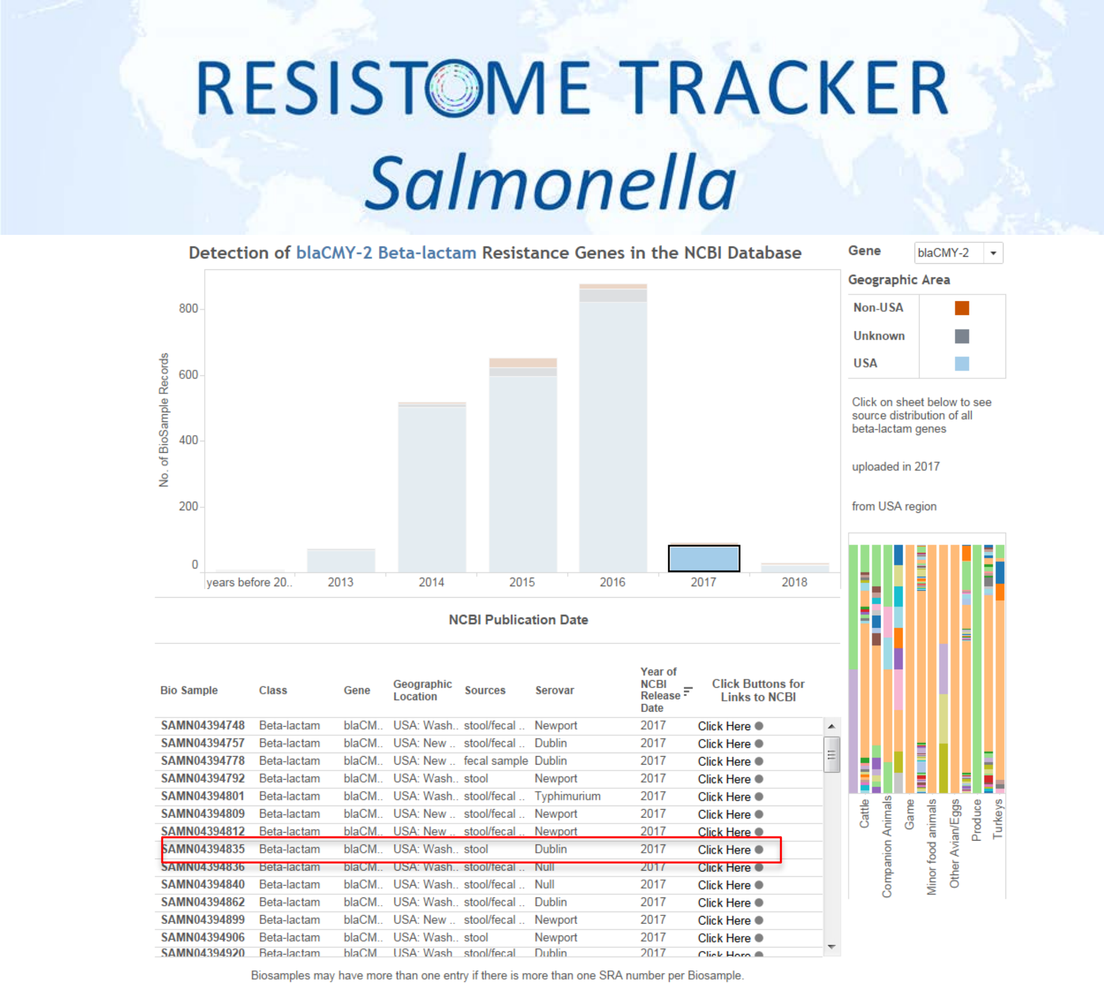
RESISTOME TRACKER
Salmonella



Return to main menu

Detection of blaCMY-2 Beta-lactam Resistance Genes in the NCBI Database

Gene: blaCMY-2



RESISTOME TRACKER

Salmonella

Detection of blaCMY-2 Beta-lactam Resistance Genes in the NCBI Database

Click on sheet below to see source distribution of all beta-lactam genes

uploaded in 2017

from USA region

Bio Sample	Class	Gene	Geographic Location	Sources	Serovar	Year of NCBI Release Date	Click Buttons for Links to NCBI
SAMN04394748	Beta-lactam	blaCM..	USA: Wash..	stool/fecal ..	Newport	2017	Click Here ●
SAMN04394757	Beta-lactam	blaCM..	USA: New ..	stool/fecal ..	Dublin	2017	Click Here ●
SAMN04394778	Beta-lactam	blaCM..	USA: New ..	fecal sample	Dublin	2017	Click Here ●
SAMN04394792	Beta-lactam	blaCM..	USA: Wash..	stool	Newport	2017	Click Here ●
SAMN04394801	Beta-lactam	blaCM..	USA: Wash..	stool/fecal ..	Typhimurium	2017	Click Here ●
SAMN04394809	Beta-lactam	blaCM..	USA: New ..	stool/fecal ..	Newport	2017	Click Here ●
SAMN04394812	Beta-lactam	blaCM..	USA: New ..	stool/fecal ..	Newport	2017	Click Here ●
SAMN04394835	Beta-lactam	blaCM..	USA: Wash..	stool	Dublin	2017	Click Here ●
SAMN04394836	Beta-lactam	blaCM..	USA: Wash..	stool/fecal ..	Null	2017	Click Here ●
SAMN04394840	Beta-lactam	blaCM..	USA: Wash..	stool/fecal ..	Null	2017	Click Here ●
SAMN04394862	Beta-lactam	blaCM..	USA: Wash..	stool/fecal ..	Dublin	2017	Click Here ●
SAMN04394899	Beta-lactam	blaCM..	USA: New ..	stool/fecal ..	Newport	2017	Click Here ●
SAMN04394906	Beta-lactam	blaCM..	USA: Wash..	stool	Newport	2017	Click Here ●
SAMN04394920	Beta-lactam	blaCM..	USA: Wash..	stool/fecal	Dublin	2017	Click Here ●

Biosamples may have more than one entry if there is more than one SRA number per Biosample.

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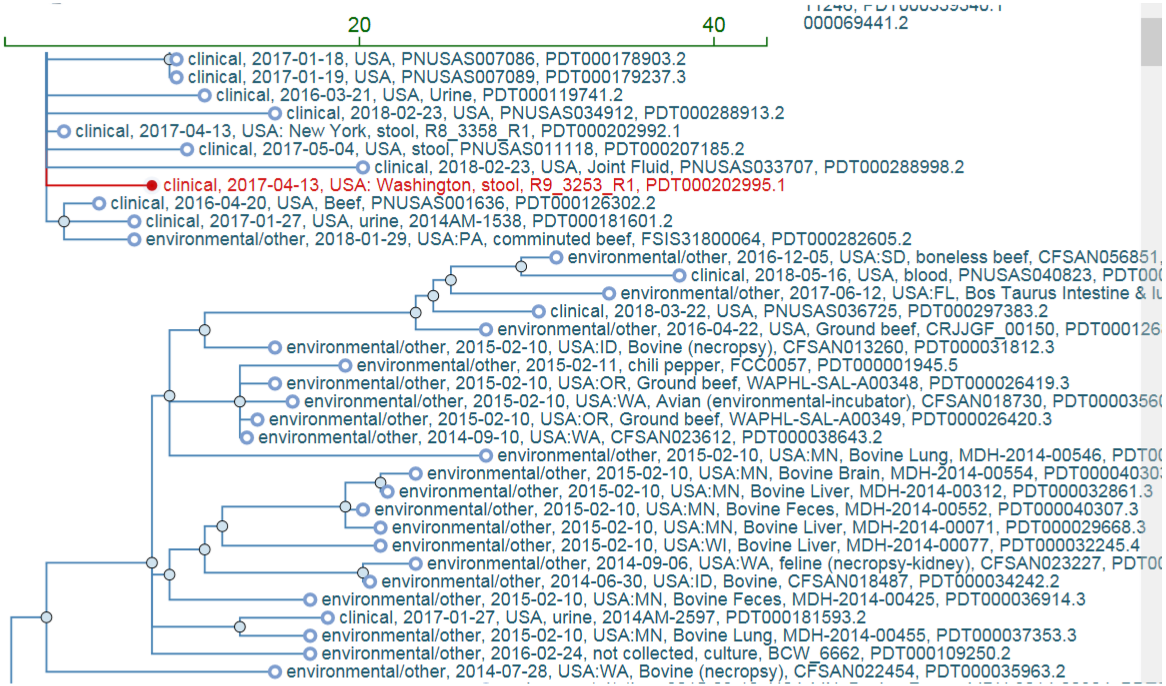
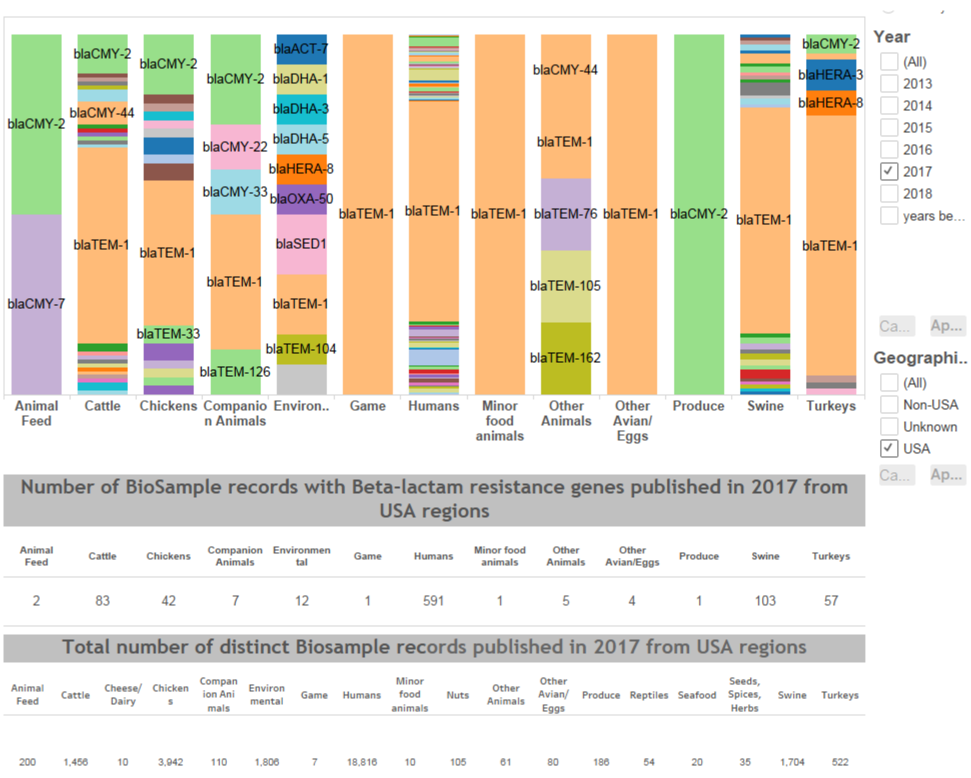


RESISTOME TRACKER

Salmonella



Year

- ☐ (All)
- ☐ 2013
- ☐ 2014
- ☐ 2015
- ☐ 2016
- ☑ 2017
- ☐ 2018
- ☐ years be...

Geographi..

- ☐ (All)
- ☐ Non-USA
- ☐ Unknown
- ☑ USA

Number of BioSample records with Beta-lactam resistance genes published in 2017 from USA regions

Animal Feed	Cattle	Chickens	Companion Animals	Environmental	Game	Humans	Minor food animals	Other Animals	Other Avian/Eggs	Produce	Swine	Turkeys
2	83	42	7	12	1	591	1	5	4	1	103	57

Total number of distinct Biosample records published in 2017 from USA regions

Animal Feed	Cattle	Cheese/ Dairy	Chickens	Companion Animals	Environmental	Game	Humans	Minor food animals	Nuts	Other Animals	Other Avian/ Eggs	Produce	Reptiles	Seafood	Seeds, Spices, Herbs	Swine	Turkeys
200	1,456	10	3,942	110	1,806	7	18,816	10	105	61	80	186	54	20	35	1,704	522