

Use of whole genome sequencing to characterize antimicrobial-resistant *Salmonella* Berta isolates from clinical and retail meat sources

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Background

Antimicrobial resistance (AMR) in foodborne pathogens of animal origin is a public health concern

- Non-typhoidal *Salmonella* (NTS) is a leading cause of gastroenteritis in humans**
 - Incidence of ~18.3/100,000 persons in the U.S. in 2018
 - ~ 1.35 million illnesses (1)
 - While most are self-limiting, some are severe including those caused by drug-resistant strains
 - Drug-resistant non-typhoidal *Salmonella* infections**
 - ~ 212,500 infections occur nationally each year
 - ~ 26,500 hospitalizations
 - ~420 deaths; ~ \$400 million in direct and indirect costs (2)
 - Source of non-typhoidal *Salmonella* infections**
 - Salmonella* lives in the intestines of many animals including poultry
 - Antimicrobial use in food animals drives emergence of resistant strains (3)
 - Humans often acquire infections through consumption of contaminated food including poultry meat
 - Antimicrobial Therapy**
 - Indicated for severe infections and at-risk patients (e.g., diabetics, geriatrics and transplant recipients)
 - National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria**
 - PA conducts integrated One Health surveillance for AMR bacteria from humans, food and animals
 - An Advanced Molecular Detection (AMD) whole genome sequencing (WGS) project with Virginia
- Study objective**
- Compare isolates from clinical and food sources to gain insights into how WGS can contribute to surveillance and antimicrobial stewardship efforts

Methods

Study design

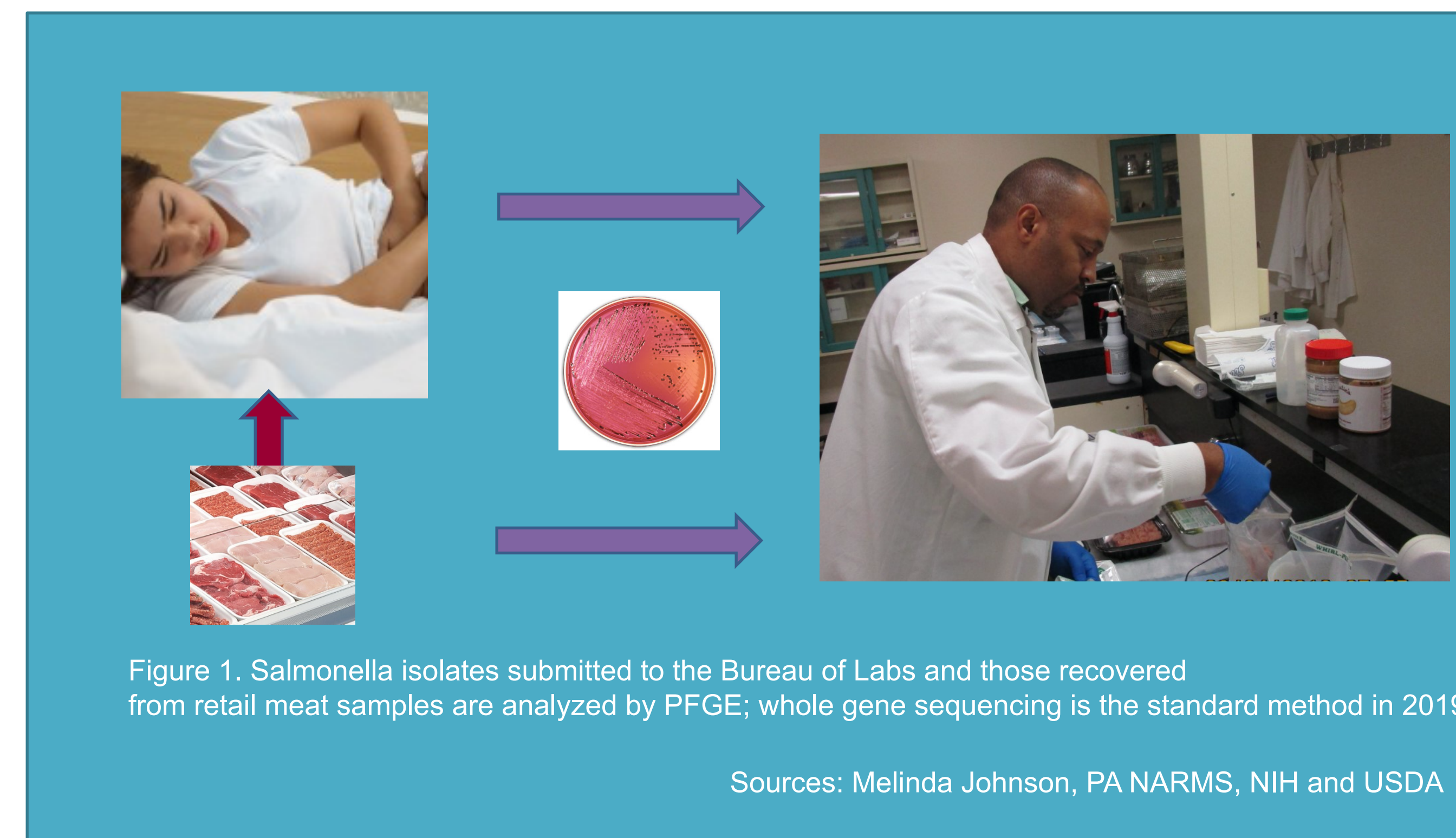
- Clinical isolates from humans**
 - Prospective testing of *Salmonella* isolates submitted to the Bureau of Labs during 2009-2014 in compliance with public health reporting requirements (4)
- Surveillance isolates from retail meat**
 - Prospective microbiological survey of *Salmonella* contamination in 2,400 retail meat samples
 - Chicken breasts, ground turkey, ground beef, and pork chops (600 of each)
 - Purchased during the same period from randomly selected retail outlets in PA (Figure 1).
 - Conducted as part of NARMS Retail Food Program coordinated by US Food and Drug Administration in multiple sites.

PFGE analysis of *Salmonella* isolates from humans and retail meat

- All isolates were analyzed by pulsed-field gel electrophoresis (PFGE) according to the PulseNet protocol for subtyping *Salmonella* (5).
 - Assayed with two enzymes (*Xba*I and *Bln*I)
 - Analysis: *BioNumerics* 6.6 (Applied Maths, Sint-Martens-Latem, Belgium)
- CDC PulseNet-assigned DNA pattern names of *Salmonella* from retail meat were compared with those from clinical isolates.

Antimicrobial susceptibility testing

- A subset of *Salmonella* isolates from clinical and all strains from retail meat sources were tested by broth microdilution method (Sensititre®, Trek Diagnostics, Westlake, OH) at the PA Veterinary Lab and the FDA NARMS lab, respectively.



Methods (Continued)

Antimicrobial susceptibility testing (Cont.)

- MICs for each of the 15 antimicrobial agents used by (NARMS) were determined and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines and consensus surveillance breakpoints (6).

Whole genome sequencing and resistance genes

- Eleven isolates with indistinguishable PFGE patterns (nine clinical and retail meat) were analyzed by WGS
 - Conducted on the Illumina MiSeq according to manufacturers specifications
- Sequence data uploaded to the FDA's GalaxyTrakr platform
 - Quality assessment genome assembly, AMR gene detection,
 - and phylogenetic inference via single-nucleotide polymorphism (SNP)

Results

- PFGE and antimicrobial resistance profiles**
 - 6644 clinical isolates received by Bureau of Labs during the study period
 - 86 (48.6%) of 177 meat isolates had PFGE matches among 1,665 clinical isolates
 - 40 distinct PFGE patterns were represented among the shared patterns.
 - 17 (43%) of the 40 shared PFGE patterns (with ≥1 isolate(s) from both sources) were multi-drug resistant (MDR)
 - MDR defined as resistant to ≥3 antibiotics in the NARMS panel
 - MDR among 48 *S. Berta* pattern JAXX01.0001 isolates (2 from meat sources):
 - Clinical 5 (10.9%); meat 2 (100%)
 - Resistance included: amoxicillin and ceftriaxone (first and second line therapeutic agents respectively)
- WGS analysis**
 - Sequence of one isolate from ground turkey meat (PNUSAS061602) had genetic related with clinical isolates
 - 9 and 11 SNPs from PNUSAS097231--- M11009873001A and PNUSAS061601 respectively (Figure 2)
 - Sequence of second isolate from meat was separated from multiple clinical isolates:
 - 14 (n=1), 17 (n=1) and ≥20 (n=7) SNPS
 - Genetic mechanisms of resistance**
 - Plasmid-mediated β-lactamase genes (*bla*_{CMY}, *bla*_{HERA}, or *bla*_{TEM})
 - 2 (100%) of isolates from meat had *bla*_{CMY-2} genes
 - 6 (13%) of isolates Associated with human illnesses
 - Five and one isolate from clinical and meat sources respectively carried *IncX1* plasmids
 - The isolate from meat had *bla*_{CMY-2} gene, and was resistant to six antimicrobials including ceftriaxone

	M1002588 3001A	M1100987 3001A	M1202244 7001A	PNUSAS0 61600	PNUSAS0 61601	PNUSAS0 61602	PNUSAS0 61603	PNUSAS0 61606	PNUSAS0 61608	PNUSAS0 62053	PNUSAS0 62054	SRR8137 075
M1002588 001A	32	43	38	31	33	31	34	25	38	33	130	
M1100987 001A	32	22	23	11	9	26	19	30	23	42	130	
M1202244 001A	43	22	32	17	24	35	29	39	35	51	139	
PNUSAS06 1600	38	23	32	21	25	21	14	36	30	37	134	
PNUSAS06 1601	31	11	17	21	14	24	17	27	23	40	128	
PNUSAS06 1602	33	9	24	25	14	28	20	32	24	44	129	
PNUSAS06 1603	31	26	35	21	24	28	17	29	23	30	127	
PNUSAS06 1606	34	19	29	14	17	20	17	32	24	32	129	
PNUSAS06 1608	25	30	39	36	27	32	29	32	28	16	122	
PNUSAS06 2053	38	23	35	30	23	24	23	24	28	36	134	
PNUSAS06 2054	33	42	51	37	40	44	30	32	16	36	129	
SRR813707 5	130	130	139	134	128	129	127	129	122	134	129	

Figure 2. Single nucleotide polymorphism (SNP) distance matrix showing relatedness in non-typhoidal *Salmonella* isolates from retail meat (n=2) and human (n=9) sources — Pennsylvania, 2010-2014. One *S. Berta* from retail meat was separated from two clinical two clinical isolates by 9 and 11 SNPs. Second isolate from meat was separated from those associated with human infections by 14 (n=1), 17 (n=1) and ≥20 (n=7).

Conclusions

- Non-typhoidal *Salmonella* from poultry meat and human sources were multi-drug resistant
 - Harbored *bla*_{CMY-2} β-lactamases genes, which confer resistance to extended-spectrum cephalosporins.
- Resistance genes identified in *Salmonella* are carried in transmissible elements (e.g., plasmids) and can be shared with other bacteria such as *E. coli* resulting in resistance to other therapeutic drugs. (8)
- One isolate from poultry meat showed high genetic relatedness to those associated with human disease
- Underscores the need for strengthening One-Health antimicrobial stewardship efforts and surveillance.

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