

Resistance to Ceftriaxone and Ciprofloxacin in Nontyphoidal *Salmonella* from Humans and Relatedness to Isolates from Animal Sources

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Background

Antimicrobial resistance in foodborne pathogens of animal origin, including nontyphoidal *Salmonella* (NTS), is a public health concern

Non-typhoidal *Salmonella* is the leading cause of gastroenteritis in humans

- *Salmonella* lives in the intestines of many animals including poultry, cattle and pigs (1)
- Humans often acquire infections through the consumption of contaminated food of animal origin
- Incidence of salmonellosis was ~14.2/100,000 persons in the U.S. in 2021 (2)
- While infections are often self-limiting, each year in the US there are:
 - ~74,000 physician visits
 - ~19,000 hospitalizations
 - ~378 deaths
 - ~\$4.14 billion in direct and indirect costs (3)

Drug-resistant non-typhoidal *Salmonella* infections

- ~200,000 infections occur in the U.S. each year (4)
- Antibiotic treatment is indicated for patients at risk of invasive disease including ceftriaxone and ciprofloxacin (5)
- Nationally, NTS isolates with decreased susceptibility to ciprofloxacin (DSC) [MIC ≥ 0.12 $\mu\text{g/mL}$] from human sources increased from 9% in 2018 to 11% in 2019 (6)
- Comparison of isolates from clinical and animal sources can facilitate outbreak investigations and antimicrobial stewardship efforts

Methods

Clinical isolates from humans

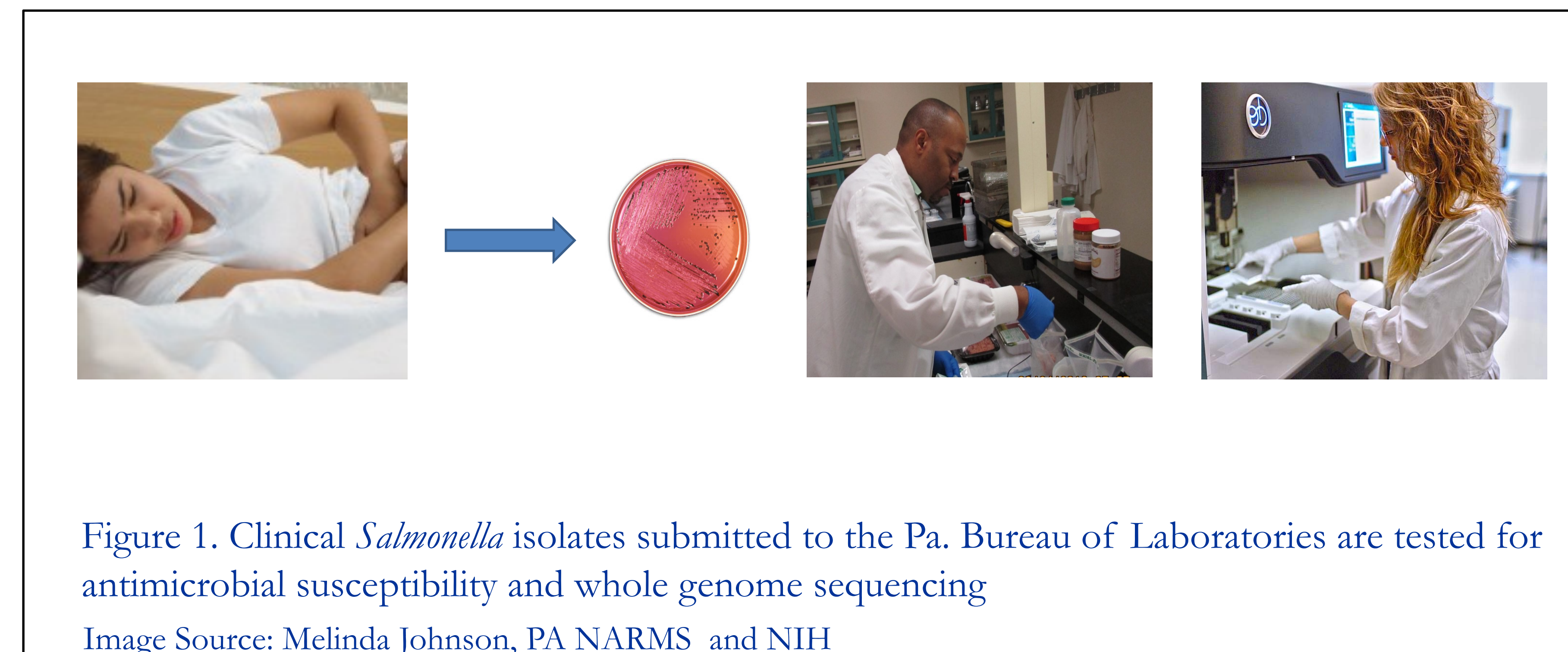
- Prospective testing of clinical NTS isolates submitted to the PA Bureau of Labs during 2018-2020 in compliance with public health reporting requirements (7)
- Isolates confirmed and serotyped by standard methods
- NTS isolates included in the National Antimicrobial Resistance Monitoring System (NARMS) frequency-based sampling sent to the CDC NARMS

Antimicrobial susceptibility testing

- Human *Salmonella* isolates (n=164) were tested by broth microdilution method (Sensititre®, Trek Diagnostics, Westlake, OH) at the Pennsylvania Veterinary Lab.
- Minimum inhibitory concentrations (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 (8-9)
- Isolates resistant to ≥ 3 antimicrobial CLSI classes considered multidrug-resistant (MDR)

Whole genome sequencing and genomic analyses

- All clinical isolates whole genome sequenced
 - Illumina short-read sequencing using PulseNet protocol (10)
- Genomes annotated using the National Center for Biotechnology Information's Prokaryotic Genome Automated Pipeline
- Resistant genes identified using a reference database at FDA, which includes publicly available resistance gene database (i.e., ResFinder)
- Resistance criteria of $\geq 85\%$ amino acid identity and $\geq 50\%$ sequence length identity to known resistance proteins
- Plasmid replicons identified using PlasmidFinder
- Genomic sequences of clinical isolates in selected serotypes compared against the PulseNet database to identify genetic relatedness within ≤ 5 allele differences to non-human sequences uploaded during 2017-2021



Results

Antimicrobial resistance in clinical non-typhoidal *Salmonella* isolates (Figure 2)

All clinical isolates (n=164)

- 16 (10%) resistant to ≥ 3 antibiotics classes
- 6 (4%) resistant to ceftriaxone
- 28 (17%) had decreased susceptibility to ciprofloxacin (DSC) [MIC ≥ 0.12 $\mu\text{g/mL}$]
- Antimicrobial resistance varied by serotype

S. Enteritidis isolates (n=38)

- 1 (3%) resistant to ≥ 3 antibiotics classes
- 0 (0%) resistant to ceftriaxone
- 16 (42%) had decreased susceptibility to ciprofloxacin

S. Typhimurium isolates (n=37)

- 5 (14%) resistant to ≥ 3 antibiotics classes
- 2 (5%) resistant to ceftriaxone
- 1 (3%) had decreased susceptibility to ciprofloxacin

S. Infantis isolates (n=16)

- 8 (50%) resistant to ≥ 3 antibiotics classes
- 3 (19%) resistant to ceftriaxone
- 8 (50%) had decreased susceptibility to ciprofloxacin

Genetic mechanisms of resistance (n=164)

- 6 ceftriaxone-resistant isolates had genetic elements that confer resistance to third generation extended-spectrum cephalosporins (ESC)
- [*bla*_{CMY-2} in Dublin and 2 Typhimurium and *bla*_{CTX-M-65} in 3 Infantis isolates]
- The 3 Infantis isolates also had a mutation in *gyrA* that results in DSC, plus a known transmissible plasmid IncFIB(pN55391) linked to multiple resistance genes (11)

Genetic relatedness (≤ 5 allele differences) of clinical isolates to isolates of animal origin

- One Infantis isolate was related to a chicken meat isolate
- One Hadar isolate was related to isolates from 3 animals and 3 meat sources
- One Dublin isolate was related to 2 isolates from beef in Pennsylvania



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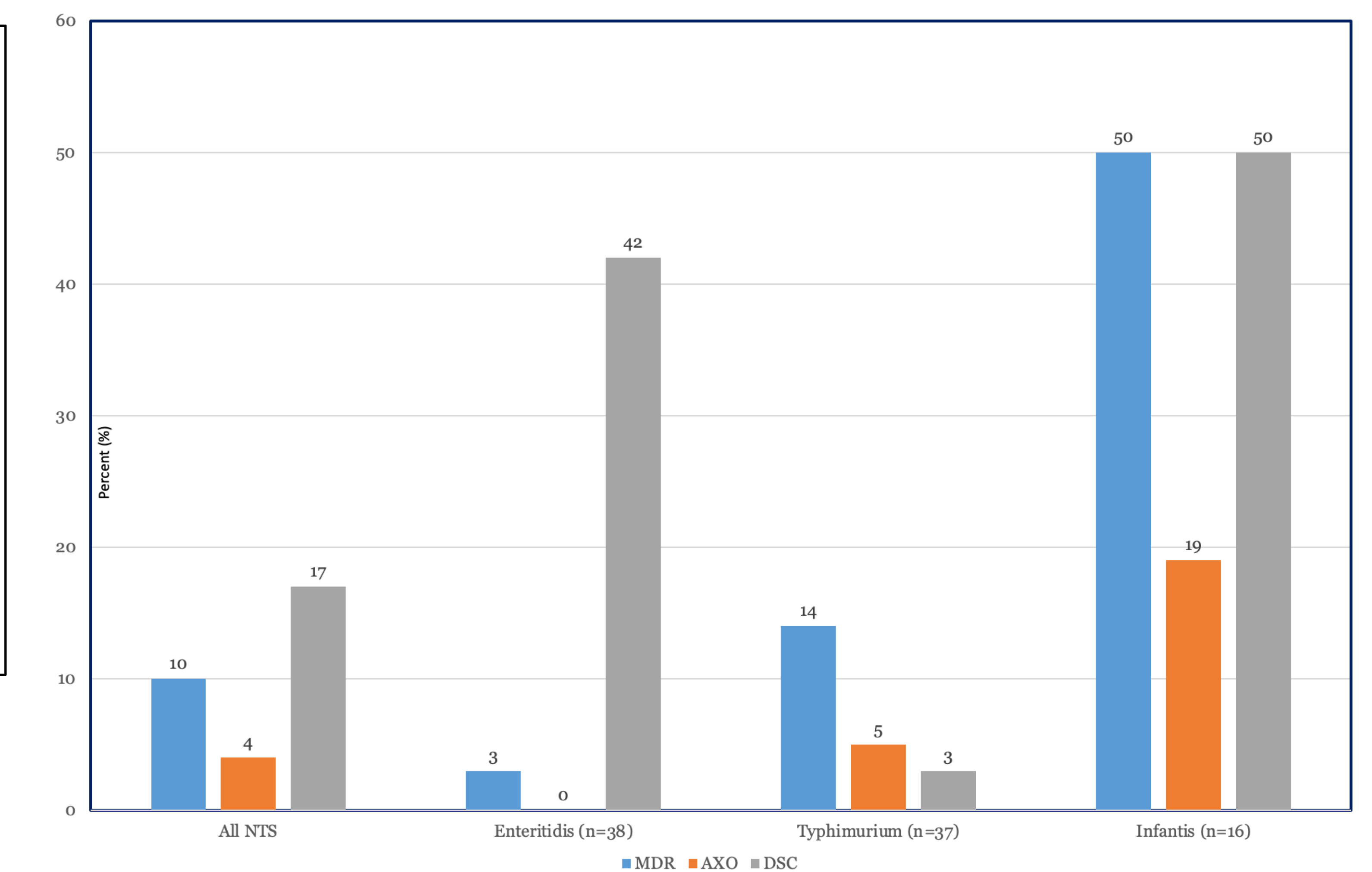


Figure 2. Percent of antimicrobial resistance in nontyphoidal *Salmonella* (NTS) clinical isolates (n=164) submitted to public health authorities by two hospital systems in Pennsylvania, 2018-2020. Multidrug resistance (MDR) was defined as resistance to ≥ 3 of the 9 antimicrobial classes tested for NTS clinical isolates. Resistance to ceftriaxone (AXO), a third-generation cephalosporin preferred for treating severe infections in children, was over 5% and 19% in serotypes *S. Typhimurium* and *S. Infantis*, respectively. Half (8/16) of Infantis isolates and 42% (16/38) of Enteritidis isolates had decreased susceptibility to ciprofloxacin (DSC)

Conclusions

- Ceftriaxone resistance and DSC in NTS from patients in Pennsylvania varied by serotype and some isolates harbored *bla*_{CMY-2} and *bla*_{CTX-M-65} genes
- Dissemination of mechanisms that confer resistance to ceftriaxone and DSC is concerning as is the genetic relatedness of isolates from human and animal sources (4)
- Study highlights the need for enhanced One Health AMR monitoring combined with a review of animal production and food processing practices

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