Antimicrobial resistance and related gene analysis of *Salmonella* from egg and chicken sources by whole-genome sequencing

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ABSTRACT Whole-genome sequencing (**WGS**) is a valuable tool in research on foodborne pathogens. In this study, a total of 143 isolates of *Salmonella* serotypes Enteritidis, Typhimurium, and Heidelberg sourced from eggs and chickens were analyzed for their antimicrobial resistance profiles using WGS data. The isolates carried high rate of genes resistant to aminoglycoside (70.63%), tetracycline (26.57%), fosfomycin (25.17%), sulfonamides (23.78%), and β-lactamases (15.38%); and *aadA* was the most frequently observed antimicrobial resistance gene (**ARG**). Antimicrobial resistance varies by *Salmonella* serotypes, with *Salmonella enterica* serovar Enteritidis (*Salmonella* ser. Enteritidis) isolates being highly resistant to aminoglycoside (particularly streptomycin); *Salmonella* ser. Typhimurium more resistant to aminoglycoside, tetracycline, and sulfonamides; and *Salmonella* ser. Heidelberg more resistant to aminoglycoside and fosfomycin. *Salmonella* ser. Typhimurium isolates presented more varieties of ARG than *Salmonella* ser. Enteritidis and *Salmonella* ser. Heidelberg. Our data showed that 5 isolates of *Salmonella* ser. Typhimurium and *Salmonella* ser. Heidelberg contained ARG resistant to $\geq$ 5 antimicrobials. In addition, 23 *Salmonella* isolates carried ARG resistant to 4 antimicrobials.

Key words: *Salmonella*, WGS, antimicrobial resistance, egg, chicken

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INTRODUCTION

Salmonella has always been a serious threat to global public health. In the United States, *Salmonella* caused approximately 1.2 million illnesses and 450 deaths annually; and about 1 million of these illnesses were due to contaminated foods (Centers for Disease Control and Prevention (CDC), 2019; Scallan et al., 2011). It was estimated that *Salmonella* serotypes Enteritidis, Typhimurium, and Heidelberg caused about 50% of the foodborne salmonellosis outbreaks in the United States and were frequently isolated from eggs and egg products, chicken meats, chicken ovaries, and feces (Schoeni et al., 1995; Chittick et al., 2006; Gast et al., 2017).

In the past 20 yr, the increasing resistance to medically important antimicrobial agents in *Salmonella* has been widely reported (Su et al., 2004). The emerging and spreading new resistance mechanisms contributed to the deteriorating situation of antimicrobial resistance and threatened the ability to treat foodborne diseases, then caused more prolonged illnesses, disabilities, and deaths (World Health Organization (WHO), 2018). The Centers for Disease Control and Prevention (**CDC**) estimated at least 2 million people are infected with antibiotic-resistant bacteria annually in the United States, leading to at least 23,000 deaths (CDC, 2018a). Furthermore, widespread of multidrug-resistance (resistance to more than 4 antibiotic classes) *Salmonella,* particularly *Salmonella* ser. Typhimurium, to the most commonly used antibiotics in human beings, has made it an even greater threat to the public health (CDC, 2014; European Center for Disease Prevention and Control (ECDC), 2009; European Food Safety Authority (EFSA) and ECDC, 2017). During 2009–2011, about 5% of nontyphoidal *Salmonella* tested by the CDC were resistant to ≥5 types of drugs (CDC, 2013). For the 2,364 *Salmonella* isolates from humans, retail meats, and food-producing animals tested in the United States in 2015, 65 of them (2.7%) were resistant

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to at least 5 antimicrobial agents: ampicillin, chloramphenicol, streptomycin, sulfonamide (sulfamethoxazole/sulfisoxazole), and tetracycline. *Salmonella* ser. Typhimurium was the serotype that most frequently presented ampicillin, chloramphenicol, streptomycin, sulfonamide (sulfamethoxazole/sulfisoxazole), and tetracycline resistance with a prevalence of 10.8% (27 of 251) (CDC, 2018b). From 1998 to 2003, the US Food Safety and Inspection Service tested 293,938 samples sourced from meat and poultry products and pasteurized egg products; of which, 136 (91.9%) of the 148 *Salmonella* ser. Enteritidis isolates from these samples were pansusceptible; 12 resistant isolates showed resistance to ampicillin (11), tetracycline (3), sulfamethoxazole (4), cephalothin (3), and ticarcillin (3); and 4 of the 12 isolates were resistant to 4 or more antimicrobials (White et al., 2007). Suresh et al. (2006) tested 492 eggs (492 eggshell and 492 egg content) and 82 egg-storing trays during 1-year period in South India; 40 *Salmonella* ser. Enteritidis isolates from the 46 *Salmonella* positive samples were all determined to be resistant to at least 4 drugs. Among the 46 positive samples, 5.3% was from eggshell, 1.8% from egg contents, and 6.1% from egg storing trays (Suresh et al., 2006). There are many mechanisms of actions that could cause antibiotic resistance, such as inhibiting an enzyme, altering the cell membrane permeability (ionophores), affecting the structure of the cell wall, interfering with DNA/protein synthesis (mutations, horizontal gene transfer) (Barbosa and Levy, 2000; Giedraitienė et al., 2011). Therefore, a collective effort should be made to limit the spread of resistance and reduce the impact of these extremely harmful bacteria.

Whole-genome sequencing (**WGS**) technology has attracted so much attention in the last decade, owing to its unique power in data generation, evolution and epidemiology study, microbiological risk assessment, and outbreak investigation. Scientists also use WGS to rapidly identify antimicrobial resistance genes (**ARG**) and gene clusters and mutations of these genetic elements in foodborne pathogens. Based on the WGS data, a novel trimethoprim-resistance gene *dfrA34* has been identified in *Salmonella* ser. Heidelberg (Tagg et al., 2018). In a Québec study, 65 of 69 (96.9%) *Salmonella* ser. Heidelberg isolates investigated contained $bla_{\text{CMY-2}}$ plasmids; 2 $bla_{\text{CMY-2}}$ plasmids were found to have been inserted into the chromosome and the CMY-2 plasmid transmission occurred among *Salmonella* ser. Heidelberg isolates with variable genetic backgrounds (Edirmanasinghe et al., 2017).

The National Center for Biotechnology Information Pathogen Detection system is a centralized Web-based portal that integrates the genomic sequence, metadata, antibiotic susceptibility and resistance gene information, and the SNP cluster information, widely used for outbreak investigation, source tracking, and epidemiologic studies of bacterial pathogens, such as *Salmonella, Campylobacter, Listeria, Escherichia coli,* and *Shigella* (https://www.ncbi.nlm.nih.gov/pathogens/).

The objectives of this study were 1) to investigate the prevalence of ARG in 143 isolates of 3 *Salmonella* serotypes (*Salmonella* ser. Enteritidis, *Salmonella* ser. Typhimurium, and *Salmonella* ser. Heidelberg) sourced from eggs and chickens, using WGS data and 2) to compare the differences regarding the occurrence and trends of ARG in these pathogens, highlighting the differences among the 3 serotypes investigated.

MATERIALS AND METHODS

Salmonella *Isolates*

A total of 143 *Salmonella* isolates from egg and chicken sources, including 64 *Salmonella* ser. Enteritidis collected from 1995 to 2016 (Table 1), 40 *Salmonella* ser. Typhimurium collected from 2001 to 2010 (Table 2), and 39 *Salmonella* ser. Heidelberg collected from 2003 to 2013 (Table 3), were used in this study. All isolates were in the collection of Division of Microbiology, Office of Regulatory Science, Center for Food Safety and Applied Nutrition (**CFSAN**), U.S. Food and Drug Administration (**FDA**). These isolates were cultured overnight at 37 ± 2°C in Trypticase Soy Broth (Becton Dickinson, Franklin Lakes, NJ) for DNA extraction.

Whole-Genome Sequencing

Isolates were cultured for 16 ± 1 h at 37 ± 2°C in Trypticase Soy Broth. Genomic DNA from each isolate was extracted and purified using the DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA). Concentrations of DNA were measured using a Qubit 3.0 fluorometer (Life Technologies, MD). Genomic DNA was sequenced on the Illumina MiSeq/NextSeq 500 platform following the manufacturer's instructions (Illumina, San Diego, CA). The Illumina reads were assembled de novo using CLC Genomics Workbench v9 (Qiagen Bioinformatics, Redwood City, CA). The WGS data of all isolates studied here can be searched and downloaded from the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) and Pathogen Detection System in the National Center for Biotechnology Information. The ARG were identified by the National Center for Biotechnology Information antimicrobial resistance finder process; all isolates were run against GenBank genomes in the Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047), which included more than 5,300 sequence data for identifying bacterial genomes with AMR genes.

RESULTS

Of the 143 *Salmonella* isolates that were investigated in this study (Tables 1–4), the highest rates of ARG were against aminoglycoside (70.63%), followed by

Table 1. Sources and antimicrobial resistance genes (ARG) of *Salmonella* ser. Enteritidis.

Isolates	Source	Location	Year	ARG
CFSAN057702	Eggs	Brazil	2000	N/A[1]
CFSAN057880	Egg	Brazil	2016	N/A
CFSAN025700	Whole eggs	US:IN	2012	N/A
CFSAN024840	Whole eggs	US:MN	2012	*aadA*
CFSAN025717	Whole eggs	US:AL	2012	*aadA*
CFSAN025708	Whole eggs	US:NJ	2012	*aadA*
CFSAN024814	Whole eggs	US:NH	2012	*aadA*
CFSAN024727	Poultry egg	Chile	2009	*qnrB19*
CFSAN024743	Poultry egg	Chile	2012	*bleO*
CFSAN017081	Frozen liquid egg	US:GA	2011	*aadA*
CFSAN057651	Cooked quail eggs	Brazil	1995	*aadA1, dfrA15, qacEdelta1, sul1*
CFSAN002042	Egg slurry	N/A	N/A	N/A
CFSAN057762	Pecked eggs/cecum	Brazil	2004	N/A
CFSAN057760	Pecked eggs/organs	Brazil	2004	N/A
CFSAN057767	Pecked eggs	Brazil	2005	N/A
CFSAN057769	Pecked eggs/yolk	Brazil	2005	N/A
CFSAN057773	Pecked eggs/yolk	Brazil	2005	N/A
CFSAN057775	Pecked eggs/organs	Brazil	2006	N/A
CFSAN057780	Pecked eggs/cecum	Brazil	2006	N/A
CFSAN057783	Pecked eggs/yolk	Brazil	2006	N/A
CFSAN057789	Pecked eggs/organs	Brazil	2007	N/A
CFSAN057791	Pecked eggs/organs	Brazil	2007	N/A
CFSAN057793	Pecked eggs/yolk	Brazil	2007	N/A
CFSAN057794	Pecked eggs/yolk	Brazil	2008	*dfrA25, sul1, tet*(A)
CFSAN057795	Pecked eggs/cecum	Brazil	2008	N/A
CFSAN057796	Pecked eggs/organs	Brazil	2008	N/A
CFSAN057812	Pecked eggs/cecum	Brazil	2009	N/A
CFSAN032971	Raw egg whites	US:IA	2012	N/A
CFSAN028530	Raw egg whites	US:NY	2012	*aadA*
CFSAN030835	Raw egg whites	US:CA	2012	*aadA*
CFSAN033541	Raw egg whites	US:PA	2013	N/A
CFSAN030081	Raw whole eggs	US:NJ	2012	N/A
CFSAN030086	Raw whole eggs	US:IA	2012	N/A
CFSAN032962	Raw whole eggs	US:IA	2012	N/A
CFSAN033543	Raw whole eggs	US:OH	2012	N/A
CFSAN035276	Raw whole eggs	US:NY	2012	*aadA*
CFSAN027378	Raw whole eggs	US:UT	2012	*aadA*
CFSAN027394	Raw whole eggs	US:NH	2012	*aadA*
CFSAN030823	Raw whole eggs	US:OH	2012	*aadA*
CFSAN035291	Raw whole eggs	US:NJ	2012	*aadA*
CFSAN034231	Raw whole eggs	US:GA	2013	N/A
CFSAN034232	Raw whole eggs	US:GA	2013	*aadA*
CFSAN035272	Raw whole eggs	US:TX	2013	*aadA*
CFSAN027377	Raw egg yolks	US:NJ	2012	*aadA*
CFSAN030066	Raw egg yolks	US:NY	2012	N/A
CFSAN030067	Raw egg yolks	US:IA	2012	*aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1}}$*, sul2, tet*(A)
CFSAN030097	Raw egg yolks	US:IN	2012	N/A
CFSAN030496	Raw egg yolks	US:GA	2012	*aadA*
CFSAN030816	Raw egg yolks	US:NJ	2012	*aadA*
CFSAN030839	Raw egg yolks	US:WA	2012	*aadA*
CFSAN030852	Raw egg yolks	US:NY	2012	*aadA*
CFSAN032958	Raw egg yolks	US:NJ	2012	N/A
CFSAN032959	Raw egg yolks	US:NJ	2012	*aadA*
CFSAN032964	Raw egg yolks	US:NY	2012	*aadA*
CFSAN032970	Raw egg yolks	US:IA	2012	*aadA*
CFSAN035289	Raw egg yolks	US:NJ	2012	*aadA*
CFSAN035308	Raw egg yolks	US:AL	2012	*aadA*
CFSAN035309	Raw egg yolks	US:IA	2012	*aadA*
CFSAN034151	Raw egg yolks	US:GA	2013	*aadA*
CFSAN057837	Chicken	Brazil	1990	N/A
CFSAN057838	Chicken	Brazil	1990	*aph(3″)-Ib, aph(6)-Id*
CFSAN057841	Chicken	Brazil	1993	N/A
CFSAN057814	Chicken	Brazil	2010	*dfrA25, qnrB2, sul1, tet*(A)
CFSAN058030	Chicken	US:NJ	2014	*aadA*

Abbreviations: AL, Alabama; CA, California; GA, Georgia; IA, Iowa; IN, Indiana; MN, Minnesota; NH, New Hampshire; NJ, New Jersey; NY, New York; OH, Ohio; PA, Pennsylvania; TX, Texas; UT, Utah; WA, Washington.

[1] No information available.

tetracycline (26.57%), fosfomycin (25.17%), sulfonamides (23.78%), and β-lactamases (15.38%) (Tables 1–3). The gene *aadA* (in 95 isolates) was the most frequently occurred ARG among the isolates, followed by gene *fosA* (36 isolates), *tet(A)* (32 isolates), and *sul2* (27 isolates). The ARG against aminoglycoside were very diverse, including genes *aadA, aadA1, aadA2, aac(3), aph(3)-IId, aph(3′)-II, aph(3′)-Ia, aph(3″)-Ib, aph(6)-Ic,*

Table 2. Sources and antimicrobial resistance genes (ARG) of *Salmonella* ser. Typhimurium.

Isolates	Source	Location	Year	ARG
CFSAN017093	Duck egg yolks (Cooked, Frozen)	China	2010	*aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1}}$*, bleO, tet*(A)
CFSAN017094	Duck egg yolks (Cooked, Frozen)	China	2010	*aadA, aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1}}$*, bleO, tet*(A)
CFSAN017095	Duck egg yolks (Cooked, Frozen)	China	2010	*aadA, aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1}}$*, bleO, tet*(A)
CFSAN015377	Frozen salted duck yolk	China	2002	N/A[1]
CFSAN015378	Frozen salted duck yolk	China	2002	*aadA, aph(3″)-Ib, aph(6)-Id, bla, bleO, tet*(A)
CFSAN015380	Frozen salted duck yolk	China	2002	N/A
CFSAN013737	Salted egg yolk	China (Taiwan)	2001	*sul2, tet*(A)
CFSAN014205	Salted duck eggs	China (Taiwan)	2004	*aadA*
CFSAN015282	Chicken jerky	China	2001	*aac(3)-IId, aadA1, aadA2, aph(3″)-Ib, aph(6)-Id, bla*$_{\text{OXA-1}}$*, catA1, dfrA12, qacEdelta1, sul1, sul2, tet*(B)
CFSAN027862	Chicken breast	US:CO	2005	*aadA*
CFSAN029083[2]	Chicken breast	US:GA	2006	*aadA, bla*$_{\text{CMY}}$
CFSAN029123	Chicken breast	US:MD	2006	*aadA, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN029101	Chicken breast	US:MD	2006	*aadA, aph(3′)-Ia, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN035417	Chicken breast	US:CA	2007	*aadA*
CFSAN035525[2]	Chicken breast	US:CT	2007	*aac(3), aadA, aadA1, bla*$_{\text{TEM-1}}$*, qacEdelta1, sul1, sul2, tet*(A)
CFSAN035560	Chicken breast	US:MD	2008	*aadA, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN035575	Chicken breast	US:NY	2008	*aadA, aph(3″)-Ib, aph(3′)-Ia, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN036172[2]	Chicken breast	US:MD	2008	*aadA, aph(3″)-Ib, aph(6)-Id, sul2, tet*(A)
CFSAN036174[2]	Chicken breast	US:NM	2008	N/A
CFSAN036179[2]	Chicken breast	US:NY	2008	*aadA, sul2, tet*(A)
CFSAN036177[2]	Chicken breast	US:NY	2008	*aph(3″)-Ib, bla*$_{\text{CMY-2}}$*, sul2, tet*(A)
CFSAN036183[2]	Chicken breast	US:PA	2008	*aadA, aph(3″)-Ib, aph(6)-Id, bla*$_{\text{CMY}}$*, sul2, tet*(A)*, tet*(B)
CFSAN036186[2]	Chicken breast	US:PA	2008	*aadA, sul2, tet*(A)
CFSAN036257[2]	Chicken breast	US:MD	2008	*aadA, aph(3′)-Ia, sul2, tet*(A)
CFSAN036272[2]	Chicken breast	US:MD	2008	*aadA, aph(3′)-Ia, sul2, tet*(A)
CFSAN036362 [2]	Chicken breast	US:NY	2008	*aadA, aph(3′)-Ia, sul2, tet*(A)
CFSAN036367[2]	Chicken breast	US:NY	2008	*aadA, aph(3″)-Ib, aph(6)-Id, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN041824[3]	Chicken breast	US:NY	2009	*aadA, sul2, tet*(A)
CFSAN041835 [3]	Chicken breast	US:NY	2009	*aadA, aph(3′)-Ia, bla*$_{\text{CMY}}$*, bla*$_{\text{TEM-1}}$*, sul2, tet*(A)
CFSAN041875[3]	Chicken breast	US:PA	2009	*sul2, tet*(A)
CFSAN041878[3]	Chicken breast	US:PA	2009	*aadA, aph(3′)-Ia, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN041887 [3]	Chicken breast	US:PA	2009	*aac(3), aadA, aadA1, qacEdelta1, sul1, sul2, tet*(A)
CFSAN040229	Chicken breast	US:MD	2009	*aadA*
CFSAN041925	Chicken breast	US:CT	2010	*aadA, aadA2, bla*$_{\text{CARB-2}}$*, floR, qacEdelta1, sul1, sul1delta, tet*(G)
CFSAN041934[3]	Chicken breast	US:GA	2010	*aadA, sul2, tet*(A)
CFSAN041947[3]	Chicken breast	US:MD	2010	*aadA, aph(3″)-Ib, bla*$_{\text{TEM-1}}$*, sul2, tet*(A)
CFSAN041940[3]	Chicken breast	US:MD	2010	*aadA, bla*$_{\text{CMY}}$*, sul2, tet*(A)
CFSAN041965[3]	Chicken breast	US:MN	2010	*aadA, sul2, tet*(A)
CFSAN041987[3]	Chicken breast	US:NY	2010	*aadA, aadA2, bla*$_{\text{CARB-2}}$*, floR, qacEdelta1, sul1, sul1delta, tet*(G)
CFSAN041662[3]	Chicken breast	US:TN	2010	*aadA, sul2, tet*(A)

Abbreviations: CA, California; CT, Connecticut; CO, Colorado; GA, Georgia; MD, Maryland; MN, Minnesota; NM, New Mexico; NY, New York; PA, Pennsylvania; TN, Tennessee.

[1] No information available.

[2] Typhimurium var. 5-.

[3] Typhimurium var. O:5-.

and aph(6)-Id. The ARG against β-lactamases included *bla, bla*$_{\text{CMY}}$*, bla*$_{\text{CMY-2}}$*, bla*$_{\text{TEM}}$*, bla*$_{\text{TEM-1}}$*, bla*$_{\text{OXA-1}}$, and *bla*$_{\text{CARB-2}}$. The ARG of *sul1, sul2, suldelta* were resistant to sulfonamides. The ARG of *tet*(A)*, tet*(B)*, tet*(C), and *tet*(G) were resistant to tetracycline.

Among the ARG studied, *Salmonella* ser. Enteritidis isolates had the highest rate of *aadA* against aminoglycoside (streptomycin). However, we did not find ARG among the *Salmonella* ser. Enteritidis isolates against fosfomycin and chloramphenicol (Table 4). Only 1 isolate of *Salmonella* ser. Enteritidis (CFSAN30067) had the β-lactamase resistant gene (*bla*$_{\text{TEM-1}}$). A few egg-sourced *Salmonella* ser. Enteritidis isolates were found to have ARG resistant to bleomycin (CFSAN024743, *BleO*) and quaternary ammonium compound (CFSAN057651, *qacEdeltal*). Only 1 egg-sourced (CFSAN024727, *qnrB19*) and 1 chicken-sourced (CFSAN057814, *qnrB2*) *Salmonella* ser. Enteritidis isolate were observed to have quinolone-associated ARG (Table 1). Other ARG, such as *tet*(A), *dfrA15, dfrA25*, were also found in *Salmonella* ser. Enteritidis isolates.

For *Salmonella* ser. Typhimurium, ARG against fosfomycin and quinolone were not detected in chicken-sourced isolates, and ARG against chloramphenicol, trimethoprim, and quaternary ammonium compound were not found in egg-sourced isolates (Table 4). The ARG *aadA, sul2,* and *tet*(A) were the most frequent ones contained in chicken-sourced isolates. We observed 8 and 5 different ARG against aminoglycoside and β-lactamases, respectively. Three chicken-soured isolates carried the *floR* (CFSAN041925 and CFSAN041987) and *catA1* (CFSAN015282) which confer resistance to

Table 3. Sources and antimicrobial resistance genes (ARG) of *Salmonella* ser. Heidelberg.

Isolates	Source	Location	Year	ARG
CFSAN015479	Egg salad	US:WA	2003	*aadA, fosA*
CFSAN024803	Egg yolks	US:IA	2012	*aadA, fosA*
CFSAN024816	Egg yolks	US:IA	2012	*aadA, fosA*
CFSAN024838	Egg yolks	US:IA	2012	*aadA, fosA*
CFSAN025710	Egg yolks	US:IN	2012	*aadA, fosA*
CFSAN024808	Whole eggs	US:SC	2012	*aadA, fosA*
CFSAN024830	Whole eggs	US:NJ	2012	*aadA, fosA*
CFSAN024821	Whole eggs	US:MI	2012	*aadA, fosA*
CFSAN025697	Whole eggs	US:AL	2012	*aadA, fosA*
CFSAN028512	Raw eggs whites	US:IA	2012	*aadA, fosA*
CFSAN033551	Raw eggs whites	US:NJ	2012	*aadA, fosA*
CFSAN033559	Raw eggs whites	US:IA	2013	*aadA, fosA*
CFSAN035286	Raw eggs whites	US:IA	2013	*aadA, fosA*
CFSAN027397	Raw whole eggs	US:SC	2012	*aadA, fosA*
CFSAN033547	Raw whole eggs	US:GA	2012	*aadA, fosA*
CFSAN034130	Raw whole eggs	US:MN	2012	*aadA, fosA*
CFSAN033536	Raw whole eggs	US:MN	2012	*aadA, fosA*
CFSAN033555	Raw whole eggs	US:GA	2013	*aadA, fosA*
CFSAN028516	Raw whole eggs	US:AL	2012	*aadA, fosA*
CFSAN035307	Raw whole eggs	US:IA	2012	*aadA, fosA*
CFSAN034223	Raw whole eggs	US:NJ	2013	N/A[1]
CFSAN035285	Raw whole eggs	US:IN	2013	*aadA, fosA*
CFSAN033552	Raw whole eggs	US:IN	2013	N/A
CFSAN034209	Raw whole eggs	US:AL	2013	N/A
CFSAN027390	Raw eggs yolks	US:IA	2012	*aadA, fosA*
CFSAN028528	Raw eggs yolks	US:IA	2012	*aadA, fosA*
CFSAN032955	Raw eggs yolks	US:AL	2012	*aadA, fosA*
CFSAN035301	Raw eggs yolks	US:IN	2012	*aadA, fosA*
CFSAN033560	Raw eggs yolks	US:IA	2013	*aadA, fosA*
CFSAN035287	Raw eggs yolks	US:AL	2013	*aadA, fosA*
CFSAN035479	Chicken breast	US:NM	2007	*aadA, fosA*
CFSAN035462	Chicken breast	US:CO	2007	*aadA, fosA*
CFSAN036211	Chicken breast	US:CT	2008	*aac(3), aadA, aadA1, fosA, qacEdelta1, sul1*
CFSAN036243	Chicken breast	US:GA	2008	*aadA, fosA*
CFSAN036278	Chicken breast	US:MN	2008	*aadA, bla*$_{\text{CMY}}$*, fosA*
CFSAN036283	Chicken breast	US:NM	2008	*aac(3), aadA, aadA1, fosA, qacEdelta1, sul1, tet*(C)
CFSAN036285	Chicken breast	US:NM	2008	*aadA, fosA*
CFSAN035554	Chicken breast	US:CA	2008	*aac(3), aadA, aadA1, aph(3″)-Ib, aph(3′)-II, aph(6)-Ic, aph(6)-Id, ble, fosA, qacEdelta1, sul1, tet*(B)
CFSAN041699	Chicken breast	US:CA	2010	*aadA, aph(3″)-Ib, aph(3′)-II, aph(6)-Ic, aph(6)-Id, ble, fosA, tet*(B)

Abbreviations: AL, Alabama; CA, California; CO, Colorado; CT, Connecticut; GA, Georgia; IA, Iowa; IN, Indiana; MI, Michigan; MN, Minnesota; NM, New Mexico; NJ, New Jersey; SC, South Carolina; WA, Washington.

[1] No information available.

chloramphenicol. And, 5 chicken-sourced isolates contained ARG *qacEdelta1.* In addition, isolates CFSAN041925 and CFSAN041987 were the only 2 isolates contained ARG *tet*(G) among all 143 isolates (Table 2).

Salmonella ser. Heidelberg isolates had high rates of ARG against aminoglycoside (90%) and fosfomycin (90%) (Table 4). A total of 27 of the 30 egg-sourced isolates only carried ARG *aadA and fosA*; 9 chicken-sourced isolates contained 14 ARG: *aadA, aadA1,aac(3),aph(3′)-II, aph(3″)-Ib, aph(6)-Ic, aph(6)-Id, bla*$_{\text{CMY}}$*, sul1,tet*(B)*, tet*(C)*, fosA, ble,* and *qacEdelta1.* Antimicrobial resistance genes *aadA* and *fos*(A) were the most prevalent ones among these *Salmonella* ser. Heidelberg isolates. However, these chicken-sourced isolates did not carry ARG against chloramphenicol, quinolone, and trimethoprim (Table 3).

Overall, more varieties of ARG were observed in chicken-soured isolates than egg-sourced isolates from *Salmonella* ser. Typhimurium (chicken: 23; egg: 8) and *Salmonella* ser. Heidelberg (chicken: 14; egg: 2). But, the opposite was true for *Salmonella* ser. Enteritidis, where chicken-soured isolates (7) contained fewer varieties of ARG than egg-sourced isolates (13) (Table 4).

There were 3 and 2 isolates with ≥ 5 ARG found from *Salmonella* ser. Typhimurium (CFSAN015282, CFSAN041925, CFSAN041987) and *Salmonella* ser. Heidelberg (CFSAN036283, CFSAN035554), respectively; there was no *Salmonella* ser. Enteritidis isolate in this category (Tables 1–3). Many isolates carried 4 ARG: 17 *Salmonella* ser. Typhimurium, 4 *Salmonella* ser. Enteritidis, and 2 *Salmonella* ser. Heidelberg isolates.

DISCUSSION

Prevalence of antimicrobial resistant bacteria has been increasing rapidly on US meat and poultry products in recent yr (North American Meat Institute (NAMI), 2019). This study investigated ARG of 3 major *Salmonella* serotypes associated with egg and chicken sources,

Table 4. Antimicrobial resistance genes of *Salmonella*.

	Salmonella ser. Enteritidis		*Salmonella* ser. Typhimurium		*Salmonella* ser. Heidelberg	
Antimicrobials	Egg and egg products (59 [1])	Chicken (5)	Egg and egg products (8)	Chicken (32)	Egg and egg products (30)	Chicken (9)

Aminoglycoside	*aadA*, 26[2];
	aadA1, 1;
	aph(3″)-Ib, 2;
	aph(6)-Id, 2
		aadA, 1;
		aph(3″)-Ib, 1;
		aph(6)-Id, 1
			aadA, 4;
			aph(3″)-Ib, 4;
			aph(6)-Id, 4
				addA, 28;
				aadA1, 3;
				aadA2, 3;
				aac(3), 2;
				aac(3)-IId, 1;
				aph(3′)-Ia, 7;
				aph(3″)-Ib, 7;
				aph(6)-Id, 4
					aadA, 27	*aadA*, 9;
						aadA1, 3;
						aac(3), 3;
						aph(3′)-II, 2;
						aph(3″)-Ib, 2;
						aph(6)-Ic, 2;
						aph(6)-Id, 2

β-Lactamases	*bla*$_{\text{TEM-1}}$, 1	0	*bla*, 1; *bla*$_{\text{TEM-1}}$, 3	*bla*$_{\text{CMY}}$, 10; *bla*$_{\text{CMY-2}}$, 1; *bla*$_{\text{TEM-1}}$, 3; *bla*$_{\text{OXA-1}}$, 1; *bla*$_{\text{CARB-2}}$, 2	0	*bla*$_{\text{CMY}}$, 1
Sulfonamides	*sul1*, 3; *sul2*, 1	*sul1*, 1	*sul2*, 1	*sul1*, 7; *suldelta*, 2; *sul2*, 25	0	*sul1*, 3
Tetracycline	*tet*(A), 2	*tet*(A), 1	*tet*(A), 5	*tet*(A), 24; *tet*(B), 2; *tet*(G), 2	0	*tet*(B), 2; *tet*(C), 1
Fosfomycin	0	0	0	0	*fosA*, 27	*fosA*, 9
Chloramphenicol	0	0	0	*floR*, 2 *catA1*, 1	0	0
Quinolone	*qnrB19*, 1	*qnrB2*, 1	0	0	0	0
Trimethoprim	*dfrA15*, 1; *dfrA25*, 2	*dfrA25*, 1	0	*dfrA12*, 1	0	0
Bleomycin	*bleO*, 1	0	*bleO*, 4	0	0	*ble*, 2
Quaternary ammonium compound (ethidium bromide)	*qacEdeltal*, 1	0	0	*qacEdeltal*, 5	0	*qacEdeltal*, 3

[1] Total number of isolates studied.

[2] Name of the antibiotics resistance gene, number of isolates containing the corresponding gene.

using WGS data. The high rates of ARG against aminoglycosides (particularly streptomycin), tetracycline, fosfomycin, and sulfonamides among these isolates are probably related to the extensive use of these antimicrobials in the poultry industry (WHO, 2011), as well as the inappropriate use of antimicrobial agents in both livestock and humans. From 2009 to 2015, in the United States, domestic sales and distribution of antimicrobials approved for use in food-producing animals increased by 24%, and tetracycline sales represent the largest volume of these domestic sales (U. S. Food and Drug Administration (FDA), 2016). The US FDA tested 4,072 imported foods in 2000, 187 *Salmonella* isolates consisting of 82 serotypes were found, 60% of the resistant isolates exhibited resistance to tetracycline, 47% to sulfonamides, and 33% to streptomycin (Zhao et al., 2003). Similar results have also been reported in other countries. For example, among nontyphoidal *Salmonella* isolates of poultry origin in Brazil (1995–2015), high frequencies of antimicrobial resistance were found for sulfonamides (44.3%), nalidixic acid (42.5%), tetracycline (35.6%), cefalotin (24.2%), and streptomycin (22.5%), and the highest resistance incidences in *Salmonella* ser. Enteritidis were against nalidixic acid (48.2%), sulfonamides (43.8%), tetracycline (32%), streptomycin (20.3%), and cefalotin (15.5%) (Voss-Rech et al., 2017). Extremely high resistance to ampicillin, sulfamethoxazole, and tetracycline was observed in monophasic *Salmonella* ser. Typhimurium isolates from carcasses of fattening pigs in Europe (EFSA and ECDC, 2017).

By binding to the bacterial 30S/50S ribosomal subunit, aminoglycosides could inhibit the translocation of the peptidyl-tRNA from A-site to P-site, causing RNA misreading and affecting protein synthesis. In bacteria, aminoglycoside resistances usually happen as the result of enzymatic inactivation by acetyltransferase (*aac*), nucleotidyltransferase/adenylyltransferases (*ant*), and phosphotransferases (*aph*) (Antibiotic Resistance Genes Database (ARDB), 2019). In this study, the genes associated with *ant* and *aph* were found in all aminoglycoside-resistant *Salmonella* isolates, but *aac* genes were only found in *Salmonella* ser. Typhimurium and *Salmonella* ser. Heidelberg from chicken-sourced isolates. Our data also showed gene *aadA* was the most frequent ARG occurring among the isolates studied, followed by gene *aph(3″)-Ib*. Chen et al. (2004) reported that among 133 *Salmonella* isolates from retail meats in the United States and China, *aadA1* gene was detected most frequently among 6 ARG against aminoglycoside (*aadA1, aadA2, aacC2, Kn, aph(3)-IIa, and aac(3)-Iva*) (Chen et al., 2004). The study also found other ARG, such as *tet*(A), *tet*(B), *dhfr1, dhfr12, dhfr13, cat1, cat2,* *bla*$_{\text{TEM-1}}$, and *bla*$_{\text{CMY-2}}$.

Our study only found quinolone-associated ARG in 1 egg-sourced and 1 chicken-sourced *Salmonella* ser. Enteritidis isolates; all isolates from *Salmonella* serotypes Typhimurium and Heidelberg did not contain

ARG against quinolone, although previous research showed a rise in quinolone resistance in *Salmonella* isolates associated with contaminated eggs and egg products in Europe during a 5-year survey from 2000 to 2004 (Meakins et al., 2008). The study also found a decreased occurrence of chloramphenicol and tetracyclines resistance in *Salmonella* ser. Typhimurium isolates. Our data indicated that resistance to chloramphenicol was associated with *Salmonella* ser. Typhimurium isolates from chicken origin only and fosfomycin with *Salmonella* ser. Heidelberg isolates from either egg or chicken origins. The use of different antimicrobials in different parts of the world may have contributed to this phenomenon, as well as the antimicrobial resistance variation by *Salmonella* serotypes (CDC, 2018b).

The present study also indicated that chicken-sourced isolates contained more diverse ARG than egg-sourced and egg products–sourced isolates among *Salmonella* ser. Typhimurium and *Salmonella* ser. Heidelberg. There were 5 *Salmonella* isolates from both chicken and egg sources with ARG against ≥5 antimicrobials in this study; none of them were from *Salmonella* ser. Enteritidis. Furthermore, among the isolates studied, many carried ARG against 4 antimicrobials, particularly the isolates from *Salmonella* ser. Typhimurium. Borges et al. (2017) analyzed 148 *Salmonella* ser. Enteritidis strains and found only 25 (16.9%) were susceptible to all antimicrobials tested, and poultry strains presented higher resistance and a greater number of multidrug resistance than those isolated from food involved in salmonellosis. Zhao et al. (2003) reported that 2 *Salmonella* ser. Typhimurium isolated from ground chicken exhibited resistance to 12 antibiotics among the 18 identified *Salmonella* resistant isolates from imported food in United States. In the European Union, 29.3% of human *Salmonella* isolates exhibited multidrug resistance, especially for the monophasic *Salmonella* ser. Typhimurium 1,4,[5],12:i:-, which had an extremely high rate of multidrug resistance of 81.1% (EFSA and ECDC, 2017). However, the relationship between antimicrobial resistance and multiresistance isolates from humans and animals are often confounded by the selected isolates with different serotypes, geographical locations, or temporal intervals (Carroll et al., 2017). The mechanisms of multiresistance are highly intricate. The use of different antibiotics in the life cycle of food animals make it more likely that *Salmonella* harbored by the animals might be resistant to common antibiotics, and the genes that encode these antibiotic resistances can also be transferred to human pathogens. With the increased resistance to conventional antibiotics (e.g., ampicillin and chloramphenicol), the extended-spectrum cephalosporins and fluoroquinolones were used more widely as the treatment of infections caused by multidrug-resistant *Salmonella* serotypes (Chen et al., 2004). Consequently, *Salmonella* is adapting to these drugs and developing resistance to them.

It would be great we could verify the ARG by traditional phenotypic antibiotic susceptibility testing and determine the minimum inhibitory concentrations for the antimicrobials. The information will help scientists better understand the expression or lack of expression of these ARG. However our WGS data were accumulated in a few years, we could not locate all the isolates anymore. This topic is worth future study as it is important to prove the correlation between the genotypic and phenotypic resistances. It has practical implications for developing prevention and control strategies for antimicrobial resistant bacteria.

In summary, among the 143 *Salmonella* isolates studied, high rates of ARG against aminoglycoside (particularly streptomycin), tetracycline, fosfomycin, sulfonamides, and β-lactamases were observed; *Salmonella* ser. Typhimurium isolates contained more variety of ARG and higher level of multiresistance than *Salmonella* ser. Enteritidis and *Salmonella* ser. Heidelberg; Chicken-sourced isolates contained more different types of ARGs than egg-sourced and egg products–sourced isolates among *Salmonella* ser. Typhimurium and *Salmonella* ser. Heidelberg. Widespread *Salmonella* antimicrobial-resistant isolates sourced from egg and chicken underline the urgent need for continued both consumer and workers/farmers education and efforts on proper animal raising and food handling/cooking to decrease/eradicate *Salmonella* in poultry and egg products. This also demonstrates the importance of One Health Initiative, which is a collaborative, multisectoral, and transdisciplinary approach, recognizing the interconnection among animals, plants, people, and their shared environment, with the collaboration at the local, regional, national, and global levels, to achieve optimal health outcomes (https://www.cdc.gov/onehealth/index.html).

DISCLOSURES

The authors declare no conflicts of interest. Mention of trade names or commercial products in the article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the US Food and Drug Administration.

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