ANTIMICROBIAL RESISTANCE PATTERNS AND PATHOGEN WHOLE GENOME SEQUENCING OF CHICKEN CARCASS RINSE SAMPLES COLLECTED DURING PROCESSING

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I. INTRODUCTION

Despite constant efforts to improve poultry food safety systems; foodborne illness, and particularly cases associated with antimicrobial resistant pathogenic strains continue to be a serious health threat associated with poultry worldwide [1]. The pathogenic diversity and antimicrobial resistance (AMR) profiles of *Salmonella* spp. and *Campylobacter* spp. linked to poultry production systems [2] and how they are affected by multiple antimicrobial interventions at different stages during processing are not fully understood and significant research gaps in understanding mechanisms and factors that contribute to microbial resistance diversity and transmission to animals and humans still exist. Hence, the objective of this study is to assess the microbial profiles and pathogen prevalence of a large commercial poultry processing plant during a typical processing day and evaluate the AMR profiles of *Salmonella* spp. and whole genome sequencing of isolated pathogens to identify the presence of AMR genes from chicken samples collected as they move through the processing value chain.

II. MATERIALS AND METHODS

In total 110 rinses (95 whole chicken carcasses and 15 chicken parts) and 6 pooled fecal samples were collected throughout processing in a poultry processing plant located in the Southeast United States. Enumeration and detection was performance based on the Microbiology Laboratory Guidebooks: 3.02 (indicator microorganisms), 4.09 (*Salmonella*), and 41.09 (*Campylobacter*). Antimicrobial susceptibility testing (AST) was performed with 58 *Salmonella* isolates following the National Antimicrobial Resistance Monitoring System protocol and Whole Genome Sequencing was conducted with isolated *Salmonella* strains. Whole genome sequencing was performed extracting and quantifying the genomic DNA. Libraries were prepared using the Nextera XT kit following the sequencing using a 2x250bp cartridge and a MiSeq Reagent kit V2. Assembled contigs files were then run into different pipelines available on the Center for Genomic Epidemiology website. Statistical analysis for microbial counts was conducted with a non-parametric test Kruskal-Wallis followed Dunn's test multiple comparison tests with a *P*-value of 0.05. Prevalence and AST of *Salmonella* and *Campylobacter* were assessed using a Chi-square test to determine statistical relationship between the processing sites. Statistics were performed using GraphPad Prism 7.03.

III. RESULTS AND DISCUSSION

The overall prevalence of *Salmonella* spp. was 55.17% (64/116; CI, 45.6 to 64.4%), and for *Campylobacter* sp. the prevalence was 12.93% (15/116; CI, 0.76 to 20.7%) (Table 1). As illustrated in Table 1, the prevalence of both pathogens decreased through harvest steps to chilling but increased on parts. From the initial bacterial load (arrival) to the final process tested (parts) the counts of the indicator organisms decreased as APC from 7.72 to 4.44 Log CFU/ml; *E. coli* from 6.16 to 1.98 Log CFU/ml, coliforms from 6.22 to 2.16 Log CFU/ml, and *Enterobacteriaceae* from 5.97 to 2.04 Log CFU/ml. The ARM patterns showed that 1.7% (1/58; CI, 0-10%) were pan-susceptible, and 98% (57/58; CI, 89-99%) of the *Salmonella* isolates presented resistance to at least one antimicrobial agent, among them 30% (17/57; CI,19- 44%) presented a multi-drug resistance phenotype (Table 2). Resistance to tetracycline 91% (52/57; CI, 80-97%), and streptomycin 89% (51/57; CI, 79-95%) represented the higher resistance patterns (Fig. 1). The WGS results of 55 *Salmonella* isolates recovered identified the presence of 15 different clones, including eight clones

attributed to the serotype Kentucky, one to Typhimurium, one to Meleagridis, one to the serotype 40:b:- of the subspecies *salamae* (II), two incomplete antigenic formulae (13:-:- and 50:-:-; S.e. 4), and two to an undetermined serotype (S.e.5 and S.e.6).

Table 1. Prevalence of *Salmonella* and *Campylobacter* recovered from fecal material and chicken carcass rinsates collected at different processing sites during a poultry processing day.

Process site	*Salmonella*		*Campylobacter*	
	No. samples positive (% positive)	95% CI	No. samples positive (% positive)	95% CI
Fecal material				
Arrival	4/6 (67)	24 - 94	6/6 (100)	54 - 100
Chicken carcass rinse				
Pre-scalder	15/15 (100)	74 - 100	0/15 (0)	0 - 25
Brushes	5/5 (100)	46 - 100	1/5 (20)	0.10 - 70
Post-sclader	13/15 (87)	58 - 98	5/15 (33)	0.13 - 61
Post-intervention	7/15 (47)	22 - 73	1/15 (7)	0.34 - 34
Post- evisceration	7/15 (47)	22 - 73	0/15 (0)	0 - 25
Pre-chiller	1/15 (7)	0.34 - 34	0/15 (0)	0 - 25
Chiller	0/15 (0)	0 - 25	0/15 (0)	0 - 25
Parts	12/15 (80)	51 - 94	2/15 (13)	0.23 - 42

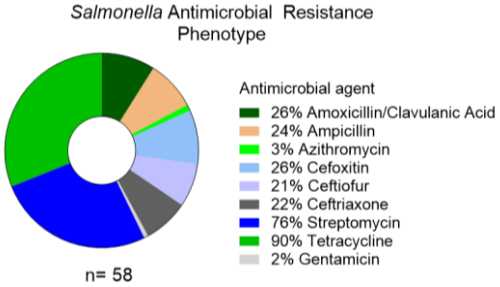

Figure 2. Antimicrobial resistance phenotypes of *Salmonella* isolates recovered throughout the poultry processing

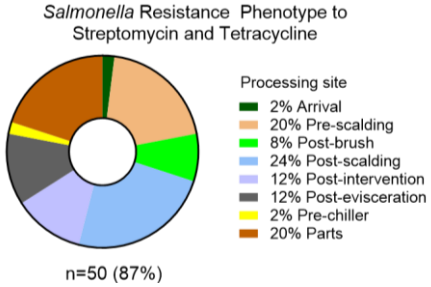

Figure 3. Antimicrobial resistance phenotypes to streptomycin and tetracycline of *Salmonella* isolates recovered throughout the different processing sites.

Table 2. *Salmonella* multi-drug resistance phenotypes (resistance to ≥ 3 antimicrobial agents). STR: Streptomycin; TET: Tetracycline; GEN: Gentamicin; AZI: Azithromycin; AUG: Amoxicillin/Clavulanic; AMP: Ampicillin; FOX: Cefoxitin; AXO: Ceftriaxone; TIO: Ceftiofur.

Multi-drug Resistance Phenotypes (% of resistance)	Processing site (No. of isolates)	Serotype	AMR genes
STR, TET, AZI (2%;1/57)	Post-scalding (n=1)	Kentucky	strA strB tet(B)
STR, TET, AUG, AMP, FOX (2%; 1/57)	Post-scalding (n= 1)	Kentucky	strA strB tet(B)
STR, TET, AUG, AMP, FOX, TIO, AXO (25%; 14/57)	Arrival (n =1)	Kentucky	strA strB blaCMY-2 tet(B)
	Pre-scalding (n= 4)	Kentucky, S.e. 5	strA strB blaCMY-2 tet(B)
	Post-scalding (n= 1)	S.e. 6	strA strB tet(B)
	Post-brush (n=1)	Kentucky	strA strB blaCMY-2 tet(B)
	Post-evisceration (n= 4)	Kentucky	strA strB blaCMY-2 tet(B)
	Parts (n= 3)	Kentucky	strA strB blaCMY-2 tet(B)
STR, TET, AUG, AMP, FOX, TIO, AXO, GEN, FIS (2%; 1/2)	Post-evisceration (n=1)	S.e. 4	blaACT-16 fosA

IV. CONCLUSION

The presence of AMR pathogens in the poultry chain remains an important public health concern. The potential presence of multi-resistance to antimicrobials in pathogens associated with poultry have motivated recent regulatory changes for the judicious use of antimicrobials in animal production. This project provides a more comprehensive understanding of the population dynamics and pathogen changes that occur in a poultry processing plant, as the process introduces a series of hurdles and potential selective pressures that modify the pathogenic profiles and antimicrobial resistance patterns of final poultry meat products.

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