Antimicrobial Resistance in *E. coli* and *Salmonella* from Small Holder Chickens

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Introduction

- Antimicrobial resistance (AMR) is commonly found in *Escherichia coli* and *Salmonella* isolated from ceca of recently slaughtered poultry (here, chickens 1-2 kg dressed).
- In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors the prevalence of AMR in generic *E. coli* and *Salmonella* of cecal origin from large holder, commercial poultry flocks slaughtered in federally inspected abattoirs.

Figure 1. A Small Holder Flock Raised in a Producer's Backyard

- No programs currently exist in Canada that routinely monitor AMR in bacteria from poultry raised using small holder (<300 birds) production practices and slaughtered in provincially inspected abattoirs.
- Until 2013, CIPARS did not routinely monitoring extended spectrum beta-lactamase (ESBL)-producing *E. coli* in abattoir cecal samples.

Objectives

- To determine the prevalence of AMR in generic *E. coli* and *Salmonella* from small holder poultry flocks, and flock-level prevalence of ESBL-Producing *E. coli*. We hypothesized that antimicrobial resistance would be more prevalent in the enteric bacteria of chickens from small holder flocks than in those from large commercial flocks, i.e., those routinely monitored by CIPARS, due to the possibility of reduced veterinary oversight and limited biosecurity.

Methods

- Chicken viscera were obtained from small holder flocks containing less than 300 birds, and slaughtered in six provincially-inspected abattoirs located in Ontario. For each flock sampled, the viscera from five birds were collected.
- Ceca were removed, incised as depicted in Figure 2, and the cecal contents frozen at -80ºC until isolation.
- Primary isolation of *E. coli* and *Salmonella* utilized enrichment, selective plating, and biochemical/confirmation testing.
- Primary isolation of ESBL-producing *E. coli* was performed by selective plating using CHROMagar™, biochemical/confirmation testing for generic *E. coli*, followed by disk diffusion using the CLSI initial screen test for ESBL-producing *E. coli* guidelines. Isolates were phenotypically characterized as ESBL-producing if susceptible to amoxicillin/clavulanic acid, and resistant to ampicillin and at least one of ceftriaxone, cefoxitin or ceftiofur.
- Overall bacterial prevalence rates and antimicrobial susceptibility results were compared to results from chickens slaughtered at 22 federally registered abattoirs in 2012 using data from the CIPARS 2012 Annual Report Government of Canada.[1]

1. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2012 Annual Report – Chapter 2. Antimicrobial Resistance. Public Health Agency of Canada, Guelph, Ontario, 2014.

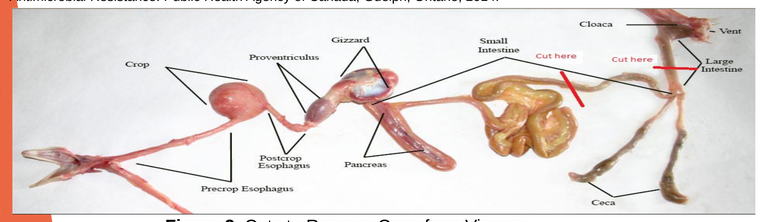

Figure 2. Cuts to Remove Ceca from Viscera

(http://www.geauga4h.org/poultry/chicken_digestion.htm)

- Isolates were sent to the CIPARS AMR Lab at the Laboratory for Foodborne Zoonoses, National Public Health Laboratories, Public Health Agency of Canada for antimicrobial susceptibility testing using the Sensititre automated broth microdilution system and the National Antimicrobial Monitoring System (NARMS) CMV3AGNF panel using established breakpoints.

Results

- 161 small holder poultry flocks were sampled and 805 cecal samples were collected.
- Recovery rates for *E. coli* and *Salmonella* are depicted in Figure 3. CIPARS recovery rates for *E. coli* was 100% (173/173) and for *Salmonella* was 18% (126/684), while our results for *E. coli* and *Salmonella* were 99% (803/805) and 0.4% (3/805) respectively.

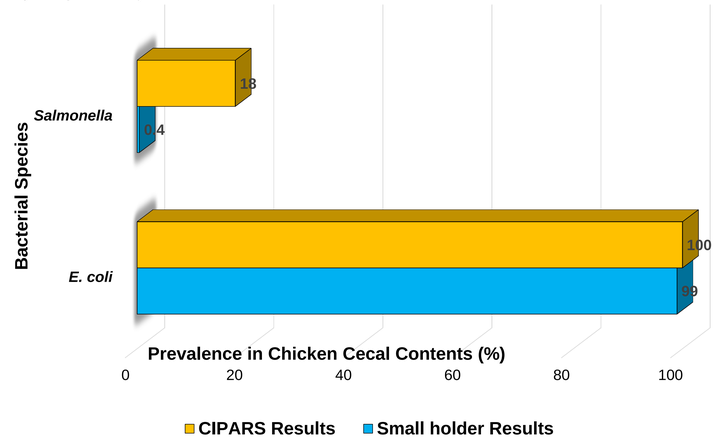

Figure 3. Recovery of *E. coli* and *Salmonella* in Chicken Cecal Contents; CIPARS Abattoir vs Chickens from Small-Holder flocks (data from CIPARS 2012 Annual Report).

- To date, antimicrobial susceptibility test results have been completed for 196 *E. coli* isolates, and the resistance data is presented in Figure 4.

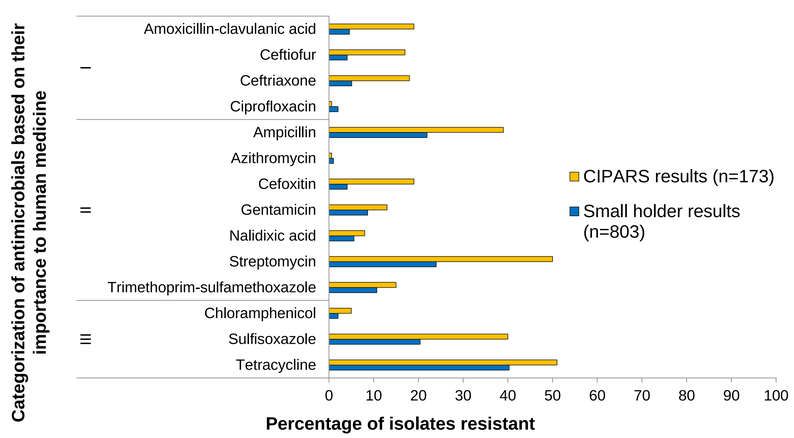

Figure 4. Antimicrobial resistance prevalences among *E. coli* Isolated from chickens from small holder flocks and commercial flocks (data from CIPARS 2012 Annual Report).

prevalence estimates are unadjusted for clustering at the flock level

- *Salmonella* (n=3) was recovered from samples from two flocks. All three isolates were identified as serovar Kentucky, also the most common serovar among the CIPARS isolates (41%) followed by Heidelberg (25%). Resistance to streptomycin and tetracycline was found in all three of our isolates. By contrast, resistance to 11/15 antimicrobials was observed among the CIPARS isolates: streptomycin & tetracycline, both 40% (51/126), ampicillin (24%), amoxicillin/clavulanic acid, ceftriaxone and ceftiofur (all 20%), cefoxitin (19%) sulfisoxazole (6%), trimethoprim-sulfamethoxazole & chloramphenicol (2%), and kanamycin (1%).
- Of the 805 ceca samples, 59 ESBL-producing presumptive *E. coli* were initially identified using the primary isolation technique.
- The phenotypic results are shown in Table 1.

Table 1. Putative ESBL-producing *E. coli* phenotypic results

	AMC	AMP	FOX	TIO	CRO
Resistant Isolates	11	59	10	59	59
Susceptible Isolates	48	0	49	0	0

- Of the 59 isolates initially submitted for susceptibility testing, 48 showed phenotypic ESBL characteristics (molecular confirmation is to be conducted).
- A total of 7% were considered possible ESBL-producing *E. coli* isolates out of the 805 samples tested.
- The possible ESBL-producing *E. coli* isolates were recovered from ceca belonging to 23 unique flocks. The distribution of cecal samples per flock is shown in Figure 5.

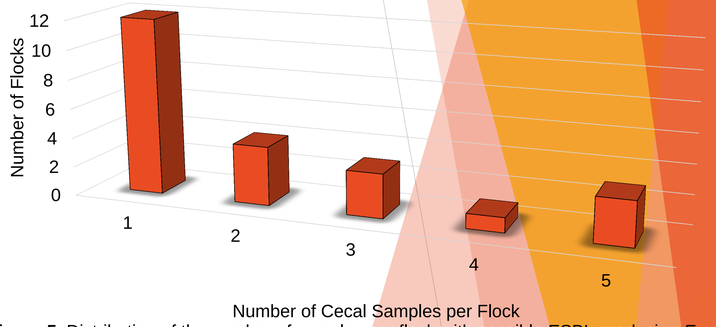

Figure 5. Distribution of the number of samples per flock with possible ESBL-producing *E.coli*

Conclusions

- The point estimate of *Salmonella* prevalence was considerably lower in the small holder flocks in our study than in the larger commercial flocks sampled for CIPARS. Similarly, point estimates of AMR prevalence among generic *E. coli* and *Salmonella* isolates from the small holder flocks are lower than seen in CIPARS results. These findings suggest that we will reject our original hypothesis. We are undertaking a survey of the small holder flock owners in order to explore risk factors for antimicrobial resistance in *E. coli* from their flocks. Sampling is currently ongoing for the 2015, the second of two planned sampling years. Our next steps will be to conduct a molecular characterization of antimicrobial resistance genes in the isolates.
- The finding, using selective media, of possible ESBL-producing *E. coli* is of some concern as they could be present in the small holder flocks with unknown use of antimicrobials.
- The use of selective media is a useful adjunct to the routine non-selective methods used for surveillance for identifying potential ESBL-producing *E. coli* that could otherwise be missed. Our next steps include molecular characterization of the potential ESBL-producing *E. coli* isolates.
- Smaller flock sizes, infrequent antimicrobial use, and free-range management may contribute to these results. Small holder management as conducted in Ontario may contribute to the low *Salmonella* prevalence, but our sample size may be too small to identify risk factors.

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