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.
- No programs currently exist in Canada that routinely report 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.
- Cecae were removed, incised as depicted in Figure 2, and the cecal contents frozen at -80°C 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 cefotaxin.
- Overall bacterial prevalence rates and antimicrobial susceptibility results were compared to results from a 2012 Canadian integrated abattoir surveillance study using data from the CIPARS 2012 Annual Report Government of Canada.¹

Results
- 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.
- 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.
- 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 cefotax (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 screening technique.
- The phenotypic results are shown in Table 1.

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 may 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 may 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.

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

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Susceptible Isolates</th>
<th>Resistant Isolates</th>
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</thead>
<tbody>
<tr>
<td>AMC</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>AMP</td>
<td>0</td>
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<tr>
<td>FOX</td>
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<tr>
<td>TIO</td>
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<td>0</td>
</tr>
<tr>
<td>CRO</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
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Figure 1. A Small Holder Flock Raised in a Producer's Backyard

Figure 2. Cuts to Remove Ceca from Viscera (http://www.geaqua4d.org/poultry/chicken_digestion.html)

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).

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

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

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