Prevalence and antimicrobial resistance among *Escherichia coli* and *Salmonella* in Ontario smallholder chicken flocks

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**Summary**

Surveillance is an important component of an overall strategy to address antimicrobial resistant bacteria in food animals and the food chain. The poultry market has many points of entry into the Canadian food chain, and some production practices are underrepresented in terms of surveillance. For example, pathogen carriage and antimicrobial resistance surveillance data are limited in smallholder chicken flocks raised for slaughter at provincially inspected abattoirs. In Canada, antimicrobial resistance in *Escherichia coli* and *Salmonella* isolated from commercial broiler chicken flocks, slaughtered at federally inspected abattoirs, is monitored by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). The objective of this study was to establish baseline information of antimicrobial resistance presence in *E. coli* and *Salmonella* isolated from smallholder flocks in Ontario, utilizing CIPARS collection and isolation methodologies, and to compare findings with CIPARS federally inspected abattoir data from Ontario, Canada. Five chickens per flock were sampled from 205 smallholder flocks. Of 1,025 samples, the *E. coli* prevalence was 99% (1,022/1,025), and 47% (483/1,022) of positive *E. coli* isolates were resistant to one or more of the 14 antimicrobials. Furthermore, as compared to results reported for the CIPARS commercial flocks, *E. coli* isolates from smallholder flocks had significantly lower resistance prevalence to six of 14 individual antimicrobials. Recovery of *E. coli* did not differ between federally inspected and provincially inspected flocks. *Salmonella* prevalence at the bird level in smallholder flocks was 0.3% (3/1,025), significantly lower ($p < 0.0001$, 95% CI 0.080%–0.86%) than federally inspected commercial flocks. The overall differences found between the commercial and smallholder flocks may be explained by differences in poultry husbandry practices and hatchery sources.

**KEYWORDS**

β-lactam, abattoir, Canadian Integrated System for Antimicrobial Resistance Surveillance, chickens, smallholder flocks, surveillance

1 | INTRODUCTION

In Ontario, producers may raise chickens for personal consumption, but they must not have a flock exceeding 300 birds, defined as being a smallholder flock (Chicken Farmers of Ontario, 2016). Smallholder flock producers source their chicks from Health Monitored Hatcheries (i.e., may have smaller incubation capacity than commercial hatcheries) and may hatch various poultry species (Government of Canada,
2016b) or source these from commercial hatcheries, controlled by the Canadian Hatcheries Federation (CHF) members (Canadian Poultry and Egg Processors Council, 2017). As in CHF member hatcheries, Health Monitored Hatcheries can source hatching eggs or chicks from imported suppliers with certain restrictions, such as the number of hatching eggs/chicks indicated in their permits (Government of Canada, 2016a).

The continuing increase in the occurrence and severity of antimicrobial resistant bacterial infections has become a global public health concern (Laxminarayan et al., 2013). Antimicrobial resistant infections can be more difficult to treat and thus may result in poorer clinical outcomes (Laxminarayan et al., 2013). Resistance to multiple antimicrobials is increasingly common, with some bacterial strains resistant to most if not all available antimicrobial classes (Liu et al., 2015). Antimicrobial resistance (AMR) in Salmonella spp. and Escherichia coli infections in people via food animals poses an additional concern as therapeutic options become limited (Beceiro, Tomás, & Bou, 2013). Commensal strains of E. coli are widely found in the gastrointestinal tracts of many animal species, including birds. Some of these strains can cause infections, as well as act as important reservoirs of resistance genes for Salmonella and other bacterial pathogens (Winfield & Groisman, 2003). Antimicrobial use in food animal production contributes to the selection pressure and spread of antimicrobial resistance in these bacteria (Marshall & Levy, 2011) which can subsequently serve as a source of resistance genes downstream of the food production continuum, ultimately posing a food safety and public health threat (Winfield & Groisman, 2003).

Escherichia coli and Salmonella are used in various surveillance programmes across the world to monitor antimicrobial resistance in the food chain, in particular, the National Antimicrobial Resistance Monitoring System (NARMS) in the USA, the Danish Programme for surveillance of antimicrobial consumption and resistance in bacteria from animals, food and humans (DANMAP) and the Canadian Integrated System for Antimicrobial Resistance Surveillance (CIPARS) in Canada (Government of Canada, 2015b). CIPARS (Government of Canada, 2015b) determines the prevalence of bacteria (E. coli and Salmonella) commonly isolated from food animals, including chickens, and monitors AMR in various species of animals through surveillance of three levels of the food chain: on-farm, abattoir and retail (Government of Canada, 2015b). On-farm surveillance monitors antimicrobial resistance in bacteria from faecal samples to provide surveillance data at the production level and to enable estimation of associations between husbandry practices, including antimicrobial use, and antimicrobial resistance (Government of Canada, 2015b). At the abattoir level, caeca are sampled in order to reflect transmission of monitored antimicrobial resistance through the food chain prior to entry into the retail segment (Government of Canada 2015b). Retail food samples are included to provide exposure estimates close to the point of consumption (Government of Canada, 2015b). Escherichia coli, and to a lesser extent Salmonella, are frequently isolated from chickens, from farm to retail, and in clinically infected birds (Salmonella) (Government of Canada, 2015b). Smallholder flocks may serve as a vehicle for the spread of foodborne bacteria such as Salmonella to people through handling and close contact to rearing facilities (Government of Canada, 2015b).

In Canada, inspection of processing of commercial and smallholder flocks typically differs; there are two levels of inspection within abattoirs: federal and provincial. Provincialy inspected abattoirs are generally smaller and have lower slaughter capacity compared to federally inspected abattoirs (Ontario Ministry of Agriculture Food and Rural Affairs, 2015). In Ontario, most of the large commercial broiler chicken flocks are slaughtered in federally inspected abattoirs. CIPARS slaughter-level samples are collected from federally inspected abattoirs, following a more traditional model of the food chain in Canada. Birds from smallholder or “backyard” flocks are typically processed under provincial inspection and therefore are not represented in national CIPARS surveillance. This may obscure the impact of raising smallholder flocks. Those who raise smallholder flocks may be in direct contact with their birds and the microbial connection is not currently observed.

Escherichia coli and Salmonella isolated from CIPARS flocks are tested for antimicrobial resistance against a panel of fourteen antimicrobials. No such testing process is currently performed on smallholder chicken flocks slaughtered in provincial abattoirs in Canada. The objectives of this study were to determine the prevalence of antimicrobial resistance among E. coli and Salmonella from smallholder chickens slaughtered in provincially inspected abattoirs in Ontario, and to make comparisons with results from CIPARS abattoir component.

**Impacts**

- Ontario smallholder chicken flocks had a significantly lower prevalence of Salmonella than commercial flocks from federally inspected abattoirs.
- β-Lactam resistance was detected from both commercial and smallholder flocks, but prevalences were not significantly different. There was no use of β-lactam or related antimicrobials to explain these findings, but operational factors (e.g., historical use, use in parent flocks) may have played a role in the dissemination and maintenance of resistance genes.
- General husbandry practices and flock densities (i.e., large commercial flocks versus smallholder flocks) may drive antimicrobial use and antimicrobial resistance development.

**2 | MATERIALS AND METHODS**

To the extent possible, the study was designed to make the results comparable to those reported by CIPARS; therefore, sampling and isolation protocols were based on the CIPARS methods used for
abattoir-level surveillance of antimicrobial resistance (Government of Canada, 2015c).

### 2.1 Study flocks and abattoirs

A list of provincially inspected abattoirs (n = 18) that process smallholder chickens, located within a 3-h driving radius of Guelph, Ontario, were invited to participate. Sampling was conducted between May and September in 2014 and 2015 during the main smallholder flock production season. During each sampling visit, five non-sequential viscera samples were collected from five chickens belonging to one flock and placed into individual leak-proof zipper bags. Caeca were separated out from the viscera upon arrival to the laboratory and caecal contents aseptically extracted.

An aliquot of each caecal sample was preserved at −80°C in Brucella broth and glycerol until testing was conducted.

### 2.2 Smallholder flock data

Farm-level data was obtained by questionnaires that were distributed to producers by mail along with stamped, self-addressed envelopes or administered in person at the abattoir if a producer was present and consented. The study protocol and questionnaire were approved by the University of Guelph Research Ethics Board. The two-page questionnaire was designed to collect basic flock- and farm-level information/operational factors (e.g., bird age, weight, breed), hatchery source, husbandry practices (e.g., vaccination history, antimicrobial use, feed sources, disinfection, pest control practices, waste disposal) and flock health.

### 2.3 E. coli isolation

Controls for bacterial cultures were prepared using small suspensions of E. coli ATCC#25922 and S. Typhimurium ATCC#14028, each in 4.5 ml of buffered peptone water (BPW). Controls were processed in the same manner as the samples.

Aliquots of thawed caecal sample were plated onto MacConkey agar incubated at 37°C for 24 hr. Suspected E. coli colonies were subcultured onto MacConkey agar and incubated at 37°C for 24 hr. One presumptive colony from each sample was further plated onto tryptic soy agar (TSA) and incubated at 37°C for 24 hr. Putative E. coli colonies were verified by positive indole and negative citrate test results.

### 2.4 Salmonella isolation and serotyping/phage typing

Once thawed, 0.5 ml of caecal content mixture was inoculated into 4.5 ml of BPW and incubated at 37°C for 24 hr. A 0.1-ml aliquot of broth was inoculated into modified semi-solid Rappaport-Vassiliadis (MSRV) agar and incubated at 42°C for 24–72 hr. Presumptive positive samples were identified by an area of white turbidity that extended out from the point of inoculation. Presumptive positive samples were inoculated onto xylose lactose tergitol-4 (XLT-4) agar and MacConkey agar and incubated at 37°C for 24 hr. Suspected Salmonella isolates were subcultured from MacConkey agar onto a second MacConkey agar plate for purity, and subsequently plated onto TSA for further biochemical testing. Colonies on XLT-4 were observed for typical Salmonella growth. Negative urease utilization, positive triple sugar iron (TSI) and positive Salmonella Poly A-1 + VI agglutination test results confirmed Salmonella isolates. Presumptive isolates were submitted to the OIE Salmonella reference laboratory at the National Microbiology Laboratory in Guelph (formerly the Laboratory for Foodborne Zoonoses), Public Health Agency of Canada. Serotyping and phage typing were carried out according to published methods (Government of Canada, 2015c).

### 2.5 Antimicrobial susceptibility testing

Escherichia coli and Salmonella isolates were sent to the National Microbiology Laboratory in Guelph, Ontario. Susceptibility testing was performed using the Sensititre automated broth microdilution system and the NARMS CMV3AGNF panel with established breakpoints (Government of Canada, 2015c). Escherichia coli ATCC#25922 and S. Typhimurium ATCC#14028 were used as quality control organisms. The 14 antimicrobials on the panel and their respective resistant minimum inhibitory concentration (MIC) break points were as follows: amoxicillin–clavulanic acid (≥32 μg/ml), ampicillin (≥32 μg/ml), azithromycin (≥32 μg/ml), cefoxitin (≥32 μg/ml), ceftriaxone (≥4 μg/ml), cefotaxime (≥4 μg/ml), ciprofloxacin (≥1 μg/ml), gentamicin (≥16 μg/ml), nalidixic acid (≥32 μg/ml), streptomycin (≥64 μg/ml), sulfisoxazole (≥512 μg/ml), tetracycline (≥16 μg/ml) and trimethoprim/sulphamethoxazole (≥4 μg/ml). Individual antimicrobial resistances and multiresistances of isolates were determined.

### 2.6 Other sources of data

The 2014 CIPARS abattoir data for Ontario on AMR for E. coli (n = 58) and Salmonella (n = 226) were used to compare AMR levels in smallholder flocks. Samples were obtained from slaughtered healthy chickens from seven federally registered abattoirs in Ontario. Samples were allocated based on the slaughter volume (i.e., plants that slaughter more birds/high capacity sampled frequently than plants that slaughter smaller volume/low capacity).

Fewer samples were collected for E. coli isolation as traditionally near 100% prevalences had been consistently obtained in the years that CIPARS has been operating (Government of Canada, 2016d).

### 2.7 Data analysis

Laboratory and flock data were entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA), and statistical analyses were conducted using SAS 9.3 Statistical software (SAS, Cary, NC). Two years of smallholder flock data was used in the analysis against the 2014 CIPARS flock data. Analysis of antimicrobial resistance E. coli isolate prevalence was conducted with a two-sample z test (Santner & Duffy, 2012). The data structure was used to get an estimate of the
logit and its standard error for each of the two flock types (smallholder or CIPARS) (Santner & Duffy, 2012). Analysis of Salmonella prevalence was conducted using Fisher’s exact test (Santner & Duffy, 2012). Random effects of abattoir and flock clustering were accounted for with flocks nested within the abattoir variable. Clustering at the flock and abattoir level was analysed to account for random effects for each of the smallholder antimicrobial resistance prevalence results. Only clustering at the abattoir level was analysed for the CIPARS antimicrobial resistance results as only one sample per flock was taken.

### 3 | RESULTS

Of the 18 provincially inspected abattoirs accepting flock sizes of <300 chickens for processing that were invited to participate, five abattoirs participated. Participating provincial abattoirs were largely from Southwestern Ontario; some flocks may have not originated from the same area, dependent on the producer’s location relative to the abattoir. There was a low (22%, 42/188) response rate to the producer surveys; but with the data available, 39 producers confirmed that they purchased their chicks from Ontario hatcheries, one producer indicated that they did not know the source (adopted the chickens from variety of sources), and two producers indicated that they were breeding their own stock (no indication of origin). The majority of chicks were obtained from two Ontario Health Monitored Hatcheries, both of which also supplied local commercial flocks, as indicated by a combined 76% of responses (32/42). At slaughter, the reported age of the chickens ranged from 4 to 52 weeks, with an average age at slaughter of 15 weeks. The weight of the chickens, as indicated on the producer surveys, ranged from 1.8 to 5.4 kg, with an average slaughter weight of 3.4 kg. White rock and white rock crosses were the predominant reported breeds, accounting for 71% of the breeds (30/42), where four other breeds were each named once (Cornish Cross, Columbian Rock, Ross 708 and an unnamed Heritage breed), two breeds were reported as being specific to the hatcheries that produced the chicks, and the remainder of producers indicated that they did not know the breed. At the hatchery level, 47% of producers (20/42) indicated that they did not know whether antimicrobials were administered to the eggs or hatched chicks before receiving them. 43% of producers (18/42) indicated that antimicrobials were not administered, and 10% of producers (4/42) affirmed that antimicrobials were administered. Several producers who returned the surveys did not complete the husbandry-level antimicrobial use section, leading to fewer responses in this category. Of the respondents, 45% (17/38) did not use antimicrobials, while 37% (14/38) reported using medicated starter rations and 18% (7/38) reported using medicated grower rations. Responding producers did not specify the names or class of antimicrobials used.

A total of 205 flocks were sampled with 157 and 48 flocks sampled in 2014 and 2015, respectively, yielding a total of 1,025 individual caecal samples. The 205 flocks belonged to 200 unique producers, with a small number of producers having more than one flock sampled. The average study flock size was 71 birds and the range was 6–367.

The prevalence of E. coli in caecal samples collected from individual birds at slaughter was 99% (1,022/1,025) and for Salmonella was 0.3% (3/1,025). At the flock level, the E. coli recovery was 100% (205/205). For Salmonella, 1% (2/205) of flocks had at least one chicken that tested positive for Salmonella.

The number of isolates resistant to one or more antimicrobials (multidrug resistant) and number of resistant isolates by antimicrobial class are shown in Table 1. Of the smallholder flocks, 29% (298/1,022) of isolates were identified as being multidrug resistant, and of the CIPARS flocks, 66% (38/58) of isolates were identified as being multidrug resistant. Phenotypic resistance profiles by antimicrobial classes are also indicated by the number of isolates identified with having resistances within each class. The classes are aminoglycosides, β-lactams, macrolides, phenicols, quinolones and tetracyclines. Within the β-lactam class, 15% of smallholder flock had resistances to ampicillin (156/1,022), 4% to amoxicillin–clavulanic acid (39/1,022), 4% to ceftriaxone (36/1,022), 3% to cefoxitin (35/1,022) and 3% to cefotaxime (33/1,022). Of the CIPARS flocks, 36% of smallholder flock had resistances to ampicillin (21/58), 9% to amoxicillin–clavulanic acid (5/58), 7% to ceftriaxone (4/58), 9% to cefoxitin (5/58) and 7% to cefotaxime (4/58). Within the quinolone class, 2% of smallholder flock isolates (22/1,022) and 5% (3/58) of CIPARS flock isolates were resistant to nalidixic acid. No isolates in CIPARS or smallholder flocks were determined to be resistant to ciprofloxacin.

The smallholder flock E. coli results indicated that tetracycline (37%), streptomycin (21%) and sulfisoxazole (16%) were the most common resistance found in isolates. Of the Category I antimicrobials (antimicrobials of very high importance to human health by Health Canada) (Health Canada, 2009), resistance was determined to be present in isolates to three of the four drugs in smallholder flocks: amoxicillin–clavulanic acid, cefotaxime and cephalexine. Of the CIPARS flock E. coli results, the most common resistances in isolates were tetracycline (57%), streptomycin (47%) and sulfisoxazole (45%). Resistance to Category I antimicrobials was present in isolates to the same three of the four drugs as in smallholder flocks: amoxicillin–clavulanic acid, cefotaxime and ceftriaxone.

Antimicrobial resistance results were adjusted for clustering at the flock level; adjustment for clustering at the abattoir did not change the prevalence values. The adjusted antimicrobial resistance prevalence values, with 95% confidence intervals for smallholder and CIPARS commercial flocks, are presented in Table 2. The antimicrobial resistance prevalence values are displayed in Figure 1, sorted by category of importance to human health. By way of comparison, CIPARS antimicrobial resistance results were adjusted for clustering at the abattoir level for six of 14 antimicrobials (amoxicillin–clavulanic acid, ampicillin, cefotaxime, cefoxitin, chloramphenicol, trimethoprim/sulphamethoxazole) while the remaining eight (tetracycline, sulfisoxazole, streptomycin, nalidixic acid, gentamicin, azithromycin, ciprofloxacin, ceftriaxone) indicated there were no effects due to clustering at the abattoir level (Government of Canada, 2016d).

At the federal level in 2014, recovery of E. coli was 100% (58/58) and recovery of Salmonella was 14.6% (33/226) in commercial sized flocks. Comparing federal Salmonella data to the
smallholder flocks, smallholder flock-level prevalences were significantly lower \( (p ≪ 0.0001, \ 95\% \ CI \ 0.080\%−0.86\%) \) than in CIPARS flocks \( (p ≪ 0.0001, \ 95\% \ CI \ 10.48\%−19.67\%) \), which held a flock-level prevalence of 14.6\% (33/226). All three \textit{Salmonella} isolates were determined to be serovar Kentucky and resistant to streptomycin and tetracycline. Among the CIPARS \textit{Salmonella} isolates in 2014, Heidelberg was the most prevalent, followed by Kentucky (Government of Canada, 2016d). The CIPARS \textit{Salmonella} isolates were resistant to eight of 14 antimicrobials (tetracycline 45\% (15/33); streptomycin 18\% (6/33); sulfisoxazole 15\% (5/33); and ampicillin, amoxicillin–clavulanic acid, cefoxitin, gentamicin, and ceftiofur each at 12\% (4/33)) (Government of Canada, 2016d).

4 | DISCUSSION

Smallholder chicken flocks have been implicated in foodborne disease outbreaks in both Canada and the United States (Centers for Disease Control and Prevention, 2016; Government of Canada, 2015a). In Canada, surveillance of foodborne pathogens and antimicrobial resistant pathogens in smallholder flocks is a knowledge gap. This study demonstrated that in general, the prevalence of resistance in the \textit{E. coli} population of smallholder chicken flocks in Ontario was lower than commercial broiler flocks. As well, there was significantly lower carriage prevalence of \textit{Salmonella} in smallholder flocks than commercial broiler flocks in the same time period. By way of comparison of the smallholder flocks, the CIPARS isolates exhibited moderate to high levels of resistance to a broader range of antimicrobials as compared to the smallholder flocks (Government of Canada, 2016d).

Compared to the 2014 CIPARS flock data, the smallholder flocks had a similar recovery rate of \textit{E. coli} in caecal samples. The prevalence of \textit{E. coli} isolates individually resistant to six of 14 antimicrobials was significantly lower among smallholder than federal flocks. For the remaining eight antimicrobials (amoxicillin–clavulanic acid, azithromycin, cefoxitin, ceftiofur, ceftriaxone, ciprofloxacin, gentamicin, nalidixic acid), there was no significant difference in antimicrobial resistance prevalence. Of the five \( \beta \)-lactam class antimicrobials, four (amoxicillin–clavulanic acid, cefoxitin, ceftiofur, ceftriaxone) were shown to have no significant differences in antimicrobial resistance prevalences between smallholder and CIPARS flocks. Similarly, smallholder and CIPARS flocks did not have significant differences in antimicrobial resistance prevalences in the two quinolone class antimicrobials (ciprofloxacin and nalidixic acid). Therefore, despite the smallholder flocks having significantly lower prevalences in six of 14 antimicrobials, the isolates from both flock types still showed similarities in phenotypic profiles to resistances of quinolone and \( \beta \)-lactam class antimicrobials.

The differences in the proportion of organisms resistant to the antimicrobials tested between our smallholder chickens and the commercial flocks monitored by CIPARS could be due to differences in antimicrobial usage. Unfortunately, antimicrobial use data from the CIPARS flocks tested at the abattoir are not available; however, the CIPARS farm programme, during the same timeframe, detected the
use of several classes of antimicrobials including trimethoprim–sulphamethoxazole, aminoglycosides, aminocyclitol-lincosamides, penicillins, tetracyclines and macrolides in Ontario sentinel farms (Government of Canada, 2016d). Our smallholder flock owners reported low use of these drugs. Of the completed surveys, nearly half of smallholder flock producers responded as not administering antimicrobials to their flocks. However, without veterinary oversight and validation of antimicrobial use recording protocol in-place in smallholder flocks, it has not been possible to conduct follow-up to confirm usage at the hatchery and farm levels. Furthermore, class data of administered antimicrobials to smallholder flocks are not well understood or described. Other factors contributing to difference between the two flock types may include general operational practices (e.g., flock density, cleaning and disinfection practices), age of the birds at slaughter and interprovincial/international exchange of poultry products. Conversely, the lack of significant difference between commercial flocks and smallholder flocks for the β-lactam and quinolone class antimicrobials may also indicate that there are factors separate from operational practices that contribute to the dissemination of resistance genes. There is evidence that resistance clones (β-lactam-resistant E. coli) and plasmids could

### TABLE 2

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Smallholder flocks</th>
<th>CI PARS flocks</th>
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<tbody>
<tr>
<td></td>
<td>Per cent Resistant (%)</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>Amoxicillin–Clavulanic acid (AMC)</td>
<td>3.7</td>
<td>2.7–5.1</td>
</tr>
<tr>
<td>Ampicillin (AMP)</td>
<td>14</td>
<td>11.6–16.9</td>
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<tr>
<td>Azithromycin (AZM)</td>
<td>0.068</td>
<td>0.0–0.29</td>
</tr>
<tr>
<td>Cefoxitin (FOX)</td>
<td>3.3</td>
<td>2.3–4.6</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>3.4</td>
<td>2.4–4.7</td>
</tr>
<tr>
<td>Chloramphenicol (CHL)</td>
<td>1</td>
<td>0.5–1.8</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
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<td>0.0–0.29</td>
</tr>
<tr>
<td>Gentamicin (GEN)</td>
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<td>7.3–11.2</td>
</tr>
<tr>
<td>Nalidixic Acid (NAL)</td>
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<td>1.3–3.2</td>
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<tr>
<td>Streptomycin (STR)</td>
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<td>17.5–23.5</td>
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<td>Sulfisoxazole (SOX)</td>
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<td>Tetracycline (TCY)</td>
<td>36.6</td>
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<td>Trimethoprim–Sulphamethoxazole (SXT)</td>
<td>4.2</td>
<td>3.0–5.9</td>
</tr>
</tbody>
</table>

*aAntimicrobials where prevalences are significantly lower in smallholder flocks.

The percentage of isolates resistant to smallholder flocks are not well understood or described. Other factors contributing to difference between the two flock types may include general operational practices (e.g., flock density, cleaning and disinfection practices), age of the birds at slaughter and interprovincial/international exchange of poultry products. Conversely, the lack of significant difference between commercial flocks and smallholder flocks for the β-lactam and quinolone class antimicrobials may also indicate that there are factors separate from operational practices that contribute to the dissemination of resistance genes. There is evidence that resistance clones (β-lactam-resistant E. coli) and plasmids could

### FIGURE 1

Percentage of resistance in *Escherichia coli* from smallholder flocks (2013–2014) and CIPARS Ontario abattoir chickens (2014). Roman numerals I–IV indicate categories of importance to human medicine as outlined by the Veterinary Drugs Directorate, Health Canada (Government of Canada, 2009). The percentage of isolates resistant to a specific antimicrobial in smallholder flocks was adjusted for clustering to account for multiple samples per flock and CIPARS abattoir was adjusted for clustering per abattoir for six of 14 antimicrobials.
spread from parent animals to broilers and then horizontally to other birds (or flocks) and maintained in poultry premises (Agersø, Jensen, Hasman, & Pedersen, 2014). Thus, resistant clones could persist even after a voluntary elimination of preventive use of β-lactam antimicrobials (e.g., ceftiofur) (Government of Canada, 2016c).

The significantly lower Salmonella prevalence observed among smallholder flocks is less easily explained. Salmonella contamination of chicken carcasses has been implicated during slaughter; however, the difference between commercial and smallholder flocks is not defined (Rasschaert et al., 2008). Due to the low prevalence of Salmonella in smallholder flocks, not enough results were obtained to estimate random effects. There may be random effects that we are not aware of occurring in CIPARS flocks, as we analysed federal data as if no random effects existed due to having too little smallholder flock data to estimate random effects. Barn- or farm-level operational factors described above including smaller flock density (Gast, Guraya, Jones, Anderson, & Karcher, 2016), and outdoor access (i.e., minimal horizontal contamination between birds) may have contributed to the lower recovery rates of Salmonella/antimicrobial resistant Salmonella. Hatchery or contamination in upper levels of production (breeder flocks) could contaminate young chicks and subsequently, the barn premises and entire flock (Liljebljekel et al., 2005); in Alberta, a hatchery was implicated in an outbreak of S. Enteritidis in people exposed to contaminated chicks (Government of Canada, 2015a). Although the chicks were not tested when placed in the barns, vertical contamination or hatchery-related contamination of Salmonella unlikely played a role in our study, as the majority of reported hatcheries were also found to supply local commercial flocks. However, as this study was limited to the Southern Ontario region of Canada, this may differ elsewhere in Canada.

Overall, we found lower prevalences of Salmonella and lower prevalences of antimicrobial resistance to six antimicrobials tested in E. coli isolated from Ontario smallholder chicken flocks, compared to current surveillance results from flocks collected from Ontario federal abattoirs. We conclude that with regard to Salmonella carriage, data from commercial flocks do not reflect the situation of smallholder chicken flocks. Smallholder flocks do share characteristics of resistance with commercial broiler flocks in some antimicrobial classes; however, they differ in others. Therefore, ongoing monitoring in smallholder flocks that would measure the impact of actions taken by the smallholder flock industry to manage AMR may reduce infections in people via other routes (e.g., handling) and inform small flock biosecurity/on-farm food safety programmes.

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