

# The Prevalence of Verocytotoxin-producing *Escherichia coli* and Antimicrobial Resistance Patterns of Nonverocytotoxin-producing *Escherichia coli* and *Salmonella* in Ontario Broiler Chickens

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## ABSTRACT

The prevalence of verocytotoxin-producing *Escherichia coli* and *Salmonella* in Ontario broiler chickens was determined by culturing cloacal samples from 500 individual birds selected from 50 poultry farms. Resistance to antimicrobials was determined for each of the isolates. In addition, abattoir and farm-level management data were obtained to evaluate variables that may be considered risk factors for infection. The variables selected included: Percentage of birds condemned at slaughter, percentage of birds dead-on-arrival, bird weight, truck number, farm size, hatchery source, litter source and type, feed source, mortality levels, type of water drinker, water sanitization, down time, barn clean out and history of antibiotic treatment. None of the cloacal samples revealed the presence of verocytotoxin-producing *E. coli*, though 19/500 (3.8%) contained *Salmonella* organisms. Nine different *Salmonella* serotypes were isolated; the most common being *S. hadar*, *S. heidelberg* and *S. mbandaka*. Resistance to tetracycline and streptomycin was common among *Salmonella* (63%) and *E. coli* (25.2%) isolates. Resistance to two or more antimicrobials occurred in 420/500 (84%) of the *E. coli* isolates. No statistically significant associations between abattoir or

farm-level management variables and the *Salmonella*-status of farms were demonstrated.

## RÉSUMÉ

Cette expérience visait à déterminer la prédominance des colibacilles et des salmonelles productrices de verocytotoxine, chez les poulets de grill, en Ontario. Les auteurs effectuèrent à cette fin l'examen bactériologique d'écouvillons du cloaque de 500 oiseaux, choisis au hasard dans 50 fermes avicoles. Ils réalisèrent aussi un antibiogramme, pour chacun des isolats. Ils obtinrent en outre des données relatives à la régie et à l'abattoir, pour évaluer les variables suivantes, considérées comme facteurs possibles d'infection: le pourcentage de poulets condamnés lors de l'abattage ou morts à leur arrivée à l'abattoir; le poids des poulets; le nombre d'expéditions par camion; l'importance des troupeaux; le couvoir d'origine; la source et la variété de litière; la source des aliments; le taux de mortalité; le type d'abreuvoir; la salubrité de l'eau; la longueur de l'occupation des poulaillers, entre deux élevages successifs; le nettoyage des poulaillers et l'historique de l'antibiothérapie. Aucun des écouvillons précités ne recela de colibacilles productrices de verocytotoxine, mais 19/500, ou 3,8%, contenaient neuf

sérotypes de salmonelles productrices de la dite toxine dont les trois plus fréquents étaient *Salmonella hadar*, *S. heidelberg* et *S. mbandaka*. La résistance à la tétracycline et à la streptomycine s'avéra fréquente, chez 63% des isolats de salmonelles et 25,2% de ceux de colibacilles. La résistance à deux antibiotiques ou plus s'observa chez 420/500, ou 84% des isolats de colibacilles. On n'enregistra pas de rapport significatif entre les variables relatives à l'abattoir ou à la régie et l'incidence de salmonelles dans les troupeaux.

## INTRODUCTION

Poultry products have gained much publicity in recent years as sources of food-borne pathogens for humans. Poultry can harbor a variety of different food-borne pathogens, notably *Salmonella* (1) and *Campylobacter* (1,2). Recently, verocytotoxin-producing *Escherichia coli* (VTEC), of serotype O157:H7, have been cultured from retail fresh poultry products (3). In the past few years, VTEC have been associated with diarrhea, hemolytic uremic syndrome, hemorrhagic colitis, and thrombotic thrombocytopenic purpura in humans (4-6). In recent outbreaks of these diseases, foods of animal origin such as hamburger (7), raw milk (8-10) and sandwich meat (5) are suspected sources of these orga-

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Financial support for this study was provided by Agriculture Canada and the Ontario Ministry of Agriculture and Food.

Submitted January 25, 1989.

nisms. Verocytotoxin-producing *E. coli* serotypes associated with human disease have been isolated from cattle (11,12), however, the importance of other food animals such as poultry, as reservoirs of VTEC for humans, is uncertain.

Not all *E. coli* carried by poultry are pathogenic to humans, but it is important to recognize the potential of nonpathogenic strains to transmit virulence or antimicrobial resistance traits to other human pathogens. Antimicrobial resistance in nonpathogenic *E. coli* harbored by poultry has been documented (13) and the risk of transmitting this resistance to suitable recipients present in the gut flora of humans has been considered (14). Much concern has been expressed in recent years that the level of antimicrobial resistance in the bacterial population in food animals has been increasing, owing to the continued use of antimicrobials at subtherapeutic levels as growth promotants or disease preventatives (15,16). There is little information available regarding the current level of antimicrobial resistance of bacteria in Ontario poultry and whether patterns of resistance in *E. coli* and *Salmonella* are associated with antimicrobial use in these animals.

Poultry products are an important source of food-borne *Salmonella* for humans, and researchers have documented the prevalence of this organism in poultry carcasses at slaughter (1,17-20). The high levels of *Salmonella* contamination in poultry carcasses (frequently greater than 50%) is considered partly due to cross-contamination during slaughter and processing. Only a few reports document the prevalence of *Salmonella* in individual live birds and at the farm level. Prescott and Gellner (21) reported that only 13 of 110 birds from 11 of 60 broiler chicken flocks tested yielded *Salmonella*. Less is known regarding the prevalence of antimicrobial resistance in *Salmonella* from poultry, and whether associations between management practices and the presence of *Salmonella* within the flock exist.

The objectives of this study were threefold: to determine the prevalence of VTEC and *Salmonella*; to determine the level of antimicrobial

resistance present in these bacteria; and to investigate the associations of selected abattoir and farm management variables with the prevalence of these organisms in Ontario broiler chickens.

## MATERIALS AND METHODS

### SAMPLE SELECTION

Broiler chickens were selected for this survey from a single Ontario slaughter establishment during a two week period in the spring of 1988. A three-stage sampling design was used with farms, truckloads of birds and individual birds as primary, secondary, and tertiary sampling units respectively. No previous estimates of the prevalence of VTEC in poultry at the farm level were available for calculating the sample size for this survey. Available resources limited the number of samples that could be processed to 500. Assuming that 10% of farms were contaminated, and that on contaminated farms, 25% of the birds were contaminated, it was decided that ten birds from each of the 50 farms should be sampled. Using this sample size, and the assumptions stated above, the probability of detecting at least one contaminated farm was 99.5%, and the probability of detecting at least one contaminated bird within a farm was 94.4%. Fifty farms were scheduled to slaughter their birds during the selected two week period, and all were included in the study. A single truckload of birds from each farm was randomly selected and ten birds per truckload were chosen using a systematic random sampling plan. Selected birds were removed from the shackles in the live receiving area at timed intervals. Cloacal samples from each bird were obtained with sterile swabs which were subsequently stored for transport in a modified Stuart medium (Starplex Scientific, Mississauga, Ontario). At the end of daily sampling, the cloacal swabs were transferred into tubes of MacConkey broth (Difco Laboratories, Detroit, Michigan) and incubated overnight at 37°C.

The average live bird weight, truck number, number of birds condemned and number found dead were obtained for each farm at the slaughter establishment.

### VEROCYTOTOXIN ASSAY

A 100 µL aliquot of MacConkey broth culture was used to inoculate 1 mL of brain heart infusion broth (Difco) which was subsequently incubated at 37°C for 6 h. This culture was centrifuged at 12,000 x g in a microcentrifuge (Beckman Microfuge 12) for 1 min. A 50 µL aliquot of supernatant was removed and used in the routine verocytotoxin assay as described by Gannon *et al* (22). *Escherichia coli* strains H30 (O26:H11 VT-1) and B2F1 (O91:H21 VT-2) were used as positive controls.

### ANTIMICROBIAL RESISTANCE OF *E. COLI*

Following incubation, the MacConkey broth cultures were plated onto MacConkey agar and incubated overnight at 37°C. Single lactose-fermenting suspect *E. coli* colonies were selected from the MacConkey plates for each of the original 500 samples. Identification was based on biochemical tests, including citrate, indole, methyl-red and voges-proskauer. Indole-negative colonies were confirmed as *E. coli* using the automated microbiological detection system, VITEK (McDonnell-Douglas Health Systems Company, Hazelwood, Missouri).

The antimicrobial resistance patterns of confirmed *E. coli* isolates were determined using the Repliscan system (Cathra International, St. Paul, Minnesota). Antimicrobial resistance was determined for the following drugs: sulfamethoxazole (SUL) (256 µg/mL), carbenicillin (CAR) (128 µg/mL), tetracycline (TET) (8 µg/mL), cephalothin (CE) (16 µg/mL), kanamycin (KAN) (32 µg/mL), streptomycin (STR) (20 µg/mL), neomycin (NEO) (16 µg/mL), nalidixic acid (NA) (16 µg/mL), trimethoprim-sulfamethoxazole (T/S) (2.0/38 µg/mL), chloramphenicol (CHL) (16 µg/mL), nitrofurantoin (NIT) (64 µg/mL), trimethoprim (TRI) (8 µg/mL), gentamicin (GEN) (8 µg/mL), ampicillin (AMP) (16 µg/mL) and furazolidone (FUR) (12 µg/mL).

### *SALMONELLA* CULTURE

A 100 µL aliquot of MacConkey broth culture was inoculated onto

**TABLE I. Principal Antimicrobial Resistance Patterns of 500 *E. coli* Isolates From Broiler Chickens in Ontario**

Resistance Pattern <sup>a</sup>	Number of Isolates (%) <sup>b</sup>
tet+str	126 (25.2)
sul+tet+str	52 (10.4)
tet	32 (6.4)
car+tet+str+amp	21 (4.2)
sul+tet+kan+str+neo	20 (4.0)
sul+tet+str+gen	18 (3.6)
str	17 (3.4)
sul+tet+str+fur	10 (2.0)
tet+kan+str+neo	10 (2.0)
sul+tet	9 (1.8)
sul+str	7 (1.4)
car+tet+amp	7 (1.4)
sul+car+tet+str+amp	7 (1.4)
tet+str+neo	5 (1.0)
car+tet+kan+str+neo+amp	5 (1.0)
sul+tet+kan+str+neo+gen	5 (1.0)
sul+tet+kan+str+neo+nit+fur	5 (1.0)

<sup>a</sup>Patterns present in five or more isolates

<sup>b</sup>tet (tetracycline), str (streptomycin), neo (neomycin), kan (kanamycin), car (carbenicillin), amp (ampicillin), sul (sulfamethoxazole), fur (furazolidone), gen (gentamicin), nit (nitrofurantoin)

modified semi-solid Rappaport Vassiliadis (MSRV) plates as described by De Smedt *et al* (23). The plates were incubated for a period of 48 h at 42°C. Positive migration was used to tentatively identify the *Salmonella* organisms. Suspect colonies were confirmed as *Salmonella* using the automated microbiological detection system, VITEK (McDonnell-Douglas Health Systems Company, Hazelwood, Missouri) and subsequently serotyped at the Health of Animals Laboratory, Winnipeg, Manitoba. The antimicrobial resistance patterns of each of the *Salmonella* isolates was determined as described above for *E. coli*.

#### MAIL SURVEY

Each of the 50 farm owner/managers was sent a mail survey containing 13 questions designed to obtain information about basic management practices, including hatchery source, litter source and type, feed source, mortality levels during the birds' growing period, type of water drinkers used, whether sanitization of water was performed, whether barns were cleaned out prior to flock placement, down time between lots of birds (the length of time the barn was

left empty before the flock was placed) and history of antibiotic treatment. Farm size was quantified as units of quota measured in five cycles per year, for example, a farm with 100,000 units of quota produces 500,000 broiler chickens per year. Nonrespondents to the mail-survey were telephoned in order to maximize the response rate. The questionnaire and abattoir data were entered directly into computer files. All observations were manually checked for errors in data entry.

#### STATISTICAL ANALYSES

The variability of the *Salmonella* prevalence estimate was expressed as the standard error of the mean (SEM) and was calculated by considering trucks as clusters of equal size (24). For the purposes of statistical analyses, farms were classified as *Salmonella*-positive or antimicrobial resistance-positive if one or more birds from a farm were positive for these characteristics. Unconditional associations between the presence of *Salmonella* and farm management or abattoir variables were assessed for statistical significance using the chi-square test for categorical and dichotomous variables, and the Student's *t*-test for continuous variables (25). Statistical analyses were performed using the Statistical Analysis System (SAS) (26).

## RESULTS

#### VEROCYTOTOXIN ASSAY

Of the 500 cloacal swab samples that were screened for the presence of verocytotoxin, all were negative. Control strains H30 and B2F1 showed positive verocytotoxin activity throughout the screening procedure.

#### ESCHERICHIA COLI ANTIMICROBIAL RESISTANCE PATTERNS

Ninety-six different resistance patterns were found among the 500 *E. coli* isolates selected. At least one *E. coli* isolate from each farm in the study was tetracycline and streptomycin resistant, therefore it was not possible to investigate associations between farm level use of these drugs and resistance patterns of the *E. coli* isolates. Patterns of resistance observed in five or more samples are listed in Table I, and the proportions of *E. coli* isolates that were susceptible, singly or multiply resistant to the selected antimicrobials are listed in Table II. The percentage of *E. coli* isolates resistant to individual antimicrobials is shown in Fig. 1.

#### SALMONELLA CULTURE AND ANTIMICROBIAL RESISTANCE

*Salmonella* spp. were recovered from 19/500 (3.8 ± 0.15%) of birds, and at least one *Salmonella*-positive chicken was identified from 7/50 (14% ± 5%) of farms. The serotypes and antimicrobial resistance patterns of the *Salmonella* isolates are listed in Table III, and the proportion of *Salmonella* isolates resistant to individual antimicrobials is displayed in Fig. 2. There were no obvious similarities in resistance patterns observed in the *E. coli* and *Salmonella* spp. isolates recovered from the same farm, or from within the same bird. All unconditional associations between farm-level or abattoir variables and the *Salmonella* status of farms were found to be statistically nonsignificant ( $p > 0.1$ ).

#### MAIL SURVEY

Of the 50 farm owner/managers that participated in this study and

**TABLE II. Percentage of 500 *E. coli* Isolates from Broiler Chickens Resistant to Antimicrobials**

	Number of Antimicrobials										
	0	1	2	3	4	5	6	7	8	9	10
Percent of <i>E. coli</i> isolates resistant	5.0	11.0	30.0	16.8	16.0	10.0	4.2	4.0	2.2	0.4	0.4

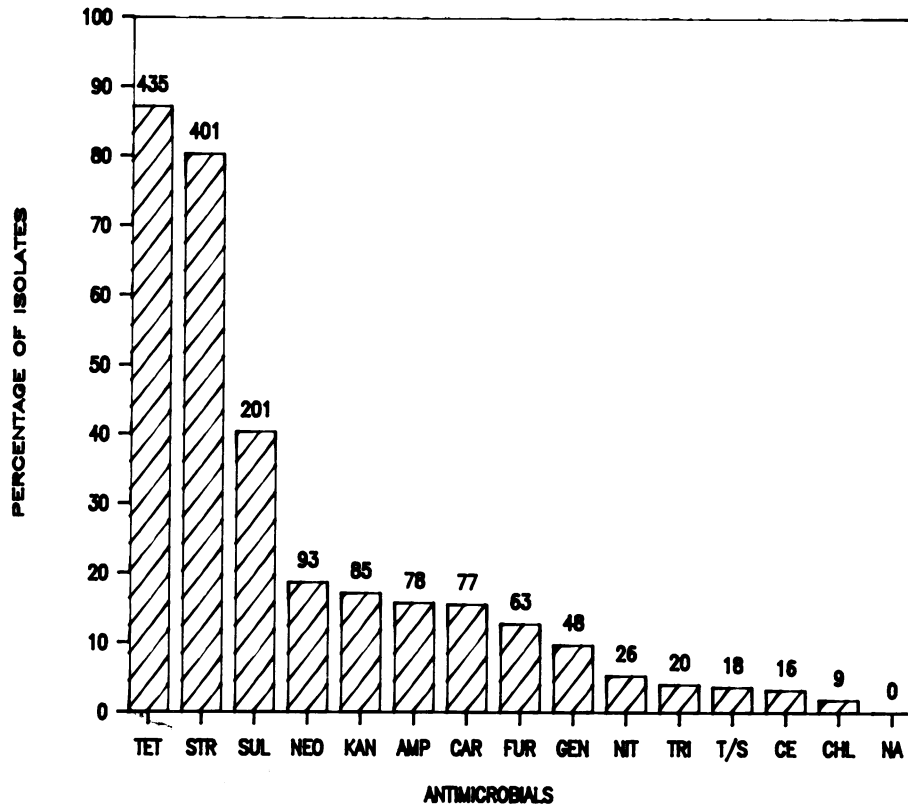


Fig. 1. Percentage of 500 *E. coli* isolates from chicken cloacal swabs resistant to individual antimicrobial drugs. The numbers above the bars indicate the actual number of isolates showing antimicrobial resistance. Antimicrobial short forms represent: TET (tetracycline), STR (streptomycin), SUL (sulfamethoxazole), NEO (neomycin), KAN (kanamycin), AMP (ampicillin), CAR (carbenicillin), FUR (furazolidone), GEN (gentamicin), NIT (nitrofurantoin), TRI (trimethoprim), T/S (trimethoprim-sulfamethoxazole), CE (cephalothin), CHL (chloramphenicol), NA (nalidixic acid).

were asked to complete a survey questionnaire, 32 (64%) replied by mail, and 11 (22%) answered the survey questions by telephone, for an overall response rate of 43/50 (86%). The distribution of selected contin-

uous and categorical variables for *Salmonella* positive and negative chicken broiler farms are presented in Tables IV and V respectively. Of the trucks transporting birds to slaughter, 27 were selected in this study.

Thirteen different hatcheries, 21 different feed companies and 38 different litter sources supplied the 50 farms. Investigations into associations between these latter variables and the *Salmonella* status of farms

TABLE III. Serotypes and Antimicrobial Resistance Patterns of *Salmonella* Isolates from Cloacal Swabs of Broiler Chickens in Ontario

Farm Number	Serotype	No. Isolates/ No. Samples	Antimicrobial Resistance Pattern <sup>a</sup>
4	<i>S. hadar</i>	4/10	tet+str
	<i>S. mbandaka</i>	1/10	tet+str
	<i>S. senftenberg</i>	1/10	tet+str
9	<i>S. indiana</i>	1/10	tet+str
	<i>S. heidelberg</i>	2/10	sensitive
15	<i>S. infantis</i>	1/10	sensitive
	<i>S. mbandaka</i>	1/10	tet+str
24	<i>S. agona</i>	1/10	tet
	<i>S. hadar</i>	2/10	tet+str
28	<i>S. hadar</i>	1/10	sul+tet+kan+str+gen+amp
	<i>S. heidelberg</i>	1/10	sensitive
43	<i>S. hadar</i>	1/10	tet+str
	<i>S. berta</i>	1/10	sensitive
49	<i>S. berta</i>	1/10	sensitive
	<i>S. typhimurium</i>	1/10	sensitive

<sup>a</sup>tet (tetracycline), str (streptomycin), sul (sulfamethoxazole) kan (kanamycin), gen (gentamicin), amp (ampicillin)

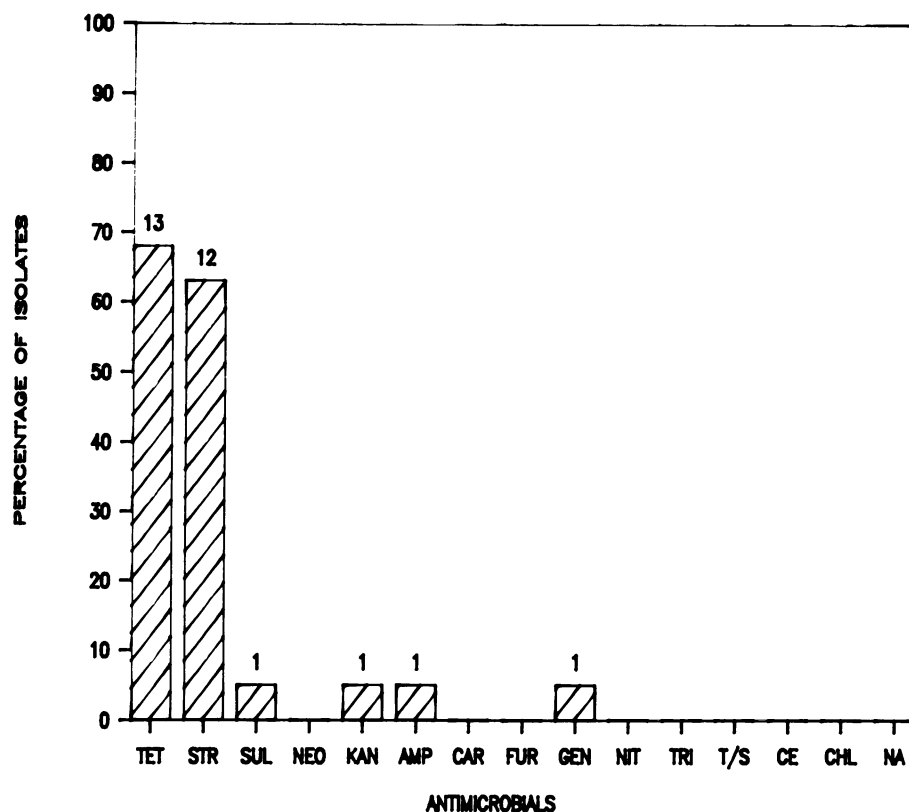


Fig. 2. Percentage of *Salmonella* isolates from chicken cloacal swabs resistant to individual antimicrobial drugs. The numbers above the bars indicate the actual number of isolates showing antimicrobial resistance. Antimicrobial short forms represent: TET (tetracycline), STR (streptomycin), SUL (sulfamethoxazole), NEO (neomycin), KAN (kanamycin), AMP (ampicillin), CAR (carbenicillin), FUR (furazolidone), GEN (gentamicin), NIT (nitrofurantoin), TRI (trimethoprim), T/S (trimethoprim-sulfamethoxazole), CE (cephalothin), CHL (chloramphenicol), NA (nalidixic acid).

were not performed because there were too many categories to be able to detect associations if indeed any existed. All farms that indicated treating with antibiotics stated that a penicillin-streptomycin combination was used.

## DISCUSSION

Verocytotoxin-producing *E. coli* were not detected in any of the 500 cloacal samples from the Ontario broiler chickens selected in this survey, therefore under the conditions of this

study, VTEC appear to be absent from the poultry population in southern Ontario. Processed poultry products, however, were shown to be a source of VTEC when Doyle *et al* (3) demonstrated that 4/263 (1.5%) of retail poultry samples in Minnesota were

TABLE IV. Distribution of Continuous Management and Abattoir Variables for *Salmonella*-positive and Negative Broiler Farms in Ontario

Variable	<i>Salmonella</i> pos. Mean (SEM) <sup>a</sup>	<i>Salmonella</i> neg. Mean (SEM)	Significance of Difference
Farm size (quota)	44,950 (7,289)	39,958 (5,266)	p = 0.2041
Down time (weeks)	3.71 (0.75)	3.63 (0.27)	p = 0.4485
Mortality during growing period (% of flock)	2.33 (0.61)	3.14 (0.31)	p = 0.6560
Bird weight (kg)	1.76 (0.06)	1.77 (0.02)	p = 0.8302
Condemned at slaughter (% of flock)	0.73 (0.13)	0.77 (0.06)	p = 1.000
Dead on arrival (% of flock)	0.27 (0.06)	0.36 (0.04)	p = 0.4017

<sup>a</sup>SEM (Standard error of the mean)

**TABLE V. Distribution of Categorical and Dichotomous Management Variables for *Salmonella*-positive and Negative Broiler Farms in Ontario**

Variable	Farm <i>Salmonella</i> Status		Significance of Difference
	Positive	Negative	
Antibiotic treatment	16.67 <sup>a</sup>	28.57	p = 0.5430
Clean out prior to flock placement	100.00	97.30	p = 0.6840
Sanitization of water	33.33	47.22	p = 0.5270
Drinker type			p = 0.7840
red plastic	66.67	51.35	
troughs	16.67	8.11	
nipples	0	8.11	
cups	0	8.11	
combination	16.67	24.32	
Litter type			p = 0.840
shavings	66.67	48.65	
straw	33.33	45.95	
sawdust	0	2.7	
combination	0	2.7	

<sup>a</sup>Numbers represent percent of farms using management practices

contaminated with *E. coli* serotype O157:H7. Their methods of detection involved the use of a hydrophobic grid membrane filter-immunoblot procedure specifically designed to recover the serotype O157:H7 while we used a screening technique to detect verocytotoxin in the fecal culture supernatant. This technique has been used successfully by Clarke *et al* (11) to detect VTEC in bovine fecal samples. Serotype O157:H7 produces substantial quantities of verocytotoxin and it is likely that our methods were sensitive enough to detect the presence of this serotype (27). If the results of the present study are precise, and the true prevalence of VTEC in southern Ontario live broiler chickens is zero, it is possible that the presence of these organisms in the processed chicken samples in Doyle's study (3) resulted from contamination of the poultry products from other meat products or humans at the processing or retail level. It is also possible that the birds sampled in Doyle's study were from a VTEC-contaminated broiler population. Under experimental conditions, *E. coli* O157:H7 is capable of colonizing chicken ceca followed by prolonged fecal shedding (28). This finding has led to the proposal that chickens may be a reservoir of the organism, although our results do not support this.

The level of antimicrobial resistance present in the nonverocytotoxin

producing *E. coli* appears to be very high, indicating that selection for multiply resistant bacteria has occurred. The high level of resistance to tetracycline (87%) and streptomycin (80.2%) may not be surprising given that these drugs are commonly used in the broiler industry. Many isolates, however, were resistant to sulfamethoxazole, kanamycin, ampicillin, carbenicillin, trimethoprim, trimethoprim/sulfa, cephalothin and chloramphenicol which are not considered to be in common use in the poultry industry. Current information on the levels of resistance in *E. coli* in Ontario broiler chickens is not available. In Japan, however *E. coli* isolated from broilers revealed similar types of resistance (13). In the Japanese study, the proportions of isolates resistant to tetracycline and ampicillin were similar to those in our study, however our results showed higher levels of resistance to streptomycin and lower levels of resistance to chloramphenicol, sulphonamides and kanamycin. In England, researchers have found levels of resistance to ampicillin and furazolidone similar to those encountered in our study, and higher levels of resistance to chloramphenicol and lower levels of resistance to tetracycline, streptomycin and neomycin (29).

In the poultry industry, the use of antimicrobials has played a significant role in controlling losses due to infectious disease, and in helping to

promote a faster growing bird (13). One disadvantage of such extensive use of these agents is highlighted by the presence of the multiply resistant isolates of *E. coli* recovered from chickens in this study.

In the present study, 7/50 (14%) of farms had at least one *Salmonella* positive bird, and *Salmonella* was recovered from 19/500 (3.8%) of the cloacal samples. The prevalence of *Salmonella* in cloacal swabs of broilers in the present study contrasts with the results of a recent national surveillance program that reported *Salmonella* contamination in 408/670 (60.9%) of broiler chicken carcass-rinse samples (1). The higher prevalence rate in carcass-rinse samples is probably due to the cross-contamination that occurs in poultry processing (18,30). Rigby *et al* (30) showed that carcass-rinse samples obtained during processing can be *Salmonella* positive, while samples obtained from the ceca are negative.

Many studies have been undertaken to assess flock level infection rates with *Salmonella* using litter (31,32), intestinal (21,30) and cecal sampling (33). Prescott *et al* (21) sampled sixty different Ontario flocks for the presence of *Campylobacter* and *Salmonella* and found that 13/110 (12%) of intestinal swabs of slaughter chickens were contaminated with *Salmonella*, indicating a farm level infection rate of 11/60 (18%). The results of our study

indicate a lower prevalence of *Salmonella*. We used cloacal swabs to assess the individual live bird's *Salmonella* status, a method which may not have been adequate to recover *Salmonella* when few organisms were present. Other studies have revealed that a preferred method of assessing flock status is to use samples from the ceca(33) or intestines(34). The use of an enrichment technique may have improved the recovery rates from the cloacal samples, however, Hinton (33) found that even when an enrichment technique was employed, the recovery of *Salmonella* remained higher from dilutions of cecal contents than from cloacal swabs. For these reasons, our *Salmonella* prevalence estimate is likely conservative.

The serotypes of *Salmonella* recovered in this study were similar in identity to those found on chilled carcasses (1). We found that 12/19 (63%) of the *Salmonella* isolates were resistant to tetracycline and streptomycin. This was also the most common multiple resistance pattern found in the *E. coli* isolates, with 126/500 (25.2%) showing this pattern. However, when comparing resistance patterns of the *Salmonella* spp. and *E. coli* isolates present on the same farm, there was no evidence of a similarity in patterns that would suggest a transfer of resistance between them. The antimicrobial resistance patterns present in the *Salmonella* isolates in this study are similar to those reported from Australia (35) and the United States (36), although higher levels of tetracycline and streptomycin resistance were found in the present study.

The unconditional associations between farm-level, or abattoir variables, and the *Salmonella* status of farms were not statistically significant. The lack of statistical association between these factors may indicate that the variables under investigation are not risk factors for *Salmonella* contamination of individual broiler chickens at slaughter. However, there is evidence that hatcheries, feed, litter, and water can provide a source of *Salmonella* contamination for the flock (37,38). Future studies should employ a larger number of *Salmonella*-positive farms

in order to detect small differences in management factors between groups of farms.

The findings of this study suggest that broiler chickens are not an important reservoir of VTEC for humans in Ontario; that non-verocytotoxin-producing *E. coli* from these birds are resistant to a wide range of antimicrobials, and that many of these organisms are multiply resistant. Also, this study has demonstrated that antimicrobial resistance is occurring among *Salmonella* from Ontario broiler chickens, although at present less extensively than among *E. coli*. This study also corroborates findings that the *Salmonella*-contamination rate of broiler chickens is much lower at the farm level than after processing.

#### ACKNOWLEDGMENTS

The cooperation of the broiler chicken producers of Ontario is gratefully acknowledged. The authors would like to thank L. Elliot and K. Rahn for their excellent technical assistance with this study.

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