Characterization of antibiotic-resistant and potentially pathogenic *Escherichia coli* from soil fertilized with litter of broiler chickens fed antimicrobial-supplemented diets

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Abstract: The objective of this study was to characterize antimicrobial resistance and virulence determinants of *Escherichia coli* from soil amended with litter from 36-day-old broiler chickens (*Gallus gallus domesticus*) fed with diets supplemented with a variety of antimicrobial agents. Soil samples were collected from plots before and periodically after litter application in August to measure *E. coli* numbers. A total of 295 *E. coli* were isolated from fertilized soil samples between August and March. Antibiotic susceptibility was determined by Sensititre, and polymerase chain reaction was performed to detect the presence of resistance and virulence genes. The results confirmed that *E. coli* survived and could be quantified by direct plate count for at least 7 months in soil following litter application in August. The effects of feed supplementation were observed on *E. coli* numbers in November and January. Among the 295 *E. coli*, the highest antibiotic resistance level was observed against tetracycline and β-lactams associated mainly with the resistance genes *tetB* and $bla_{\text{CMY-2}}$, respectively. Significant treatment effects were observed for phylogenetic groups, antibiotic resistance profiles, and virulence gene frequencies. Serotyping, phylogenetic grouping, and pulsed-field gel electrophoresis confirmed that multiple-antibiotic-resistant and potentially pathogenic *E. coli* can survive in soil fertilized with litter for several months regardless of antimicrobials used in the feed.

Key words: field application, broiler bedding, antimicrobial resistance, soil, *E. coli*.

Résumé : L'objectif de cette étude était de caractériser les déterminants de la résistance aux antimicrobiens et de la virulence de la bactérie *Escherichia coli* isolée du sol amendé avec la litière de poulets à griller (*Gallus gallus domesticus*) nourris avec des diètes supplémentées avec une variété d'agents antimicrobiens. Des échantillons de sol ont été récoltés à partir de lopins avant et après l'application de la litière au mois d'août pour quantifier le nombre de la bactérie *E. coli*. Au total, 295 spécimens d'*E. coli* ont été isolés d'échantillons de sol fertilisé du mois d'août au mois de mars. La susceptibilité aux antibiotiques a été déterminée by Sensititre et des gènes de résistance et de virulence ont été détectés par amplification en chaine par polymérase « PCR ». Des résultats ont confirmé que la bactérie *E. coli* survit et peut être quantifiée dans le sol par la méthode de numération directe sur Pétri pendant au moins 7 mois après l'application de la litière au mois d'août. Les effets de la supplémentation des moulées étaient observés sur le nombre de bactéries *E. coli* en novembre et janvier. Le niveau de résistance aux antibiotiques le plus élevé parmi les 295 échantillons d'*E. coli* était observé envers la tétracycline et les β-lactames, essentiellement à cause des gènes de résistance *tetB* et $bla_{\text{CMY-2}}$, respectivement. Des effets significatifs étaient observés au plan de la distribution des groups phylogénétiques, des profils de résistance aux antibiotiques et de la fréquence des gènes de virulence. Le sérotypage et l'électrophorèse en champ pulsé « PFGE » confirmaient que la bactérie *E. coli* multi-résistante et potentiellement pathogène peut survivre plusieurs mois dans le sol fertilisé avec la litière, indépendamment des antimicrobiens utilisés dans la moulée.

Mots-clés : application sur le terrain, litière de poulet, résistance antimicrobienne, sol, *E. coli*.

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Introduction

Poultry litter, a mixture of bedding, feces, feathers, insects, and other small invertebrates, is a valuable soil amendment that is rich in nutrients and can improve soil physical, chemical, and biological properties for agricultural crops (Brye et al. 2004). However, poultry litter also harbours a large number of enteric bacteria, including *Escherichia coli* and *Enterococcus* spp., which could be potentially pathogenic (Diarra et al. 2007; Diarrassouba et al. 2007; Lu et al. 2003). The amount of poultry litter produced in British Columbia (Canada) is about 320 000 t (1 tonne = 1000 kg) annually, most of which is applied directly to land; very little is composted or digested before use (Timmega and Associates Inc. 2003). It has been reported that total enteric bacteria and ceftiofur-resistant bacteria are significantly higher in soil samples from farm than in non-farm environments (Yang et al. 2010). Therefore, for environmental and public health reasons it is important to characterize the persistence of bacteria that may cause human disease or propagate antibiotic-resistant determinants in soil after litter application.

Pathogenic *E. coli* often possess and express various virulence genes, whereas commensal *E. coli* lack the complement of virulence genes required to cause disease (Chapman et al. 2006; Kaper et al. 2004). In pathogenic strains, including avian pathogenic *E. coli* (APEC), virulence and antibiotic resistance genes are often carried on the same genetic elements (Johnson et al. 2007; Skyberg et al. 2006). Furthermore, *E. coli* can be classified into 4 different phylogenetic groups: A, B1, B2, and D (Clermont et al. 2000). Groups A and B1 are generally commensal strains but may harbour some antibiotic resistance and virulence genes, whereas groups B2 and D are usually pathogenic strains, and the virulent extraintestinal strains are more commonly found in group B2 than D (Clermont et al. 2000; Cortés et al. 2010). Comparing *E. coli* isolates from human urinary tract infections and from cecal contents of slaughtered animals showed that chickens are a reservoir for extraintestinal pathogenic *E. coli* for humans (Bergeron et al. 2012).

Owing to the increase of antibiotic resistance in bacteria, the use of antibiotics in agricultural production represents a serious threat to public health, especially at a time when there is little discovery and development of new antimicrobial agents (Mulvey and Simor 2009; Witte 2000). For example, ceftiofur is a common broad-spectrum β-lactam antibiotic used in veterinary medicine to treat respiratory infections in animals (Hornish and Kotarski 2002). Bacteria that express extended spectrum β-lactamases can inactivate such broad-spectrum antibiotics (Kolar et al. 2010). Tetracycline is also commonly used in the treatment of animal infections because it is inexpensive, but because of the increasing prevalence of tetracycline-resistant pathogens, its use is being compromised (Chopra and Roberts 2001; Diarra et al. 2009).

Survival of *E. coli* in soils and sediments has been previously studied (McElhany and Pillai 2011). However, there is little information about the molecular ecology of different genotypes (virulence and resistance) of specific *E. coli* strains from poultry litter when applied as a fertilizer to soil. Knowledge of the survival and dynamics of such *E. coli* in soil after litter application will help in understanding their prevalence, dissemination, and role in the emergence of specific pathogenic strains. The aim of the present study was to characterize *E. coli* isolated over time from soil fertilized with litter from broiler chickens fed diets supplemented with virginiamycin, monensin, narasin, and chlortetracycline. The distribution of serotypes, phylogenetic groups, and antibiotic resistance among recovered *E. coli* was determined to assess their genotype and phenotype.

Material and methods

Study design and litter application

Ross 308 1-day-old male broiler chicks were obtained from a local commercial hatchery (Abbotsford, British Columbia, Canada) and were vaccinated for Marek's disease and infectious bronchitis. The broiler chicken trial was performed from July to August 2005 in a barn where chickens were placed in 18 physically separated pens (50 birds/pen) measuring 1.2 m × 3.0 m each and were randomly allocated to 6 experimental diets (3 pens/diet) (Bonnet et al. 2009; Diarra et al. 2007). The clean and disinfected concrete floor pens were bedded with approximately 3 inches (1 inch = 25.4 mm) of fresh clean softwood shavings from Visscher Shavings Inc. (Chilliwack, British Columbia, Canada). The bird density was approximately 0.75 ft^2 (0.07 m^2) per bird, and high hygienic and biosecurity practices were used before and throughout the experimental protocol. The starter, grower, and finisher diets were formulated in accordance with the broiler diet used in Western Canada, with wheat, barley, and corn as the principal cereals, and soybean and canola meals as protein concentrates. Details of raising conditions have been previously described (Bonnet et al. 2009; Diarra et al. 2007). The 6 experimental groups consisted of a control group fed without antibiotics and 5 groups fed rations containing the following per kilogram of feed: chlortetracycline (110 mg), monensin (99 mg), narasin (70 mg), and virginiamycin at 11 and 22 mg (Bonnet et al. 2009). When the birds had reached 36 days of age (August), litter (a mixture of manure and bedding materials) was collected from each pen and placed in individual containers to avoid cross-contamination. Pooled litter from the 3 pens for each treatment group was then applied by hand at agronomic rates to simulate mechanical methods used by farmers in quadruplicate to the surface of grass plots (2 m^2 treatment area) at a rate of 27.5 t/ha according to industry practices. The grass sward was a pure stand of orchardgrass (*Dactylis glomerata* L.), which is the most common seeded grass in the region and very widely used internationally. In the Lower Fraser Valley region, application of chicken litter on orchardgrass is a common practice. These plots had not been fertilized with manure for the previous 3 years. The 24 fertilized plots (6 treatments × 4 replicates) and 4 unfertilized plots were arranged in a randomized complete block design. Each plot was separated by a 1 m strip on all 4 sides of unfertilized grass, and during setup, application, and sampling, care was taken to prevent cross-contamination of the test plots. Temperature and rainfall data were recorded at an Environment Canada weather station located about 300 m away from the experimental plots.

Sampling, *E. coli* isolation, and serotyping

The initial *E. coli* population in litter from each pen was determined before application, as previously reported (Furtula et al. 2010). Three core samples (10 cm diameter and 10 cm

deep) were collected from each treatment plot (4 plots per treatment) using a sanitized tulip bulb planter. Samples were collected from day 0 (before and after application) and at intervals of 2–4 weeks thereafter. The 3 samples from each plot were combined and thoroughly mixed manually to ensure homogeneity, and 25 g of this mixture was suspended in 225 mL of buffered peptone water (EMD, Mississauga, Ontario, Canada). Serial dilutions were made from each mixed sample, and 1 mL of each was pipetted onto *E. coli*/coliform Petrifilm (3M, London, Ontario) and incubated overnight for 20 h at 37 °C. After incubation, *E. coli* colonies were counted to determine the number of colony-forming units (cfu) per gram of wet soil according to manufacturer recommendations. From each sample, 3–5 presumptive *E. coli* (blue with gas) colonies were randomly selected and frozen at –80 °C in tryptic soy broth (Becton Dickinson, Mississauga, Ontario) containing 25% glycerol for characterization (Diarra et al. 2009 and 2007). The identities of all *E. coli* isolates were confirmed by polymerase chain reaction (PCR) using the specific primer sets targeting the *uidA* gene and using *E. coli* ATCC 25922 and *Escherichia fergusonii* ATCC 34569 as positive and negative controls, respectively (Maheux et al. 2009). Before the fresh shaving were used as bedding in the chicken trial, their microbiological quality was evaluated by Petrifilm method, and no *E. coli* were found.

Somatic (O) and flagellar (H) antigens were identified by tube agglutination methods for identification of O1–O181 and H1–H56 (Ewers et al. 2009; Kaper et al. 2004).

Antimicrobial susceptibility profile

Minimum inhibitory concentrations were determined for all isolates using the Sensititre broth microdilution automated system (Trek Diagnostic System, Cleveland, Ohio, USA), according to Clinical Laboratory Standards Institute (1999) guidelines with *E. coli* ATCC 25922 as the control. The following 15 antibiotics were included in the test panel: amikacin, amoxicillin, ampicillin, ceftiofur, ceftriaxone, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim–sulfamethoxazole. The antibiotic susceptibilities were interpreted according to the breakpoints of the Clinical Laboratory Standards Institute (1999) and the Canadian Integrated Program for Antimicrobial Resistance Surveillance (2009) guidelines.

PCR detection of antibiotic resistance genes

The DNA isolation was performed according to Bonnet et al. (2009). The presence of tetracycline resistance (*tetA* and *tetB*), extended spectrum β-lactamase ($bla_{\text{CMY-2}}$, bla_{SHV}, and bla_{TEM}), streptomycin resistance (*strA* and *strB*), sulfonamide resistance (*sul1*), and chloramphenicol resistance (*floR*) genes was determined in corresponding resistant *E. coli* isolates using specific primers (Invitrogen, Burlington, Ontario) by PCR (Briñas et al. 2005; Diarrassouba et al. 2007). The positive control *E. coli* strains for all of the above genes were from our collection (Bonnet et al. 2009; Diarra et al. 2007; Diarrassouba et al. 2007). The PCR products were separated on a 2% Tris–acetate–EDTA buffer agarose electrophoresis gel stained with ethidium bromide (1 μL/10 mL), and bands were referenced to a 1 kb gene ruler (Fermentas, Burlington, Ontario, Canada) to size the amplicons.

Virulence genotyping and pulsed-field gel electrophoresis

The exact set of virulence factors of APEC has not yet been clearly defined. However, based on the literature of virulence factors related to APEC (Johnson et al. 2003; Rodriguez-Siek et al. 2005), isolates were considered APEC if they contained at least 4 of the following 7 genes: *iss* (increased serum survival protein), *iucA* (aerobactin operon gene), *traT* (surface exclusion), *tsh* (temperature-sensitive hemagglutinin), *pap* (P fimbrae), *kpsMT II* (group II capsule synthesis), or *ompT* (outer-membrane protein 3b). In addition, isolates that had 3 of the above traits were considered "potentially APEC"; isolates with fewer than 3 were regarded as nonpathogenic (commensal). A series of multiplex PCRs were run on the isolates following the protocol outlined by Rodriguez-Siek et al. (2005) using positive control isolates from our collection (Bonnet et al. 2009). The method developed by Clermont et al. (2000) based on the presence or absence of the *chuA* and *yjaA* genes and the TSPE4.C2 DNA fragment was used to assign isolates to phylogenetic groups A, B1, B2, or D. All isolates of group D were subtyped by pulsed-field gel electrophoresis (PFGE) according to the Centers for Disease Control and Prevention PulseNet protocol (http://www.cdc.gov/pulsenet/protocols.htm) to assess their diversity.

Statistical analyses

Data on *E. coli* enumerations were log transformed and analyzed using a repeated measurement analysis of SAS (SAS Inc., Cary, North Carolina, USA) with the individual soil plots as experimental units (4 plots per treatment group). Least significant difference was used to separate treatment means on individual dates when the F value for the effect of treatment was significant. The association test of Cochran–Mantel–Haenszel was used to determine the relationship between bacterial characteristics (resistance phenotype and genotype) and treatment and collection time using the FREQ procedure in SAS. Associations between phenotype and genotype were determined using Pearson's chi-square and Fisher's exact test (Bonnet et al. 2009, Diarra et al. 2007). A P value of 0.05 was used to declare significance.

Results

Escherichia coli population size in soil

Temperature and rainfall data recorded during the study period are presented in Fig. 1. The lowest temperature and the highest rainfall were observed from December 2005 to March 2006. The pH of the soil samples varied from 5.4 to 6.5 and was below neutral throughout the study period for both fertilized and unfertilized plots. Following application of fresh litter containing approximately 8.3 log cfu *E. coli*/g in August, generic *E. coli* populations in the soil decreased steadily in all treatments until November ($P < 0.05$), at which point the total *E. coli* population remained constant at approximately 3.0 log cfu/g until March (Fig. 2). Our data demonstrated that *E. coli* could be quantified at least 7 months after the litter application in August. Direct plating showed that the unfertilized plots had no quantifiable *E. coli* compared with plots receiving litter (data not shown). In November, the lowest *E. coli* number was observed in soil fertilized with litter from the monensin and virginiamycin

Fig. 1. Temperature and rainfall data collected by a local weather station during the study period from August 2005 to March 2006.

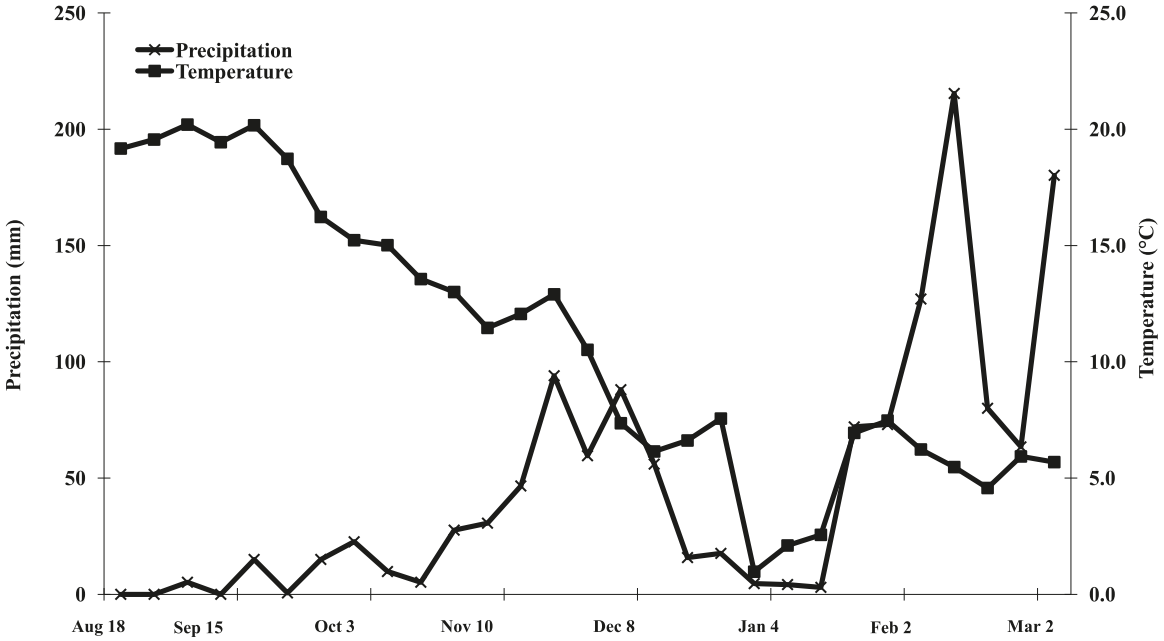
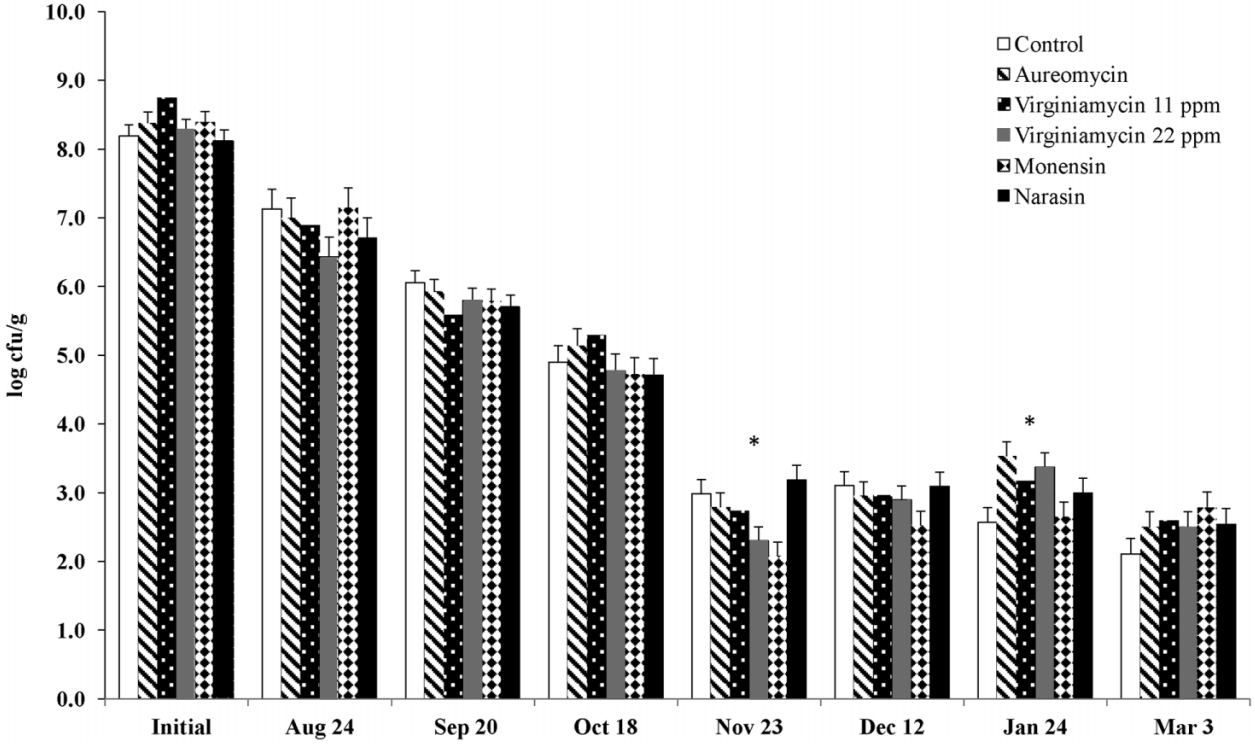


Fig. 2. Temporal trends of means (standard error bars are presented) for *Escherichia coli* populations recovered from soil fertilized with poultry litter. Initial *E. coli* populations in the litter before application are presented. No *E. coli* were recovered by direct plate count from unfertilized soils during the trials. Asterisks (*) indicate the collection dates at which the treatment effects were statistically different ($P < 0.05$).



(22 ppm) treatments ($P = 0.08$). In January, *E. coli* populations in the soil receiving litter from the control and monensin-treated birds were significantly lower than populations in the other treatments ($P = 0.03$). A total of 295 presumptive *E. coli* were isolated for characterization from treated plots in August ($n = 44$), September ($n = 43$), October ($n = 44$), November ($n = 46$), December ($n = 38$), January ($n = 38$), and March ($n = 42$). Before application and at all sampling dates, no *E. coli* were obtained using direct plating Petrifilm of the unfertilized plots.

Antibiotic susceptibility

Of the 295 *E. coli* isolates recovered, 229 (77.6%) were resistant to at least 1 of the antibiotics tested. The highest percentages of resistance were against tetracycline (74.6%), followed by ampicillin (70.8%), amoxicillin – clavulanic acid (65.8%), cefoxitin (65.1%), and ceftiofur (29.5%). Overall, resistance levels were highest in August and decreased thereafter, and for most of the antibiotics tested, the level of resistance fluctuated between high and low from September to March (Table 1). In isolates from the final collection, a total of 237 (80.3%) presented intermediate resistance to streptomycin and no resistance to ceftriaxone, gentamicin, or chloramphenicol (Table 1). The soil fertilized with litter from the monensin treatment showed the highest number of isolates resistant to ceftiofur (44.6%), followed by those fertilized with litters from the control (31.5%), virginiamycin 11 ppm (26.1%), and narasin (25.0%) treatments ($P < 0.05$) (Table 2). The isolates from soil fertilized with litter from the control nonmedicated diet showed the highest resistance level to tetracycline (94.4%), followed by isolates from soil fertilized with litter from chlortetracycline (82.7%), monensin (71.4%), and virginiamycin 11 (69.6%) treatments. Differences in percentage resistance among treatments were statistically significant ($P < 0.05$) for amoxicillin – clavulanic acid, ceftiofur, ampicillin, cefoxitin, sulfisoxazole, tetracycline, and chloramphenicol (Table 2). Multidrug resistance was evident, and the most prevalent resistance spectrum was amoxicillin – clavulanic acid – ampicillin–cefoxitin–tetracycline, which was found in 92 of 229 (40.2%) isolates. Interestingly, ≥94.0% of the *E. coli* isolates from soil receiving litter from the control treatment (no antibiotics) presented this resistance pattern.

PCR detection of antibiotic resistance genes

Of the 229 *E. coli* isolates that were found to be resistant to at least 1 antibiotic, 96.9% had at least 1 antibiotic resistance gene. Overall, 220 (74.6%) of the total isolates were resistant to tetracycline; of these 220 isolates, 206 (93.6%) and 76 (34.5%) harboured *tetB* and *tetA*, respectively. Among the 211 isolates resistant to at least 1 β-lactam antibiotic, 95 (45.0%), 28 (13.3%), and 16 (7.6%) were found to carry $bla_{\text{CMY-2}}$, bla_{TEM}, and bla_{SHV}, respectively. Of the 59 aminoglycoside-resistant isolates, 21 (35.6%), 24 (40.7%), and 20 (33.9%) were positive for *strA*, *strB*, and *strA*+*strB*, respectively. Only 8 (28.6%) and 3 (16.7%) of the 28 sulfonamide-resistant isolates and 18 of the chloramphenicol-resistant isolates harbored *sul1* and *floR* genes, respectively. The tetracycline and β-lactam resistance genes were found in combination with *strA*, *strB*, *sul1*, and *florR* at varying degrees (Table 3). Isolates from September, October, and November contained the most $bla_{\text{CMY-2}}$, whereas isolates from December and January collections contained the most *tetA* ($P < 0.05$). Isolates containing $bla_{\text{CMY-2}}$ were more prevalent in the virginiamycin (72.0%) and the control (58.0%) treatments, whereas isolates containing bla_{SHV} were more prevalent in the virginiamycin 11 (20.0%) and virginiamycin 22 (16.0%) treatments ($P < 0.05$). The *tetB* gene was found to be most prevalent in the monensin (100%) and narasin (100%) treatments ($P < 0.05$).

Serotypes

Out of the 229 resistant *E. coli* isolates, only 193 (84.3%) were serotyped because of their resistance to at least 4 anti-

Table 1. Antibiotic resistance level in 295 *Escherichia coli* isolates by sampling date.

		Antibiotic resistance (%)						
Antibiotic	Phenotype[a]	24 Aug. 2005 ($n = 44$)	20 Sep. 2005 ($n = 43$)	18 Oct. 2005 ($n = 44$)	23 Nov. 2005 ($n = 46$)	12 Dec. 2005 ($n = 38$)	24 Jan. 2006 ($n = 38$)	3 Mar. 2006 ($n = 42$)
Amoxicillin – clavulanic acid	R	75.0	60.5	61.4	71.7	73.7	55.3	64.3
Ceftiofur*	R	47.7	25.6	22.7	41.3	28.9	21.1	16.7
	I			2.3	2.2		2.6	
Ceftriaxone	R			2.3				
	I	11.4	4.7	6.8	10.9	13.2	5.3	9.5
Ampicillin	R	84.1	60.5	72.7	80.4	73.7	60.5	71.4
Cefoxitin	R	79.5	60.5	56.8	71.7	73.7	55.3	64.3
	I	2.3					2.6	
Gentamicin*	R	6.8	7.0	4.5	6.5	13.2	10.5	0.0
Streptomycin	R	31.8	18.6	13.6	17.4	18.4	21.1	16.7
	I	68.2	81.4	86.4	82.6	81.6	78.9	83.3
Sulfisoxazole	R	13.6	9.3	11.4	6.5	13.2	10.5	2.4
Trimethoprim – sulfamethoxazole	R	6.8	7.0	4.5	2.2	0.0	0.0	2.4
Tetracycline	R	81.8	76.7	72.7	76.1	76.3	76.3	66.7
Chloramphenicol	R	6.8	7.0	4.5	4.3	13.2	10.5	0.0
	I	2.3	2.3	2.3	2.2	2.6		
Nalidixic acid	R	2.3	2.3	0.0	0.0	5.3	0.0	9.5

Note: Antibiotics marked with an asterisk (*) showed resistance percentages that were statistically different ($P \leq 0.05$) between sampling dates.

[a]R, resistant; I, intermediate.

Table 2. Antibiotic resistance level in the 295 *Escherichia coli* isolates by treatment.

		Antibiotic resistance (%)					
Antibiotic	Phenotype[a]	Control (n = 54)	Chlortetracycline (n = 52)	Virginiamycin 11 ppm (n = 23)	Virginiamycin 22 ppm (n = 54)	Monensin (n = 56)	Narasin (n = 56)
Amoxicillin – clavulanic acid*	R	96.3	61.5	65.2	59.3	64.3	48.2
Ceftiofur*	R	31.5	30.8	26.1	11.1	44.6	25.0
	I	1.9	1.9				
Ceftriaxone	R						1.8
	I	5.6	9.6	8.7	5.6	12.5	8.9
Ampicillin*	R	100.0	65.4	69.6	59.3	67.9	64.3
Cefoxitin*	R	94.4	63.5	65.2	59.3	64.3	46.4
	I	1.9					
Gentamicin	R	9.3	15.4	8.7	1.9	1.8	3.6
Streptomycin	R	25.9	26.9	17.4	5.6	17.9	23.2
	I	74.1	73.1	82.6	94.4	82.1	76.8
Sulfisoxazole*	R	13.0	21.2	8.7	3.7	1.8	8.9
Trimethoprim – sulfamethoxazole	R	3.7	5.8	0.0	1.9	0.0	3.6
Tetracycline*	R	94.4	82.7	69.6	63.0	71.4	66.1
Chloramphenicol*	R	7.4	15.4	8.7	1.9	1.8	3.6
	I	1.9	0.0	0.0	1.9	3.6	1.8
Nalidixic acid	R	0.0	5.8	4.3	1.9	1.8	3.6

Note: Antibiotics marked with an asterisk (*) showed resistance percentages that were statistically different ($P \leq 0.05$) between treatments.
[a]R, resistant; I, intermediate.

biotics. Based on somatic and flagellar antigens, 14 serotypes were found. Among these multiresistant isolates, the most prevalent serotypes were O8:H19 (105 isolates), O178:H2 (32 isolates), O82:H8 (16 isolates), and O86:H51 (16 isolates). The different serotypes were detected in similar proportion at all collection dates ($P > 0.05$). There was no significant correlation between the presence of serotypes and the different treatments received except that the 3 O23:H16 isolates were only found in soil fertilized with litter from the chlortetracycline treatment (data not shown). In addition, O8:H19 isolates tended to be more prevalent in soils receiving litter from virginiamycin (81.3% and 60.0% for 22 and 11 ppm treatments, respectively), control (71.2%), and narasin (50.0%) treatments ($P = 0.07$) than in the soils fertilized with litter from the other treatments. Significant differences in resistance level and pattern were noted among the different serotypes ($P < 0.05$).

Phylogenetic groups

Analysis of the 193 resistant *E. coli* isolates by PCR showed (Table 4) that group A was the most prevalent (106 isolates), followed by group B1 (75 isolates), and group D (12 isolates). No isolates of group B2 were found. Except for 2 isolates (serotype O117:H34) from the March collection of chlortetracycline treatment, all isolates (104) of group A were of serotype O8:H19 from August to March. The 75 isolates of group B1 belonged to 10 serotypes, with O178:H2 (35 isolates), O82:H8 (10 isolates), and O86:H51 (10 isolates) being the most prevalent detected at all sampling times. Seven, 4, and 1 of the 12 isolates of group D were of serotypes O15:H45, O1:H45, and O124:H25, respectively. The most prevalent serotype O15:H45 was detected at the first (August) and last (March) collection time. Serotyping data indicated that the studied isolates overtime come from litter.

The different phylogenetic groups were found at similar proportions over time, and significant differences in the distribution of phylogenetic groups were found between soils receiving litter from treated birds ($P < 0.05$). Group A isolates were most frequent in soil fertilized with litter from the control (36 isolates) and from virginiamycin 22 ppm (26 isolates) treatments, whereas only 9 isolates of this group were found in each chlortetracycline and virginiamycin 11 ppm treatment. Isolates from group B1 were most common in soil fertilized with litter from chlortetracycline-treated (22 isolates) and monensin-treated (20 isolates) birds. Sixteen and 9 isolates of group B1 were found in the soil fertilized with litter from control and narasin treatments, respectively, whereas only 6 of them were found in virginiamycin treatments.

Significant differences in antibiotic resistance were found between the phylogenetic groups ($P < 0.05$). At least 83.0% of isolates from all of the phylogenetic groups were resistant to amoxicillin – clavulanic acid and cefoxitin; the majority of group B1 (95.0%) and D (75.0%) isolates were resistant to ceftiofur and cefoxitin. The 14 isolates that were resistant to 9 antibiotics were all associated with phylogenetic group B1, and 13 of those isolates were serotype O86:H51.

Virulence genes and pathotypes

PCR analysis of the 193 multiresistant *E. coli* isolates was performed to detect virulence genes to assign them to specific pathotypes. The most prevalent virulence genes were *traT* (97.9%) and *iss* (87.0%). The genes *iucA*, *tsh*, *kpsMT II*, *malX*, *papC*, *papG II*, and *ompT* were found in 16.0%, 11.9%, 2.1%, 2.1%, 1.5%, 1.5%, and 0.5% of the 193 isolates, respectively. The most common virulence gene pattern was *iss-traT* and was found in 61% of isolates (Table 5). No effect of collection time was noted for the number of virulence genes detected ($P > 0.05$). However, *tsh* was most prevalent in the November collection ($P < 0.05$). The virulence genes *iucA*, *tsh*, and *papC* were more commonly found in the chlortetracycline and monensin treatments, whereas *papC* and *papG II* genes were only found in the chlortetracycline treatment ($P < 0.05$). In addition, the 4 isolates of serotypes O1:H45 (phylogenetic group D) contained 5 virulence genes: *iucA*, *malX*, *kpsMT II*, *traT*, and *tsh*. Three isolates of serotypes O23:H16 belonging to phylogenetic group B1 con-

Table 3. Distribution of resistance genes in resistant *Escherichia coli* isolates.

Sampling date	Gene pattern[a]	No. of isolates positive for screened genes[b]						
		Control (n = 51)	Chlortetracycline (n = 43)	Virginiamycin 11 ppm (n = 14)	Virginiamycin 22 ppm (n = 35)[c]	Monensin (n = 39)[d]	Narasin (n = 37)[d]	Total (n = 219)
24 Aug. 2005 (n = 36)	*bla*$_{CMY-2}$-*strA-strB-tetA-tetB*	2			1			3
	bla$_{CMY-2}$-*strA-strB-tetB*	1						1
	bla$_{CMY-2}$-*tetA*	1						1
	bla$_{CMY-2}$-*tetA-tetB*	1					1	2
	bla$_{CMY-2}$-*tetB*	1						1
	bla$_{SHV}$-*bla*$_{CMY-2}$-*tetB*				1			1
	strA-strB-tetB					2		2
	bla$_{TEM}$-*bla*$_{CMY-2}$-*tetA-tetB*		1			1		2
	bla$_{TEM}$-*strA-strB-tetB*					1		1
	tetA		1					1
	tetA-tetB		1				3	4
	tetB	1	5	3	2	3	2	16
20 Sep. 2005 (n = 32)	*bla*$_{CMY-2}$-*floR-strA-strB-sul1-tetB*	1						1
	bla$_{CMY-2}$-*strA-strB-tetB*	1						1
	bla$_{CMY-2}$-*tetA-tetB*	3			2	1		6
	bla$_{CMY-2}$-*tetB*				1			1
	bla$_{CMY-2}$-*bla*$_{SHV}$-*sul1-tetB*						1	1
	bla$_{SHV}$-*tetA-tetB*						1	1
	strA-strB-tetB					2		2
	bla$_{CMY-2}$-*bla*$_{TEM}$-*tetA-tetB*	1					1	2
	tetA-tetB				1			1
	tetB	2	7		2	3	1	15
18 Oct. 2005 (n = 33)[c]	*bla*$_{CMY-2}$-*floR-strA-strB-sul1-tetA-tetB*	1						1
	bla$_{CMY-2}$-*strA-strB-sul1*	1						1
	bla$_{CMY-2}$-*strA-strB-sul1-tetB*	1						1
	bla$_{CMY-2}$-*strA-strB-tetA*	1						1
	bla$_{CMY-2}$-*tetA-tetB*	1	1					2
	bla$_{CMY-2}$-*tetB*	3			3	4	2	12
	bla$_{CMY-2}$-*bla*$_{TEM}$-*tetA-tetB*		3		1			4
	tetA-tetB			1				1
	tetB		2	1	1	1	5	10
23 Nov. 2005 (n = 35)[c]	*bla*$_{CMY-2}$-*strA-strB-tetB*	1						1
	bla$_{CMY-2}$-*strA-tetB*			1				1
	bla$_{CMY-2}$-*tetA*	1						1
	bla$_{CMY-2}$-*tetA-tetB*	2				1		3
	bla$_{CMY-2}$-*tetB*	2	1	1	3		4	11
	bla$_{CMY-2}$-*bla*$_{TEM}$				1			1

Table 3 (*continued*).

Sampling date	Gene pattern[a]	No. of isolates positive for screened genes[b]						
		Control (n = 51)	Chlortetracycline (n = 43)	Virginiamycin 11 ppm (n = 14)	Virginiamycin 22 ppm (n = 35)[c]	Monensin (n = 39)[d]	Narasin (n = 37)[d]	Total (n = 219)
	bla$_{CMY-2}$-*bla*$_{TEM}$-*tetA*-*tetB*	1				1		2
	bla$_{TEM}$-*strA*-*strB*-*tetB*			1				1
	bla$_{TEM}$-*sul1*-*tetA*-*tetB*		1					1
	bla$_{TEM}$-*tetA*-*tetB*			1		1		2
	tetA		1					1
	tetB		2	1		4	2	9
12 Dec. 2005 (n = 29)	*bla*$_{CMY-2}$-*strA*-*strB*-*tetA*	1						1
	bla$_{CMY-2}$-*strA*-*strB*-*tetB*						1	1
	bla$_{CMY-2}$-*tetA*-*tetB*	1	1		2	1		5
	bla$_{CMY-2}$-*tetB*					1		1
	bla$_{CMY-2}$-*bla*$_{SHV}$-*sul1*-*tetB*		1					1
	bla$_{CMY-2}$-*bla*$_{SHV}$-*tetA*-*tetB*				2			2
	bla$_{SHV}$-*tetA*-*tetB*		1					1
	strB-*tetB*						1	1
	bla$_{CMY-2}$-*bla*$_{TEM}$-*tetA*-*tetB*	1				2		3
	bla$_{TEM}$-*bla*$_{SHV}$-*tetA*			1				1
	bla$_{TEM}$-*tetB*	1						1
	tetA-*tetB*		1			1		2
	tetB	4	2		1	1	1	9
24 Jan. 2006 (n = 29)	*bla*$_{CMY-2}$-*floR*-*sul1*-*tetB*		1					1
	bla$_{CMY-2}$-*strA*-*strB*-*tetA*-*tetB*						1	1
	bla$_{CMY-2}$-*tetA*-*tetB*	1			2			3
	bla$_{CMY-2}$-*tetB*		1					1
	bla$_{CMY-2}$*bla*$_{SHV}$-*tetA*-*tetB*			1				1
	bla$_{CMY-2}$*bla*$_{SHV}$-*tetB*				1			1
	bla$_{SHV}$-*tetA*-*tetB*	2			1			3
	strB-*tetA*-*tetB*						2	2
	bla$_{TEM}$-*tetA*		2					2
	tetA		1					1
	tetA-*tetB*					1	1	2
	tetB	4	1		2	3	1	11
3 Mar. 2006 (n = 28)	*bla*$_{CMY-2}$-*tetB*		2		2			4
	bla$_{CMY-2}$-*bla*$_{SHV}$-*tetB*				1			1
	bla$_{SHV}$-*tetA*-*tetB*			1				1
	bla$_{TEM}$-*bla*$_{SHV}$-*tetA*-*tetB*			1				1
	bla$_{TEM}$-*strB*-*tetB*						1	1
	bla$_{TEM}$-*tetB*						1	1
	tetA	1						1

Table 3 (*concluded*).

Sampling date	Gene pattern[a]	No. of isolates positive for screened genes[b]						
		Control ($n = 51$)	Chlortetracycline ($n = 43$)	Virginiamycin 11 ppm ($n = 14$)	Virginiamycin 22 ppm ($n = 35$)[c]	Monensin ($n = 39$)[d]	Narasin ($n = 37$)[d]	Total ($n = 219$)
	tetA-tetB		1					1
	tetB	5	2		2	4	4	17
Total ($n = 219$)		51	43	14	35	39	37	219

[a]Isolates were screened for tetracycline resistance (*tetA* and *tetB*), extended spectrum β-lactamase resistance (bla_{CMY-2}, bla_{SHV}, and bla_{TEM}), streptomycin resistance (*strA* and *strB*), sulfonamide resistance (*sul1*), and chloramphenicol resistance (*floR*).

[b]No screened genes were detected in 3 isolates.

[c]The bla_{CMY-2} gene was the most prevalent (41.9%) β-lactam resistance gene and significant differences ($P < 0.05$) of the distribution of this gene among isolates were observed between treatments (highest prevalence of 65.7% from virginiamycin 22 ppm) and sampling dates (highest prevalence of 66.7% from October 18).

[d]The *tetB* gene was the most prevalent tetracycline resistance (93.2%) gene and was found significantly more frequently ($P < 0.05$) in the monensin and narasin treatment (100%) when compared with other treatment groups.

tained 6 (*iss*, *iucA*, *papC*, *papG*, *traT*, and *tsh*) of the virulence genes (Table 6). Further, 23 (11.9%), 9 (4.6%), and 161 (83.9%) of the 193 isolates were classified as APEC, potentially APEC, and commensal, respectively. The majority of classified APEC strains were of phylogenetic group B1 (19 isolates) and 4 isolates were group D. Among the potentially APEC isolates, 8 were of phylogenetic group D, 1 isolate was of phylogenetic group B1, and the majority of the commensal strains were of groups A or B1. Additionally, 140 (87.0%) of the 161 commensal isolates contained *iss* and *traT*, whereas 31 (96.9%) and 23 (71.9%) of the 32 APEC and potentially APEC isolates contained *iucA* and *tsh*, respectively. There was no correlation between the detection of the pathotype and the treatment received.

Discussion

Our data in the present study showed that *E. coli* can survive for months in an oligotrophic soil during a period (August to March) of varying temperatures, moisture content, and different intensities of solar radiation as previously reported (Ishii and Sadowsky 2008; Reddy et al. 1981). Jamieson et al. (2002) reviewed the factors affecting survival of *E. coli* in soil. The high moisture and warm temperature in August could have contributed to the initial survival and persistence of *E. coli* in soil during this time period. The difference between treatments (diets with different antibiotics) tended to be significant in November, and significant differences were noted between treatments in January, when *E. coli* populations were lower in control plots than in all other treatments except monensin. These differences could be due to the antimicrobial agents altering sensitivity to changes in the chemical, physical, or biological properties of litter and (or) soil that were not elucidated. Indeed, moisture levels of soil can fluctuate because of drying and precipitation that could affect our viable counts. The difference in *E. coli* numbers seen between treatments specifically in January needs further investigation to fully understand how exposure to antibiotics may alter the ability of *E. coli* populations to survive in soil, especially during periods that would appear to be unfavourable for growth.

Studies from Zhou et al. (2010) suggested that tetracycline persists in soil after amendment with manure from antibiotic-fed swine. Antimicrobial residues were reported in broiler litter used in this present study (Furtula et al. 2010). However, persistence of antimicrobial residues in soil was not determined. In addition to the reduction in viable *E. coli* population from August through March, the percentage of isolates expressing resistance also declined. Several factors could have contributed to this decline, including a cost to fitness of maintaining antibiotic resistance. However, our results showed significant differences between treatments on antibiotic resistance, but no specific antimicrobial contributed to the emergence, persistence, and spread of antibiotic-resistant *E. coli* in soil after litter applications. We do not know the history of antibiotic use in the hatchery, and the origin of resistance in the control is unknown and could be derived from diverse sources. The selection and maintenance of resistant *E. coli* may be due to environmental components independent of antibiotic selection, which could explain the presence of resistant *E. coli* in the control (Diarra et al. 2007).

Table 4. Phylogenetic grouping of 193 multiresistant *Escherichia coli* isolated from soil fertilized with poultry litter from August 2005 to March 2006.

		No. of isolates in phylogenetic group per treatment						
Sampling date	Group	Control (n = 52)[a]	Chlortetracycline (n = 34)[b]	Virginiamycin 11 ppm (n = 15)[a]	Virginiamycin 22 ppm (n = 32)[a]	Monensin (n = 34)[b]	Narasin (n = 26)	Total (n = 193)
24 Aug. 2005 (n = 33)	A	3		1	2	2	2	10
	B1	4	6	3	2	4	3	22
	D		1					1
20 Sep. 2005 (n = 26)	A	7	2		4	1	2	16
	B1	1	2			5	1	9
	D			1				1
18 Oct. 2005 (n = 25)	A	5	1	2	5	2	1	16
	B1	3	3			1	1	8
	D						1	1
23 Nov. 2005 (n = 32)	A	5	1	3	1	3	2	15
	B1	2	3	2	1	4	2	14
	D				2		1	3
12 Dec. 2005 (n = 29)	A	6	2	1	5	3	1	18
	B1	2	4			2		8
	D					1	2	3
24 Jan. 2005 (n = 20)	A	4			5	2		11
	B1	2	4				3	9
	D							0
3 Mar. 2005 (n = 28)	A	6	3	2	4		4	19
	B1	2				4		6
	D		2		1			3
Total (n = 193)		52	34	15	32	34	26	193

[a]The group A was the most prevalent and was significantly ($P < 0.05$) more frequently detected in the control and virginiamycin 22 ppm groups.

[b]The group B1 was significantly ($P < 0.05$) more frequently detected in the chlortetracycline and monensin treatments.

Table 5. Virulence gene patterns found in 193 multiresistant *Escherichia coli* isolated from soil fertilized with poultry litter from August 2005 to March 2006.

		No. of isolates in phylogenetic group per treatment						
Sampling date	Virulence genes[a]	Control (n = 52)[b]	Chlortetracycline (n = 34)	Virginiamycin 11 ppm (n = 15)	Virginiamycin 22 ppm (n = 32)[b]	Monensin (n = 34)	Narasin (n = 26)[c]	Total (n = 193)
24 Aug. 2005 (n = 33)	*iss*				3			3
	iss-iucA-papC-papG II-traT-tsh		2					2
	iss-iucA-traT		1					1
	iss-iucA-traT-tsh		1			1		2
	iss-traT	7	2	3		5	4	21
	traT			1				1
	tsh		1					1
20 Sep. 2005 (n = 26)	*iss*				4			4
	iss-iucA-traT			1				1
	iss-iucA-traT-tsh		1			1		2
	iss-traT	8	3			4	2	17
	traT					1	1	2
18 Oct. 2005 (n = 25)	*iss*				5			5
	iss-iucA-traT-tsh		3					3
	iss-ompT						1	1
	iss-traT	7	1	2		3	2	15
23 Nov. 2005 (n = 32)	*iss*				1			1
	iss-iucA-papC-papG II-traT-tsh		1				1	2
	iss-iucA-traT-tsh	1	1			4		6
	iss-traT	6	2	4		3	4	19
	iucA-malX-kpsMT II-tsh				2			2
	traT			1				1
12 Dec. 2005 (n = 29)	*iss*				5			5
	iss-iucA-traT						2	2
	iss-iucA-traT-tsh					2		2
	iss-traT	6	2	1		3	1	13
	iucA-malX-kpsMT II-traT					1		1
	traT	2	4					6
24 Jan. 2006 (n = 20)	*iss*				5			5
	iss-iucA-traT-tsh	1						1
	iss-traT	5	1			2	3	11
	traT		3					3

Table 5 (*concluded*).

Sampling date	Virulence genes[a]	No. of isolates in phylogenetic group per treatment: Control (n = 52)[b]	Chlortetracycline (n = 34)	Virginiamycin 11 ppm (n = 15)	Virginiamycin 22 ppm (n = 32)[b]	Monensin (n = 34)	Narasin (n = 26)[c]	Total (n = 193)
3 Mar. 2006 (n = 28)	*iss*				4			4
	iss-iucA				1			1
	iss-iucA-traT	1	2					3
	iss-traT	6	3	2		4	4	19
Total (n = 193)		50	34	15	30	34	25	188

[a]Eleven resistance gene patterns were detected with *iss-traT* being the most prevalent found in 115 (61.2%) isolates. The *iucA* and *tsh* genes were significantly more prevalent in the chlortetracycline and monensin treatments. The *tsh* gene was detected significantly more frequently in isolates from 23 November.

[b]No screened genes were found in 2 isolates.

[c]No screened genes were found in 1 isolate.

Table 6. Relationship of serotype, phylogenetic group, and virulence genes in the 193 multiresistant *Escherichia coli* isolates.

Serotype[b]	Phylogenetic group	No. (%) of isolates that are positive for genes[a]: *iss*	*iucA*	*kpsMT II*	*malX*	*ompT*	*papC*	*papG II*	*traT*	*tsh*
O1:H45 (n = 4)	D	0	4 (100)	4 (100)	4 (100)	0	0	0	4 (100)	4 (100)
O117:H34 (n = 2)	A	2 (100)	0	0	0	0	0	0	2 (100)	0
O124:H25 (n = 1)	D	1 (100)	0	0	0	1 (100)	0	0	1 (100)	0
O15:H45 (n = 7)	D	7 (100)	7 (100)	0	0	0	0	0	7 (100)	0
O178:H2 (n = 32)	B1	32 (100)	0	0	0	0	0	0	32 (100)	0
O103:H19 (n = 1)	B1	1 (100)	0	0	0	0	0	0	1 (100)	0
O23:H16 (n = 3)	B1	3 (100)	3 (100)	0	0	0	3 (100)	3 (100)	3 (100)	3 (100)
O25:H28 (n = 1)	B1	1 (100)	0	0	0	0	0	0	1 (100)	0
O43:H34 (n = 1)	B1	1 (100)	1 (100)	0	0	0	0	0	1 (100)	0
O8:H19 (n = 104)	A	104 (100)	0	0	0	0	0	0	104 (100)	0
O82:H8 (n = 16)	B1	16 (100)	16 (100)	0	0	0	0	0	16 (100)	16 (100)
O86:H51 (n = 16)	B1	0	0	0	0	0	0	0	16 (100)	0

[a]All isolates were negative for the virulence *hlyD*, *kpsMT*, *papA*, *papEF*, and *sfa* genes.

[b]The serotypes O166:H49 and O6:H49 were negative for all screened genes.

In *E. coli*, resistance to β-lactam antibiotics can be intrinsic or acquired through plasmids or transposons and resistance to extended spectrum β-lactam antibiotics may be due to the presence of the transferable β-lactamase enzyme bla_{CMY-2} gene (Poole et al. 2009; Rice and Bonomo 2005). Production of extended spectrum β-lactamase by Gram-negative bacteria such as *E. coli* constitutes a significant public health concern. The products of the class A β-lactamase bla_{TEM} gene (plasmid-encoded found in *E. coli*) and bla_{SHV} gene (plasmid-encoded or chromosomally encoded found in *Klebsiella pneumoniae*) hydrolyse ceftazidime, ceftriaxone, cefotaxime, and aztreonam, while those of the bla_{CMY-2} gene, the most commonly encountered plasmid-mediated class C β-lactamase, which is often found in *E. coli* and *Salmonella enterica* serovars, hydrolyses extended-spectrum cephalosporins, including ceftiofur (used only in animals) and cephamycin (Rice and Bonomo 2005; Zhou et al. 2010). In the present study, *E. coli* was frequently resistant to the β-lactam antibiotics amoxicillin – clavulanic acid and ceftiofur. About 45.0% of β-lactam-resistant isolates harbored bla_{CMY-2} alone or in combination with bla_{TEM} and bla_{SHV} genes, confirming that raw untreated broiler litter could be a significant source of β-lactam-resistant *E. coli* in agricultural soils. The source of β-lactam-resistant isolates, including ceftiofur- and cefoxitin-resistant isolates, in this study is unknown; however, it has been suggested that ceftriaxone-resistant bacteria in farm environments could be associated with resistance to multiple antibiotics and that pine wood shavings used as bedding can be a potential reservoir for such multidrug-resistant bacteria (Yang et al. 2006).

Tetracycline ranks second (847 000 kg) among the antibiotics used in Canadian animal production (2006 data; Canadian Integrated Program for Antimicrobial Resistance Surveillance 2009). The most prevalent tetracycline resistance gene was *tetB*, predominantly detected in isolates classified as APEC (95.7%). The predominance of *tetB* in APEC suggests a possible direct or indirect link between APEC genetic determinants (virulence genes) and this tetracycline resistance gene. On the other hand, 55 ampicillin-resistant *E. coli* isolates were also resistant to streptomycin and tetracycline, suggesting that the use of tetracycline for treatment of growth promotion would select for isolates resistant to these other antibiotics as well.

Multiresistant *E. coli* were isolated from each collection date; about 20.0% of the overall isolates were resistant to at least 6 of the 12 antibiotics, and 14 isolates were resistant to 9 antibiotics. Multiple resistance genes, including *floR*, *strA*, *strB*, and *sul1*, were also detected most of the time in combination with *bla*, *tetA*, and (or) *tetB*, suggesting that application of litter could contribute to the accumulation of antibiotic resistant genetic determinants in soil. Most isolates contained ≤6 virulence genes, suggesting that the majority of them are less pathogenic. However, there were 23 isolates classified as APEC, and the majority of the isolates in which virulence capability needs to be established in vivo were of phylogenetic group B1. Similarly, *E. coli* isolates of phylogenetic groups B1 and D were found to be APEC (Bonnet et al. 2009; Rodriguez-Siek et al. 2005). In addition, of the 9 isolates classified as potentially APEC, 8 were of the phylogenetic group D and 1 was of group B1. The virulence gene *tsh*, indicator of avian *E. coli*, was found in all of the collection dates. It is important to note that one cannot be certain that an isolate is truly APEC unless it has been proven to be infectious in vivo (Bonnet et al. 2009).

The serotyping of *E. coli* is used to classify types of bacteria, to trace infectious outbreaks, to detect disease reservoirs, and to follow the emergence and spread of antibiotic-resistant pathogens (Cortés et al. 2010). In the present study, significant differences in resistance level and pattern were noted between the different serotypes ($P < 0.05$). All 16 isolates of serotype O86:H51 were multiresistant to ceftiofur, cefoxitin, gentamicin, chloramphenicol, and tetracycline, while the 32 isolates of serotype O178:H2 were resistant to amoxicillin – clavulanic acid, ceftiofur, cefoxitin, and tetracycline. All 4 isolates of serotype O1:H45 and all 16 isolates of O82:H8 showed the ceftiofur–cefoxitin–tetracycline resistant pattern, and all isolates from serotypes O8:H19 and O23:H16 were resistant to cefoxitin and tetracycline.

Among the 14 serotypes found in the present study, APEC strains were detected in serotypes O1, O23, and O82. Additionally, the 4 most prevalent serotypes (mentioned above) presented all nonvirulent characteristics belonging to either phylogenetic groups A or B1. Furthermore, 2 serotypes O1:H45 and O124:H25, both of phylogenetic group D, had the *tetB* and bla_{CMY-2} genes present in all of the isolates. Four O1:H45 isolates (3 in November and 1 in December) were not present in the first 3 soil collections (August, September, and October) along with the virulence genes *kpsMT II* and *malX* genes. The PFGE analysis followed by cluster analysis showed that these 4 O1:H45 isolates of group D clustered together, as did the 7 O15:H45 isolates from August (1 isolate), September (1 isolate), December (2 isolates), and March (3 isolates), suggesting their respective clonality. The serotype O124:H25 detected in November could come from different sources (wildlife for example) or was missed during other collections. However, serotyping of, phylogenetic grouping of, and PFGE on a subset (group D) of our isolates showed that multiple-antibiotic-resistant and potentially pathogenic *E. coli* from raw poultry litter can persist up to 7 months in agricultural soils. This persistence could represent a serious problem because of the potential contamination of ground or surface water and crops (Jamieson et al. 2002).

In conclusion, more research is needed to determine the mechanism behind the establishment of poultry litter *E. coli* populations in soil and their pathogenicity. The movement of antibiotic resistance genes between *E. coli* and indigenous soil bacteria also deserves more attention. Antibiotic-resistant bacteria appear to be environmentally adaptable, and isolates with the potential to cause serious, life-threatening infections that are difficult to control were identified in this work. The detection of extended spectrum β-lactamases and tetracycline resistance genes in combination with other resistance genes in all treatment groups suggests that care should be taken for the cropping and harvesting of crops from soils fertilized with raw broiler litter and that there remains a need to develop better guidelines concerning the use of such litter as fertilizers to protect human and environment health. Litter application to agricultural land can have a significant impact on the chemical, physical, and biological properties of soil, and as a result, management of litter needs to be updated regularly to ensure its safe use. Estimating survival of antibiotic-resistant and potential pathogenic bacteria in soil amended

with raw litter from broiler fed antimicrobial supplemented diets is essential for developing intervention strategies against resistant pathogens and towards pathogen control.

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