Antimicrobial resistance in generic *Escherichia coli* isolated from swine fecal samples in 90 Alberta finishing farms

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Abstract

The objective of this study was to determine the prevalence of antimicrobial resistance in generic *Escherichia coli* isolates obtained from 90 Alberta finisher swine farms. Up to 5 isolates were obtained from each of 269 pooled fecal samples and were classified as susceptible or resistant according to Clinical and Laboratory Standards Institute guidelines. Of the 1322 isolates, 166 (12.6%) were susceptible to all 15 antimicrobials. No resistance to amikacin, ceftiofur, ceftriaxone, or ciprofloxacin, antimicrobials of importance in human medicine, was observed. Relatively low frequencies of resistance were observed to gentamicin (1.1%), amoxicillin/clavulanic acid (0.7%), and cefoxitin (0.7%). Higher frequencies of resistance were observed for tetracycline (78.9%), sulfisoxazole (49.9%), streptomycin (49.6%), ampicillin (30.6%), chloramphenicol (17.6%), kanamycin (10%), and trimethoprim/sulfamethoxazole (6.4%). Among the isolates resistant to ≥ 2 antimicrobial classes, 20.8%, 20.6%, 18.2%, 7.0%, 1.8%, 0.2%, and 0.2% were resistant to 2, 3, 4, 5, 6, 7, and 8 antimicrobials, respectively. The most common multidrug-resistance patterns (resistance to ≥ 2 antimicrobial classes) were streptomycin-tetracycline (9.4%), streptomycin-sulfisoxazole-tetracycline (6.2%), and ampicillin-streptomycin-sulfisoxazole-tetracycline (6.1%). More clustering (higher intra-class correlation coefficients) in antimicrobial resistance was observed for isolates at the same visit than for isolates from different visits in the same farm, indicating that sampling more farms, testing fewer isolates per visits, and taking longer periods between visits may be appropriate and more efficient for a better understanding of potential shifts in resistance over time.

Résumé

*L'objectif de la présente étude était de déterminer la prévalence de résistance antimicrobienne chez des isolats d'*Escherichia coli *génériques obtenus de 90 fermes albertaines de porcs en finition. Un maximum de 5 isolats a été obtenu de chacun des 269 échantillons de pool de fèces et étaient classés comme sensible ou résistant selon les recommandations du «Clinical and Laboratory Standards Institute». Parmi les 1322 isolats, 166 (12,6 %) étaient sensibles aux 15 antimicrobiens. Aucune résistance à l'amikacine, au ceftiofur, à la ceftriaxone ou au ciprofloxacin, des antimicrobiens importants en médecine humaine, n'a été observée. Des fréquences relativement peu élevées de résistance ont été notées pour la gentamycine (1,1 %), l'amoxicilline/acide clavulanique (0,7 %) et le cefoxitin (0,7 %). Des fréquences plus élevées de résistance ont été notées pour la tétracycline (78,9 %), le sulfisoxazole (49,9 %), la streptomycine (49,6 %), l'ampicilline (30,6 %), le chloramphénicol (17,6 %), la kanamycine (10 %) et le trimethoprime/sulfaméthoxazole (6,4 %). Parmi les isolats résistants à 2 classes ou plus d'antimicrobiens, 20,8 %, 20,6 %, 18,2 %, 7,0 %, 1,8 %, 0,2 % et 0,2 % étaient résistants respectivement à 2, 3, 4, 5, 6, 7 et 8 antimicrobiens. Les patrons les plus communs de multi-résistance (résistance à 2 classes ou plus d'antimicrobien) étaient : streptomycine-tétracycline (9,4 %), streptomycine-sulfisoxazole-tétracycline (6,2 %) et ampicilline-streptomycine-sulfisoxazole-tétracycline (6,1 %). Plus de regroupements (coefficient de corrélation intra-classe plus élevé) de résistance antimicrobienne ont été observés pour les isolats provenant d'échantillons pris lors d'une même visite que pour les isolats obtenus lors de visites différentes sur la même ferme, montrant ainsi qu'il serait plus approprié et plus efficace pour mieux comprendre les changements de résistance dans le temps, d'échantillonner plus de fermes, de tester moins d'isolats par visite et d'espacer les visites.*

(Traduit par Docteur Serge Messier)

Introduction

The appearance of antimicrobial resistant bacteria in humans, animals, and the environment has raised both public and animal health concerns (1,2). *Escherichia coli* are among the most prevalent enteric bacteria in humans and animals, with the capability of acquiring and preserving resistance genes found in other organisms, the environment, and animal populations (3). Antimicrobial resistance (AMR) in these organisms, most strains of which are nonpathogenic, is considered to be a good indicator of the selective pressure resulting from antimicrobial use in target populations and a potential reservoir of resistance genes (4–5).

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Longitudinal monitoring of antimicrobial use and resistance has been recommended in order to gain a better understanding of the overall prevalence of antimicrobial resistance in targeted populations, and for the timely detection of emerging resistance trends (6). The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was initiated in 2002 to meet this need in Canada (7). An important component of this program is the integration of ongoing targeted research studies and routine surveillance activities in animals and humans, as well as generating longitudinal data on resistance specific to various food animal populations (7).

Previous Canadian studies that have described AMR in generic *E. coli* isolated from pigs have been conducted only in Ontario and British Columbia (8–10). These studies reported that in finishing swine, resistance was frequently observed for ampicillin, kanamycin, sulfamethoxazole, streptomycin, and tetracycline (8).

To the best of our knowledge no other study has investigated resistance in *E. coli* isolated from finishing swine in Alberta using a large number of swine farms. Thus, the objectives of this study were to investigate the prevalence of AMR in generic *E. coli* isolates obtained from 90 Alberta finishing pig farms, to evaluate clustering in the occurrence of resistance, and to provide retrospective data for longitudinal analysis of AMR in the swine population in Canada as an adjunct to CIPARS activities.

Materials and methods

Farm and fecal sampling

A retrospective cross-sectional study was conducted using pen-level pooled fecal samples collected for a study of *Salmonella* spp. in finishing swine (11). For that study, fecal samples were collected from finishing swine in 90 farms that produced at least 2000 pigs annually. Farms were selected by veterinarians from their client lists, based on the producers' willingness to participate in the study. The farms were visited by veterinarians 3 times at approximately 4- to 6-wk intervals from May to September 2000, with the exception of 1 farm that, for logistical reasons, was visited only twice. Five pens were randomly selected at each visit, and a pool of 25 g fecal material was collected from each pen in 5 g portions from different locations within the pen (5 × 5 g of feces). All pooled samples were thoroughly mixed using a sterile spatula. On average, 15 pooled fecal samples were collected per farm, and 2 g of each pooled sample were frozen for subsequent analysis.

Bacterial isolation

All laboratory work was conducted by the Agri-Food Laboratories Branch, Food Safety Division of Alberta Agriculture and Food (Edmonton, Alberta). Upon thawing, the samples for each farm were pooled by visit for a total of 269 farm-visit pools. A swab of the pooled sample was streaked onto a MacConkey's agar plate, and incubated at 35°C for 24 h. Suspect colonies were plated onto blood and MacConkey's agar plates to harvest a maximum of 5 presumptive colonies from each visit pool. These colonies were confirmed to be *E. coli* using EC MEDIUM with MUG (4-methylumbelliferyl-β-D-glucuronide), indole, methyl red, Voges-Proskauer, and citrate methods (12).

Table I. Summary of the 15 antimicrobials present on the Sensititre Custom Plate 2004 Panel (CMV1AGNF), used for antimicrobial resistance testing, and the corresponding breakpoints according to the NCCLS and NARMS guidelines[a,b,c]

Antimicrobials	Resistant (μg/mL)
Amikacin (AMI)	≥ 64
Amoxicillin/Clavulanic acid (AUG)	≥ 32
Ampicillin (AMP)	≥ 32
Cefoxitin (FOX)	≥ 32
Ceftiofur (TIO)	≥ 8
Ceftriaxone (AXO)	≥ 64
Chloramphenicol (CHL)	≥ 32
Ciprofloxacin (CIP)	≥ 4
Gentamicin (GEN)	≥ 16
Kanamycin (KAN)	≥ 64
Naladixic acid (NAL)	≥ 32
Streptomycin (STR)	≥ 64
Sulfisoxazole (FIS)	≥ 512
Tetracycline (TET)	≥ 16
Trimethoprim/Sulfamethoxazole (SXT)	≥ 4

[a] CLSI M100-S15 Table 2A. M7-A-MIC Testing Section.

[b] CDC. National Antimicrobial Resistance Monitoring System for Enteric Bacteria.

[c] NARMS: 2001 Annual Report. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2003.

Antimicrobial susceptibility testing

All isolates were tested for antimicrobial susceptibility using a broth microdilution technique and following Clinical and Laboratory Standards Institute (CLSI) guidelines (13,14) and the National Antimicrobial Resistance Monitoring System (NARMS) *E. coli* 2004 report guidelines for streptomycin (15). Isolates underwent susceptibility testing for 15 antimicrobials utilizing the Sensititre NARMS Gram-negative minimum inhibitory concentration (MIC) plate (CMV1AGNF, Sensititre; TREK Diagnostic Systems, Westlake, Ohio, USA). Sensititre panels were read using the Sensititre Automated Reading and Incubation System (ARIS; TREK Diagnostic Systems) and results were transferred to the Sensititre for Windows (SWIN) software for interpretation. Isolates with resistance to ≥ 2 classes of antimicrobials were defined as multidrug-resistant.

The antimicrobials tested, MIC breakpoints for resistance, and reference guidelines are shown in Table I.

Data analysis

Antimicrobial susceptibility data were transferred into a spreadsheet (Microsoft Excel 2000; Microsoft, Redmond, Washington, USA). The dataset was reviewed for missing values, proper coding, and distribution of values. Data were subsequently imported into a statistical software package (Intercooled Stata 9.1; Stata Corporation, College Station, Texas, USA) for descriptive analyses. Isolates with intermediate susceptibility to the tested antimicrobials were considered "susceptible" for analysis. Frequency tabulations for the categorical outcome variable ("susceptible" or "resistant") were determined for each of the 15 antimicrobials. Prevalence estimates

Table II. Frequency of antimicrobial resistance in generic *E. coli* isolates from 90 finishing swine farms in Alberta (*n* = 1322 isolates)

Antimicrobials[a,b]	Number of resistant isolates (%)	95% binomial exact confidence interval
AUG	9 (0.7)	0.3–1.3
AMP	404 (30.6)	28.1–33.1
FOX	9 (0.7)	0.3–1.3
CHL	233 (17.6)	15.6–19.8
GEN	14 (1.1)	0.6–1.8
KAN	132 (10.0)	8.4–11.8
STR	655 (49.6)	46.8–52.3
FIS	660 (49.9)	47.2–52.7
TET	1044 (78.9)	76.7–81.1
SXT	85 (6.4)	5.2–7.9

[a] AUG — amoxicillin/clavulanic acid; AMP — Ampicillin; FOX — cefoxitin; CHL — chloramphenicol; GEN — gentamicin; KAN — kanamycin; STR — streptomycin; FIS — sulfisoxazole; TET — tetracycline; SXT — trimethoprim/sulfamethoxazole.

[b] No organisms were resistant to amikacin (AMI), ceftiofur (TIO), ceftriaxone (AXO), ciprofloxacin (CIP), and nalidixic acid (NAL).

Table III. Frequencies of the most common multidrug-resistant (resistance to ≥ 2 antimicrobial classes) patterns among 1322 generic *E. coli* isolates from 90 finishing swine farms in Alberta

AMR Pattern[a]	Number of isolates (%)
STR-TET	124 (9.4)
FIS-TCY	57 (4.3)
AMP-TET	49 (3.7)
STR-FIS-TET	82 (6.2)
AMP-STR-TET	77 (5.8)
CHL-FIS-TET	52 (3.9)
KAN-STR-TET	5 (0.4)
AMP-STR-FIS-TET	81 (6.2)
CHL-STR-FIS-TET	64 (4.8)
KAN-STR-FIS-TET	30 (2.3)
AMP-CHL-STR-FIS-TET	36 (2.7)
AMP-STR-FIS-TET-SXT	18 (1.4)
AMP-KAN-STR-FIS-TET	16 (1.2)

[a] AMP — ampicillin; CHL — chloramphenicol; KAN — kanamycin; STR — streptomycin; FIS — sulfizoxazole; SXT — trimethoprim/sulfamethoxazole; TET — tetracycline.

were computed for each antimicrobial by dividing the total number of resistant isolates by the total number of isolates tested, and exact binomial 95% confidence intervals (CI) for each prevalence estimate were calculated.

Correlations among resistances to individual antimicrobials were investigated at the isolate level using Spearman's rank correlation coefficients (16). A *P*-value ≤ 0.05 was used to indicate statistically significant correlations.

To evaluate clustering in AMR, a 3-level random intercept logistic regression model [Generalized Linear Latent and Mixed Model (GLLAMM)] with adaptive quadrature and 16 integration points was used (17,18). Only antimicrobials where the frequency of resistance among *E. coli* isolates was ≥ 5% (subjectively chosen cut-off value) were included to have enough variability in the models. The outcome variable was the prevalence of AMR. Farm visits and farms were included in the model as random effects (17,18). Based on the estimated variance components for the unexplained variation at each level of the model (isolates, visits and farms), intra-class correlation coefficients (ICC) were calculated for isolates at the same visit, and for isolates at different visits in the same farm. The ICC was computed, by assuming that level 1 variance on the logit scale was:

$$\pi^2 \div 3 = 3.29,\ \pi = 3.1416 \quad (19) \qquad \textit{(Equation 1)}$$

The formulae used for ICC calculations for the 3 level models were (20):

$$\text{ICC (isolates at the same visit)} = \frac{\sigma^2_{visit} + \sigma^2_{farm}}{\sigma^2_{visit} + \sigma^2_{farm} + 3.29} \qquad \textit{(Equation 2)}$$

$$\text{ICC (isolates at different visits in the same farm)} = \frac{\sigma^2_{farm}}{\sigma^2_{visit} + \sigma^2_{farm} + 3.29} \qquad \textit{(Equation 3)}$$

The adjusted prevalence of resistance among *E. coli* isolates, accounting for farm and farm visit level clustering, was calculated using the aforementioned multilevel model structure. Only antimicrobials with a frequency of resistance ≥ 5% were included. The formula used was (20):

$$P = \frac{e^{\beta_0}}{1 + e^{\beta_0}} \qquad \textit{(Equation 4)}$$

where: β_0 = coefficient for the intercept.

Results

A total of 1322 *E. coli* isolates were recovered from 269 farm visit pooled fecal samples representing 90 farms. From 1 sample, just 2 isolates were recovered, from 3 pooled samples no *E. coli* were recovered, and from 5 samples just 4 isolates were recovered; 5 isolates were recovered from the remaining pooled samples. The prevalence of resistance to each antimicrobial is shown in Table II. No *E. coli* isolates were resistant to amikacin, ceftiofur, ceftriaxone, ciprofloxacin, or nalaxidic acid. A low frequency (< 2%) of resistance was observed to gentamicin (1.1%), amoxicillin/clavulanic acid (0.7%), and cefoxitin (0.7%). Less than 11% resistance was observed to kanamycin (10%) and trimethoprim/sulfamethoxazole (6.4%). Relatively higher frequencies of resistance (> 15%) were observed to ampicillin (30.6%), chloramphenicol (17.6%), streptomycin (49.6%), sulfisoxazole (49.9%), and tetracycline (78.9%). The frequencies of the most common resistance patterns among the 1322 *E. coli* isolates are shown in Table III.

The statistically significant ($P \leq 0.05$) Spearman's rank correlation coefficients among the individual antimicrobials are presented in Table IV. The hierarchical data structure of the 3-level multivariable model used for the analysis of variance of AMR in *E. coli* isolates consisted off 1322 isolates at the lowest level, 265 farm visits at the next level, and 90 farms at the highest level. Analysis of the variance components for the unexplained variation in AMR and the

Table IV. Spearman's rank correlation coefficients for antimicrobial resistance to different antimicrobials in 1322 generic fecal *E. coli* isolates from 90 finishing swine farms in Alberta

Antimicrobials[a]	AUG	AMP	FOX	CHL	GEN	KAN	STR	FIS	TET	SXT
AUG	x	—	—	—	—	—	—	—	—	—
AMP	0.12[b]	x	—	—	—	—	—	—	—	—
FOX	—	0.12	x	—	—	—	—	—	—	—
CHL	—	—	—	x	—	—	—	—	—	—
GEN	0.35	0.09	0.35	—	x	—	—	—	—	—
KAN	—	0.06	—	0.10	—	x	—	—	—	—
STR	—	0.24	—	0.06	—	0.12	x	—	—	—
FIS	0.06	0.18	0.06	0.46	0.06	0.19	0.26	x	—	—
TET	—	0.21	—	0.19	—	0.10	0.27	0.28	x	—
SXT	—	0.17	—	0.11	—	—	—	0.26	0.07	x

[a] AUG — amoxicillin/clavulanic acid; AMP — Ampicillin; FOX — cefoxitin; CHL — chloramphenicol; GEN — gentamicin; KAN — kanamycin; STR — streptomycin; FIS — sulfisoxazole; TET — tetracycline; SXT — trimethoprim/sulfamethoxazole.

[b] Statistically significant ($P \leq 0.05$) Spearman's rank correlation coefficients.

ICC indicated that higher ICC values (clustering) were present for isolates at the same visit than for isolates at different visits in the same farm (Table V). The adjusted prevalence of resistance in *E. coli* isolates, accounting for farm-visit and farm-level clustering, are presented in Table VI. Higher prevalence of resistance was observed for tetracycline, sulfisoxazole, streptomycin, and ampicillin than for the other antimicrobials tested.

Discussion

Results from different studies investigating AMR in *E. coli* from swine are difficult to compare due to differences in study design, particularly the population of swine sampled as well as sampling and testing protocols used. Discussion of comparisons is therefore restricted to those studies that investigated AMR in generic fecal *E. coli* in apparently healthy pigs on-farm or at slaughter.

Little or no resistance to the tested antimicrobials classified in Canada as of very high importance (21) (the 3rd generation cephalosporins ceftiofur and ceftriaxone, and the fluoroquinolone ciprofloxacin) was observed in this study; these are encouraging results from a human health perspective. The relatively high resistance levels observed to tetracycline and sulfisoxazole were expected findings. Dunlop et al (8) reported similar resistance levels to tetracycline (71%) and sulfisoxazole (38.9%) in generic *E. coli* isolates from 34 farrow-to-finish swine farms in Ontario. High levels of resistance to tetracycline (~ 60–95%) have also been detected in *E. coli* isolates recovered from apparently healthy swine on-farm or at slaughter in Japan (22), Spain (23), and England (24). The prevalence of resistance to streptomycin in Japan was 43% in isolates from healthy swine on-farm (22), compared with 30.6% found in this study. The prevalence of resistance to sulfonamides was 50% in this study, 64.8% in Japan (22), and 87.8% in Spain (23). A relatively high prevalence of resistance to ampicillin ranging from 22.6% to 29% was also reported in other studies (8,22) and is consistent with the 30% prevalence seen in this work. Almost 70% of all isolates were resistant to 2 or more antimicrobial classes in our study.

Substantial pair-wise correlation in AMR between chloramphenicol-sulfisoxazole, streptomycin-tetracycline, sulfisoxazole-streptomycin, and tetracycline-streptomycin patterns might have several explanations including plasmid (25,26) or class 1 integron-mediated resistance (27,28), co-selection, and selection pressure due to the use of these antimicrobials as feed additives (29). Using pathogenic *E. coli* isolates from swine, Bischoff et al (25) demonstrated that a chloramphenicol resistance gene was situated on the same plasmid as resistance genes for sulfamethoxazole, kanamycin, and tetracycline, and that these genes could be transmitted and preserved together. This might explain the relatively high prevalence of chloramphenicol resistance and the 52 isolates with the chloramphenicol-sulfisoxazole-tetracycline resistance pattern observed in our study. Molecular epidemiological studies are warranted to demonstrate this assumption.

A previous study of AMR in fecal *E. coli* in swine (30) demonstrated the utility of using pooled fecal samples instead of individual animal testing as an unbiased estimate of the prevalence. We attempted to evaluate aspects of clustering and found that the ICC of AMR for 7 of the antimicrobials studied was lower for isolates obtained at different visits in the same farm than for isolates at the same visit. In future studies, it might be more efficient to conduct fewer visits and sample more farms. On the other hand, the period between visits could be made longer in order to derive unbiased estimates of shifts in AMR prevalence over time. The relatively high level of clustering observed for isolates recovered at the same visit suggests that future studies using the pooled sample approach could test fewer than 5 isolates per sample without any substantial information loss.

The study population represented approximately 25% of annual swine production in Alberta in 2000 (11). Although participating farms were not selected randomly, the results of this study should be generally representative of larger swine farms in Alberta. Farms producing less than 2000 market pigs per year, however, were not included in the study, and the results should only be extrapolated to smaller operations with appropriate caution. The results demonstrate a wide distribution of AMR in *E. coli* isolates across the farms used in this study.

The results of this study will provide a benchmark for ongoing monitoring of AMR in fecal *E. coli* isolates from Alberta swine, and

Table V. Prevalence variance component at farm visits and farm level and ICC of antimicrobial resistance in 1322 generic fecal *E. coli* isolates from 90 finishing swine farms in Alberta

Antimicrobials[a]	Visit variance	Farm variance	ICC[b]	ICC[c]
AMP	2.06	1.55	0.52	0.22
CHL	2.50	4.13	0.67	0.42
KAN	3.82	0.68	0.58	0.08
STR	1.74	0.81	0.44	0.14
TET	3.91	3.76	0.70	0.34
SXT	4.10	4.23	0.72	0.36
FIS	2.96	2.11	0.61	0.25

[a] AMP — ampicillin; CHL — chloramphenicol; KAN — kanamycin; STR — streptomycin; TET — tetracycline; FIS — sulfizoxazole; SXT — trimethoprim/sulfamethoxazole.

[b] Intra-class Correlation Coefficient for isolates at the same visit.

[c] Intra-class Correlation Coefficient for isolates at different visits in the same farm.

Table VI. Adjusted prevalence of antimicrobial resistance, accounted for farm-visit and farm-level clustering, for antimicrobials with ≥ 5% frequency of resistance in generic *E. coli* isolates from 90 finishing swine farms in Alberta using the generalized linear latent and mixed model (GLLAMM) procedure (*n* = 1322 isolates)

Antimicrobials[a]	Percent resistant (%)	95% Confidence interval
AMP	21.5	16.0–28.3
CHL	5.2	2.9–9.4
KAN	2.8	1.6–4.9
STR	49.8	43.0–56.6
FIS	50.8	41.28–60.7
TET	93.2	88.2–96.2
SXT	0.6	0.19–1.86

[a] AMP — ampicillin; CHL — chloramphenicol; KAN — kanamycin; STR — streptomycin; FIS — sulfizoxazole; TET — tetracycline; SXT — trimethoprim/sulfamethoxazole.

complement CIPARS (7) antimicrobial use and resistance initiatives and other research studies in swine in western Canada.

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