Exploring Methodological Issues in Modelling Antimicrobial Resistance using Generic *Escherichia coli* Isolates from Chicken Abattoir and Retail Meat Surveillance in Canada

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ABSTRACT

EXPLORING METHODOLOGICAL ISSUES IN MODELLING ANTIMICROBIAL RESISTANCE USING GENERIC *ESCHERICHIA COLI* ISOLATES FROM CHICKEN ABATTOIR AND RETAIL MEAT SURVEILLANCE IN CANADA

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Comparisons were made of the performance of different regression models for analysis of annual variation in susceptibility of generic *Escherichia coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken surveillance. Secondly, impacts of using different multidrug resistance (MDR) classification metrics for the analysis of annual variation in MDR were evaluated using generic *E.coli* isolates from chicken abattoir surveillance. Antimicrobial susceptibility data were obtained from the Canadian Integrated Program for Antimicrobial Resistance Surveillance. Model assumptions were met using logistic and multinomial regression. Linear, tobit, ordinal and complementary log-log regression did not meet model assumptions and/or did not perform well. Significant annual variation in susceptibility to all three antimicrobials was identified with multinomial regression, whereas logistic regression only identified significant annual variation in susceptibility to ceftiofur. Both the prevalence of MDR and interpretation of the association between MDR, and year and region differed depending on the MDR classification metric used.

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STATEMENT OF WORK

The data for chapters 2 and 3 were obtained from the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), which is led by the Public Health Agency of Canada. The research plan was designed and data analysis was performed by Melissa MacKinnon. The thesis was written in its entirety by Melissa MacKinnon. Contributions to study conception and design, guidance and critical revision of the thesis were provided by the members of the advisory committee: Dr. Scott McEwen; Dr. David Pearl; Dr. Carolee Carson; and Dr. Jane Parmley.

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CHAPTER 1

Introduction, Literature Review and Study Rationale

Introduction

Antimicrobial resistance (AMR) is an urgent global public health crisis (World Health Organization, 2014, 2015). AMR can occur in any bacterial organism whether commensal or pathogenic and affects both humans and animals. An increase in the burden of illness for humans with resistant infections has been documented (World Health Organization, 2014). The correlation between AMR in bacteria from animals and AMR in human infections has been demonstrated in the literature (Dutil et al., 2010). This concept of transmission of AMR through the food chain to humans is well described for enteric bacteria (Kittl et al., 2013; Hoffmann et al., 2014; Jonas et al., 2015). Surveillance of AMR is an integral aspect of understanding the scope of the AMR issue and identifying interventions to improve the AMR crisis (World Health Organization, 2017). The enteric bacteria that are part of active AMR surveillance in Canada are *Salmonella, Campylobacter,* and generic *E. coli (Government of Canada, 2015a).* This program is called the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), which is led by the Public Health Agency of Canada (Government of Canada, 2015a).

A numeric minimum inhibitory concentration (MIC) is obtained when a bacterial isolate is tested for antimicrobial susceptibility. The MIC values are discrete, interval-censored and ordinal, and commonly have a bimodal or skewed distribution (Wagner et al., 2003; Sanchez, 2007; Otto, 2011). These characteristics can make statistical modelling of MIC data challenging. AMR surveillance programs are particularly interested in identifying temporal trends in the prevalence of resistance. A common approach, in light of the challenges noted above, is to dichotomize the data using established breakpoints into resistant (R) and susceptible (S) categories, then a logistic regression modelling approach is used

(Government of Canada, 2015a). However, in creating categories there is a loss of information and subtle yet important changes in bacterial populations' MICs could be missed, when logistic regression analysis is used (Wagner et al., 2003; Sanchez, 2007; Otto, 2011). There have been other statistical models applied to MIC data, but the performance of the various models has not been compared.

Multidrug resistance (MDR) is also an emerging issue. The genes that confer resistance to antimicrobials can occur on mobile genetic elements and can be located close to other resistance genes. This facilitates transfer of resistance genes between bacteria (e.g., *E. coli* and *Salmonella*) and allows the selection of resistance to one antimicrobial by exposure to a different antimicrobial (Allocati et al., 2013; Toombs-Ruane et al., 2017). It is difficult to compare MDR between different studies and surveillance programs because there is not a standard agreed upon definition of MDR (Falagas et al., 2006; Magiorakos et al., 2012). AMR surveillance programs, including CIPARS, are interested in monitoring the emergence and temporal trends in MDR, because monitoring resistance to only one antimicrobial drug in isolation loses critical information. Descriptive analysis of the prevalence of MDR and components of the resistance patterns are often performed; however, statistical analysis of temporal trends of the prevalence of MDR has only been performed on a limited basis in the literature.

The objectives of the following review are:

1) To describe the importance of AMR in enteric bacteria, especially *E. coli.*

2) To review the elements of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) related to *E. coli* isolates from chicken abattoir cecal and retail meat samples.

3) To explore the characteristics of MIC data and summarize literature related to statistical modelling of MIC data.

4) To discuss the definitions and importance of MDR and explore approaches for statistical modelling of MDR data.

Literature Review

The epidemiology of antimicrobial resistance in Escherichia coli

The importance of antimicrobial resistance

Currently, one of the most important and potentially devastating global public health crises is antimicrobial resistance (AMR) (World Health Organization, 2014, 2015). The AMR crisis affects people and animals across the world. With the rapidly increasing and evolving levels of AMR, very important antimicrobials are becoming less effective against prominent human and animal bacterial pathogens. AMR threatens to turn back the clock on modern medicine to a time that resembles the pre-antimicrobial era where a simple bacterial infection could be fatal (Aarestrup et al., 2008; World Health Organization, 2014, 2015). The epidemiology of AMR is complex with numerous points of transmission between humans, animals and the environment (Aarestrup et al., 2008). One of the main drivers of the development, propagation and maintenance of AMR is antimicrobial use (AMU), both in humans and animals. Unfortunately, antimicrobials have for decades been overused and misused, which further contributes to the development of resistance (Aarestrup et al., 2008; Verraes et al., 2013). Action plans to limit the exponential growth of the AMR problem and preserve the remaining effectiveness of current antimicrobials have been produced both at national and global levels (Public Health Agency of Canada, 2015; World Health Organization, 2015, 2017). The Canadian federal action plan on antimicrobial resistance and use in Canada has three main pillars: surveillance, stewardship and innovation (Public Health Agency of Canada, 2015).

Characteristics of Escherichia coli

Antimicrobial resistance can occur in any bacteria, including commensals and pathogens, and this amplifies the scope of the AMR issue. The focus of this review will be *Escherichia coli (E. coli)* and its

associated AMR, supplemented where necessary with relevant literature on nontyphoidal *Salmonella*. Both *E. coli* and nontyphoidal *Salmonella* belong to the family of Gram-negative bacteria called *Enterobacteriaceae* and are considered enteric bacteria (Allocati et al., 2013; Exner et al., 2017). *Escherichia coli* are typically considered commensal bacteria that are members of normal healthy gastrointestinal flora in humans and animals (White, 2006), but in some situations *E. coli* are also pathogenic. *Escherichia coli* isolates can be serotyped based on the O (somatic lipopolysaccharide), H (flagellar), F (fimbrial), and K (capsular) antigens. Pathogenic *E. coli* are further classified by their virulence mechanisms, including toxins, adhesions and invasiveness, and their clinical manifestations (White, 2006; Allocati et al., 2013). Broadly, pathogenic *E. coli* are either enteric *E. coli* (causing enteric disease including diarrhea) or extraintestinal *E. coli* (causing invasive diseases including urinary tract infections, blood stream infections, and meningitis). Pathogenic *E. coli* are transmitted through fecal-oral transmission as a result of poor hygiene or ingestion of contaminated food or water (Allocati et al., 2013).

Burden of illness of Escherichia coli infections in humans

Escherichia coli is a common cause of urinary tract infections and blood stream infections in humans (Allocati et al., 2013; World Health Organization, 2014). The antimicrobials that are frequently used to treat these infections are fluoroquinolones and third generation cephalosporins (World Health Organization, 2014). Third generation cephalosporins and fluoroquinolones are considered critically important (World Health Organization, 2016) or of very high importance (Government of Canada, 2009) for human medicine, depending on the classification scheme, both are the most important, highest, category in their respective classification scheme. In 2014, the World Health Organization (WHO) released an extensive global report on AMR surveillance. Within this report, results of studies on the burden of illness from resistance to third generation cephalosporins and fluoroquinolones in human *E. coli* infections were synthesized (World Health Organization, 2014). Typically, these studies determine

the added burden of illness from resistant infections compared to that from susceptible infections. Indicators of burden of illness include duration of illness, severity of illness, length of hospital stay and mortality, along with measures of economic impact (Cosgrove and Carmeli, 2003; Kaye et al., 2004; World Health Organization, 2014). The WHO systematic review and meta-analysis revealed that patients with third-generation cephalosporin or fluoroquinolone resistant *E. coli* infections had significant increases in their overall mortality and 30-day mortality. There was not a significant increase in their length of hospital stay. The economic burden of resistant *E. coli* infections has not been extensively studied (World Health Organization, 2014). The literature clearly demonstrates the importance of *E. coli* infections in humans and the critical need to maintain effectiveness of current antimicrobials (Pepin et al., 2009; Camins et al., 2011; Doi et al., 2013; Nussbaum et al., 2013; Martelius et al., 2016).

The connection between AMR and AMU in food animals and AMR in humans

There are many potential origins of *E. coli* infections in humans and sometimes it is difficult to identify the precise source of the infection. Humans are believed to be important reservoirs of resistant *E. coli* infections, and two of the more common transmission routes of resistant *E. coli* infections are auto-infection or transmission from another human (Allocati et al., 2013). There is however, some controversy regarding the importance of food-producing animals as reservoirs and the foodborne route of transmission for human *E. coli* infections (Carmo et al., 2014; Manges, 2016). The theory, which has more supportive evidence with nontyphoidal *Salmonella* and *Campylobacter* infections in humans, is that AMU in food production animals leads to the selection and spread of AMR. Then humans can be exposed to the resistant bacteria and resistance genes either through direct contact or consumption of animal products (Dutil et al., 2010; Kittl et al., 2013; Hoffmann et al., 2014; Jonas et al., 2015). Modifications in the use of antimicrobials in food animals may result in a change in spread of resistant bacteria to humans. The extend of the contribution of AMU in animals to the burden of AMR in humans

has not been definitively documented (McEwen, 2012). For example, CIPARS demonstrated a decrease in ceftiofur-resistant *Salmonella* Heidelberg from retail chicken meat and human clinical samples and subsequent increase in Québec during the voluntary withdrawal followed by the reintroduction of ceftiofur use in chicken hatcheries (Dutil et al., 2010). There was a corresponding decrease followed by an increase in ceftiofur-resistant *E. coli* from retail chicken meat samples, however, human clinical *E. coli* samples were not available for comparison (Dutil et al., 2010).

Surveillance of AMR in enteric bacteria

Integrated AMR surveillance

Surveillance is the cornerstone of efforts to identify trends in AMR in enteric bacteria. The WHO is encouraging all countries to develop and implement national surveillance programs for AMR in enteric bacteria (World Health Organization, 2017). The WHO recommends that the national surveillance programs should integrate AMR data from the main food animal species, retail meats, and clinical human samples. They should also collect and integrate data on AMU from the main food animal species and humans. The bacteria targeted in these integrated surveillance programs are the enteric bacteria that have been demonstrated to pass through the food chain to humans. After testing the bacterial isolates for susceptibility, the trends in AMR and AMU are analyzed and interpreted with an integrated approach (World Health Organization, 2017). Through these integrated and active surveillance programs, it is hoped that emerging and evolving AMR issues can be identified quickly with subsequent initiation of appropriate and timely interventions. The WHO released a guidance document on the integrated surveillance of antimicrobial resistance with the goal of harmonizing the methods used for sampling, susceptibility testing, and reporting (World Health Organization, 2017). Through harmonized methods, the results from different countries could be meaningfully compared.

In Canada, since 2002, surveillance of AMR in enteric bacteria has been conducted by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) led by the Public Health Agency of Canada. The CIPARS program includes three enteric bacteria; *Salmonella, Campylobacter* and generic *E. coli*; however not all bacterial species are isolated from the various surveillance components (Government of Canada, 2015a). Generic *E. coli* are included as a sentinel Gram-negative bacteria, in which resistance is likely representative of the general antimicrobial selection pressure on Gram-negative enteric bacteria (World Health Organization, 2017). *E. coli* is also an effective scavenger and reservoir of genetic material, which could include AMR genes (Allocati et al., 2013; Exner et al., 2017; World Health Organization, 2017). Samples are actively collected along the farm-to-fork continuum. On-farm surveillance of AMR and AMU occurs on grower-finisher pig farms, broiler chicken farms, and turkey farms. Sampling of broiler chickens, finished swine and cattle occurs at abattoirs. Retail meat samples of chicken, turkey, pork and beef are included. Clinical *Salmonella* isolates from animals and humans are collected passively. The design and methods employed by CIPARS have been previously documented (Government of Canada, 2015a) and are similar to the equivalent program in the United States of America, the National Antimicrobial Resistance Monitoring System (NARMS), which facilitates comparison of the results between CIPARS and NARMS (The National Antimicrobial Resistance Monitoring System, 2014). The chicken abattoir and retail chicken meat components of the CIPARS program will be focused on during this review.

Retail chicken meat component

The objective of the retail chicken meat surveillance component is to provide regional level information on the prevalence of and annual variation in AMR of nontyphoidal *Salmonella, Campylobacter* and generic *E. coli* (Government of Canada, 2015a). The chicken retail meat samples comprise chicken legs or wings with skin. In brief, the sampling plan includes dividing the participating regions by census division into strata based on population, then selecting a predetermined number of census divisions

within each stratum using stratified random selection with weighting by population. The population size is also used to weight the number of sampling days for each stratum.

Chicken abattoir component

The objective of the chicken abattoir component is to provide national level information on the prevalence of and annual variation in AMR of nontyphoidal *Salmonella*, *Campylobacter* and generic *E. coli* (Government of Canada, 2015a). A sample of cecal contents is obtained from the chickens during slaughter. Cecal contents are obtained to provide an indication of AMR in the bacteria on the farm and only chickens shipping from Canadian farms are considered for sampling. Federally inspected abattoirs participate voluntarily. In brief, a 2-stage sampling protocol is used. In the first stage, the abattoirs are randomly selected with a probability proportional to their slaughter volume. In the second stage, chickens are systematically selected for sampling. The number of chickens sampled per year at each abattoir is proportional to the slaughter volume (Government of Canada, 2015a).

Antimicrobial susceptibility testing of E. coli isolates

The methods for isolation and susceptibility testing of the generic *E. coli* isolates obtained from the samples have been previously documented and are based on established methods from the Clinical and Laboratory Standards Institute (CLSI) (Government of Canada, 2015a). One *E. coli* isolate from each sample is tested for antimicrobial susceptibility. The automated method of broth microdilution is used to obtain a minimum inhibitory concentration (MIC) value for each antimicrobial on the microtiter plate (Government of Canada, 2015a). NARMS designs the microtiter plates, which are used for susceptibility testing of *E. coli* and *Salmonella* isolates, and they are updated over time (The National Antimicrobial Resistance Monitoring System, 2016a). From 2004-2015, there were three different plates used for susceptibility testing and only three antimicrobials were not present on the plates for the entire time period (Table 1.1) (Government of Canada, 2015a). The microtiter plates contain successive double-

dilutions of the specified antimicrobials within a predetermined range of concentration and this facilitates determination of the MIC (Annis and Craig, 2005).

The MIC is a numeric value representing the lowest concentration of the tested antimicrobial that completely inhibits growth of the bacterial isolate (Annis and Craig, 2005). In general terms, an isolate that is more resistant to the antimicrobial being tested will take a higher concentration of antimicrobial for inhibition of growth compared to a more susceptible isolate. Interpretive criteria are needed to classify the isolate. CIPARS uses CLSI clinical breakpoints specific to each antimicrobial to classify the MIC values into susceptible (S), intermediate (I) and resistant (R) (Government of Canada, 2015a). For surveillance analysis purposes, CIPARS groups the I isolates with the S isolates (Government of Canada, 2015a). Clinical breakpoints are determined using pharmacokinetic and pharmacodynamic characteristics of the antimicrobial; this is because the original purpose of the breakpoints was to predict the success of clinical therapy with the antimicrobial (Rubin, 2013). Alternatively, interpretative criteria derived using observed susceptibility data can be used. These are called epidemiological breakpoints or cut-offs (ECOFFs) and their development and use was pioneered by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2017). ECOFFs classify isolates into wild-type (MIC below the ECOFF value) and non-wild-type (MIC value above the ECOFF value). The non-wild-type isolates have acquired resistance mechanisms that decrease their susceptibility to the antimicrobial compared to the wild-type isolates (Rubin, 2013; EUCAST, 2017). The ECOFF approach is useful for research and surveillance purposes but it not predictive of clinical therapeutic success (Rubin, 2013). If a CLSI clinical breakpoint is not available for an antimicrobial tested (e.g., streptomycin), then an approach similar to ECOFF determination is used with CIPARS and NARMS MIC data to determine an appropriate interpretive cut-off value (Government of Canada, 2015a).

Statistical modelling with MIC data

Characteristics of MIC data

MIC values are discrete, interval-censored and ordinal and these characteristics can make statistical modelling challenging (Sanchez, 2007; Otto, 2011). As well, it is not uncommon for the MIC data to be bimodal or skewed in distribution and to be sparse for certain dilutions (Wagner et al., 2003; Sanchez, 2007). These specific characteristics and distributional challenges need to be considered when analyzing the data using statistical models. One solution is to dichotomize the data using established breakpoints into S and R categories, then analyze the data using a logistic regression approach. However, by dichotomizing the MIC data, outcome information will be lost (Wagner et al., 2003; Sanchez, 2007; Otto, 2011). It has been suggested that this loss of information could result in failing to identify subtle changes in MIC, including MIC creep, which could be very important to surveillance programs and even trigger decision-making (Otto, 2011). MIC creep is when there is a progressive shift of MICs toward the breakpoint MIC value, within the susceptible MIC values (Yeh et al., 2012). In other words, the susceptible isolates are becoming less susceptible over time and moving close to the threshold for resistance. The concept of MIC creep has been well documented for vancomycin susceptibility in methicillin-resistant *Staphylococcus aureus* (Yeh et al., 2012).

Temporal analysis of AMR trends by surveillance programs

For AMR surveillance programs, evaluating temporal trends in susceptibility is part of routine data analysis. Annually, CIPARS performs temporal analysis of the AMR data for generic *E. coli* isolates from the chicken retail and abattoir components (analyzed separately) as part of the data analysis for the entire program (Government of Canada, 2015a). One antimicrobial is selected from each antimicrobial class for modelling. If an antimicrobial has a very low or zero prevalence of resistance, is cross-resistant to a selected antimicrobial or is banned for use in food animals, the antimicrobial is not considered for

modelling. Individual univariable logistic regression models are built for each antimicrobial selected using a dichotomous S/R outcome and categorized year as the predictor variable (Government of Canada, 2015a). CIPARS compares the prevalence of resistance in the current year to that in the previous year and initial year of surveillance implementation to identify statistically significant differences. For the retail chicken component, this temporal comparison is performed for each province/region where samples were collected (Government of Canada, 2015a). In the NARMS annual report, the trends in susceptibility for fluoroquinolone and cephalosporin resistance in generic *E. coli* isolates are presented descriptively (The National Antimicrobial Resistance Monitoring System, 2014). NARMS has an online interactive data display that allows users to select the bacterial species, sample source and antimicrobials of interest, which then can be used to explore temporal trends (The National Antimicrobial Resistance Monitoring System, 2016b). The European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) compile summary reports on AMR surveillance data from the European Union (EU) (European Food Safety Authority and European Centre for Disease Prevention and Control, 2016). The report includes a detailed description of the comparative prevalence of resistance in generic *E. coli* isolates between the participating countries. To analyze the temporal trends within individual countries for each antimicrobial separate logistic regression models are used (European Food Safety Authority and European Centre for Disease Prevention and Control, 2016).

Approaches to statistical modelling of MIC data in the literature

Statistical analysis of AMR data beyond the logistic regression approach is limited within the literature. The statistical modelling approaches other than logistic regression that have been reported include: linear regression; tobit regression; multinomial regression; ordinal regression; Poisson regression; accelerated time failure regression; Cox proportional hazards regression (complementary log-log); and mixture models (Tiemersma et al., 2004; Stegeman et al., 2006; Sanchez, 2007; Aerts et al., 2011; Otto,

2011; Jaspers et al., 2014; Bjork et al., 2015; Jaspers et al., 2016; Zawack et al., 2016). There is often a lack of independence and corresponding hierarchical structure to AMR data and therefore modelling approaches that account for the hierarchical structure should be used, which includes multilevel models (Wagner et al., 2003; Sanchez, 2007; Otto, 2011; Bjork et al., 2015). Important variables that have been included in models as random effects to account for clustered AMR data include: laboratory performing the testing; geographical location of sample collection; and the isolate itself to account for lack of independence among resistance genes and phenotypes (Stegeman et al., 2006; Otto, 2011; Bjork et al., 2015). When temporal trends in the prevalence of resistance are analyzed, the possibility of additional important predictor variables should be explored through the use of multivariable models. Significant predictor variables, in addition to year, for the analysis of the prevalence of resistance that have been identified previously in the literature include: animal species; multidrug resistance (MDR) indicator; age of patient isolate originated from; season or month of sample collection; and surveillance component sample source (Sanchez, 2007; Otto, 2011; Bjork et al., 2015; Zawack et al., 2016). Bjork et al. (2015) compared mixed multivariable logistic and accelerated failure time frailty (AFT-frailty) regression models for the analysis of the prevalence of resistance and association with time, animal species and MDR indicator for *Salmonella* isolates from the NARMS animal clinical component, while accounting for clustering by laboratory. They concluded that the AFT-frailty model was superior to the logistic regression model for identifying non-linear temporal trends and significant differences between animal species. However, if the data were highly skewed, then the AFT-frailty model did not reliably produce interpretable results. Results for evaluation of model fit or assumptions were not reported (Bjork et al., 2015).

Characteristics of select regression models

Based on the previous literature, the following regression models warrant further exploration: linear; tobit; logistic; multinomial; ordinal; and Cox proportional hazards regression models. For each

regression model, the characteristics and application to modelling MIC data will be described. For linear and tobit regression models the MIC outcome is modelled as a continuous numeric variable (Twisk and Rijmen, 2009; Dohoo et al., 2014). The tobit regression model is an extension of the linear regression model that accounts for right and left censoring of the outcome variable (Twisk and Rijmen, 2009). Due to the interval censored nature and frequently non-normal distribution of the MIC data, the models will likely have difficulty meeting the model assumptions of normality and homoscedasticity of the residuals even with logarithm base 2 transformation of the MIC data (Wagner et al., 2003; Otto, 2011).

The logistic regression model with R/S outcome data models the odds of an isolate being resistant. It tends to be a relatively robust method that applies readily to the AMR data and the odds ratio results are easy to communicate (Bjork et al., 2015). If a fixed effects logistic regression model is used, a goodness-of-fit (GOF) test can be used to assess model fit. There are GOF tests available for binary and binomial data and a nonsignificant ($p > 0.05$) result signifies that the model fits the data (Dohoo et al., 2014). If a mixed effects logistic regression model is used, the best linear unbiased predictions (BLUPs) should be assessed for homoscedasticity and normality (Dohoo et al., 2014). The multinomial regression model is an extension of the logistic regression model that allows more than two outcome categories. With a multinomial regression model the outcome categories are nominal (Dohoo et al., 2014). AMR data could be applied to multinomial regression using S/I/R categories, with MIC values as individual categories or another approach can be used to categorize the MIC data (Aerts et al., 2011; Otto, 2011). The results obtained are relative risk ratios for each predictor variable, which are commonly referred to as odds ratios, relating the odds of being in a given outcome category compared to the base or referent outcome category (Dohoo et al., 2014). In order to thoroughly interpret the multinomial regression model results all possible two-way comparisons between the outcome categories must be considered. Therefore, if individual MIC values are used as outcome categories, the number of comparisons necessary is very large and this significantly impacts the ability to effectively communicate the results. The methods for

assessment of model fit for fixed effects models and model assumptions for mixed effects models are the same as those described for the logistic regression model (Dohoo et al., 2014). The ordinal regression model is also an extension of the logistic regression model that allows multiple outcome categories that are ordered. The results for each predictor variable represent the odds of being at or above a given outcome category compared to being below the given outcome category (Dohoo et al., 2014). The ordinal regression model in theory appears to extend nicely to MIC data because it accounts for the discrete, ordinal and interval-censored nature of MIC data (Sanchez, 2007). However, the assumption of proportional odds must be met and this has been an issue with MIC data (Sanchez, 2007). The assumption of proportional odds means that the impact of the predictor variable on the outcome is equal for each outcome category. This assumption is evaluated for each predictor variable graphically or by using a Brant test. A non-significant ($p > 0.05$) Brant test signifies that the assumption of proportional odds has been met (Sanchez, 2007). If a mixed effects ordinal model is used, then the homoscedasticity and normality of the BLUPs should be evaluated.

The Cox proportional hazards regression model can be applied to MIC data using a complementary log-log (clog-log) regression model, which is equivalent to a Cox proportional hazards model but for discrete data. These are both semi-parametric survival methods and make no assumption about the distribution of the survival times. Ordinarily, survival models analyze the time-to-event and in the context of MIC data, the 'time' is the MIC value (concentration) and the 'event' is inhibition of bacterial growth (Sanchez, 2007; Otto, 2011). The assumption of proportional hazards must be met by the model. The assumption of proportional hazards means that across all MIC concentrations the effect of the predictor variable is equal. This assumption can be assessed by including two-way interactions between each significant predictor variable and the MIC concentration variable in the final model. If the interaction terms are statistically significant ($p \leq 0.05$) then the assumption of proportional hazards is violated and in order to meet the assumption, the interactions terms must be included in the model

(Sanchez, 2007; Dohoo et al., 2014). Issues related to parameter estimation with the clog-log regression model due to sparseness of data have been noted (Sanchez, 2007; Otto, 2011). The BLUPs should be evaluated for homoscedasticity and normality, if a mixed effects clog-log regression model is used.

Evaluating MDR in AMR surveillance data

The importance of MDR

A continually evolving and emerging issue related to AMR is MDR. Infection with multidrug resistant bacteria can lead to treatment challenges, delays in effective antimicrobial therapy and increased burden of illness (Roberts et al., 2009; Magiorakos et al., 2012). In *Enterobacteriaceae*, MDR is a significant issue because resistance genes are commonly on mobile genetic elements that can be transferred between different bacterial species and resistance genes can co-locate on mobile genetic elements (Allocati et al., 2013; Toombs-Ruane et al., 2017). A common cause of MDR in *E. coli* are β-lactamase enzymes, the genes for which are commonly located on plasmids and confer resistance to β-lactams and cephalosporins (Allocati et al., 2013; Toombs-Ruane et al., 2017). An emerging MDR issue in *E. coli* are carbapenemase enzymes that are frequently plasmid-encoded and confer resistance to carbapenems, as well as β-lactams and cephalosporins (Allocati et al., 2013; Nordmann, 2014). Resistance to fluoroquinolones in *E. coli* can be located on plasmids or chromosomes. It is not uncommon for resistance to fluoroquinolones to occur concurrently with β-lactamase enzymes (Allocati et al., 2013). There are a number of genes that confer resistance to aminoglycosides with some causing resistance to the entire aminoglycoside class, and these genes are commonly located near the carbapenemase gene (Allocati et al., 2013). *Escherichia coli* isolates with resistance to fluoroquinolones and aminoglycosides, and in possession of β-lactamase enzymes and/or carbapenemase enzymes highlight the issue of co-selection of resistance or co-resistance (Exner et al., 2017). Co-resistance can have very important implications for the selection and spread of resistance. For example even if use of one

antimicrobial is suspended or significantly reduced, resistance to that antimicrobial may be maintained or increased due to use of another antimicrobial.

MDR classification metrics

There is a lack of consistency in the MDR classification metrics used in the literature (Falagas et al., 2006; Magiorakos et al., 2012; German et al., 2016; Paramythiotou and Routsi, 2016). There are several elements that need to be considered when evaluating a study using an MDR classification metric: first, the actual antimicrobials assessed for susceptibility; second, the interpretive criteria used to classify resistant isolates [clinical breakpoints vs ECOFFs (Government of Canada, 2015a; EUCAST, 2017)]; third, whether individual antimicrobial drugs, antimicrobial categories or antimicrobial classes are being used to classify the antimicrobials assessed for susceptibility; and fourth, the number of antimicrobial drugs, categories or classes being used as the threshold for classification as multidrug resistant (Magiorakos et al., 2012). The criteria used to classify the antimicrobials into categories or classes are also not standardized (Magiorakos et al., 2012; The National Antimicrobial Resistance Monitoring System, 2014; Government of Canada, 2015b), nor is the threshold for MDR used in the literature. Resistance to three antimicrobial drugs, categories or classes is the most commonly used threshold for MDR, but resistance to two antimicrobial drugs, categories or classes is also used (M'Ikanatha et al., 2010; Glenn et al., 2013; Mainali et al., 2013; Medalla et al., 2013; Rzewuska et al., 2015; Guo et al., 2016). All of these factors affect the validity of comparing results between different studies (Falagas et al., 2006; Magiorakos et al., 2012; German et al., 2016). Another approach for classifying isolates as multidrug resistant, is to assess resistance to a preselected list of important antimicrobials and evaluate using a predetermined cut-off for MDR (German et al., 2016). An important point to consider when using MDR classification metrics for analysis of AMR data is that the composition of the resistance patterns for the multidrug resistant isolates are not all the same. The classification of an isolate as multidrug resistant does not provide insight into the importance of the isolate's resistance pattern to

human and animal health (Magiorakos et al., 2012). One multidrug resistant isolate may include resistance to a combination of antimicrobials that are highly important to human medicine and another may include resistance to antimicrobials that are of lower importance to human medicine.

The multidrug resistant isolates can be further characterized as extensively drug resistant (XDR) and pandrug resistant (PDR). There is no consensus for the definition of XDR or PDR (Falagas et al., 2006; Magiorakos et al., 2012; German et al., 2016; Paramythiotou and Routsi, 2016). XDR is commonly defined as susceptibility to one or fewer, or two or fewer of the antimicrobial drugs, categories or classes tested (Magiorakos et al., 2012; The National Antimicrobial Resistance Monitoring System, 2014). PDR is routinely defined as resistance to all of the antimicrobial drugs, categories or classes tested (Magiorakos et al., 2012). When classifying isolates as XDR or PDR, it matters whether the isolates are tested for susceptibility to a reasonable number and diversity of antimicrobials (Magiorakos et al., 2012). Magiorakos et al. (2012) suggested a list of antimicrobial categories that should be tested for susceptibility to *E. coli* isolates and if all of the categories are not tested then the isolates can only be considered possible extensively resistant (pXDR) or possible pandrug resistant (pPDR). The consensus statement for definitions of MDR, XDR, and PDR by Magiorakos et al. (2012) was intended for application to clinical human medicine; it would be ideal if an international consensus statement for MDR, XDR, and PDR classification metrics related to AMR surveillance in enteric bacteria could be completed.

Analysis of MDR trends in surveillance programs

As part of routine data analysis, AMR surveillance programs are increasingly interested in exploring MDR within their AMR surveillance data. CIPARS provides a descriptive analysis of MDR using an antimicrobial class approach. The number of generic *E. coli* isolates resistant to each possible numeric sum of antimicrobial classes and the corresponding antimicrobial classes contained in the resistance pattern are presented for each sample source component by animal species (Government of Canada,

2015b). The NARMS annual report provides the annual prevalence of MDR in generic *E. coli* isolates for each sample source component by animal species. An antimicrobial class approach for MDR classification is used. The presence of specific resistance patterns and XDR generic *E.coli* isolates are presented descriptively (The National Antimicrobial Resistance Monitoring System, 2014). The NARMS interactive display does not currently include MDR in generic *E. coli* (The National Antimicrobial Resistance Monitoring System, 2016b). The summary report on AMR surveillance in the EU includes a thorough description of MDR. The prevalence of MDR in generic *E. coli* isolates is presented for each country and sample source component by animal species. The MDR classification used is based on an antimicrobial category approach modelled after Magiorakos et al. (2012). The resistance patterns are described with special focus on isolates that are phenotypically resistant to third generation cephalosporins, carbapenems and fluoroquinolones (European Food Safety Authority and European Centre for Disease Prevention and Control, 2016). Statistical analyses of temporal trends in MDR are not currently reported by CIPARS, NARMS or EFSA/ECDC.

Approaches to statistical modelling of MDR data in the literature

Reporting the prevalence of MDR and/or description of the resistance patterns present is the most common approach to the analysis of MDR in studies evaluating AMR in *Salmonella* or *E. coli* isolates (M'Ikanatha et al., 2010; Lindsey et al., 2011; Sheikh et al., 2012; Cummings et al., 2013; Mainali et al., 2013). Statistical analysis of MDR is very limited in the literature. Simple statistical analyses (chi square) to compare MDR in two time periods have been used (Rzewuska et al., 2015). Logistic regression models were used to analyze temporal trends in MDR for nontyphoidal *Salmonella* clinical human samples (Medalla et al., 2013). A Poisson regression model was used to assess the temporal trends in MDR for *E. coli* F4, *Pasteurella multocida* and *Steptococcus suis* clinical porcine samples (Glass-Kaastra et al., 2014).

Study Rationale and Objectives

Integrated surveillance programs for antimicrobial resistance (AMR) are of the utmost importance for addressing the AMR crisis. The analysis performed by national AMR surveillance programs, including the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), are extensive; however, as AMR continues to evolve there is the need for new analytic techniques to be explored. Annual variations in the prevalence of resistance in monitored bacteria are currently modelled using logistic regression, but this limits the ability to identify subtle changes in MIC, including MIC creep. Other approaches to statistical analysis have been used in the literature, but there has not been a systematic comparison of the performance of different statistical regression models for the analysis of annual variation in the prevalence of resistance. Multidrug resistance (MDR) in bacteria is another emerging issue requiring analysis within AMR surveillance programs. Currently, a major challenge related to MDR analysis is the absence of an accepted classification metric or definition for MDR. The use of varying definitions of MDR limits ability to make meaningful comparisons between different studies and surveillance program data. There has not been a study to compare the impact of using different MDR classification metrics on statistical analysis of resistance data. Therefore, the objectives of this research project are:

1) To systematically compare the performance of various types of regression models using MIC and breakpoint (R/S) data for the analysis of annual variation in susceptibility of generic *Escherichia coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat surveillance samples while accounting for clustering of the samples (Chapter 2).

2) To use the best performing models to compare the similarities and differences in the results for all three antimicrobials (Chapter 2).

3) To describe the most common resistance patterns present in generic *Escherichia coli* isolates from chicken abattoir surveillance in Canada, as well as their variation over the study period and between sampling regions. In particular resistance patterns involving quinolones, and third generation cephalosporins and/or β-lactams with β-lactamase inhibitors will be described (Chapter 3).

4) To compare different multidrug resistance classification metrics, and use them to compare the annual and region variation in the prevalence of MDR using generic *Escherichia coli* isolates from chicken abattoir surveillance samples while accounting for clustering of the samples (Chapter 3).

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Table 1.1 – Summary of the antimicrobials included on the NARMS plates used by CIPARS for susceptibility testing of *E. coli* and *Salmonella* isolates from 2004-2015.

Antimicrobial	NARMS Plate and Time Period used by CIPARS		
	CMV1AGNF 2004-2010[1]	**CMV2AGNF 2011-2013[1]**	**CMV3AGNF 2014-2015[1]**
Amoxicillin-clavulanic acid (Amc)	Y	Y	Y
Amikacin (Amk)	Y	N	N
Ampicillin (Amp)	Y	Y	Y
Azythromycin (Azm)	N	Y	Y
Chloramphenicol (Chl)	Y	Y	Y
Ciprofloxacin (Cip)	Y	Y	Y
Ceftriaxone (Cro)	Y	Y	Y
Cefoxitin (Fox)	Y	Y	Y
Gentamicin (Gen)	Y	Y	Y
Kanamycin (Kan)	Y	Y	N
Nalidixic acid (Nal)	Y	Y	Y
Sulfisoxazole (Sss)	Y	Y	Y
Streptomycin (Str)	Y	Y	Y
Trimethoprim-sulfamethoxazole (Sxt)	Y	Y	Y
Tetracycline (Tet)	Y	Y	Y
Ceftiofur (Tio)	Y	Y	Y

[1] Yes (Y) and no (N) signify if the antimicrobial was present (Y) or absent (N) on the plate

CHAPTER 2

Comparison of model performance for evaluation of annual variation in susceptibility of generic *Escherichia coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat in Canada

Abstract

Statistical modelling of antimicrobial resistance (AMR) data is an important aspect of AMR surveillance programs; however, minimum inhibitory concentration (MIC) data can be quite challenging to model. The conventional approach is to dichotomize the data into resistant (R) and susceptible (S) categories using established breakpoints, then use a logistic regression model for analysis. A disadvantage of this approach is a loss of information created by dichotomizing the data. The objectives of the study were to compare the performance and results of different regression models for the analysis of annual variation in susceptibility of generic *E. coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat surveillance samples. *Escherichia coli* susceptibility data for ceftiofur, ampicillin and nalidixic acid from retail chicken meat samples from 2007-2014 were obtained from the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Annual variation in susceptibility for each antimicrobial was evaluated using multivariable linear, tobit, logistic, multinomial, ordinal and complementary log-log regression models. MIC ($\log_2$), censored MIC ($\log_2$), R/S, and categorized MIC (3 or 4 categories) data were used as outcome variables for the appropriate statistical models. Year and region were modeled as categorical predictor variables. Random effects were included in the models for ceftiofur and ampicillin to account for clustering by retail establishment. The nalidixic acid models with random effects for retail establishment either would not converge or the variance components for retail establishment were extremely small; therefore fixed effects only models were presented. Model

assumptions were evaluated and only met by the logistic and multinomial models. Significant annual variation in susceptibility to all three antimicrobials was identified by the multinomial regression models, whereas the logistic regression models only identified significant annual variation in susceptibility to ceftiofur. The logistic and multinomial regression models for all three antimicrobials identified significant regional variation in susceptibility. The multinomial regression model consistently identified additional significant annual variation in susceptibility compared to the logistic regression model. The multinomial modelling approach was able to identify differences between MIC categories within susceptible MIC values, which were below the breakpoint (R) detection level. Given the convention of dichotomizing susceptibility data, the logistic regression approach is likely to remain the standard method of analysis for AMR surveillance data; however, the results of this study demonstrate that multinomial regression should be considered for the analysis of AMR surveillance data since it provides more information along the range of MIC values.

Introduction

Antimicrobial resistance (AMR) is a 21st century global public health crisis (Marshall and Levy, 2011; World Health Organization, 2014, 2015; Zawack et al., 2016) that seriously threatens the successful treatment of bacterial infections (Aarestrup et al., 2008; World Health Organization, 2014, 2015). The selection and spread of acquired bacterial resistance to antimicrobials is an issue for both humans and animals, and has been driven by increasing antimicrobial use (AMU) and misuse (Molbak, 2005; Aarestrup et al., 2008; Schechner et al., 2013; Verraes et al., 2013). The correlation between antimicrobial-resistant bacteria in animals or animal products, and humans has been demonstrated (Aarestrup et al., 2008; Dutil et al., 2010); however, the magnitude of the impact of AMU in animals on the burden of AMR in humans has not been definitively documented (McEwen, 2012). An important aspect of dealing with the AMR crisis is surveillance (Aarestrup et al., 2008; World Health Organization, 2017). In Canada, surveillance of AMR in enteric bacteria is performed by the Canadian

Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), which is led by the Public Health Agency of Canada (Government of Canada, 2015a). CIPARS samples various points along the farm-to-fork continuum for the main food animal species, and also includes human and animal clinical samples. The enteric bacteria included in the surveillance program are *Salmonella*, generic *Escherichia coli* and *Campylobacter* (Government of Canada, 2015a). Generic *E. coli* is included in surveillance programs because it reflects the general antimicrobial selection pressures on commensal bacteria (Hanon et al., 2015; World Health Organization, 2017) and it is efficient at scavenging external DNA material, which could include AMR genes (Allocati et al., 2013; Exner et al., 2017; World Health Organization, 2017).

One goal of surveillance is to analyze the temporal trends in AMR with the aim of identifying issues early. In the surveillance context, AMR data typically take the form of a numeric minimum inhibitory concentration (MIC) value for each combination of bacterial isolate and antimicrobial tested. Modelling the MIC values is challenging due to the fact they are discrete, interval-censored and ordinal, and the distribution of the MIC values is generally skewed or bimodal (Sanchez, 2007; Aerts et al., 2011; Otto, 2011). The standard approach to deal with these issues is to dichotomize the MIC values into resistant (R) and susceptible (S) categories using established clinical breakpoints or epidemiological cutoffs, and then use logistic regression for modelling (Bjork et al., 2015; Government of Canada, 2015a; Hanon et al., 2015). This strategy is effective at producing easy to interpret odds ratios; however, it has been suggested that this is not an ideal approach since there is a loss of information from the MIC values when they are dichotomized (Sanchez, 2007; Aerts et al., 2011; Zawack et al., 2016). It is possible that subtle changes in MIC values over time including MIC creep could be missed when logistic regression modelling is used (Wagner et al., 2003; Aerts et al., 2011; Bjork et al., 2015). MIC creep is described as a gradual increase in MIC within the susceptible range of MIC values, which alternatively can be described as the gradual shift of susceptible MIC values towards the resistant breakpoint MIC value

(Yeh et al., 2012). The concept of MIC creep has been well documented with vancomycin susceptibility in methicillin-resistant *Staphylococcus aureus* infections in humans (Yeh et al., 2012).

There is interest in using statistical models other than logistic regression for the analysis of trends in AMR over time, but this has not been extensively explored. Some studies have utilized accelerated failure time, Cox proportional hazards, multinomial logistic, ordinal logistic, linear and tobit regression models (Stegeman et al., 2006; Sanchez, 2007; Aerts et al., 2011; Otto, 2011; Bjork et al., 2015). In well-established national surveillance programs like CIPARS, bacterial isolates are often accompanied by basic epidemiological data in addition to MIC values and date of collection, and therefore multivariable models can be used to analyze these data. However, the nature of AMR surveillance data can pose challenges to analysis and affect the suitability of various types of models. For example, there is a need to address lack of independence (clustering) in the data that can be accounted for by utilizing mixed effects regression modelling (Dohoo et al., 2014). Examples of multivariable and mixed effects modelling approaches for analysis of trends in AMR over time are limited within the literature (Otto, 2011; Bjork et al., 2015; Martelius et al., 2016). In addition, assessment of the resulting models for goodness-of-fit and whether they meet the required model assumptions is not routinely reported in studies; this assessment is important because of the MIC data characteristics and non-normal distributions.

The first objective of the study was to systematically compare the performance of various types of regression models using MIC and breakpoint (R/S) data for the analysis of annual variation in susceptibility of generic *Escherichia coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat surveillance samples while accounting for clustering of the samples. The second objective was to use the best performing models to compare the similarities and differences in the results for all three antimicrobials.

Methods

Surveillance data and susceptibility testing

The generic *E. coli* isolates were obtained from retail chicken meat samples as part of the CIPARS active retail component from 2007-2014 using conventional methods for isolation (Government of Canada, 2015a). An automated broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) standards was used to obtain the MIC of the generic *E. coli* isolates for 14-15 antimicrobials, including the antimicrobials of interest for this study, ceftiofur, ampicillin and nalidixic acid (Government of Canada, 2015a). These three antimicrobials were selected for analysis since their respective prevalence of resistance differed (Government of Canada, 2015b). The range of antimicrobial concentrations tested for each antimicrobial were as follows: ceftiofur (0.12 – 8 μg/ml), ampicillin (1 – 32 μg/ml) and nalidixic acid (0.5 – 32 μg/ml) (Government of Canada, 2015a). The breakpoints used to characterize resistance of the generic *E. coli* isolate to the antimicrobial being tested were from CLSI defined clinical breakpoints: ceftiofur (R ≥ 8 μg/ml), ampicillin (R ≥ 32 μg/ml) and nalidixic acid (R ≥ 32 μg/ml) (Government of Canada, 2015a). The data available related to each isolate included MIC value (μg/ml) for each antimicrobial of interest, and the year, region and coded retail establishment of sample origin.

Statistical Methods

STATA / SE 14.2 was used to perform all statistical analyses (StataCorp LLC, 2015). Descriptive statistics were performed to summarize the overall prevalence of resistance and distribution of the isolates within each MIC category for the three antimicrobials of interest. The following regression models (STATA codes - fixed effects, mixed effects) were used to analyze the susceptibility data for the three antimicrobials: linear (regress, mixed), tobit (tobit, xttobit), logistic (logit, melogit), multinomial (mlogit, gllamm with binomial family and mlogit link), ordinal (ologit, meologit), and Cox proportional

hazards for discrete data [complementary log-log regression model (clog-log), cloglog, mecloglog] (Skrondal and Rabe-Heskath, 2003; StataCorp LLC, 2015). All of either the fixed or mixed effects regression models for each antimicrobial were evaluated further. The results of each mixed effects model were presented unless the models failed to converge, the random effects variance component was not statistically significant and the variance component was extremely small ($< 1.0X10^{-3}$), or the Akaike information criterion (AIC) and Bayesian information criterion (BIC) were more than 10 points greater than that of the fixed effects model (Dohoo et al., 2014).

The outcome variables were modelled as appropriate for each type of model. For linear and clog-log regression, log base 2 transformation of the MIC value was used. The "time-to-event" for the clog-log regression model was characterized by the concentration of antimicrobial ("time") when there was inhibition of bacterial growth ("event"). The censored log base 2 transformed MIC value was used for the tobit regression model. The left (lower limit, ll) and right (upper limit, ul) censored values were specific to each antimicrobial: ceftiofur ($ll \leq 0.12$, $ul > 8$ μg/ml), ampicillin ($ll \leq 1$, $ul > 32$ μg/ml) and nalidixic acid ($ll \leq 0.5$, $ul > 32$ μg/ml). For logistic regression, the outcome variable was dichotomized based on the previously noted CLSI clinical breakpoints in to R and S categories. Categorized MIC data were used for the multinomial and ordinal regression models. The number of categories was based on the distribution of the MIC data for the antimicrobial of interest; the MIC values representing 'resistant', based on the clinical breakpoint, were maintained as a category and the susceptible MIC values were divided into 2 or 3 categories (Table 2.1).

Several predictor variables were considered for inclusion in the models. Year of sampling (year) was considered for inclusion as a continuous variable. It was assessed for linearity using a locally weighted regression (lowess or lowess logit) of the outcome variable and year (Dohoo et al., 2014). If the relationship was non-linear, the suitability of a quadratic relationship was assessed graphically. If suitable the squared year term was included in the model and assessed for significance ($p \leq 0.05$). If not

suitable or non-significant, year was categorized by individual years and modeled as a hierarchical categorical variable. Province/region of sample origin (region) was modelled as a categorical variable and included as a fixed effect to account for possible clustering by region. The regions included were British Columbia (BC), Prairies (PR, including Manitoba, Saskatchewan, and Alberta), Ontario (ON) and Québec (QC). The isolates from the Maritimes region were excluded due to missing data. Random intercepts (shared frailty for clog-log) for retail establishment of origin were included to account for possible clustering.

Multivariable regression models were built using a backward stepwise manual approach. Fixed effects with p-values of ≤ 0.05 for the likelihood ratio test were retained in models. Since one of the main objectives of the study was to evaluate year as a predictor variable in the models, it was always forced into the final model regardless of statistical significance. Any non-significant predictor variables were assessed for confounding and were included in the final model if they caused a >20% change in a coefficient for another variable. The final multinomial model was executed with each outcome category as the base referent category, to allow complete representation of all comparisons between outcome categories.

The performance of the regression models was compared using the criteria described below. The model assumptions and goodness-of-fit were evaluated as appropriate for each model (Table 2.2) (Dohoo et al., 2014). To assess the assumption of proportional hazards for the clog-log model, 2-way interactions between the MIC concentration variable and all significant predictor variables were evaluated in the final model. If the interactions were statistically significant ($p \leq 0.05$), then the assumption of proportional hazards was violated and to meet the assumption the interactions were included in the final model (Sanchez, 2007; Dohoo et al., 2014). Additional diagnostics were performed on models that fit the data or met model assumptions to assess individual observations or covariate patterns for fit and influence on the model.

Results

General descriptive results

There were 3243 generic *E. coli* isolates from retail chicken meat surveillance from 2007-2014 included in the study. The distributions of the MIC values for ceftiofur, nalidixic acid and ampicillin are presented in Table 2.3. The distributions of MIC values were bimodal in shape for ceftiofur and ampicillin, and right skewed for nalidixic acid.

Model characteristics

The relationships between year and the outcome variables were non-linear and not quadratic for ceftiofur and ampicillin. There was a linear relationship between year and the outcome variable for nalidixic acid; however, to maintain consistency of the model results year was modeled as a hierarchical categorical variable for all models. For ceftiofur and ampicillin, the final multivariable models included random effects for retail establishment and fixed effects for year and region. The final multivariable models for nalidixic acid contained fixed effects for year and region. The nalidixic acid models with random effects for retail establishment either would not converge (clog-log) or the variance components for retail establishment were extremely small (all other models).

Overall, the linear, tobit and ordinal regression models did not meet model assumptions and will not be discussed further (Tables 2.4 and 2.5). The performance of the clog-log regression models was variable between the three antimicrobials (Tables 2.4 and 2.5). In order for the clog-log regression models for ceftiofur and nalidixic acid to meet the assumption of proportional hazards, two-way interactions between both year and region, and MIC concentration had to be included in the model (Tables 2.4a and 2.5). The inclusion of the interaction terms confined interpretation of the model results to graphical presentations. The right skewed distribution of the nalidixic acid data impacted the ability to interpret the model results, since sparseness of data led to failure to calculate certain coefficients and large

standard errors. The mixed effects ampicillin clog-log model violated the assumption of proportional hazards, but the model would not converge when interactions between MIC concentration, and both year and region were included. The logistic and multinomial regression models met model assumptions and performed well for all three antimicrobials (Tables 2.4 and 2.5). There were several covariate patterns that had standardized residuals with extreme values or had large influence on the models, however, there was no justification to remove the covariate patterns and they were all included in the models.

Logistic and multinomial models for ceftiofur

The logistic and multinomial regression models identified significant annual and regional variation in susceptibility to ceftiofur. When the results of these models related to annual variation in susceptibility are compared (Table 2.6), it is clear that there are both similarities and differences in the significant results of the two models. In the logistic model, an isolate in 2008 had significantly increased odds of being resistant compared to 2007 (Table 2.6). In the multinomial regression model, compared to 2007, there were significantly increased odds of an isolate from 2008 being in category 3 compared to category 0 (Table 2.6). An isolate in 2014 had significantly decreased odds of being resistant compared to 2013, in the logistic regression model (Table 2.6). In the multinomial regression model, when comparing 2014 to 2013, there were significantly decreased odds of an isolate being in categories 1 and 3 compared to category 0, and category 3 compared to categories 1 and 2 (Table 2.6).When comparing 2009 to 2008, 2010 to 2009, and 2013 to 2012, no significant difference in susceptibility was identified with logistic regression (Table 2.6). However, with the multinomial regression model, when comparing 2009 to 2008, there were significantly increased odds of an isolate being in category 1 compared to category 0 (Table 2.6). When comparing 2010 to 2009, with the multinomial regression model, there were significantly decreased odds of an isolate being in category 1 compared to category 0 and significantly increased odds of an isolate being in category 3 compared to category 1 (Table 2.6). There were significantly increased odds of an isolate being in categories 1 and 3 compared to category 0, when

comparing 2013 to 2012, with the multinomial regression model (Table 2.6). The significant results related to region were similar between the logistic and multinomial regression models (Table 2.6), with the exception that results for MIC category 3 compared to category 2 in the multinomial regression model were different than the logistic regression model when comparing ON to BC (Table 2.6). The variance components for retail establishment were small and non-significant in both models but met the criteria, outlined in the methods, to be included (Table 2.6).

Logistic and multinomial models for ampicillin

The logistic and multinomial regression models identified significant regional variation in susceptibility to ampicillin. However, only the multinomial regression model identified significant annual variation in susceptibility. When the results of the logistic and multinomial regression models related to regional variation in susceptibility are compared (Table 2.7), it is clear that there are similarities in the significant results of the two models. The significantly decreased odds of an isolate being resistant from ON, PR and QC compared to BC in the logistic model is represented in the multinomial model by decreased odds of an isolate from the same regions being in category 2 (resistant) compared to both categories 0 and 1 (Table 2.7). In the multinomial regression model, significant annual variation in susceptibility was identified. When comparing 2012 to 2011, there were significantly increased odds of an isolate being in category 1 compared to category 0 and significantly decreased odds of an isolate being in category 2 compared to category 1 (Table 2.7). There were significantly increased odds of an isolate being in categories 1 and 2 compared to category 0, when comparing 2013 to 2012 (Table 2.7). When comparing 2014 to 2013, there were significantly decreased odds of an isolate being in categories 1 and 2 compared to category 0 (Table 2.7). In both the logistic and multinomial models, the variance components for retail establishment were small and non-significant but met the criteria, outlined in the methods, to be included (Table 2.7).

Logistic and multinomial models for nalidixic acid

The logistic and multinomial fixed effects regression models both identified significant regional variation in susceptibility; however significant annual variation in susceptibility was only identified with the multinomial regression model. When 2012 was compared to 2011, there were significantly increased odds of an isolate being in categories 1 and 2 compared to category 0 (Table 2.8). There were significantly decreased odds of an isolate being in categories 1, 2 and 3 compared to category 0 and category 2 compared to category 1, when comparing 2014 to 2013 (Table 2.8). With respect to regional variation in susceptibility, there was a significant decrease in the odds of an isolate being resistant from ON and QC compared to BC in the logistic model and this was also demonstrated in the multinomial model by decreased odds of an isolate from the same regions being in category 3 (resistant) compared to both categories 0 and 1 (Table 2.8). In the multinomial regression model there was also significantly decreased odds of an isolate from ON and QC compared to BC being in category 2 compared to category 1 (Table 2.8). There was no significant difference identified in the logistic regression model when PR was compared to BC. In the multinomial model, compared to BC, an isolate from PR had significantly decreased odds of being in category 2 compared to categories 0 and 1 and significantly increased odds of being in category 3 compared to category 2 (Table 2.8).

Discussion

In this study, a systematic approach was used to compare the performance of six different regression models for the analysis of annual variation in susceptibility of generic *E. coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat surveillance samples. These three antimicrobials were selected to demonstrate model performance over a range of resistance prevalences (5.0-44.0%, Table 2.2) that would be commonly encountered when analyzing AMR surveillance data (Government of Canada, 2015b). Two types of regression models were consistently appropriate for modelling the

susceptibility data for the three antimicrobials examined: logistic regression and multinomial regression models. When the results for analysis of annual variation in susceptibility were compared from these two models, there was consistently additional significant annual variation in susceptibility identified with the multinomial regression model. These are important results to note because awareness of changes in the distribution of isolates within the susceptible MIC values is valuable information for surveillance programs (Wagner et al., 2003). Using the approach from the current study for categorizing MIC, it would be possible to identify MIC creep within susceptible MIC values prior to a significant change in the prevalence of resistance; therefore implementation of control strategies could occur sooner.

Multivariable and mixed effects were important aspects of the regression models that we used. When susceptibility data are being analyzed it is important to consider and account for the hierarchical structure of the data by using mixed effects models when appropriate (Otto, 2011; Bjork et al., 2015). Bjork et al. (2015), demonstrated that the laboratory performing the testing was a significant and important random effect for their regression models analyzing factors associated with level of resistance to various antimicrobials in livestock and poultry clinical *Salmonella* isolates. In the current study, mixed effects model results were presented for ceftiofur and ampicillin models. The random intercepts of retail establishment only explained a small amount of variation in the data and were not significant. The notable cluster variable in the data for our study was region of sample origin. Since there were only four regions, it was modelled as a fixed categorical variable. When the results for regional variation in susceptibility were compared for the logistic and multinomial regression models, there was additional significant regional variation identified in the nalidixic acid model. The regression models in the current study were multivariable including year and region. Depending on available data and type of surveillance program, previous literature has demonstrated the importance of including other fixed effects predictor variables when analyzing annual variation in susceptibility including: species of animal

sampled, multidrug resistance indicator, quarter of year, month and age of subject (Sanchez, 2007; Otto, 2011; Bjork et al., 2015).

The logistic regression model has been the conventional approach to evaluate annual variation in susceptibility for surveillance data (Government of Canada, 2015a). It embodies many desirable characteristics that have been demonstrated in the current study; it is a robust method where the model fits the data or model assumptions are met, and the odds ratios obtained as results are easy to interpret and present. However, especially in the context of surveillance, there are some limitations with using logistic regression modelling. By dichotomizing the data to S and R categories there is a loss of information and all that can be identified are significant changes in the prevalence of resistance (Sanchez, 2007; Aerts et al., 2011; Otto, 2011; Bjork et al., 2015). Thus, there is no information available for changes within the susceptible or resistant MIC values and issues will only be detected when there is potential for clinical problems. An important point to consider is the impact of the value used to dichotomize the data (e.g., breakpoint) on the final model results (Aerts et al., 2011). The data for this study were dichotomized using CLSI clinical breakpoints (Government of Canada, 2015a); however, epidemiological cut-offs (ECOFFs) are another common value used to dichotomize susceptibility data (EUCAST, 2017). The CLSI clinical breakpoints are higher than the ECOFFs for ceftiofur ($R \geq 8$ μg/ml vs ECOFF ≥ 2 μg/ml) and ampicillin ($R \geq 32$ μg/ml vs ECOFF ≥ 16 μg/ml) (Government of Canada, 2015a; EUCAST, 2017). The CLSI clinical breakpoint and ECOFF for nalidixic acid are the same (Government of Canada, 2015a; EUCAST, 2017). If the ECOFF values were used to dichotomize the data, then the results for the ceftiofur and ampicillin models could have been different.

The multinomial regression model has not been widely used to analyze annual variation in susceptibility with surveillance data (Aerts et al., 2011). The multinomial models performed well with the data in this study for all three antimicrobials and provided additional information compared to the dichotomized

outcome of the logistic regression model. However, with additional outcome categories, the presentation of results is more complicated than with logistic regression models. The desire to use all MIC concentration values as separate categories to prevent any loss of information has been highlighted by others (Sanchez, 2007; Aerts et al., 2011; Otto, 2011; Bjork et al., 2015; Hanon et al., 2015), but this could not be achieved in this study. We used 3-4 pre-determined outcome categories for the multinomial models instead of all of the individual MIC concentration values for two main reasons: firstly, by increasing the number of outcome categories the complexity of interpretation and difficulty of presenting results increases due to the exponential rise in the number of comparisons required to fully analyze the data; and secondly, the sparseness of data in some MIC concentration values would negatively impact the model performance necessitating merging of some categories. The optimal number of outcome categories maximizes both the interpretability of the results and model performance, and this will vary based on the MIC distribution of the antimicrobial susceptibility data being analyzed. Even if information on the entire MIC distribution could not be modelled with the multinomial regression modelling approach used in this study, there was consistently additional significant annual variation in susceptibility identified and this could be very valuable information for a surveillance program.

There were four types of regression models that for varying reasons did not perform well with the data in this study. The linear and tobit regression models consistently failed to meet model assumptions with all three antimicrobials studied. Our observation that the skewed or bimodal distribution and interval censored nature of the MIC data does not extend well to modelling as a continuous outcome variable has been reported previously (Sanchez, 2007; Otto, 2011). The ordinal logistic regression model accounts for the ordered structure of the MIC concentration value categories, however to accommodate this structure the assumption of proportional odds must be met (Sanchez, 2007; Dohoo et al., 2014). With all three antimicrobials, the assumption of proportional odds was violated, which limited the usefulness of

the model. The performance of the clog-log regression model was not consistent. It would not converge with the ampicillin data and the assumption of proportional hazards was violated with the models for ceftiofur and nalidixic acid which necessitated the inclusion of two-way interactions between both year and region, and MIC concentration (Otto, 2011; Dohoo et al., 2014). The inclusion of the interactions made the models complicated to interpret and limited the presentation of results to graphs that required descriptions for each MIC value. The inconsistencies in model performance and complicated interpretation make the clog-log regression model less desirable for surveillance purposes than the logistic or multinomial regression models.

There are many factors that could influence the performance of a regression model while analyzing annual variation in susceptibility. As demonstrated in this study, the distribution of the MIC values is important. If the MIC value distribution is distinctly bimodal then a multinomial regression model probably offers no added value compared to a logistic regression model. If there is a very small prevalence of resistance, the logistic regression model may not accurately estimate parameters and the multinomial regression model could provide a more informative approach to detect MIC creep. There are also likely to be differences with the prevalence of resistance and MIC value distribution with different bacterial-antimicrobial combinations, species of animal sampled, types of samples, and testing methodologies. All of these could impact regression model performance. There are limitations in extrapolating our study results to other AMR surveillance datasets, since our study was limited to the analysis of susceptibility data for generic *E. coli* isolates from chicken meat samples for ceftiofur, ampicillin and nalidixic acid. As well, our final regression models, although multivariable, were relatively simple and did not include interactions unless necessary to meet model assumptions. This was done to facilitate comparability of the results between the model types. Therefore, future research should explore the performance of logistic and multinomial regression models using susceptibility data for other bacterial-antimicrobial combinations, animal species sources and sample types to address these

limitations. When considering the complexity and diversity of AMR surveillance data, it is likely impossible to find a regression model that would be ideal in all situations.

In conclusion, the logistic and multinomial regression models consistently performed well for the analysis of annual variation in susceptibility of generic *E. coli* isolates from retail chicken meat surveillance samples to ceftiofur, ampicillin and nalidixic acid. The mixed effects multivariable regression models facilitated the inclusion of random effects. Logistic regression modelling for analysis of annual variation in susceptibility is likely to remain the conventional model for surveillance data, but multinomial regression modelling is also useful and is a relatively straightforward approach to identify shifts in MIC distribution, including MIC creep. Analyzing the AMR surveillance data using a multinomial regression modelling approach would allow surveillance programs to produce and interpret results using information related to a wider range of available MIC values.

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Table 2.1 – The relationship between the outcome categories for the logistic and multinomial regression models.

2.1a – Ceftiofur models

Logistic Regression Outcome Categories							
Susceptible (S)						Resistant (R)	
≤0.12 μg/ml	0.25 μg/ml	0.5 μg/ml	1.0 μg/ml	2.0 μg/ml	4.0 μg/ml	8.0 μg/ml	>8.0 μg/ml
Category 0		Category 1		Category 2		Category 3	
Multinomial Regression Outcome Categories							

2.1b – Ampicillin models

Logistic Regression Outcome Categories						
Susceptible (S)					Resistant (R)	
≤1.0 μg/ml	2.0 μg/ml	4.0 μg/ml	8.0 μg/ml	16.0 μg/ml	32.0 μg/ml	>32.0 μg/ml
Category 0		Category 1			Category 2	
Multinomial Regression Outcome Categories						

2.1c – Nalidixic acid models

Logistic Regression Outcome Categories							
Susceptible (S)						Resistant (R)	
≤0.5 μg/ml	1.0 μg/ml	2.0 μg/ml	4.0 μg/ml	8.0 μg/ml	16.0 μg/ml	32.0 μg/ml	>32.0 μg/ml
Category 0		Category 1	Category 2			Category 3	
Multinomial Regression Outcome Categories							

Table 2.2 – Summary of assumptions or goodness-of-fit tests evaluated for the specific fixed effects or mixed effects regression models. [a]

Regression Model	**Assumptions or goodness-of-fit tests evaluated for fixed effects models**	**Assumptions evaluated for mixed effects models**
Linear	Homoscedasticity of residuals Normality of residuals	Homoscedasticity of residuals and BLUPs Normality of residuals and BLUPs
Tobit	Homoscedasticity of residuals Normality of residuals	Homoscedasticity of residuals and BLUPs Normality of residuals and BLUPs
Logistic	Goodness-of-fit test	Homoscedasticity of BLUPs Normality of BLUPs
Multinomial	Goodness-of-fit test	Homoscedasticity of BLUPs[b] Normality of BLUPs[b]
Ordinal	Proportional odds – Brant test	Proportional odds – graphically Homoscedasticity of BLUPs Normality of BLUPs
Complementary log-log	Proportional hazards	Proportional hazards Homoscedasticity of BLUPs Normality of BLUPs

BLUPs – best linear unbiased predictions

[a] Data lacked independence and to account for possible clustering, fixed effects for region and random intercepts or shared frailty for retail establishment were considered for inclusion in the models.

[b] BLUPs were evaluated for models with each outcome category as the base referent category.

Table 2.3 – Summary of the prevalence of resistance and distribution of minimum inhibitory concentration (MIC) values for ceftiofur, ampicillin and nalidixic acid for 3243 generic *Escherichia coli* isolates from retail chicken meat samples.

Antimicrobial	**%R[a]**	**Distribution (% of isolates tested) of MICs (µg/ml)**									
		0.12	0.25	0.5	1	2	4	8	16	32	64
Ceftiofur	24.4	1.8	29.3	39.0	2.2	0.7	2.6	**15.5**	**8.9**		
Ampicillin	44.0				9.7	30.5	15.2	0.5	0.1	**0.1**	**43.9**
Nalidixic Acid	5.0			0.5	15.1	71.4	8.7	0.2	0.1	**0.6**	**3.4**

[a] R=resistant

Bolded numbers represent MIC categories that are considered resistant based on CLSI clinical breakpoints.

Boxes that are shaded grey are outside of the range of MIC values tested for the specified antimicrobial.

Table 2.4 – Results of the assessment of model assumptions appropriate for each mixed effects model analyzing the associations between ceftiofur (2.4a) and ampicillin (2.4b) susceptibility of generic *E. coli* isolates, and region and year.

2.4a – Ceftiofur

Model	Assumption met?			Overall model met assumptions?
	Homoscedasticity of BLUPs (residuals)	Normality of BLUPs (residuals)	Proportional odds (hazards)	
Linear	No (No)	No (No)	n/a	No
Tobit	- - (No)	- - (No)	n/a	No
Logistic	Yes (n/a)	Yes (n/a)	n/a	Yes
Multinomial	Yes (n/a)	Yes (n/a)	n/a	Yes
Ordinal	No (n/a)	No (n/a)	No	No
Cloglog	No (n/a)	Yes (n/a)	(Yes with inclusion of interactions between both year and region, and MIC concentration)	No

2.4b – Ampicillin

Model	Assumption met?			Overall model met assumptions?
	Homoscedasticity of BLUPs (residuals)	Normality of BLUPs (residuals)	Proportional odds (hazards)	
Linear	No (Yes)	No (No)	n/a	No
Tobit	- - (Yes)	- - (No)	n/a	No
Logistic	Yes (n/a)	Yes (n/a)	n/a	Yes
Multinomial	Yes (n/a)	Yes (n/a)	n/a	Yes
Ordinal	No (n/a)	No (n/a)	No	No
Cloglog				Model did not converge

n/a – not applicable for the regression model

Table 2.5 – Results of the assessment of model assumptions appropriate for each fixed effects model analyzing the associations between nalidixic acid susceptibility of generic *E. coli* isolates, and region and year.[1]

Model	Assumption met?			Goodness-of-fit test (Yes, p > 0.05)	Overall model met assumptions?
	Homoscedasticity of residuals	Normality of residuals	Proportional odds (hazards)		
Linear	No	No	n/a	n/a	No
Tobit	Yes	No	n/a	n/a	No
Logistic	n/a	n/a	n/a	Yes	Yes
Multinomial	n/a	n/a	n/a	Yes	Yes
Ordinal	n/a	n/a	No	n/a	No
Cloglog	n/a	n/a	(Yes with inclusion of interactions between both year and region, and MIC concentration)	n/a	Yes

n/a – not applicable for the regression model

[1] The mixed effects nalidixic acid models either would not converge (clog-log) or the variance components for retail establishment were extremely small (all other models); therefore, the fixed effects models were presented.

Table 2.6 – The adjusted odds ratios (95% confidence interval) describing the annual and regional variation in susceptibility of generic *E. coli* isolates to ceftiofur from the multivariable mixed effects logistic and multinomial regression models.

Predictor Variable		**Multinomial[b]**					
	Logistic[a]	1 vs 0	2 vs 0	3 vs 0	2 vs 1	3 vs 1	3 vs 2
Year	**p = 0.012**	**p < 0.0001**					
08 vs 07	**1.44 * ↑ (1.02-2.03)**	1.36 (0.99-1.86)	0.87 (0.43-1.78)	**1.63* ↑ (1.12-2.39)**	0.64 (0.32-1.31)	1.21 (0.83-1.76)	1.87 (0.89-3.92)
09 vs 08	0.92 (0.67-1.27)	**1.61+ ↑ (1.16-2.23)**	1.41 (0.76-2.93)	1.25 (0.86-1.81)	0.87 (0.42-1.78)	0.77 (0.55-1.08)	0.89 (0.42-1.87)
10 vs 09	1.15 (0.83-1.60)	**0.51+ ↓ (0.37-0.72)**	0.70 (0.34-1.48)	0.78 (0.53-1.14)	1.38 (0.67-2.86)	**1.53* ↑ (1.07-2.18)**	1.09 (0.52-2.30)
11 vs 10	0.99 (0.71-1.38)	1.00 (0.71-1.41)	0.91 (0.42-1.96)	0.98 (0.67-1.43)	0.91 (0.42-1.95)	0.98 (0.67-1.42)	1.09 (0.50-2.40)
12 vs 11	0.93 (0.66-1.30)	1.19 (0.85-1.68)	0.54 (0.22-1.36)	0.98 (0.67-1.43)	0.45 (0.18-1.13)	0.82 (0.56-1.18)	1.83 (0.72-4.61)
13 vs 12	1.21 (0.87-1.70)	**2.22+ ↑ (1.54-3.19)**	1.26 (0.39-4.01)	**2.02+ ↑ (1.34-3.05)**	0.56 (0.18-1.77)	0.91 (0.64-1.30)	1.59 (0.50-5.08)
14 vs 13	**0.57+ ↓ (0.40-0.80)**	**0.63* ↓ (0.44-0.90)**	1.55 (0.53-4.52)	**0.43+ ↓ (0.28-0.66)**	2.44 (0.86-6.96)	**0.68* ↓ (0.48-0.97)**	**0.28* ↓ (0.095-0.81)**
Region	**p < 0.0001**	**p < 0.0001**					
British Columbia	referent	referent	referent	referent	referent	referent	referent
Ontario	**0.34+ ↓ (0.27-0.43)**	**0.70* ↓ (0.53-0.95)**	**0.13+ ↓ (0.076-0.24)**	**0.25+ ↓ (0.19-0.34)**	**0.19+ ↓ (0.11-0.33)**	**0.36+ ↓ (0.28-0.46)**	**1.91* ↑ (1.08-3.38)**
Prairies	**0.31+ ↓ (0.23-0.41)**	0.76 (0.55-1.05)	**0.18+ ↓ (0.096-0.34)**	**0.24+ ↓ (0.17-0.34)**	**0.24+ ↓ (0.13-0.44)**	**0.32+ ↓ (0.23-0.43)**	1.36 (0.71-2.59)
Québec	**0.32+ ↓ (0.25-0.41)**	**0.68* ↓ (0.50-0.91)**	**0.20+ ↓ (0.12-0.33)**	**0.24+ ↓ (0.17-0.32)**	**0.29+ ↓ (0.17-0.48)**	**0.34+ ↓ (0.26-0.45)**	1.22 (0.72-2.05)

* p = 0.01 – 0.05

+ p < 0.01

↑ Represents significantly increased odds

↓ Represents significantly decreased odds

[a] Variance (SE) of random intercept – 0.15 (0.11)

[b] Variance (SE) of random intercept for model with each outcome category as the referent outcome category: 0 = 0.046 (0.091); 1 = 0.056 (0.081); 2 = 0.33 (0.50); 3 = 0.15 (0.11)

Table 2.7 – The adjusted odds ratios (95% confidence interval) describing the annual and regional variation in susceptibility of generic *E. coli* isolates to ampicillin from the multivariable mixed effects logistic and multinomial regression models.

Predictor Variable	**Logistic[a]**	**Multinomial[b]**		
		1 vs 0	2 vs 0	2 vs 1
Year	p = 0.315	**p < 0.0001**		
08 vs 07	1.06 (0.80-1.41)	1.16 (0.75-1.77)	1.09 (0.81-1.47)	0.95 (0.61-1.49)
09 vs 08	1.07 (0.82-1.41)	1.41 (0.96-2.11)	1.18 (0.88-1.58)	0.82 (0.54-1.24)
10 vs 09	1.19 (0.90-1.58)	0.87 (0.57-1.32)	1.14 (0.84-1.55)	1.33 (0.88-2.04)
11 vs 10	0.88 (0.65-1.18)	0.80 (0.50-1.26)	0.83 (0.61-1.13)	1.04 (0.65-1.65)
12 vs 11	0.99 (0.74-1.33)	**1.89+ ↑ (1.22-2.92)**	1.19 (0.87-1.63)	**0.62* ↓ (0.40-0.96)**
13 vs 12	1.18 (0.88-1.59)	**1.60* ↑ (1.06-2.40)**	**1.43* ↑ (1.02-2.00)**	0.88 (0.60-1.30)
14 vs 13	0.81 (0.60-1.08)	**0.41+ ↓ (0.28-0.62)**	**0.59+ ↓ (0.43-0.62)**	1.45 (0.98-2.16)
Region	**p < 0.0001**	**p < 0.0001**		
British Columbia	referent	referent	referent	referent
Ontario	**0.35+ ↓ (0.28-0.44)**	1.07 (0.75-1.54)	**0.36+ ↓ (0.29-0.46)**	**0.33+ ↓ (0.24-0.47)**
Prairies	**0.31+ ↓ (0.24-0.41)**	1.02 (0.69-1.50)	**0.32+ ↓ (0.24-0.42)**	**0.31+ ↓ (0.21-0.45)**
Québec	**0.40+ ↓ (0.32-0.50)**	0.87 (0.60-1.26)	**0.39+ ↓ (0.31-0.50)**	**0.44+ ↓ (0.31-0.63)**

* p = 0.01 – 0.05

+ p < 0.01

↑ Represents significantly increased odds

↓ Represents significantly decreased odds

[a] Variance (SE) of random intercept – 0.048 (0.082)

[b] Variance (SE) of random intercept for model with each outcome category as the referent outcome category: 0 = 0.023 (0.076); 1 = 0.23 (0.16); 2 = 0.023 (0.076)

Table 2.8 – The adjusted odds ratios (95% confidence interval) describing the annual and regional variation in susceptibility of generic *E. coli* isolates to nalidixic acid from the multivariable fixed effects logistic and multinomial regression models.

Predictor Variable	**Logistic**	**Multinomial**					
		1 vs 0	2 vs 0	3 vs 0	2 vs 1	3 vs 1	3 vs 2
Year	p = 0.53	**p < 0.0001**					
08 vs 07	1.53 (0.79-2.93)	1.05 (0.71-1.54)	1.30 (0.70-2.41)	1.61 (0.78-3.34)	1.24 (0.73-2.11)	1.54 (0.80-2.97)	1.24 (0.55-2.81)
09 vs 08	0.68 (0.37-1.26)	1.09 (0.74-1.60)	0.99 (0.54-1.79)	0.72 (0.36-1.46)	0.91 (0.73-1.50)	0.67 (0.36-1.24)	0.73 (0.34-1.58)
10 vs 09	0.94 (0.47-1.90)	0.99 (0.66-1.49)	1.43 (0.79-2.59)	0.97 (0.44-2.12)	1.45 (0.89-2.36)	0.98 (0.48-1.99)	0.68 (0.29-1.55)
11 vs 10	1.11 (0.53-2.32)	0.68 (0.46-1.02)	0.64 (0.36-1.13)	0.80 (0.36-1.79)	0.94 (0.58-1.51)	1.18 (0.56-2.47)	1.26 (0.54-2.94)
12 vs 11	0.76 (0.36-1.63)	**1.71* ↑ (1.13-2.58)**	**1.97* ↑ (1.11-3.51)**	1.22 (0.53-2.82)	1.16 (0.72-1.85)	0.72 (0.33-1.54)	0.62 (0.26-1.47)
13 vs 12	1.18 (0.55-2.55)	1.45 (0.88-2.38)	1.74 (0.94-3.22)	1.69 (0.69-4.12)	1.20 (0.78-1.85)	1.16 (0.54-2.53)	0.97 (0.41-2.28)
14 vs 13	0.72 (0.33-1.56)	**0.23+ ↓ (0.15-0.35)**	**0.10+ ↓ (0.05-0.18)**	**0.20+ ↓ (0.08-0.47)**	**0.42+ ↓ (0.08-0.21)**	0.86 (0.40-1.88)	2.04 (0.82-5.05)
Region	**p = 0.0002**	**p = 0.0001**					
British Columbia	referent	referent	referent	referent	referent	referent	referent
Ontario	**0.49+ ↓ (0.29-0.82)**	1.08 (0.79-1.46)	0.70 (0.46-1.07)	**0.49* ↓ (0.28-0.88)**	**0.65* ↓ (0.46-0.91)**	**0.46+ ↓ (0.27-0.77)**	0.71 (0.39-1.29)
Prairies	1.17 (0.71-1.92)	0.97 (0.69-1.36)	**0.53* ↓ (0.32-0.87)**	1.07 (0.60-1.89)	**0.54+ ↓ (0.36-0.82)**	1.11 (0.67-1.83)	**2.03* ↑ (1.10-3.78)**
Québec	**0.51* ↓ (0.30-0.85)**	0.83 (0.62-1.12)	**0.57+ ↓ (0.37-0.86)**	**0.41+ ↓ (0.23-0.74)**	**0.68+ ↓ (0.48-0.96)**	**0.50+ ↓ (0.30-0.84)**	0.73 (0.40-1.33)

* p = 0.01 – 0.05

\+ p < 0.01

↑ Represents significantly increased odds

↓ Represents significantly decreased odds

CHAPTER 3

Comparison of the annual and regional variation identified using various multidrug resistance classification metrics for generic *Escherichia coli* isolates from chicken abattoir surveillance samples in Canada

Abstract

Antimicrobial resistance (AMR) and multidrug resistance (MDR) are important global public health issues. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) performs surveillance of AMR in enteric bacteria. AMR surveillance programs are interested in identifying temporal trends in MDR. The analysis of MDR is complicated by the lack of consensus for MDR definitions. The objectives were to describe the most common resistance patterns in generic *E. coli* isolates from chicken cecal samples and determine the impact of using different MDR classification metrics for analysis of annual and regional variation in MDR. From 2006-2015, there were 1598 *E. coli* isolates from chicken cecal samples collected at federally inspected abattoirs for CIPARS. The isolates were tested for susceptibility to 13 antimicrobials consistently during the study. Three MDR classification metrics were used: MDR-drug, MDR if the isolate was resistant to three or more of the 13 antimicrobials included in the study; MDR-cat, MDR if it was resistant to three or more of the nine antimicrobials categories; and MDR-class, MDR if it was resistant to three or more of the six antimicrobial classes. The most frequent resistance patterns overall, and by year and region were extracted along with resistance patterns that included resistance to quinolones, and third generation cephalosporins and/or β-lactams with β-lactamase inhibitors. Logistic regression models for each MDR classification metric were used to analyze the association between prevalence of MDR, and year and region. Interaction effects between year and region were evaluated. The prevalence of MDR was lowest with MDR-class and highest with MDR-drug. Overall, and in all years and regions, pansusceptible was

the most common susceptibility pattern. Resistance patterns that included third generation cephalosporins and β-lactams with β-lactamase inhibitors were common; however, those that included quinolones were uncommon. Significant annual variation in the prevalence of MDR was identified with MDR-drug and MDR-class univariable logistic regression. Significant regional variation was identified in the univariable logistic regression models for all three MDR classification metrics. There were significant interaction effects between year and region with MDR-drug and MDR-cat multivariable logistic regression. Both the prevalence of MDR and interpretation of the association between the prevalence of MDR, and year and region differed depending on the MDR classification metric used. These results are supportive of the previous concerns that caution must be taken when comparing MDR results from different studies. Global consensus is needed for the optimal MDR classification metric for non-clinical enteric bacteria surveillance.

Introduction

Antimicrobial resistance (AMR) is quickly reaching very concerning levels in bacterial-antimicrobial combinations that are important to human and animal health (Marshall and Levy, 2011; World Health Organization, 2014, 2015; Zawack et al., 2016; Exner et al., 2017). If the current trends in AMR continue, the approaching reality, which has been realized in certain situations already, suggests the future of medicine will be defined by decreased effectiveness or ineffectiveness of antimicrobials to treat and prevent not only complicated but also simple bacterial infections (World Health Organization, 2014, 2015). Multidrug resistant bacteria are a major component of the global AMR public health issue (Exner et al., 2017). Multidrug resistance (MDR) in the gram negative family of bacteria, Enterobacteriaceae, which includes both commensal and pathogenic bacteria such as *Salmonella, E. coli,* and *Klebsiella,* is complicated by the presence of mobile genetic elements which can confer resistance and co-resistance (Ingram et al., 2013; Mainali et al., 2013; Chalmers et al., 2017; Exner et al., 2017). Selection pressure from antimicrobial use (AMU) including misuse in both humans and animals propagates the AMR and

MDR issues (Marshall and Levy, 2011; Zawack et al., 2016). In order to maintain the effectiveness of current antimicrobial agents and improve the AMR crisis, surveillance is one critical aspect that is required (World Health Organization, 2014, 2015; Exner et al., 2017). In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) provides surveillance of AMR in enteric bacteria (*Escherichia coli*, *Salmonella*, and *Campylobacter*) (Government of Canada, 2015a). The chicken abattoir component of CIPARS includes isolation of *E. coli* from cecal content samples collected at federally inspected abattoirs and testing them for susceptibility to an established panel of antimicrobials (Government of Canada, 2015a).

Surveillance programs such as CIPARS or the American equivalent [The National Antimicrobial Resistance Monitoring System (NARMS)] identify temporal trends in both AMR of specific bacterial-antimicrobial combinations and MDR (The National Antimicrobial Resistance Monitoring System, 2014; Government of Canada, 2015b). MDR temporal trends are generally presented descriptively and through simple graphical presentations. As well, the frequency of specific resistance patterns is often presented (The National Antimicrobial Resistance Monitoring System, 2014; Government of Canada, 2015b). Moreover, MDR involving resistance to third generation cephalosporins and fluoroquinolones in *E. coli* is of particular importance (World Health Organization, 2014).

MDR is not consistently defined in the literature (Falagas et al., 2006; Magiorakos et al., 2012; Exner et al., 2017) with variations regarding the individual antimicrobials, antimicrobial categories or antimicrobials classes tested and considered (Medalla et al., 2013). There is also a lack of consistency related to which antimicrobial classes are presented, especially regarding β-lactams and cephalosporins (Medalla et al., 2013; The National Antimicrobial Resistance Monitoring System, 2014; Government of Canada, 2015b). Resistance to the minimum number of antimicrobials, categories or classes to qualify as multidrug resistant is also variable in the literature, with resistance to 2 or 3 often being used (Magiorakos et al., 2012; Glenn et al., 2013). Another approach to define MDR is based on resistance to

a number of antimicrobials specifically important for the treatment of the bacterial infection of concern (German et al., 2016). Several consensus statements have attempted to unify the definitions for MDR in important human bacterial pathogens (Magiorakos et al., 2012; German et al., 2016). The importance of characterizing the MDR patterns in addition to analyzing the presence of MDR has also been highlighted (Magiorakos et al., 2012). The statistical analysis of MDR in AMR surveillance data has not been extensively explored (Medalla et al., 2013; Glass-Kaastra et al., 2014). To our knowledge, the impact of using different MDR classification metrics on the results obtained with statistical analysis has not been previously studied.

The first objective of this study was to describe the most common resistance patterns present in generic *E. coli* isolates from chicken abattoir surveillance in Canada, as well as their variation over the study period and between sampling regions. In particular, resistance patterns involving quinolones, and third generation cephalosporins and/or β-lactams with β-lactamase inhibitors were described. The second objective of the study was to compare different multidrug resistance classification metrics, and use them to compare the annual and region variation in the prevalence of MDR using generic *E.coli* isolates from chicken abattoir surveillance samples while accounting for clustering of the samples.

Methods

Surveillance data and susceptibility testing

Generic *E. coli* isolates for this study were obtained using conventional methods from chicken cecal content samples collected at federally inspected abattoirs for CIPARS from 2006 to 2015. The details for the design of the active surveillance have been previously reported (Government of Canada, 2015a). Briefly, two stage sampling was used: in the first stage, abattoirs were randomly selected proportional to their annual slaughter volume and in the second stage, chickens were systematically selected with the number of chickens sampled per year for each enrolled abattoir proportional to each abattoir's slaughter

volume (Government of Canada, 2015a). The generic *E. coli* isolates were tested for susceptibility to 13 antimicrobials consistently throughout the study period using an automated method of broth microdilution (Table 3.1) (Government of Canada, 2015a). The Clinical and Laboratory Standards Institute (CLSI) defined clinical breakpoints were used to classify each isolate as resistant or susceptible to the antimicrobials, except for streptomycin where a CLSI clinical breakpoint was not available and a CIPARS / NARMS harmonized interpretive cut-off value was used (Government of Canada, 2015a). The category of importance to human medicine according to Health Canada was recorded for each antimicrobial (Table 3.1) (Government of Canada, 2009). The 13 antimicrobials represented nine of the epidemiologically significant antimicrobial categories described by Magorikos et al. (2011) and six antimicrobial classes according to the classification used by CIPARS (Table 3.1) (Government of Canada, 2015b). Susceptibility patterns were classified using three different MDR classification metrics: first, MDR-drug, an isolate was considered multidrug resistant if it was resistant to 3 or more of the 13 antimicrobials included in the study; second, MDR-cat, an isolate was considered multidrug resistant if it was resistant to 3 or more antimicrobial categories (Magiorakos et al., 2012); and third, MDR-class, an isolate was considered multidrug resistant if it was resistant to 3 or more antimicrobial classes (Table 3.1). An isolate was considered resistant to an antimicrobial category or class if it was resistant to at least one antimicrobial in the category or class. Isolates were considered possible extensively drug resistant (pXDR) if resistant to 11 or more of the 13 antimicrobials included in the study (pXDR-drug); 7 or more antimicrobial categories (pXDR-cat) (Magiorakos et al., 2012); and 4 or more antimicrobial classes (pXDR-class) (Table 3.1). An isolate was considered possible pandrug resistant (pPDR) if it was resistant to all antimicrobials (pPDR-drug), categories (pPDR-cat) or classes (pPDR-class) included in the study (Table 3.1). The data available for each isolate were year of sample collection, region where the animal was located prior to arrival at the abattoir, and coded abattoir identifier.

Descriptive statistics

The prevalence of MDR and 95% confidence interval were calculated using the three MDR classification metrics and the distribution of isolates in each number of resistant antimicrobials, categories or classes was tabulated. The prevalence of pXDR and 95% confidence interval were calculated for each of the three pXDR classification metrics. Cohen's kappa was calculated to assess the strength of the agreement for all two-way comparisons between the three MDR classification metrics, and interpreted as follows: almost perfect (κ = 0.81-1.0); substantial (κ = 0.61-0.8); moderate (κ = 0.41-0.6); fair (κ = 0.21-0.4): slight (0.01-0.2); and poor (κ = ≤0) (Landis and Koch, 1977).

Resistance patterns

The 20 most frequent resistance patterns for the dataset were extracted and for each the numbers of isolates tabulated and percentages of total isolates were calculated. The number of antimicrobials, categories and classes were counted for each resistance pattern and subjected to criteria for MDR-drug, MDR-cat or MDR-class. The six most frequent resistance patterns for each of the years and regions were extracted. The six most frequent were chosen for practical reasons to allow presentation of pansusceptible and the 5 most frequent resistance patterns for each of the years and regions. Resistance patterns that included Category I antimicrobials were noted (Government of Canada, 2009). Resistance patterns that included combinations of resistance to quinolones [ciprofloxacin (Cip) and nalidixic acid (Nal)], and third generation cephalosporins [ceftiofur (Tio) and ceftriaxone (Cro)] and/or β-lactams with β-lactamase inhibitors [amoxicillin clavulanic acid (Amc)] were extracted and the number of isolates with each resistance pattern was recorded.

Statistical analysis

Statistical analyses were performed using STATA / SE 14.2 (StataCorp LLC, 2015). A separate logistic regression model was built for each MDR classification metric: MDR-drug; MDR-cat; and MDR-class.

The dichotomous outcome variables were coded to represent the odds of an isolate being multidrug resistant. Three predictor variables were considered for inclusion in the logistic regression models. The linearity assumption for year of sample origin (year) was assessed by examining a locally weighted regression (lowess) of the log odds of the MDR outcome and year (Dohoo et al., 2014). If it was not appropriate to model year as a continuous variable, a quadratic relationship was assessed by adding a squared year term to the model. If a quadratic relationship was not appropriate, year was modelled as a hierarchical categorical variable. Region of animal origin (region) was included as a fixed categorical variable to account for possible clustering by region. The regions included were British Columbia (BC), the Prairies (PR, including Alberta, Saskatchewan and Manitoba), Ontario (ON) and Québec (QC). Ontario was used as the referent. Isolates from the Maritime region were excluded due to a small sample size during the study period (69 isolates). Random intercepts for abattoir were considered for inclusion to account for possible clustering by abattoir.

Univariable logistic regression models with random intercepts for abattoir were built for year and region. The mixed effects model was presented if the likelihood ratio test (LRT) for the random effects was statistically significant ($p \leq 0.05$). If the LRT was not significant, then the fixed effects model was presented. Year and region were forced into the final multivariable model regardless of level of significance in the univariable models. Multivariable logistic regression models with random intercepts for abattoir were built with year and region as fixed predictor variables and interaction effects between year and region. Interaction effects between year and region were included in the final multivariable model, if the interaction terms were statistically significant ($p \leq 0.05$), using a LRT. The same criteria for presentation of the mixed or fixed effects model were used as described for the univariable models. If the final multivariable logistic regression model only included fixed effects, a goodness-of-fit (GOF) test appropriate for the data was performed (Dohoo et al., 2014). The level of significance was noted and a non-significant GOF test ($p > 0.05$) indicated the model fit the data (Dohoo et al., 2014). The Pearson

standardized residuals were assessed and any outliers were noted. Covariate patterns with large leverage were noted. The impact of covariate patterns on the model was assessed using delta-beta, delta-chi-square and delta-deviance and those with large impacts were noted. If the final multivariable logistic regression model included random effects, then the best linear unbiased predictions (BLUPs) were assessed for homoscedasticity and normality. The Pearson residuals from the final models were assessed and any outliers were noted. If there were interaction terms containing continuous variables in the final multivariable model, predicted margins plots were used to visualize the relationship between year and region, and the MDR outcome variable. For final multivariable logistic regression models that included interaction terms, regardless of whether continuous or categorical variables were included in the interaction term, contrasts were performed to estimate the odds of an isolate being multidrug resistant for BC, PR and QC compared to ON in 2006, 2011 and 2015.

Results

Descriptive statistics

There were 1598 generic *E. coli* isolates from chicken cecal content samples obtained at abattoirs from 2006-2015 included in the study. The prevalence of MDR isolates was lowest when estimated with MDR-class and highest with MDR-drug (Table 3.2). The prevalence of pXDR isolates was variable depending on which pXDR classification metric was used: pXDR-drug 0.3% (95% CI 0.1-0.7%); pXDR-cat 8.4% (95% CI 7.1-9.8%); and pXDR-class 16.5% (95% CI 14.7-18.4%). There were isolates that were resistant to all 9 antimicrobial categories (pPDR-cat) and all 6 antimicrobial classes (pPDR-class) included in the study (Table 3.2). However, no isolates were resistant to all 13 antimicrobials (Table 3.2). The prevalence of MDR isolates by year was variable, but there was a general trend for an increase in the prevalence of MDR during the study period regardless of the MDR classification metric used (Table 3.3). There was almost perfect agreement between MDR-drug and MDR-cat ($\kappa = 0.92$, SE =

0.025, p-value ≤ 0.0001). MDR-drug and MDR-class (κ = 0.71, SE = 0.024, p-value ≤ 0.0001), and MDR-cat and MDR-class (κ = 0.78, SE = 0.024, p-value ≤ 0.0001) had substantial agreement.

Resistance patterns

Pansusceptible was the most frequent pattern overall, and when stratified by year and region (Tables 3.4, 3.5 and 3.6). The 20 most frequent resistance patterns represented 73.8% of the isolates in the study. Tetracycline was the most common antimicrobial appearing in the resistance patterns (13 of 20 patterns, Table 3.4). Six of the 20 most frequent resistance patterns included three Category I antimicrobials (Amc, Cro and Tio) (Table 3.4). The 20 most frequent resistance patterns did not include any quinolones (Cip or Nal). There were four resistance patterns in the 20 most frequent, where the MDR classification metrics disagreed regarding whether the resistance pattern should be considered multidrug resistant or not (Table 3.4).

When the frequencies of resistance patterns were evaluated for individual years, three of the six most frequent patterns appeared in all years (Table 3.5). The Amc-Amp-Cro-Fox-Tio (Amp – ampicillin and Fox – cefoxitin) resistance pattern appeared in the six most frequent from 2006 to 2013 (Table 3.5). In 2014 and 2015, patterns in the six most frequent included resistance to Gen (gentamicin) (Table 3.5). There were only two patterns in the six most frequent that appeared in all regions (Table 3.6). Three of the six most frequent resistance patterns in BC included resistance to three Category I antimicrobials (Amc, Cro and Tio) (Table 3.6). Conversely, QC did not have any patterns that included resistance to Category I antimicrobials in the six most frequent patterns, but QC did have one resistance pattern that included resistance to Gen (Table 3.6). The six most frequent resistance patterns by year and region did not include any resistance to quinolones (Cip or Nal).

The resistance patterns for 22 isolates that included resistance to quinolones (Cip or Nal), and third generation cephalosporins (Cro or Tio) and/or β-lactams with β-lactamase inhibitors (Amc) can be found

in Table 3.7. The most common resistance backbone when considering the combination of resistance to quinolones, and third generation cephalosporins and/or β-lactams with β-lactamase inhibitors was Amc-Cro-Nal-Tio appearing in 18 isolates (Table 3.7). When considering these combinations for resistance backbones, resistance to ciprofloxacin was infrequent (2 of 22 isolates) (Table 3.7).

Mixed effects univariable logistic regression models

There were 136 (8.5%) isolates where the region was unknown and data related to these isolates were not included in the logistic regression models. Therefore, data from 1462 (1598-136) isolates were used to build the logistic regression models. There was a linear relationship between year and the log odds of being multidrug resistant for the three MDR classification metrics; therefore, year was modelled as a continuous predictor variable. Year was statistically significant in the MDR-drug and MDR-class models but was not statistically significant in the MDR-cat model (Table 3.8). Region was statistically significant in all three models (Table 3.8).

Mixed effects multivariable logistic regression models for MDR-drug and MDR-cat

The final multivariable logistic regression models for MDR-drug and MDR-cat included fixed effects for year (continuous) and region (categorical) and a statistically significant interaction effect between year and region (Table 3.9). Random intercepts for abattoir only explained a small amount of variation in the data, but were statistically significant (Table 3.9). The predicted margins plots used to visualize the relationship between year and region for the final multivariable model were similar using both MDR classification metrics (Figure 3.1). The PR and QC had a positive trend in MDR over the 10 year study period and QC had a steeper slope than the PR (Figure 3.1). For both models the BLUPs were homoscedastic and normal. There were no outliers identified.

The predicted odds ratios from the contrasts were similar from the MDR-drug and MDR-cat models (Table 3.10). QC was generally more likely to have multidrug resistant isolates than ON (statistically

significant in 2011 and 2015) (Table 3.10). Compared to ON, BC was statistically significantly more likely to have multidrug resistant isolates (Table 3.10). The PR were less likely to have multidrug resistant isolates than ON [statistically significant in 2006 (MDR-drug and MDR-cat) and 2011 MDR-drug)] (Table 3.10). The trends identified with the contrast statements were generally consistent with the predicted margins plots (Table 3.10 and Figure 3.1).

Mixed effects multivariable logistic regression model for MDR-class

The final multivariable logistic regression model for MDR-class included fixed effects for year (continuous) and region (categorical) (Table 3.11). There was not a statistically significant interaction effect between year and region ($p = 0.091$). The random intercepts for abattoir were statistically significant but only explained a small amount of the variation in the data (Table 3.11). However, the random intercepts should be interpreted cautiously because the BLUPs were heteroscedastic. The BLUPs did meet the assumption of normality. There were no outliers identified. There was a statistically significant increased odds of an isolate being multidrug resistant with each additional year (Table 3.11). Over the 10 year study period, there was a 2.05 (1.44-2.90) times increase in the odds of an isolate being multidrug resistant. Isolates from BC and QC had statistically significant increased odds of being multidrug resistant compared to isolates from ON (Table 3.11).

Discussion

This study took a multifaceted approach to evaluating MDR in *E. coli* isolates from chicken abattoir surveillance samples: first, the composition of the resistance patterns was explored; and second, the effect of using different MDR classification metrics on the analysis of the associations between the prevalence of MDR, and annual and regional variation was investigated. The most common susceptibility pattern overall, in each year and in each region, was pansusceptible. Nevertheless, the prevalence of *E. coli* MDR from the chicken cecal samples was moderately-high across the metrics used

in this study (38.5% - 53.3%). The precise value for the prevalence of MDR was variable depending on the MDR classification metric used. Resistance to third generation cephalosporins and β-lactams with β-lactamase inhibitors was not uncommon, appearing in the 4th, 7th, 12th, 14th, 18th and 20th most frequent resistance patterns. There was variability in the frequency of resistance patterns with resistance to third generation cephalosporins and β-lactams with β-lactamase inhibitors over the study period and between regions. Resistance to the fluoroquinolone, ciprofloxacin, was infrequent (0.2%). The annual and regional variation in the prevalence of MDR identified was dependent on the MDR classification metric used. The prevalence of MDR was variable and depended on which MDR classification metric was used, which supports the concerns raised previously in the literature regarding caution when comparing MDR results from different studies (Falagas et al., 2006; Magiorakos et al., 2012; German et al., 2016). The MDR-drug and MDR-cat classification metrics had the highest level of agreement.

Comparing the prevalence of MDR in Canada with the other national AMR surveillance programs would be interesting. However, several factors would have to be considered: first, the value used to classify an isolate as resistant to an antimicrobial could alter the interpretation (CLSI vs epidemiological cutoff (ECOFF)) (Government of Canada, 2015a; EUCAST, 2017) and second, the definition of MDR including the antimicrobials tested with the corresponding antimicrobial classes or categories used would need to be examined to evaluate the validity of comparing the results (Medalla et al., 2013; Government of Canada, 2015b). Although the prevalence of MDR is generally presented at least descriptively in annual national surveillance reports, it tends not to be the main focus and therefore access to the results necessary for comparison is limited (The National Antimicrobial Resistance Monitoring System, 2014; Government of Canada, 2015b; European Food Safety Authority and European Centre for Disease Prevention and Control, 2016; The Danish Intergrated Antimicrobial Resistance Monitoring and Research Programme, 2016). In 2014, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) were able to compare the

prevalence of MDR among the countries contributing data to their AMR summary document (European Food Safety Authority and European Centre for Disease Prevention and Control, 2016). They used a MDR classification metric based on Magiorakos et al. (2011), which is most similar to the MDR-cat in this study, however, there are important differences: they used ECOFFs to classify resistance to 11 antimicrobial categories tested, only 7 of which were the same as in this study. In 2014, the overall prevalence of MDR in *E. coli* isolates from chicken cecal samples in the European Union (EU) summary document was 54.6% with a wide range (1.5% to 86.4%) among individual countries (European Food Safety Authority and European Centre for Disease Prevention and Control, 2016). Canada's prevalence of MDR in *E. coli* isolates from chicken cecal samples in 2014 using MDR-cat was 53.7% (95% CI 45.7-61.6%), which is similar to the EU mean value, but the differences in methodology mentioned previously should be considered.

Other important aspects of classification of multidrug resistant isolates are the concepts of extensively drug resistant (XDR) and pandrug resistant (PDR). Similar to the variability of MDR classification metrics, there are no uniform definitions for XDR or PDR; however some have been suggested for clinically important human bacterial infections (Falagas et al., 2006; Magiorakos et al., 2012; German et al., 2016). In order to classify an isolate as XDR or PDR, all relevant classes or categories of antimicrobials should be tested and this is a limitation in non-clinical based surveillance programs. If any of the antimicrobial classes or categories are not tested then the identified isolates should be considered possible XDR or possible PDR; alternatively, further susceptibility testing could be performed on the pXDR or pPDR isolates for the antimicrobial classes or categories not included on the standard panel (Magiorakos et al., 2012; German et al., 2016). There were isolates in this study that were resistant to all antimicrobials categories (7 isolates) and classes (8 isolates), and these isolates are pPDR. Confirmation of PDR requires additional susceptibility testing, which could include assessing

susceptibility to antipseudomonal penicillins with β-lactamase inhibitors, carbapenems, glycylcyclines, monobactams, phosphonic acids and polymixins (Magiorakos et al., 2012).

The interpretation of the relationship between year, region and the prevalence of MDR in this study was dependent on the MDR classification metric used. There were both similarities and differences between the results from the logistic regression models for MDR-drug, MDR-cat and MDR-class. In the mixed effects univariable logistic regression models for year, year was only statistically significant in the MDR-drug and MDR-class models. With the MDR-class mixed effects multivariable logistic regression model, there was statistically significant annual and regional variation in the prevalence of MDR identified but there was not a statistically significant interaction effect between year and region. Comparatively with MDR-drug and MDR-cat, there was a statistically significant interaction effect between year and region. With the three MDR classification metrics used in this study, different logistic regression results were obtained. The results of this study substantiate the previous warnings that caution should be taken when comparing MDR results from different studies (Falagas et al., 2006; Magiorakos et al., 2012; German et al., 2016). There is a need for a consensus statement on the appropriate MDR classification metric for non-clinical AMR surveillance data. The MDR-cat classification metric was based on antimicrobial categories that were epidemiologically significant in human medicine and it is easily applied in the context of non-clinical surveillance (Magiorakos et al., 2012). It provides a balanced approach between considering resistance to every antimicrobial tested where multiple antimicrobials in the same antimicrobial class might be tested and considered individually (MDR-drug) and only considering resistance to antimicrobial classes, which can group large numbers of antimicrobials into classes depending on how the classes are defined (MDR-class). For susceptibility testing of *E. coli,* Magiorakos et al. (2012) recommended testing 17 antimicrobial categories; in this study data from 9 of the antimicrobial categories were included. The differences in antimicrobial categories tested for susceptibility are related to the purpose of the susceptibility testing being

performed: the data for the study are from a CIPARS active surveillance program that uses an established susceptibility plate that is designed for surveillance purposes (Government of Canada, 2015a), whereas treatment of clinical human infections was the focus of the paper by Magiorakos et al. (2012). Through consensus of AMR surveillance experts, the antimicrobial categories necessary for MDR determination in non-clinical surveillance could be established. Nonetheless, using logistic regression modelling facilitated the analysis of annual and regional variation in the prevalence of MDR. In 2013, CIPARS added on-farm surveillance for broiler chickens which provides information on AMR and AMU for sentinel farms (Government of Canada, 2015a). This AMU information could help inform the interpretation of the trends identified over time and by region. It highlights the critical nature of integrated active surveillance programs that pair the AMR data with AMU information. The interaction effects identified between year and region were interesting and emphasized the importance of exploring potential interaction between predictor variables. The hierarchical nature of the data was considered during model development. Abattoir was a significant random effect but only explained a small amount of variation in the data. Abattoir must represent unmeasured predictor variables that influence the prevalence of MDR in generic *E. coli* from chickens. Due to restrictions related to confidentiality agreements, additional information for abattoirs was not available. The more notable cluster variable was region where the chicken was located prior to arrival at the abattoir. It appears that region is an important predictor variable to consider in future studies evaluating the prevalence of MDR.

When MDR classification metrics are used to create a dichotomous MDR outcome, it is critical to consider that not all multidrug resistant isolates carry the same implications or potential importance to human or animal health. This issue is addressed by pairing statistical analysis of the prevalence of MDR with descriptive analysis of the resistance patterns present. Resistance to third generation cephalosporins and fluoroquinolones is classified as critically important to human health (World Health Organization, 2016) or very high importance to human health, depending on the classification scheme (Government of

Canada, 2009). Third generation cephalosporins are often used to treat severe human *E. coli* infections, including septicemia and resistance is commonly conferred through extended spectrum β-lactamases (ESBL), which are easily transferred between the same or different species of gram-negative bacteria (World Health Organization, 2014; Chalmers et al., 2017). Fluoroquinolones are commonly used to treat *E. coli* infections in the community, including urinary tract infections (World Health Organization, 2014). Resistance to ciprofloxacin was rare in the isolates included in the study and that is consistent with NARMS findings (The National Antimicrobial Resistance Monitoring System, 2014). Resistance patterns that included third generation cephalosporins (Cro and Tio) and β-lactams with β-lactamase inhibitors (Amc) were not uncommon in the isolates included in the study. The Chicken Farmers of Canada banned the preventive use of ceftiofur in 2014 (Chalmers et al., 2017), and it will be interesting to analyze the data after 2015 to see if a reduction in resistance to third generation cephalosporins is realized, similar to the reduction seen during the voluntary withdrawal of ceftiofur use from 2005 to 2007 (Dutil et al., 2010). It is noteworthy that the six most frequent resistance patterns in 2014 and 2015 did not include resistance to third generation cephalosporins or β-lactams with β-lactamase inhibitors, which were present within the six most frequent resistance patterns from 2006 to 2013. Resistance to antimicrobials without previous or with limited exposure can be driven by co-selection of AMR genes and this has been demonstrated in *E. coli* from chicken samples (Ingram et al., 2013; Chalmers et al., 2017). Chalmers et al. (2017) demonstrated likely co-selection of gentamicin resistance in *E. coli* through the selection pressure of spectinomycin use in broiler chickens. The recent surge in the application of whole genome sequencing to AMR surveillance samples will further inform our understanding of co-selection of AMR genes.

There are some limitations associated with the study. Not testing antimicrobials in all of the antimicrobial categories (Magiorakos et al., 2012) could have resulted in increased false negatives and non-differential misclassification bias. This could have led to underestimating the prevalence of MDR

with MDR-cat and underestimating the strength of the associations identified. There were three different MDR classification metrics used in the study and these do not represent all of the possible MDR classification metrics. These three were selected to provide a range of metrics for comparison and were aligned with the CIPARS definitions where possible (MDR-class) (Government of Canada, 2015b). The results of the study encourage the development of an internationally accepted consensus statement on an optimal MDR classification metric for non-clinical surveillance purposes.

There are several possibilities that can be pursued for future research. It would be very interesting to statistically model specific important resistance patterns or backbones to assess for temporal trends and regional variation. Depending on the origin of the data there may be issues with a lack of statistical power due to small sample size. There are also other statistical modelling approaches that could be explored for the analysis of factors associated with the prevalence of MDR. They include multinomial logistic and Poisson regression models; both of these approaches retain more information in the outcome than the dichotomized MDR outcome for logistic regression. The multinomial logistic regression model could model three outcome categories (not MDR, MDR and pXDR). This would provide more information for risk factors or trends that could differ between MDR and pXDR isolates. The Poisson regression approach models the number of antimicrobials, categories or classes to which each isolate is resistant as the outcome and has been previously used to model multiple-class antimicrobial resistance in swine clinical samples (Glass-Kaastra et al., 2014).

In conclusion, the MDR classification metric used affected the interpretation of the relationship between the prevalence of MDR, and annual and regional variation. The results from the study are supportive of previous concerns that caution needs to be taken when comparing the analysis of prevalence of MDR between studies. For future generalizability and comparability of results between studies, there needs to be consensus on the optimal MDR classification metrics for each bacterial species or family. The development of MDR classification metrics for non-clinical surveillance purposes will need to consider

the impact of the species of origin of the sample and the source of the sample on the metrics. In generic *E. coli* isolates from chicken cecal surveillance samples in Canada, resistance to fluoroquinolones was rare and resistance to third generation cephalosporins and β-lactams with β-lactamase inhibitors appeared to be decreasing in later years of the study. Analysis of the association between the prevalence of MDR, and annual and regional variation and characterization of the associated resistance patterns of the isolates provides interesting and informative results for AMR surveillance programs.

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Table 3.1 – Categories and classes of the 13 antimicrobials included in the study that were used for susceptibility testing of generic *E. coli* isolates from chicken abattoir surveillance samples.[1]

Antimicrobial ([2])	**Antimicrobial Category[3]**	**Antimicrobial Class[4]**
Gentamicin (II)	Aminoglycosides	Aminoglycosides
Streptomycin (II)		
Ceftriaxone (I)	Extended Spectrum Cephalosporins (3^{rd} and 4^{th} generation)	β-lactams
Ceftiofur (I)		
Cefoxitin (II)	Cephamycins	
Ampicillin (II)	Penicillins	
Amoxicillin-clavulanic acid (I)	Penicillins with β-lactamase inhibitors	
Ciprofloxacin (I)	Quinolones	Quinolones
Nalidixic acid (II)		
Sulfisoxazole (III)	Folate pathway inhibitors	Folate pathway inhibitors
Trimethoprim-sulfamethoxazole (II)		
Chloramphenicol (III)	Phenicols	Phenicols
Tetracycline (III)	Tetracyclines	Tetracyclines

[1] Amikacin, kanamycin and azithromycin were not considered since they were not included in the susceptibility panel for the entire 10 year period.

[2] Category of importance to human health (Government of Canada, 2009)

[3] Magiorakos et al., 2012

[4] Government of Canada, 2015b

Table 3.2 – Comparison of the prevalence of multidrug resistance (MDR) and distribution using three MDR classification metrics for 1598 generic *E. coli* isolates from chicken abattoir surveillance.

Metric	Prevalence (%) of MDR (95% CI)	Number of antimicrobial drugs, categories or classes													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13
MDR-drug	53.3% (50.8-55.8%)	398	163	185	**251**	**127**	**168**	**105**	**55**	**58**	**57**	**26**	**4***	**1***	**0**$^{+}$
MDR-cat	49.4% (46.9-51.9%)	398	171	240	**298**	**184**	**101**	**72**	**78***	**49***	**7**$^{+}$				
MDR-class	38.5% (36.1-41.0%)	398	273	311	**353**	**180***	**75***	**8**$^{+}$							

Bolded numbers represent isolates that are considered MDR with the classification metric.

***** Represents MDR isolates that are also possible extensively drug resistant (pXDR).

$^{+}$ represents MDR isolates that are also pXDR and possible pandrug resistant (pPDR).

Grey shading represents a number of antimicrobial categories or classes beyond those included in the study.

Table 3.3 – Comparison of the prevalence of multidrug resistance (MDR, %) with 95% confidence interval for each year using three MDR classification metrics for 1598 generic *E. coli* isolates from chicken abattoir surveillance.

Year	MDR-drug	MDR-cat	MDR-class
2006	47.5% (39.6-55.5%)	45.1% (37.2-53.1%)	32.7% (25.6-40.5%)
2007	48.3% (40.6-56.0%)	46.0% (38.4-53.7%)	33.9% (26.9-41.5%)
2008	49.1% (41.2-57.0%)	46.7% (38.9-54.6%)	32.1% (25.1-39.8%)
2009	53.3% (45.4-61.1%)	49.7% (41.8-57.6%)	36.4% (29.0-44.2%)
2010	61.6% (51.9-70.6%)	60.7% (51.0-69.8%)	42.0% (32.7-51.7%)
2011	54.2% (46.0-62.2%)	49.0% (40.9-57.2%)	37.4% (29.8-45.5%)
2012	52.1% (44.3-59.8%)	49.7% (41.9-57.5%)	42.0% (34.5-49.8%)
2013	53.2% (45.4-60.9%)	46.7% (39.0-54.6%)	37.3% (30.0-45.0%)
2014	58.0% (50.0-65.7%)	53.7% (45.7-61.6%)	46.9% (39.0-54.9%)
2015	58.8% (50.9-66.3%)	50.3% (42.4-58.2%)	46.1% (38.3-54.0%)

Table 3.4 – The 20 most frequent antimicrobial resistance patterns from generic *E. coli* isolates from chicken abattoir surveillance samples and comparison of classification using three multidrug resistance (MDR) classification metrics.

Resistance Pattern	Number of Isolates (%)	Number of Antimicrobials in Resistance Pattern	MDR-drug Y/N	Number of Categories in Resistance Pattern	MDR-cat Y/N	Number of Classes in Resistance Pattern	MDR-class Y/N
Pansusceptible	398 (24.9%)	0	N	0	N	0	N
Sss-Str-Tet	93 (5.8%)	3	Y	3	Y	3	Y
Tet	91 (5.7%)	1	N	1	N	1	N
Amc-Amp-Cro-Fox-Tio (I)	**87 (5.4%)**	**5**	**Y**	**4**	**Y**	**1**	**N**
Str-Tet	73 (4.6%)	2	N	2	N	2	N
Amp-Str-Tet	55 (3.4%)	3	Y	3	Y	3	Y
Amc-Amp-Cro-Fox-Sss-Str-Tet-Tio (I)	38 (2.4%)	8	Y	7	Y	4	Y
Gen-Sss-Str-Tet	37 (2.3%)	4	Y	3	Y	3	Y
Str	33 (2.1%)	1	N	1	N	1	N
Amp-Sss-Str-Sxt-Tet	29 (1.8%)	5	Y	4	Y	4	Y
Sss-Tet	28(1.7%)	2	N	2	N	2	N
Amc-Amp-Cro-Fox-Str-Tet-Tio (I)	28 (1.7%)	7	Y	6	Y	3	Y
Amp-Tet	27 (1.7%)	2	N	2	N	2	N
Amc-Amp-Chl-Cro-Fox-Sss-Str-Tet-Tio (I)	26 (1.6%)	9	Y	8	Y	5	Y
Sss-Str-Sxt-Tet	25 (1.6%)	4	Y	3	Y	3	Y
Gen-Sss-Str	**24 (1.5%)**	**3**	**Y**	**2**	**N**	**2**	**N**
Amp-Str	23 (1.4%)	2	N	2	N	2	N
Amc-Amp-Cro-Fox-Tet-Tio (I)	**22 (1.4%)**	**6**	**Y**	**5**	**Y**	**2**	**N**
Amp	22 (1.4%)	1	N	1	N	1	N
Amc-Amp-Cro-Fox-Str-Tio (I)	**20 (1.2%)**	**6**	**Y**	**5**	**Y**	**2**	**N**
Total for 20 Most Frequent	1179 (73.8%)						

Bolded lines signify disagreement for classification of resistance pattern as MDR between the MDR classification metrics

(I) Represents that at least 1 Category I antimicrobial included in resistance pattern

Amc – amoxicillin-clavulanic acid; Amp – ampicillin; Chl – chloramphenicol; Cro – ceftriaxone; Fox – cefoxitin; Gen – gentamicin; Sss – sulfisoxazole; Str – streptomycin; Sxt – trimethoprim-sulfamethoxazole; Tet – tetracycline; Tio – ceftiofur

Table 3.5 – The 6 most frequent resistance patterns by year from generic *E. coli* isolates from chicken abattoir surveillance samples.

Resistance Pattern	**Ranking of the 6 most frequent resistance patterns for each year**									
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Pansusceptible	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Tet	**2**	**3**	**2**	**=5**	**3**	**=5**	**3**	**3**	**=5**	**2**
Amc-Amp-Cro-Fox-Tio (I)	3	4	=3	2	2	=3	4	5		
Amc-Amp-Chl-Cro-Fox-Sss-Str-Tet-Tio (I)	=4									
Amc-Amp-Cro-Fox-Tet-Tio (I)	=4									
Sss-Str-Tet	**6**	**=5**	**=3**	**=3**	**4**	**=3**	**5**	**2**	**2**	**=4**
Str-Tet		2	=3			2	2		3	=6
Sss-Tet		=5								
Amc-Amp-Cro-Fox-Sss-Str-Tet-Tio (I)		=5			5			4		
Str			6	=5						
Amc-Amp-Cro-Fox-Str-Tet-Tio (I)				=3						
Amp-Str-Tet					6	=5			4	=6
Sss-Str-Sxt-Tet							6			
Amp-Sss-Str-Sxt-Tet								6	=5	
Gen-Sss-Str									=5	=4
Gen-Sss-Str-Tet										3

Bolded lines signify that the pattern appeared in the 6 most frequent resistance patterns in all years

(I) Represents that at least 1 Category I antimicrobial included in resistance pattern

= Denotes that the resistance patterns had the same number of isolates

Amc – amoxicillin-clavulanic acid; Amp – ampicillin; Chl – chloramphenicol; Cro – ceftriaxone; Fox – cefoxitin; Gen – gentamicin; Sss – sulfisoxazole; Str – streptomycin; Sxt – trimethoprim-sulfamethoxazole; Tet – tetracycline; Tio – ceftiofur

Table 3.6 – The 6 most frequent resistance patterns by region from generic *E. coli* isolates from chicken abattoir surveillance samples.

Resistance Pattern	**Ranking of the 6 most frequent resistance patterns**			
	British Columbia	Prairies	Ontario	Québec
Pansusceptible	**1**	**1**	**1**	**1**
Amc-Amp-Cro-Fox-Tio (I)	2	6	5	
Amc-Amp-Cro-Fox-Sss-Str-Tet-Tio (I)	3			
Amc-Amp-Cro-Fox-Str-Tet-Tio (I)	4			
Amp-Str-Tet	5	5		6
Tet	**6**	**4**	**2**	**=4**
Sss-Str-Tet		2	4	3
Str-Tet		3	3	
Str			6	
Sss-Str-Sxt-Tet				2
Gen-Sss-Str-Tet				=4

Bolded lines signify that the pattern appeared in the 6 most frequent resistance patterns in all regions

(I) Represents that at least 1 Category I antimicrobial included in resistance pattern

= Denotes that the resistance patterns had the same number of isolates

Amc – amoxicillin-clavulanic acid; Amp – ampicillin; Cro – ceftriaxone; Fox – cefoxitin;
Gen – gentamicin; Sss – sulfisoxazole; Str – streptomycin; Sxt – trimethoprim-sulfamethoxazole;
Tet – tetracycline; Tio – ceftiofur

Table 3.7 - Patterns with a combination of resistance to quinolones, and third generation cephalosporins and/or β-lactams with β-lactamase inhibitors from generic *E. coli* isolates from chicken abattoir surveillance samples.[1]

Resistance Backbone	Frequency	Resistance Pattern (Number of antimicrobials)	Frequency
Amc-Cip-Cro-Nal-Tio	1	Amc-Amp-Chl-Cip-Cro-Fox-Gen-Nal-Sss-Str-Tet-Tio (12)	1
Cip-Cro-Nal-Tio	1	Amp-Chl-Cip-Cro-Nal-Sss-Str-Tet-Tio (9)	1
Amc-Cro-Nal-Tio	18	Amc-Amp-Chl-Cro-Fox-Nal-Sss-Str-Tet-Tio (10)	3
		Amc-Amp-Cro-Fox-Nal-Str-Tet-Tio (8)	2
		Amc-Amp-Cro-Fox-Gen-Nal-Sss-Str-Tet-Tio (10)	2
		Amc-Amp-Cro-Fox-Nal-Sss-Str-Tet-Tio (9)	2
		Amc-Amp-Chl-Cro-Fox-Gen-Nal-Sss-Str-Tet-Tio (11)	2
		Amc-Amp-Chl-Cro-Fox-Nal-Sss-Str-Sxt-Tet-Tio (11)	1
		Amc-Amp-Cro-Fox-Nal-Sss-Str-Sxt-Tet-Tio (10)	1
		Amc-Amp-Cro-Fox-Gen-Nal-Sss-Str-Tio (9)	1
		Amc-Amp-Cro-Fox-Nal-Sss-Tio (7)	1
		Amc-Amp-Cro-Fox-Nal-Str-Tio (7)	1
		Amc-Amp-Cro-Fox-Nal-Tio (6)	1
		Amc-Amp-Cro-Fox-Gen-Nal-Sss-Str-Sxt-Tio (10)	1
Cro-Nal-Tio	1	Amp-Cro-Gen-Nal-Sss-Str-Tio (7)	1
Amc-Nal	1	Amc-Amp-Nal-Sss (4)	1

Amc – amoxicillin-clavulanic acid; Amp – ampicillin; Chl – chloramphenicol; Cip – ciprofloxacin; Cro – ceftriaxone; Fox – cefoxitin; Gen – gentamicin; Nal – nalidixic aicd; Sss – sulfisoxazole; Str – streptomycin; Sxt – trimethoprim-sulfamethoxazole; Tet – tetracycline; Tio – ceftiofur

[1] Individual antimicrobial prevalence of resistance (%): Amc 23.7%; Cip 0.2%; Cro 23.3%; Nal 4.6%; Tio 21.5%

Table 3.8 – The odds ratios (95% confidence intervals and p-values) for the mixed effects univariable logistic regression models describing either the annual or regional variation in multidrug resistance of generic *E. coli* isolates from chicken abattoir surveillance using different MDR classification metrics.

Predictor Variable	**MDR-drug**		**MDR-cat**		**MDR-class**	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Year	1.06 (1.01-1.10)	0.006	1.03 (0.99-1.07)	0.16	1.07 (1.04-1.12)	<0.001
Region		<0.0001		<0.0001		0.0001
ON	Referent		Referent		Referent	
BC	2.59 (1.75-3.84)	<0.001	2.68 (1.77-4.06)	<0.001	0.61 (0.40-0.91)	0.016
PR	0.63 (0.41-0.96)	0.031	0.66 (0.42-1.03)	0.068	0.52 (0.32-0.84)	0.008
QC	2.28 (1.53-3.42)	<0.001	1.66 (1.08-2.54)	0.020	1.34 (0.86-2.10)	0.19

ON – Ontario; BC – British Columbia; PR – Prairies; QC - Québec

Table 3.9 – The adjusted coefficients (95% confidence intervals and p-values) for the mixed effects multivariable logistic regression models describing the annual and regional variation in multidrug resistance of generic *E. coli* isolates from chicken abattoir surveillance using different MDR classification metrics.

Predictor Variable	**MDR-drug**		**MDR-cat**	
	β (95% CI)	p-value	β (95% CI)	p-value
Year	0.023 (-0.035, 0.081)	0.78	0.00030 (-0.059, 0.059)	0.99
Region		<0.0001		<0.0001
Ontario (ON)	Referent		Referent	
British Columbia (BC)	1.06 (0.48, 1.64)	<0.001	1.11 (0.51, 1.70)	<0.001
Prairies (PR)	-0.96 (-1.72, -0.19)	0.014	-0.85 (-1.63, -0.076)	0.031
Québec (QC)	0.27 (-0.32, 0.86)	0.37	-0.0070 (-0.62, 0.60)	0.98
Year & Region Interactions		0.022		0.039
Year X ON	Referent		Referent	
Year X BC	-0.024 (-0.12, 0.076)	0.63	-0.027 (-0.13, 0.073)	0.60
Year X PR	0.088 (-0.034, 0.21)	0.16	0.082 (-0.041, 0.20)	0.19
Year X QC	0.13 (0.030, 0.23)	0.012	0.12 (0.017, 0.12)	0.022
Random Intercept – Abattoir				
Variance (95% CI)	0.042 (0.0087, 0.20)	0.029	0.058 (0.014, 0.23)	0.0094
ICC (95% CI)	1.26% (0.26-5.80%)		1.72% (0.44-6.52%)	

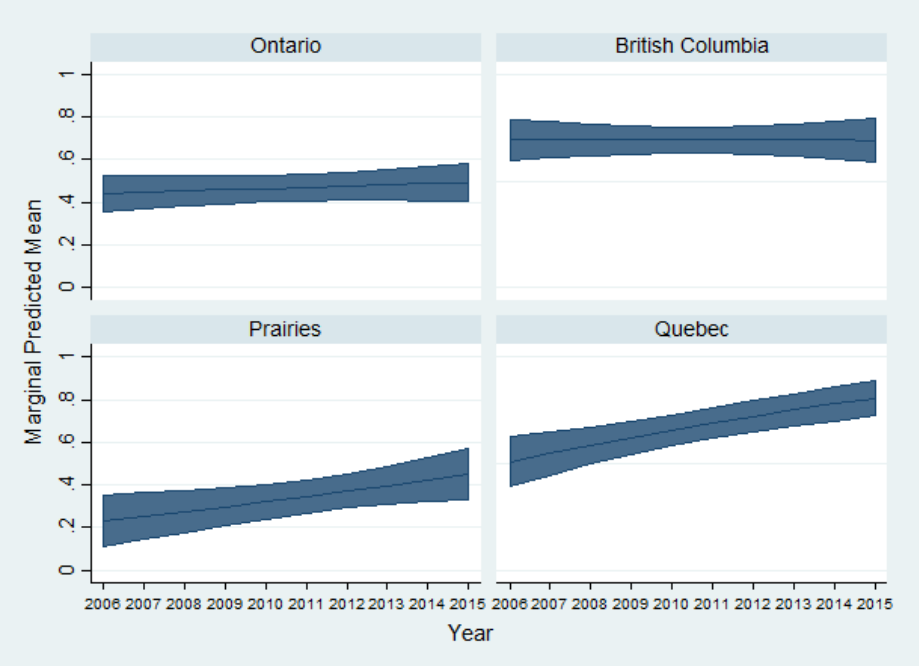
Table 3.10 – The predicted odds ratios (95% confidence intervals and p-values) for contrasts comparing specified years and regions from the mixed effects multivariable models describing the annual and regional variation in multidrug resistance of generic *E. coli* isolates from chicken abattoir surveillance using different MDR classification metrics.

Region and Year for Comparison	**MDR-drug**		**MDR-cat**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Ontario (ON) in 2006	Referent		Referent	
British Columbia (BC) in 2006	2.89 (1.62-5.16)	<0.001	3.03 (1.67-5.50)	<0.001
Prairies (PR) in 2006	0.38 (0.18-0.82)	0.014	0.42 (0.19-0.93)	0.031
Québec (QC) in 2006	1.31 (0.72-2.37)	0.37	0.99 (0.54-1.83)	0.98
ON in 2011	Referent		Referent	
BC in 2011	2.56 (1.72-3.80)	<0.001	2.64 (1.74-4.02)	<0.001
PR in 2011	0.59 (0.39-0.91)	0.016	0.64 (0.41-1.00)	0.052
QC in 2011	2.54 (1.67-3.87)	<0.001	1.77 (1.15-2.74)	0.010
ON in 2015	Referent		Referent	
BC in 2015	2.32 (1.26-4.28)	0.007	2.37 (1.27-4.43)	0.007
PR in 2015	0.84 (0.45-1.57)	0.59	0.89 (0.46-1.70)	0.71
QC in 2015	4.31 (2.26-8.24)	<0.001	*2.82* (1.50-5.30)	0.001

Table 3.11 – The adjusted odds ratios (95% confidence intervals and p-values) for the mixed effects multivariable logistic regression model describing the annual and regional variation in multidrug resistance of generic *E. coli* isolates from chicken abattoir surveillance based on MDR-class.

Predictor Variable	**MDR-class**	
	OR (95% CI)	p-value
Year	1.08 (1.04–1.13)	<0.001
Region		0.0001
Ontario	Referent	
British Columbia	1.65 (1.10–2.49)	0.017
Prairies	0.80 (0.50–1.27)	0.34
Québec	2.19 (1.42–3.40)	<0.001
Random Intercept – Abattoir		
Variance (95% CI)	0.066 (0.018–0.24)	0.0062
ICC (95% CI)	1.97% (0.54-6.90%)	

3.1a



3.1b

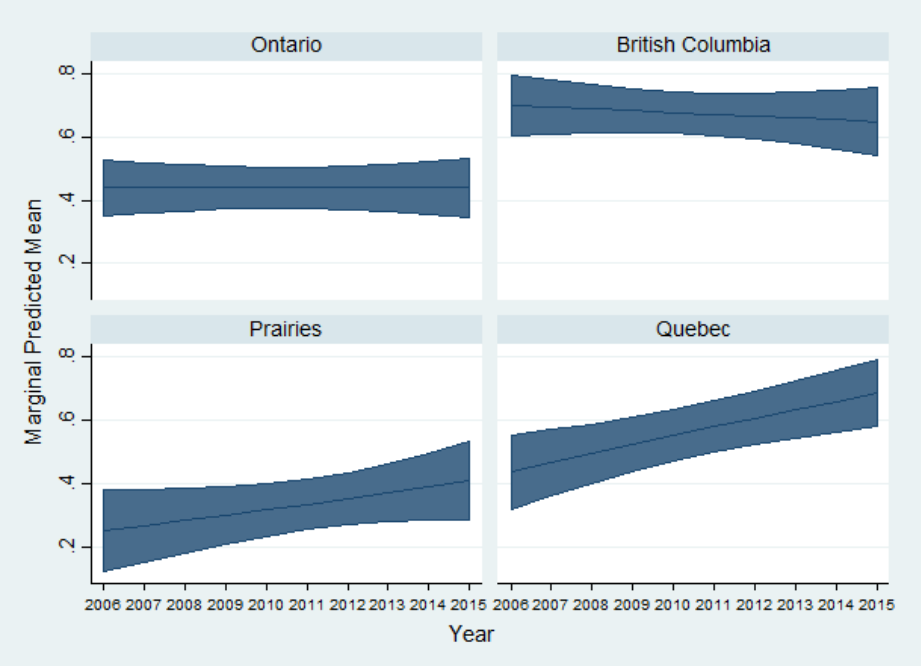


Figure 3.1 – Margins plots of the predicted multidrug resistance (MDR) probabilities for the relationship between year and region from the final mixed multivariable logistic regression models for MDR-drug (3.1a) and MDR-cat (3.1b).

CHAPTER 4

Summary Discussion and Conclusions

Currently, bacterial antimicrobial resistance (AMR) is a global public health issue of the utmost importance (Marshall and Levy, 2011; World Health Organization, 2014, 2015; Zawack et al., 2016). An emerging issue within AMR is multidrug resistance (MDR) (Exner et al., 2017). Due to resistance, antimicrobials are less effective against bacterial infections and it therefore becomes more difficult to treat these infections in humans and animals (Aarestrup et al., 2008; World Health Organization, 2014, 2015). Antimicrobial use in any sector (e.g., humans, animals, plants) can select for AMR in that sector, but there may also be AMR spread to other sectors (Molbak, 2005; Aarestrup et al., 2008; Schechner et al., 2013; Verraes et al., 2013). For example, antimicrobial use in animals may select for AMR in animal pathogens, but there is also evidence that antimicrobial-resistant enteric bacteria can spread from animals or animal products to humans (Aarestrup et al., 2008; Dutil et al., 2010). In order to understand the breadth of the AMR issue, discover emerging trends and inform policy decisions, surveillance programs are needed (Aarestrup et al., 2008; World Health Organization, 2017). In Canada, surveillance of AMR in enteric bacteria along the farm-to-fork continuum is performed by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), which is led by the Public Health Agency of Canada (Government of Canada, 2015a).

An important aspect of AMR surveillance programs, including CIPARS, is to analyze temporal trends in AMR. However, modelling minimum inhibitory concentration (MIC) data can be challenging because MIC data are discrete, interval censored and ordinal, and often do not have a normal distribution (Sanchez, 2007; Aerts et al., 2011; Otto, 2011). To overcome these

challenges, the MIC data are commonly dichotomized using established breakpoints in to susceptible (S) and resistant (R) categories and modelled using logistic regression (Bjork et al., 2015; Government of Canada, 2015a; Hanon et al., 2015). There is a loss of information when the MIC data is dichotomized and subtle temporal trends may not be identified (Wagner et al., 2003; Sanchez, 2007; Aerts et al., 2011; Bjork et al., 2015). Exploration of alternatives to logistic regression for modelling MIC data from AMR surveillance programs would be informative and beneficial by allowing AMR surveillance programs to utilize information along a wider range of MIC values. Another aspect of interest to AMR surveillance programs, including CIPARS, is the evaluation of temporal trends in MDR and the composition of resistance patterns (Government of Canada, 2015b). There is not an accepted definition of MDR for non-clinical surveillance samples and this makes analyzing MDR difficult. Due to the variety of MDR classification metrics used, the validity of comparing MDR results from different surveillance programs and studies must be considered (Falagas et al., 2006; Magiorakos et al., 2012; Exner et al., 2017). In order to catalyze the creation of a harmonized MDR classification metric for non-clinical surveillance programs, it would be advantageous to assess the impact of using different MDR classification metrics on the analysis of temporal trends in MDR.

Therefore, the objectives of this research project were:

1) To systematically compare the performance of various types of regression models using MIC and breakpoint (R/S) data for the analysis of annual variation in susceptibility of generic *Escherichia coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat surveillance samples while accounting for clustering of the samples (Chapter 2).
2) To use the best performing models to compare the similarities and differences in the results for all three antimicrobials (Chapter 2).

3) To describe the most common resistance patterns present in generic *Escherichia coli* isolates from chicken abattoir surveillance in Canada, as well as their variation over the study period and between regions. In particular resistance patterns involving quinolones, and third generation cephalosporins and/or β-lactams with β-lactamase inhibitors were described (Chapter 3).
4) To compare different multidrug resistance classification metrics, and use them to compare the annual and region variation in the prevalence of MDR using generic *Escherichia coli* isolates from chicken abattoir surveillance samples while accounting for clustering of the samples (Chapter 3).

The analysis of annual and regional variation in susceptibility of 3243 generic *E. coli* isolates to ceftiofur, ampicillin and nalidixic acid from retail chicken meat surveillance samples was compared using six different regression models (Chapter 2). Only the logistic and multinomial regression models consistently met model assumptions. The multinomial regression model regularly identified additional statistically significant annual variation compared to the logistic regression model. Region was an important (statistically significant) variable in all of the models. Logistic regression modelling of MIC surveillance data was confirmed to be a relatively robust method with results that are easy to communicate. However, the concerns regarding loss of information through dichotomizing the MIC data with logistic regression were supported through the multinomial regression model results. The multinomial regression approach used more information along the range of MIC values and allowed additional statistically significant annual variation in susceptibility to be identified, which could be valuable information for CIPARS and other AMR surveillance programs. However, with the additional outcome categories of the multinomial regression model, the results cannot be communicated as concisely as with the logistic regression model.

MDR was evaluated in 1598 *E. coli* isolates from chicken abattoir surveillance samples using two approaches (Chapter 3): first, the composition of the resistance patterns was explored; and second, the impact of using different MDR classification metrics on the analysis of associations between the prevalence of MDR, and annual and regional variation was investigated. Pansusceptible was the most common susceptibility pattern overall, and in each year and region. Nevertheless, there was a moderately-high prevalence of *E. coli* MDR (38.5% - 53.3%), which varied depending on the MDR classification metric used. Resistance to third generation cephalosporins and β-lactams with β-lactamase inhibitors was common within resistance patterns, however, resistance to quinolones was uncommon. The choice of MDR classification metric impacted the interpretation of the associations between the prevalence of MDR, and year and region, echoing previously raised concerns. Pairing the statistical analysis of the prevalence of MDR with descriptive analysis of the composition of the resistance patterns allowed the temporal trends to be evaluated while providing context of the potential importance of the MDR *E. coli* isolates to human and animal health. The identification of moderately-high prevalence of MDR in the *E. coli* isolates from chicken abattoir surveillance samples, together with resistance patterns that commonly included resistance to third generation cephalosporins and β-lactams with β-lactamase inhibitors, highlights potentially serious threats to animal and human health, and points to the value of continued surveillance.

There were limitations with this research. First, since Chapter 2 was limited to the analysis of susceptibility data for generic *E. coli* isolates from chicken meat samples for ceftiofur, ampicillin and nalidixic acid, the results cannot be directly generalized to other AMR surveillance datasets. Second, the regression models for Chapter 2 were simple multivariable models to facilitate comparison between model types, but these models did not evaluate interaction effects unless

they were necessary to meet model assumptions. Third, regarding MDR classification in Chapter 3, limitations posed by the range of antimicrobials included on test panels may have led to increased false negatives and non-differential misclassification bias with MDR-cat (Magiorakos et al., 2012). The prevalence of MDR with MDR-cat and strength of associations identified could have been underestimated due to this bias. Fourth, only three of the numerous possible MDR classification metrics were compared in Chapter 3 and therefore, further variation in the results may have been realized if other MDR classification metrics were used.

These limitations point to opportunities for future research. The performance of logistic and multinomial regression models could be assessed using susceptibility data for other bacterial-antimicrobial combinations, animal sample sources and sample types. More complex regression models could be considered, including evaluating possible interaction effects between predictor variables. As whole genome sequencing becomes more frequently available within AMR surveillance programs, its application to the analysis of temporal trends will need to be explored. Related to the exploration of MDR in surveillance data, if sufficient power was available in a dataset, logistic regression models could be used to analyze annual and region variation for the prevalence of specific important resistance patterns or backbones. The analysis of factors associated with the prevalence of MDR could be explored with regression models beyond logistic regression, including multinomial and Poisson regression models.

In conclusion, AMR surveillance programs, including CIPARS, collect data in MIC format and then routinely dichotomize the data for analysis; the results of Chapter 2 provided a supplemental or alternative statistical approach, multinomial regression, which would allow more of the MIC information to be used. By using the multinomial regression approach, surveillance programs could identify changes within susceptible MIC values, including MIC creep, before a significant

change in the prevalence of resistance occurred. The approaches for the descriptive and statistical analysis of MDR in AMR surveillance data used in Chapter 3 could be extended to the analyses routinely performed by AMR surveillance programs. This would provide a multifaceted approach to characterizing the evolution and emergence of MDR within the AMR surveillance programs, which could highlight areas for further investigation and policy development. The results of Chapter 3 clearly show the need for an internationally accepted consensus statement on an optimal MDR classification metric for non-clinical surveillance purposes. This would facilitate meaningful comparisons of MDR results between different AMR surveillance programs and studies.

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