An Examination of Data and Analyses for Antimicrobial Resistance
Surveillance of Clinical Bacterial Isolates from Ontario Swine

by

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ABSTRACT

AN EXAMINATION OF DATA AND ANALYSES FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE OF CLINICAL BACTERIAL ISOLATES FROM ONTARIO SWINE

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Surveillance of antimicrobial resistance (AMR) is critical to inform the appropriate choice of antimicrobials for treatment, developing antimicrobial use policies and assessing stewardship interventions. This work assessed the quality and quantity of clinical bacterial isolate antimicrobial susceptibility data from a comprehensive diagnostic veterinary laboratory for the development of an AMR surveillance system for swine in Ontario. Analysis options were evaluated and recommendations were developed to improve laboratory information systems (LIMSs) in terms of quality and quantity of surveillance data. In addition, Ontario Swine Veterinary-based Surveillance System (OSVS) data were used to explore relationships between reported failure of antimicrobial treatment, antimicrobial use, diagnosis and affected body systems in Ontario swine.

Although missing data, recording consistency and temporal changes in susceptibility panels were found within the diagnostic data, temporal scan statistics and visualization methods were found to be appropriate for presenting meaningful results to surveillance stakeholders. Furthermore, the use of prospective scan statistics for assessing increases in multiple-class resistance were shown to have potential for the syndromic
surveillance of emerging viral diseases (e.g., Porcine Circovirus). These results can
provide useful estimates of the prevalence of resistance for practitioners in Ontario
treating ill animals and for assessing the impact of antimicrobial stewardship
interventions. Moreover, if increases in multiple-class resistance can be detected in
clinical pathogens before large animal and/or economic losses occur, it may be possible
to impose interventions to mitigate the impact of future novel viral outbreaks.

Reported treatment failure in the OSVS dataset was found to occur at a
significantly lower rate when the gastrointestinal (GI) system was affected as compared
to conditions affecting other body systems. Therefore, a more holistic approach to herd
health may be advantageous over antimicrobial use when the treatment of non-GI
infections becomes problematic. Appropriate measures may include re-visiting
vaccination, management and biosecurity protocols.

Based upon this work, recommendations for future LIMSs development in
support of AMR surveillance include the use of electronic submission forms, production
system profiles for submitting owners and practitioners, limiting checkboxes and free-
text fields, and the use of a consistent panel for antimicrobial susceptibility testing.
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STATEMENT OF WORK

The following individuals were involved with writing the initial proposal and acquiring Animal Health Strategic Investment (AHSI) funding for this project: Drs. David Pearl, Scott McEwen, Richard Reid-Smith, Beverly McEwen, Durda Slavic, Jane Parmley, David Léger, Agnes Agunos, and Jim Fairles. The AHSI is managed by the Animal Health Laboratory (AHL) of the University of Guelph and supported by the agreement between the Ontario Ministry of Agriculture and Foods (OMAF), the Ministry of Rural Affairs (MRA), and the University of Guelph.

All bacterial isolations and antimicrobial susceptibility tests were performed by personnel at the AHL. Resulting data were acquired from the AHL. All data cleaning, merging, assessment, and analyses were performed by Shiona Glass-Kaastra. Writing and manuscript generation was performed by Shiona Glass-Kaastra.

Ontario Swine Veterinary Surveillance System data were acquired from participating veterinarians, and funding was acquired from Agriculture and Agri-Food Canada, Food Safety Initiative (FSI), OMAF, MRA, and the AHSI.

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CHAPTER ONE

INTRODUCTION, LITERATURE REVIEW, STUDY RATIONALE AND OBJECTIVES
INTRODUCTION

Antimicrobial resistance (AMR) is a growing concern for the health of humans and animals alike. With increasing rates of resistance in pathogenic bacteria, the choice of antimicrobial for treatment becomes more difficult; choice of an antimicrobial for which the pathogen is resistant may result in treatment failure and increased morbidity and mortality (Walsh and Fanning, 2008). Moreover, the use of antimicrobials is commonly cited as a contributing factor for the development and maintenance of resistance in a bacterial population (Livermore, 2012; Mathew et al. 2007). Therefore, practitioners, including physicians and veterinarians, are first tasked with determining whether or not antimicrobial treatment is the appropriate choice for the affected individual, and if so, then with the choice of an appropriate compound or drug.

In order to make an informed decision regarding the best compound or drug for treatment, knowledge of the prevalence of resistance in the pathogen of interest is required. For example, an intensive care unit (ICU) physician in Canada is unlikely to treat a new *Staphylococcus aureus* infection with methicillin, given that the rate of methicillin resistance in nosocomial *S. aureus* infections is high in Canadian hospitals (Simor et al., 2005). However, if the prevalence of resistance within given pathogens is unknown (or unreported in the literature), the practitioner is left to make a treatment decision blindly or based on experience within a particular practice.

Antimicrobial resistance surveillance systems track the prevalence of resistance within target pathogens and therefore provide data that may be used to assist in the decision for treatment options. As surveillance systems for livestock pathogens are rare, it was originally intended that this thesis would describe the suitability of developing a real-
time surveillance system for AMR in all livestock commodities in Ontario. Susceptibility results from the Animal Health Laboratory (AHL) for all livestock commodities were received; however, it was found that the volume of susceptibility data for horses, dairy cattle, beef cattle, and poultry was insufficient to reach statistical power to assess differences in the rate of AMR over time. A subset of these data is described in Appendix A. However, sufficient data volume was available for swine. Therefore, data assessment and analyses were limited to pathogens of swine. Three pathogens with the highest volume of susceptibility data were then chosen for the assessment: *Escherichia coli* F4 (previously referred to as subtype K88), *Pasteurella multocida* and *Streptococcus suis*.

The objectives of this literature review are to:

1) Summarize background information on the development of AMR.
2) Describe the use of antimicrobials in livestock production, specifically in swine.
3) Describe current AMR surveillance systems in humans and livestock, in terms of the pathogens under investigation and data presentation.
4) Describe analytical options for AMR surveillance data, including:
   a. The Cochran-Armitage trend test, logistic regression, control charts, cumulative sum (CUSUM),
   b. Space, time and space-time cluster detection methodology; and
   c. A new biosurveillance program entitled “What’s Strange About Recent Events?” (WSARE).
5) Provide the current status of knowledge surrounding AMR in the following pathogens of swine:
   a. *Escherichia coli* F4,
   b. *Streptococcus suis*, and
   c. *Pasteurella multocida.*
LITERATURE REVIEW

Antimicrobial resistance

The increasing ability of bacterial pathogens to resist the effects of antimicrobial treatment is of great concern. Through gene mutation and transfer, bacterial pathogens have acquired the ability to survive in the presence of antimicrobial agents by modification of target sites, production of antimicrobial-degrading enzymes and up-regulation of efflux pumps, resulting in pathogens that are resistant to antimicrobial treatment (Gootz, 2010; Gold and Moellering, 1996). Antimicrobial resistance reduces the ability of antimicrobial agents to treat infections of humans and animals, leading to prolonged morbidity, as well as increasing the treatment-associated costs and the risk of mortality. It imaginable that we may soon face a post-antimicrobial era (Alanis, 2005; Cohen, 1992), as resistance rates are ever-increasing while the production of new antimicrobials, especially those with novel modes of action, has stalled (Talbot et al., 2006). Therefore, in order to maintain treatment options for infectious diseases for future generations, containment and reduction of AMR must be a priority for health research.

Although antimicrobial resistance is a natural phenomenon, the most often cited driver for the emergence of resistant pathogens as predominant over susceptible wild-types and the dissemination resistant populations, is the use of antimicrobials. Therefore, judicious and appropriate use of these products is required in order to reduce the pressure for selection of resistant pathogens (Fishman, 2006). Judicious and appropriate use requires first the confirmation of a bacterial infection, and second, the choice of an antimicrobial with suitable activity against the pathogen in question. Furthermore, in gram positive infections, the use of narrow spectrum agents is preferable to broad
spectrum agents when the treatment outcomes are comparable, as broad spectrum agents are expected to pose a greater selection pressure for resistance. Determining whether an antimicrobial is a suitable choice requires knowledge of the susceptibility of the organism to the primary treatment options, as well the potential for cross-resistance to other agents (Fishman, 2006).

**Use of antimicrobials in livestock**

The use of antimicrobials in livestock production is currently a topic of great interest. Consumers are becoming more aware of the use of antimicrobials within the production of meat and dairy commodities. The growing popularity of organic and antibiotic-free products are a testament to the expanding concerns over antimicrobial residues in consumer products, AMR and for food chain transmission of AMR bacteria to humans (Jacob et al., 2008), regardless of the validity of these concerns (Harper and Makatouni, 2002).

In order to assess the risks posed by livestock antimicrobial use upon human health, risk assessments have been performed, and furthermore, frameworks for conducting future risk assessments have been detailed by a number of organizations (OIE, 2012; Codex, 1999). However, in order to develop the most accurate figures for risk, a measure of the volume of antimicrobials used in livestock is often required. Intuitively, this seems like something that could be measured easily; however, measuring use in livestock species is a difficult task. Although human antimicrobials in Canada are regulated by physician prescription and dispensed by pharmacies, antimicrobials for livestock use is more complex. In Canada, animal antimicrobials may be purchased OTC
(over-the-counter), or included in pre-mixed medicated feeds, and in some cases do not require a prescription from a veterinarian (with the exception of the province of Québec, where prescriptions for all veterinary antimicrobial use is mandatory). Although producers are required to comply with mandatory withdrawal times (a drug-specific amount of time required between treatment and animal slaughter), there are no requirements for producers to report their use of antimicrobials. Therefore, it is rarely possible to get an accurate measurement of the volume of antimicrobials used on-farm, especially at a broad scale (e.g., at the provincial level).

The task of monitoring AMU in livestock in Canada was undertaken by the Public Health Agency of Canada as part of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Due to the above-mentioned issues in obtaining accurate use measures, CIPARS adopted a sentinel farm strategy, and is currently collecting data from swine and poultry farms. In 2008, 95 grower-finisher swine farms were included in the sentinel program from the five major pork producing provinces in Canada (Alberta, Saskatchewan, Manitoba, Ontario, and Québec) and the vast majority reported use of antimicrobials (Government of Canada, 2008). Indications for use included respiratory disease, enteric disease, prevention of disease, and growth promotion. Twenty-two different antimicrobials were used among these herds, at varying levels. The most commonly used drugs were penicillin G, tylosin, lincomycin, and chlortetracycline.

The route of administration of antimicrobials is also a topic of interest in regards to AMR selection pressure, as evidenced by its inclusion in risk assessment frameworks (OIE, 2012). Due to the large scale nature of swine rearing, it may be difficult to single
out ill individuals for treatment. As such, in-feed or in-water antimicrobials may be used for therapeutic treatment (defined here as antimicrobial therapy for disease treatment, but not for prophylaxis). In-feed or in-water administration of antimicrobials exposes the entire group to antimicrobials, in comparison to individual level treatment (often by injection). Administering group-level antimicrobial through feed or water is expected to produce a higher potential for selection of resistant pathogens or flora, due to a larger population being exposed. Furthermore, the dosage received by each individual within the herd cannot be measured directly; each animal will receive product proportional to their feed and water intake. Some individuals will consume more than the recommended dosage, while others may receive far less; particularly ill individuals who tend to go off-feed. In addition, non-therapeutic antimicrobial use occurs within swine production for purposes of enhancing feed efficiency and disease prophylaxis. Non-therapeutic use can occur at lower dosages than therapeutic uses. This has been raised as a cause for concern, as low dosage treatment may be more likely to select for resistant bacteria. Indeed, it has been shown that sub-therapeutic dosing of tetracycline antimicrobials can select for the carriage of resistant bacterial species in beef and poultry (Inglis et al., 2005; Ladley et al., 2007). For 2008, CIPARS reported that three of the top four antimicrobials (tylosin, lincomycin, and chlortetracycline) used in pigs were most likely to be administered at the herd level through feed, while penicillin G was most likely to be administered at the individual-pig level by injection.
**Antimicrobial resistance surveillance**

Currently, there are a number of AMR surveillance programs in different countries that focus on enteric and nosocomial bacteria of humans. Many of these programs also perform integrated surveillance of these pathogens in animal populations, animal environments and agri-food commodities. For example, CIPARS and the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) collect samples from humans presenting with food-borne illness symptoms, healthy and diseased animals, as well as from animal products entering the food chain at abattoirs and retail outlets (DANMAP, 2008; Government of Canada, 2010a). Other programs, including the National Antimicrobial Resistance Monitoring System in the USA, include isolates from agricultural, abattoir, and food sources along with clinical isolates from humans, and yet others, such as the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM), focus solely on animal samples (Tamura, 2003; Centers for Disease Control and Prevention, 2010a - c). These systems have emerged in response to increasing human health concerns about antimicrobial use in the agri-food sector and its possible impact on resistance in zoonotic and enteric pathogens of humans. As such, pathogens and commensals included in these surveillance systems include *Campylobacter* spp., *Enterococcus* spp., untyped *Escherichia coli*, and *Salmonella*. In some cases, the bacteria are chosen for AMR surveillance because they are important food-borne pathogens (e.g., *Salmonella*, *Campylobacter* spp.) that may acquire resistance to antimicrobials of importance to human health. In other cases (e.g., untyped *E. coli*, *Enterococcus* spp.), these bacteria are commensals that are readily isolated and are used as indicators of resistance selection pressures. In addition, they may cause opportunistic
infections in humans, and may act as donors of resistance genes to (other) pathogenic or zoonotic bacteria. Although certain subtypes of these bacteria (e.g., *Salmonella, E. coli*) may cause clinical disease in animals, other subtypes may be of human health importance, and there are many species of bacteria that are important from an animal health perspective that are not included in these surveillance programs.

**Antimicrobial resistance surveillance in livestock**

The need for surveillance of AMR in animal pathogens has been highlighted recently by the European Parliament, which has called for “regular systematic surveillance and monitoring of AMR in both food producing animals and companion animals... [and] prudent and responsible use of antimicrobials and animals and for more information to veterinarians and farmers to minimize the development of AMR” (De Castro, 2011). Furthermore, Health Canada’s “Final Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health” makes the recommendation to “[d]evelop a coordinated, ongoing, national surveillance system for antimicrobial resistance in the major pathogens affecting food animals” in a chapter describing the impacts of AMR upon animal health (Health Canada, 2002). These calls clearly state that surveillance for AMR is necessary and that the results of surveillance efforts must be disseminated to practitioners on a timely basis.

Currently, there are several organizations dedicated to the surveillance of AMR within animal and zoonotic pathogens at the national level, with varying sources of samples and bacteria of interest. In Canada, surveillance of AMR in the foodchain is performed by CIPARS. CIPARS describes the prevalence of, and changes in AMR over
time for zoonotic pathogens; *Salmonella* spp., *Escherichia coli*, *Enterococcus* spp., and *Campylobacter* are isolated from human and animal fecal samples, diagnostic samples from animals, cecal swabs from abattoirs, and retail meat samples. These bacteria were chosen due to their impact on human health through the food chain, as well as being indicator species for resistance. However, there are certainly other bacterial species that have developed AMR, including clinical pathogens, which affect the health and productivity of livestock.

There is currently no ongoing surveillance in Canada concerning the development of AMR in other animal pathogens that affect the health and productivity of livestock. At the current time, DANMAP, the Swedish Veterinary Antimicrobial Resistance Monitoring program (SVARM), and the Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents program (FINRES-Vet) are the only national groups tracking AMR from pathogens isolated from livestock diagnostic samples. DANMAP reports data concerning swine isolates of *E. coli* O149 and *Staphylococcus hyicus*, as well as bovine isolates of *E. coli* F5 (previously known as K99) (DANMAP, 2011). FINRES-Vet tracks resistance in clinical *E.coli* enteritis of swine (FINRES-Vet, 2011). SVARM has a more comprehensive report, including AMR in *E. coli* from swine, cattle, and horses, *Pasteurella* spp. in swine and cattle, *Brachyspira hyodysenteriae, Brachyspira pilosicoli, Actinobacillus pleuropneumoniae*, in swine, and *Aeromonas salmonicida* subspecies achromogenes, *Flavobacter columnare*, and *Flavobacter psychrophilum* in farmed fish. Additional projects within swine, dairy herds, sheep, and broiler chickens were instigated in 2012 (SWEDRES-SVARM, 2012).
Surveillance of livestock antimicrobial consumption occurs through many of the AMR surveillance programs as well. For example, CIPARS describes the use of antimicrobials in sentinel swine herds by class, weight of animals and route of administration (Government of Canada 2010; Government of Canada, 2009). DANMAP reports the volume of antimicrobial consumption by the weight of sold products (in kilograms of active ingredient) and by defined animal daily doses per kilogram of body weight (ADDkg) broken down by agent, commodity group and reason for treatment (DANMAP, 2011). However, the antimicrobial stewardship interventions evolving from these surveillance programs focus on pathogens with the ability to affect human health (zoonotic), rather than clinical pathogens of animals alone. For example, one of the most cited interventions from the CIPARS program was the voluntary withdrawal of ceftiofur use in broiler chicken hatcheries in Québec. Upon the withdrawal of use of ceftiofur, CIPARS reported reduced rates of ceftiofur resistance within *Salmonella* Heidelberg isolates from humans and retail chicken swabs, and within *E. coli* isolates from retail chicken swabs (Government of Canada, 2007). These data, while providing key information for human health, do not provide direct information to producers and veterinarians faced with treating ill animals or looking to improve/maintain the health status of their herd/flock, given that *Salmonella* Heidelberg and commensal *E. coli* are not necessarily pathogenic in animals.

**Presentation and analysis of AMR surveillance data**

Currently, methods for displaying results from AMR surveillance systems are mostly descriptive, but analytical options for the analysis and presentation of results are
available. These include Cochran-Armitage trend tests, logistic regression models, threshold techniques, control charts, cumulative sum charts, cluster detection via scan statistics, and surveillance algorithms such as “What’s Strange About Recent Events?”.

*Descriptive data presentation*

AMR surveillance results are commonly presented as figures displaying the raw proportions of isolates that are resistant in a given year, over time (where longitudinal data are available) and tables describing the isolates resistant to antimicrobials tested. Results are presented in a manner that is easy to understand and therefore accessible to a variety of users; however, the statistical significance of changes are often overlooked or not considered.

AMR phenotype data are commonly presented using susceptible-intermediate-resistant (S-I-R), or simply susceptible-resistant (S-R) classifications, which are based upon standardized minimum inhibitory concentration (MIC) or disk-diffusion measurement breakpoints (Government of Canada, 2008). Proportions of resistant results are displayed graphically by year or quarter if data are sufficient (CIPARS, NARMS, EARS-Net). The proportions of isolates resistant to >1 drug, or >1 class of antimicrobial are also reported by many systems (Government of Canada, 2011; Government of Canada, 2010; Government of Canada, 2009; FDA, 2012; EARSS, 2009).

*Cochran-Armitage trend test and logistic regression models*

To date, formal statistical modeling for assessing trends in dichotomized AMR data has also been pursued using the Cochran-Armitage trend test and logistic regression
models (Aerts, 2011). In a comparison of the Cochran-Armitage test to logistic regression models for dichotomized AMR data, the latter were deemed to be the superior choice (Aerts, 2011). The Cochran-Armitage trend test assesses whether the slope of the time-dependent data is equal to zero (no trend) and is equivalent to the Wald or Likelihood ratio test for the slope of the time variable in a logistic regression model (Aerts, 2011). However, the logistic regression model provides information not provided by the Cochran-Armitage trend test, including the magnitude and direction of the slope (if one exists) and has many extensions that make it a more flexible tool, such as the ability to include other predictor or confounding variables, cyclical time components and random effects to deal with clustered data (Aerts, 2011; Dohoo et al., 2009).

**Threshold techniques**

Identification of trends in the proportion of resistant isolates over time alone may not fulfill the needs of an AMR surveillance system. In cases where the system is also designed to detect outbreaks, a number of techniques have been employed. The most basic technique compares the observed to the expected number of occurrences in a given time frame, using an “alarm threshold”. The expected number of occurrences is often based on the historical mean and the statistical significance of the observed number of cases is assessed using a Z-statistic, therefore assuming that the “disease” rate follows a normal distribution. A critical cut-off value for the Z-statistic is used to produce an alarm when the number of observed cases is higher than expected (Weatherall and Haskey, 1976). However, the choice of the expected number of cases, as well as the critical cut-off value, affects the sensitivity, specificity and predictive values of the produced alarm.
For example, a low cut-off value will result in alarms being produced more frequently than a high cut-off value, and consequently, alarms may be produced when there is no true outbreak occurring. In this instance, the sensitivity of the alarm (the probability of an alarm sounding when an outbreak is occurring) would be high, but the positive predictive value of an alarm (the probability that an alarm reflects a true outbreak) would be low. For this reason, although the threshold technique is relatively simple statistically, the choice of the expected number (threshold) value can be quite difficult. This choice requires balancing the impacts of a missed outbreak with the costs of investigating a false-positive alarm, which vary based upon the disease in question (Stoto et al., 2004). Furthermore, a considerable baseline of historical data may be required to make this decision, especially when the outcome under investigation is affected by seasonal or other temporal variables (Berger et al., 2006).

Control charts

A similar threshold method is the Shewhart control chart, proposed originally in the field of manufacturing quality control (Shewhart, 1926). Although originally developed to assess changes in a continuous mean, the Shewhart control charts are not based on any particular distribution. Therefore, Shewhart control charts may be used to assess Poisson or binomial processes, such as a disease count or the proportion of resistant isolates (Nelson, 1999). When applied to disease surveillance, the Shewhart control chart graphically compares the number of cases or proportion of resistant cases over time to warning limits and decision rules to determine whether the process is “predictable” or “unpredictable”. The terms “in-control process” and “out-of-control”
process are also used in the literature, although inconsistently, to describe “predictable” and “unpredictable” processes (Woodall, 2000). When a decision rule for signaling an “unpredictable process” alarm is broken, this suggests that a disease outbreak may be occurring, and investigations are initiated. The decision rules for signaling an “unpredictable process” alarm are chosen \textit{a priori} to ensure that the charts are interpreted by all users in a uniform manner. Commonly employed sets of decision rules have been developed and presented by a number of individuals (Nelson, 1984; Wheeler, 1983; Bissell, 1978; Western Electric, 1959; Roberts, 1958; Page, 1955).

\textit{Cumulative sum charts}

Cumulative sum charts (CUSUM) constitute another technique using decision rules similar in application to the Shewhart control chart that were also first described for assessing output quality within industrial manufacturing (Page, 1954). The CUSUM chart plots the cumulative sum of the difference between the observed value and a target value (e.g., the historical mean, \(H\)) against the number of values that make up the cumulative sum (Ewan, 1963). For example, on day 1, the historical mean \(H\) would be subtracted from the disease count on day 1 of surveillance (\(C_1\)) and the difference plotted against the \(x\) value of 1. On day 2, the sum of \((C_1 - H) + (C_2 - H)\) would be plotted against an \(x\) value of 2. In general terms, the cumulative sum for day \(N\) is \(\sum_{i=1}^{N} (C_i - H)\), and would be plotted against the \(x\) value equal to \(N\) (Ewan, 1963). As with the Shewhart control chart, decision rules are then used to determine when the CUSUM falls outside of its expected range. Because the historical mean is subtracted from each observation, the CUSUM chart for a predictable process will be maintained around zero, and will have a
mean slope of zero. When the line deviates from the origin dramatically, the process becomes unpredictable. Much like the Shewhart control table, decision rules are available to discern when the process is deemed unpredictable (Ewan WD and Kemp, 1960; Barnard, 1959). Modifications to the basic CUSUM methodology have allowed for other variables to be assessed. For example, risk-adjusted CUSUM has been applied in the surveillance of surgical outcomes, which has allowed investigators to control for the differences in risk among patients with varying characteristics (i.e., comorbidities, type of surgery, age, sex) (Grigg et al., 2003). The CUSUM technique has also been applied for surveillance of Salmonella within animal diagnostic laboratory data (Carpenter, 2002).

*Cluster detection*

Although the control chart methods are easy to implement and use, these methods do not provide a measure of statistical significance for the observed patterns. Furthermore, these methods are limited to the analysis of temporal data, where surveillance systems may also seek to detect aberrant disease patterns in space (over geographical areas) as well. Cluster detection methods using scan statistic methodology have been developed for this purpose and have been effectively employed to identify disease clusters for a number of pathogens in both humans and animals (Pearl et al. 2006; Guerin et al., 2004; Ward, 2002; Ward and Carpenter, 2000). Scan statistics use a moving window of various sizes and positions within the dataset. The maximum size of the scanning window may be set, usually to 50%, such that clusters may range from very localized and/or short periods of time to half of the geographic area and/or half of the time frame covered. The data within the window is then compared to the baseline process.
using a likelihood ratio test (Kulldorf, 1997). Statistically significant differences infer times, areas, or times in an area when the disease rate is higher or lower than expected; these are known as “clusters”. As this methodology employs repeated testing, p-value adjustments are required in order to reduce bias; thus, standard and sequential Monte Carlo hypothesis testing and the Gumbel approximation are used in concert to calculate unbiased p-values (Dwass, 1957; Abrams et al. 2008). Scan statistics have been developed for binomial (Bernoulli model), count (Poisson model), ordinal, exponential, and continuous data. Furthermore, multivariate models are available, as well as the ability to adjust for covariates. The application of scan statistics to AMR data is directly comparable to that of disease detection, and therefore employs the Bernoulli model. With AMR data, a resistant isolate becomes the positive case, and a susceptible (or intermediately susceptible) isolate becomes the non-case (Otto, 2011). Although examples of using this technique for AMR data are rare in the literature at this time, it has been successfully used to detect significant spatial and temporal clusters of human ciprofloxacin-resistant *Campylobacter jejuni* infections in Saskatchewan, Canada (Otto, 2011).

An additional strength of the scan statistic for AMR cluster detection is the detection of the most likely (primary), and other (secondary) clusters within the same analysis. With purely temporal data, an iterative process is used to detect these secondary clusters. This option identifies the most likely cluster present within the data, as well as secondary clusters. The secondary clusters are identified by removing the most likely cluster, and reanalyzing the remaining data (Zhang, 2010). Interestingly, secondary
clusters can be identified without the iterative function in the purely space and space-time functions.

“What’s Strange about Recent Events?” (WSARE version 3.0)

New programs are being developed for the analysis of surveillance data, including an algorithm entitled “What’s Strange About Recent Events?” (WSARE) (Wong et al., 2002; Wong et al., 2003a; Wong et al. 2003b). WSARE is an anomaly detection algorithm that focuses on anomalous patterns, rather than anomalous observations (the focus of traditional anomaly detection algorithms) (Wong et al., 2002). WSARE version 3.0 uses a Bayesian network to produce a baseline distribution of the available data by taking the joint distribution of the parameters and conditioning on attributes responsible for trends (Wong et al., 2003a). Using this methodology, WSARE 3.0 develops a background expectation for each parameter alone and every combination of the parameters being evaluated (Wong et al., 2003b). The data are then scanned prospectively at the daily (or other predetermined interval) level and the observed values for the parameters and parameter combinations are compared to their expected values. Therefore, the WSARE 3.0 program is able to detect unexpected levels of resistance or susceptibility to a single antimicrobial or to groups of antimicrobials. When an unexpected level of resistance or susceptibility is found, WSARE 3.0 produces a “rule” and this rule is reported to the user with its statistical significance.

WSARE 3.0 has been shown to be able to detect disease outbreaks in several of applications, with a high specificity (low rate of false positive “rules”). For example, outbreak data from Walkerton, Ontario in 2000 was retrospectively scanned using
WSARE 3.0 and it was determined that the *E. coli* O157:H7 outbreak may have been identified a day earlier using this methodology than when the alarm threshold technique is used (alarm threshold reached when >2 positive tests were reported) (Sabhanani *et al.*, 2005).

Using susceptible-intermediate-resistant (S-I-R) resistance results, WSARE 3.0 may be applied to resistance data in a similar fashion as used in cluster detection methods. In addition to determining times when resistance exceeds expected levels, WSARE 3.0 methodology allows for the user to detect unexpected combinations in resistance (new resistance profiles) as they appear in the dataset.

The strategies discussed above may be useful in determining whether statistically significant trends or clusters appear in AMR data. However, the validity of these tests should be considered in light of the sampling strategy of the surveillance system and the population at risk. In the case of a passive surveillance system using laboratory data (as proposed in this thesis), two assumptions must be made: 1) the volume of submissions for AMR susceptibility testing received by the laboratory is relatively constant over the study time frame and 2) the population from which these submissions are derived is relatively constant over the study time frame. For example, it is has been shown that submission rates for swine diagnostic tests may be influenced by the strength of the Canadian dollar, higher auction prices and the outbreak of a new disease (O’Sullivan, 2012). Therefore, any trends or clusters identified within such passive laboratory surveillance data must be assessed in light of known fluctuations in the submission rate or population at risk.
**Escherichia coli F4**

*Escherichia coli* is a pathogen, some strains of which are of considerable concern in the swine industry. As an important cause of postweaning diarrhea (PWD), *E. coli* infections are responsible for considerable economic losses in the industry through increased morbidity, mortality, cost of medications, and decreased growth rates (Fairbrother *et al.*, 2005). PWD cases are commonly caused by *E. coli* strains carrying the F4 adhesin (Fairbrother *et al.*, 2005), a structure on the cell wall that mediates the attachment of the *E. coli* bacterium to receptor molecules on the walls of cells lining the intestinal tract in the postweaning pig (enterocytes) (Beachey, 1981). Interestingly, *E. coli* with the F4 adhesin only cause diarrhea illness in swine but not in other mammals, as the adhesin is specific to receptors upon swine enterocytes (Beachey, 1981; Jones *et al.*, 1974) and produces enterotoxins. Enterotoxins act upon enterocytes, resulting in increased secretion of water and electrolytes and reduced nutrient absorption, which may be fatal even before clinical manifestations of postweaning diarrhea occur (Amezcua *et al.*, 2002).

Given the implications of postweaning diarrhea, AMR within *E. coli* F4 isolates is concerning for producers, because antimicrobials are the common treatment and resistance may adversely affect treatment efficacy. Previous research by Amezcua *et al.* (2002) reported resistance to spectinomycin and tetracycline in all 75 isolates tested from 20 F4-positive farms in Ontario (Amezcua *et al.*, 2002). Furthermore, >50% of isolates were resistant to ampicillin, carbenicillin and neomycin, ≥20% resistance was seen for sulfamethoxazole, cefadroxil, gentamicin, tobramycin, and apramycin (Amezcua *et al.*, 2002).
Low levels of resistance to ceftiofur were reported, while 100% susceptibility was found for amikacin, enrofloxacin, ciprofloxacin, and polymixin B (Amezcuca et al., 2002).

Traditionally, sub-therapeutic doses of antimicrobials have been included in feed rations for prophylactic control of *E. coli* diarrhea (Bhandari et al., 2008). However, due to increasing rates of AMR and consumer opposition (Heuer et al., 2006), research efforts into the development of feed supplements (Bhandari et al., 2008) vaccines and bacteriophage therapies (Jamalludeen et al., 2008) for enterotoxigenic *E. coli* have been increasing.

*Pasteurella multocida*

*Pasteurella multocida* is a non-motile gram-negative coccobacillus that places considerable strain on the swine industry as the necessary cause of progressive atrophic rhinitis (PAR) and pneumonic pasteurellosis (Straw, 2006). PAR is a disease of economic importance, often resulting in reduced feed utilization and therefore growth retardation (Straw, 2006). Similarly, pneumonic pasteurellosis has been reported to significantly reduce growth rates and increase time to reach market weight (Straw, 2006).

Antimicrobials are often used for treating PAR and pneumonic pasteurellosis. However, *P. multocida* has evolved resistance to a number of antimicrobials, which limits treatment options (Straw, 2006). Resistance rates are quite variable for various antimicrobial agents across jurisdictions. For example, trimethoprim-sulfamethoxazole resistance rates were reported from a low of 3% in Hungary (2005-2008) (Sellyei et al., 2009), to a high of 74.2% in China (2003-2007) (Tang et al., 2009). Similarly, the same studies reported rates of tetracycline resistance of 6% and 58%, respectively (Sellyei et
al., 2009; Tang et al., 2009). In contrast, reports of low resistance rates to penicillin and ceftiofur are consistent (Lizarazo et al., 2006; Sellyei et al., 2009).

**Streptococcus suis**

*Streptococcus suis* is an important pathogen in the swine industry that causes high mortality rates characterized by meningitis, endocarditis, pneumonia, arthritis, and septicaemia (Straw et al., 2006). In addition to health effects for infected animals, high mortality rates result in significant economic losses for producers. Human meningitis caused by *S. suis* infection has also been reported, and human infection is becoming more prevalent in Southeast Asian countries (Gottschalk et al., 2007; Lun et al., 2007). These infections are often epidemiologically linked to swine exposure (Arends et al., 1988; Halaby et al., 2000). Interestingly, very few cases of human *S. suis* meningitis have been reported in North America (Segura, 2009); however, it has been shown that individuals with exposure to swine in the United States have higher antibody titres to *S. suis* than individuals without exposure to swine, suggesting that the rate of infection may be higher than currently reported (Smith et al., 2008). Therefore, *S. suis* is a pathogen of interest in both the animal and public health sectors and has the potential to become a problematic zoonotic disease worldwide.

Reports of AMR within swine *S. suis* isolates are frequent, with especially high rates of tetracycline resistance commonly documented (Aarestrup et al., 1998; Kataoka et al., 2000; Martel et al., 2001; Marie et al., 2002; Vela et al., 2005; Princivalli et al., 2009). Low prevalence of resistance to penicillins are also commonly reported (Aarestrup et al., 1998; Kataoka et al., 2000; Martel et al., 2001; Marie et al., 2002; Vela et al.,
2005; Princivalli et al., 2009). Low levels of penicillin resistance are encouraging, as penicillins are the first line choice for treatment of swine S. suis infections (Gottschalk et al., 1991; Kahn, 2005). However, penicillin is a commonly used antimicrobial in the swine industry and ongoing use may increase selection pressure for the development of resistance over time; therefore disrupting treatment routines.

**STUDY RATIONALE**

The use of antimicrobials is the most commonly cited factor for the development, dissemination and persistence of AMR. Therefore, responsible antimicrobial use and choices for treatment are of paramount importance. In order to make the best possible treatment decisions, a practitioner requires information regarding the susceptibility profile of the pathogen in question. For this reason, pre-treatment susceptibility testing has been suggested for clinically ill animals. However, it is unlikely that pre-treatment testing will become the norm, given that susceptibility testing may be costly and may take weeks and practitioners and owners want to treat ill animals as quickly as possible (Kehrenberg et al., 2001). Therefore, in order to provide informed timely treatment, practitioners must rely on resistance reports from textbooks, current literature, or surveillance programs. Access to this information is not readily available however; currently, there is little published information regarding AMR rates within animal pathogens in Ontario.

Without accessible information regarding current geographically-relevant AMR patterns, Ontario veterinarians are left to make treatment decisions based on past experience. However, with continuous evolution of bacterial pathogens, treatment options
are expected to become more limited. With increasing resistance and a reduction in antimicrobial choices, treatment failure will probably increase as well, leading to multiple treatment courses, prolonged morbidity and increased costs.

A surveillance system tracking AMR in veterinary clinical pathogens may help address this information gap. Furthermore, as the funding for a de novo active animal health AMR surveillance system is not available in Ontario, there is value in examining the usefulness and quality of existing passive data. The Animal Health Laboratory (AHL) at the Ontario Veterinary College (OVC) of the University of Guelph is a potential source of such passive data that may be suitable for the development of a surveillance system for AMR in pathogens from Ontario livestock. If data are suitable for the development of a surveillance system, suitable analyses must be determined and preliminary results reported to stakeholders.

THESIS OBJECTIVES

In light of the need for information regarding antimicrobial resistance in clinical pathogens of livestock, the objectives of this thesis are as follows:

1. Assess the quality and quantity of swine antimicrobial resistance data available from the Animal Health Laboratory at the University of Guelph and highlight key areas for improving data management and/or acquisition for the potential development of a surveillance system using these data.

2. Describe variations in resistance to ampicillin, tetracycline, tiamulin, and trimethoprim-sulfamethoxazole among *P. multocida* and *S. suis* using a variety of
temporal analysis methods and to assess whether these results could be potentially important for informing practitioners and surveillance stakeholders.

3. To assess the significance of the Porcine Circovirus outbreak upon multiclass resistance within *E. coli* F4, *P. multocida* and *S. suis* from Ontario swine.

4. Summarize and describe the data acquired by the Ontario Veterinary Swine Surveillance program in terms of the frequency of antimicrobial treatment failure.
REFERENCES


CHAPTER TWO

ASSESSMENT OF ANTIMICROBIAL SUSCEPTIBILITY DATA FOR

*ESCHERICHIA COLI F4*, *PASTEURELLA MULTOCIDA* AND *STREPTOCOCCUS SUIS* ISOLATES FROM A COMPREHENSIVE DIAGNOSTIC VETERINARY LABORATORY AND RECOMMENDATIONS FOR SURVEILLANCE SYSTEM DEVELOPMENT

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ABSTRACT

Antimicrobial susceptibility data were acquired from a comprehensive diagnostic veterinary laboratory in Ontario, Canada. These data consisted of *Escherichia coli* F4, *Pasteurella multocida*, and *Streptococcus suis* isolates from Ontario swine (January 1998 – October 2010). In relation to the possible development of a surveillance system for antimicrobial resistance, data were assessed for ease of management, completeness, consistency and applicability for temporal and spatial statistical analyses. Limited farm location data precluded spatial analyses and missing demographic data limited their use as predictors within multivariable statistical models. Changes in the standard panel of antimicrobials used for susceptibility testing reduced the number of antimicrobials available for temporal analyses. Data consistency and quality could improve over time in this and similar diagnostic laboratory settings by encouraging complete reporting with sample submission and by modifying database systems to limit free-text data entry. These changes could make more statistical methods available for disease surveillance and cluster detection.

INTRODUCTION

Surveillance of antimicrobial resistance (AMR) in bacterial pathogens is becoming increasingly important in the face of rising resistance and reduced availability of effective antimicrobial drugs. Antimicrobial resistance surveillance may include detection of temporal trends and identification of opportunities for interventions to mitigate dissemination and increases in the prevalence of resistance. These interventions may include efforts intended to reduce the use of specific antimicrobials and/or replacing
the use of drugs of concern with alternatives showing high in-vitro or in-vivo effectiveness. Furthermore, AMR surveillance provides information about the prevalence of resistance in relevant pathogens, which may be useful to practitioners making treatment decisions for animals affected by these pathogens.

Currently, there are a number of AMR surveillance programs operating in different countries that focus on human enteric pathogens and foodborne commensal bacteria from various sources (1-6). These systems have emerged in response to increasing human health concerns about antimicrobial use in the agri-food sector and its possible impacts on resistance in zoonotic and enteric pathogens of humans. Other AMR surveillance systems focus on non-enteric (e.g., nosocomial, respiratory and sexually-transmitted) human pathogens (7-9). However, programs that focus on AMR in target pathogens of animals are rare. This is an important gap in AMR surveillance, as bacterial species that routinely affect animals clinically have developed resistance (10). Resistant animal pathogens are potential reservoirs of resistance genes for human pathogens, can serve as indicators of antimicrobial use in a population and may be drivers of antimicrobial use as resistance to one drug begets increased use of second and third line options.

The need for surveillance of AMR in animal pathogens was in a May 2011 motion by the European Parliament, which called for “regular systematic surveillance and monitoring of AMR in both food producing animals and companion animals... prudent and responsible use of antimicrobials in animals and for more information to [be provided to] veterinarians and farmers to minimize the development of AMR” (11). Furthermore, the Advisory Committee on Animal Uses of Antimicrobials and Impact on
Resistance and Human Health included the design and implementation of AMR surveillance in food animal production as a key recommendation in their 2009 report (12). The development of a surveillance system for resistance in isolates associated with clinical disease from food-animals would help to identify whether resistance is a problem in important animal pathogens, trends in resistance prevalence and potentially provide veterinarians and farmers with information they can use to increase the responsible use of antimicrobials in farm animal production.

Surveillance systems generally follow one of two methods of data collection: active or passive (13). In the former, samples are obtained for the express purpose of surveillance; the surveillance team actively seeks out samples and the required supporting data. In contrast, a passive system obtains data on cases or submissions indirectly from sources such as laboratories or practitioners upon diagnosis or at regular intervals, or may be acquired from databases storing information for other purposes (13). Passively collected data are typically limited to the information collected for their original purpose such as clinical diagnosis. Passive methods can require less labour and financial input to implement and maintain than active methods because of the primary purpose of the database and therefore may be favoured over active methods when a suitable passive data source is available. This is particularly the case for human diseases, where sample acquisition may be difficult due to compliance (e.g., individuals refusing stool sample submission) or invasiveness (e.g., tissue samples). However, passively collected data may have more missing epidemiological information than that collected actively, may not be available in a format appropriate for surveillance analysis and may be limited in the availability of epidemiological information by the data requirements of the original user.
Therefore, careful scrutiny of the quality and applicability of the available passive data is warranted before they are used as the basis for a surveillance system.

The Animal Health Laboratory (AHL) of the Laboratory Services Division of the University of Guelph is a source of such passive data that may be suitable for the development of a surveillance system for AMR in agricultural animal pathogens in Ontario.

The objective of this research was to assess the quantity, consistency and quality of antimicrobial susceptibility data available from selected swine pathogens isolated at the AHL and to evaluate their potential for analyses in a semi-automated surveillance system. The pathogens included were: *Escherichia coli* F4 (previously K88), *Streptococcus suis* and *Pasteurella multocida*. These pathogens were chosen through a pre-study review of available data. They were the three most frequently isolated bacterial pathogens from swine in the AHL data and monthly isolation counts for these pathogens remained > 0 throughout the study period. Furthermore, these pathogens are important in Ontario swine production as causes of post-weaning diarrhea, neurological and respiratory infections, and secondary bacterial respiratory infections following viral infection, respectively (14). The data characteristics assessed for these pathogens included ease of data management, completeness of reporting and availability of consistent antimicrobial susceptibility testing over time, with respect to statistical analyses such as cluster detection or multivariable logistic regression. A discussion of limitations/issues identified in this assessment is used to make suggestions for improving data quality and for the development of automated protocols to maximize the quality of data storage for use in a prospective quantitative AMR surveillance system.
MATERIALS AND METHODS

Data detailing sample submissions, resulting isolations and susceptibility tests were obtained from the AHL from January 1998 – October 2010 for *E. coli* F4, *S. suis* and *P. multocida*. Isolation and antimicrobial susceptibility testing were performed according to the standard operating procedures of the AHL. All data pertaining to isolates were requested, including location and name for the clinic and farm submitting the sample, case demographic information, and all antimicrobial susceptibility results.

During the study period, the AHL used one laboratory information system (LIMS) from January 1998 to May 2007 [i.e., the Veterinary Animal Disease Diagnostic System © (VADDS) (Advanced Technology Corp, 2007)] and another LIMS from May 2007 to October 2010, which continues to be used in 2013 (i.e., Sapphire ©, LabVantage Solutions Inc., 2008). Data fields to be assessed were determined from the swine submission form from the AHL: case type (diagnostic, research, or monitoring), number of animals at risk, number sick, number dead, weight and age of animal, submitting clinic telephone number, name, and postal code; and owner contact information including farm/owner name, unique identification number, and postal code (15). A confidentiality agreement was signed regarding farm and veterinary clinic information such that individuals are not identified in results, and that data will be destroyed upon project completion.

To avoid potential biases, data from ongoing research programs and duplicate samples were excluded before any assessments or analyses were performed. Data pertaining to isolates not subjected to susceptibility testing were also removed. When the numbers of animals at risk, sick, and dead were all reported as zero, we assumed that the
data were missing. Furthermore, when number at risk was not reported and number sick
and number dead were reported as zero, an assumption was made that the number sick
and number dead values were missing. Recording consistency for the new LIMS data was
compared to all data using the Z-test for proportions. Similarly, recording consistencies
for the top 4, 8, and 10 submitting practices were compared to the recording consistency
for all data using the Z-test for proportions.

As it was not deemed feasible to assess susceptibility to every tested antimicrobial
within each pathogen, an \textit{a priori} decision was made to identify suitable
pathogen/antimicrobial combinations for surveillance; antimicrobials were identified as
suitable for continued surveillance for each pathogen if they were on the 2010 testing
panel, displayed \(\geq2\) years of historical data, and \(\geq1\) susceptibility test was performed per
month on average.

Potential sentinel practices were identified by enumerating the number of
observations acquired from each practice after cleaning the veterinary practice field.
Three groups of potential sentinel practices were identified; those with \(>400\), \(>200\), and
\(>150\) observations, which reflect major groupings in the practice list.

All data merging, cleaning, protocol development, graphical methods, and
analyses were performed using Stata/MP 11.0 (StataCorp 2009, College Station, TX).

\textbf{RESULTS}

\textit{Ease of data management}

The AHL used two LIMSs during the study period of January 1998 – October
2010. For both systems, demographic and isolate information was provided in separate
datasets for all pathogens. A third dataset was acquired detailing *E. coli* F4 results, as these results could not be extracted with the isolate information. Each case and sample in these datasets was provided with a submission identification number unique to the farm and date of submission, but not to the isolate. As such, multiple isolates from a single case may have shared submission identification numbers. Demographic variables (e.g., farm and practice data) were merged to the isolate data by the submission identification number. A single dataset was developed by merging all common data fields from the two LIMSs, and a semi-automated protocol was developed for future merging. Similarly, a semi-automated protocol was developed to append new data from the current LIMS on a prospective basis.

Unfortunately, semi-automated protocols for identifying the specific *E. coli* isolates positive for the F4 antigen could not be developed because serotyping information was linked to the submission identification without an isolate-specific identifier. Therefore, in cases where multiple *E. coli* isolates were obtained from a single submission, it was not possible to identify the specific isolate (and associated susceptibility results) that was tested for F4 reactivity. In these instances, the raw diagnostic laboratory reports for the submissions were reviewed to determine the isolate of interest.

Due to free-text entry of veterinary clinic name, and farm/owner name data fields, a considerable amount of cleaning was required to ensure that all data were in the same format. For example, variations of veterinary clinic name could appear due to spelling or abridgement inconsistencies. Similarly, the recording of antimicrobial susceptibilities as upper- or lower- case text was variable from the VADDS LIMS, which disrupts analyses.
In order to address these issues, an automated protocol was developed to ensure that upper-case text was used for all susceptibility recordings. This approach was not sufficient for editing the clinic or farm/owner names however, as there is a potentially infinite number of combinations of recording inconsistencies in these fields. To address this issue, changes were recorded manually and added to an automatic protocol to correct these errors in subsequent runs; however, as isolates are entered into the LIMS and accessed for surveillance, unique recording inconsistencies could be added to the database at any time.

**Consistency of recording**

Following merging of all data into a common working database, they were assessed for consistency of recording and missing values. The recording consistency of each variable is displayed in Table 2.1 for all data (both LIMSs), and for the current LIMS alone. Data regarding the veterinary clinic was more consistently available in the new LIMS as compared to all data. The clinic identification number was recorded at a significantly higher rate (p < 0.001) in the new system than all data. However, the identification number was not consistent within a clinic itself; multiple identification numbers were associated with each clinic, both within and between LIMSs. Moreover, farm demographic and herd health information was recorded in a significantly lower proportion of data from the current system than in all the data (p < 0.001) (Table 2.1).

Completeness of recording (based upon the sample submission form) was further assessed by veterinary clinic to determine whether data from sentinel practices might be better suited for use in a surveillance system than all available data. Approximately 150
practices were represented within the dataset; however, a small number of practices accounted for a disproportionate amount of data within the system. Ten practices had > 150 observations (> 71% of the total), eight practices had > 200 observations (~65%), and four practices had > 400 observations (~45%). Recording consistency for the top four, eight, and ten submitting practices did not differ significantly from the recording consistency for all variables (p > 0.5; Table 2.2).

Consistency of susceptibility testing over time

Over the study time frame, there were 1323 E. coli F4, 2549 S. suis and 1464 P. multocida isolates with ≥1 antimicrobial susceptibility test result. Among the pathogens, the number of isolates with susceptibility results was quite variable over time (Figure 2.1); S. suis isolates had the highest number of susceptibility tests performed each year, while E. coli F4 and P. multocida varied in their relative ranking. From 2008 on, more susceptibility tests were performed on E. coli F4 isolates than P. multocida (Figure 2.1). Among all three pathogens, the monthly number of isolates tested stabilized during the 2008 – 2010 time period at approximately 8-10 isolates per pathogen per month.

Twenty-eight different antimicrobials were used for susceptibility testing over the study period. The numbers of isolates tested for susceptibility to each antimicrobial per year are displayed in Tables 2.3 – 2.5. Changes in the most commonly used susceptibility panel occurred with the introduction or withdrawal of drugs over time (Table 2.6).

The antimicrobials deemed suitable for surveillance in E. coli F4 were: ampicillin, apramycin, ceftiofur, gentamicin, kanamycin, spectinomycin, sulfisoxazole, tetracycline, and TMS (Table 2.3). The antimicrobials deemed suitable for surveillance in P.
**multocida** were: ampicillin, ceftiofur, florfenicol, penicillin G, spectinomycin, sulfisoxazole, tetracycline, tiamulin, TMS, and tulathromycin (Table 2.4). The antimicrobials deemed suitable for surveillance in *S. suis* were: ampicillin, ceftiofur, penicillin G, spectinomycin, sulfisoxazole, tetracycline, tiamulin, and TMS (Table 2.5).

**DISCUSSION**

Antimicrobial resistance data for selected swine pathogens isolated at the AHL at the University of Guelph has potential for use in a prospective surveillance system. It is believed that the AHL handles a larger portion of the clinical submissions from Ontario livestock than other laboratories, as a result of the partnership of the University of Guelph with the Ontario Ministry of Agriculture and Food and Ministry of Rural Affairs (OMAF & MRA). Therefore, data from the AHL are expected to be fairly representative of those requested by veterinarians serving the Ontario livestock industry as a whole. However, it should be noted that swine submissions received at the AHL have been shown to be affected by factors other than disease incidence, including the presence of disease outbreaks, as well as economic factors including the value of the Canadian dollar, and auction prices (16-17). Although the data present some limitations, such as missing covariate data and some recording inconsistency, that limit analyses to temporal options, the authors believe that knowledge of the prevalence of resistance within these isolates may be informative to veterinary practitioners facing decisions for treatment of clinically ill animals.

The availability of representative data, the high training and expertise of AHL personnel and ease of data acquisition are factors that make a passive surveillance system
for antimicrobial resistance in Ontario livestock using AHL data an attractive option. Through automated protocols for data analysis and online reporting of results, an efficient system could be developed to provide useful information for veterinary practitioners in the province. Semi-automated protocols have been generated for *S. suis* and *P. multocida* isolates, and could be implemented into a surveillance program with current AHL protocols. Manual evaluation of the *E. coli* diagnostic reports was an extensive task, however. It was confirmed with laboratory personnel that susceptibility reports for *E. coli* are primarily made for the F4 positive isolates, unless otherwise requested by the referring veterinarian. Therefore, with a small decrease in the specificity of F4 results, F4 *E. coli* data are also suitable for semi-automated AMR surveillance using the AHL data. However, isolate specific identifier data are suggested for future LIMS development.

Missing data were commonly encountered, reflecting incomplete form submission by the submitting veterinarian. Missing farm location limits the application of spatial statistical methods to the level of the veterinary clinic. Veterinary clinic level data are not the ideal resolution for swine data in Ontario, as most swine herds are served by a limited number of veterinarians who cover large territories. Amezcua et al. (18) found that seven Ontario practitioners visited 23.6% of Ontario swine herds in 2006, and that these farms were located in four of the five Ontario agricultural regions (all but Northern Ontario, which was estimated to represent only 0.5% of all herds in 2006). For this reason, a clinic may serve herds separated by large distances (>100 kilometres), which makes clinic location an epidemiologically unimportant and potentially spurious predictor variable in a statistical model. Therefore, in the current state of data management and collection, temporal analyses are more appropriate for these AMR data than spatial or temporal-
spatial analyses. For example, cluster detection methods may be used to indicate periods in time when the proportion of resistance is statistically higher (or lower) than expected, while logistic regression models with year and season as predictor variables or the application of time-series modeling may allow for the visualization of statistically significant trends. The consistency of recording of herd health information (herd size, number sick, number dead) was also found to be lacking, limiting the use of these factors as predictors or offset values within statistical models. Missing information for these potential covariates (e.g., production system or age of animal) may pose an issue when considering the confounding effects of uncontrolled variables (19), and changes in background populations may result in biased p-values if an incorrect assumption of stable background populations is made (20). As infectious agents and their resistance profiles may be particularly influenced by demographic factors such as animal age/stage of production, the statistical models produced with these data may be limited in their predictive abilities without these parameters. The availability of these predictors would allow surveillance personnel to determine whether an increase in resistance is true in the general swine population, or if it reflects an increase in submissions from (or growth within) a given level of the production process compared to other production levels.

Stage of production information should be added to the submission form and veterinarians and producers submitting samples should be encouraged to be diligent in providing complete information in order to develop a complete database and support the use of AHL data for surveillance purposes. Emphasis must be placed upon completeness of recording of data on intake forms, which may include continuing education for submitting veterinarians/owners about the importance of surveillance data and how they
may benefit from these results. Alternatively, as AHL livestock and poultry testing are subsidized, it may be possible to make the preferential food animal pricing contingent on completion of submission forms with the required demographic data. Furthermore, simply informing practitioners of the data requirements may influence response as a form of stewardship to the industry (21), that is, practitioners accessing data may be more likely to submit samples with the knowledge that results may be improved with more samples.

Observations from high-submitting practices were evaluated in comparison to the average in regards to recording consistency, as these practices could potentially be used as sentinels for the province. Although no significant differences in completeness of records were found for these most frequently submitting practices as compared to all practices, previous research has shown that Ontario swine clinics were willing to participate/comply with pilot testing of surveillance initiatives (18). Therefore, consideration should be given to requesting that these clinics provide all submission details on a prospective basis, providing an avenue for increasing data quality and consistency. With high compliance to this request, the consistency of the demographic data might be improved for sentinel practices as compared to all Ontario practitioners. Alternatively, future work could assess whether resistance data from the top submitting practices differs significantly from all Ontario data. If resistance patterns from potential sentinel clinics are representative of all resistance patterns, sentinel clinic data may provide an option for temporal AMR surveillance.

Another common occurrence that may hinder data analysis in a semi-automated surveillance system is recording inconsistency. In many instances, a single clinic or farm
name was recorded in multiple formats within the data acquired. Thus, one clinic might be treated as multiple clinics in a statistical model, purely due to formatting problems (either at data entry when specimens arrive at the lab or by the individuals submitting specimens). A standardized recording method should be used for clinic and farm names such as a unique identification code or by use of a drop-down menu approach in place of current free-text fields. Although the unique identification code field has a high compliance for recording, it currently does not fulfill the requirements for use in a surveillance system because each clinic has multiple “unique” codes recorded. It may be suitable to develop a system such that clinics are registered into the LIMS database using a standardized format, which would provide an opportunity to check new registrations against those that already exist within the data.

Although the availability of susceptibility results for antimicrobial/pathogen combinations limits the choice of drugs to include on the panel, the choice should be based on defined criteria that include use in therapy of swine diseases and the importance for human medicine. The AHL data fulfills these criteria without adaptation. As the AHL aims to provide susceptibility information that is practical to the practitioner, susceptibility is tested to antimicrobials used in swine to treat the organism in question. The most commonly employed panel of antimicrobials for each of the pathogens assessed in this study match with the Canadian Veterinary Medical Association’s (CVMA) antimicrobial prudent use guidelines (22), and include antimicrobials in each category of the Veterinary Drug Directorate (VDD) Categorization of Antimicrobial Drugs Based on Importance in Human Medicine (23). Accordingly, these antimicrobials are also those used in Canadian swine production (24).
Additions or removal of antimicrobials from the commonly tested panels, and changes in interpretive criteria present a challenge for an ongoing surveillance system. Trends in resistance (including multidrug and multiclass resistance) cannot be reliably tracked for a pathogen/antimicrobial combination if susceptibility testing for the given antimicrobial changes over time. When these changes occur, documentation must be provided to stakeholders, along with the impact of the change upon interpretation of the data. An example of suitable documentation is provided online by CIPARS (25). During the study period, different panels were used over time for each of the pathogens. If possible, a standard minimum panel for each pathogen should be implemented such that a set of antimicrobials is available for which resistance rates could be followed over time. Changes to these panels may be appropriate in situations where new drugs are made available for agricultural use, or changes to antimicrobial labels including times when *E. coli*, *P. multocida*, or *S. suis* are added as targeted pathogens. However, removal of drugs from agricultural use may not warrant the removal of these drugs from the panel used for surveillance. Depending on the circumstances and the potential for reinstatement over time, such changes would require discussion at regular meetings between AHL staff and surveillance personnel. Similarly, changes in breakpoints for resistance that affect R/I/S interpretive criteria may be conveyed at these meetings. This highlights a key conflict between the uses of laboratory data; within a laboratory, the focus is on identifying the susceptibility results for the individual case/veterinarian/producer. Therefore, testing susceptibility of an antimicrobial no longer used in practice is illogical, and logical changes in breakpoints may occur over time as pathogens evolve. However, to individuals conducting surveillance, continued monitoring of a product that has been
removed from the market provides key information, particularly about the rate at which AMR prevalence declines or persists upon the removal of a particular antimicrobial drug or class. Furthermore, surveillance personnel need to have breakpoint changes documented, as these changes affect the comparability of data.

It became apparent through this work that the particular data management system used at the AHL requires significant technological expertise to retrieve data in a suitable format for surveillance. The system performs well when generating individual reports for farm owners or practitioners; however, retrieval of large datasets is difficult. The current system requires multiple searches and extraction/merging steps to develop a database that lists submission demographic information and results in formatting with one row per isolate. A system or subroutine that allows extraction/export in this format, in a single file, would increase efficiency with regards to speed, number of files saved, and possible points of error.

With knowledge of the current trends in resistance and predictors for specific animal pathogens, veterinarians may be able to make more informed decisions regarding their use of antimicrobials and its potential to increase selection pressure for AMR. Furthermore, knowledge of the current trends in AMR prevalence may allow for routine treatment options to be assessed in order to reduce the occurrence of treatment failure, to guide the development of provincial regulations and empirical treatment guidelines for antimicrobial use in livestock, and to examine the impacts of interventions to alter antimicrobial use in the province. Moreover, two of the three pathogens assessed in this paper (P. multocida and S. suis) displayed an increase in isolation from 2004 – 2006, concurrent with an increase in submissions to the AHL and the Ontario Porcine
Circovirus type 2 outbreak (16). These results suggest that a system for AMR surveillance may also allow for a form of syndromic surveillance for emerging diseases whereby increased submissions to the AHL, requests for antimicrobial susceptibility testing, or isolation of other pathogens affecting the anatomical system of interest may indicate times when a novel disease is circulating in the source population.

Therefore, the development of an AMR surveillance system for clinical isolates from Ontario swine would be an asset for local veterinarians and researchers. This surveillance system could be used to promote the health of swine herds in Ontario, to improve and monitor antimicrobial stewardship efforts in the province, and potentially identify unique or novel infections. Current data from the AHL will support a system using temporal statistical techniques, and with the adoption of improved recording practices, adjustment for covariates and spatial or temporal-spatial analyses may be supported in the future.

ACKNOWLEDGEMENTS

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Table 2.1: Recording consistency (percentage of submissions with values recorded) for data fields from clinical swine submissions to the Animal Health Laboratory, displayed for all data (January 1998 – October 2010) and data from the current LIMS (May 2007 – October 2010).

<table>
<thead>
<tr>
<th>Data field</th>
<th>Escherichia coli F4</th>
<th>Streptococcus suis</th>
<th>Pasteurella multocida</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All data</td>
<td>Current LIMS</td>
<td>All data</td>
</tr>
<tr>
<td>Submission number</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
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<td>Submission date</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Clinic name</td>
<td>100</td>
<td>100</td>
<td>99.96</td>
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<tr>
<td>Clinic postal code</td>
<td>100</td>
<td>100</td>
<td>15.81</td>
</tr>
<tr>
<td>Clinic number</td>
<td>77.40</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
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<td>Case type</td>
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<td>100</td>
<td>98.94</td>
</tr>
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<td>Breed</td>
<td>78.91</td>
<td>69.96</td>
<td>79.95</td>
</tr>
<tr>
<td>Owner or farm name</td>
<td>15.95</td>
<td>80.23&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Owner unique id</td>
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<td>2.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.71</td>
</tr>
<tr>
<td>Owner postal code</td>
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<tr>
<td>Number at risk</td>
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<td>37.71&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Number sick</td>
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<td>26.27&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Number dead</td>
<td>44.62</td>
<td>26.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.52</td>
</tr>
</tbody>
</table>

<sup>a</sup>Recording consistency significantly higher from the new system than all data, p < 0.001 by Z-test

<sup>b</sup>Recording consistency significantly higher in all data as compared to new system data, p < 0.001 by Z-test
Table 2.2: Percent of isolates, maximum distance between practices, and demographic variable recording consistency for the highest 4, 8, and 10 submitting practices within swine antimicrobial susceptibility testing data from the Animal Health Laboratory (January 1998 – October 2010) compared to the overall data.

<table>
<thead>
<tr>
<th></th>
<th>Top 4</th>
<th>Top 8</th>
<th>Top 10</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of isolates</td>
<td>44.94</td>
<td>65.01</td>
<td>71.38</td>
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<td>Approximate maximum</td>
<td>57.5km</td>
<td>196.0km</td>
<td>196.0km</td>
<td>675.0km</td>
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<td>distance between practices</td>
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<td></td>
</tr>
<tr>
<td>Recording consistency (%)(a):</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owner or farm name</td>
<td>12.80</td>
<td>13.75</td>
<td>12.94</td>
<td>12.99</td>
</tr>
<tr>
<td>Unique identification number</td>
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<td>55.90</td>
<td>57.71</td>
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</tr>
<tr>
<td>Number sick</td>
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<td>33.71</td>
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</tr>
<tr>
<td>Number dead</td>
<td>37.53</td>
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</tr>
<tr>
<td>Number at risk</td>
<td>52.21</td>
<td>52.26</td>
<td>59.57</td>
<td>51.12</td>
</tr>
</tbody>
</table>

\(a\)No significant differences in the recording consistency between the top 4, 8, or 10 submitting practices and all data.
Table 2.3: Number of susceptibility tests performed on *Escherichia coli* F4 isolates at the Animal Health Laboratory by year (January 1998 – October 2010).

<table>
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</thead>
<tbody>
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<td>Ampicillin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27</td>
<td>91</td>
<td>207</td>
<td>176</td>
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<td>79</td>
<td>60</td>
<td>64</td>
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<td>80</td>
<td>60</td>
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<td>-</td>
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<td>79</td>
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<td>2</td>
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<td>3</td>
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<td>207</td>
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<td>64</td>
<td>72</td>
<td>80</td>
<td>60</td>
</tr>
</tbody>
</table>

<sup>a</sup>Chosen for inclusion in a potential AMR surveillance system

<sup>b</sup>Trimethoprim-sulfamethoxazole
Table 2.4: Number of susceptibility tests performed on *Pasteurella multocida* isolates at the Animal Health Laboratory by year (January 1998 – October 2010).

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</table>

<sup>a</sup>Chosen for inclusion in a potential AMR surveillance system

<sup>b</sup>Trimethoprim-sulfamethoxazole
Table 2.5: Number of susceptibility tests performed on *Streptococcus suis* isolates at the Animal Health Laboratory by year (January 1998 – October 2010).

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*a* Chosen for inclusion in a potential AMR surveillance system  
*b* Trimethoprim-sulfamethoxazole
Table 2.6: Most commonly tested antimicrobial susceptibility panels for *Escherichia coli* F4, *Streptococcus suis* and *Pasteurella multocida*, 1998 – 2010.

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<th>Most commonly tested susceptibility panel for <em>Escherichia coli</em> F4</th>
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<td>AMP + CEF + GEN + KAN + SPE + SUL + TET + TMS</td>
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<td>2007 - 2010</td>
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</table>

<table>
<thead>
<tr>
<th>Years</th>
<th>Most commonly tested susceptibility panel for <em>Pasteurella multocida</em></th>
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<table>
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<tr>
<th>Years</th>
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<tr>
<td>2000</td>
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<td>2002 – 2003</td>
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<td>AMP + CEF + GEN + NEO + PEN + SPE + SUL + TET + TIA + TIL + TMS</td>
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<td>2007 - 2010</td>
<td>AMP + CEF + PEN + SPE + SUL + TET + TIA + TMS</td>
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AMK = amikacin, AMP = ampicillin, APR = apramycin, CEF = ceftiofur, CEP = cephalothin, CLI = clindamycin, FLO = florfenicol, GEN = gentamicin, KAN = kanamycin, NEO = neomycin, OXA = oxacillin, PEN = penicillin G, SPE = spectinomycin, SUL = sulfisoxazole, TET = tetracycline, TIA = tiamulin, TMS = trimethoprim & sulfamethoxazole, TUL = tulathromycin
Figure 2.1: Smoothed\(^a\) number of isolates per month that underwent ≥1 antimicrobial susceptibility test at the Animal Health Laboratory from January 1998 – October 2010.

\(^a\)Smoothing was performed by using the current observation, 6 lagged, and 5 forward observations.
CHAPTER THREE

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN CLINICAL SWINE

PASTEREURILLA MULTOCIDA AND STREPTOCOCCUS SUIS ISOLATES

FROM ONTARIO

Accepted for publication in the Canadian Journal of Veterinary Research on September 9th, 2013.
ABSTRACT

Susceptibility results for *Pasteurella multocida* and *Streptococcus suis* isolated from swine clinical samples were obtained from January 1998 – October 2010 from the Animal Health Laboratory at the University of Guelph. These data were used to describe variation in antimicrobial resistance (AMR) to four drugs of importance within the Ontario swine industry: ampicillin, tetracycline, tiamulin, and trimethoprim-sulfamethoxazole. Four temporal data analysis options were employed: visualization of trends using 12-month rolling averages, temporal scan statistics, logistic regression modeling, and the “What’s Strange About Recent Events” (WSARE) algorithm. AMR trends varied among antimicrobial drugs within a pathogen and between pathogens for a single antimicrobial, suggesting that pathogen-specific AMR surveillance may be preferable to indicator data. The four methods provided complementary and, at times, redundant results. The most appropriate combination of analysis methods for surveillance using these data included temporal scan statistics with a visualization method (rolling average or predicted probability plots following logistic regression models). The WSARE algorithm provided interesting results for quality control purposes and has the potential for the detection of new resistance patterns; however, missing data created problems for displaying these results in a way that would be meaningful to all surveillance stakeholders.
INTRODUCTION

*Pasteurella multocida* and *Streptococcus suis* are clinical pathogens of swine that place considerable strain upon the industry. *Pasteurella multocida* is the necessary cause of progressive atrophic rhinitis (PAR) and pneumonic pasteurellosis (1). PAR is a disease of economic importance, often resulting in reduced feed utilization and growth retardation (1). Similarly, pneumonic pasteurellosis has been reported to significantly reduce growth rates and increase time to reach market weight (1). *Streptococcus suis* infection results in high mortality rates characterized by meningitis, endocarditis, pneumonia, arthritis, and septicaemia (1). In addition to these health implications for infected animals, high mortality rates result in significant economic losses for producers. Therefore, effective treatment of both of these pathogens is of interest to swine practitioners and producers.

Antimicrobials are often used for treating clinical infections with *P. multocida* and *S. suis* in swine. However, antimicrobial resistance (AMR) has been reported in both of these pathogens, threatening treatment options (2-10). The Canadian Veterinary Medical Association (CVMA) has produced guidelines for prudent antimicrobial use in livestock, including the treatment of these two pathogens in swine (11), in order to limit the further development of antimicrobial resistance. These guidelines provide an important reference for appropriate antimicrobial options. However, reported resistance rates within these pathogens have varied by jurisdiction. For example, trimethoprim-sulfamethoxazole resistance rates in *P. multocida* were reported from a low of 3% in Hungary (2005-2008) (9), to a high of 74.2% in China (2003-2007) (10). Therefore, a single reference for treatment options across Canada may not be suitable for practitioners.
faced with treating clinically ill animals and potential treatment failures due to resistance. Accessible data concerning the local prevalence of resistance in clinical pathogens may provide more informative resources for practitioners and policy-makers.

Currently, there are no reports in the literature about resistance rates of *P. multocida* and *S. suis* in Ontario, or current surveillance efforts to characterize trends in resistance rates at the provincial level. Previous work has assessed the quality and quantity of clinical isolate AMR data from the Animal Health Laboratory at the University of Guelph to develop a *de novo* surveillance system for Ontario swine (12). The objective of this research is to use those data to assess variation in resistance within *P. multocida* and *S. suis* isolates to four antimicrobials of importance within the Ontario swine industry: ampicillin, tetracycline, tiamulin, and trimethoprim-sulfamethoxazole (TMS). Data analyses suitable for temporal analyses include visualization of trends using 12-month rolling average, temporal scan statistics (13-14), logistic regression models, and the “What’s Strange About Recent Events” algorithm; a biosurveillance program for pattern abnormality detection (15-17). In addition to the description of trends, models and significant clusters/rules for resistance, we considered the usefulness and complementary results from these analyses in the context of surveillance system development. These results are important for understanding how AMR in these pathogens changes over time, for highlighting significant trends and clusters in resistance and to assess whether these results could be potentially important for informing practitioners and surveillance stakeholders.
MATERIALS AND METHODS

Susceptibility results for *P. multocida* and *S. suis* isolated from swine clinical samples were obtained from January 1998 – October 2010 from the Animal Health Laboratory (AHL) at the University of Guelph. All bacterial cultures and bacterial identifications were performed according to the standard operating procedures of the AHL. Susceptibility testing was performed following CLSI guidelines. Isolates were considered to have some form of resistance if susceptibility testing results reported intermediate or resistant classifications. These classifications were combined and termed “resistant” for ease of analysis and comparison.

Trends in the monthly resistance rates (i.e., resistant classification/all susceptibility tests for that antimicrobial) were calculated and visualized using 12-month rolling averages with 6 prior months, the current month and 5 forward months using Stata/MP 11.2 (StataCorp LP, 2009, College Station, TX). Logistic regression models were built using Stata/MP 11.2 for antimicrobials showing variation within the rolling average plots. Year and season were assessed as predictors within these models. Year was modeled as a categorical variable due to non-linearity with the log odds of resistance to the antimicrobial. Similarly, season was assessed as a categorical variable, with the months March – May describing ‘spring’, June – August described ‘summer’, September – November described ‘fall’, and December – February described ‘winter’. Goodness of fit of the logistic models was assessed using the Pearson chi-square goodness of fit test. Standardized residuals, influence by delta-beta and leverage were visualized using scatter plots and any covariate patterns showing anomalous values were recorded. Models were re-run with the exclusion of these noted covariate patterns to assess any dramatic changes
in the coefficients. Predicted values with their 95% confidence limits were calculated and graphs were produced using Microsoft Excel (2010).

Detection of the most likely temporal clusters (of high or low resistance rates) was performed in SatScan™ v9.1.1 (13). Retrospective purely temporal Bernoulli models were run with 9999 replications and a maximum cluster size equal to 50% of the study period. In order to detect secondary clusters, an iterative function was employed. The iterative function detects the most likely cluster on the first iteration, then removes all of the case and control included in the most likely cluster and re-scans the data in order to find the next most likely cluster within the remaining data (14). Scans continue until the most likely cluster found is not significant (p-value > 0.05) or if the maximum number of iterations are met (9999). Scans were employed for each pathogen/antimicrobial combination.

Finally, the WSARE version 3.1 algorithm was applied in order to detect anomalous patterns within the susceptibility and pathogen isolation data (15-17). All *P. multocida* and *S. suis* AMR results were presented to WSARE (separately) to detect anomalous patterns within the data. For each month over the time frame, patterns in resistance, susceptibility, or non-testing were compared to baseline data for that event (determined from past data). Data supplied to WSARE were limited to the 2004 – 2010. This was done to avoid significant rules for missing tiamulin results for the 1998 – 2004 time period, as testing for tiamulin began in 2004 and the WSARE algorithm recognizes when non-testing occurs at a higher rate than expected.
RESULTS

Susceptibility test results were acquired for 1464 and 2549 isolates of *P. multocida* and *S. suis*, respectively. The majority of these isolates were tested for susceptibility to ampicillin, tetracycline and TMS (Table 3.1). The majority of isolates from 2004 – 2010 were also tested for tiamulin susceptibility (Table 3.1).

Rolling average plots

Trends in the proportion of resistant isolates differed by pathogen and antimicrobial (Figure 3.1). Resistance to ampicillin remained low in both pathogens from 1998 to 2007; however, a dramatic increase in resistance occurred in the 2007 – 2010 period in *P. multocida* (Figure 3.1a). Patterns in resistance to tetracycline varied dramatically between the two pathogens examined; a high rate of resistance was displayed in *S. suis* throughout the time frame, while resistance in *P. multocida* varied from 10-40% from 1998 to 2010 (Figure 3.1b). Tiamulin resistance patterns followed roughly similar trends between the pathogens, though with much more dramatic changes occurring within the *P. multocida* data; a decline in resistance was visible from 2004 to 2008, followed by an increase 2008 to 2010 (Figure 3.1c). In contrast, resistance to TMS was not similar between the two pathogens; the highest rates of resistance within *S. suis* isolates occurred between 2000 and 2007, concurrent with the lowest rates of resistance within *P. multocida* isolates, which increased as *S. suis* resistance declined 2007 - 2008 (Figure 3.1d).
Logistic regression models

For the \textit{P. multocida} data, year and season were found to be significant predictors for ampicillin and TMS resistance (Table 3.2). Peaks in resistance to both of these antimicrobials were seen during the summer months (Figure 3.2a). While no linear secular trend was seen for TMS resistance, a pattern of increasing resistance from 2007 to 2010 was apparent from the model predictions (Figure 3.2a). Year was a significant predictor for resistance to tetracycline and tiamulin (Table 3.2). No obvious trend in tetracycline resistance was apparent from model predictions; however, a notable decrease in tiamulin resistance occurred in 2007 (Figure 3.2b).

For the \textit{S. suis} data, year and season were found to be significant predictors for tetracycline resistance (Table 3.3). Peaks in tetracycline resistance occurred during the summer months (Figure 3.3a). Although resistance rates were consistently high, a peak in 2004 was followed by a dramatic drop in the spring of 2005 (Figure 3.3a). Year was a significant predictor for the ampicillin and TMS models (Table 3.3). Resistance to ampicillin peaked in 2009 with the odds of resistance in 2009 being 12.10 times higher than in 2002 (\(p < 0.001\)) (Table 3.3; Figure 3.3b). Year (\(p = 0.521\)) and season (\(p = 0.264\)) were not significant predictors for the tiamulin data.

Temporal scan statistics

Within the \textit{P. multocida} data, significant clusters of high resistance were found for ampicillin and tetracycline, while significant clusters of low resistance (high susceptibility) were found for ampicillin, tiamulin and TMS (Table 3.4). A cluster of resistance to ampicillin was found from May 2007 to June 2010 and a significant cluster
of susceptibility was detected from March 1999 to April 2002. Tetracycline resistance clusters were found for July 2000 through May 2005 and from August to September 2010 (Table 3.4). A significant cluster of susceptibility to tiamulin was detected from February 2007 - May 2008 (Table 3.4). Similarly, clusters of TMS susceptibility were detected August 1998 to May 2000 and July 2002 to February 2005.

Within the S. suis data, significant clusters of high resistance were found for ampicillin, tetracycline and TMS. A cluster of ampicillin resistance was found from July 2001 to October 2007, followed by a cluster of susceptibility July to September 2009 (Table 3.4). A long cluster of tetracycline resistance was detected May 2001 to March 2005. Similarly, a long cluster of TMS resistance was found October 2000 to March 2007, which was bookended by clusters of susceptibility (May 1998 to April 2000 and January to November 2008) (Table 3.4).

**WSARE**

Significant rules for P. multocida data from the WSARE algorithm involved tiamulin and TMS resistance (Table 3.5). High resistance to tiamulin was found as a significant rule March 2005 and high resistance to TMS was a significant rule in November 2005 (Table 3.5). Significant rules for S. suis data from the WSARE algorithm mainly reflected the results for tiamulin (Table 3.5). In February and April 2004, the observed ratio of missing tiamulin results was lower than expected; that is, testing was higher than expected, likely due to low testing in January 2004. In March of 2004, susceptibility to tiamulin was higher than expected from the data. Similarly, susceptibility to tetracycline was higher than expected in April 2005 and dual susceptibility to tiamulin
and TMS was higher than expected May 2005 (Table 3.5). In March 2006, tiamulin susceptibility was lower than expected and in May 2006, testing for tiamulin lower than expected (Table 3.5).

DISCUSSION

Antimicrobial resistance among clinical P. multocida and S. suis from Ontario swine displayed varying trends from 1998 – 2010. Trends differed among antimicrobial drugs within a pathogen and between pathogens for a single antimicrobial; this could be the result of a number of factors. Practitioners and producers typically have standard treatment routines that they follow for given disease presentations; these routines may differ from those of other practitioners or producers. Therefore, selection pressures for resistance may vary at the level of the production system, or veterinary practitioner. Although it is known that some veterinary clinics submit samples to the AHL at a higher rate than others, practitioner or producer-level data were not available for analysis (12). Selection pressures, and therefore AMR, may also vary based on the production level of the animals from which clinical samples are submitted, as the prevalence of various diseases varies by production level (1). The production level and age of animals were not currently available in the AHL data. However, avenues for improving data acquisition at the AHL have been suggested by our previous work (12) and therefore, there may be an opportunity for research on sources of AMR variation using the AHL data in the future. Pathogen-level changes may also produce changes in resistance patterns over time. For example, MacInnes et al. (2008) reported that the prevalence of toxigenic strains of P. multocida has declined in Ontario relative to non-toxigenic strains (18). If toxigenic and
non-toxigenic strains have different resistance profiles, a shift in population dynamics would result in changes to the proportion of resistant isolates over time. As no differentiation is made between toxigenic and non-toxigenic strains of *P. multocida* within the AHL data analysed here, the effect of this shift could not be assessed. However, this presents an avenue for future work, possibly with a focus from a resistance perspective on the non-toxigenic strain of *P. multocida*.

Four methods suitable for temporal AMR data analysis were employed here, with outputs providing some redundant and some complementary information. Twelve-month rolling average plots of proportion resistant are an attractive option for use in an AMR surveillance program due to their simplicity. Trends in resistance over time within a pathogen are easily visualized with these plots and plots can be generated using automated sub-routines. Furthermore, these plots provide a quick method for visualizing similarities and differences in resistance between pathogens. However, small monthly values for the denominator can create large fluctuations in the proportion of resistance. Large fluctuations can be difficult to interpret and therefore the implications of an increase or decrease in the proportion of resistance may be challenging to assess. The effect of a short period with low denominator values may be dampened by the smoothing function; however, over longer periods, unreliable plots may result. Similarly, low isolation numbers affect the user’s ability to visually assess differences in resistance to various antimicrobials within a pathogen, as well as differences in resistance to single antimicrobials among pathogens. Methods that provide a clear-cut means of assessing changes in resistance can provide complementary information (i.e., methods that produce a p-value for statistical significance).
Temporal scan statistics provide this clear means of assessing changes in the proportion of resistant isolates over time. Clusters of resistance and susceptibility were found that matched peaks in resistance and susceptibility displayed in the rolling-average plots, thereby providing p-values for assessing the significance of these changes over time. These methods may be accessed by automated sub-routines developed in statistical packages and are therefore suitable for surveillance system development.

Logistic regression models using year and season as predictors for resistance similarly provided the statistical significance of changes over time. Predicted probability plots from the logistic regression models were comparable to the rolling average graphs, albeit with dramatically less noise, and allowed for visual assessment of significant changes over time. With the limited covariate information available at this time, automated sub-routines for model-building within statistical packages may be suitable for surveillance system development. However, as covariate data collection is improved, manual model-building is preferable, as epidemiological concepts such as confounding may not be adequately assessed in an automated fashion. As the cluster detection and logistic regression models provided similar results in terms of statistical significance, and predicted probability plots provided analogous results to the rolling averages, the use of all three of these methods in combination may be superfluous for the needs of an AMR surveillance system.

The WSARE algorithm provided a means of assessing anomalous patterns in the susceptibility data. In the data assessed here, one significant rule described a month where concurrent susceptibility to two antimicrobials was detected more often than expected. Although concurrent susceptibility may not be an extraordinary result,
significant rules for the detection of new concurrent resistance patterns would provide practical information for all AMR stakeholders. However, missing results for individual antimicrobials was found to produce significant rules, limiting the use of this software to times when all antimicrobials were tested on the majority of isolates. Although knowledge of months when data are missing may be informative for quality control purposes, the suitability of presenting these results to practitioners is questionable.

The AMR in the clinical pathogens analyzed here highlights the necessity for including clinical veterinary pathogens in AMR surveillance. Although there are currently a number of AMR surveillance programs operating that involve swine, the main focus of these programs is on human enteric pathogens and foodborne commensal bacteria with potential exposure sources (19-24). Data regarding AMR for generic *Escherichia coli*, *Campylobacter* spp. and *Salmonella* spp. are available through these programs; however, AMR results data for target pathogens of animals are rare. As the clinical isolates assessed here displayed widely varying patterns of resistance, bacteria such as *E. coli*, *Campylobacter* and *Salmonella* may not be appropriate indicators of AMR in clinical swine isolates. Therefore, practitioners currently do not have access to AMR data suitable for informing their decisions among antimicrobials to treat clinically ill animals. Furthermore, the availability of AMR data from clinical isolates of swine would allow tracking of changes in resistance following interventions to antimicrobial use. As recommendations have been made for a change to prescription-only use of antimicrobials for disease treatment in animals (25), it will be important to assess the impact of these changes.
Finally, the potential impact on human health cannot be ignored in regards to AMR in livestock production. Given that sick animals are presumably exposed to more antimicrobials than well animals, clinical pathogens of these livestock are likely to display resistance earlier than the indicator commensals. Resistance genes can be potentially shared among clinical pathogens and commensal bacteria that can cause human illness. Therefore, surveillance of AMR in veterinary pathogens may provide an early warning system for resistances that may arise in human pathogens.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to Dr. Jane Parmley, Dr. David Léger and Dr. Agnes Agunos for their contributions to the conceptual framework for this project and for their guidance in the preparation of this manuscript.
REFERENCES


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Statens Serum Institut, Danish Veterinary and Food Administration, Danish Medicines Agency, and Danish Institute for Food and Veterinary Research, 2012.


Table 3.1: Number of susceptibility tests and proportion of resistant results for *Pasteurella multocida* and *Streptococcus suis* 1998–2010, by antimicrobial.

<table>
<thead>
<tr>
<th>Year</th>
<th><strong>Pasteurella multocida</strong></th>
<th></th>
<th></th>
<th><strong>Streptococcus suis</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP (%)</td>
<td>TET (%)</td>
<td>TIA (%)</td>
<td>TMS (%)</td>
<td>AMP (%)</td>
<td>TET (%)</td>
</tr>
<tr>
<td>1998</td>
<td>174 (8.1)</td>
<td>174 (5.7)</td>
<td>.</td>
<td>172 (5.8)</td>
<td>246 (4.8)</td>
<td>241 (89.6)</td>
</tr>
<tr>
<td>1999</td>
<td>183 (1.6)</td>
<td>183 (10.9)</td>
<td>.</td>
<td>182 (0.5)</td>
<td>226 (3.1)</td>
<td>225 (89.8)</td>
</tr>
<tr>
<td>2000</td>
<td>134 (0.8)</td>
<td>134 (22.4)</td>
<td>.</td>
<td>134 (9.7)</td>
<td>194 (3.6)</td>
<td>195 (90.8)</td>
</tr>
<tr>
<td>2001</td>
<td>122 (0)</td>
<td>122 (20.5)</td>
<td>.</td>
<td>122 (5.7)</td>
<td>204 (4.4)</td>
<td>203 (94.6)</td>
</tr>
<tr>
<td>2002</td>
<td>102 (2.0)</td>
<td>102 (21.6)</td>
<td>.</td>
<td>102 (9.8)</td>
<td>249 (0.8)</td>
<td>249 (96.4)</td>
</tr>
<tr>
<td>2003</td>
<td>89 (4.5)</td>
<td>89 (22.5)</td>
<td>.</td>
<td>88 (4.5)</td>
<td>194 (2.6)</td>
<td>194 (97.4)</td>
</tr>
<tr>
<td>2004</td>
<td>99 (4.0)</td>
<td>99 (13.1)</td>
<td>82 (34.1)</td>
<td>99 (5.0)</td>
<td>216 (1.9)</td>
<td>216 (97.2)</td>
</tr>
<tr>
<td>2005</td>
<td>205 (2.0)</td>
<td>205 (16.6)</td>
<td>205 (30.7)</td>
<td>204 (11.8)</td>
<td>319 (0.9)</td>
<td>319 (90.3)</td>
</tr>
<tr>
<td>2006</td>
<td>126 (4.8)</td>
<td>126 (11.9)</td>
<td>68 (32.3)</td>
<td>126 (6.3)</td>
<td>219 (1.4)</td>
<td>219 (93.6)</td>
</tr>
<tr>
<td>2007</td>
<td>79 (19.0)</td>
<td>79 (15.2)</td>
<td>77 (11.7)</td>
<td>79 (19.0)</td>
<td>149 (1.3)</td>
<td>149 (90.6)</td>
</tr>
<tr>
<td>2008</td>
<td>48 (29.2)</td>
<td>49 (8.2)</td>
<td>49 (16.3)</td>
<td>49 (14.3)</td>
<td>119 (1.7)</td>
<td>118 (91.5)</td>
</tr>
<tr>
<td>2009</td>
<td>53 (22.6)</td>
<td>53 (17.0)</td>
<td>53 (28.3)</td>
<td>53 (1.9)</td>
<td>112 (8.9)</td>
<td>112 (92.9)</td>
</tr>
<tr>
<td>2010a</td>
<td>49 (36.7)</td>
<td>49 (20.4)</td>
<td>49 (14.3)</td>
<td>49 (26.5)</td>
<td>97 (3.1)</td>
<td>97 (93.8)</td>
</tr>
</tbody>
</table>

AMP = ampicillin, TET = tetracycline, TIA = tiamulin, TMS = trimethoprim-sulfamethoxazole

*Results obtained in 2010 were limited to January - October*
Table 3.2: Odds ratios and p-values from logistic regression models describing antimicrobial susceptibility results from *Pasteurella multocida* isolates.

<table>
<thead>
<tr>
<th>Year</th>
<th>Ampicillin OR</th>
<th>P-value</th>
<th>Tetracycline OR</th>
<th>P-value</th>
<th>Tiamulin OR</th>
<th>P-value</th>
<th>TMS OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>0.20</td>
<td>0.01</td>
<td>2.01</td>
<td>0.08</td>
<td>0.09</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>0.08</td>
<td>0.02</td>
<td>4.73</td>
<td>&lt;0.01</td>
<td>1.73</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>N/Aa</td>
<td>N/A</td>
<td>4.23</td>
<td>&lt;0.01</td>
<td>1.04</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>0.23</td>
<td>0.06</td>
<td>4.51</td>
<td>&lt;0.01</td>
<td>1.87</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>0.57</td>
<td>0.34</td>
<td>4.75</td>
<td>&lt;0.01</td>
<td>0.84</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>0.47</td>
<td>0.19</td>
<td>2.48</td>
<td>0.04</td>
<td>Ref</td>
<td>.</td>
<td>0.85</td>
<td>0.78</td>
</tr>
<tr>
<td>2005</td>
<td>0.23</td>
<td>0.01</td>
<td>3.26</td>
<td>&lt;0.01</td>
<td>0.86</td>
<td>0.58</td>
<td>2.22</td>
<td>0.04</td>
</tr>
<tr>
<td>2006</td>
<td>0.59</td>
<td>0.30</td>
<td>2.22</td>
<td>0.06</td>
<td>0.92</td>
<td>0.82</td>
<td>1.14</td>
<td>0.79</td>
</tr>
<tr>
<td>2007</td>
<td>2.78</td>
<td>0.01</td>
<td>2.94</td>
<td>0.02</td>
<td>0.26</td>
<td>&lt;0.01</td>
<td>3.95</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2008</td>
<td>5.39</td>
<td>&lt;0.01</td>
<td>1.46</td>
<td>0.54</td>
<td>0.38</td>
<td>0.03</td>
<td>3.04</td>
<td>0.04</td>
</tr>
<tr>
<td>2009</td>
<td>3.61</td>
<td>0.03</td>
<td>3.36</td>
<td>0.01</td>
<td>0.76</td>
<td>0.48</td>
<td>0.35</td>
<td>0.32</td>
</tr>
<tr>
<td>2010</td>
<td>7.43</td>
<td>&lt;0.01</td>
<td>4.21</td>
<td>&lt;0.01</td>
<td>0.32</td>
<td>0.02</td>
<td>6.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>Ref</td>
<td>.</td>
<td>NS</td>
<td>N/A</td>
<td>Ref</td>
<td>.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>2.36</td>
<td>0.01</td>
<td></td>
<td></td>
<td>1.99</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>1.93</td>
<td>0.05</td>
<td></td>
<td></td>
<td>1.81</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>1.31</td>
<td>0.40</td>
<td></td>
<td></td>
<td>0.95</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data for ampicillin model dropped for 2001 as all isolates from this year were susceptible
Table 3.3: Odds ratios and p-values from logistic regression models describing antimicrobial susceptibility results from *Streptococcus suis* isolates.

<table>
<thead>
<tr>
<th>Year</th>
<th>Ampicillin OR</th>
<th>P-value</th>
<th>Tetracycline OR</th>
<th>P-value</th>
<th>Tiamulin OR</th>
<th>P-value</th>
<th>TMS OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Ref 0.63</td>
<td>0.34</td>
<td>Ref 1.06</td>
<td>0.85</td>
<td>NS</td>
<td>N/A</td>
<td>Ref</td>
<td>0.87</td>
</tr>
<tr>
<td>1999</td>
<td>0.74</td>
<td>0.53</td>
<td>2.10 1.11</td>
<td>0.05</td>
<td>1.98</td>
<td>0.05</td>
<td>3.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2000</td>
<td>0.91</td>
<td>0.84</td>
<td>3.15 1.05</td>
<td>0.01</td>
<td>4.67</td>
<td>&lt;0.01</td>
<td>3.18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2001</td>
<td>0.37</td>
<td>0.09</td>
<td>3.14 1.14</td>
<td>&lt;0.01</td>
<td>3.12</td>
<td>&lt;0.01</td>
<td>3.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2002</td>
<td>0.16</td>
<td>0.02</td>
<td>3.15 1.05</td>
<td>&lt;0.01</td>
<td>4.67</td>
<td>&lt;0.01</td>
<td>3.18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2003</td>
<td>0.52</td>
<td>0.23</td>
<td>1.73 1.11</td>
<td>0.11</td>
<td>2.13</td>
<td>0.04</td>
<td>2.28</td>
<td>0.01</td>
</tr>
<tr>
<td>2004</td>
<td>0.34</td>
<td>0.16</td>
<td>1.25 1.18</td>
<td>0.58</td>
<td>2.04</td>
<td>0.07</td>
<td>2.13</td>
<td>0.04</td>
</tr>
<tr>
<td>2005</td>
<td>0.37</td>
<td>0.09</td>
<td>1.80 1.54</td>
<td>0.31</td>
<td>1.40</td>
<td>0.46</td>
<td>2.04</td>
<td>0.07</td>
</tr>
<tr>
<td>2006</td>
<td>0.63</td>
<td>0.48</td>
<td>1.80 1.54</td>
<td>0.31</td>
<td>1.40</td>
<td>0.46</td>
<td>2.04</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Season</th>
<th>Ampicillin OR</th>
<th>P-value</th>
<th>Tetracycline OR</th>
<th>P-value</th>
<th>Tiamulin OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Ref 1.94</td>
<td>&lt;0.01</td>
<td>Ref 1.45</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>1.34</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.4: Significant clusters of resistance/susceptibility within swine *Pasteurella multocida* and *Streptococcus suis* isolates obtained from the Animal Health Laboratory (January 1998 – October 2010).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Antimicrobial</th>
<th>Cluster time frame(s)a</th>
<th>Observed Cases</th>
<th>Expected Cases</th>
<th>Relative Risk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pasteurella multocida</em></td>
<td>Ampicillin</td>
<td>May 2007 – Jun 2010</td>
<td>59</td>
<td>13.13</td>
<td>9.92</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mar 1999 – Apr 2002</td>
<td>3</td>
<td>13.94</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Tetracycline</td>
<td>Jul 2000 – May 2005</td>
<td>126</td>
<td>87.37</td>
<td>2.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aug – Sep 2010</td>
<td>5</td>
<td>0.55</td>
<td>9.55</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Tiamulin</td>
<td>Feb 2007 – May 2008</td>
<td>8</td>
<td>26.07</td>
<td>0.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>TMS</td>
<td>Aug 1998 – May 2000</td>
<td>2</td>
<td>25.40</td>
<td>0.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jul 2002 – Feb 2005</td>
<td>10</td>
<td>25.53</td>
<td>0.33</td>
<td>0.04</td>
</tr>
<tr>
<td><em>Streptococcus suis</em></td>
<td>Ampicillin</td>
<td>Jul 2001 - Oct 2007</td>
<td>19</td>
<td>38.28</td>
<td>0.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jul – Sep 2009</td>
<td>6</td>
<td>0.93</td>
<td>7.23</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Tetracycline</td>
<td>May 2001 – Mar 2005</td>
<td>828</td>
<td>794.34</td>
<td>1.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>TMS</td>
<td>Oct 2000 – Mar 2007</td>
<td>242</td>
<td>185.88</td>
<td>2.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jan – Nov 2008</td>
<td>0</td>
<td>7.36</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May 1998 – Apr 2000</td>
<td>19</td>
<td>34.44</td>
<td>0.39</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Sorted by most likely cluster*
Table 3.5: Significant rules from WSARE scan of *Pasteurella multocida* and *Streptococcus suis* susceptibility tests obtained from the Animal Health Laboratory (January 1998 – October 2010).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Date</th>
<th>Observed ratio</th>
<th>Expected ratio</th>
<th>First antimicrobial</th>
<th>First value</th>
<th>Second antimicrobial</th>
<th>Second value</th>
<th>Score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pasteurella multocida</em></td>
<td>Mar-05</td>
<td>55</td>
<td>26.16</td>
<td>TIA</td>
<td>R</td>
<td></td>
<td></td>
<td>-0.00334</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Nov-05</td>
<td>29.17</td>
<td>7.35</td>
<td>TMS</td>
<td>R</td>
<td></td>
<td></td>
<td>-0.00134</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Streptococcus suis</em></td>
<td>Feb-04</td>
<td>39.13</td>
<td>91.25</td>
<td>TIA</td>
<td>Missing</td>
<td></td>
<td></td>
<td>-6.46E-10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Mar-04</td>
<td>87.50</td>
<td>24.45</td>
<td>TIA</td>
<td>S</td>
<td></td>
<td></td>
<td>-6.63E-13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Apr-04</td>
<td>0</td>
<td>43.19</td>
<td>TIA</td>
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<td></td>
<td></td>
<td>-4.32E-5</td>
<td>&lt;0.01</td>
</tr>
<tr>
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<td>Apr-05</td>
<td>16.67</td>
<td>3.29</td>
<td>TET</td>
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<td></td>
<td></td>
<td>-1.10E-3</td>
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</tr>
<tr>
<td></td>
<td>May-05</td>
<td>90</td>
<td>59.34</td>
<td>TIA</td>
<td>S</td>
<td>TMS</td>
<td>S</td>
<td>-6.30E-4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Mar-06</td>
<td>46.15</td>
<td>76.95</td>
<td>TIA</td>
<td>S</td>
<td></td>
<td></td>
<td>-1.90E-4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>May-06</td>
<td>100</td>
<td>8.11</td>
<td>TIA</td>
<td>Missing</td>
<td></td>
<td></td>
<td>-1.32E-24</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Figure 3.1: 12-month rolling average graphs of the proportion of resistance to ampicillin (A), tetracycline (B), tiamulin (C), and trimethoprim-sulfamethoxazole (TMS) (D) within swine *Pasteurella multocida* and *Streptococcus suis* isolates obtained from the Animal Health Laboratory (January 1998 – October 2010).
Figure 3.2: Predicted probabilities with 95% confidence intervals from the logistic regression models describing (A) ampicillin and TMS resistance, and (B) tetracycline and tiamulin resistance in *Pasteurella multocida* isolates from Ontario swine.
Figure 3.3: Predicted probabilities with 95% confidence intervals from the logistic regression models describing (A) tetracycline and (B) ampicillin and TMS resistance in *Streptococcus suis* isolates from Ontario swine.
CHAPTER FOUR

MULTIPLE-CLASS ANTIMICROBIAL RESISTANCE SURVEILLANCE IN SWINE ESCHERICHIA COLI F4, PASTEURELLA MULTOCIDA AND STREPTOCOCCUS SUIS ISOLATES FROM ONTARIO AND THE IMPACT OF THE 2004 – 2006 PORCINE CIRCOVIRUS TYPE-2 ASSOCIATED DISEASE OUTBREAK

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ABSTRACT

The objective of this work was to describe trends in multiple-class antimicrobial resistance present in clinical isolates of *Escherichia coli* F4, *Pasteurella multocida* and *Streptococcus suis* from Ontario swine 1998 – 2010. Temporal changes in multiple-class resistance varied by the pathogens examined; significant yearly changes were apparent for the *E. coli* and *P. multocida* data. Although not present in the *E. coli* data, significant increases in multiple-class resistance within *P. multocida* isolates occurred from 2003 – 2005, coinciding with the expected increase in antimicrobials used to treat clinical signs of Porcine Circovirus Associated Disease (PCVAD) before it was confirmed. Prospective temporal scan statistics for multiple-class resistance suggest that significant clusters of increased resistance may have been found in the spring of 2004; months before the identification of the PCVAD outbreak in the fall of 2004.

**Keywords:** multiple-class antimicrobial resistance, swine, PCVAD
INTRODUCTION

Antimicrobial resistance (AMR) is a serious topic of concern in both the human and veterinary medical communities. Recommendations have been made for continued and improved surveillance of AMR and antimicrobial use, as well as to change to prescription-only use of antimicrobials for disease treatment in animals (Health Canada, 2002). Currently, there are no ongoing efforts to provide surveillance data from isolates from clinical diseases, as opposed to those from healthy swine in Ontario. Given the variability in AMR by location and the lack of ongoing surveillance, locally relevant AMR information is not available for practitioners looking for informed treatment decisions for clinically ill animals. Previous work has assessed the quality and quantity of clinical isolate AMR data from the Animal Health Laboratory (AHL) at the University of Guelph to develop a de novo surveillance system for Ontario swine (Glass-Kaastra et al., 2013a), and produced reports of the trends in resistance to four antimicrobials within *Escherichia coli*, *Pasteurella multocida*, and *Streptococcus suis* isolates (Glass-Kaastra et al., 2013b).

Given high rates (40%) of multiple-class resistance within the zoonotic pathogen *Salmonella* from ongoing surveillance of AMR in Canadian swine (Government of Canada, 2010), it is reasonable to assume that multiple-class resistance is also present within clinical isolates. Therefore, practitioners may be faced with treating infections that are resistant to more than one of the first-line antimicrobial choices. As the data are available, analyses of multiple-class resistance may be implemented within an AMR surveillance system using AHL data from Ontario swine. These results would enhance single-antimicrobial resistance trends, allow researchers to assess linkages between
resistance in these pathogens, and provide data for assessing the effect of interventions to reduce inappropriate use of antimicrobials in livestock.

Furthermore, surveillance of multiple-class resistance may support syndromic surveillance for new viral diseases. Counts of the number of submissions and the probability of positive results for a particular viral test have been shown to be associated with new viral disease outbreaks (O’Sullivan et al., 2012a; O’Sullivan et al. 2012b), and we suggest that multiple-class resistance may present another option when these measures are not available. In the late fall of 2004, the Ontario swine industry was challenged by a rapidly-spreading outbreak of disease of an unknown etiology (Carman et al., 2008). A wide range of severe symptoms were associated with the outbreak, including fertility problems and wasting/failure to thrive in the nursery and grower stages (jaundice, weight loss, diarrhea, dyspnea, lymphadenopathy) (Sorden, 2000; Carman et al., 2008). Given the dramatic health impact upon affected animals, and the failure of antimicrobials used for treatment, this outbreak was cause of significant economic concern. Now identified as Porcine Circovirus Associated Disease (PCVAD), caused by porcine circovirus type-2 (PCV-2), this outbreak was estimated as being responsible for economic losses exceeding $200 million in hog sales in Canada (Agriculture and Agri-Food Canada, 2011). With no clear treatment plan available, we expect that antimicrobial use was increased before a vaccine became available in March 2006 (O’Sullivan et al., 2012). Furthermore, the authors expect that herds were exposed to multiple classes of antimicrobials following failure of first-line antimicrobial options. Therefore, the selection pressures for multiple-class resistance were likely high during the outbreak.
The objective of this work was to describe trends in multiple-class resistance present in clinical isolates of *Escherichia coli* F4, *Pasteurella multocida* and *Streptococcus suis* from 1998 – 2010. These trends were assessed to determine whether an increase in multiple-class resistance occurred during, or following the 2004 - 2006 outbreak of PCVAD in Ontario. A secondary objective was to evaluate whether an ‘outbreak’ of multiple-class resistance could be detected earlier in the PCVAD outbreak than detected by traditional lab-based surveillance.

**METHODS**

As part of a larger project assessing antimicrobial resistance in Ontario swine, passive surveillance data describing AMR susceptibility were obtained from clinical submissions to the AHL between January 1998 and October 2010. All clinical swine isolates of *E. coli*, *P. multocida*, and *S. suis* and their respective susceptibility testing results were used for these analyses. All bacterial cultures and bacterial identifications were performed according to the standard operating procedures of the AHL. Susceptibility testing was performed following CLSI guidelines.

Antimicrobials were chosen based on the quantity and quality of available data, as described in Glass-Kaastra et al. (2013a). Isolates were considered to have some form of resistance if susceptibility testing results reported intermediate or resistant classifications. These classifications were combined and termed “resistant” for ease of analysis and comparison. Due to inconsistency in panels tested over time, and changes in breakpoints for classifying isolates as resistant/intermediate/susceptible, data availability changed over time (Glass-Kaastra et al., 2013a). Due to these changes, susceptibility test results...
for *E. coli* isolates were available for ampicillin, gentamicin, spectinomycin, sulfisoxazole, tetracycline, and TMS from 1998 – 2010, apramycin from 2007 – 2010, ceftiofur from 2002 – 2010, and kanamycin from 2006 – 2010. For *P. multocida* isolates, susceptibility results were available for ampicillin, ceftiofur, tetracycline, and TMS results from 1998 – 2010, penicillin G and spectinomycin results from 2001 – 2010, sulfisoxazole and tiamulin results from 2004 – 2010, and florfenicol and tulathromycin results from 2007 - 2010. Finally, for *S. suis* isolates, susceptibility results were available for ampicillin, ceftiofur, penicillin G, spectinomycin, tetracycline, and TMS results from 1998 – 2010, and sulfisoxazole and tiamulin results from 2004 – 2010. Susceptibility results were then collapsed by antimicrobial class, resulting in the representation of 9 classes as follows: aminoglycoside (kanamycin, gentamicin, spectinmycin, apramycin); penicillin (penicillin G, ampicillin); sulphonamides (sulfisoxazole); tetracycline (tetracycline); cephalosporin (ceftiofur); pleuromutilin (tiamulin); phenicol (florfenicol); macrolie (tulathromycin); diaminopyrimidine-sulfonamide (trimethoprim-sulfamethoxazole). Two count variables were developed to support model development: the number of antimicrobial classes that isolates were resistant to (the outcome variable for the models) and the natural logarithm of the number of susceptibility tests performed for each isolate (the offset variable). Resistance to an antimicrobial class was defined as a resistant result for ≥1 antimicrobial within the respective class.

Poisson regression models were built using Stata/MP 11.2 to assess whether the number of classes per classes tested changed over time. Year and season were assessed as predictors within these models. Year was modeled as a categorical variable due to non-linearity with natural log of the proportion of resistant results (number of resistant
classifications / number of susceptibility tests for the isolate). Similarly, season was assessed as a categorical variable, with the months March – May describing ‘spring’, June – August described ‘summer’, September – November described ‘fall’, and December – February described ‘winter’. Goodness of fit was first tested by assessment of the overdispersion parameter; if significant, a negative binomial model was run to control for the overdispersion present. Standardized residuals, leverage, deviance and Pearson chi-square residuals were visualized using scatter plots, and any covariate patterns showing anomalous values were recorded. Pearson goodness-of-fit tests were assessed for the final Poisson or negative binomial models, and normality of the Anscombe residuals was assessed visually using a normal quantile plot. Predicted values with their 95% confidence limits were calculated using the average number of susceptibility tests for the given year as the offset. Differences in the predicted rate of multiple-class resistance between years were calculated using contrast statements. Graphs were produced using Microsoft Excel (2010), and all other analyses were performed in Stata MP/11.2 (StataCorp LP, 2009, College Station, TX).

Detection of the most likely temporal clusters (of high or low counts of resistant results) was performed in SatScanTM v9.1.1 (Kulldorf, 1997). Retrospective purely temporal Poisson models were run with 9999 replications and a maximum cluster size equal to 50% of the study period. In order to detect secondary clusters, an iterative function was employed. The case file included the number of resistant results for each isolate, and the population file included the number of susceptibility tests for each isolate. Both files included a submission number in order to link the results and number of tests. The iterative function detects the most likely cluster on the first iteration, then removes
all of the isolates included in the most likely cluster and re-scans the data in order to find the next most likely cluster within the remaining data (Zhang, 2010). Scans continue until the most likely cluster found is not significant (p-value > 0.05) or if the maximum number of iterations were met (9999). Prospective purely temporal Poisson scans using all background data from 1998 forward were run on a monthly basis from January 2004 to December 2004 to determine if a significant cluster of resistant results could be identified prior to the PCVAD outbreak detection in the fall of 2004. In comparison to retrospective scans, where clusters may be identified at any point within the dataset, prospective scans are interested in detecting clusters that are “active”, that is, they include the most recent data (Kulldorf, 2001).

RESULTS

Multiple-class resistance

More than 97.7% of E. coli isolates were resistant to at least one antimicrobial class tested, and more than 91.3% were resistant to ≥ 2. The most commonly seen multiple-class resistance patterns in E. coli were aminoglycoside, sulfonamide, and tetracycline resistance together (22.5% of isolates) and aminoglycoside, penicillin, sulfonamide, and tetracycline resistance together (20.6% of isolates) (Table 4.1). For P. multocida isolates, 41.5% displayed resistance to at least one antimicrobial tested, with 17.1% of isolates displaying resistance to ≥ 2 classes. The most commonly seen multiple-class resistance patterns for P. multocida isolates were sulfonamide and tiamulin resistance together (2.9% of isolates), and concurrent sulfonamide and sulfonamide combination (TMS) resistance (1.5% of isolates) (Table 4.1). Finally, more than 93.3% of
*S. suis* isolates displayed resistance to at least one antimicrobial tested, with >51.6% displaying multiple-class resistance. The most common multiple-class resistance patterns in the *S. suis* isolates were sulfonamide with tetracycline, and aminoglycoside with tetracycline (12.4% and 7.8%, respectively) (Table 4.1).

**Poisson regression models**

Year was found to be a significant predictor for the number of resistant results within the Poisson model developed for the *E. coli* data (Table 4.2). Although an increasing trend in the number of classes of antimicrobials to which *E. coli* isolates were resistant was present from 1998 to 2007 (Figure 4.1), the only significant difference occurred between 2007 and 2001 (IRR = 1.22; p = 0.007). Therefore, no significant increase in the rate of resistant test results was seen within the PCVAD outbreak time frame.

Year was also found to be a significant predictor for the number of resistant results within the negative binomial model developed for the *P. multocida* data (Table 4.2). In contrast to the *E. coli* results, a significant increase occurred in 2004 over the previous years (Figure 4.1). Although the increase from 2004 to 2005 was not significant, the increase seen in these two years was maintained 2005 – 2010 (Figure 4.1). Neither year (p = 0.503) or season (p = 0.110) were found to be significant predictors of multiple-class resistance within the *S. suis* model.
Temporal scan statistics

i) Retrospective:

The retrospective scan of all *E. coli* data found a cluster of resistance from August 1999 to July 2003 (Table 4.3). Two secondary clusters were identified: the first spanned November 2006 and indicated a time of lower multiple-class resistance than expected, and the second spanned August 2004 to March 2008, and indicated a time of increased multiple-class resistance than the expectation (Table 4.3).

The retrospective scan of all *P. multocida* data found two significant clusters of decreased resistance rates, book-ending the PCVAD outbreak: January 1998 to January 2004, and July 2007 to July 2010 (Table 4.3).

ii) Prospective:

The prospective *E. coli* scans found times where the resistance rates were lower than expected; spanning from August or December of 2003 to the final month of the respective scans (Table 4.4).

The prospective scans for March 2004 and April 2004 within the *P. multocida* data found significant clusters from February – March, and February – April, respectively (Table 4.4).

DISCUSSION

Antimicrobial resistance poses challenges for the treatment of bacterial infections, resulting in increased mortality and duration of infection. The existence of pathogens displaying multiple-class resistance, and a limited array of treatment products compounds
this problem. Furthermore, as antimicrobial treatment itself increases selection pressure for resistance, the choice faced by practitioners and producers is an important one. By providing information regarding multiple-class resistance prevalence at the local level, Ontario swine practitioners will have a source of data upon which to make informed decisions regarding the care of affected animals.

Isolates of *E. coli* F4, *P. multocida*, and *S. suis* from clinical samples originating from Ontario swine farms commonly displayed resistance to at least one antimicrobial class. Furthermore, multiple-class resistance was not uncommon. Previous reports of multiple-class resistance have been similar in other jurisdictions (Chen et al., 2013). Interestingly, in contrast to the *E. coli* and *S. suis* isolates, no single multiple-class resistance pattern in *P. multocida* displayed a dramatically larger prevalence than the remaining patterns. However, when multiple-class resistance was present within the *P. multocida* isolates, resistance to sulfisoxazole was common, which was common within the *E. coli* isolates as well. These results mirror those found by the 2008 Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) report; resistance to sulfisoxazole was commonly reported in *Salmonella* and *E. coli* isolates from the farm surveillance program (Government of Canada, 2011).

Differences in the prevalence of multiple-class resistance among the three pathogens may reflect a number of factors, including treatment preferences at the producer/practitioner level, and the stage of production of the animals (i.e., nursery, grower, finisher). Given that practitioners/producers likely follow typical treatment routines for given disease presentations; selection pressures for multiple-class resistance are expected to vary between production systems and/or veterinary practitioners.
Although it is known that some veterinary clinics submit samples to the AHL at a higher rate than others, practitioner or producer-level data are not available at this time (Glass-Kaastra et al., 2013b). Furthermore, the prevalence of various diseases varies by stage of production (Straw, 2006). Therefore, selection pressure for resistance and multiple-class resistance may also vary based on the production stage of the animals from which clinical samples are submitted. Similar to practitioner and producer-level data, the stage of production and age of animals are not currently available from the AHL data. However, avenues for improving data acquisition at the AHL have been suggested by previous work (Glass-Kaastra et al., 2013b), and sources of multiple-class resistance variation may be an opportunity for research in the future.

Results here indicate that temporal changes in multiple-class resistance vary by the pathogens examined. Significant yearly changes in multiple-class resistance were apparent for the *E. coli* and *P. multocida* data. A peak in multiple-class resistance occurred in 2007 for *E. coli* F4, which does not match with the expectation of increased multiple-class resistance during the PCVAD outbreak in Ontario 2004 – 2006. The temporal changes in *E. coli* multiple-class resistance are expected to reflect relative changes in the prevalence of different *E. coli* F4 strains. In contrast, significant increases in multiple-class resistance within *P. multocida* isolates occurred from 2003 – 2005, coinciding with the expected increase in antimicrobial use to treat symptoms of PCVAD before a viral etiology was confirmed. Interestingly, the increase in multiple-class resistance in *P. multocida* isolates was sustained following the PCVAD outbreak, with no break down in the following years, which contrasts with the breakdown seen in *E. coli* isolates following the peak in 2007. These results suggest that the loss of resistance genes
may occur at a slower rate in *P. multocida* than in *E. coli*, or may reflect selection pressure differences, as treatment failure has been shown to be higher in respiratory than digestive conditions of swine (Ontario Pork, 2010).

Given the significant increase in multiple-class resistance seen during the PCVAD outbreak, the application of prospective temporal scan statistics to historical data were employed to determine whether significant clusters in multiple-class resistance could be identified prior to the identification of the outbreak. Interestingly, results suggest that prospective temporal scan statistics may have found significant clusters of increased resistance in the spring of 2004, months before the identification of the PCVAD outbreak in the fall of 2004 (Carman et al, 2008). This early detection of multiple-class resistance suggests that surveillance of similar results may support syndromic surveillance for new viral disease outbreaks; an increase in multiple-class resistance suggests an increase in the use of multiple antimicrobial classes. As multiple classes of antimicrobials are expected to be employed during times of treatment failure, it follows that an increase in multiple-class resistance suggests a decrease in the health status of the industry resulting from a non-bacterial (likely viral) pathogen. As an increase was not found within the data for all pathogens examined, the choice of pathogen(s) to monitor for multiple-class resistance requires further research. Although PCVAD affects all body symptoms (Dorr et al., 2007; Segales et al., 2005), and the Ontario outbreak was first described due to an increase in pneumonia and diarrhea (Carman et al., 2008), an increase in multiple-class resistance was only present in a respiratory pathogen (*P. multocida*), and not within *E. coli* F4, which is a major cause of diarrhea in post-weaning pigs. Therefore,
representative pathogens for each body system may not be suitable, or required for surveillance purposes.

Conclusion

This work supports the addition of multiple-class resistance to the surveillance of AMR in clinical pathogens of swine, using data from the AHL at the University of Guelph. Significant temporal changes in multiple-class resistance and differences between pathogens highlight the need to update practitioners and policy makers of the current AMR/multiple-class resistance status of pathogens. These ongoing updates will support evidence-based treatment choices and provide the means for assessing the impact of proposed changes to antimicrobial use in the Ontario swine industry. Furthermore, these results support the use of multiple-class resistance as a potential avenue for surveillance of emerging diseases in swine; if increases in multiple-class resistance can be detected in clinical pathogens in tandem with severe clinical signs, it may be possible to initiate warnings, and potential interventions, to mitigate the impact of future novel viral outbreaks within the Ontario swine industry.

Acknowledgements

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the study design, collection, analysis or interpretation of data, or manuscript preparation/submission.
REFERENCES


Table 4.1: Five most frequently occurring resistance patterns (intermediate or resistant results) among clinical *Escherichia coli* F4, *Pasteurella multocida*, and *Streptococcus suis* isolates from the Animal Health Laboratory (January 1998 - October 2010).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Resistance pattern</th>
<th>Number of isolates</th>
<th>Proportion of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> F4</td>
<td>AMI + SUL + TET</td>
<td>298</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>AMI + PEN + SUL + TET</td>
<td>273</td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>AMI + PEN + SUL + TET + TMS</td>
<td>198</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>AMI + SUL + TET + TMS</td>
<td>84</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>PEN + TET</td>
<td>72</td>
<td>0.054</td>
</tr>
<tr>
<td><em>Pasteurella multocida</em></td>
<td>SUL + TIA</td>
<td>42</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>SUL + TMS</td>
<td>22</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>SUL + TET</td>
<td>19</td>
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</tr>
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<td></td>
<td>TET + TMS</td>
<td>18</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>SUL + PEN</td>
<td>15</td>
<td>0.010</td>
</tr>
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<td><em>Streptococcus suis</em></td>
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<td>315</td>
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</tr>
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<td>AMI + TET</td>
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<tr>
<td></td>
<td>AMI + TET + PEN</td>
<td>100</td>
<td>0.039</td>
</tr>
</tbody>
</table>

AMI = aminoglycosides, PEN = penicillins, SUL = sulfonamides, TET = tetracyclines, TIA = tiamulin, TMS = trimethoprim-sulfamethoxazole (sulfonamide combination)
Table 4.2: Incidence rate ratios and p-values from Poisson and negative binomial regression models describing the number of resistant results within *Escherichia coli* and *Pasteurella multocida* isolates from Ontario swine (January 1998 – October 2010).

<table>
<thead>
<tr>
<th>Year</th>
<th><em>Escherichia coli</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th><em>Pasteurella multocida</em>&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IRR</td>
<td>P-value</td>
<td></td>
<td>IRR</td>
<td>P-value</td>
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<tr>
<td>1998</td>
<td>Ref</td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>Ref</td>
<td>.</td>
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</tr>
<tr>
<td>1999</td>
<td>1.15</td>
<td>0.259</td>
<td></td>
<td>0.63</td>
<td>0.084</td>
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</tr>
<tr>
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<td>0.248</td>
<td></td>
<td>1.56</td>
<td>0.053</td>
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</tr>
<tr>
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<td></td>
<td>1.01</td>
<td>0.960</td>
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</tr>
<tr>
<td>2002</td>
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<td>0.266</td>
<td></td>
<td>1.15</td>
<td>0.557</td>
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</tr>
<tr>
<td>2003</td>
<td>1.08</td>
<td>0.504</td>
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<td>1.11</td>
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<tr>
<td>2004</td>
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<td>2.40</td>
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<td>0.696</td>
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</tr>
<tr>
<td>2009</td>
<td>0.89</td>
<td>0.339</td>
<td></td>
<td>2.04</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>0.85</td>
<td>0.224</td>
<td></td>
<td>2.82</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Alpha</td>
<td></td>
<td></td>
<td></td>
<td>0.094</td>
<td>0.028</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Poisson model  
<sup>b</sup>Negative binomial model  
<sup>c</sup>P-value for the partial F-test  
<sup>d</sup>Overdispersion parameter for the negative binomial model
Table 4.3: Significant clusters of higher or lower resistance than expected from antimicrobial class susceptibility testing within swine *Escherichia coli* F4, and *Pasteurella multocida* isolates from Ontario swine (January 1998 – October 2010), as detected by temporal retrospective scans of the Poisson data.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Cluster Time Frame</th>
<th>Relative Risk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Aug 1999 – July 2003</td>
<td>1.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Nov 2006</td>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Aug 2004 – Mar 2008</td>
<td>1.20</td>
<td>0.008</td>
</tr>
<tr>
<td><em>P. multocida</em></td>
<td>Jan 1998 – Jan 2004</td>
<td>0.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Jul 2007 – Jul 2010</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 4.4: Significant clusters of resistance from antimicrobial class susceptibility testing within Ontario swine *Escherichia coli* F4, and *Pasteurella multocida* isolates obtained from the Animal Health Laboratory, as detected by monthly prospective temporal scans of the Poisson data January to December 2004.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Scan</th>
<th>Time</th>
<th>Relative Risk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Jan 1998 – Feb 2004</td>
<td>Dec 2003 – Feb 2004</td>
<td>0.57</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Jan 1998 – Mar 2004</td>
<td>Dec 2003 – Mar 2004</td>
<td>0.63</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Jan 1998 – Apr 2004</td>
<td>Aug 2003 – Apr 2004</td>
<td>0.83</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Jan 1998 – May 2004</td>
<td>Dec 2003 – May 2004</td>
<td>0.75</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Jan 1998 – Jul 2004</td>
<td>Aug 2003 – Jul 2004</td>
<td>0.82</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Jan 1998 – Aug 2004</td>
<td>Aug 2003 – Aug 2004</td>
<td>0.83</td>
<td>0.005</td>
</tr>
<tr>
<td><em>P. multocida</em></td>
<td>Jan 1998 – Mar 2004</td>
<td>Feb – Mar 2004</td>
<td>2.56</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Jan 1998 – Apr 2004</td>
<td>Feb – Apr 2004</td>
<td>2.32</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Figure 4.1: Predicted number of antimicrobial classes with 95% confidence intervals to which *Escherichia coli* F4 and *Pasteurella multocida* clinical isolates, from Ontario swine, were resistant (January 1998 – October 2010).
CHAPTER FIVE

DESCRIBING ANTIMICROBIAL USE AND REPORTED TREATMENT EFFICACY IN ONTARIO SWINE USING THE ONTARIO SWINE VETERINARY-BASED SURVEILLANCE (OSVS) PROGRAM

Formatted for publication in BMC Veterinary Research and submitted in July 2013.
ABSTRACT

Background

The objective of this work was to retrospectively assess records received through the Ontario Swine Veterinary-based Surveillance program July 2007 – July 2009 to describe and assess relationships between reported treatment failure, antimicrobial use, diagnosis and body system affected.

Results

Antimicrobial use occurred in 676 records, 80.4% of all records recording treatment (840). The most commonly used antimicrobials were penicillin (34.9%), tetracyclines (10.7%) and ceftiofur (7.8%), and the use of multiple antimicrobials occurred in 141/676 records (20.9%). A multi-level logistic regression model was built to describe the probability of reported treatment failure. The odds of reported treatment failure were significantly reduced if the record indicated that the gastro-intestinal (GI) system was affected, as compared to all other body systems (p < 0.05). In contrast, the odds of reported treatment failure increased by 1.98 times if two antimicrobials were used as compared to one antimicrobial (p = 0.009) and by 6.52 times if three or more antimicrobials were used as compared to one antimicrobial (p = 0.005). No significant increase in reported treatment failure was seen between the use of two antimicrobials and three or more antimicrobials. No other antimicrobials were significantly associated with reported treatment failure after controlling for body system and the number of antimicrobials used.
Conclusions

Failure of antimicrobial treatment is more likely to occur in non-GI conditions, as compared to GI conditions and the use of multiple antimicrobial products is also associated with an increased probability of antimicrobial treatment failure. The authors suggest that a more preventative approach to herd health should be taken in order to reduce antimicrobial inputs on-farm, including improved immunity via vaccination, management and biosecurity strategies. Furthermore, improved immunity may be viewed as a form of antimicrobial stewardship to the industry by reducing required antimicrobial inputs and consequently, reduced selection pressure for AMR.

Keywords: Treatment failure, treatment efficacy, antimicrobial use, swine

BACKGROUND

Antimicrobial resistance (AMR) threatens the efficacy of antimicrobial drugs for treating infections in humans and animals alike. Antimicrobial resistance emerges in the presence of antimicrobial products and the transfer of resistance genes among bacteria may occur [1]. As clinical infection with resistant pathogens may lead to prolonged morbidity, increased costs and increased risk of mortality, AMR is a serious concern for food-animal production. Recently, it has been reported that multiple-class resistance within clinical pathogens of swine in Ontario is not uncommon [2]. However, it is not known if this has led to increased antimicrobial treatment as there is currently no accurate measurement of the volume of antimicrobial use in Ontario swine. Furthermore, these resistance data do not include information regarding the treatment provided, or efficacy
of the treatment. Given that in-vitro resistance does not necessarily predict failure of antimicrobial treatment in-vivo [3], industry stakeholders may also benefit from the knowledge of significant predictors for reporting treatment failure.

In July 2007, the Ontario Swine Veterinary-based Surveillance (OSVS) program was initiated to assess the potential for developing a practitioner-based syndromic health surveillance system for swine in Ontario [4]. Recruited practitioners were asked to record and report on all farm visits or calls related to swine. These reports requested information regarding suspected diagnosis, the body system affected, treatment(s) provided, and the efficacy of treatments. Consequently, these records may provide a valuable source of antimicrobial use and in-vivo efficacy data for Ontario; a complement to the available in-vitro resistance data from the Animal Health Laboratory [5]. Furthermore, these data allow for the examination of associations between treatment with certain antimicrobials and reported treatment failure. Therefore, the objective of this work was to retrospectively assess records received through the OSVS program from July 1, 2007 through June 30, 2009 to describe and assess relationships between reported treatment failure, antimicrobial use, diagnosis, and body system affected.

METHODS

A full description of the OSVS pilot project is available elsewhere [4]. Briefly, the OSVS pilot program was funded by the Ontario Ministry of Agriculture Food & Rural Affairs (OMAFRA) and the Ontario Animal Health Strategic Investment (AHSI) fund. During the July 1 2007 to June 30 2009 time period, reports were received from up to ten practitioners representing five clinics known to service most of the swine industry.
in Ontario. During this period, practitioners recorded data for all daily swine-related farm visits and calls using either a paper or electronic submission via personal digital assistants (PDA) or an internet-based form. Data collected included whether the visit/call was a disease or routine visit, unique practitioner ID, unique farm ID, signs/symptoms displayed, diagnosis, body system affected, whether it was an incident or ongoing condition, farm history of the condition, diagnosis, veterinarian-prescribed treatment, and efficacy of treatment.

A database was created through the electronic form submissions and manual input of the paper forms, using Microsoft Access (2003). Data cleaning, tabulations and multi-level logistic regression modeling were performed in Stata/MP 12.1 (Stata Corporation, College Station, Texas, USA). Manual mining of free-text fields was performed in order to determine the most commonly used antimicrobials and common diagnoses. Treatment failure was deemed to have occurred if practitioners recorded a treatment as being not efficacious, or “occasionally” efficacious. Due to small numbers of observations, the nervous, integument and reproductive body systems were combined into a single ‘other’ category. The treatment variable was searched to create binary variables for each antimicrobial used and a variable describing the number of antimicrobials used was developed by adding across these binary variables. Therefore, multiple antimicrobial treatment was defined as any record with >1 antimicrobial listed within the treatment text field. Multiple antimicrobial treatments may not have been initiated at the time of the record, but were either 1) in use concurrently, or 2) recently used to treat/control the specific condition in the animals being seen at the time of the visit. As only a single
record was found with more than 3 antimicrobials used, a “3 or more” category was created in the number of antimicrobials variable.

Multilevel logistic regression models were built to describe the probability of treatment failure, given antimicrobial treatment. Two- and three-level models were built using practitioner and farm as random intercepts, and the inclusion of a random slope for practitioner was also tested. Fixed effect predictors examined were diagnosis, body system affected, the number of antimicrobials used and each of the individual antimicrobial use variables. A manual backwards-selection process was used to build the model; all predictors were added to the model initially and removed one at a time based on the highest p-value. Categorical variables with > 2 levels were assessed for significance using the likelihood ratio test [6]. As predictors were removed, their impact on all other statistically significant coefficients was assessed to ensure that confounding variables remained in the model; a 30% change in any significant coefficient resulted in the removed variable being replaced in the model [6]. All two-way interaction terms were generated between all significant main-effects and tested within the model at p < 0.05. Where more than one model fit the data, the model with the most negative Akaike information criteria was chosen [6]. At the record level, Pearson and deviance residuals were visualized and any covariate patterns showing anomalous values were recorded. The normality of the best linear unbiased predictors (BLUPS) was assessed visually with normal quantile plots. Models were re-run with the exclusion of noted covariate patterns to assess any dramatic changes in the coefficients. Contrast statements were used to make comparisons between dummy variable categories within the body system and number of
antimicrobials variables and the latent variable technique was employed to calculate variance components at each level [7].

RESULTS

In total, 3691 records were received by the OSVS program from July 2007 – July 2009. Antimicrobial use was reported in 676 of these records. These reflected reports from nine practitioners, submitting 7 to 255 records each and reflected data from 335 farms with 1 to 14 records each. A number of records indicated that an antimicrobial was used, without naming the product (182/676). When drug names were included, the most commonly used antimicrobials were penicillin (34.9%), tetracyclines (10.7%) and ceftiofur (7.8%) (Table 5.1). Use of multiple antimicrobials in a single record was not uncommon; 141 (20.6%) records indicated that treatment included ≥ 2 antimicrobials (Table 5.2). The most common combinations of antimicrobials used for treatment were penicillin with tetracyclines (24 records), penicillin with ceftiofur (16 records) and penicillin with a sulfonamide product (13 records) (Table 5.3).

In records with antimicrobial treatments recorded, the recorded body systems affected were: respiratory, GI, musculoskeletal, multisystemic, or other. More than 27% (185/676) of records with antimicrobial use indicated that multiple systems were affected. Furthermore, 78.9% (146/185) of these records indicated treatment failure. The second and third most commonly noted body systems affected were respiratory and GI. Treatment failure was reported in 74.3 and 52.9% of these records, respectively. The most commonly noted diagnoses were *Streptococcus suis* infection (94 records, 13.9%) and porcine reproductive and respiratory syndrome (PRRS) (93; 13.8%) (Table 5.4).
Interestingly, antimicrobials were used in records where diagnoses included non-bacterial conditions (e.g. porcine circovirus infection and influenza) (Table 5.4).

Significant predictors within the final multi-level logistic regression model describing treatment failure included body system affected, the number of antimicrobials used and the use of neomycin (Table 5.5). Practitioners and farm were included as random effects, accounting for the 3-level nested structure of the data (reports from farms within a veterinarian’s practice) (Table 5.5). No significant interaction terms were found. Records indicating GI disease were at significantly decreased odds of treatment failure as compared to multisystemic, musculoskeletal and respiratory body systems (Table 5.5). No other significant differences in treatment failure were present between body systems. As the number of antimicrobials used increased, so did the odds of treatment failure. The odds of failure increased by 2.29 times if two antimicrobials were used as compared to one antimicrobial (p < 0.01) and by 7.56 times if three or more antimicrobials were used as compared to one antimicrobial (p = 0.01). No significant increase in treatment failure was seen between the use of two antimicrobials and three or more antimicrobials (OR = 1.19; p = 0.15; CI: -0.45 – 2.84). Finally, reduced odds of treatment failure was found in records where neomycin was used (OR = 0.34; p = 0.02). No other antimicrobials were significantly associated with treatment failure after controlling for body system affected.

The variance partition coefficient indicated that the majority of variation in reported treatment failure occurred at the report level after accounting for fixed effects (70.0%) as compared to the practitioner (17.2%) and farm levels (17.8%).
The BLUPS at the farm and practitioner levels were normally distributed by visual assessment. No anomalous values for the Pearson or deviance residuals were apparent at the report level.

DISCUSSION

This work presents an assessment of the use of antimicrobials in the Ontario swine industry and the frequency of treatment failure when antimicrobials were used for treating disease. Given that antimicrobial use has been reported as a common occurrence within the swine industry [1,8], it was not surprising to find that 50% of OSVS records with a treatment recorded indicated that an antimicrobial was employed. The most commonly reported antimicrobials were penicillin, tetracyclines and ceftiofur, consistent with the three highest-use injectable antimicrobials reported by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) for 2008 [9]. Although the administration route for antimicrobials is available within the CIPARS data, the reason for use are not available. However, the OSVS dataset may provide insight on the proportion of antimicrobial use for disease treatment as compared to growth promotion or prophylaxis, as OSVS participants were requested to only record treatments (not routine use). Use of multiple antimicrobials was not uncommon within the reports assessed here; more than 20% of records indicated that ≥ 2 antimicrobials were used for treatment. Furthermore, these data displayed that antimicrobials were used in cases where the expected diagnosis was viral (e.g., Porcine Circovirus infection and influenza) or non-infectious (e.g., injury), either as a precautionary measure if a suspected viral infection is actually bacterial, or to prevent/treat secondary bacterial infections.
In reports indicating antimicrobial use, treatment failure was surprisingly high (78.9%). To assess predictors for treatment failure within these records, a multi-level logistic regression model was built with the assumption that the reported treatment failure reflected failure of the antimicrobial mentioned in the treatment field. Results of this model indicated that the odds of treatment failure was associated with the body system affected, the number of antimicrobials used in treatment and use of the antimicrobial neomycin. The odds of treatment failure was significantly lower when the GI system was affected as compared to respiratory, multisystemic, musculoskeletal or other body system conditions, and the odds of treatment failure with multisystemic conditions was significantly higher than in reports with no body system recorded.

Significant differences in treatment failure among body systems affected likely reflected the etiology of common swine conditions. Many respiratory and multi-systemic conditions in swine have a viral etiology (e.g., Porcine Circovirus infection, influenza, PRRS) and antimicrobial treatment is not expected to resolve the primary viral infection. This is not to say that antimicrobial treatment is always inappropriate however, as secondary bacterial infections or co-infections may also occur and antimicrobial treatment may prevent the exacerbation of clinical signs [10-11]. Accordingly, prudent use guidelines for antimicrobial use in swine encourage veterinarians to determine the causative agent of disease while recognizing the potential for secondary bacterial infections [12].

The differences in the probability of treatment failure among the body systems may also reflect the route of administration of antimicrobials in swine production. Due to large herd or group sizes, treatment of individual animals by injection can be difficult and
impractical. Therefore, mass medication of herds through water or feed is a common practice [9]. Enteric bacterial infections are expected to be effectively treated with this method, given that no metabolism or uptake of the drug into the blood stream/tissue is required. As such, failure of orally administered treatment is expected to occur less often than when other body systems are affected. Furthermore, ill animals are likely to go off-feed, which can result in under-dosing of infections requiring drug metabolism or blood stream uptake. The observed sparing effect of neomycin use may also be explained by body system and route of administration; neomycin is supplied through feed or water in order to treat bacterial enteritis caused by *Escherichia coli* and *Salmonella* spp. [13].

The odds of treatment failure increased significantly with the use of multiple antimicrobials. Although practitioners were requested to record data only pertaining to the current visit, the data suggested that records listing multiple antimicrobials for treatment may have reflected either concurrent use of multiple products, or successive use following failure of the primary treatment. However, these results suggest that it may be prudent to explore non-bacterial etiologies and preventative approaches to swine health when the use of two or more antimicrobials is being considered.

Given that the variance in reported treatment failure was greater at the report level than the farm or practitioner level, it may be assumed that the disease in question has greater influence on the probability of treatment failure than farm- or practitioner-level factors. Therefore, the potential impact of prescription-only standards for accessing antimicrobials on-farm is great, as suggested by the Veterinary Drugs Directorate “Uses of antimicrobials in food animals in Canada” report [14]. These standards would require producers to obtain a prescription for all antimicrobial use, which is not a current practice
in Ontario. Upon the adoption of this recommendation, a shift in the influence may occur towards practitioners. The impact of this shift upon the frequency of treatment failure presents an interesting topic for follow-up studies.

In instances of non-GI conditions or failure of first-line antimicrobial treatment, a review of the vaccination, biosecurity, artificial insemination, and air quality strategies used on-farm may provide a more effective means of improving and maintaining herd health. Vaccines are available and a topic of ongoing research for many common pathogens of swine, including *S. suis* [15-16], PRRS [17-18], Porcine Circovirus [19-20], and *E. coli* [21-22]. Given that the majority of antimicrobial use reported in the OSVS reports reflected the treatment of conditions caused by these pathogens, successful vaccination strategies are expected to lower the probability of antimicrobial use and treatment failure alike. Similarly, two manageable biosecurity measures, the presence of a shower on-site and limited access to main entrances by rendering trucks, have been shown to be associated with reduced probability of positive PRRS virus status on-farm [23]. Other management practices such as the use of semen from specific-pathogen free boars for artificial insemination [24], weaning at 28 days of age or later [25] improving ventilation [26-27], reducing group sizes to decrease density [28] and switching to all-in-all-out flow systems [28] have been shown to be associated with reduced probability of positive viral status. However, the practicality of some of these changes is questioned, as many may require large economic inputs by producers (e.g., changes requiring renovations to facilities). Therefore, it may be prudent for practitioners, producers, and policy makers to reassess the current guidelines around vaccinations, and the use and acquisition of antimicrobials in swine production. In order to reduce the volume of
antimicrobial product being used to treat non-bacterial infections, priority should be
given to research that focuses on assessing the health and economic impacts of
vaccination, prescription-only standards for antimicrobial use, or increasing the
frequency of contact between producers and practitioners.

Due to the nature of the data collection, it should be noted that there are some
potential biases present in this dataset. There is some potential for over-estimation of the
use of antimicrobials for treatment of disease, given that the reporting veterinarian(s) may
have recorded antimicrobials used for growth promotion and/or prophylaxis in the
treatment field. Furthermore, some diagnostic misclassification may have occurred
between diseases that present similarly, as laboratory confirmation was not linked to
these records.

This work suggests that failure of antimicrobial treatment is more likely to occur
in non-GI conditions, as compared to GI conditions. Furthermore, the use of multiple
antimicrobial products is also associated with an increased probability of antimicrobial
treatment failure. Improved immunity via vaccination, management and biosecurity
strategies may be viewed as a form of antimicrobial stewardship to the industry by
reducing required antimicrobial inputs and consequently, reduced selection pressure for
AMR. Furthermore, further research is suggested surrounding the economic and health
impacts of changes to guidelines surrounding vaccination, antimicrobial acquisition and
use, as well as increasing the frequency of contact between producers and practitioners.
REFERENCES


Table 5.1: Number of OSVS records where treatment with each antimicrobial was reported (July 1, 2007 – June 30, 2009).

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Frequency</th>
<th>Percent of records with antimicrobial treatment (N = 676)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>236</td>
<td>34.91</td>
</tr>
<tr>
<td>Specific drug not recorded</td>
<td>182</td>
<td>26.92</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>72</td>
<td>10.65</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>53</td>
<td>7.84</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>41</td>
<td>6.07</td>
</tr>
<tr>
<td>Neomycin</td>
<td>41</td>
<td>6.07</td>
</tr>
<tr>
<td>Tylosin</td>
<td>36</td>
<td>5.33</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>28</td>
<td>4.14</td>
</tr>
<tr>
<td>Tulathromycin</td>
<td>26</td>
<td>3.85</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>22</td>
<td>3.25</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>20</td>
<td>2.96</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>19</td>
<td>2.81</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>18</td>
<td>2.66</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>14</td>
<td>2.07</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>11</td>
<td>1.63</td>
</tr>
<tr>
<td>Apramycin</td>
<td>10</td>
<td>1.48</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>8</td>
<td>1.18</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>1</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Table 5.2: Number of OSVS records where treatment efficacy or failure was reported by the number of antimicrobials used for treatment (July 1, 2007 – June 30, 2009).

<table>
<thead>
<tr>
<th>Number of antimicrobials used</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment failure</td>
<td>360</td>
<td>86</td>
<td>26</td>
<td>1</td>
<td>473</td>
</tr>
<tr>
<td>Efficacious treatment</td>
<td>175</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>203</td>
</tr>
<tr>
<td>Total</td>
<td>535</td>
<td>112</td>
<td>28</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.3: Most frequent antimicrobial pairings as reported within OSVS records (July 1, 2007 – June 30, 2009).

<table>
<thead>
<tr>
<th>Combination</th>
<th>Number of records (N = 676)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin + tetracycline</td>
<td>24</td>
</tr>
<tr>
<td>Penicillin + ceftiofur</td>
<td>16</td>
</tr>
<tr>
<td>Penicillin + sulfonamide</td>
<td>13</td>
</tr>
<tr>
<td>Sulfonamide + tetracycline</td>
<td>9</td>
</tr>
<tr>
<td>Neomycin + tetracycline</td>
<td>8</td>
</tr>
<tr>
<td>Tulathromycin + ceftiofur</td>
<td>8</td>
</tr>
<tr>
<td>Sulfonamide + trimethoprim</td>
<td>8</td>
</tr>
<tr>
<td>Penicillin + sulfonamide + tetracycline</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 5.4: Diagnoses recorded in OSVS records that indicated antimicrobial use, when antimicrobials were used in treatment (July 1, 2007 – June 30, 2009).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Records</th>
<th>Proportion of records</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus suis</em></td>
<td>94</td>
<td>13.91</td>
</tr>
<tr>
<td>PRRS</td>
<td>93</td>
<td>13.76</td>
</tr>
<tr>
<td>Scours&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49</td>
<td>7.25</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>45</td>
<td>6.66</td>
</tr>
<tr>
<td>Circovirus</td>
<td>43</td>
<td>6.36</td>
</tr>
<tr>
<td>Arthritis</td>
<td>40</td>
<td>5.92</td>
</tr>
<tr>
<td>Ileitis</td>
<td>31</td>
<td>4.59</td>
</tr>
<tr>
<td>Influenza</td>
<td>30</td>
<td>4.44</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>29</td>
<td>4.29</td>
</tr>
<tr>
<td>Glassers</td>
<td>28</td>
<td>4.14</td>
</tr>
<tr>
<td>Greasy pig&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19</td>
<td>2.81</td>
</tr>
<tr>
<td><em>Actinobacillus pleuropneumonia</em></td>
<td>19</td>
<td>2.81</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>18</td>
<td>2.66</td>
</tr>
<tr>
<td>Lameness</td>
<td>17</td>
<td>2.51</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>17</td>
<td>2.51</td>
</tr>
<tr>
<td>Coccidiosis</td>
<td>10</td>
<td>1.48</td>
</tr>
</tbody>
</table>

<sup>a</sup>Specific pathogen not indicated
Table 5.5: Odds ratios and p-values for the mixed logistic regression model describing the effect of body system treated, number of antimicrobials used and the use of neomycin upon treatment failure in OSVS records, when antimicrobials were used in treatment (July 1, 2007 – July 30, 2009).

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Odds Ratio</th>
<th>Standard Error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Referent</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>Multisystemic</td>
<td>3.14</td>
<td>0.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>2.39</td>
<td>1.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Other</td>
<td>1.96</td>
<td>0.76</td>
<td>0.08</td>
</tr>
<tr>
<td>Respiratory</td>
<td>2.40</td>
<td>0.75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Not recorded</td>
<td>1.47</td>
<td>0.78</td>
<td>0.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of antimicrobials used</th>
<th></th>
<th></th>
<th>0.01&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Referent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2.29</td>
<td>0.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3 or more</td>
<td>7.56</td>
<td>6.10</td>
<td>0.01</td>
</tr>
</tbody>
</table>

| Neomycin                    | 0.34    | 0.15           | 0.02              |
| Intercept                   | 1.02    | 0.41           | 0.97              |

<table>
<thead>
<tr>
<th>Random intercepts</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Practitioner</td>
<td>0.81</td>
<td>0.62</td>
<td>&lt;0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Farm</td>
<td>0.60</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>P-value for the likelihood ratio test comparing the model with and without the system variable

<sup>b</sup>P-value for the likelihood ratio test comparing the random effects model to the fixed-effects model alone
CHAPTER SIX

SUMMARY OF FINDINGS
INTRODUCTION

The work described in this thesis examines the potential for using clinical isolate data from the Animal Health Laboratory (AHL) at the University of Guelph to provide a platform for a surveillance system for antimicrobial resistance (AMR). The original scope of the project included data from all livestock commodities including broiler and layer chickens, turkeys, beef and dairy cattle, sheep, goats, horses, and swine; however, the scope quickly narrowed to swine alone. Due to a small number of isolates and an even smaller number of susceptibility tests performed, the statistical power of the data from the remaining commodities was deemed to be too low for use at this time. A suitable number of susceptibility tests were available for three clinical pathogens affecting swine: *Escherichia coli* with the F4 adhesin, *Streptococcus suis* and *Pasteurella multocida*. Therefore, isolation, demographic and susceptibility test data for these pathogens became the new starting point for assessing the potential for development of a novel surveillance system for AMR in clinical pathogens. Future work is required to determine why data are so sparse for other commodity groups and into ways of increasing the data volume.

The advantages and disadvantages of using passive data sources for surveillance have been discussed in many sources (Salman, 2003; Teusch and Churchill, 2000). In comparison with actively collected data, passive data may provide a less labour- and cost-intensive strategy for surveillance efforts. A commonly cited disadvantage for the use of clinical laboratory data is missing information: non-response to important predictors and covariates for which information are not collected. Therefore, an assessment of passively acquired data is necessary before use.
In Chapter 1, the quantity and quality of isolation, demographic and susceptibility testing data for the three swine pathogens were examined. Through this work, a number of issues were brought to light including insufficient information for merging exported files, missing data, recording inconsistencies, and temporal changes in the commonly used panel for susceptibility testing. These issues persisted following a change in the laboratory information system (LIMS) and it became apparent that although both LIMSs employed during the study time frame were suitable for the laboratory setting, the collected data required considerable merging and cleaning before statistical analyses could be performed for surveillance. However, this was not an unexpected finding as the database requirements of a surveillance system differ from those of a clinical laboratory. The former requires access to large numbers of records over a period of time, with comparable recording among the records. In contrast, as the goal of the clinical laboratory is diagnosis of an individual’s condition (or multiple individuals from the same source), the desired output is at the sample submission level and consistency of data recording among records is not a priority. Similarly, missing data, recording consistency and temporal changes in common susceptibility panels are surveillance-specific issues. These issues are rarely expected to affect the day-to-day functioning of the laboratory given that isolation, susceptibility testing and diagnosis may be made regardless of covariate information or differences between records. Recommendations to improve the quality and quantity of antimicrobial susceptibility data were developed based upon this assessment and are described fully in Appendix B.
Using analytical methods to describe susceptibility results for surveillance

Following from the assessment of AHL data, *P. multocida* and *S. suis* data were used to explore options for analytical AMR surveillance methods. As current AMR surveillance systems focus on descriptive statistics to present results, the goal of work here was to employ analytical options, while still providing results that are accessible to users less familiar with statistical analyses. The four techniques employed were: 12-month rolling average smoothers of the proportion of resistant results out of all susceptibility tests, logistic regression models and predicted value graphs, temporal scan statistics, and the “What’s Strange About Recent Events?” (WSARE) algorithm. Furthermore, using these techniques, trends and significant changes in resistance to ampicillin, tetracycline, tiamulin, and trimethoprim-sulfamethoxazole were highlighted for these two pathogens.

The methods employed were found to provide complementary and, at times, redundant results. The most appropriate combination of analysis methods for surveillance using these data included temporal scan statistics with a visualization method. Although the WSARE algorithm provided interesting results for quality control purposes and has the potential for detecting new resistance patterns, missing data created problems for displaying these results in a meaningful way.

With respect to the susceptibility results, varying trends were apparent from visual methods (rolling average smoothers of the proportion of resistant results out of all susceptibility tests), displaying that resistance patterns differed between the two pathogens and among the four antimicrobial drugs assessed. These results highlight the importance of surveillance at the clinical pathogen level; differing patterns in
susceptibility between pathogens and among antimicrobials suggests that surveillance of indicator bacteria may not be enough. There are currently a number of AMR surveillance programs operating that involve swine, the main focus of these programs is on human enteric pathogens and foodborne commensal bacteria with potential exposure sources (DANMAP, 2012; Government of Canada, 2010; CDC, 2010a; CDC, 2012b; CDC, 2010c; Government of Japan, 2009). Data regarding AMR within generic Escherichia coli, Campylobacter spp. and Salmonella spp. are available through these programs; however, AMR results from target pathogens of animals are rare. As the clinical isolates assessed in this work displayed widely varying patterns of resistance, the use of these indicator bacteria may not be appropriate indicators of AMR in clinical pathogens. Therefore, the addition of clinical isolate surveillance to current AMR surveillance programs is strongly suggested, in order to provide access to suitable AMR data for informing decisions regarding the treatment of ill animals. Furthermore, the availability of AMR data from clinical isolates would allow tracking of changes following interventions to antimicrobial use. These changes are expected in the future, as recommendations have been made to change the prescribing and use of antimicrobials in agriculture use in Canada, including moving to prescription-only use and the development of policy around extra-label use (Health Canada, 2002).

Using multiple-class antimicrobial resistance as a syndromic surveillance tool for emerging diseases in swine

As susceptibility tests to multiple antimicrobials are performed upon the majority of isolates from clinical laboratories, multiple-class resistance can also be evaluated using
the AHL data. Using the three clinical pathogens of swine and the antimicrobials deemed suitable for surveillance identified in Chapter 1, Poisson and negative-binomial models were used to assess the significance of temporal changes in the number of resistant results out of all susceptibility tests for each pathogen. A major finding from this work was that significant increases in multiple-class resistance occurred from 2003 – 2005 within *P. multocida* isolates. This increase coincided with an outbreak of disease in the Ontario swine industry of unknown etiology (at the time). During the outbreak, it is expected that producers and practitioners used considerable volumes of antimicrobial drugs, therefore increasing the selection pressure for AMR. The outbreak was identified as Porcine Circovirus Type-2 Associated Disease (PCVAD), caused by porcine circovirus type-2 (PCV-2) in the fall of 2004 and a vaccine became available in March 2006. Given the significant increase in multiple-class resistance seen during the PCVAD outbreak for *P. multocida*, prospective temporal scan statistics were employed to determine whether significant clusters in multiple-class resistance could be identified prior to the identification of the outbreak. Interestingly, the prospective temporal scans suggest that a significant increase in multiple-class resistance would have been identified in the spring of 2004, months before the identification of the PCVAD outbreak in the fall of 2004.

Results of this work suggest that multiple-class resistance could provide an opportunity for surveillance of emerging diseases in swine; if increases in multiple-class resistance can be detected in clinical pathogens before large losses occur, it may be possible to impose interventions to mitigate the impact of future novel viral outbreaks within the Ontario swine industry.
Antibiotic use and treatment efficacy in Ontario swine

The Ontario Swine Veterinary-based Surveillance (OSVS) program database was assessed in order to better understand the use of antimicrobials to treat clinical conditions in Ontario swine and the efficacy of these treatments. Using reports from swine veterinarians, multi-level logistic regression modeling was used to describe the relationship between treatment failure, body system affected in the treated animal and antibiotic use. Significant predictors included body system affected, whether multiple antibiotics were used (as compared to a single product) and the use of neomycin. The odds of treatment failure were significantly reduced if the record indicated that the gastrointestinal (GI) system was affected, as compared to all other body systems. This finding likely reflects improved performance of feed- and water-administered antibiotics upon gastrointestinal pathogens as compared to pathogens affecting other body systems, for which pharmacokinetics and pharmacodynamics may play a stronger role. In contrast, the odds of treatment failure were significantly increased if >1 antibiotic was used; likely reflecting the treatment of non-bacterial pathogens, or the presence of pathogens that display multiple resistances.

This work provides some valuable information for stakeholders in the Ontario pork industry surrounding the use of antibiotics. In the case of non-GI infections in swine, which are at higher odds of antimicrobial treatment failure, a more holistic approach to herd health may be advantageous over antimicrobial use. These measures may include re-visiting vaccination, management and biosecurity protocols. In addition to improving health at the herd level, these changes may be viewed as a form of
antimicrobial stewardship for the industry by reducing required antimicrobial inputs and consequently, selection pressure for AMR.

**FINAL REMARKS**

In conclusion, the work described in this thesis supports the continuing enhancement of AMR surveillance systems to improve the health of livestock in Ontario. Collaboration between clinical veterinary laboratories, epidemiologists, regulators, veterinary practitioners, and computer scientists is required in order for surveillance systems to stay ahead of the continual evolution of pathogens. High quality, consistent data, appropriate and accessible analytical methods, and innovation in terms of syndromic data and dissemination strategies are major factors to be considered. With careful design, a surveillance system may be designed to provide information to aid practitioners in selecting appropriate antimicrobials for the treatment of clinically ill animals, as well as to regulators aiming to reduce selection pressure for AMR and ensure that these products are efficacious well into the future.
REFERENCES


Danish Integrated Antimicrobial Resistance Monitoring and Research Programme 2011 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. Copenhagen, Denmark: Statens Serum Institut, Danish Veterinary and Food Administration, Danish Medicines Agency, and Danish Institute for Food and Veterinary Research, 2012.


APPENDIX A

SUMMARY OF DATA AVAILABILITY FOR CLINICAL PATHOGENS OF CATTLE AND CHICKEN
Table A.1: Number of susceptibility tests (with ≥1 antimicrobial tested) performed upon clinical pathogens of Ontario cattle at the Animal Health Laboratory, by year.

<table>
<thead>
<tr>
<th>Year</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007*</th>
<th>2008</th>
<th>2009</th>
<th>2010#</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli F4</em></td>
<td>7</td>
<td>33</td>
<td>70</td>
<td>83</td>
<td>81</td>
<td>78</td>
<td>49</td>
<td>42</td>
<td>43</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus somnus/Histophilus somni</em></td>
<td>21</td>
<td>16</td>
<td>15</td>
<td>22</td>
<td>13</td>
<td>16</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>11</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td><em>Mannheimia/Pasteurella haemolytica</em></td>
<td>41</td>
<td>60</td>
<td>58</td>
<td>81</td>
<td>53</td>
<td>57</td>
<td>51</td>
<td>33</td>
<td>36</td>
<td>33</td>
<td>20</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>14</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><em>Pasteurella multocida</em></td>
<td>32</td>
<td>36</td>
<td>42</td>
<td>72</td>
<td>45</td>
<td>44</td>
<td>40</td>
<td>23</td>
<td>26</td>
<td>24</td>
<td>23</td>
<td>18</td>
<td>12</td>
</tr>
</tbody>
</table>

*Data up to May 2007
#Data up to July 14th, 2010
Table A.2: Number of susceptibility tests (with ≥1 antimicrobial tested) performed upon clinical pathogens of Ontario chicken at the Animal Health Laboratory, by year.

<table>
<thead>
<tr>
<th>Year</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>270</td>
<td>370</td>
<td>405</td>
<td>276</td>
<td>279</td>
<td>295</td>
<td>395</td>
<td>364</td>
<td>311</td>
<td>127</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>29</td>
<td>50</td>
<td>69</td>
<td>34</td>
<td>41</td>
<td>47</td>
<td>27</td>
<td>51</td>
<td>43</td>
<td>13</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>81</td>
<td>72</td>
<td>49</td>
<td>45</td>
<td>35</td>
<td>30</td>
<td>22</td>
<td>28</td>
<td>26</td>
<td>7</td>
</tr>
</tbody>
</table>

*Data up to May 2007*
APPENDIX B

RECOMMENDATIONS FOR DESIGNING LABORATORY INFORMATION SYSTEMS AND SUBMISSION FORMS TO BENEFIT SURVEILLANCE
Recommendations are made in five areas that may improve LIMSs and submission forms to improve the quality and quantity of antimicrobial susceptibility data for use in surveillance:

1. Electronic submission forms
2. Elements used for data collection
3. Isolate-specific identifiers
4. Data retrieval
5. Antimicrobial panels

In addition, this summary will comment on the presentation of AMR surveillance results to stakeholders, the use of analytical methods to describe susceptibility results for surveillance, the use of multiple-class susceptibility testing data as a syndromic surveillance tool, and the relationship between antimicrobial use and treatment efficacy in Ontario swine.

**Recommendations for improving laboratory information systems and submission forms to benefit surveillance**

Each of the issues encountered in this work could be resolved by changes to the LIMSs and submission forms. It cannot be assumed that the programmers who create LIMSs are aware of the data requirements and best practices for data collection. As the use of particular design elements can benefit or hinder surveillance, clear direction from surveillance stakeholders should occur in order for the design elements to be implemented. Although recommendations for the objectives, operations, conceptual designs and characteristics of regional, national and global animal health surveillance
systems are available and well developed (Food and Agriculture Organization of the United Nations, 2011), to our knowledge, there are no published resources describing specific design elements for LIMS and submission forms to support the use of laboratory data for passive surveillance. Even a faultless system in terms of objectives, operations, analysis and reporting is limited by the quality and quantity of the data available.

Therefore, suggestions for LIMS and submission form design are made here using concrete examples from AHL data. The goal of these suggestions is to assist in the development of LIMSs and submission forms to improve the quality and quantity of data such that the data could be easily used for passive surveillance, with minimal impact on day-to-day function at the laboratory level. Although a number of these suggestions may seem simplistic from the viewpoint of a surveillance-focused epidemiologist, care must be taken to recognize *a posteriori* knowledge gained through immersion in epidemiological work. Finally, although the examples described here are pulled from a veterinary laboratory with a three-tier data complexity (animal, owner/producer and practitioner), they can be applied to human LIMS with two-tier data complexity (patient and practitioner) as well.

*Electronic submission forms*

Given the choice, the authors suggest that electronic submission forms be employed preferentially over paper forms in order to take advantage of the time saving potential and the added functionality. Considerable time savings for laboratory personnel are available with the use of electronic submission forms, as the submission form itself may be used to populate the database directly, thereby reducing the workload required by
laboratory personnel by eliminating the transposing step. Furthermore, electronic submission allows for the development of submitter profiles, which could reduce the workload for repeat submitters while concurrently reducing recording inconsistencies for submitter demographic information. Unique profiles for each submitter (e.g., practitioner or producer) may be saved and used to self-populate submission forms on an ongoing basis. The use of profiles allows repeat users to skip large portions of submission forms without decreasing the quality or quantity of data submitted to the laboratory or surveillance system. For example, a profile may include the submitter’s name and contact information (address, phone number) and other information that may be informative for surveillance or targeted interventions (e.g., sex, birth date and educational institution of the practitioner, given that these factors may influence submission patterns). Finally, the use of profiles will assist in eliminating recording inconsistencies for submitting users. For example, the AHL data were found to include multiple spellings and formats for veterinary practice names (Chapter 1). A profile system would eliminate these inconsistencies and limit manual correction of practitioner/practice names for the surveillance team, as well as streamline contact information for laboratory personnel.

In addition, the functionality of electronic submission forms provides an opportunity to reduce missing data for all required variables. To improve data recording consistency, electronic forms may be coded such that questions are mandatory. Mandatory questions require the user to provide a response for all variables required by both the laboratory and the surveillance system. For example, information regarding the age, weight and production level of the animal from which samples were collected were commonly missing from the AHL data (Chapter 1). An electronic form may be coded so
that a prompt occurs when no response is given for these variables, asking for the user to respond. A “not applicable” option should be available for each of these mandatory elements however, such that users in unique situations may bypass the requirements when necessary.

Electronic forms also prevent submitters from checking boxes that are contradictory/mutually exclusive. For example, a user may accidentally check both “yes” and “no” for the same prompt on a paper form. However, electronic forms are coded such that only one of these boxes may be checked. Therefore, the quality of data may be improved through electronic submission forms.

If it is not possible to switch to electronic submission forms, it is recommended that the LIMS data entry displays be designed as a replica of the paper form to limit the potential for transposition errors or omissions (Jarossi, 2006).

Elements used for data collection

The use of various elements on submission forms can contribute to the quality of data collected. A properly designed form may increase the sensitivity and specificity of questions, resulting in a more informative database with reduced misclassification. Elements that should be avoided are checkboxes, free-form entry fields.

Checkboxes are a commonly used design element on submission forms due to their small size and, generally, these elements are simple to understand. The assumption of checkboxes is that the submitter will check the box when the answer to the given question is “true” or “yes”. Thus, the resulting assumption is that an empty checkbox is a “no” response. However, there is another function that may lead to an empty checkbox -
skipped questions. Given the fast pace of veterinary practice and the number of forms and surveys that individuals encounter on a day-to-day basis, skipped questions are expected to be a common occurrence due to survey fatigue or indifference (Porter et al., 2004). Therefore, empty checkboxes may also reflect a non-answer and the resulting responses may have a low negative predictive value, which can lead to non-informative data at best, or incorrect interpretation of results. The use of bullets may be a more appropriate option for yes/no questions. Bullets have both “yes” and “no” options. Therefore, if no mark is made to either bullet, a non-answer can be assumed.

Similarly, problems at the point of data analysis occur with free-form entry boxes. These boxes allow the user to input strings of characters without any restrictions (with the exception of length in an electronic form, if a maximum is set). Therefore, if these elements are used for inputting producer/practitioner/clinic names, the responses may differ between submissions by the same producer/practitioner/clinic, merely due to typographical or formatting differences and/or errors. The previous suggestion for profile use may reduce or eliminate this problem. Although not encountered within the AHL data, another example where free-form entry may result in data inconsistencies is the date field; dates may be recorded numerically or alphanumerically and the year/month/day values may be transposed, causing confusion at the analysis stage. On paper forms, replacing one free-form entry element for date with three clearly labeled separate elements is suggested to improve data recording quality. As with the other elements discussed, additional control is available with the electronic forms; date fields may be set to display prompts if the date entered is not appropriate (e.g., a yyyy/mm/dd field would not allow a user to input values >12 in the month portion of the field). This would not
prevent transposition between the month and date portions for the days 1 through 12 in each month, however. An alternative option is using a date field that requires text for the month portion, or three separate drop-down menus with month names in text.

Although these are just a few examples of issues that may be faced when analyzing data from an electronic database developed without surveillance efforts in mind. As such, the authors suggest that surveillance personnel assess the most appropriate elements for use when new submission forms and entry displays are designed.

In addition to the element suggestions made for submission forms, the elements chosen for use within entry display for laboratory personnel provides an avenue for improving data quality for surveillance purposes. The preferential use of drop-down menus over free-text fields is suggested, particularly for fields such as the bacterial pathogen isolated and the results of susceptibility testing if resistant/intermediate/susceptible (R/I/S) coding is used. Drop-down menus limit the response to options that are pre-formatted, therefore eliminating the requirement for data cleaning in these fields at the time of analysis, which is especially important in the context of real-time surveillance, where timeliness is crucial.

Isolate-specific identifiers

A major hurdle faced when assessing AHL swine data was linking specific Escherichia coli isolates with susceptibility results to the F4 typing tests. Although it was an uncommon occurrence, submissions were found that included > 1 E. coli isolate and > 1 set of susceptibility testing results. As only F4 positive isolates (not all E. coli) were
desired for surveillance purposes, the isolation and susceptibility results needed to be merged with a separate dataset that included the F4 typing results. Unfortunately, while the F4 susceptibility dataset included a unique isolate identifier, this identifier could not be extracted with the isolation and susceptibility results data. Therefore, direct merging of these datasets was not possible and we were forced to revisit the individual pathology reports for all submissions with > 1 *E. coli* isolate and > 1 set of susceptibility testing results to identify which susceptibility results belonged to the F4 positive isolate. Had an isolate-specific identifier been available for extraction within all of the datasets, data merging would have been straight-forward and semi-automated for future use. Therefore, in order to support a surveillance program, we suggest that isolate-specific identifiers be used (and are available for extraction) when multiple isolates are possible within a single submission, with particular emphasis on use when sub-typing occurs and multiple datasets are required to contain all information relating to a submission.

*Data retrieval*

The format of extracted data from the LIMS may affect the workload required by surveillance personnel in terms of merging, formatting and data cleaning. For surveillance analysis purposes, the most convenient data display consists of a spreadsheet with variables across the columns and a unique row for each isolate. Therefore, submissions with > 1 isolate would have repeated data within the producer/practitioner/clinic data columns. Although this may seem intuitive for those familiar with surveillance databases, it is worth noting before preparations are made for new system development, as duplicated data are expected to be actively avoided when
designing an efficient data storage system and unnecessary for the output required by the laboratory. The output required by laboratory personnel/veterinarians accessing laboratory services is quite different from the ideal surveillance output; these stakeholders require a single record with all pertinent information from a single submission for diagnosis and treatment. Therefore, a system that supports both diagnostic laboratory work and surveillance would allow for single-submission reports, as well as large database extraction of numerous submissions. Ideally for surveillance, the export function would allow for retrieval by date ranges, animal species and pathogen(s) isolated.

*Antimicrobial panels*

Specific to AMR surveillance systems is the choice of the standard panel of antimicrobials used for susceptibility testing. Currently, AMR systems using passive laboratory data can only track changes in antimicrobials that are currently tested by the laboratory and/or have been tested in the past (in order to make comparisons or visualize trends over time). Therefore, a consistent panel and interpretive criteria are required to support efficient surveillance. Additions or removal of antimicrobials from the commonly tested panels and changes in interpretive criteria make interpretation difficult, or impossible. For example, the removal of neomycin from the AHL’s *E. coli* panel in 2005 caused the rolling-average plots to appear unstable due to small denominators. Furthermore, a change in the interpretive criteria for spectinomycin in *P. multocida* caused the rolling average plot to drop dramatically in 2000. Without the knowledge of
lab-level changes such as these, surveillance results may be misinterpreted and believed to reflect real changes in the prevalence of resistance.

Although trends in resistance cannot be reliably tracked for a pathogen/antimicrobial combination if susceptibility testing changes over time, at the laboratory level, changes may occur relatively frequently. The antimicrobials tested are based upon the label claims of particular products and recommended use guidelines, as these are the antimicrobials most immediately applicable to the diagnosis and treatment information required by the submitter. Therefore, although testing for susceptibility of an antimicrobial no longer used in practice is illogical at the laboratory level, to individuals conducting surveillance, continued monitoring of a product that has been removed from the market provides key information, particularly about the rate at which AMR prevalence declines or persists upon the removal of a particular antimicrobial drug or class. Considerable communication between laboratory and surveillance personnel may be required in order to come to a consensus about the most advantageous standard panel for susceptibility testing.

An issue that may require discussion between laboratory and surveillance personnel is that of the ethics surrounding a common panel and how susceptibility results are displayed on reports to the submitters. For example, if the common panel includes antimicrobials classified as critically important by the World Health Organization (WHO) (such as the third and fourth generation cephalosporins) (World Health Organization, 2011), is it ethical to list that an isolate is susceptible to these products, considering that they are highly discouraged from use? Given that a susceptible result does not necessarily mean that the product is a good choice for treatment, it may be prudent to discuss
whether susceptibility reports should include a note about these products, describing that they should be used with caution, only as a third- or fourth-line treatment option. Another option would be to include all antimicrobials for surveillance purposes, but to limit the scope of susceptibility results printed on individual reports sent to practitioners, or to indicate on reports that the third- and fourth-line antimicrobials are only displayed for information purposes. This final option allows veterinarians and producers to be aware of how closely they/their animals are linked to seriously resistant organisms.

We recognize that changes will be inevitable, given that logical changes in breakpoints will occur over time as pathogens evolve, and that new products will be added to the market. Therefore, we suggest that documentation occur at the time of these changes and that this documentation includes an explanation of how the change impacts the interpretation of results. An example of suitable documentation is provided online by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (Government of Canada, 2010).
REFERENCES


