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Comparison of mixed effects models of antimicrobial resistance metrics of livestock and poultry *Salmonella* isolates from a national monitoring system

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ABSTRACT

Antimicrobial use in agriculture is considered a pathway for the selection and dissemination of resistance determinants among animal and human populations. From 1997 through 2003 the U.S. National Antimicrobial Resistance Monitoring System (NARMS) tested clinical *Salmonella* isolates from multiple animal and environmental sources throughout the United States for resistance to panels of 16–19 antimicrobials. In this study we applied two mixed effects models, the generalized linear mixed model (GLMM) and accelerated failure time frailty (AFT-frailty) model, to susceptible/resistant and interval-censored minimum inhibitory concentration (MIC) metrics, respectively, from *Salmonella enterica* subspecies *enterica* serovar Typhimurium isolates from livestock and poultry. Objectives were to compare characteristics of the two models and to examine the effects of time, species, and multidrug resistance (MDR) on the resistance of isolates to individual antimicrobials, as revealed by the models. Fixed effects were year of sample collection, isolate source species and MDR indicators; laboratory study site was included as a random effect. MDR indicators were significant for every antimicrobial and were dominant effects in multivariable models. Temporal trends and source species influences varied by antimicrobial. In GLMMs, the intra-class correlation coefficient ranged up to 0.8, indicating that the proportion of variance accounted for by laboratory study site could be high. AFT models tended to be more sensitive, detecting more curvilinear temporal trends and species differences; however, high levels of left- or right-censoring made some models unstable and results uninterpretable. Results from GLMMs may be biased by cutoff criteria used to collapse MIC data into binary categories, and may miss signaling important trends or shifts if the series of antibiotic dilutions tested does not span a resistance threshold. Our findings demonstrate the challenges of measuring the AMR ecosystem and the complexity of interacting factors, and have implications for future monitoring. We include suggestions for future data collection and analyses, including alternative modeling approaches.

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1. Introduction

Antimicrobial resistance (AMR) is a serious global threat to animal and human health. Antimicrobial use in agriculture is considered a pathway for the selection and dissemination of determinants of resistance; consequently, the purposes and uses of antimicrobials in animal agriculture have raised concerns among stakeholders and prompted multiple agencies to respond. The U.S. Department of Agriculture (USDA) established an Action Plan for AMR monitoring and response (USDA, 2014), and the U.S. White House has implemented a multi-agency National Action Plan for Combating Antibiotic Resistant Bacteria (U.S., 2015). The U.S. Food and Drug Administration (FDA) has issued guidances for food animal and veterinary pharmaceutical industries seeking to eliminate the use of medically important antimicrobials for growth promotion in animal agriculture and to bring the remaining therapeutic uses under veterinary oversight (FDA, 2012, 2013). The U.S. Centers for Disease Control and Prevention's most recent report on antibiotic resistance in the United States (CDC, 2013) categorized drug resistant non-typhoidal *Salmonella* and *Campylobacter* as serious threats. Each of these reports and initiatives cites gaps in knowledge and emphasizes the importance of surveillance and monitoring, research and development, and the need to strengthen capacity for detection and response to urgent and emerging threats. Analyses of

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data collected systematically and targeting animals, food products and consumers across broad geographies are essential components of monitoring and response systems.

The ability to discern trends in AMR monitoring data may be influenced by the AMR metric. Quantitative metrics such as minimum inhibitory concentrations (MICs) are often categorized into susceptible (S), resistant (R), or intermediate (I, usually combined with S) and interpreted via internationally accepted breakpoints or epidemiologic cutoffs. Investigators modeling dichotomous S/R values or interval-bounded MIC values via logistic regression, survival analyses, and a Bayesian implementation of a multilevel regression have demonstrated shifts and differences in susceptibility (Stegeman et al., 2006; van de Kassteele et al., 2012; CDC, 2013).

In this study we investigated mixed effects regression modeling of AMR metrics of clinical isolates from livestock and poultry specimens tested as part of the U.S. National Antimicrobial Resistance Monitoring System (NARMS) (CDC, 2014a,b). The first objective was to compare generalized linear mixed models (GLMM) and accelerated failure time frailty (AFT-frailty) models to ascertain similarities and differences between the two approaches. The second objective was to examine trends and influential covariates for *Salmonella* isolate AMR fixed effects while adjusting for random variability within a national laboratory network. We describe results obtained with the two approaches and the complexity of measuring influential factors for AMR, and suggest improvements in data collection and analyses.

2. Materials and methods

2.1. Data

From 1997 to 2003 a component of NARMS' surveillance activities was directed at testing *Salmonella* isolates for antimicrobial susceptibility from diagnostic fecal samples of clinically ill domestic animals. A subset of isolates submitted by practitioners to State veterinary diagnostic laboratories (VDLs) was tested for susceptibility at the USDA's Agricultural Research Service (ARS) laboratory in Athens, GA. Isolates either came directly from State VDLs or were recovered from a bank of stored isolates at the USDA's National Veterinary Services Laboratories (NVSL) in Ames, IA. Because NVSL isolates also originated from VDLs, isolates from the VDLs that submitted directly to the ARS were excluded when isolates were selected from the NVSL bank. Isolates were randomly sampled, stratified by source. Susceptibility testing was performed according to NARMS protocols. The number of antimicrobials in test panels varied from 16 to 19. Serial dilutions within panels depended on the specific antimicrobial and could vary over time. MICs were determined by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) standards, with dilutions and number of wells per antimicrobial selected to span a range containing expected breakpoints (USDA, 1998a,b, 1999, 2000, 2001, 2002, 2003).

The NARMS dataset contained 215 *Salmonella* serotypes. *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) isolate data were selected for modeling based on the serotype's broad host range. According to the NARMS program, in 1998–2000, *S.* Typhimurium represented 10–20% of *Salmonella* isolations from food animal carcasses, and in the U.S. in 2000 accounted for 19% of human *Salmonella* infections (Rigney et al., 2004).

Covariates were based on available data and included the isolate source species, year of specimen collection, the study site (laboratory) of isolate testing, and an indicator of multidrug resistance (MDR). MDR is a major animal and public health concern and is characteristic of some phage types of *S.* Typhimurium, e.g., DT 104, which began circulating globally in the 1990's (McDermott, 2006). MDR phenotypes, such as the ACSSuT phenotype for the chromosomal genotype that encodes resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, are clinically important for both animals and humans and are specifically monitored by NARMS (Greene et al., 2008; USDA, 2003).

2.2. Statistical analysis

2.2.1. Mixed effects logistic regression

GLMMs assume a conditional response distribution from the exponential family and accommodate fixed and random effects (McCulloch and Searle, 2001). In this study, S/R responses were modeled via the binomial distribution with a logit link. For each isolate (i) and antimicrobial ($a = 1,\ldots,A$), fixed effects were source species of isolate (s), time (t = year of isolate collection−1997), and an MDR indicator (m), the number of other (A-1) antimicrobials the isolate was resistant to. Study site (l) was included as a random intercept. In our implementation, $y_{ia}|\gamma_l = 1$ if isolate i was resistant to antimicrobial a and zero else, $\gamma \sim \text{Normal}\left(0, \sigma_G^2\right)$.

Parametric estimation of GLMM coefficients is complex, with the computational method dependent on data characteristics, model specification, and estimates needed (Bolker et al., 2008). Because one objective of our study was to compare parameter estimates between GLM and AFT-frailty models, and because some study sites and source species had small numbers of isolates, we used maximum likelihood (ML)-Laplace approximation for parameter estimation. However, ML estimation requires integration of likelihoods over all possible values of random effects, with residual error variance, $\hat{\sigma}_R^2$, not estimable concurrently with the intra-class (study site) covariance, $\hat{\sigma}_G^2$. We used residual pseudolikelihood (RSPL) to obtain the intra-class correlation coefficient, $\widehat{\text{IC}}_a$, the proportion of random study site variance to total variance (McCulloch and Searle, 2001).

For each antimicrobial, individual covariates were tested in the univariate and those significant at $p \leq 0.20$ were kept for multivariable modeling. In each candidate model, $m = 0,\ldots, A\text{-}1$, and $t = 0,\ldots,6$, were entered as linear and quadratic terms. Fixed effects, including linear and quadratic terms, were maintained in multivariable models if significant (at $p \leq 0.05$ level), and if the inclusion of the term contributed to the best fit of the model based on Akaike information criteria (AIC) values and model parsimony. Because we were interested in comparing coefficient estimates for trends between the two model types, we report $\hat{\beta}$ estimates and plots of the linear components (where $\text{LC} = \hat{\beta}_1 x + \hat{\beta}_2 x^2; x = 0, \ldots, 6$, for time and $x = 0,\ldots,A\text{-}1$, for the MDR indicator) for individual antimicrobials for comparison with AFT-frailty model $\hat{\alpha}$-coefficients and linear components. Analyses were conducted with R and SAS statistical software (R Core Team, 2013; SAS Institute Inc., 2008).

2.2.2. Accelerated failure time-frailty model

The AFT-frailty model is a parametric survival model with fixed effect covariates multiplicative with respect to MIC values and a shared frailty, an unobserved random effect, appropriate for modeling interval censored data (Collett, 2003; Lindsey and Ryan, 1998). AFT-frailty models require assumptions for distributions for the hazard and the random effect. The Weibull distribution allows a monotonically increasing or decreasing hazard over the series of concentrations of antimicrobials tested. The general Weibull AFT model for MIC_{ia} with a shared frailty is:

$$h_{ij}\left(\text{MIC}_{ia}\right) = e^{-\eta\_iaj}\lambda p(e^{-\eta\_iaj}\text{MIC}_{ia})^{p-1}$$

with linear component, $\eta_{iaj} = \alpha \prime x_{iaj} + \tau_j$ for isolate i, antimicrobial a, in study site group j, $\tau \sim \text{Gaussian}\left(1, \theta\right)$ (Collett, 2003). The $\hat{\alpha}$