

RESEARCH ARTICLE

A hierarchical Bayesian latent class mixture model with censorship for detection of linear temporal changes in antibiotic resistance

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Data Availability Statement: The CDC NARMS data (for application examples 1 and 2) can be found either in the "Download" panel of the website <https://wwwn.cdc.gov/narmsnow/>, or in the Github repository https://github.com/MinZhang95/AMR-Linear/blob/master/IsolateData_all%20NARMS%20Now%20data.csv. Please note that the data on the CDC website can be updated by the NARMS program, while the Github data was downloaded from the CDC website in March 2019. The ISU VDL data (for application example 3) is also available in the Github repository

Abstract

Identifying and controlling the emergence of antimicrobial resistance (AMR) is a high priority for researchers and public health officials. One critical component of this control effort is timely detection of emerging or increasing resistance using surveillance programs. Currently, detection of temporal changes in AMR relies mainly on analysis of the proportion of resistant isolates based on the dichotomization of minimum inhibitory concentration (MIC) values. In our work, we developed a hierarchical Bayesian latent class mixture model that incorporates a linear trend for the mean \log_2 MIC of the non-resistant population. By introducing latent variables, our model addressed the challenges associated with the AMR MIC values, compensating for the censored nature of the MIC observations as well as the mixed components indicated by the censored MIC distributions. Inclusion of linear regression with time as a covariate in the hierarchical structure allowed modelling of the linear creep of the mean \log_2 MIC in the non-resistant population. The hierarchical Bayesian model was accurate and robust as assessed in simulation studies. The proposed approach was illustrated using *Salmonella enterica* I,4,[5],12:i:- treated with chloramphenicol and ceftiofur in human and veterinary samples, revealing some significant linearly increasing patterns from the applications. Implementation of our approach to the analysis of an AMR MIC dataset would provide surveillance programs with a more complete picture of the changes in AMR over years by exploring the patterns of the mean resistance level in the non-resistant population. Our model could therefore serve as a timely indicator of a need for antibiotic intervention before an outbreak of resistance, highlighting the relevance of this work for public health. Currently, however, due to extreme right censoring on the MIC data, this approach has limited utility for tracking changes in the resistant population.

Introduction

Rationale

Identifying and controlling the emergence of antimicrobial resistance (AMR) is a high priority for researchers and public health officials. A critical component of this control effort is

https://github.com/MinZhang95/AMR-Linear/blob/master/Reduced%20Food%20Animal%20Susceptibility%20and%20Salmonella%20NVSL%20from%202003_used%20for%20PLOS%20ONE%20linear%20paper.xlsx

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surveillance for emerging or increasing resistance, as evidenced by the number and scale of surveillance programs around the world [1] [2]. The aims of these surveillance programs are to enable detection of emerging resistance in a timely manner and to facilitate antimicrobial stewardship programs to be implemented properly and accurately [3]. Currently, detection of temporal changes in AMR relies primarily on analysis of the proportion of resistant isolates based on the dichotomization of minimum inhibitory concentration (MIC) [3]. The MIC can be obtained from laboratory methods or machine-learning approaches [3] [4]. Regardless of the approach to MIC determination or the breakpoint used, dichotomization results in a loss of information.

Previous work and challenges

To date, the predominant approach to assessing changes in AMR has focused on evaluation of changes in the proportion of isolates resistant to a particular antibiotic over time. Several statistical methods have been employed, including the Cochran-Armitage trend test, logistic regression model with time as a co-variate [5] [6] [7], and the Mann-Kendall non-parametric method to test monotonic trends over time [8]. These statistical methods are based on MIC data that are dichotomized to resistant and non-resistant. Mazloom *et al.* [9] pointed out that methods based on categorizations cause information loss. As such, a focus on changes in proportion of bacteria that are categorized as resistance means that changes in the mean MIC of isolates above or below the resistant breakpoint, a phenomena referred to as MIC creep/decline, are not included in the current surveillance monitoring [10]. Similarly, reliance on dichotomized MIC data prevents monitoring of correlations in mean MIC, despite the fact that such information would aid in the identification of emerging joint resistance patterns.

Mean MIC estimation must address the natural characteristics of MIC values, which are measured from serial dilution experiments [11] or obtained using the whole-genome sequencing based machine learning method [4]. Regardless of the method for obtaining MIC, currently observed MIC values are all censored. For example, an observed or predicted MIC of 8 for the organism A actually implies that the true MIC is >4 , ≤ 8 , and ultimately unknown. Estimation of the mean MIC without adjusting for censorship is biased and likely to overestimate the bacterial resistance to an antibiotic [12]. An additional issue is modeling of the underlying distribution of the true unknown MIC values. With respect to MIC data, bacteria isolates typically consist of a mixture of two components. Depending upon the focus (or region), these two populations may be considered resistant and non-resistant populations or wild-type and non-wild-type populations; however, the presence of two overlapping populations is commonly considered reasonable for these bacteria. For each component, the true concentration of antibiotic required to inhibit bacterial growth (i.e., the true MIC value) is believed to follow a log-normal distribution [13]; hence, the \log_2 MIC follows a normal distribution.

Statistical approaches for estimation of the mean MIC have been proposed previously. Kastele *et al.* [14] suggested a model for mean \log_2 MIC estimation that incorporated the censored nature of MIC data and adjusted for such bias using the interval-censored normal distribution as the underlying distribution. This model is a reasonable accommodation for censorship; however, the approach did not address the mixture of resistant and non-resistant populations in the observed data. Craig [15] proposed that the underlying distribution of \log_2 MIC can be modeled by a mixture of Gaussian distributions with resistant and non-resistant populations. Jaspers *et al.* [16] [17] [18] [19] modeled the full continuous MIC distribution for wild-type and non-wild-type bacteria populations determined by epidemiological cut-off rather than clinical breakpoints. According to their definition of bacterial categorization, the non-wild-type population has less informative distributions and was therefore estimated in

non-parametric ways. These previously published approaches suggest that \log_2 MIC mean can be estimated, although none of the above mentioned studies evaluated an approach for estimating a temporal trend in mean \log_2 MIC, and such an approach is clearly a critical need for AMR surveillance programs. Therefore, building upon these previous studies, we sought to describe an approach to estimate the mean \log_2 MIC and describe temporal changes in AMR, while simultaneously addressing the censored nature of MIC data and the mixed distributions of populations.

Contribution

In this paper, we proposed a hierarchical Bayesian latent class mixture model with censoring to detect temporal changes in AMR. This proposed model was applied to data from human samples from the Center for Disease Control National Antimicrobial Resistance Monitoring System (NARMS) surveillance program and swine samples from the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). The human data consisted of *Salmonella* I,4, [5],12:i:- tested with ceftiofur (TIO) and chloramphenicol (CHL). The swine data included *Salmonella* I,4,[5],12:i:- tested with TIO. Our applications revealed some interesting patterns in the Results Section. Simulation studies showed that our model was accurate and robust in the estimation of the mean \log_2 MIC in non-resistant populations, the linear temporal trend in non-resistant populations, and the proportion of resistant bacteria over time. Future work stemming from our model is also discussed in the Discussion Section. Inclusion of such analyses into current surveillance programs would provide additional insight for monitoring of temporal changes in AMR and would increase the value of information extracted from such surveillance systems.

Methods

Model notation and assumptions

Our hierarchical Bayesian model for detection of linear temporal changes in AMR must take into account the censored nature of the data and the underlying mixed distribution of the observations. The commonly used approach for analysis of two-fold serial dilution observations is to transform to base 2 logarithm. To account for censoring statistically, each observed MIC value was assumed to represent an interval of true MIC values rather than a single discrete point value. With the following notations, Table 1 explains the conversion between the observed \log_2 MIC values and a continuous scale interval (l_{ij}, u_{ij}) for each isolate and each antibiotic.

y_{ij}^* : observed value of \log_2 MIC for isolation j in year i ;

y_{ij} : latent true value of \log_2 MIC for isolation j in year i ;

Table 1. Conversion table between observed and latent \log_2 MIC.

y_{ij}^*	sensor type	l_{ij}	u_{ij}	$y_{ij} \in (l_{ij}, u_{ij})$
$\leq a$	left censored	$-\infty$	a	$-\infty < y_{ij} \leq a$
$= a$	interval censored	$a - 1$	a	$a - 1 < y_{ij} \leq a$
$> a$	right censored	a	∞	$a < y_{ij} < \infty$

The observed records of MIC from the CDC NARMS dataset range from = 0.016 to >256. Hence, the censored \log_2 MIC data, a , is commonly observed as integer numbers ranging from $\log_2(0.016) \approx -6$ to $\log_2(256) = 8$.

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l_{ij}, u_{ij} : lower bound and upper bound of the true value $y_{ij}, y_{ij} \in (l_{ij}, u_{ij})$; and c_{ij} : latent indicator of the bacterial population from which the observation was drawn, where $i = 1, 2, \dots, I$, and $j = 1, 2, \dots, n_i$. Here, I is the total number of years, and n_i is the total number of isolates tested in the i th year.

Denote S as the conversion function between y_{ij}^* and y_{ij} , and therefore, $y_{ij}^* = S(y_{ij})$, which is depicted in Fig 1 by the step-like plot. The distribution of the latent true value of \log_2 MIC was assumed to be a bimodal Gaussian mixture model, which corresponds to the underlying mixture of resistant and non-resistant populations.

We proposed a model with a linear trend in the mean \log_2 MIC over time. The motivation for this model stemmed from the results of a naïve analysis of *Salmonella enterica* I,4,[5],12:i:- and antibiotic CHL in the CDC NARMS dataset (the grey line displayed in Fig 2). Since the true \log_2 MIC cannot be observed with the raw data due to censoring, the naïve mean \log_2 MIC was calculated and presented over time. The naïve analysis for mean \log_2 MIC ignored the nature of censoring of MIC data, resulting in calculation of the arithmetic average of \log_2 MIC each year. For example, if the observed MIC value was ≤ 2 , then \log_2 MIC = $\log_2(2) = 1$ was treated as the corresponding \log_2 MIC value and was therefore used for the naïve mean calculation. Although mathematically this approach has some issues, it served to illustrate the linear

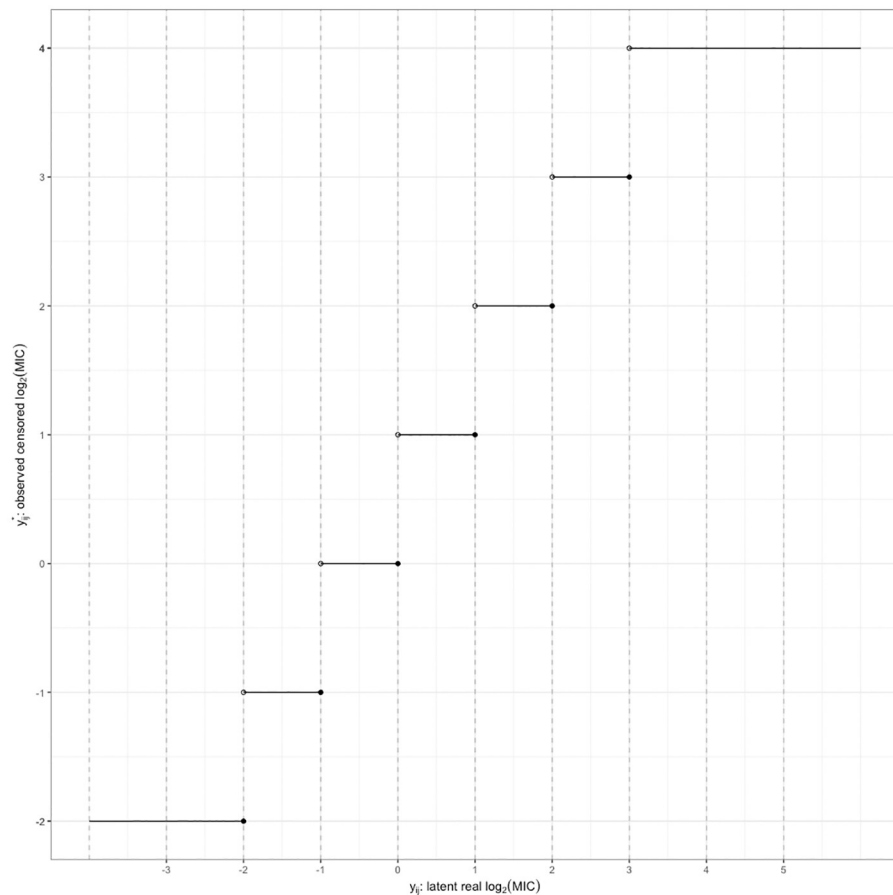


Fig 1. Conversion plot between observed and latent \log_2 MIC. In this example, the serial dilution experiment starts at $\text{MIC} = 2^{-2} = 0.25$ and ends at $\text{MIC} = 2^4 = 16$.

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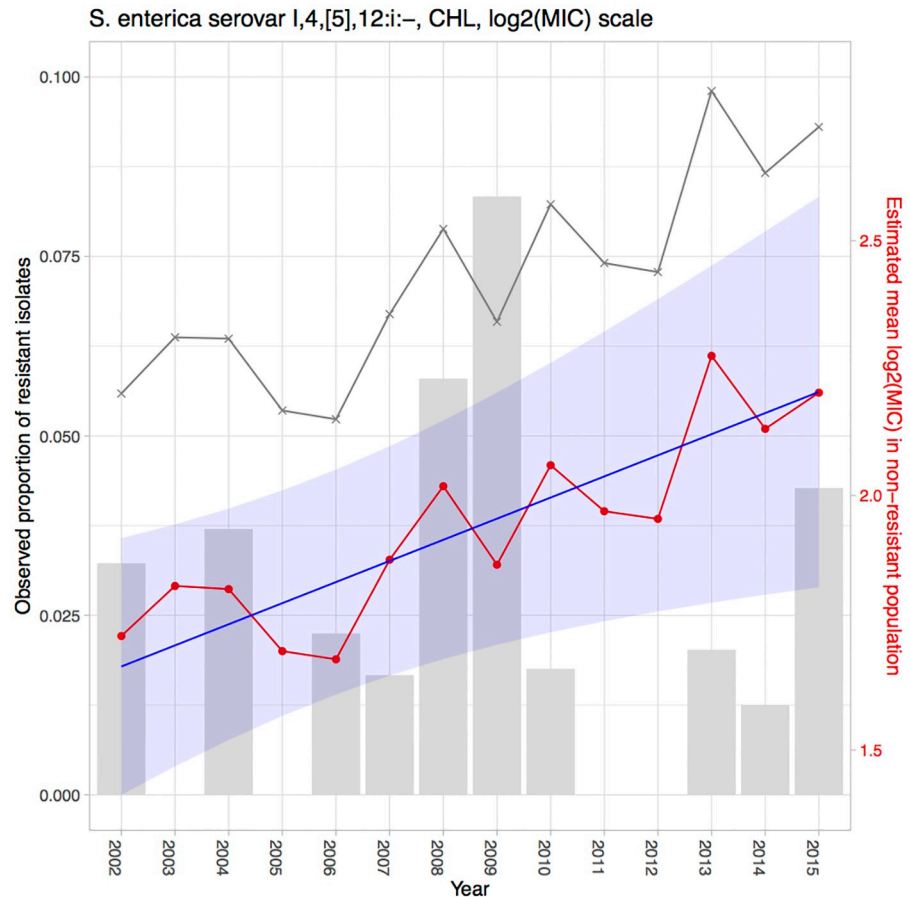


Fig 2. Estimation results for *Salmonella enterica* I,4,[5],12:i:- tested with CHL in the CDC NARMS dataset. The grey bars represent the observed proportions of resistant bacteria (left y-axis). The grey line indicates the naïve mean of log₂MIC in the non-resistant population. The red line connects the point estimates of the mean log₂MIC in the non-resistant population. The blue line represents the estimated linear trend, shaded with its 95% CI.

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trend in the non-resistant population. The potential to observe and assess the presence of a linear trend is less useful in the resistant population, because the majority of the observed log₂-MIC results are right censored at the highest concentration of the serial dilutions. As a consequence, in the resistant population, the naïve mean log₂MIC barely changed over time. Our assumption about the linear trend in the naïve mean log₂MIC was only applicable to the non-resistant population.

Model description

Below, we describe in detail the approaches we used; however, as an introduction, we first provide a brief lay summary of the approach. In the first level of the model, we modeled the data with a mixture Gaussian distribution within each year. This level of model did not allow for estimation of a time effect, because each year was analyzed separately without being imposed by any pattern in time. The second level added complexity to the model via regression of the yearly mean log₂MIC towards a line. For the resistant population, we assumed a flat line and that yearly randomness arose around this line. For the non-resistant population, we assumed a non-flat line that is a linear function of time. This time effect was estimated by a slope parameter in the model (described below): a positive result implies that the AMR is increasing with

time, while a negative result implies that the AMR is decreasing with time. We used the real data for such a model with two levels and showed that the linear trend model was able to quantify the year effect observed in the year-to-year mode. Based on the notations and assumptions described in the beginning of the Methods Section, the construction procedure of the hierarchical Bayesian latent class mixture model with censoring and linear trends is described as follows:

$$c_{ij} | p_i \stackrel{ind}{\sim} Ber(p_i), \tag{1}$$

$$y_{ij} | c_{ij}, \beta_{0i}, \beta_{1i}, \sigma_0^2, \sigma_1^2 \stackrel{ind}{\sim} \begin{cases} N(\beta_{0i}, \sigma_0^2), c_{ij} = 0 \\ N(\beta_{1i}, \sigma_1^2), c_{ij} = 1 \end{cases}, \tag{2}$$

where $i = 1, 2, \dots, I; j = 1, 2, \dots, n_i$. The variable before the pipe (“|”) was modeled with some distribution parameterized by the variable(s) behind the pipe. $Ber(p)$ denotes a Bernoulli distribution with probability p , and $N(\beta, \sigma^2)$ denotes a normal distribution with mean β and variance σ^2 . In the i th year, the j th isolate comes from the resistant population with probability p_i and from the non-resistant population with probability $1 - p_i$. The parameters β_{0i} represent the mean \log_2 MIC for the non-resistant group in i th year, and the parameters β_{1i} represent the mean \log_2 MIC for the resistant group in the i th year. The variances for both components, σ_0^2 and σ_1^2 , were set as invariant across the years, because we expected the spread of the observations within one population to be consistent over time. Using a predetermined cutoff value to categorize isolates into resistant and non-resistant can be problematic, because the threshold is not always consistent over years for some datasets (e.g., ISU VDL dataset contains examples of inconsistent threshold). With the Gaussian mixture model described in (1) and (2), the isolates for each year are separated into two components based on the posterior probabilities.

So far, the model allowed estimation of the mean \log_2 MIC for each year but has not imposed any constraints on the yearly means. Considering the heterogeneity of bacteria isolates in the MIC dataset, due perhaps to different sampling collection methods from year to year or different labs used to test isolates (e.g., CDC NARMS dataset contains data collected from multiple institutes), a hierarchical modeling strategy was adopted to borrow information about mean \log_2 MIC values across years and to integrate uncertainty from each individual year.

Based on the descriptive naïve means of \log_2 MIC in the non-resistant population, we proposed to incorporate a linear trend into the model above to describe the temporal changes of the mean \log_2 MIC in the non-resistant group for the organisms and antibiotics that appeared to be candidates for formal assessment of a linear pattern. First, we modeled the yearly mean \log_2 MIC of the non-resistant population by introducing the hyper-parameters γ_0 and γ_1 , with a simple linear model as follows:

$$\beta_{0i} = \gamma_0 + \gamma_1 t_i + \epsilon_i, \tag{3}$$

where $i = 1, 2, \dots, I, \epsilon_i \stackrel{iid}{\sim} N(0, \tau_0^2)$. Time (year) was used as a covariate with $t_i = i$. For the first year of our observation $t_i = i = 1$. This is equivalent to

$$\beta_{0i} | \gamma_0, \gamma_1, \tau_0^2 \stackrel{iid}{\sim} N(\mu_{0i}, \tau_0^2), \tag{4}$$

where $\mu_{0i} = \gamma_0 + \gamma_1 t_i$. Second, we modeled the yearly mean \log_2 MIC of the resistant population, using the hyper-parameter μ_1 which is a constant:

$$\beta_{1i} | \mu_1, \tau_1^2 \stackrel{iid}{\sim} N(\mu_1, \tau_1^2). \tag{5}$$

This model implied that the yearly mean of $\log_2\text{MIC}$ in the non-resistant population is distributed around a straight line with intercept γ_0 and slope γ_1 , with normally distributed error and variance quantified by τ_0^2 , while the yearly mean of $\log_2\text{MIC}$ in the resistant population is distributed around a constant μ_1 , also with normally distributed error and variance quantified by τ_1^2 . We modeled the yearly mean $\log_2\text{MIC}$ of the resistant population with a constant instead of a linear trend because the MIC determination of the observed $\log_2\text{MIC}$ is often highly right censored, meaning that we do not have enough information to estimate β_{1i} or its trend. If more studies reported end-point dilutions for resistant isolates, modeling the time trend in the resistant population would likely be feasible.

Further modeling on top of the first level, i.e. Eqs (1) and (2), involved addition of more hyper-parameters in the hierarchical structure for the proportion of the resistant population in the i th year, p_i . This parameter was modeled with a normal distribution through a logit link function:

$$\alpha_i := \text{logit}(p_i) = \log\left(\frac{p_i}{1 - p_i}\right), \tag{6}$$

$$\alpha_i | \theta, v^2 \stackrel{iid}{\sim} N(\theta, v^2); i = 1, 2, \dots, I. \tag{7}$$

Let Θ be the vector of all unknown parameters $(\gamma_0, \gamma_1, \mu_1, \theta, \tau_0^2, \tau_1^2, \sigma_0^2, \sigma_1^2, v^2)^T$; f be a generic expression for probability density function (pdf) or probability mass function (pmf). Also, $\mathbf{y}^* = (\mathbf{y}_1^*, \dots, \mathbf{y}_I^*)$, where $\mathbf{y}_i^* = \mathbf{y}_{i1}^*, \dots, \mathbf{y}_{in_i}^*$; $\boldsymbol{\beta}_0 = (\beta_{01}, \dots, \beta_{0I})$; $\boldsymbol{\beta}_1 = (\beta_{11}, \dots, \beta_{1I})$; $\mathbf{p} = (p_0, \dots, p_I)$; $i = 1, 2, \dots, I$. The joint likelihood function was used as follows:

$$f(\mathbf{y}^* | \Theta) = \prod_{i=1}^I \prod_{j=1}^{n_i} f(y_{ij}^* | \Theta), \tag{8}$$

$$\Leftrightarrow f(\mathbf{y}_{ij}^* | \Theta) = \int_{l_{ij}}^{u_{ij}} f(y_{ij} | \Theta) dy_{ij} = \int_{l_{ij}}^{u_{ij}} \sum_{c_{ij}=0,1} f(y_{ij}, c_{ij} | \Theta) dy_{ij}, \tag{9}$$

$$\Leftrightarrow f(y_{ij}, c_{ij} | \Theta) = \int_0^1 \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} f(y_{ij}, c_{ij}, \beta_{0i}, \beta_{1i}, p_i | \Theta) d\beta_{0i} d\beta_{1i} dp_i, \tag{10}$$

Based on densities and masses from Eqs (1) to (7):

$$\begin{aligned} &\Leftrightarrow f(y_{ij}, c_{ij}, \beta_{0i}, \beta_{1i}, p_i | \Theta) \\ &= f(y_{ij}, c_{ij}, \beta_{0i}, \beta_{1i}, p_i | \gamma_0, \gamma_1, \mu_1, \theta, \tau_0^2, \tau_1^2, \sigma_0^2, \sigma_1^2, v^2) \\ &= f(y_{ij} | c_{ij}, \beta_{0i}, \beta_{1i}, \sigma_0^2, \sigma_1^2) \times f(c_{ij} | p_i) \times f(\beta_{0i} | \gamma_0, \gamma_1, \tau_0^2) \times f(\beta_{1i} | \mu_1, \tau_1^2) \times f(p_i | \theta, v^2). \end{aligned} \tag{11}$$

For Eq (9), our latent variables y_{ij} and c_{ij} were integrated (summed) over their possible range (values) to obtain the likelihood function of the observed data. In Eq (10), the mean and proportion parameters were also integrated over their supports. Eq (11) shows the derivation of the likelihood of latent variables and parameters from the data model.

Prior distribution for hierarchical model parameters

The full Bayesian analysis required a joint prior distribution of all unknown parameters in the model. In our model setting, the vector of unknown parameters was

$\Theta = (\gamma_0, \gamma_1, \mu_1, \theta, \tau_0^2, \tau_1^2, \sigma_0^2, \sigma_1^2, v^2)^T$. Furthermore, we assumed independent prior

distribution for each parameter. The inverse gamma distribution was assigned to each variance of the data model and hierarchical part, due to their positive supports. The normal distribution was assigned to each linear parameter and each mean parameter of the hierarchical part, due to their supports on the whole real line. Since we did not have sufficient prior knowledge about these parameters, non-informative priors were used within the conjugate family. In particular, small values were chosen for the shape and scale parameters of the inverse gamma prior distribution; and large variance was chosen for the normal prior distribution.

$$\tau_0^2, \tau_1^2, \sigma_0^2, \sigma_1^2, v^2 \stackrel{iid}{\sim} IG(10^{-4}, 10^{-4}), \tag{12}$$

$$\gamma_0, \gamma_1, \mu_1, \theta \stackrel{iid}{\sim} N(0, 10^6). \tag{13}$$

Using the Bayesian rule, our goal was to obtain samples and draw inference from the posterior distribution, which can be expressed based on densities from (8) to (13):

$$\begin{aligned} & f(\Theta | \mathbf{y}^*) \\ \propto & f(\mathbf{y}^* | \Theta) f(\Theta) \\ \propto & f(\mathbf{y}^* | \Theta) f(\gamma_0) f(\gamma_1) f(\mu_1) f(\theta) f(\tau_0^2) f(\tau_1^2) f(\sigma_0^2) f(\sigma_1^2) f(v^2) \end{aligned} \tag{14}$$

The posterior distribution did not have a closed form, and we illustrated the sampling approach in the following section with some real data applications.

Application to real datasets

The goal of the assessment of the linear trend was to add a new dimension to understanding the temporal changes of AMR in both humans and livestock. To illustrate this, we applied the proposed model to human data from the CDC NARMS surveillance program and swine data submissions to the ISU VDL. The CDC NARMS data included *Salmonella* I,4,[5],12:i:- tested with CHL and TIO, while the swine data included *Salmonella* I,4,[5],12:i:- tested with TIO.

Description of the CDC NARMS data used

NARMS program collects isolates of *Salmonella* spp., *Escherichia coli* and *Campylobacter* spp. The AMR data collected for the CDC surveillance program were obtained from bacteria isolated from patients who attend public health departments or hospitals that are part of the CDC NARMS surveillance network between 1996 and 2015. In the CDC NARMS dataset, the most abundant species was *Salmonella enterica*, which accounted for 58.70% of the 54351 total isolates. Serotype I,4,[5],12:i:- had 892 records in the dataset, accounting for 2.79% of the *Salmonella enterica* isolates. We chose *Salmonella enterica* I,4,[5],12:i:- because this strain is an emerging pathogen with public health implications for both hosts. The antibiotics CHL and TIO were selected for evaluation of a linear trend based on evidence of a linear trend in the naïve mean log₂MIC in the non-resistant population descriptive analysis. For each of the two organism-antibiotic combinations, the distribution of the observed MIC was a mixture of two components: resistant and non-resistant populations. Therefore, the model assumptions were satisfied for these two combinations. Prior to the linear trend analysis, we discarded records before 2002, because the data were sparse for the first six years of the surveillance dataset (fewer than 10 isolates).

Description of the VDL data used

Organisms isolated from livestock and submitted to veterinary diagnostic laboratories offer insight into the emergence and patterns of AMR. This population of organisms could offer different and unique information about AMR, providing insight into the implications for the spread of resistance through the local environment as well as occupational exposure. Yuan *et al.* (2018) reported that the swine population submitted to the ISU VDL suggested emergence of the pathogen *Salmonella* I,4,[5],12:i:- sooner than the NARMS data. For comparison with the analysis for the CDC NARMS data and also for the sake of model assumptions, we applied the proposed model to a subset of ISU VDL data: *Salmonella enterica* I,4,[5],12:i:- tested with TIO from swine submissions. We did not evaluate CHL as for the CDC NARMS data, as this antibiotic was not used by the ISU VDL.

The ISU VDL dataset provided surveillance data on MIC from 2003 to 2018, comprising 93,634 isolates in total in the swine subset. Of these, 21,392 isolates are *Salmonella enterica*. Our target subset, *Salmonella enterica* serotype I,4,[5],12:i:- in the swine submissions included 1,967 isolates. We removed data obtained in 2018, because records in 2018 were not complete and still being processed in the diagnostic laboratory at the time of our analysis. We also excluded data from the first 8 years and focused on data obtained between 2011 and 2017, due to the sparsity of isolates observed in the first few years.

The implementation of the Bayesian model to the CDC NARMS dataset and the VDL dataset followed the same procedure. We describe the determination of the initial values of the Markov Chain Monte Carlo (MCMC) chain and calculation for inference in the next subsection.

Implementation

Our proposed hierarchical Bayesian latent class mixture model with censoring and linear trend was implemented using the MCMC Gibbs sampling method. The Gibbs sampling algorithm was adapted for censorship in a finite mixture model [20] [21]. The algorithm of the Gibbs sampler is provided in S1 Appendix. All computation was implemented using R 3.3.5. All R scripts (including data cleaning, model construction, model implementation, results extraction, and results visualization) are provided on Github repository: <https://github.com/MinZhang95/AMR-Linear>.

The initial values for MCMC were obtained from the raw data, so that the MCMC chain converged soon. For one combination of organism and antibiotic, the naïve mean of \log_2 MIC in the non-resistant population in each tested year with censorship was calculated as the initial values for β_{0i} . Similarly, the naïve mean of \log_2 MIC values in the resistant population in each year was calculated as initial values for β_{1i} , $i = 1, 2, \dots, I$. For the CDC NARMS dataset, $I = 14$, and for the VDL dataset, $I = 7$. The initial values for the linear parameters γ_0 and γ_1 were obtained from fitting β_{0i} and years $i = 1, 2, \dots, I$ with simple linear regression. The estimated standard deviation of the error term of the simple linear regression was used as the initial value for τ_0 . The sample mean and sample standard deviation of β_{1i} ($i = 1, 2, \dots, I$) were calculated as the initial values for μ_1 and τ_1 , respectively. The initial values for the proportion of the resistant population p_i were calculated by dividing the number of resistant isolates by the total number of isolates in each year. The initial values of α_i ($i = 1, 2, \dots, I$) were obtained by performing a logit transformation on the proportions p_i .

Ten thousand iterations were performed, and the remaining 6,000 iterations after the 4,000 burn-in iterations were collected to make inferences. The parameters in the model were estimated by the mean of posterior distribution. The 2.5th and 97.5th percentiles of those 6,000

samples of posterior draws were used for determination of the 95% credible interval (CI). Results are presented and interpreted in the following section.

Results

Using the proposed hierarchical Bayesian latent class mixture model with censoring and linear trend, we analyzed the resistance level of *Salmonella enterica* I,4,[5],12:i:- tested with CHL and TIO in the CDC NARMS human data and also *Salmonella enterica* I,4,[5],12:i:- tested with TIO in the ISU VDL swine dataset. The goal of the analyses was to illustrate the evaluation of increasing or decreasing trends in mean log₂MIC over time in order to identify trends with important public health implications. The estimates of the yearly mean log₂MIC in both the non-resistant ($\hat{\beta}_{0i}$) and resistant populations ($\hat{\beta}_{1i}$) and estimates of the proportions of the population designated as resistant (\hat{p}_i), along with their 95% CIs, are presented in Tables 2, 3 and 4. A linear model was fitted to the mean log₂MIC in the non-resistant population to borrow information across years and to reveal a potential linear trend. The intercept (γ_0) was interpreted as the baseline of the mean log₂MIC in the non-resistant population, while the slope (γ_1) was interpreted as an increase in the resistance from the previous year (for $i = 2, \dots, I$) or from the baseline (for $i = 1$). The point and interval estimates of the linear model parameters for each example are presented in Table 5. Among all the above-mentioned estimates, the estimates for the yearly mean log₂MIC ($\hat{\beta}_{0i}$) and the linear parameters ($\hat{\gamma}_0, \hat{\gamma}_1$) of the non-resistant population are of the greatest interest, as the main objective of our study was to use the proposed model to detect linear temporal changes in AMR in the susceptible group. If $\hat{\beta}_{0i}$ shows an increase over time and the estimated slope $\hat{\gamma}_1$ is positive, then this result could signify increasing resistance for the organism to the antibiotic. The estimates for these parameters are also presented in Figs 2, 3 and 4 for better visualization. The yearly mean log₂MIC in the resistant population ($\hat{\beta}_{1i}$) was not our priority in this study, as most of the observations in the resistant population are right censored and thus do not provide enough information for parameter estimations. This right censoring also underlay the wide credible intervals of $\hat{\beta}_{1i}$.

Table 2. Point estimates and 95% CIs for mean and proportion parameters for *Salmonella* I,4,[5],12:i:- tested with (CHL) from the CDC NARMS dataset.

Year	Estimated mean log ₂ MIC in the non-resistant population		Estimated mean log ₂ MIC in the resistant population		Estimated proportion of resistant population	
	$\hat{\beta}_{0i}$	95% CI of $\hat{\beta}_{0i}$	$\hat{\beta}_{1i}$	95% CI of $\hat{\beta}_{1i}$	\hat{p}_i	95% CI of \hat{p}_i
2002	1.7238	(1.5553, 1.8918)	14.7834	(7.9651, 22.0001)	0.0263	(0.0128, 0.0444)
2003	1.8222	(1.6545, 1.9887)	14.6345	(7.8851, 21.8576)	0.0252	(0.0084, 0.0384)
2004	1.8161	(1.6386, 1.9864)	14.4699	(8.4943, 21.7372)	0.0265	(0.0133, 0.0468)
2005	1.6942	(1.5195, 1.8615)	14.6031	(7.8794, 21.8553)	0.0245	(0.0067, 0.0333)
2006	1.6782	(1.5772, 1.7793)	14.2337	(8.6058, 22.2089)	0.0252	(0.0109, 0.0346)
2007	1.8737	(1.7584, 1.9877)	14.6124	(7.2965, 21.3269)	0.0253	(0.0096, 0.0370)
2008	2.0180	(1.9090, 2.1258)	14.8148	(8.0501, 22.2385)	0.0287	(0.0188, 0.0590)
2009	1.8640	(1.7291, 1.9975)	14.6725	(8.2464, 22.1023)	0.0301	(0.0210, 0.0711)
2010	2.0592	(1.9447, 2.1789)	14.7990	(8.4321, 21.7882)	0.0253	(0.0083, 0.0351)
2011	1.9689	(1.8522, 2.0900)	14.5946	(7.9317, 21.9562)	0.0239	(0.0061, 0.0308)
2012	1.9539	(1.8505, 2.0570)	14.6309	(7.9532, 22.0486)	0.0239	(0.0058, 0.0316)
2013	2.2740	(2.1836, 2.3674)	14.8033	(7.8064, 21.4324)	0.0253	(0.0128, 0.0336)
2014	2.1307	(2.0357, 2.2311)	14.5674	(8.3267, 21.7343)	0.0248	(0.0085, 0.0348)
2015	2.2017	(2.1175, 2.2860)	14.4117	(8.2791, 21.2827)	0.0275	(0.0185, 0.0478)

<https://doi.org/10.1371/journal.pone.0220427.t002>

Table 3. Point estimates and 95% CIs for mean and proportion parameters for *Salmonella* I,4,[5],12:i:- tested with (TIO) from the CDC NARMS dataset.

Year	Estimated mean log ₂ MIC in the non-resistant population		Estimated mean log ₂ MIC in the resistant population		Estimated proportion of resistant population	
	$\hat{\beta}_{0i}$	95% CI of $\hat{\beta}_{0i}$	$\hat{\beta}_{1i}$	95% CI of $\hat{\beta}_{1i}$	\hat{p}_i	95% CI of \hat{p}_i
2002	-1.3309	(-1.4990, -1.1657)	12.7589	(5.6945, 22.6436)	0.0415	(0.0246, 0.0596)
2003	-1.1844	(-1.3495, -1.0210)	12.7476	(5.6395, 22.6380)	0.0422	(0.0258, 0.0624)
2004	-1.3890	(-1.5780, -1.2017)	12.5196	(5.2118, 22.6231)	0.0431	(0.0271, 0.0654)
2005	-1.2564	(-1.4195, -1.0979)	12.7641	(5.6486, 22.7206)	0.0415	(0.0256, 0.0583)
2006	-1.1858	(-1.2808, -1.0922)	12.8693	(5.8527, 22.5644)	0.0423	(0.0287, 0.0586)
2007	-0.8702	(-0.9801, -0.7550)	12.7401	(5.5777, 22.7752)	0.0418	(0.0242, 0.0603)
2008	-0.9226	(-1.0280, -0.8147)	12.7304	(5.6264, 22.6573)	0.0427	(0.0274, 0.0622)
2009	-0.7899	(-0.9158, -0.6651)	12.7352	(5.6360, 22.6448)	0.0413	(0.0246, 0.0588)
2010	-0.8039	(-0.9231, -0.6844)	12.7766	(5.7197, 22.6692)	0.0417	(0.0250, 0.0576)
2011	-0.9334	(-1.0518, -0.8116)	12.7302	(5.5366, 22.6482)	0.0432	(0.0269, 0.0622)
2012	-0.6249	(-0.7338, -0.5156)	12.5240	(5.1599, 22.6408)	0.0404	(0.0218, 0.0562)
2013	-0.5035	(-0.5995, -0.4095)	12.7588	(5.5134, 22.7535)	0.0398	(0.0203, 0.0547)
2014	-0.8214	(-0.9197, -0.7222)	12.8010	(5.5933, 22.8088)	0.0424	(0.0242, 0.0604)
2015	-0.7063	(-0.7908, -0.6185)	12.7208	(5.8189, 22.6522)	0.0458	(0.0309, 0.0736)

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From the first row in Table 5, which shows the estimations of the linear model parameters for *Salmonella* I,4,[5],12:i:- tested with CHL in the CDC NARMS dataset, the 95% CI for the slope was (0.0088, 0.0773), indicating a significantly positive slope estimation and therefore a significantly increasing trend in the mean log₂MIC of the non-resistant population. Fig 2 depicts this increasing pattern by plotting the fitted regression line (blue line) through the

Table 4. Point estimates and 95% CIs for mean and proportion parameters for *Salmonella* I,4,[5],12:i:- tested with (TIO) in the ISU VDL dataset.

Year	Estimated mean log ₂ MIC in the non-resistant population		Estimated mean log ₂ MIC in the resistant population		Estimated proportion of resistant population	
	$\hat{\beta}_{0i}$	95% CI of $\hat{\beta}_{0i}$	$\hat{\beta}_{1i}$	95% CI of $\hat{\beta}_{1i}$	\hat{p}_i	95% CI of \hat{p}_i
2011	-1.5938	(-2.0234, -1.1577)	3.0631	(2.6063, 3.5410)	0.2214	(0.1648, 0.2733)
2012	-1.4962	(-1.6860, -1.3145)	3.3031	(3.0435, 3.6241)	0.2126	(0.1574, 0.2536)
2013	-1.0193	(-1.1410, -0.8924)	3.3055	(3.0956, 3.5459)	0.2100	(0.1645, 0.2472)
2014	-0.9867	(-1.1124, -0.8591)	3.2542	(3.0673, 3.4813)	0.2100	(0.1627, 0.2428)
2015	-0.8984	(-1.0210, -0.7761)	3.0831	(2.9517, 3.2393)	0.2341	(0.2011, 0.2842)
2016	-0.9169	(-0.9952, -0.8391)	3.1126	(3.0195, 3.2254)	0.2288	(0.2043, 0.2628)
2017	-0.6747	(-0.7633, -0.5882)	3.0487	(2.9413, 3.1770)	0.2137	(0.1804, 0.2411)

<https://doi.org/10.1371/journal.pone.0220427.t004>

Table 5. Point estimates and 95% CIs for linear model parameters in the three examples.

Data set	Antibiotic	Estimated intercept for the linear trend of non-resistant mean log ₂ MIC		Estimated slope for the linear trend of non-resistant mean log ₂ MIC	
		$\hat{\gamma}_0$	95% CI of $\hat{\gamma}_0$	$\hat{\gamma}_1$	95% CI of $\hat{\gamma}_1$
CDC NARMS	CHL	1.6225	(1.3416, 1.8861)	0.0415	(0.0088, 0.0773)
CDC NARMS	TIO	-1.2297	(-1.6223, -0.7820)	0.0369	(-0.0168, 0.0844)
ISU VDL	TIO	-1.6391	(-2.2577, -1.0546)	0.1388	(0.0049, 0.2713)

<https://doi.org/10.1371/journal.pone.0220427.t005>

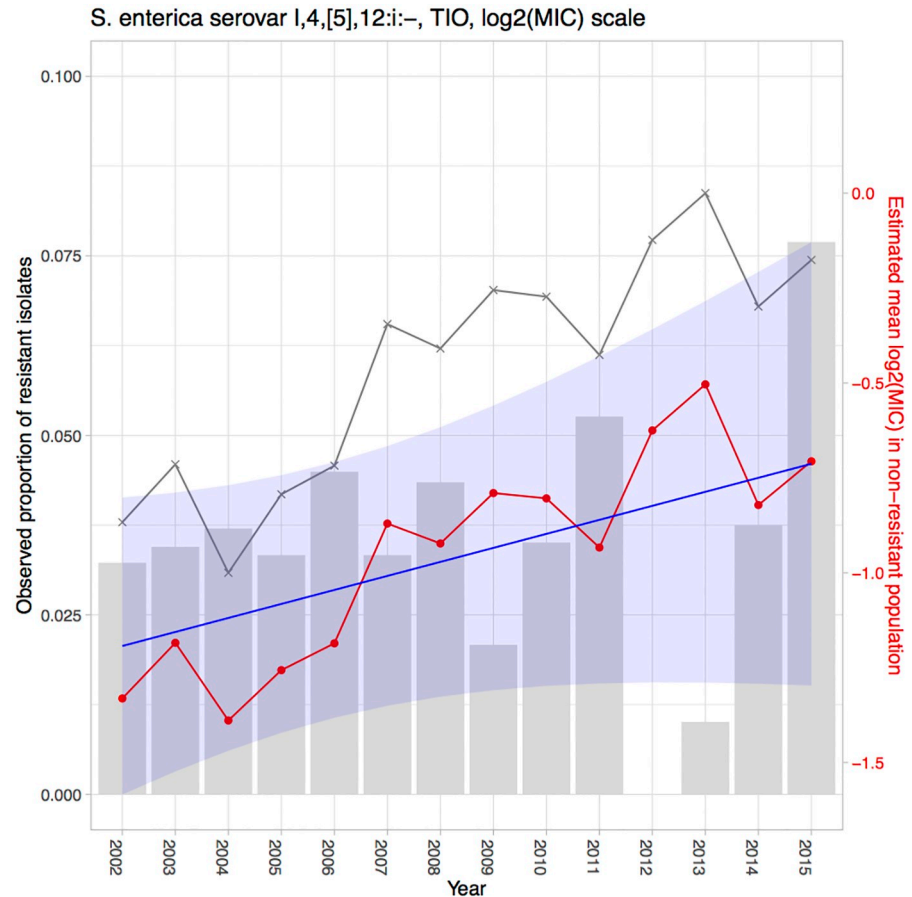


Fig 3. Estimation results for *Salmonella enterica* I,4,[5],12:i:- tested with TIO in the CDC NARMS dataset. The grey bars represent the observed proportions of resistant bacteria (left y-axis). The grey line indicates the naive mean of log₂MIC in the non-resistant population. The red line connects the point estimates of the mean log₂MIC in the non-resistant population. The blue line represents the estimated linear trend, shaded with its 95% CI.

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estimated non-resistant means (red points). The yearly non-resistant means were scattered around the regression line in a random pattern, in agreement with the linear model assumption of independence. The grey histogram in Fig 2 shows the observed proportions of the resistant isolates. Notably, no *Salmonella* I,4,[5],12:i:- isolate resistant to CHL was observed for some years, while the mean of log₂MIC below the resistance threshold was estimated to be increasing constantly. This example demonstrated a situation in which analysis based on dichotomized MIC alone would misleadingly indicate a decreasing level of resistance from 2009 to 2012 and neglect the increasing level of resistance below the break point. Based on these results, we concluded that an intervention for use of CHL for *Salmonella* I,4,[5],12:i:- in human is suggested to prevent a possible outbreak of resistance if the linearly increasing pattern is allowed to continue in the following years.

In the example of *Salmonella* I,4,[5],12:i:- tested with TIO in the CDC NARMS dataset, we found an insignificant slope estimation, with a 95% CI of (−0.0168, 0.0844) (second row in Table 5). Despite the notion the true value of the slope parameter is within an interval that contains zero, our best estimation was positive, and the major coverage of the CI was greater than zero. No organism exhibited resistance to TIO above the threshold in 2012, reflecting a rapid

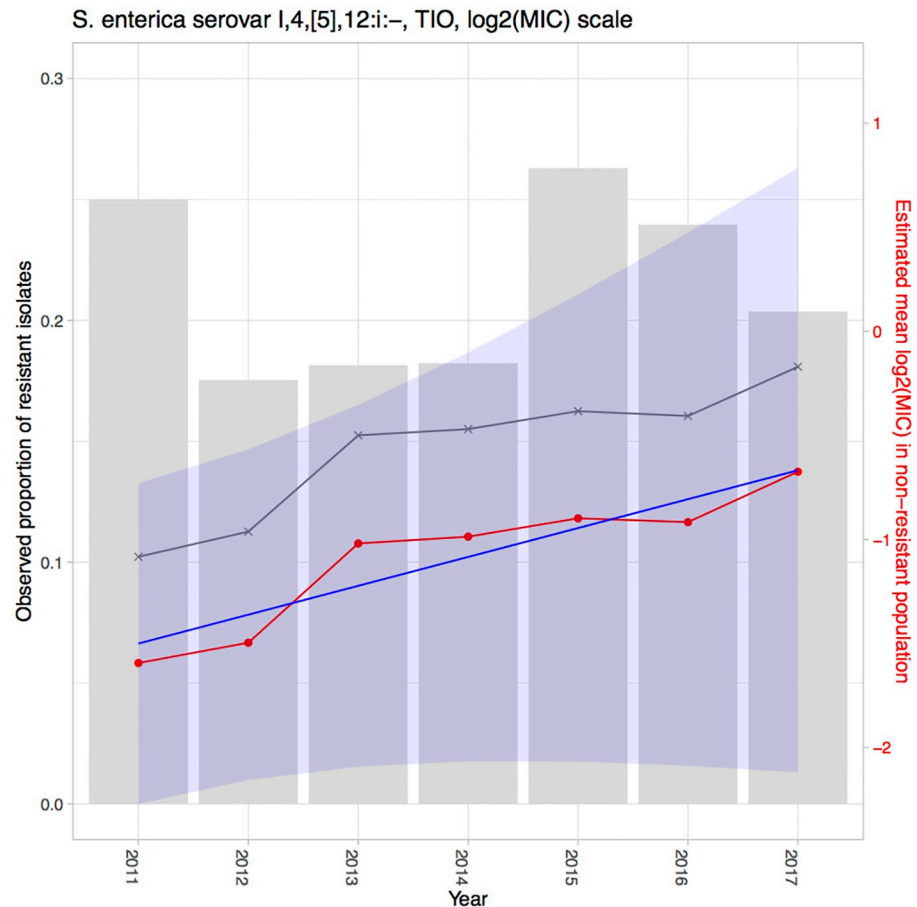


Fig 4. Estimation results for *Salmonella enterica* I,4,[5],12:i:- tested with TIO in the ISU VDL dataset. The grey bars represent the observed proportions of resistant bacteria (left y-axis). The grey line indicates the naïve mean of log₂MIC in the non-resistant population. The red line connects the point estimates of the mean log₂MIC in the non-resistant population. The blue line represents the estimated linear trend, shaded with its 95% CI.

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decrease from more than 5% in 2011. This phenomenon was accompanied by a stable MIC increase in the non-resistant population.

For the ISU VDL dataset, we detected a significantly increasing pattern in the non-resistant means for *Salmonella* I,4,[5],12:i:- tested with TIO, with a 95% CI of (0.0049, 0.2713) (third row in Table 5). As shown in Fig 4, the 95% CI of the estimated regression line (the shaded area) is rather wide compared with those in Figs 2 and 3, and the difference likely resulted from the limited number of observations in this example. In this VDL swine dataset, the observed resistant proportions exhibited an abrupt decrease in 2012, an abrupt increase in 2015, and a continuous decrease since that time. Our estimated regression line added more dimensions in the non-resistant population by revealing the creep in its mean log₂MIC.

Model evaluation with simulation

In order to assess the performance of the proposed hierarchical Bayesian latent class mixture model with censoring and linear trend, a simulation study was conducted based on the CDC NARMS-CHL example. In our model, the parameters of interest were $\gamma_0, \gamma_1, \mu_1, \theta, \tau_0^2, \tau_1^2, \sigma_0^2, \sigma_1^2$,

and v^2 . In the simulation study, these parameters were predetermined according to the estimation results from the example and were denoted with a “hat” on the Greek letter. Also, we simulated the same number of observations for the i th year as the total number of isolates (n_i) in the CDC NARMS-CHL dataset. The data generation process is described in the following steps.

For $i = 1, 2, \dots, I; j = 1, 2, \dots, n_i$; and $t_i = i$:

1. Generate $\alpha_i \stackrel{iid}{\sim} N(\hat{\theta}, \hat{v}^2)$.
2. Calculate p_i by performing an expit transformation (inverse of logit) on α_i , $p_i = \frac{1}{1 + \exp(-\alpha_i)}$.
3. Generate the latent class indicator $c_{ij} \stackrel{iid}{\sim} Ber(p_i)$.
4. Generate the mean \log_2 MIC in the non-resistant population $\beta_{0i} \stackrel{iid}{\sim} N(\hat{\gamma}_0 + \hat{\gamma}_1 t_i, \hat{\tau}_0^2)$.
5. Generate the mean \log_2 MIC in the resistant population $\beta_{1i} \stackrel{iid}{\sim} N(\hat{\mu}_1, \hat{\tau}_1^2)$.
6. Generate the \log_2 MIC for isolate j in year i with $y_{ij} \stackrel{iid}{\sim} \begin{cases} N(\beta_{0i}, \hat{\sigma}_0^2), c_{ij} = 0 \\ N(\beta_{1i}, \hat{\sigma}_1^2), c_{ij} = 1 \end{cases}$.

The simulated p_i and β_{0i} were treated as the real proportion of resistant population and the real yearly mean of \log_2 MIC, which were compared with estimations based on the simulated observations subsequently. Until this point, the simulated y_{ij} were continuous values drawn from a mixture of Gaussian distributions. To mimic the censoring nature of \log_2 -MIC, y_{ij} was also censored according to its value. We previously defined l_{ij} and u_{ij} to be the lower bound and upper bound of y_{ij} . For CHL, the starting dilution was 2 mg/ml, and the ending dilution was 32 mg/ml for both serotypes. This indicated that if $y_{ij} \leq \log_2(2)$, then it will be left censored as $y_{ij} \leq 1$ with $l_{ij} = -\infty$ and $u_{ij} = 1$. Similarly, if $y_{ij} > \log_2(32)$, it will be right censored as $y_{ij} > 5$, with $l_{ij} = 5$ and $u_{ij} = \infty$. If $\log_2(2) < y_{ij} \leq \log_2(32)$, then y_{ij} will be interval censored with l_{ij} as its nearest integer to the left and u_{ij} as its nearest integer to the right. This censoring operation corresponds to step 7.

7. Censor the underlying true values of \log_2 MIC, y_{ij} , to the observed values y_{ij}^* with

$$y_{ij}^* = \begin{cases} 1, & y_{ij} \leq 1 \\ \lceil y_{ij} \rceil, & 1 < y_{ij} \leq 5, \text{ where } \lceil y_{ij} \rceil \text{ represents rounding up to the nearest integer.} \\ 5, & y_{ij} > 5 \end{cases}$$

End of simulation.

As a single set of observed \log_2 MIC (i.e., y_{ij}^*) could be generated by completing steps 1 to 7, we simulated 100 datasets by repeating the above procedure 100 times. With each simulated dataset, estimation was conducted using the proposed hierarchical Bayesian model, which produced a set of estimations: $\tilde{p}_i, \tilde{\beta}_{0i}, \tilde{\gamma}_0, \tilde{\gamma}_1$, etc., for $i = 1, 2, \dots, I$.

As a comparison, parameters were also estimated with the naïve estimation method, where \tilde{p}_i is nothing but the yearly proportion of resistant component; $\tilde{\beta}_{0i}$ is the yearly arithmetic mean of the observed \log_2 MIC in the non-resistant component; and the $\tilde{\gamma}_0, \tilde{\gamma}_1$ are estimations obtained from simple linear regression on the $\tilde{\beta}_{0i}$ against time. The naïve estimation method does not resolve the censorship issue, and separate the isolates with a hard threshold.

In order to assess and compare the performance of our hierarchical Bayesian model and the naïve approach, two metrics were calculated. The first was the mean bias over the 100

Table 6. The mean bias and RMSE of the proportion and mean parameters from the simulation study—A comparison between the proposed model and the naïve approach.

Year	Proposed hierarchical Bayesian model				Naïve approach			
	Mean bias		RMSE		Mean bias		RMSE	
	p_i	β_{0i}	p_i	β_{0i}	p_i	β_{0i}	p_i	β_{0i}
2002	0.00018	-0.00181	0.00682	0.07521	0.00533	0.42282	0.02933	0.46958
2003	0.00069	-0.00522	0.00685	0.08694	0.00067	0.35529	0.02818	0.40492
2004	0.00097	0.00266	0.00685	0.10295	0.00383	0.41238	0.03005	0.45091
2005	-0.00025	0.00289	0.00729	0.07703	-0.00155	0.58917	0.02530	0.62948
2006	0.00063	-0.00034	0.00643	0.04481	0.00203	0.64111	0.01725	0.66823
2007	0.00003	-0.00119	0.00735	0.05521	-0.00199	0.49288	0.01775	0.53106
2008	-0.00011	-0.00125	0.00704	0.04911	-0.00712	0.37628	0.02086	0.41558
2009	0.00018	-0.00152	0.00739	0.06671	-0.00672	0.60513	0.02272	0.63279
2010	-0.00014	0.00186	0.00667	0.06255	0.00053	0.44058	0.02068	0.48096
2011	0.00002	0.00847	0.00696	0.06332	-0.00058	0.58104	0.02107	0.62239
2012	0.00085	-0.00129	0.00654	0.05962	0.00326	0.65920	0.01896	0.69075
2013	-0.00009	-0.00834	0.00664	0.04629	0.00123	0.36472	0.01612	0.41247
2014	-0.00003	0.00078	0.00697	0.05114	-0.00281	0.53222	0.01701	0.56277
2015	-0.00009	0.00031	0.00685	0.04405	-0.00383	0.53804	0.01430	0.56901

The parameter p_i represents the proportion of the resistant population, and β_{0i} represents the mean \log_2 MIC in the non-resistant population of the i th year.

<https://doi.org/10.1371/journal.pone.0220427.t006>

Table 7. The mean bias and RMSE of the linear model parameters from the simulation study—A comparison between the proposed model and the naïve approach.

	Mean bias		RMSE	
	γ_0	γ_1	γ_0	γ_1
Proposed model	-0.02105	0.00330	0.20174	0.02669
Naïve approach	0.47159	0.00398	0.48441	0.01387

The parameters γ_0 and γ_1 represent the intercept and slope of the linear trend in the proposed model.

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simulations, and the second was the root of mean squared error (RMSE). Mean bias measures how close the estimations are relative to the true parameter values, while RMSE measures the variation of estimates around the true parameters.

Table 6 shows the mean bias and RMSE for the yearly parameters, and Table 7 shows the two metrics for the linear parameters, both of which compare between the proposed hierarchical Bayesian model and the naïve approach. The mean biases and RMSE for p_i , β_{0i} , and γ_0 from our proposed model were closer to 0 compared with those from the naïve approach. The mean biases and RMSE for γ_1 produced by the two approaches were both fairly small with similar values. This simulation study indicates that the estimations for the most relevant parameters are precise and robust by utilizing our proposed model.

Discussion

Our goal with this study was to address a deficiency in the available approaches for analysis of AMR data using MIC values. AMR is a serious public health issue worldwide, and enormous resources have been devoted to monitoring the changes in MIC over the years. We proposed a Bayesian hierarchical model with a linear trend and demonstrated that this model enables

additional information about the linear patterns in the mean \log_2 MIC in the non-resistant population in addition to the proportion of resistant bacteria. The linear changes in the non-resistant population may be linear creep that signals a need for intervention or a linear decline that implies a successful intervention. Therefore, our proposed model offers a tool to a more complete picture of the resistance level of organisms against various antibiotics, providing valuable information for the surveillance programs.

The proposed approach was founded on the concept of identifying a valid and robust mean \log_2 MIC estimate that addresses the latent nature of the MIC data. Under our framework, there were two sources of latent parameters. One resulted from censorship, as the true underlying continuous values for the censored observed MIC values are unknown. The other involved the population from which the bacterial isolates arose (resistant or non-resistant population). By tackling the censorship problem and incorporating the mixed components of the data, our Bayesian hierarchical model corrected the systematic bias of the mean MIC estimations and separated the isolates from different groups. We then added a higher level of complexity to this fundamental model setup: linear regression in the hierarchical model.

Considering the variation of the MIC values shown in the dataset, we allowed the mean \log_2 MIC to vary across different years. The Bayesian hierarchical model yielded a more robust estimation by shrinking the mean estimates towards a linear regression line in the non-resistant population and towards a common constant in the resistant population. In this way, we were able to quantify the linear pattern in the mean \log_2 MIC in the group of isolates that are often undervalued by researchers. Compared with regressing the mean \log_2 MIC to a constant in the non-resistant population, a linear trend with a non-zero slope provided a better fit to the datasets and satisfied our model assumptions.

Our model relied on several assumptions. We assumed normal distributions for resistant and non-resistant populations. This assumption was supported by the observed MIC distribution for the examples used in this paper; however, under violation of this assumption, non-parametric methods, such as spline fitting, could be used to replace the normality assumption [16]. For both resistant and non-resistant populations, we also assumed invariant variances across years, following the principle of parsimonious models. This assumption could be important when there are observations from many years but not enough observations within each year. In addition, we assumed that the proportion of the resistant population was independent across all years. We also assumed the mean \log_2 MIC had independent errors in the linear model and the constant mean model in the sub-populations. Violation of this assumption would require inclusion of a correlation structure in the proportions or the errors terms.

In conclusion, we proposed a framework of analysis of longitudinal \log_2 MIC data using a Bayesian hierarchical approach with linear trend. We not only estimated the mean of \log_2 MIC values properly and accurately but also detected a significant linear increase in the mean \log_2 MIC in the non-resistant population for some given organisms and antibiotics, potentially signaling the need for intervention. Additional directions from this proposed framework include studying the correlations among multiple antibiotics, between human and animal resistance, and between different surveillance programs for the same population. In addition, analysis of the relationship between clinical interventions and the MIC responses to the interventions based on these models is of interest.

Supporting information

S1 Appendix. Gibbs sampling procedure.
(PDF)

S1 File. Editing certificate. The certificate of English editing is attached in the Supporting information as an external file.
(PDF)

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Validation: Min Zhang.

Visualization: Min Zhang.

Writing – original draft: Min Zhang, Annette O'Connor.

Writing – review & editing: Min Zhang, Chong Wang, Annette O'Connor.

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