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Application of mixed-effects models to study the country-specific outpatient antibiotic use in Europe: a tutorial on longitudinal data analysis

Girma Minalu[1]*, Marc Aerts[1], Samuel Coenen[2,3], Ann Versporten[3], Arno Muller[3], Niels Adriaenssens[2,3], Philippe Beutels[4], Geert Molenberghs[1,5], Herman Goossens[3] and Niel Hens[1,4]

[1]Interuniversity Institute for Biostatistics and Statistical Bioinformatics (I-BIOSTAT), University of Hasselt, Hasselt, Belgium; [2]Centre for General Practice, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium; [3]Laboratory of Medical Microbiology, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium; [4]Centre for Health Economics Research and Modelling Infectious Diseases (CHERMID), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium; [5]Interuniversity Institute for Biostatistics and Statistical Bioinformatics (I-BIOSTAT), Catholic University of Leuven, Leuven, Belgium

*Corresponding author. Tel: +32-1-126-8283; Fax: +32-1-126-8299; E-mail: girma.minaluayele@uhasselt.be

Resistance to antibiotics is a major public health problem and antibiotic use is being increasingly recognized as the main selective pressure driving this resistance. Yearly and quarterly data on outpatient antibiotic use were collected by the European Surveillance of Antimicrobial Consumption (ESAC) project for the period 1997–2009 from 33 and 27 European countries, respectively, and expressed in defined daily doses per 1000 inhabitants per day. Since repeated measures were taken for the countries, correlation has to be taken into account when analysing the data. This paper illustrates the application of mixed-effects models to the study of country-specific outpatient antibiotic use in Europe. Mixed models are useful in a wide variety of disciplines in the biomedical, physical and social sciences. In this application for outpatient antibiotic use, the linear mixed model is extended to a non-linear mixed model, allowing analysis of seasonal variation on top of a global trend, with country-specific effects for global mean use and amplitude, and trends over time in use and in amplitude.

Keywords: linear mixed models, non-linear mixed models, antibiotic use, ambulatory care, seasonal variation

Introduction

Antibiotic resistance is a major European and global public health problem and international efforts are needed to counteract the emergence of resistance. Antibiotic use is increasingly recognized as the main driver of resistance,[1,2] and differential selection pressure of antibiotic agents may be responsible for some of the observed differences.[3] Specific actions, such as campaigns aimed at general practitioners as well as the public, appeared essential because antibiotic use in outpatients accounts for a large part of overall antibiotic usage, but evaluating their impact is not straightforward.[4]

Yearly and quarterly data on outpatient antibiotic use were available from 33 and 27 European countries, respectively, for the period 1997–2009 within the European Surveillance of Antimicrobial Consumption (ESAC) project.[5–10] The fact that measurements are taken from the same country makes them interdependent and clustered within the country. Mixed(-effects) models are therefore the most appropriate tools for analysis of these datasets.[11,12] The unique aspect of the mixed-effects model is the inclusion of both fixed and random effects, which leads to the name 'mixed model'. Fixed effects lead to descriptions of population-averaged responses, as in a conventional regression model, i.e. the average for Europe, while random effects account for the natural heterogeneity in the responses of different European countries and allow estimation of country-specific means.

For the yearly antibiotic use data, a two-stage model and a linear mixed model can be used to assess country-specific trends in Europe. Linear mixed models provide the flexibility of modelling variances and covariances of variables in addition to means specified in a cross-sectional regression model, and hence can be used to model data that show correlation and non-constant variability. For the quarterly antibiotic use data, a non-linear mixed model is used to assess country-specific trends while accounting for country-specific global use as well as country-specific seasonal variation. Non-linear mixed models, in addition to the aforementioned features of the linear mixed models, allow non-linear terms in the model.

The aim of this paper is to illustrate the application of mixed-effects models to study country-specific outpatient antibiotic use in Europe, using yearly and quarterly antibiotic use

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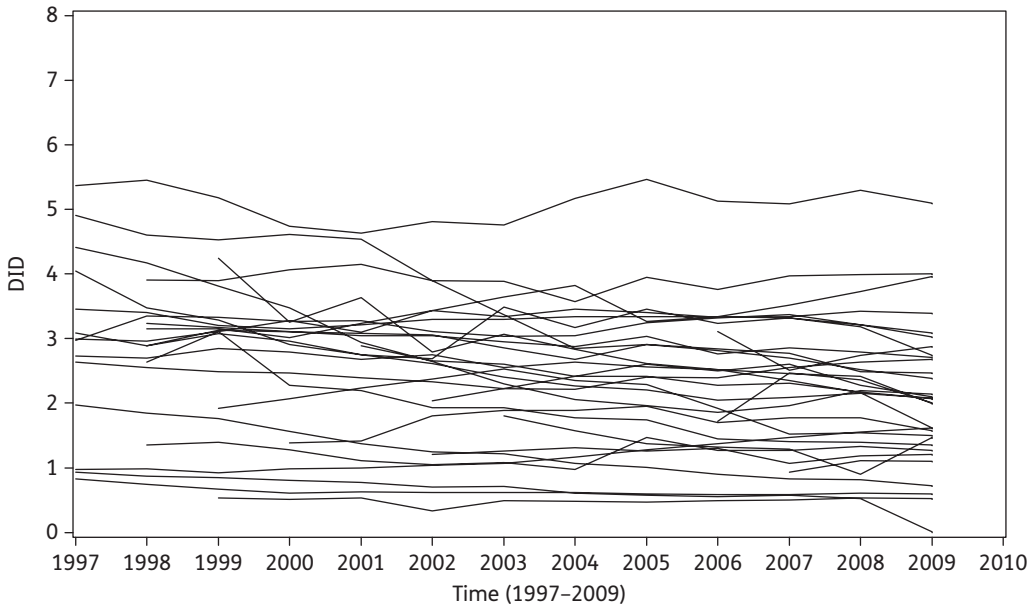

Figure 1. Observed country-specific changes in yearly use of tetracyclines expressed in DID in 33 European countries.

data. The application of mixed-effects models yields important new insights into the patterns of outpatient antibiotic use in Europe. This paper comprises an initial description of the two datasets analysed, an introduction to the mixed-effects models and presentation of the results. Finally, discussions and concluding remarks are included. The data structure and the procedures used to fit the models are presented in Appendix 1 (available as Supplementary data at *JAC* Online).

Data

Yearly ESAC data on total outpatient antibiotic use from 33 European countries and quarterly data on total outpatient antibiotic use from 27 European countries were collected for the period 1997–2009 within ESAC, an international network of surveillance systems. Since Romania and Switzerland had data for only one year, they were not included in the analysis of yearly antibiotic use data. The methods of data collection and processing for the ESAC project have been described in detail elsewhere[5] and are also available on the ESAC web site (www.esac.ua.ac.be). Antibiotic use data are expressed as the number of defined daily doses (DDD) per 1000 inhabitants per day (DID).

This paper focuses on the outpatient use of tetracyclines for the period 1997–2009,[10] with the observed country-specific trends for yearly and quarterly tetracycline use in DID being shown in Figures 1 and 2, respectively. As can be seen in Figure 1, there is variability across repeated measurements from the same country (i.e. within-country variability) as well as variability between countries (i.e. between-country variability), which suggests that country-specific intercepts and slopes should be incorporated into the model to account for heterogeneity across countries. The longitudinal profiles for quarterly tetracycline use (Figure 2) show clear seasonal variation of total outpatient tetracycline use in all countries, with upward peaks in the winter season. Thus, a non-linear model needs to be adopted to take the seasonality into account. Figure 2 also shows within-country variability and between-country variability. From the longitudinal profiles it can clearly be seen that countries with higher tetracycline use at the baseline (in 1997) have a higher amplitude (higher seasonal variation).

Statistical analysis

Given that repeated measures were taken for each country, intra-country correlation has to be taken into account when analysing the data. The application of the mixed-effects models is illustrated using a two-stage model and a linear mixed-effects model for the yearly tetracycline use data (a two-stage model), and using a non-linear mixed model for the quarterly tetracycline use data (non-linear mixed model). All analyses were conducted using SAS®9.2 software. The SAS codes used to fit the models are included in Appendix 1.

A two-stage model

In this section the two-stage model and the linear mixed model for the yearly tetracycline use data are considered in order to assess whether there has been a decrease or an increase in tetracycline use in Europe.

Two-stage analysis

The model was fitted in two stages. First, a linear regression model was fitted separately for each country. Afterwards, regression methods were used to model the variability of country-specific regression coefficients.

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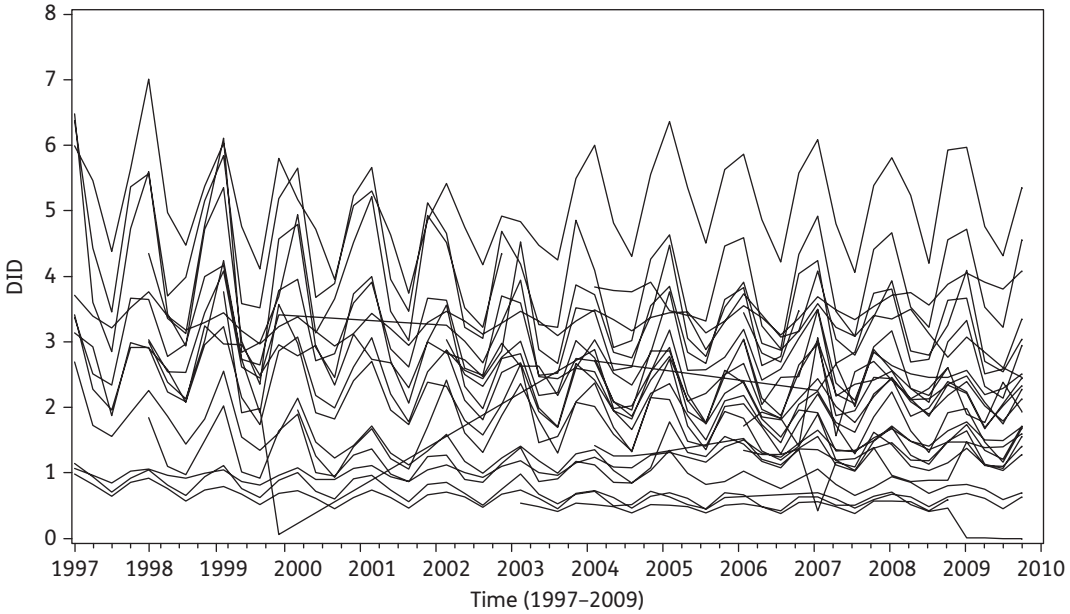

Figure 2. Observed country-specific changes in quarterly use of tetracyclines expressed in DID in 27 European countries.

Stage 1: linear regression model for each country separately

In the first stage, a linear regression model (1) is used to summarize the observations of country i ($i=1, 2, \ldots, N$) by their regression parameters:

$$Y_{ij} = \beta_{0i} + \beta_{1i} t_{ij} + \varepsilon_{ij}, \quad (1)$$

where Y_{ij} is tetracycline use in DID for country i at timepoints $t_{ij} = (j = 1, 2, \ldots, n_i)$, n_i is the number of observations from the ith country, time=1 corresponds to the start of the study (year 1997), β_{0i} and β_{1i} are unknown country-specific regression coefficients and ε_i is an n_i-dimensional vector of unexplained error terms ε_{ij}. ε_i is assumed to follow a normal distribution with mean vector zero and $n_i \times n_i$ covariance matrix Σ_i. To account for the serial correlation of the error terms, a first-order autoregressive structure was used for the variance structure for the error terms. The (co)variance of the errors at timepoints j and j' for country i equals

$$(\sigma_{jj'})_i = {\sigma_i}^2 {\rho_i}^{|j-j'|}, \quad j, j' = 1, 2, \ldots, 13$$

where ${\sigma_i}^2$ is the error variance for country i and ρ_i is the AR-1 parameter (correlation parameter) for country i.

The scatter plot of the estimated country-specific regression coefficients is presented in Figure 3, which shows variation across countries (as expected from the observed country-specific changes shown in Figure 1), indicating that the majority of countries have a negative slope, indicating decreasing outpatient tetracycline use from 1997 to 2009. It can also be clearly seen that there is a negative relationship between the country-specific slopes and country-specific intercepts (so countries with a high level of tetracycline use in 1997 tend to have the largest decrease in use over time).

Stage 2: modelling the variability of country-specific regression coefficients

In the second stage, a multivariate model of the form

$$(\beta_{0i}, \beta_{1i}) = (\beta_0, \beta_1) + (b_{0i}, b_{1i}) \quad (2)$$

is used to explain the observed variability between the countries with respect to their country-specific regression coefficients (β_{0i} and β_{1i}). β_0 and β_1 are unknown regression parameters and b_{0i} and b_{1i} are country-specific random effects, where b_{0i} expresses how much the intercept of country i deviates from the global intercept (β_0) and b_{1i} expresses how much the slope of country i deviates from the global slope (β_1). The random effects are assumed to follow a normal distribution with mean vector zero and general covariance matrix D, with elements $d_{ij}=d_{ji}$:

$$D = \begin{pmatrix} d_{11} & d_{12} \\ d_{12} & d_{22} \end{pmatrix} \quad \text{and} \quad \rho_{12} = corr(b_{0i}, b_{1i}) = \frac{d_{12}}{\sqrt{d_{11}}\sqrt{d_{22}}} \quad (3)$$

where d_{11} is the variance of the random intercept b_{0i}, d_{22} is the variance of the random slope b_{1i}, d_{12} is the covariance of the random intercept and the random slope, and ρ_{12} is the correlation between the random intercept and the random slope.

Table 1 shows the parameter estimates and standard errors for the global intercept (β_0) and the global slope (β_1). The parameter β_0 can be interpreted as the average response at the baseline or the average outpatient tetracycline use in DID in 1997, whereas the parameter β_1 represents the average linear time effect or the average change in outpatient tetracycline use in DID per year from 1997 to 2009. The results in Table 1 indicate that there is an overall significant decrease in the trend of outpatient tetracycline use (slope −0.0401). The

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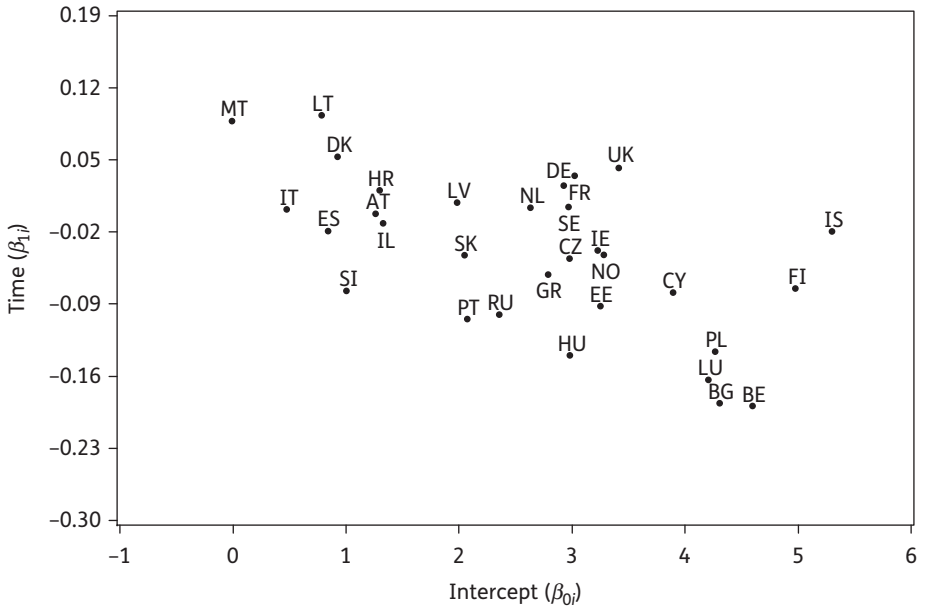

Figure 3. Scatter plot of country-specific slopes (β_{1i}) and country-specific intercepts (β_{0i}) obtained by fitting the two-stage model. AT, Austria; BE, Belgium; BG, Bulgaria; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; ES, Spain; FI, Finland; FR, France; GR, Greece; HR, Croatia; HU, Hungary; IE, Ireland; IL, Israel; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MT, Malta; NL, Netherlands; NO, Norway; PL, Poland; PT, Portugal; RU, Russian Federation; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom.

estimate for the general covariance matrix D is

$$D = \begin{pmatrix} 1.957 & \\ -0.0623 & 0.0056 \end{pmatrix}.$$

The estimated variance of the random intercept is 1.957, the estimated variance of the random slope is 0.0056 and the correlation between the random effects is −0.5959 (negative, as expected from Figure 3).

In the two-stage analysis, information is lost in summarizing the observed measurements for the ith country by the country-specific regression coefficients, the number of observations per country is not taken into account when analysing the estimated regression coefficients (in the second stage) and random variability is introduced by replacing β_{0i} and β_{1i} with their estimates. These drawbacks can be avoided by combining the two stages into one model, the so-called linear mixed(-effects) model.[11]

Combining stages 1 and 2: linear mixed-effects model

Linear mixed models provide a very flexible environment for modelling data with many types of repeated measurements, whether repeated in time, space or both. Correlations among measurements made on the same subject or experimental unit can be modelled using random effects and through the additional specification of a covariance structure. Observations from different countries are assumed to be independent and observations within countries are expected to be correlated. The model is defined as

$$Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})t_{ij} + \varepsilon_{ij}, \quad (4)$$

Table 1. Parameter estimates (and standard errors) for the parameters obtained by fitting the two-stage model

Parameter	Estimate (standard error)	*P* value
β_0	2.6258 (0.2512)	<0.0001
β_1	−0.0401 (0.0134)	0.0056

where β_0 is the global intercept (average outpatient tetracycline use in Europe in 1997), β_1 is the global slope describing the marginal linear time trend (t) of the time series (average change in outpatient tetracycline use in DID per year in Europe from 1997 to 2009), $b_i = (b_{1i}, b_{0i})$ is a vector of country-specific random effects (for intercept and slope) and we assume $b_i \sim N(0, D)$. The matrix D is an unstructured covariance matrix defined as in (3). The unstructured covariance matrix allows all of the parameters of the variance–covariance matrix to be different. A likelihood ratio test was used to compare the unstructured covariance matrix with various more parsimonious covariance matrices, e.g. a compound symmetry structure (see Appendix 2; available as Supplementary data at *JAC* Online). The likelihood ratio test supports the unstructured covariance structure.

ε_i is a vector of unexplained error terms ε_{ij}. It is usually assumed that all ε_i are independent and normally distributed with mean vector zero and covariance matrix Σ_i. Often, Σ_i is assumed equal to $\sigma^2 I_{ni}$, where I_{ni} is the n_i-dimensional identity matrix. This structure is often referred to as the simple covariance structure. Many possible covariance structures are available for the covariance matrix for the error components. A likelihood ratio test was used to contrast the simple covariance structure

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with the first-order autoregressive structure (see Appendix 2). The likelihood ratio test supports the first-order autoregressive structure (AR-1). In this case, the (co)variance of the errors at timepoints j and j' equals

$$\sigma_{jj'} = \sigma^2 \rho^{|j-j'|}, \quad j, j' = 1, 2, \ldots, 52$$

where σ^2 is the error variance and ρ is the correlation parameter, also known as the autocorrelation coefficient, which reflects the degree to which the errors are autocorrelated. The correlation decreases exponentially across the lags of the timepoints. We tried to extend the assumption of constant within-country variability across all countries, but the model did not converge, probably due to parameter redundancy, as was the case for several other more general covariance matrices.

The need for the inclusion of the random effects was tested using a likelihood ratio test, by comparing the log-likelihoods of models with and without the appropriate random effect. The asymptotic null distribution of the likelihood ratio test statistic for testing the significance of random effects in a linear mixed model is a mixture of χ^2 distributions, the mixing proportions of which depend on the number of random effects present in the model as well as on their variance–covariance structure.[11,13] The results of this analysis indicate that the covariance structure should not be simplified by deleting the random effects from the model (see Appendix 2).

Table 2 shows the parameter estimates and standard errors for the fixed-effects parameters using the random-effects model with random intercept and random slope. The results show there is an overall significant decrease in the trend of outpatient tetracycline use in DID (slope −0.0481). The parameter estimate for the marginal linear time trend (β_1) in the two-stage model (Table 1) is slightly higher than the estimate in the linear mixed model (Table 2).

The estimates for the variance, covariance and correlation components are

$$D = \begin{pmatrix} 1.2034 & \\ -0.0309 & 0.0022 \end{pmatrix}, \quad \rho = 0.9511 \text{ and } \sigma^2 = 0.4485.$$

The estimated variance of the random intercept is 1.2034 and the estimated variance of the random slope is 0.0022. The correlation coefficient between the random intercept and the random slope is −0.5952, and it indicates that countries with higher tetracycline use in DID at the baseline (in 1997) have a lower slope (decreasing use over time), while countries with lower tetracycline use at the baseline have a higher slope (increasing use over time).

The within-country variability in DID, which is assumed to be constant across all countries, is estimated to be 0.4485 (σ^2) and

Table 2. Parameter estimates (and standard errors) for the fixed-effects parameters obtained by fitting the linear mixed model

Parameter	Estimate (standard error)	*P* value
β_0	2.7282 (0.2420)	<0.0001
β_1	−0.0481 (0.0137)	0.0015

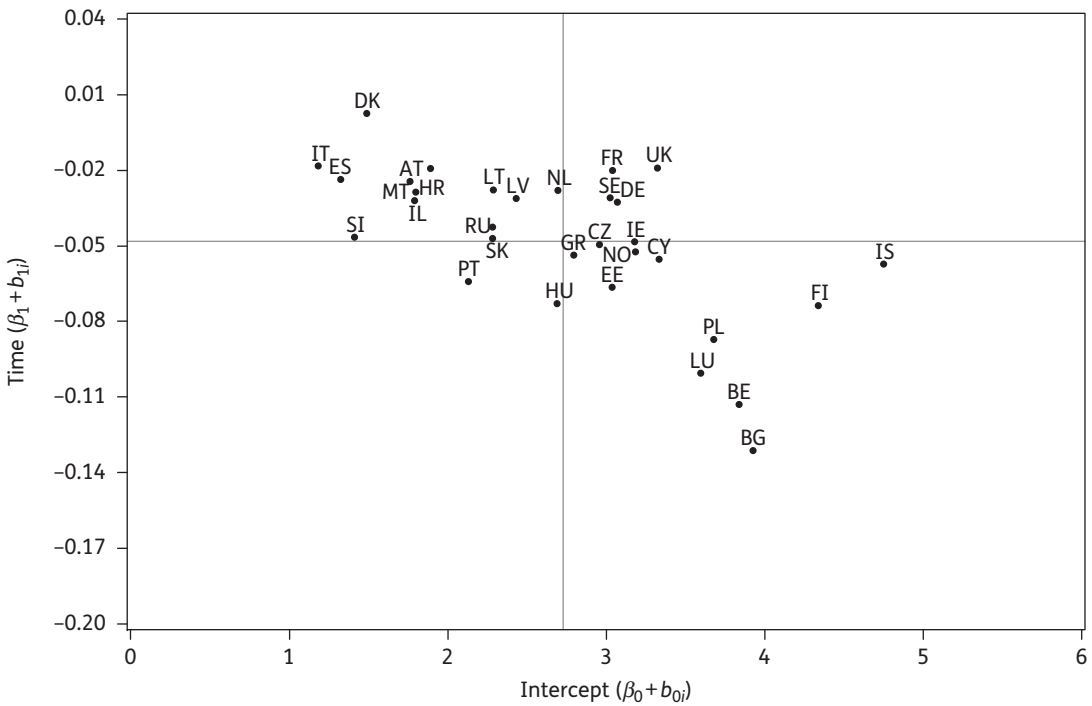

Figure 4. Scatter plot of slopes for time (fixed effect + random effects) and intercepts (fixed effect + random effects) obtained by fitting the linear mixed model. AT, Austria; BE, Belgium; BG, Bulgaria; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; ES, Spain; FI, Finland; FR, France; GR, Greece; HR, Croatia; HU, Hungary; IE, Ireland; IL, Israel; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MT, Malta; NL, Netherlands; NO, Norway; PL, Poland; PT, Portugal; RU, Russian Federation; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom.

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the correlation parameter is 0.9511 (ρ). The value of error variance is small, indicating that much of the total variability is captured by the between-country variability.

Figure 4 shows again the strong negative relationship between the intercepts and slopes. Denmark has the highest slope (slope is positive), while Belgium has the smallest slope (slope is negative). Figure 4 also shows the negative relationship between the random intercept and the random slope from the very similar scatter plot of country-specific slopes and country-specific intercepts (Figure 3). The vertical line (=2.6938) is the estimate for the global intercept (β_0; average outpatient tetracycline use in Europe in 1997) and the horizontal line (=−0.0453) is the estimate for the global linear time effect (β_1; average change in outpatient tetracycline use in DID per year in Europe from 1997 to 2009).

Attempts to extend model (4) by including quadratic and cubic time effects did not identify any significant effects. Residuals and influential diagnostic measures were used to examine model assumptions and to detect outliers and potentially influential observations. From the influential measures, observations of Belgium and Iceland have a slight effect on the estimates of the fixed-effect parameters, but are not considered influential.

Table 3. Parameter estimates (and standard errors) for the fixed-effects parameters obtained by fitting the non-linear mixed model

Parameter	Estimate (standard error)	*P* value
β_0	2.6041 (0.2510)	<0.0001
β_1	−0.0091 (0.0033)	0.0111
${\beta_0}^S$	0.6225 (0.0717)	<0.0001
${\beta_1}^S$	−0.0064 (0.0015)	<0.0003
δ	0.4947 (0.0235)	<0.0001

Non-linear mixed model

This section illustrates the application of the non-linear mixed model (6) for using the quarterly tetracycline use data to assess country-specific trends while accounting for country-specific seasonal effects. This seasonal-trend model is defined as

$$Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})t_{ij} + (\beta_0{}^S + b_{0i}{}^S + (\beta_1{}^S + b_{1i}{}^S)t_{ij})\mathrm{Sin}(\omega t_{ij} + \delta) + \varepsilon_{ij}, \quad (6)$$

where Y_{ij}, β_0, β_1 and ε_{ij} are defined as before, time=1 corresponds to the start of the study (first quarter of 1997), ${\beta_0}^S$ is the amplitude of the seasonal variation, ${\beta_1}^S$ is the change in amplitude over time, ω (in radians) is the frequency, which is a constant ($=2\pi/T$) where T (=4) is the period for the sine curve, δ (in radians) is the phase shift or phase angle, which is an unknown parameter and is estimated by the fitting algorithm, $b_i = (b_{i0}, b_{i1}, {b_{0i}}^S, {b_{1i}}^S)$ is a vector of random effects, where b_{i0} is the country-specific random intercept, b_{i1} is the country-specific random slope for time, ${b_{0i}}^S$ is the country-specific random slope for amplitude and ${b_{1i}}^S$ is the country-specific damping effect on the seasonal variation, and we assume $b_i \sim N(0, D)$ where now D is a 4×4 covariance

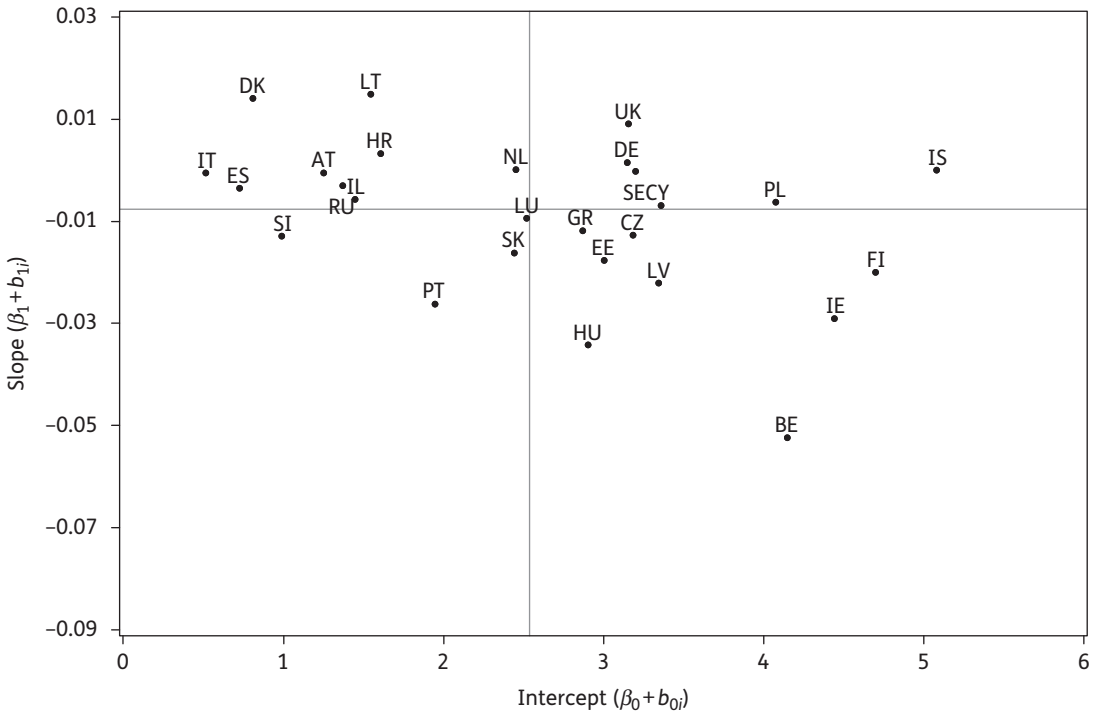

Figure 5. Scatter plot of slopes for time (fixed effect+random effects) and intercepts (fixed effect+random effects) obtained by fitting the non-linear mixed model. AT, Austria; BE, Belgium; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; ES, Spain; FI, Finland; GR, Greece; HR, Croatia; HU, Hungary; IE, Ireland; IL, Israel; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; NL, Netherlands; PL, Poland; PT, Portugal; RU, Russian Federation; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom.

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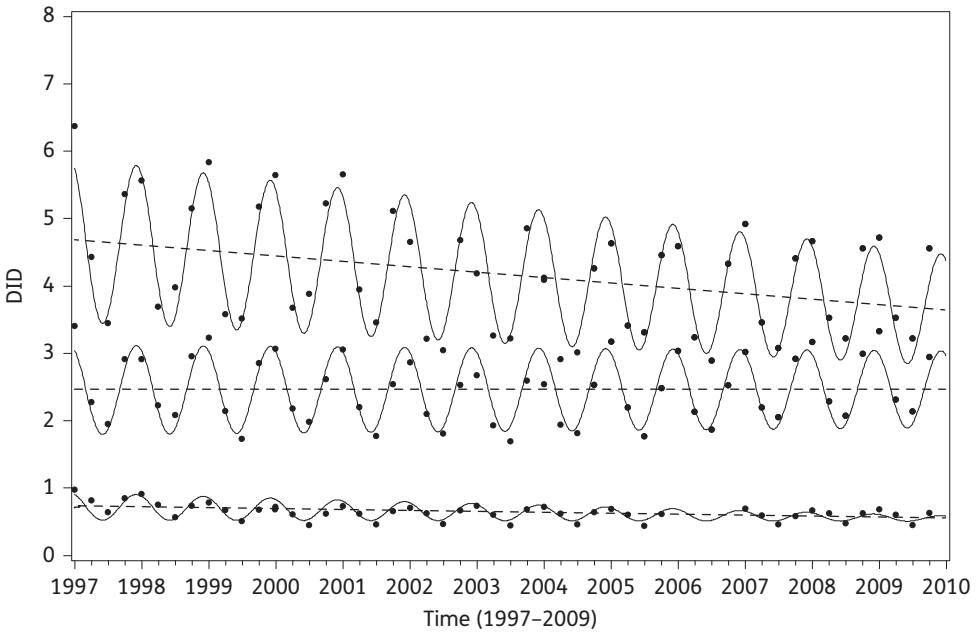

Figure 6. The predicted country-specific profiles (continuous lines), country-specific predicted linear trends (broken lines) and observed country-specific DID (dots) for three selected countries (Finland, the Netherlands and Spain from top to bottom).

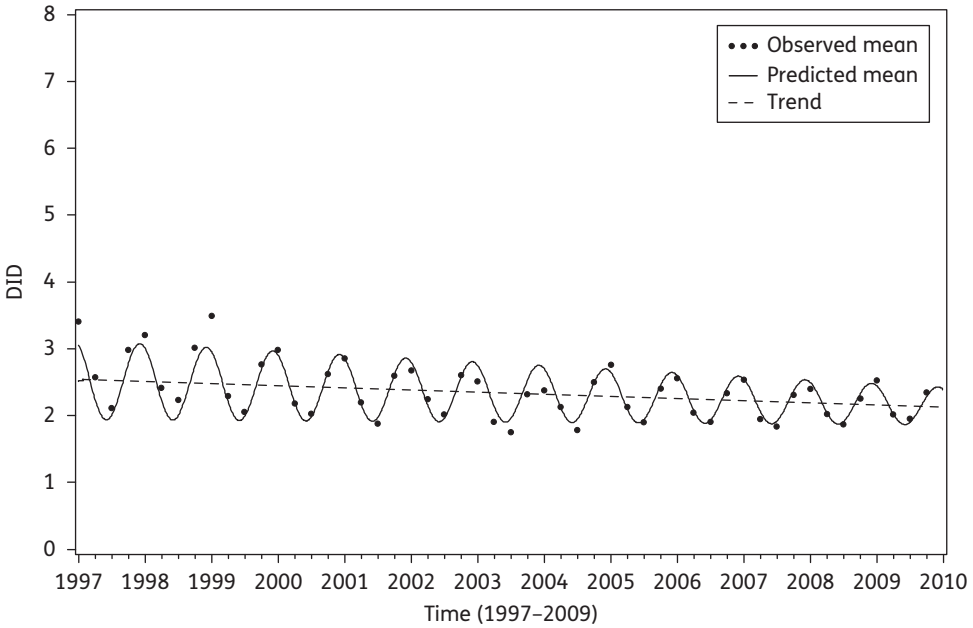

Figure 7. The predicted mean profile (continuous line), predicted trend (broken line) and observed mean (dots) DID.

matrix. No convergence was obtained when we fitted the model with an unstructured covariance matrix for the random effects. To obtain convergence, we simplified the covariance structure by setting the covariance between $b_{1i}{}^{S}$ and b_{i0}, b_{i1} and $b_{0i}{}^{S}$ equal to 0. For non-linear mixed models, SAS provides no straightforward way to assess the serial correlation of the residuals. To get insight into the residual serial correlation, we plotted the residuals versus time (see Appendix 2). The plot (i.e. Figure S1 in Appendix 2) shows there is essentially no systematic structure in the residual profiles. This supports the assumption that the time dependency and correlation are accounted for by the random effects and the sinusoidal component. Furthermore, the heterogeneity of the structure of the error component across the countries (such as heteroscedasticity) is accounted for by the country-specific seasonal variation (amplitude). In the final model all assignable sources of heterogeneity and variability have been formulated in the mean structure of the model, which allows the model to be used for accurate predictions.

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The results given in Table 3 suggest again that there is an overall significant decrease in the use of tetracyclines over time (slope −0.0091). There is also a significant seasonal variation ($P<0.0001$).

The estimates for the variance components are

$$D = \begin{pmatrix} 1.6350 & & & \\ -0.0093 & 0.0003 & & \\ 0.3411 & -0.0030 & 0.1037 & \\ 0 & 0 & 0 & 0.00003 \end{pmatrix} \text{ and}$$

$$\sigma_\varepsilon{}^2 = 0.0757.$$

The correlation coefficient between the random effects was estimated to be −0.4199 (random intercept and random slope for time; negative, as expected from Figures 3 and 4), 0.8284 (random intercept and random slope for amplitude) and −0.5379 (random slope for time and random slope for amplitude), respectively. The high correlation coefficient between the random intercept and random slope for amplitude indicates that countries with higher tetracycline use at the baseline have higher seasonal variation. A similar relationship was also observed between the random intercept and the random slope for amplitude from the observed country-specific profiles (Figure 2).

From the scatter plot of slopes and intercepts (Figure 5) we can see by how much the country-specific estimates deviate from the overall estimates for the intercept (β_0) and the linear time effect (β_1). After correcting for seasonal variation, the vertical line (=2.6041) is the estimate for the intercept (β_0) and the horizontal line (=−0.0091) is the estimate for the linear time effect (β_1). From the plot, we again observe that there is a strong negative linear relationship between the random intercepts and random slopes for time. As can be seen from Figure 6, which shows the observed country-specific profiles and the predicted country-specific profiles for three selected countries (Finland, the Netherlands and Spain), the predicted country-specific profiles are quite close to the observed country-specific DID.

The estimated linear trend (broken line), the estimated seasonal-trend model (continuous line) and the observed average DID for Europe are shown in Figure 7, again indicating that the model describes the data very well.

Discussion

Longitudinal data are common in many disciplines where repeated measurements on a response variable are collected for all units. Mixed-effects models provide a very flexible approach for analysing longitudinal data. The linear mixed-effects model is often used to analyse continuous longitudinal data and the generalized linear mixed model is the most frequently used random-effects model for discrete repeated measurements.

This paper describes a two-stage model and a linear mixed-effects model for yearly tetracycline use data to assess country-specific trends in Europe. For quarterly tetracycline use data, a non-linear mixed model was used to assess country-specific trends while accounting for country-specific seasonal effects. The latter model was applied to total outpatient antibiotic use data[5] as well as to data on outpatient use of: penicillins; cephalosporins; macrolides, lincosamides and streptogramins; quinolones; and tetracyclines, sulphonamides and trimethoprim, and other antibacterials.[6–10] Like the compositional data analysis described in a complementary tutorial paper,[14] this analysis can be performed at several levels within the Anatomical Therapeutic Chemical (ATC) classification.[15] In this paper, the analysis was conducted at the pharmacological subgroup (ATC-3) level, but the analysis could be performed at the therapeutic subgroup (ATC-2) level or the chemical subgroup (ATC-4) or substances (ATC-5) level as well.

An extension of the non-linear mixed model with multiple unknown fixed change points and country-specific random change points, described by Minalu *et al*.,[16] can be applied to investigate changes in slope. Similar techniques could be adopted to assess the impact of public campaigns, like the ones organized in Belgium and France,[17–19] or the European Antibiotic Awareness Day organized by the European Centre for Disease Prevention and Control (ECDC).[20]

From the results, there is an overall significant decrease in the trend of tetracycline use, and there is a strong positive relationship between the random intercept and random slope for amplitude, indicating that countries with higher tetracycline use at the baseline are observed to have high seasonal variation. We also identified significant differences in total outpatient tetracycline use between countries in Europe.

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Disclaimer

The information contained in this publication does not necessarily reflect the opinion/position of the European Commission or the ECDC.

Supplementary data

Appendix 1 and Appendix 2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org).

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