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Use of generalized ordered logistic regression for the analysis of multidrug resistance data

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ABSTRACT

Statistical analysis of antimicrobial resistance data largely focuses on individual antimicrobial's binary outcome (susceptible or resistant). However, bacteria are becoming increasingly multidrug resistant (MDR). Statistical analysis of MDR data is mostly descriptive often with tabular or graphical presentations. Here we report the applicability of generalized ordinal logistic regression model for the analysis of MDR data. A total of 1,152 *Escherichia coli*, isolated from the feces of weaned pigs experimentally supplemented with chlortetracycline (CTC) and copper, were tested for susceptibilities against 15 antimicrobials and were binary classified into resistant or susceptible. The 15 antimicrobial agents tested were grouped into eight different antimicrobial classes. We defined MDR as the number of antimicrobial classes to which *E. coli* isolates were resistant ranging from 0 to 8. Proportionality of the odds assumption of the ordinal logistic regression model was violated only for the effect of treatment period (pre-treatment, during-treatment and post-treatment); but not for the effect of CTC or copper supplementation. Subsequently, a partially constrained generalized ordinal logistic model was built that allows for the effect of treatment period to vary while constraining the effects of treatment (CTC and copper supplementation) to be constant across the levels of MDR classes. Copper (Proportional Odds Ratio [Prop OR] = 1.03; 95% CI = 0.73–1.47) and CTC (Prop OR = 1.1; 95% CI = 0.78–1.56) supplementation were not significantly associated with the level of MDR adjusted for the effect of treatment period. MDR generally declined over the trial period. In conclusion, generalized ordered logistic regression can be used for the analysis of ordinal data such as MDR data when the proportionality assumptions for ordered logistic regression are violated.
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1. Introduction

Epidemiological studies of antimicrobial resistance (AMR) typically involve testing of isolated bacterial strains against a panel of various antimicrobial agents which results in a multivariate outcome (Agga, 2013). However, statistical analysis of phenotypic AMR data are largely univariate focusing on individual outcomes of the antimicrobials tested (Scott et al., 2005; Alali et al., 2008). Such univariate analysis however, fails to account for the pharmacological, biological (i.e. the outcomes were derived from same isolate) or genetic dependences of the outcomes. Multivariate analysis on the other hand accounts for such co-dependences among multiple AMR outcomes. Bivariate and multivariate probit models (Agga et al., 2014, 2015) for multivariate analysis of multiple binary outcomes, and multivariate linear regression model for multiple quantitative outcomes (Agga et al., 2015) were previously applied for the analysis of AMR data. Other multivariate approaches (Agga, 2013) that were used for the analysis of AMR data include cluster analysis (Berge et al., 2003; Alali et al., 2010), factor analysis (Wagner et al., 2003) and more recently Bayesian networks (Ludwig et al., 2013; Ward and Lewis, 2013).

Bacterial multidrug resistance (MDR), due to cross resistance between pharmacologically similar antimicrobial agents or through co-resistance of genetically linked resistance determinants, is an increasing problem. Considering MDR as an outcome can detect emerging resistance profiles, that may not be easily detected by univariate analysis of individual AMR results (Wagner et al., 2003). For example, *Salmonella* isolates exhibiting a penta-resistance profile (ACSSuT) against five different antimicrobial classes (ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline) has significant epidemiological relevance. MDR can be defined in three ways: (1) as the number of antimicrobial agents to which bacterial strain is resistant to, (2) as the number of antimicrobial classes to which each bacterial strain is resistant and (3) as resistance to ≥3 antimicrobial classes commonly used

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by National Antimicrobial Resistance Monitoring System (NARMS) (FDA, 2013). Defining MDR based on the number of antimicrobial classes overcomes the problem of pharmacological dependence between related antimicrobial agents that arises as a result of testing multiple antimicrobial agents in each class. However this does not solve the problem of biological dependence between multiple classes since such data arise from testing a single bacterial isolate.

Analysis of MDR data is mostly descriptive and is presented as frequency distribution in bar graphs or tables (Scott et al., 2005; Lowrance et al., 2007; Alali et al., 2008; Platt et al., 2008; Tadesse et al., 2012; FDA, 2013) sometimes combined with univariate analysis using a χ^2 test on binary classified MDR data. Such univariate analysis does not allow for simultaneous evaluation of more than one risk factor on the occurrence of MDR. Furthermore it suffers from a problem of multiple comparisons and such binary categorization can result in loss of information. Poisson regression defining MDR as the number of antimicrobial agents to which an individual isolate was resistant potentially ranging from zero to the maximum number of antimicrobial agents tested was used (Varga et al., 2009). Multinomial logistic regression also can be used to analyze the different established dichotomies comparing each of the MDR categories to a selected baseline category (Ananth and Kleinbaum, 1997; Hosmer and Lemeshow, 2000; Varga et al., 2009). However, all of the methods described above do not consider the generally ordinal nature of the MDR counts.

Ordered logistic regression takes the natural ordering of MDR data into account to examine the effect of different risk factors on MDR count (Hosmer and Lemeshow, 2000). When the proportional odds assumptions (i.e., the equality of the log-odds across the different cut points (categories) of the outcome variable) are met, a cumulative logit model (proportional odds model) can be used for the analysis of such ordered data (McCullagh, 1980; Hosmer and Lemeshow, 2000). However, it is exceedingly rare that these parallel line assumptions are met. Unconstrained ordinal logistic regression model, a form of generalized ordered logistic regression (gologit) model, can be used to relax the proportionality assumptions (Williams, 2006). Partial proportional odds model, is another form of gologit model in which coefficients of variables for which the proportionality assumptions are met are constrained while allowing the coefficients of the variables for which proportionality assumptions are not met to vary without any constraint.

We have previously used ordinal logistic regression model to evaluate the effect of treatment on the number of tetracycline resistance gene determinants detected from swine fecal metagenome (Agga et al., 2015). However, to the best of our knowledge, there are no published papers that used generalized ordinal logistic regression model for the analysis of MDR data. Therefore the objective of this paper was to show the applicability of generalized ordinal logistic regression model for the analysis of MDR data. The MDR data used for the present analysis is based on susceptibility results of *Escherichia coli* isolates from a previously reported trial conducted to investigate the impact of copper and CTC supplementation of weaned pigs on antimicrobial resistance of fecal bacteria (Agga et al., 2014).

2. Materials and methods

2.1. Phenotypic antimicrobial resistance data

Phenotypic AMR data used in this paper was originated from *E. coli* isolates obtained from experimental study conducted to investigate the impact of CTC and copper supplementation on the gut bacterial flora of weaned pigs. A full description of the study design, study population, sampling scheme, and determination of phenotypic antimicrobial resistance of the *E. coli* isolates is available elsewhere (Agga et al., 2014). Briefly, the study design was a full factorial cluster randomized trial in which 32 pens (each with five pigs) were randomly allocated to control, CTC, copper or copper plus CTC groups. Pens were supplemented with experimental doses of copper, CTC or their combination continuously for 21 days. This was followed by post-treatment period of 14 days. A total of 576 fecal samples were collected weekly from three pigs per pen for six weeks. Two non-type specific *E. coli* isolates were isolated per fecal sample giving rise to 1,152 isolates. Each isolate was then characterized for phenotypic and genotypic antimicrobial resistance.

Antimicrobial susceptibility testing was done using broth microdilution following Clinical Laboratory Standards Institute (CLSI) Veterinary Antimicrobial Susceptibility Testing standards (CLSI, 2008). Minimum inhibitory concentration (MIC) was determined for 15 antimicrobial agents for each isolate by using Sensititre™ semi-automated system (Trek Diagnostic Systems, Cleveland, OH) using NARMS custom panels (CMV1AGNF and CMV2AGNF). The MIC values of each *E. coli* isolate were categorized as resistant or susceptible (including intermediate MIC results) based on CLSI breakpoints for all but streptomycin for which NARMS consensus breakpoint was used. The 15 antimicrobial agents were categorized into eight classes according to CLSI definition (CLSI, 2008). The antimicrobial classes were: aminoglycosides, β-lactam/β-lactamase inhibitor combinations, cephems, folate pathway inhibitors, penicillins, phenicols, quinolones and tetracyclines. The macrolides class, represented by azithromycin, was excluded since all the isolates were not tested for azithromycin resistance (Agga et al., 2014).

2.2. Statistical approaches

Sampling days (d0–d35) were categorized into treatment periods as pre-treatment, during treatment and post-treatment. A full factorial model approach was used in the analysis to examine the effects of copper and CTC supplementations. In this approach, the copper plus CTC group was categorized as treatment group when evaluating copper and CTC effects independently. Moreover, the copper group was used as a control when evaluating the CTC effect; and the CTC group was used as a control when evaluating copper effect. Full factorial approach increases power by doubling the sample size both in the treatment and control groups. Control group (in which CTC or copper was not given) and the pre-treatment period were considered as referent groups; and $P < 0.05$ was considered significant. Data analysis was carried out in STATA 12 (STATA Corp LP, College Station, TX). For this paper we defined MDR as the number of antimicrobial classes to which each isolate was resistant.

2.3. Proportional ordered logistic regression model: assessing assumptions and model selection

In a proportional ordered logistic regression, the log-odds, and thus the odds ratios, are assumed to be constant across the ordered categories of the outcome and assumed only to differ by the levels of explanatory variable. However, the intercepts are allowed to vary across the categories of the outcome variable thus giving a series of parallel lines with constant slope but with different intercepts (Hosmer and Lemeshow, 2000; Dohoo et al., 2009). First proportional ordinal logistic regression model was fitted to assess the significance of each term (treatment, treatment period and all 2- and 3-way interactions) and to assess the proportionality assumption. This model accounts for the ordinal nature of the MDR outcome ranging potentially from 0 to 8 antimicrobial classes. Three isolates in the pan-susceptible category (resistant to 0 antimicrobial classes) were re-categorized to a category with resistance to one antimicrobial class. Moreover, all isolates were