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Inferring the interaction structure of resistance to antimicrobials

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ABSTRACT

The growth of antimicrobial resistance presents a significant threat to human and animal health. Of particular concern is multi-drug resistance, as this increases the chances an infection will be untreatable by any antibiotic. In order to understand multi-drug resistance, it is essential to understand the association between drug resistances. Pairwise associations characterize the connectivity between resistances and are useful in making decisions about courses of treatment, or the design of drug cocktails. Higher-order associations, interactions, which tie together groups of drugs can suggest commonalities in resistance mechanism and lead to their identification. To capture interactions, we apply log-linear models of contingency tables to analyze publically available data on the resistance of *Escheresia coli* isolated from chicken and turkey meat by the National Antimicrobial Resistance Monitoring System. Standard large sample and conditional exact testing approaches for assessing significance of parameters in these models breakdown due to structured patterns inherent to antimicrobial resistance. To address this, we adopt a Bayesian approach which reveals that *E. coli* resistance associations can be broken into two subnetworks. The first subnetwork is characterized by a hierarchy of β-lactams which is consistent across the chicken and turkey datasets. Tier one in this hierarchy is a near equivalency between amoxicillin-clavulanic acid, ceftriaxone and cefoxitin. Susceptibility to tier one then implies susceptibility to ceftiofur. The second subnetwork is characterized by more complex interactions between a variety of drug classes that vary between the chicken and turkey datasets.

1. Introduction

Antimicrobial resistance is a serious threat to human and animal health garnering much attention domestically and internationally. In the US more than 2 million people a year contract an antibiotic resistant infection (CDC, 2013). In the EU more than 25,000 people a year die due to antibiotic resistant bacteria, based on data from the European Commission Directorate-General on Health and Food Safety (2011). In 2011 The EU issued an action plan on antibiotic resistance, and the US followed suit in 2015. Within the domain of antibiotic resistance an especially concerning problem is that of multi-drug resistance as it may increase the chances that there will be no therapeutic agent available to treat a given infection, as in the case for several strains of gram negative bacteria (Falagas et al., 2008). It is therefore essential to understand not only resistance, but the dynamics of multi-drug resistance.

To gain a more complete picture of multi-drug resistance it is important to interrogate the associations between the various drug resistances. One way to do this is to use genomic sequence data. Not all resistance genes in a microbial genome are expressed, however, so genetic linkage does not necessarily guarantee phenotypic linkage. Similarly, not all resistance genes are yet known so a lack of genotypic linkage does not necessarily guarantee a lack of phenotypic linkage. Consequently, it is important to also investigate phenotypic associations. An understanding of phenotypic associations would provide useful information for evaluating phenotypic information in the clinic. It could also inform investigation of the biochemical and genetic mechanisms of multidrug resistance as well as shedding light on environmental factors driving the proliferation of the co-resistance phenotypes such as the joint resistance to cephalosporins resulting from their common use in feedlots (Wagner et al., 2003).

One of the most comprehensive sources of data on antimicrobial resistance in the United States is that collected by the National Antimicrobial Resistance Monitoring System (NARMS) (NARMS, 2014). Since 1996, NARMS, a collaboration among the United States Department of Agriculture, Food and Drug Administration and Center for Disease Control and Prevention, has been monitoring antimicrobial

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resistance in slaughter houses, retail meat, and human enteric bacteria. It monitors antibiotic resistance of *Escherichia, Enterococcus, Campylobacter* and *Salmonella* isolated from beef, chicken, turkey and pork at slaughter and retail. Resistance is also monitored in human enteric *Campylobacter* and *Salmonella*. NARMS reports resistance as Minimum Inhibitory Concentrations along with guidelines for setting resistance thresholds.

The NARMS findings are published each year in a summary report (NARMS, 2014). This report covers overall trends in single drug resistance, highlighting case studies of particular importance to human and veterinary health. The report also follows multidrug resistance as measured by resistance to three or more drugs regardless of the drugs involved, and as the prevalence of known multidrug resistance phenotypes like ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline (ACSSuT). The report does not include any inference of novel associations.

Because of the significance of the NARMS data our group has undertaken a systematic reanalysis. Our first paper carried out an exploratory analysis of single drug resistance as measured both by continuous MIC and by dichotomous susceptibility/resistance showing that resistance trends depended extensively on the source, antibiotic and microbe being examined (Zawack et al., 2016). It documented the differences between modeling the data as dichotomous vs continuous as well as the effect of using different resistance thresholds. Finally, we undertook a power analysis to determine how long it would take to detect a change in resistance given current data. Our second paper made use of Markov networks to infer associations between resistance in *Escherichia coli* isolated from chicken (Love et al., 2016). This work identified two subnetworks, one for the β-lactams and a second covering a mixture of drug classes.

In addition to our work using Markov networks a number of other approaches have been applied to understanding phenotypic associations among resistances, often focusing on resistance of *E. coli*. An experimental determination of relationships between resistance in *E. coli* was carried out by selecting cultures for resistance to a single antibiotic and then measuring the resistance level of the resulting population to other antibiotics (Imamovic and Sommer, 2013). Factor analysis and principal component analyses have been used to identify relationships between resistances in *E. coli* isolated from feedlot cattle (Wagner et al., 2003). Additive Bayesian models have been applied to further resolve the structure and direction of pairwise relationships between resistances of *E. coli* isolated from pig pens (Ludwig et al., 2013) as well as to learn relationships among resistance genes and resistance phenotypes in *Enterococcus faecalis* isolated from retail chicken (Hidano et al., 2015). All of these approaches have shown that multidrug resistance is highly structured uncovering associations both within and between drug classes. These include associations between the fluoroquinolones ciprofloxacin and naladixic acid (Wagner et al., 2003) as well as between the amphenicol and tetracycline drug classes (Imamovic and Sommer, 2013). Many of these relationships are also well supported with previous genetic work such as that between the drugs sulfisoxazole and streptomycin which are known to be combined in integrons (Ludwig et al., 2013). While these methods allow for the discovery of associations between antibiotics they do not provide information on the structure of these associations. As an example, they do not differentiate whether three drugs have independent pairwise associations or are all jointly associated with one another.

Inferring both the identify of associations between resistances and their structure directly from phenotypic data can be accomplished by returning to first principals and comparing the probability of resistance under various combinations of the resistance status of other drugs. If, as shown in Table 1a, a microbe is resistant to drug X 10 out the 200 or 5% of the time when it is susceptible to drug Y, but 100 out of the 200 or 50% of the time when it is resistant to drug Y, then we can conclude there is a 2-way association between drugs X and Y. Such associations can be visualized using a network or graph with drugs as nodes and edges between drugs that share a 2-way association. By looking at combinations of resistance and susceptibility for multiple microbes we can infer associations between more than two variables. This is the case in Table 1 if Table 1a is taken to be data for microbes susceptible to drug Z and Table 1b is taken to be microbes resistant to drug Z. In Table 1a, among isolates that are susceptible to drug Z, a microbe is resistant to drug X 5% of the time when it is susceptible to drug Y and 50% of the time when it is resistant to drug Y. In Table 1b, among isolates resistant to drug Z, a microbe is resistant to drug X 185 out of 200 or 92.5% of the time when it is susceptible to drug Y and 4 out of 4 or 100% of the time when it is resistant to drug Y. Since drug Z modifies the relationship between drugs X and Y we conclude there is a 3-way association between drugs X, Y, and Z. When there is an association involving more than 2 drugs it is called an interaction.

Table 1
Hypothetical contingency tables representing the counts for various susceptible/resistant (S/R) combinations of three drugs X, Y and Z. Table 1a is for when drug Z is susceptible and Table 1b is for when drug Z is resistant.

a)		Y	
		S	R
X	S	190	100
	R	10	100

b)		Y	
		S	R
X	S	15	0
	R	185	4

The above approach provides a straightforward and intuitive way to infer both associations and interactions. The tradeoff is that it requires categorical data that can be arranged into contingency tables of counts. The NARMS data can be put in such a form by making use of relevant resistance cutoffs. On the one hand, such a categorization obscures information about resistance away from the selected breakpoint. On the other hand, it provides focused information about resistance at clinically and/or epidemiologically relevant levels.

This paper examines the interaction structure of phenotypic antibiotic resistance patterns of *E. coli* isolated from chickens, and turkeys. It does so by making use of log-linear models for contingency tables, exact conditional testing, and Bayesian inference.

2. Materials and methods

2.1. The data set

For the purposes of this study the publically available data sheets were downloaded from the NARMS website. Susceptibility status was determined using the NARMS guidelines. The dataset consisted of isolates collected from chickens and turkeys in the years 2011 through 2013. *E. coli* was chosen as the bacteria of interest since this both maximized sample size and allowed for comparison with previous work. In order to appear in the analysis a drug had to be present in more than 80% of all samples, and a sample had to be tested for each such drug. This resulted in a collection of 2601 isolates from chickens and 1133 isolates from turkeys. All analyses were carried out in Python (Van Rossum, 1995). Regressions were done using the Statsmodels package (Seabold and Perktold, 2010). Hypothesis tests were done using Scipy (Perez et al., 2011).

2.2. Log linear model

One standard way to infer interactions like that in Table 1 is using log-linear models for contingency tables (Agresti, 2002, pp. 314). In these models, the expected count in each cell of the table is modeled as a function of the resistance and susceptibility pattern among the drugs. For example, if there are three drugs (X, Y and Z), the most general