Analysis methods for evaluating bacterial antimicrobial resistance outcomes

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Antimicrobial resistance of bacterial isolates in human and veterinary medicine has become an issue of critical importance. Ultimately, the concern has been focused on the potential for treatment failure and the selection of bacteria that no longer respond to currently available antimicrobial agents. The debate continues on the roles of prescription practices in humans, antimicrobial use in animals (production and companion) and plants, and a declining infrastructure for control of infection.[1-9] However, the general goal of researchers should be to provide a better understanding of the emergence, dissemination, and maintenance of resistant bacterial isolates in human and animal populations and the environment.

These general goals entail a number of specific research questions and objectives. An initial research objective is to quantify the amount (prevalence) of resistance or susceptibility that a bacterial species has to a single antimicrobial or a panel of antimicrobials, usually within a specific source population. Detection of changes in prevalence, such as pattern identification, is an important extension of point-in-time prevalence estimates. Identification of emerging resistance is a second objective of this type of research. This objective is essentially an evaluation of patterns in which the focus is detecting the initial increase in resistance prevalence above some minimum threshold or, perhaps, a substantial shift in the distribution of the **minimum inhibitory concentration (MIC)** values. The purpose of surveillance activities (ie, establishing baseline resistance values and identifying changes in resistance patterns over time)[10] is a combination of the first 2 research objectives. A third research objective is to identify risk factors that are associated with identified resistance patterns.

A number of epidemiologic research approaches have been used to address these objectives, including case studies,[2,11,12] controlled designed studies,[13,14] cohort studies,[15-18] and cross-sectional studies.[19-21] Many investigations have relied on evaluation of clinical isolates obtained from humans and other animals.[3,22]

Predictably, a diverse battery of statistical methods has been used to analyze resistance data. The objective of the information reported here is to review some general considerations when analyzing data on antimicrobial resistance, delineate analytic methods that have been used in the analysis of antimicrobial resistance of bacteria, and identify additional methods that may have application. For purposes of this report, the analytic methods will be grouped into the following 6 general categories: descriptive, indices, categorical, nonparametric, multivariate, and analysis of clustered data.

General Considerations

A detailed exploration of the impacts of experimental design and outcome measurement on analysis methods is beyond the scope of this manuscript. However, some common considerations are needed as a starting point to aid readers when considering appropriate selection of statistical methods. When using statistical analyses, investigators need to make certain assumptions about the data for the methods to allow appropriate inferences. The required assumptions vary according to the analysis technique used. Most commonly, there must be an assumption of independence of observations and, for parametric tests, a normal distribution (ie, normality) for the measured outcome. The assumption of independence is often violated when multiple samples are collected from a group of animals or multiple bacterial isolates are derived from a single sample. The fact that MIC values constitute discrete data (rather than continuous data), as well as the typical detection or reporting of only a few MIC values, may lead to violation of the normality assumption.

Beyond the statistical assumptions, there are a number of other general considerations that need equal attention. Among these considerations is the epidemiologic nature of the data. Prevalence data arise from a single point-in-time estimate of the proportion of the population that has an outcome of interest (eg, in the context of antimicrobial resistance, resistance [or susceptibility] to an antimicrobial). Data collected longitudinally over time from the same population are often used to examine antimicrobial susceptibility or outcome incidence. Incidence or incidence rate can be defined as the number of event onsets in a population divided by the sum of the time period of observations for all individuals in the population.[23] The strength of incidence data is that risk (ie, the probability of developing disease or antimicrobial resistance in a specified time period) can be calculated. When prevalence is low, the value can be used to approximate risk.

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Different analytic approaches will be appropriate for differing types of data.[24]

The analytic approach used will also be dependent on the definition of the unit of observation. The unit of observation can be a bacterial isolate, a sample that was submitted for microbial culture, the individual from which the sample or samples were obtained, or the unit (eg, pen, building, or hospital) in which the individual or individuals reside. Occasionally, sample collection may be more efficient when the individuals are evaluated as a group, such as steers in a feedlot pen. Summary statistics, such as the **50th percentile for the MIC (median MIC [MIC_{50}])**, can be used to represent values for the group but not each animal from which samples were obtained. Investigators must also consider the impact of the sample collection protocols, such as selecting multiple isolates cultured from a single fecal sample, collecting multiple fecal samples from an individual, or collecting multiple fecal samples from individuals that are part of a group. Increasing the number of isolates obtained from a single fecal sample or increasing the number of samples obtained from the animals within a single management group may improve precision associated with point estimates. However, the analytic approach should account for the sample collection protocol (ie, nonindependence of observational units within a group).

Collecting samples from every animal might be the most complete method of evaluating a population; however, for the sake of efficiency, samples are often collected from a subset of the population, and results are assumed to be representative of the entire population. Using a representative method (eg, randomization) to determine the animals that will be targeted for sample collection will help to ensure that these extrapolations are valid.[24] Similarly, the use of appropriate statistical methods for analysis will ensure reliability of the results. The goal of most statistical analyses is to assist in making inferences from a subset of a population and then extrapolate results to the entire population or the universe of items of interest. Conclusions drawn from these analyses rely on the assumption that the sample population does indeed represent the entire population. Validity of these conclusions also depends on how well the reference population represents other populations of interest. For example, any inference regarding the bacterial population in healthy animals that is determined solely on the basis of antimicrobial-resistance patterns for bacterial isolates obtained from clinical submissions should be questioned. Inferences from clinical specimens submitted to 1 or more laboratories could, under some circumstances, be used to make inferences regarding clinical specimens submitted to other laboratories, but such inferences are less likely to be valid when results for clinical specimens are extended to make inferences about bacterial populations in healthy animals.

Descriptive Methods

Descriptive statistical methods have been widely applied to data categorized into **susceptible-intermediate-resistant (SIR)** categories and to MIC data. Such SIR data are usually provided in tables, graphs (histograms and line charts), or the text as the proportion of isolates that are resistant or susceptible. Data on antimicrobial resistance provided in this manner are common in the human and veterinary medical literature to describe resistance to specific antimicrobials.[25-33] Antibiograms are a tabulation of resistance phenotypes of bacterial isolates. Isolates are grouped on the basis of shared phenotypic resistance patterns as a way of descriptively reporting proportions of multiple resistance.[8,19,26,33-35]

The use of MIC data to describe patterns of resistance is widespread in the literature. Commonly, MIC values are summarized in a frequency cross-tabulation of the antimicrobials and their concentrations.[36-38] Cumulative distributions have also been provided in cross-tabulations.[39] Furthermore, frequency distributions and cumulative distributions have been combined into a single table.[31,40]

Values for MIC are summarized in other formats in addition to frequency tabulations. An alternative format is to report the MIC data by use of summary descriptive statistics.[39] The MIC_{50} and **90th percentile for the MIC (MIC_{90})** are reported along with the maximum and minimum values, which are sometimes referred to as the range.[41-43] The geometric mean is used as a measure of central tendency for MIC data.[44] Another alternative is to graphically depict the MIC data, typically by use of histograms.[45] In 1 study,[10] investigators reported a histogram of MIC data but also demarcated the SIR boundaries, which clearly illustrated the microbial susceptibility patterns. A cumulative distribution plot has been suggested as a simple alternative that is useful and easy to understand, providing numerous distributions are not depicted in a single graph.[39]

Cumulative distribution plots may be of use in comparing antimicrobials with regard to their respective breakpoint values. Defined by national standards, breakpoints are MIC cutoff values at which an organism is considered to be susceptible, intermediate, or resistant to a drug. The MIC values for antimicrobials can be standardized by use of the following equation:

$$MIC_{standard} = \frac{\ln(MIC/breakpoint)}{\ln(2)}$$

where ln is the natural logarithm.

The **National Antimicrobial Resistance Monitoring System–Enteric Bacteria (NARMS-EB)** provides information regarding the occurrence of resistance in isolates of *Salmonella* spp from various animal species[46]; this information can be used to document use of this standardization method. In 1997, NARMS-EB reported MIC results for isolates obtained from clinically affected cattle. We selected data for 4 antimicrobials (cefoxitin, cephalothin, nalidixic acid, and streptomycin) for use in providing graphic documentation of this method. The nonstandardized MIC values for ceftiofur, nalidixic acid, and streptomycin define fairly distinct peaks, whereas MIC values for cephalothin tend to be more broadly distributed (**Fig 1**). When the MIC values are standardized relative to their respective

resistance breakpoints, the graphic portrayal changes substantially such that the cumulative distributions of 3 antimicrobials (ie, ceftiofur, cephalothin, and nalidixic acid) appear extremely similar, even though the broad distribution of cephalothin is still reflected in its slightly less steep increase to a value of 100% **(Fig 2)**. The distribution of standardized MIC values for streptomycin is shifted to the right and still reflects resistance. The percentage of bacteria susceptible to streptomycin (72.7%) is the value of the cumulative distribution when the standardized MIC value is zero. Correspondingly, the value for resistant bacteria is 27.3%.

The advantage of reporting data in a descriptive manner is that large amounts of data can be summarized and displayed in a format that is understandable by a wide range of interested groups. The SIR data have additional advantages of being relatively easily understood, compact for reporting, and more readily related to in vitro interpretation of resistance. Antibiograms have the advantage of allowing for examination of the multivariate nature of SIR data, including evaluating the data for groupings and multiple resistance, as well as identification of rare resistance phenotypes or a high prevalence of resistance phenotypes.

Reporting data in a descriptive manner also has disadvantages. Categorization of MIC data (or zone-diameter data) into SIR data can result in the loss of some information. Subtle shifts in distributions of MIC values that are below the breakpoint are not detectable. The SIR data would not necessarily be the most appropriate data structure when the objective of testing is to serve as an early warning of slight progressions, perhaps all of which are below the resistance breakpoint, in MIC values. In 1 study,[39] it was reported that the dynamic nature of breakpoints over time may lead to problems with interpreting SIR data. It was suggested that investigators rely on the distribution of MIC data to allow for consistency over time. The use of MIC values removes subjectivity associated with the choice of breakpoints and SIR categorization. However, MIC values determined by standard microbroth dilution methods do not form a truly continuous distribution because of the scale (doubling of dilutions) and potential for truncation of values at both extremes. Analyses that assume continuous data should be used cautiously.

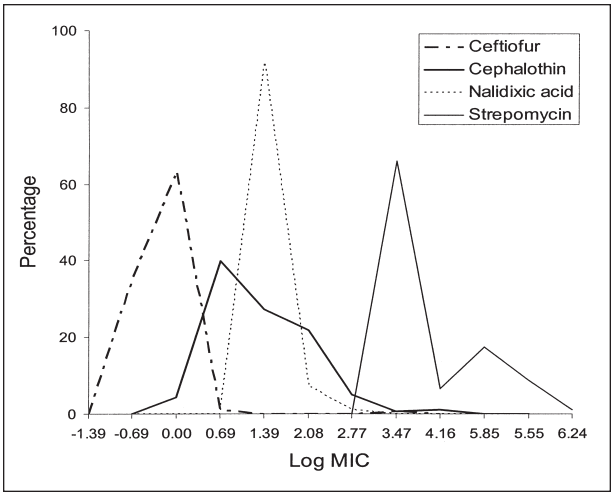

Figure 1—Nonstandardized, logarithmically transformed minimum inhibitory concentration (MIC) values for 4 antimicrobials tested against diagnostic isolates of *Salmonella* from cattle.[46]

Antibiograms serve as a good example of the problems that can be encountered when relying solely on SIR data. For example, the MIC value in an isolate could be 1 value below the breakpoint, whereas in another isolate the MIC value is at the breakpoint. These 2 isolates would appear as different phenotypes because of the SIR categorization, although the difference may not be important biologically. Another concern with the use of antibiograms is that they can be difficult to interpret when relationships of > 2 antimicrobials are considered at 1 time.

Summary descriptive analyses also have advantages and disadvantages. **Cumulative distribution functions (CDFs)** allow for visual reporting of quantiles, including MIC_{50} and MIC_{90} values as well as comparison of several antimicrobials or bacterial species. When only a few MIC categories are included in the data, then the CDFs and MIC quantiles may not be informative. Also, the CDF will not be meaningful when only a few isolates are included.

Indices

Ecologists have long sought indices that could represent a single quantitative measure of species diversity.[47] In ecologic studies, species diversity is considered to be a function of the number of species in the setting (ie, species richness) and the evenness with which individuals of the various species are distributed.[48] Translating the concept of a single comprehensive value that represents the diversity of animal species to

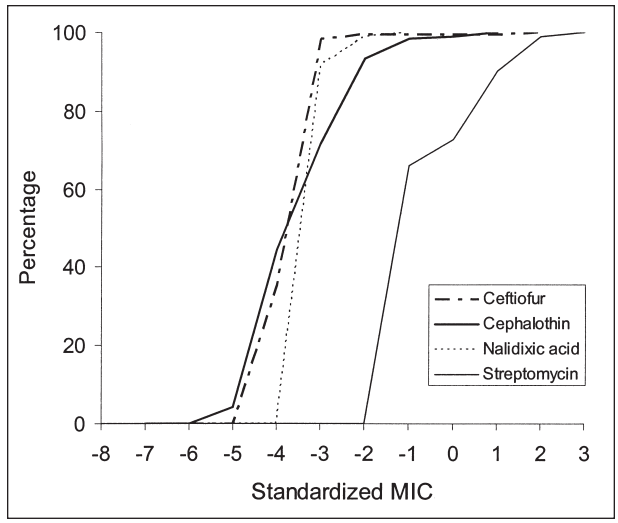

Figure 2—Standardized cumulative MIC distributions for 4 antimicrobials tested against diagnostic isolates of *Salmonella* cultured from cattle.[46] The logarithmic breakpoint value for each of the antimicrobials, based on guidelines used by the National Antimicrobial Resistance Monitoring System for evaluation of the susceptibility of enterbactereciae of veterinary origin and on guidelines established by the National Committee for Clinical Laboratory Standards, were as follows: ceftiofur, ≥ 2.08; cephalothin, ≥ 3.47; nalidixic acid, ≥ 3.47; and streptomycin, ≥ 4.16.

obtaining a single value that represents the diversity of susceptibility-resistance data for a panel of antimicrobials has intuitive appeal.

Perhaps the simplest index is species richness, which has been adopted somewhat indirectly as an index of antimicrobial resistance. In the context of antimicrobial susceptibility, species richness would be interpreted as the number of antimicrobials to which a single isolate is resistant. Penta-resistance, although often applied to specific clones, essentially is an example of richness of antimicrobial resistance or an index of multiple resistance. In 1 study,[49] investigators used the multiple-resistance index, which they termed multiple antibiotic resistance, to compare patterns among a number of bacterial species. They constructed histograms of multiple resistance for 8 species of bacteria to allow visual comparison of the frequency distributions. In another study,[33] researchers compared the distribution of the number of antimicrobials to which bacterial isolates were resistant for isolates cultured from humans and 3 species of production animals.

The number and selection of antimicrobials in the test panel can impact the multiple-resistance index. The maximum value of the index can be limited by selection of a small test panel. Also, when related antimicrobials are placed on the panel, the index value can be artificially manipulated, depending on the resistance status of the antimicrobials. The **multiple antibiotic resistance (MAR)** index, defined as the proportion of antimicrobials to which an isolate is resistant or the number of antimicrobials to which an isolate is resistant divided by the number of antimicrobials that the isolate was tested against,[20] adjusts for the size of the test panel but not for the diversity of the antimicrobials selected for the panel. The MAR index (also referred to as the **antibiotic resistance index [ARI]**)[50] can be used to summarize the proportion of resistance among several isolates. The MAR index has been used to identify sources of fecal contamination in food[20] and water[51] and for tracking changes in patterns of antimicrobial resistance in calves over time.[50]

With the exception of species richness and the MAR or ARI, it appears that studies of antimicrobial susceptibility have not used the many indices available from the ecologic sciences. The Simpson index,[52] which is a measure of evenness, may have application as an index of diversity for analysis of antimicrobial-resistance patterns. The Simpson index has been used to summarize and compare the diversity of bacterial strains identified by different typing methods.[53] The index can be used to compare 2 hypothetical bacterial samples, each with a size of 100 isolates, which are individually tested for susceptibility. Assume that 10 antimicrobial-resistance phenotypes are evident in each of the samples. Of the 100 isolates in the first sample, assume there were 10 isolates that had each of phenotypes 1 through 4, 1 isolate that had each of phenotypes 5 through 9, and 55 isolates that had the last phenotype. Also assume that the distribution of the second 100 isolates was equivalently distributed among the 10 phenotypes (ie, 10 isolates had each phenotype). The Simpson index would be represented by the following equatioion:

$$D = 1 - \sum_{i=1}^{k} \frac{n_i(n_i-1)}{N(N-1)}$$

where, in the context of the hypothetical analysis of the described antibiogram, k is the number of phenotypes, n_i is the number of isolates that had the ith phenotype, and N is the total number of isolates used to construct the antibiogram. The Simpson index would yield a value of 0.664 for the first sample of 100 isolates and 0.909 for the second sample of 100 isolates. The evenness of the distribution of the phenotypes in the second sample is reflected in the higher index value, compared with the index value for the first sample.

The principal advantage of the use of indices is that they provide a mechanism for incorporating multiple outcomes into a single measure. However, there are disadvantages to consolidating all of the outcomes into a single value. First, specific values for the indices do not necessarily represent a unique or comparable set of conditions. For instance, a value of 3 for richness in each of 2 isolates can be based on 2 differing sets of antimicrobials to which the isolates are resistant. This problem points to the second disadvantage of indices, which is the loss of information when the data are distilled into a single number. Use of SIR data to calculate an index will likely cause an additional loss of information, and specific trends or changes in individual antimicrobial patterns will not be readily discernible. Lastly, results from indices can be dependent on the panel of antimicrobials selected. For example, a panel of 5 antimicrobials has a maximum richness value of 5, whereas a panel of 17 antimicrobials can readily have richness values in excess of 5. Despite these limitations, indices may have use in conjunction with other descriptive measures and inferential analysis to help describe patterns of antimicrobial resistance.

Categorical and Nonparametric Methods

Data on antimicrobial resistance in the form of SIR or MIC data have been analyzed by use of a wide range of parametric and nonparametric categorical statistical methods. Categorical methods that have been or may be used range from univariate estimation of a single proportion and its associated confidence interval to multivariable techniques, such as logistic regression. Contrary to descriptive methods and indices, the goal for categorical analysis of antimicrobial-resistance data is to make inferences to the population of interest.

At a minimum, point estimates should be reported with an associated measure or indicator of error, such as the SE or confidence interval. However, the proportion of resistant (or susceptible) bacteria in a sample is frequently reported without an associated measure of error or confidence interval. When the assumptions of the binomial probability distribution are appropriate and the sample size is reported, the SE and confidence interval can be calculated by readers. Given the assumption that the binomial probability distribution is appropriate and the population size from which the sample was obtained is large, the SE for a proportion can be calculated by use of the following equation[54]:

$$SE(p) = \sqrt{\frac{p \times q}{n}}$$

where p is the proportion of interest, q is the value 1 – p, and n is the sample size. For example, when 12 isolates from a sample of 85 isolates are resistant to an antimicrobial, then the point estimate is 0.141 and the estimated SE is 0.038. Use of the normal approximation yields a 95% confidence interval (0.067, 0.215); however, in this example, the exact confidence interval (0.075, 0.234) is not symmetric. Exact confidence intervals can be calculated by many statistical software packages.

The χ^2 test is probably the most common statistical test used to compare 2 proportions. Proportions that have been compared include the frequency of bacterial species,[55] frequency of resistance to a specific antimicrobial in bacterial populations,[31,41] changes in frequency of resistance between 2 time periods,[10,56] and frequency of multiple-resistant isolates versus nonmultiple-resistant isolates.[57] The Fisher exact test can be used for similar types of comparisons when values in the cells of tables are expected to be low.[18,21,42,58] For paired or matched data, more appropriate tests are available, such as the McNemar test[54] or Mantel-Haenszel χ^2 test.[59] In 1 study,[15] researchers used an adjusted χ^2 test (described in detail in another report[60]) to account for the lack of independence among isolates originating from the same poultry flock when they were examining the association between use of avoparcin and the proportion of samples with vancomycin-resistant *Enterococcus faecium*.

An extension of the χ^2 test (ie, the χ^2 test for trend or linearity[54]) has been used primarily to evaluate changes in prevalence of resistance over time.[5,56,59] However, the test could be applied to other quantitatively ordered variables, such as age group, when investigators are interested in looking for linear trends in proportions for > 2 groups. Researchers in 1 study[61] looked for patterns in the proportion of resistant isolates to 13 antimicrobial agents by use of the SIR categories for penicillin. Resistance to 12 of the 13 antimicrobials increased linearly with increasing resistance (ie, susceptible to intermediate to resistant) to penicillin.

The χ^2 and Fisher exact tests are intended to measure the significance of association. In addition to significance of association, a number of measures of association have been derived to describe the degree of association.[54] Two measures of association that have been used to analyze antimicrobial data include the κ statistic and odds ratios. The κ value measures the amount of agreement after correcting for agreement attributable to chance alone. In 1 study,[19] investigators calculated the κ value for pairs of antimicrobials to assess susceptibility relationships between various antimicrobials among *Salmonella* isolates.

Odds ratios are typically used to assess the relationship of resistance relative to an exposure of interest (ie, identifying risk factors). Typically, odds ratios are derived from multivariable logistic regression models, which allows for adjustment of other variables in the model as well as confounding variables and potential interactions. Logistic regression has been used[61] to examine risk factors for fatalities associated with invasive disease attributable to *Streptococcus pneumoniae* that were resistant to penicillin among all patients with invasive pneumococcal disease. Adjusted odds ratios were obtained from the model for the independent variables of age, race, state, and hospitalization status. In another study,[58] investigators used odds ratios to document the increased likelihood of isolating choloramphenicol-resistant salmonella from cows on a dairy that had exposure to chloramphenicol during the recent past, compared dairies that were not recently exposed.

Odds ratios also can be used for matched samples. Exact conditional logistic regression was used[59] to investigate 2 risk factors (ie, foreign travel and recent use of quinolones) for development of an infection with quinolone-resistant *Campylobacter jejuni*, compared with development of an infection with quinolone-susceptible *C jejuni*. Relative risk, the measure the odds ratio is usually intended to approximate, was used to examine the relationship between administration of avoparcin and the prevalence of vancomycin-resistant *E faecium* on swine farms in Denmark.[15] Contrary to the aforementioned χ^2 test, relative risk and the associated confidence interval were not adjusted for a lack of independence of samples, which may result in incorrect analysis and interpretation.

A measure that, to our knowledge, has not been applied to antimicrobial-resistance data is the **population attributable fraction (PAF)**. The PAF can be defined as the fraction of the overall occurrence of the outcome that can be attributed uniquely to the risk factor.[62] The PAF allows investigators to combine prevalence information with odds ratios (or relative risk) into a single measure. The interpretation of PAF results has intuitive appeal. For example, PAF results may suggest that eliminating the effects associated with a specific factor prevents a portion of the existing antimicrobial resistance. Univariate and multivariable methods for calculating PAF are described elsewhere.[54,63]

Nonparametric statistics have been applied primarily to bacterial counts associated with antimicrobial resistance. In 1 study,[64] investigators determined the number of viable bacteria on coryneform agar that contained furazolidone (to inhibit staphylococci) and differential counts of viable bacteria for the same media after the addition of erythromycin (5 mg/L) or tetracycline (10 mg/L). They compared bacterial counts for erythromycin-resistant bacteria and tetracycline-resistant bacteria cultured from samples obtained from a single location by use of the Wilcoxon test for matched pairs, and they used a Wilcoxon-Mann-Whitney test to compare the proportion of resistant bacteria among locations.

Data on MIC values have been handled descriptively (eg, mean, quantiles, and CDFs) or categorically because of the semicontinuous nature of the data. Investigators in 1 study[65] used a Wilcoxon-Mann-Whitney 2-sided exact test to document that cyclohexane-resistant organisms were more resistant (measured by use of MIC values) to several antimicrobials,

compared with cyclohexane-susceptible organisms. A second nonparametric test, the Kolmogorov-Smirnov test, can be used to compare the CDFs of MIC values. The CDFs of isolates cultured from samples obtained for different sample collection protocols have been compared by use of the Kolmogorov-Smirnov test.[66]

The aforementioned categorical and nonparametric methods have the principal advantage of making inferences for the sampled populations. The availability of the common statistical procedures, including proportion estimates, χ^2 tests, and Fisher exact tests, on many statistical software packages is another important advantage. The Fisher exact test is appropriate for sparse data in that it uses an exact calculation of the probability value.[54]

Logistic regression is an analytic method used in epidemiologic studies, and it has several important advantages, including allowing for comparison of a dichotomous outcome with 1 or more independent variables, enabling conversion of regression coefficients to odds ratios that are adjusted for other independent variables in the model, and allowing for examination of relationships among independent variables (eg, confounding or effect modification).[67] Nonparametric tests have the advantage of relaxing the assumption of an underlying assumed probability distribution for the data.[68]

Independence of observations is an assumption that is common to all of these inferential statistical methods. However, independence of observations may be the assumption that is most likely to be violated in studies of antimicrobial susceptibility. For example, bacterial isolates from production animals within herds or flocks, or even those from the same animal, may have patterns of antimicrobial resistance that are more similar than those for single isolates from a randomly selected group of animals. This similarity within a group is often reflected in smaller variance than might be expected had the grouping not happened.[69] Thus, failing to account for a lack of independence of observations can result in smaller SEs than the true SEs for group effects; this could increase the probability of a type-I error (concluding significant differences exist when, in fact, they do not exist). Methods exist for adjusting for the lack of independence of observations.

Individually, the procedures have other disadvantages that should be mentioned. Categorization of MIC data into SIR data for the purpose of estimating resistance prevalence or use in χ^2 tests may result in loss of information contained in the data. The χ^2 test evaluates SIR data for each antimicrobial; therefore, a panel of 17 antimicrobials would require 17 separate tests. The Fisher exact test works well with sparse data in a 2×2 contingency table, but it can be a difficult procedure to implement when contingency tables are larger than 2×2. The standard test to detect trends (ie, Cochran-Armitage trend test) considers only linear trends in the proportion in relation to a quantitative variable and thus does not test for curvilinear trends.[54] The Cochran-Armitage trend test can be modified to assess the significance of polynomial (curvilinear) trends in the data, but we are not aware of the application of this approach for data on antimicrobial resistance. Also, tests for trends can only be interpreted when the data collection, testing methods, and sampled population are the same throughout the time frame of interest. The κ statistic is affected by the prevalence of the response being measured.[54] The McNemar test is used for bivariate matched data. When > 2 levels for a variable or more than paired observations are included, then other methods such as the Stuart χ^2 test or Cochran Q test may be valid alternatives.[54]

Multivariate Statistics

The use of multivariate statistics for the analysis of antimicrobial resistance has been limited. However, testing multiple antimicrobials against individual isolates results in multiple outcomes per isolate, which makes multivariate statistics a possible alternative for analysis. Multivariate methods that have been applied include cluster, correspondence, discriminant, and factor analyses.

A hierarchical cluster analysis, based on average linkage (ie, the **unweighted pair group method with arithmetic mean [UPGMA]**), and squared Euclidean distances, apparently based on MIC data, have been reported.[57] In addition to distance, similarities can also be used as a basis for cluster analysis. A number of similarity coefficients can be used for cluster analysis of binary outcomes.[70] We are not aware of the use of these or other similarity measures in studies of antimicrobial resistance. Typically, cluster analysis has been used to group isolates on the basis of results of genetic testing, such as evidence or lack of evidence of specific bands on ribotyping analysis. In 1 study,[71] investigators used a Dice coefficient (ie, an ecologic index measure) to construct a dendrogram by use of UPGMA. After construction of the dendrogram, they attached additional information to each of the isolates, including the resistance pattern, geographic and animal species origin, and restriction fragment-length polymorphism category for erythromycin-resistant methylase B.

Correspondence analysis is an exploratory analytic method for describing complex relationships among qualitative outcome variables.[72] Principally, the objective is to summarize the variables in fewer dimensions (commonly called ranks) and represent the data graphically. Correspondence analysis has been used to examine host and geographic influences on antimicrobial-resistance patterns.[49] In that study, the first 2 ranks from the correspondence analysis explained 26.8% of the observed variation in resistance for the original panel of 13 antimicrobials. Those investigators graphically displayed the mean and SD values for ranks 1 and 2 for each host species and the geographic area of interest. Additionally, they used the values from rank 1 as the dependent variable and host and geographic categories as independent variables in an ANOVA.

Discriminant analysis is a multivariate classification procedure whereby the primary objective is to separate groups of objects and allocate new observations to the existing groups.[70] In the context of antimicrobial resistance, the objective of discriminant analysis has been to examine the potential to create a discriminant function based on antimicrobial-resistance data that can be used to reliably distinguish groups of isolates on

the basis of their sources. The objective of discriminant analysis in 3 studies[73-75] was to identify the source (human or other animal) of fecal streptococci on the basis of patterns of antimicrobial resistance. The binary outcome of growth or no growth in specified concentrations of the antimicrobials of interest was used to construct the discriminant function.

Contrary to discriminant analysis, factor analysis is oriented toward understanding underlying patterns in data. This method assumes that there is a set of interpretable factors that can be computed as a function of the original variables based on the underlying covariance or correlation structure.[70,72] In 1 study,[76] factor analysis was used to describe underlying patterns in MIC data. In that study, investigators identified 6 factors that could be interpreted on the basis of class of antimicrobial, prevalence of susceptibility or resistance, and previously described associations.

When panels of antimicrobials are tested against a group of isolates, it can be extremely difficult to summarize patterns of association by use of SIR or MIC data. Multivariate methods are appropriate for use in the analysis of data with multiple outcomes and do not have the pitfall of oversummarizing the data, such as when indices are used. Multivariate methods can be used to reduce the number of outcomes being analyzed. In this sense, the output from multivariate approaches is used in further analysis. This type of analysis has been performed by use of correspondence results in an ANOVA.[49]

A number of disadvantages are inherent when using multivariate methods to analyze data on antimicrobial resistance. First, the methods have not been widely used to analyze antimicrobial data, and readers may have some difficulty in understanding the methods, results, and interpretations. Second, multivariate normality is an assumption of most multivariate techniques.[70] This assumption may not be plausible when MIC data are used because of the discrete nature of the data and relatively few MIC values that may be determined in studies of antimicrobial resistance. Multivariate measures of central tendency, such as a multivariate ANOVA, may be difficult to interpret in the context of antimicrobial resistance.

Analysis of Clustered Data

Mixed-model ANOVAs and other methods for analyzing clustered data have not been widely used to analyze data on antimicrobial resistance, despite the potential use of these techniques. A detailed discussion of the need for appropriate design and analytic methods is reported elsewhere.[69] Investigators in 1 study[77] used a multiple-stage, sample collection design to investigate the prevalence of vancomycin resistance in *E faecium* in pigs. They calculated the effect associated with the design of their sample collection protocol. Design effect is a measure of the change in the variance attributable to the sample collection design relative to the variance for a simple, random, sample collection design.[78] In that study, confidence intervals for farm-level prevalence were adjusted to account for the design effect. In another study,[79] investigators used a logistic regression model to estimate the effects of independent variables (eg, room, pen, and sex) on the proportion of resistant *E coli* found on swine farms. They corrected for clustering (ie, overdispersion) by scaling the model deviances by the residual deviance divided by the number of degrees of freedom.[80] This correction factor adjusted the likelihood ratio test statistics of 2 models (1 for tetracycline and another for gentamicin) by factors of 47.26 and 13.55, respectively. They also adjusted the SEs associated with their models by factors of 6.87 and 3.68, respectively. In both models, the proportion of resistance between rooms of pigs or between pigs of differing sex were not significantly different when the correction factor was used to account for clustering.

In 1 study,[66] investigators calculated SEs associated with the proportion of resistance by use of the delta method (a first-order Taylor series approximation). This accounted for the lack of independence of multiple isolates obtained from a sample and for nesting of fecal samples within a feedlot pen.

Mixed models are another method for accounting analytically for the sample design. They have potential for use in analyzing MIC or SIR data. Mixed models allow for inclusion of random and fixed effects. Additionally, variance components can be evaluated to assess the importance of sources of variation in the data. A random-effects ANOVA has been used to examine the proportion of total and tetracycline-resistant *E coli* concentrations attributable to the hierarchical levels associated with the sample-collection technique.[79] For the proportion of tetracycline-resistant *E coli*, investigators in that study documented that most of the variance was in the between-pig, within-pen component, whereas the between-pen, within-room, and between-room components were relatively small.

The design-based structure of data on antimicrobial resistance is often overlooked during the analysis phase in many studies. Methods for analyzing clustered data, principally multiple-level models, can account for the structure of the data by accounting for dependencies, random effects, and hierarchical nesting.[81,82] Mixed models can be used to evaluate the contribution of variance from various sources (eg, herd, flock, pen, or each animal). Knowledge of the important sources of variance can be important when designing studies and considering interventions.[66,79]

Mixed models for discrete outcomes, such as the binary susceptible-resistance response, can be relatively difficult to understand, implement, and interpret. Mixed models for discrete outcomes rely on hierarchical generalized linear models; research is continuing in this area, and researchers continue to develop software to implement statistical analyses.[82]

Conclusions

Many methods have been used to analyze data on antimicrobial resistance. We have described various methods that have been applied to this type of data, including some of the advantages and disadvantages. We believe there are several issues that should be considered when analyzing data on antimicrobial resistance.

First, the conversion of the MIC (or size of the disk-diffusion zone) to SIR data must be carefully con-

sidered. Categorizing data has the advantage of distilling information into a form that is easier to report and can be more quickly assimilated by the intended audience. However, summarizing the MIC or size of the disk-diffusion zone can result in loss of information. We recommend that the MIC and data on the zone size be reported whenever possible. A similar recommendation was made in another report.[39] The authors of that report recognized the fact that a lack of internationally uniform breakpoints for resistance hinders interpretation. Furthermore, those authors emphasized that reporting data, such as the MIC or zone size, will enable ease and accuracy of comparison of data in future studies. Reporting the MIC and data on zone size does not have to exclude analysis or reporting of SIR data.

A second issue for consideration relates to the assumption of independent observations that is typical of inferential statistical techniques.[54,68] When multiple isolates are obtained from the same animal or source, such as a hospital or feedlot pen, the assumption of independence may be inappropriate. For example, gram-negative isolates were obtained from intensive-care units in hospitals in Turkey.[83] Investigators in that study obtained 749 isolates from 473 patients. Of those isolates, 128 (17.1%) were derived from polymicrobial growths on the same culture, and 160 (21.4%) were obtained from repeat cultures, which were interpreted as persistent colonization. When observations are assumed to be independent but actually are correlated, then the subsequent analysis may be flawed.[69] Underestimating variance may lead to accepting the alternative hypothesis in cases in which it should not be accepted (ie, rejecting the null hypothesis). The degree to which the lack of independence can alter results is evident by the size of the adjustment factors that were used in an aforementioned study.[79] Methods used for inferential analysis of data on antimicrobial resistance must account for the lack of independence or correlated data structures to avoid erroneous conclusions. Many of the common analytic methods, including prevalence estimates and χ^2 tests, can be used while accounting for clustering through post hoc variance adjustments or the use of procedures to estimate alternative variance estimates.

An important issue in antimicrobial resistance is strains of bacteria with resistance to multiple antimicrobials. Almost exclusively, patterns of multiple-drug resistance have been examined by use of SIR data, which are usually collapsed into resistant-nonresistant categories to create antibiograms or descriptions of multiple-resistant phenotypes. Multivariate methods provide an alternative method for use in assessing patterns of resistance-susceptibility by use of MIC data. We recommend that analysts consider use of multivariate techniques for exploring and describing potentially complex relationships among antimicrobials. The use of multivariate techniques should not preclude the use of other methods of reporting multiple-resistant results that may be more widely understood.

One additional issue for consideration is the selection of the appropriate analytic method. The selection of analytic methods should reflect clearly stated research goals and be appropriate for the study design and data structure, as well as the intended audience. These criteria make it implausible that a single analytic method will be most appropriate for all situations. Similarly, there likely will be a need to use several analytic methods to display, summarize, and test hypotheses to meet these criteria.

References

1. Bywater RJ, Casewell MW. An assessment of the impact of antibiotic resistance in different bacterial species and of the contribution of animal sources to resistance in human infections. *J Antimicrob Chemother* 2000;46:643–645.
2. Fey PD, Safranek TJ, Rupp ME, et al. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. *N Engl J Med* 2000; 342:1242–1249.
3. Finch RG. Antibiotic resistance. *J Antimicrob Chemother* 1998; 42:125–128.
4. Low DE. Appropriate antibiotic use: reducing the emergence and dissemination of resistance. *Int J Clin Pract* 2000;115:106–110.
5. Normand EH, Gibson NR, Reid S, et al. Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. *Prev Vet Med* 2000;46:267–278.
6. Rice LB. Emergence of vancomycin-resistant enterococci. *Emerg Infect Dis* 2001;7:183–187.
7. Stobberingh EE, van den Bogaard AE. Spread of antibiotic resistance from food animals to man. *Acta Vet Scand Suppl* 2000; 93:47–50.
8. Threlfall EJ, Ward LR, Frost JA, et al. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int J Food Microbiol* 2000;62:1–5.
9. White DG, McDermott PF. Emergence and transfer of antibacterial resistance. *J Dairy Sci* 2001;84:E151–E155.
10. Sahm DF, Karlowsky JA, Kelly LJ, et al. Need for annual surveillance of antimicrobial resistance in *Streptococcus pneumoniae* in the United States: 2-year longitudinal analysis. *Antimicrob Agents Chemother* 2001;45:1037–1042.
11. Holmberg SD, Osterhom MT, Senger KA, et al. Drug-resistant Salmonella from animals fed antimicrobial. *N Engl J Med* 1984; 311:617–622.
12. Tacket CO, Dominguez LB, Fisher HJ, et al. An outbreak of multiple-drug-resistant *Salmonella enteritis* from raw milk. *JAMA* 1985;253:2058–2060.
13. Dawson KA, Boling JA. Monensin-resistant bacteria in the rumens of calves on monensin-containing and unmedicated diets. *Appl Environ Microbiol* 1983;46:160–164.
14. Ebner PD, Mathew AG. Effects of antibiotic regimens on the fecal shedding patterns of pigs infected with *Salmonella* Typhimurium. *J Food Prot* 2000;63:709–714.
15. Bager F, Madsen M, Christensen J, et al. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev Vet Med* 1997;31:95–112.
16. Donskey CJ, Chowdhry TK, Hecker MT, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *N Engl J Med* 2000; 343:1925–1932.
17. Kapperud G, Skjerve E, Vik L, et al. Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. *Epidemiol Infect* 1993;111:245–255.
18. Mathew AG, Saxton AM, Upchurch WG, et al. Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. *Appl Environ Microbiol* 1999;65:2770–2772.
19. Dargatz DA, Fedorka-Cray PJ, Ladely SR, et al. Antimicrobial susceptibility patterns of *Salmonella* isolates from cattle in feedlots. *J Am Vet Med Assoc* 2002;221:268–272.
20. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl Environ Microbiol* 1983;46:165–170.
21. Tsolia M, Kouppari G, Zaphiropoulou A, et al. Prevalence and patterns of resistance of *Streptococcus pneumoniae* strains isolated from carriers attending day care centers in the area of Athens. *Microb Drug Resist* 1999;5:271–278.

22. Aminov RI, Garrigues-Jeanjean N, Mackie RI. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Appl Environ Microbiol* 2001;67:22–32.

23. Rothman KJ. Measures of disease frequency. In: *Modern epidemiology*. Boston: Little, Brown & Co, 1986;23–34.

24. Zar JH. Chapters 1 and 2. In: *Biostatistical analysis*. 4th ed. Englewood Cliffs, NJ: Prentice-Hall Inc, 1999;1–19.

25. Benbachir M, Benredjeb S, Boye CS, et al. Two-year surveillance of antibiotic resistance in *Streptococcus pneumoniae* in four African cities. *Antimicrob Agents Chemother* 2001;45:627–629.

26. Breuil J, Brisabois A, Casin I, et al. Antibiotic resistance in salmonellae isolated from humans and animals in France: comparative data from 1994 and 1997. *J Antimicrob Chemother* 2000;46:965–971.

27. Brophy PO, Caffrey PJ, Collins JD. Sensitivity patterns of *Escherichia coli* isolated from calves during and following prophylactic chlortetracycline therapy. *Br Vet J* 1977;133:340–345.

28. Engberg J, Aarestrup FM, Taylor DE, et al. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerg Infect Dis* 2001; 7:24–34.

29. Millar MR, Walsh TR, Linton CJ, et al. Carriage of antibiotic-resistant bacteria by healthy children. *J Antimicrob Chemother* 2001;47:605–610.

30. Saenz Y, Zarazaga M, Lantero M, et al. Antibiotic resistance in Campylobacter strains isolated from animals, foods, and humans in Spain in 1997–1998. *Antimicrob Agents Chemother* 2000;44:267–271.

31. Sahm DF, Jones ME, Hickey ML, et al. Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in Asia and Europe, 1997–1998. *J Antimicrob Chemother* 2000;45:457–466.

32.Sanches IS, Mato R, De Lencastre H, et al. Patterns of multidrug resistance among methicillin-resistant hospital isolates of coagulase-positive and coagulase-negative staphylococci collected in the International Multicenter Study RESIST in 1997 and 1998. *Microb Drug Resist* 2000;6:199–211.

33. Seyfarth AM, Wegener HC, Frimodt-Moller N. Antimicrobial resistance in *Salmonella enterica* subsp. *enterica* serovar typhimurium from humans and production animals. *J Antimicrob Chemother* 1997;40:67–75.

34. Rajashekara G, Haverly E, Halvorson DA, et al. Multidrug-resistant *Salmonella* Typhimurium DT104 in poultry. *J Food Prot* 2000;63:155–161.

35. Stephan R, Schumacher S. Resistance patterns of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from animals, food and asymptomatic human carriers in Switzerland. *Lett Appl Microbiol* 2001;32:114–117.

36. Aarestrup FM, Agerso Y, Ahrens P, et al. Antimicrobial susceptibility and presence of resistance genes in staphylococci from poultry. *Vet Microbiol* 2000;74:353–364.

37. Malorny B, Schroeter A, Helmuth R. Incidence of quinolone resistance over the period 1986 to 1998 in veterinary *Salmonella* isolates from Germany. *Antimicrob Agents Chemother* 1999;43:2278–2282.

38. Wiuff C, Madsen M, Baggesen DL, et al. Quinolone resistance among *Salmonella enterica* from cattle, broilers, and swine in Denmark. *Microb Drug Resist* 2000;6:11–17.

39. Jones RN. Detection of emerging resistance patterns within longitudinal surveillance systems: data sensitivity and microbial susceptibility. MYSTIC Advisory Board. Meropenem Yearly Susceptibility Test Information Collection. *J Antimicrob Chemother* 2000;46(suppl T2):1–8.

40. Thornsberry C, Jones ME, Hickey ML, et al. Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in the United States, 1997–1998. *J Antimicrob Chemother* 1999;44:749–759.

41. Arvand M, Hoeck M, Hahn H, et al. Antimicrobial resistance in *Streptococcus pyogenes* isolates in Berlin. *J Antimicrob Chemother* 2000;46:621–623.

42. Butaye P, Devriese LA, Haesebrouck F. Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. *Antimicrob Agents Chemother* 2001;45:1374–1378.

43. Zhanel GG, Karlowsky JA, Palatnick L, et al. Prevalence of antimicrobial resistance in respiratory tract isolates of *Streptococcus pneumoniae*: results of a Canadian national surveillance study. The Canadian Respiratory Infection Study Group. *Antimicrob Agents Chemother* 1999;43:2504–2509.

44. Cavallo JD, Fabre R, Leblanc F, et al. Antibiotic susceptibility and mechanisms of beta-lactam resistance in 1310 strains of *Pseudomonas aeruginosa*: a french multicentre study (1996). *J Antimicrob Chemother* 2000;46:133–136.

45. Bruun MS, Schmidt AS, Madsen L, et al. Antimicrobial resistance patterns in Danish isolates of *Flavobacterium psychrophilum*. *Aquaculture* 2000;187:201–212.

46. National Antimicrobial Resistance Monitoring Program—enteric bacteria. *Veterinary isolates final report*. Athens, Ga: USDA—Agricultural Research Service, 1997;50–52.

47. Hurlbert SH. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* 1971;52:577–586.

48. Kricher JC. Bird species diversity: the effect of species richness and equitability on the diversity index. *Ecology* 1972;53:278–282.

49. Sherley M, Gordan DM, Collignon PJ. Variations in antibiotic resistance profile in Enterobateriaceae isolated from wild Australian mammals. *Environ Microbiol* 2000;2:620–631.

50. Hinton M, Hedges AJ, Linton AH. The ecology of *Escherichia coli* in market calves fed a milk-substitute diet. *J Appl Bacteriol* 1985; 58:27–35.

51. Kaspar CW, Burgess JL, Knight IT, et al. Antibiotic resistance indexing of *Escherichia coli* to identify sources of fecal contamination in water. *Can J Microbiol* 1990;36:891–894.

52. Simpson EH. Measurement of diversity. *Nature* 1949; 163:688.

53. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol* 1988;26:2465–2466.

54. Fleiss JL. *Statistical methods for rates and proportions*. New York: John Wiley & Sons Inc, 1981;1–321.

55. Barisic Z, Punda-Polic V. Antibiotic resistance among enterococcal strains isolated from clinical specimens. *Int J Antimicrob Agents* 2000;16:65–68.

56. Davis MA, Hancock DD, Besser TE, et al. Changes in antimicrobial resistance among *Salmonella enterica* serovar Typhimurium isolates from humans and cattle in the Northwestern United States, 1982–1997. *Emerg Infect Dis* 1999;5:802–806.

57. London N, Nijsten R, Bogaard AVD, et al. Carriage of antibiotic-resistant *Escherichia coli* by health volunteers during a 15-week period. *Infection* 1994;22:187–192.

58. Spika JS, Waterman SH, Hoo GW, et al. Chloramphenicol-resistant *Salmonella newport* traced through hamburger to dairy farms. A major persisting source of human salmonellosis in California. *N Engl J Med* 1987;316:565–570.

59. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. Investigation Team. *N Engl J Med* 1999;340:1525–1532.

60. Donner A. The comparison of proportions in the presence of litter effects. *Prev Vet Med* 1993;18:17–26.

61. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000;343:1917–1924.

62. Wagner BA, Wells SE, Kott PS. Variance estimation for population attributable risk in a complex cross-sectional animal health survey. *Prev Vet Med* 2001;48:1–13.

63. Bruzzi P, Green SB, Byar DP, et al. Estimating the population attributable risk for multiple risk factors using case-control data. *Am J Epidemiol* 1985;122:904–914.

64. Eady EA, Coates P, Ross JI, et al. Antibiotic resistance patterns of aerobic coryneforms and furazolidone-resistant gram-positive cocci from the skin surface of the human axilla and fourth toe cleft. *J Antimicrob Chemother* 2000;46:205–213.

65. Randall LP, Cooles SW, Sayers AR, et al. Association between cyclohexane resistance in *Salmonella* of different serovars and increased resistance to multiple antibiotics, disinfectants and dyes. *J Med Microbiol* 2001;50:919–924.

66. Wagner BA, Dargatz DA, Salman MD, et al. Comparison of sampling techniques for measuring antimicrobial susceptibility of enteric *Escherichia coli* recovered from feedlot cattle. *Am J Vet Res* 2002;63:1662–1670.

67. Hosmer DW, Lemeshow S. Interpretation of the coefficients of the logistic regression model. In: *Applied logistic regression*. 2nd ed. New York: John Wiley & Sons Inc, 2000;38–81.

68. Conover WJ. Statistical inference. In: *Practical nonparametric statistics*. 2nd ed. New York: John Wiley & Sons Inc, 1980; 60–93.

69. McDermott JJ, Schukken YH, Shoukri MM. Study design and analytical methods for data collected from clusters of animals. *Prev Vet Med* 1994;18:175–191.

70. Johnson RA, Wichern DW. *Applied multivariate statistical techniques*. 3rd ed. Englewood Cliffs, NJ: Prentice-Hall Inc, 1992;1–642.

71. Boerlin P, Burnens AP, Frey J, et al. Molecular epidemiology and genetic linkage of macrolide and aminoglycoside resistance in *Staphylococcus intermedius* of canine origin. *Vet Microbiol* 2001; 79:155–169.

72. Dohoo IR, Ducrot C, Fourichon C, et al. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Prev Vet Med* 1997;29:221–239.

73. Harwood VJ, Whitlock J, Withington V. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Appl Environ Microbiol* 2000;66:3698–3704.

74. Wiggins BA. Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and animal sources of fecal pollution in natural waters. *Appl Environ Microbiol* 1996;62:3997–4002.

75. Wiggins BA, Andrews RW, Conway RA, et al. Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution. *Appl Environ Microbiol* 1999;65:3483–3486.

76. Wagner BA, Salman MD, Dargatz DA, et al. Factor analysis of minimum inhibitory concentrations for *Escherichia coli* isolated from feedlot cattle to model relationships among antimicrobial outcomes. *Prev Vet Med* 2003;57:127–139.

77. Herrero IA, Teshager T, Garde J, et al. Prevalence of vancomycin-resistant *Enterococcus faecium* (VREF) in pig faeces from slaughterhouses in Spain. *Prev Vet Med* 2000;47:255–262.

78. Dargatz DA, Hill GW. Analysis of survey data. *Prev Vet Med* 1996;28:225–237.

79. Dunlop RH, McEwen SA, Meek AH, et al. Sampling considerations for herd-level measurement of faecal *Escherichia coli* antimicrobial resistance in finisher pigs. *Epidemiol Infect* 1999;122:485–496.

80. Aitkin M, Anderson D, Francis B, et al. Section 4.9: overdispersion, omitted variables and model mis–specification. In: Francis B, ed. *Statistical modeling in GLIM*. Oxford, England: Clarendon Press, 1989;213–216.

81. Hox JJ, Kreft IGG. Multilevel analysis methods. *Sociologic Methods Res* 1994;22:283–299.

82. Snijders TAB, Bosker RJ. Chapters 1 to 5. *Multilevel analysis: an introduction to basic and advanced multilevel modeling*. London: Sage Publications Ltd, 1999;1–85.

83. Aksaray S, DokuzoGuz B, Guvener E, et al. Surveillance of antimicrobial resistance among gram-negative isolates from intensive care units in eight hospitals in Turkey. *J Antimicrob Chemother* 2000;45:695–699.