Special Report

Survey of antimicrobial susceptibility testing practices of veterinary diagnostic laboratories in the United States

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Objective—To describe antimicrobial susceptibility testing practices of veterinary diagnostic laboratories in the United States and evaluate the feasibility of collating this information for the purpose of monitoring antimicrobial resistance in bacterial isolates from animals.

Design—Cross-sectional study.

Procedures—A questionnaire was mailed to veterinary diagnostic laboratories throughout the United States to identify those laboratories that conduct susceptibility testing. Nonrespondent laboratories were followed up through telephone contact and additional mailings. Data were gathered regarding methods of susceptibility testing, standardization of methods, data management, and types of isolates tested.

Results—Eighty-six of 113 (76%) laboratories responded to the survey, and 64 of the 86 (74%) routinely performed susceptibility testing on bacterial isolates from animals. Thirty-four of the 36 (94%) laboratories accredited by the American Association of Veterinary Laboratory Diagnosticians responded to the survey. Laboratories reported testing > 160,000 bacterial isolates/year. Fifty-one (88%) laboratories reported using the Kirby-Bauer disk diffusion test to evaluate antimicrobial susceptibility; this accounted for 65% of the isolates tested. Most (87%) laboratories used the NCCLS (National Committee for Clinical Laboratory Standards) documents for test interpretation. Seventy-five percent of the laboratories performed susceptibility testing on bacterial isolates only when they were potential pathogens.

Conclusions—The veterinary diagnostic laboratories represent a comprehensive source of data that is not easily accessible in the United States. Variability in testing methods and data storage would present challenges for data aggregation, summary, and interpretation. (J Am Vet Med Assoc 2003;222:168–173)

Monitoring trends in antimicrobial susceptibility of bacterial isolates from animals is becoming increasingly important in public and animal health. The Swann Committee report, issued by the British government in 1969, evaluated the risk of transmitting bacteria resistant to antimicrobial drugs from animals to humans and recommended that antimicrobials used to treat infections in humans not be used as feed additives for animals. The United States General Accounting Office (GAO) questioned the human health implications of using antimicrobials in animals in 1977. At that time, the GAO concluded that there were too many gaps in the data to allow for conclusions and recommended that more research be conducted. The World Health Organization, National Research Council, Food and Drug Administration, and US Department of Agriculture have also called for more data.

Many questions about the magnitude of the antimicrobial resistance problem and the factors most likely to affect development of resistance remain unanswered. To provide a more thorough assessment of risk and improve decision making in the development of policies for future regulation of antimicrobial drugs, it is imperative to have efficient methods for detecting the emergence of antimicrobial resistance and for tracking changes in antimicrobial susceptibility.

Several public and private organizations have established programs to monitor antimicrobial resistance of bacterial isolates from humans. The Surveillance Network Database-USA, operated by Focus Technologies, is an example of a large private-sector antimicrobial resistance surveillance effort that targets human bacterial isolates in the United States. The system has recruited multiple human hospitals throughout the United States to submit susceptibility data electronically on a daily basis. Similarly, the World Health Organization has developed the WHONET software, which can be downloaded by laboratories at no cost, to assist in characterization and analyses of susceptibility profiles. The software uses a standardized data format for all centers, which allows comparisons to be easily made among participating centers. Private companies supplying automated susceptibility testing systems also provide software for interpretation and analyses of susceptibility results.
Most published summaries of antimicrobial susceptibility data have been derived from point-in-time projects focused on particular subsets of organisms or classes of antimicrobial drugs. Because of the possibility that resistant organisms will be transferred from animals to humans, government organizations have begun monitoring information on antimicrobial susceptibility of enteric isolates from animals. The National Antimicrobial Resistance Monitoring System for Enteric Bacteria was established in 1996 as a joint effort of the US Department of Agriculture, Food and Drug Administration, and Centers for Disease Control and Prevention and is the only federally sponsored program for ongoing monitoring of antimicrobial susceptibility in enteric isolates from humans and animals. The program has been successful in establishing new thematic areas of research to determine the mechanisms of antimicrobial resistance and risks posed by resistant bacteria from animals.

Although the information gathered by the National Antimicrobial Resistance Monitoring System is beneficial, the scope of the program has been relatively narrow because of limited resources. In 1998, the system evaluated 4,000 bacterial isolates from animals, but this number may not be sufficient to effectively detect small changes in antimicrobial susceptibility patterns or to reliably identify rare but important resistance phenotypes. In addition, the system relies heavily on passive surveillance to obtain bacterial isolates, which may affect the ability to adequately characterize antimicrobial resistance among healthy animal and human populations. Additionally, the system is able to report its findings only months after collection of the data, which could limit its use as an early warning system.

An optimal system for monitoring changes in antimicrobial susceptibility should include many species of bacterial isolates (pathogens and non-pathogens), evaluate a large number of isolates from a broad and diverse number of sources, characterize susceptibility to an adequate number of antimicrobial drugs that are important therapeutically in both animals and humans, process and report the data in a timely manner, and establish strict quality control procedures to ensure that data are uniform and externally comparable. Unfortunately, no system currently available in the United States meets all of these criteria. An alternative to establishing a new system de novo would be to integrate susceptibility data that are already being collected by the state and regional veterinary diagnostic laboratories throughout the country. These laboratories evaluate most of the biologic specimens submitted for bacterial culture from animal sources in the United States. Benefits obtained by compiling this susceptibility information from these laboratories would include improved quality care for patients, informed policy decisions, and a foundation of understanding that would allow for targeting critical areas of research.

Unfortunately, there is no existing monitoring system in place to aggregate and summarize the data generated by these laboratories. Further, it is not clear if the data from the various laboratories are so dissimilar as to prevent their compilation. The purpose of the study reported here was to describe antimicrobial susceptibility testing practices of veterinary diagnostic laboratories in the United States and evaluate the feasibility of collating this information for the purpose of monitoring antimicrobial resistance in bacterial isolates from animals.

Materials and Methods

Study overview—A self-administered questionnaire was mailed in May 2000 to veterinary diagnostic laboratories throughout the United States. The questionnaire was designed to collect information on laboratory demographics, antimicrobial susceptibility testing protocols, and data management for each laboratory. Returned questionnaires were reviewed for completeness, and laboratories were contacted by telephone for missing data or clarification of answers. A second questionnaire was mailed to nonrespondent laboratories in February 2001 after a telephone call was made to confirm address information. Data were collected from mail surveys and telephone conversations between May 2000 and June 2001.

Laboratory participation—The sampling frame for the study consisted of the 1995 Directory of Animal Disease Diagnostic Laboratories, published by the USDA:APHIS-VS National Veterinary Services Laboratories in Ames, Iowa, and a list of all laboratories in the United States accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). Enrollment was limited to laboratories in the United States that received isolates from domestic species (agricultural or nonagricultural species) and did not restrict their diagnostic work to a single program (eg, aquarium) or bacterial species (eg, Brucella abortus). Satellite laboratories were not enrolled separately, because information for satellite laboratories was collected from the parent laboratories.

Survey design—Information was gathered about methods used to determine antimicrobial susceptibility during the previous year (eg, broth dilution or disk diffusion) and how results were reported and archived (eg, susceptible-intermediate-resistant interpretation, zone sizes, and minimum inhibitory concentrations [MICs]). Data were also collected regarding number of isolates tested per animal species and types of diagnostic services routinely provided. Information was gathered regarding standardization protocols that were used in evaluating antimicrobial susceptibility. Questions were also asked about whether methods of testing (eg, broth dilution and disk diffusion) were altered in some circumstances or for certain organisms. In addition, data were requested on recognized nosocomial agents (eg, Salmonella spp, Staphylococcus spp) from those laboratories associated with a hospital. Epidemiologic investigators and laboratory diagnosticians previewed the survey for thoroughness, clarity, usefulness, and applicability to the objectives. The survey was pretested at a veterinary diagnostic laboratory to ensure that instructions were clear and data requested were easily accessible by laboratory personnel.

Data analysis—Data were entered into a database and validated to ensure accuracy and completeness. Descriptive statistics were calculated. Data were stratified for analysis by laboratory size (small, < 2,000 isolates tested/yr; large, ≥ 2,000 isolates tested/yr) and AAVLD accreditation status (yes vs no). χ² Tests of association were used to determine whether laboratory size or accreditation status was associated with each survey response. Analyses were performed with standard software; for all analyses, a value of P < 0.05 was considered significant.
Results

Surveys were initially mailed to 181 laboratories. However, 41 (23%) of these were found to not be eligible for enrollment, because they did not meet the criteria for participation, and 27 laboratories could not be contacted. Eighty-six of the remaining 113 (76%) laboratories responded to the survey and were eligible for inclusion. Of these 86 laboratories, 22 (26%) were not able to complete the survey, as they did not perform susceptibility testing. Of the 64 respondents that did perform susceptibility testing, 6 (9%) could not complete the section asking for demographics of animals tested, as this information was not retrievable from their database. Thirty-four of the 36 (94%) AAVLD-accredited laboratories in the United States responded to the survey.

Respondents from the 64 participating laboratories estimated that a total of 164,283 bacterial isolates were evaluated per year. Thirty-three percent (54,603) of the isolates were from canines, 19% (32,017) were from bovines, 12% (20,440) were from equines, 10% (16,106) were from felines, 10% (15,158) were from exotic animal species, and the remaining 16% (25,959) were from swine, poultry, ovines, and caprines. Seventeen laboratories reported testing samples (14,421) from patients at affiliated hospitals.

Considerable variability among laboratories was found in the number of isolates tested (Table 1). One laboratory reported evaluating 18 isolates during the previous year, whereas 7 laboratories each reported testing >5,000 isolates in the previous year. The median number of antimicrobial susceptibility tests performed annually for all laboratories was 1,185 (interquartile range, 600 to 2,700). Median number of antimicrobial susceptibility tests performed by the AAVLD-accredited laboratories was 2,049 (interquartile range, 1,000 to 3,443).

The Kirby-Bauer disk diffusion method was the predominant method used for susceptibility testing. The disk diffusion method was used by 51 laboratories to evaluate 65% of all isolates. Broth dilution methods were used in 21 laboratories to evaluate 33% of all isolates. The remaining 2% of isolates were evaluated by other methods (eg, E test and agar dilution method).

The disk diffusion test was the predominant method used for isolates from all animal species, except for swine and canines, for which broth dilution and disk diffusion were used with approximately equal frequency (Fig 1). Fifty-one percent of the laboratories reported that the method of susceptibility testing they performed (broth dilution or disk diffusion) did not vary with the type of organism identified. Laboratories typically reported the susceptible-intermediate-resistant interpretation for disk diffusion results and the MIC for broth dilution results (Table 2).

Thirty-three of 58 (57%) laboratories performed susceptibility testing on isolates from all of the host species identified in the survey. Ten (17%) performed susceptibility testing on isolates from 4 or fewer animal species identified in the survey. Forty-eight of 64 (75%) laboratories performed susceptibility testing on bacterial isolates only when they were potential pathogens, 10 of 64 (16%) routinely tested most isolates, and 6 of 64 (9%) tested isolates only when requested by the attending clinician. Fifty-six of 64 (88%) laboratories reported using performance standards described in the National Committee for Clinical Laboratory Standards (NCCLS) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals.

Some isolates are not covered in the NCCLS standards document, in which case most laboratories reported using drug manufacturer recommendations or generated their own guidelines on the basis of results of previous testing of the organisms in question. Eighty-one percent (52/64) of respondent laboratories reported using quality control protocols described in the NCCLS standards document.

Sixty-one percent (39/64) of laboratories reported that they did not summarize antibiogram statistics on a regular basis. Of the 25 laboratories that did produce summaries of susceptibility patterns, 56% (14/25) summarized data on an annual basis, and 44% (11/25) summarized data less often. Seventeen percent (11/64) of laboratories performed susceptibility testing on anaerobic isolates. Ten of the 17 laboratories that reported testing isolates from patients at an affiliated veterinary hospital reported that they routinely monitored for nosocomial organisms. Escherichia coli was of concern at 7 of these 10 hospital-affiliated laboratories, Pseudomonas spp and Staphylococcus spp were of concern at 5 laboratories each, Salmonella enterica was of concern at 4 laboratories, and Klebsiella spp and Enterococcus spp were of concern at 2 laboratories each.

Fifty-three percent (34/64) of respondent laboratories reported testing specific groups of drugs on the

<table>
<thead>
<tr>
<th>No. of isolates tested/y</th>
<th>No. of laboratories</th>
<th>No. of isolates tested</th>
<th>Percentage of all isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–999</td>
<td>23</td>
<td>9,893</td>
<td>6%</td>
</tr>
<tr>
<td>1,000–1,999</td>
<td>13</td>
<td>16,383</td>
<td>10%</td>
</tr>
<tr>
<td>2,000–2,999</td>
<td>10</td>
<td>24,657</td>
<td>15%</td>
</tr>
<tr>
<td>≥ 3,000</td>
<td>12</td>
<td>113,350</td>
<td>69%</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>164,283</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure 1—Methods used for antimicrobial susceptibility testing of 164,283 bacterial isolates from various species by 58 veterinary diagnostic laboratories in the United States.
basis of type of bacteria isolated (eg, gram-staining characteristic). Eighty-four percent (54/64) of laboratories reported testing specific groups of drugs on the basis of animal species from which the isolate was obtained, and 40% (26/64) reported testing specific groups of drugs on the basis of type of disease (eg, abortion or respiratory tract disease).

A laboratory accredited by the AAVLD was 10 times as likely (odds ratio, 10.0; 95% confidence interval, 1.1 to 232.3) to use the NCCLS standards document as was a laboratory that was not accredited. No other factors (eg, use of particular drug groups and compilation of antibiograms) studied in this survey were significantly associated with laboratory size or accreditation status.

Discussion

Results of the present study suggest that veterinary diagnostic laboratories could be used as a vital source of data for monitoring antimicrobial susceptibility. However, many aspects that affect the quality of susceptibility data would need to be addressed and standardized before an effective monitoring system could be developed. Laboratory identification and recruitment, standardization of testing protocols, variability between tests, data storage and retrieval, duplicate samples, clinical status, and surveillance efficiency are areas to consider when using these types of data.

The population used for the present survey was all veterinary diagnostic laboratories in the United States that perform antimicrobial susceptibility testing on bacterial isolates from animals. The AAVLD is the only organization that maintains current address information for accredited veterinary diagnostic laboratories in the United States. However, there are only 36 AAVLD-accredited laboratories, which is a minority of the total number of veterinary diagnostic laboratories in the United States. We could identify only 1 other source for a comprehensive list of veterinary laboratories and their addresses, which was a directory published in 1995 by the USDA’s National Veterinary Services Laboratories in Ames, Iowa. Unfortunately, that directory was not current, which complicated efforts to conduct this survey. Nonrespondent laboratories were contacted by telephone to verify address information when possible.

Compilation of susceptibility information from multiple diagnostic laboratories would require standardization of testing methods so that data would be comparable. But standardization of susceptibility testing is challenging for several reasons. It is not possible to devise ideal culture media for all organisms, and debate continues as to the best inoculum size, disk content, atmosphere for incubation, method of reading results, and interpretation of those results in microbiologic, clinical, and epidemiologic contexts. For this reason, use of the NCCLS-recommended methodology would greatly facilitate production of susceptibility data under uniform conditions. It has been suggested that only laboratories demonstrating adequate microbiologic proficiency and good standards of practice should be included in monitoring systems. Accreditation, along with internal and external quality control analyses, could assist with selection of laboratories for such a system. To evaluate the standardization of methods, the present survey included questions on the use of the NCCLS testing protocols that allow for comparability of susceptibility data.

Although laboratories accredited by the AAVLD were more likely to use the NCCLS testing protocols and quality control procedures than were other laboratories, most laboratories that responded to the present survey used the NCCLS procedures, suggesting that results for various laboratory systems should be comparable. Laboratory size and AAVLD accreditation status were not associated with responses to other questions in the survey, suggesting that these classifications were not related to testing procedures used in the respondent laboratories.

The broth dilution method of assessing antimicrobial susceptibility yields a quantitative result: the concentration of antimicrobial drug necessary to inhibit bacterial growth. However, although results of the broth dilution method are quantitative, they are limited by the number of dilutions performed. Breakpoints

Table 2—Information routinely reported and archived by 58 veterinary diagnostic laboratories in the United States performing antimicrobial susceptibility testing of 164,283 bacterial isolates from animals

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates tested</th>
<th>No. of laboratories performing test</th>
<th>No. of laboratories routinely reporting information</th>
<th>No. of laboratories routinely archiving information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Broth diffusion Disk diffusion</td>
<td>Broth diffusion Disk diffusion</td>
<td>Broth diffusion Disk diffusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIC BP Both NR SIR Zone Both NR</td>
<td>MIC BP Both NR SIR Zone Both NR</td>
<td>MIC BP Both NR SIR Zone Both NR</td>
</tr>
<tr>
<td>Bovine</td>
<td>32,017</td>
<td>15 47</td>
<td>9 2 3 1 39 2 2 4</td>
<td>4 0 6 5 15 0 18 14</td>
</tr>
<tr>
<td>Swine</td>
<td>14,921</td>
<td>12 37</td>
<td>6 2 2 2 28 1 2 6</td>
<td>4 0 4 4 11 0 14 12</td>
</tr>
<tr>
<td>Oxine</td>
<td>1,949</td>
<td>14 37</td>
<td>9 2 2 1 30 2 2 3</td>
<td>4 0 5 5 11 0 16 10</td>
</tr>
<tr>
<td>Poultry</td>
<td>8,089</td>
<td>12 38</td>
<td>8 1 3 0 32 1 2 3</td>
<td>4 0 3 5 13 0 14 11</td>
</tr>
<tr>
<td>Equine</td>
<td>20,440</td>
<td>16 47</td>
<td>11 1 3 1 36 2 2 7</td>
<td>4 0 7 5 13 0 17 17</td>
</tr>
<tr>
<td>Canine</td>
<td>54,603</td>
<td>19 44</td>
<td>13 0 5 1 35 2 2 5</td>
<td>4 0 8 7 12 0 17 15</td>
</tr>
<tr>
<td>Feline</td>
<td>16,106</td>
<td>17 40</td>
<td>11 0 6 0 33 1 2 4</td>
<td>4 0 7 6 12 0 15 13</td>
</tr>
<tr>
<td>Exotic</td>
<td>16,158</td>
<td>15 39</td>
<td>11 1 3 0 34 1 2 2</td>
<td>4 0 6 5 13 0 16 10</td>
</tr>
</tbody>
</table>

MIC = Minimum inhibitory concentration. BP = Breakpoint. NR = Not reported by the laboratory. SIR = Susceptible-intermediate-resistant interpretation. Zone = Zone diameter.
that help to simplify interpretation of broth dilution results have been established by combining information on the distribution of MICs for at least 300, and preferably 600, susceptible and resistant strains of various bacterial species or for 100 to 300 strains of single species; blood and tissue antimicrobial concentrations achieved following administration of conventional doses in a range of healthy subjects, as well as infected and special patients; and the comparability of in vitro and in vivo susceptibility results.

The disk diffusion method, on the other hand, is an assay that uses disks impregnated with precise concentrations of specific antimicrobial drugs that will diffuse away from the disk over a predetermined period of time. This yields zones with lower concentrations of drug as distance from the disk increases. There are many aspects to the disk diffusion method that allow variability to be introduced, but when the multiple variables that can affect the outcome of the test are standardized, results are assumed to be comparable. Results of the disk diffusion method have been shown to be 95% reproducible among laboratories when procedures were standardized. Minimum inhibitory concentrations can be extrapolated from zone diameters with regression analyses so that results of the disk diffusion method can be compared with those of broth dilution.

Nineteen laboratories in the present study could give only general estimates of the number of isolates tested annually. This suggests that these laboratories cannot produce annual reports for performance evaluation. For the purpose of this study, respondents were allowed to report general estimates of the number of tests performed to obtain a proper representation of testing methodologies among the various laboratories. However, for a monitoring program to be successful, laboratory computer information systems will need to be more robust to allow extraction of information needed to identify changes in the susceptibility of bacterial populations.

It is common for veterinary diagnostic laboratories to test multiple samples from a single animal or farm. A surveillance system should be able to extract data for a representative isolate from a single animal or farm to ensure that resistance estimates are not biased. The Surveillance Network by Focus Technologies, which compiles susceptibility profiles for 1 million isolates each month, adjusts for this problem by identifying organisms as duplicate when they are from the same patient, have the same MIC profile, and are received within the same 7-day period.

Specimens submitted to veterinary diagnostic laboratories are typically obtained from clinically ill patients and often do not reflect the general population of bacterial isolates. In many cases, empirical treatment of animals with antimicrobial drugs has been initiated prior to obtaining specimens for microbiologic testing. As such, resistance profiles for populations of isolates may be skewed and may not accurately reflect the distribution of susceptibility for populations of bacteria in healthy untreated animals. Nevertheless, although results from treated animals may not perfectly reflect susceptibility patterns in untreated animals, use of these results increases the likelihood of early detection of important trends in susceptibility and detection of important resistance phenotypes before these strains become established in the population at large. It is important to recognize that these types of results do not represent the general population, and that these data would be most useful if differences in susceptibility patterns for these 2 populations were characterized and well understood. In this sense, these data are not necessarily useful for determining whether resistance has become established in healthy animals, but rather provide data on a sentinel population and might be useful in making informed decisions regarding the regulation of antimicrobial drugs before resistance mechanisms have become widely established. In summary, information gathered from monitoring both healthy and clinically sick animals is helpful in developing a more complete understanding of antimicrobial susceptibility in animal populations.

The efficiency of a surveillance network is critical in providing timely information to clinicians, scientists, and policy makers. The ideal system should be sustainable for long-term surveillance of susceptibility trends, alert health care professionals to novel susceptibility profiles, be sensitive enough to detect small changes in susceptibility patterns, and grant both scientists and prescribing clinicians access to the database at any time for comprehensive review and analyses. Such a system would provide resistance data that when linked with supportive research programs in infection control and pharmaceutical usage, would allow for development of practical measures to limit and ultimately reduce the burden of antimicrobial resistance at the local and national levels.

References

*Survey available on request to the corresponding author.
†EpInfo, version 6, Statcalc module, Centers for Disease Control and Prevention, Atlanta, Ga.

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