Antibiotics are collections of antimicrobial susceptibility data for a particular bacterial organism and host species. Antibiotics are important tools for antimicrobial stewardship, as they may be used to guide empirical antimicrobial therapy and assess trends in antimicrobial resistance, maximizing treatment success and preserving the efficacy of currently available pharmaceuticals. Targeted use of antimicrobials is critically important to minimize the spread of antimicrobial resistance, which may be conveyed between animals and humans directly but may also be spread through the environment and ecological niches, such as soil, water, and wildlife reservoirs. To effectively utilize antibiotic data of infectious diseases, veterinarians need to know data characteristics, including the source population, body site (when possible), and number of isolates included, in addition to the animal species and bacterial organisms for which each breakpoint was developed. Although widely used in human health systems, antibiotic data are not often available in veterinary medicine. This paper describes antibiotic development by US veterinary diagnostic laboratories, and shares California’s process to create and promote livestock antibiotic data. The companion Currents in One Health article by Burbick et al, AJVR, September 2023, addresses the benefits and challenges associated with developing veterinary antibiotic data.
Antibiograms provide information about the proportion of tested bacterial isolates that are interpreted to be susceptible to the drugs tested. They should include a minimum of 30 isolates to ensure statistical validity. The Clinical and Laboratory Sciences Institute (CLSI) provides guidance for antibiogram development in human and animal diagnostic laboratories. The CLSI approves breakpoints, which are concentrations of antimicrobial used to categorize bacteria as susceptible, intermediate, or resistant. Breakpoints are established on the basis of a specific dosage regimen (dose, frequency, duration, and route of administration) and are developed utilizing pharmacokinetic/pharmacodynamic, microbiological, and clinical response data. When a breakpoint for a particular bacterial or host species has not been developed, breakpoints may be extrapolated from similar bacterial species, host species, indications, or infection sites, although this process results in lower confidence in the prediction of clinical correlation. When available, breakpoints defined in the same host species (eg, breakpoints established for dogs applied to bacterial isolates recovered from dogs) are more reliable than those extrapolated from humans or other species (eg, breakpoints established for humans that are extrapolated to dogs or antimicrobial breakpoints for dogs extrapolated to cats). Veterinarians should use the same dosage regimen (dose, route, frequency, and duration) as was used to establish the breakpoints, or those breakpoints may not correlate with anticipated clinical outcomes and are not applicable.

Antibiograms should be used to aid drug selection in the context of other patient considerations, including disease factors (eg, site of infection and severity), expected bacterial pathogen(s), potential for polymicrobial infection, other diagnostic test results (eg, cytology and PCR), patient factors (eg, contraindications and comorbidities), dosing and route of administration, antimicrobial agent factors (eg, sites of metabolism and excretion), client factors (eg, feasibility of therapeutic plan and resource availability), and status as a drug of human medical importance.

When carefully curated, updated, and correctly applied, antibiograms can be used to support initial therapeutic decisions, describe local susceptibilities, and, when data are available from multiple time points, monitor in vitro resistance trends. When applied to an epidemiologically similar population, up-to-date antibiograms complement individual culture and susceptibility testing and prescribing protocols (eg, label restrictions and best practice recommendations) to guide decision-making. Consideration should be given to choosing the most current antibiogram available that is representative of the same animal species, suspected organism(s), and same geographic area or hospital as the patient population. Antibiograms created from and used for a single hospital or clinic, group of hospitals or clinics, or production system are preferred, since the patient population will have more in common with the historical source of the patient data.

Introduction

Given their importance to antimicrobial stewardship, individual clinical decision-making, and tracking of susceptibility patterns over time, the California Department of Food and Agriculture Antimicrobial Use and Stewardship program (CDFA AUS) has made efforts to support the development and use of antibiograms in animal agriculture. The CDFA AUS is a first-in-the-nation program that promotes antimicrobial stewardship and assesses AMR and use trends in livestock at the state level. Program veterinarians, epidemiologists, and specialists work to preserve the efficacy of antimicrobial agents through innovative approaches to AMR and responsible antimicrobial use associated with livestock in California. The California Animal Health and Food Safety (CAHFS) Laboratory System at the University of California-Davis, which comprises 4 individual laboratories spanning the state, safeguards animal and public health through rapidly, reliable testing and diagnoses for livestock diseases, including those associated with pathogens that impact humans by threatening food safety or causing zoonotic diseases. As part of this work, we assessed antibiogram development by US veterinary diagnostic laboratories and implemented a program to develop and promote livestock antibiograms for California veterinarians.

Methods

National survey of veterinary antibiogram development

To better understand the current use of antibiograms in veterinary medicine, the CDFA administered an electronic survey to veterinary diagnostic laboratories (VDLs) that had American Association of Veterinary Laboratory Diagnosticians accreditation, were members of the National Animal Health Laboratory Network, or were associated with veterinary school teaching hospitals (Supplementary Material S1). Laboratory directors and personnel in the bacteriology or microbiology sections were identified using laboratory websites.

California livestock antibiograms

Under the shared goal of promoting antimicrobial stewardship for livestock species, the CDFA AUS partnered with the CAHFS Laboratory System to create livestock antibiograms for veterinarians licensed in California, along with educational resources to ensure optimal clinical application (Figure 1). The resulting antibiograms adhered to standardized guidance for veterinary antibiogram development and interpretation provided by the CLSI, unless otherwise indicated.
Data

Cumulative susceptibility data were generated from AST performed at CAHFS. Samples for bacterial culture and AST were from either samples collected and submitted by a client or samples collected by CAHFS’s pathologists from animals received for necropsy. Client information was removed prior to data aggregation. In 2021, approximately 37% of susceptibility tests performed by CAHFS were from client-submitted samples, while 63% were from necropsy samples. Either submitting veterinarians requested AST or CAHFS pathologists provided samples for AST when evidence of disease was found during necropsy and only for submissions listing a reporting veterinarian.

Per CLSI guidelines, samples included in an antibiogram should be associated with disease. Attribution of organisms to disease in the absence of sufficient clinical history or associated lesions at necropsy can be challenging, particularly for organisms that may also be present as commensals (eg, *Pasteurella multocida* from a nasal swab). Necropsy isolates provided the highest confidence of an organism’s association with disease; those with clinical history gave the next highest confidence. Disease status could not be assessed when clinical history was not provided. Often occurring at other diagnostic laboratories, CAHFS receives a subset of samples without any clinical history but for which AST is requested. As an example, 12% of isolates included in the bovine respiratory disease antibiograms did not have associated data on clinical history, and an additional 4% of isolates were obtained from specimens submitted for routine surveillance. These samples were included in the cumulative susceptibility data, while acknowledging in a disclaimer included with the antibiograms that those samples may have been collected from healthy animals and intended for surveillance purposes.

At the time of publication, CAHFS has released antibiograms for bovine respiratory pathogens (*Mannheimia haemolytica*, *P. multocida*, and *Histophilus somni*), ovine and caprine respiratory pathogens (*M. haemolytica* and *P. multocida*), and equine *Streptococcus equi* subsp *zooepidemicus*. These were selected on the basis of the availability of adequate isolates (Figure 2) analyzed in a 1-year period and their relative importance to California’s animal industries. *Mannheimia* spp, *Pasteurella* spp, and *Streptococcus* spp are among the most frequent organisms for which AST is performed by CAHFS. In addition, respiratory disease was the third most reported reason for individual animal antimicrobial therapy (bolus/injectable) in cows and replacement heifers and the second most reported reason for similar antimicrobial therapy in calves according to a 2017 survey of California cow-calf operations. Respiratory disease therapy was the most reported reason for use of oral and injectable antimicrobials for both ewes and lambs in a 2018 survey of California commercial sheep operations. In equine submissions requesting culture and AST at CAHFS, *S. equi* subsp *zooepidemicus* was one of the most commonly recovered organisms.

Common reasons why bacteria were not included in antibiograms were the following: lack of species-specific breakpoints, insufficient numbers of isolates from a single host species, and recovery from feces or intestinal sites (eg, bovine *Salmonella* spp), where it is known that the susceptibility of Enterobacteriales isolated does not reliably predict the clinical response of treatment for enteric infections.
Sample processing and analysis

Sample source and clinical history informed CAHFS’s sample processing and culture procedures. Prior to AST, organism identification was verified by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry and biochemical testing methods (Supplementary Material S2).

Approved CLSI breakpoints were used for susceptibility interpretations. Where breakpoints were not approved for a specific pathogen-host combination, breakpoints were either extrapolated on the basis of CLSI recommendations or interpretations were not provided. When extrapolated, breakpoint source used for interpretations was provided with the antibiogram.

Focus group and expert opinion

A multidisciplinary focus group of California-based large animal clinical veterinarians, academicians, university extensionists, and state-level agriculture and public health veterinarians (n = 16) was recruited to provide guidance on antibiogram design and content. Members were chosen on the basis of their experience with antibiograms, engagement in antimicrobial stewardship programs, and experience with clinical utilization of veterinary medical drugs. Five additional veterinary clinicians at University of California-Davis, selected for their host species expertise, provided feedback on content clarity prior to releasing the final antibiograms.

Registration survey

Veterinarians registered to receive CAHFS livestock antibiograms through an online survey. Promotion of antibiogram availability to veterinarians treating livestock species occurred through social media, newsletters, and veterinary medical associations.

Since 2018, California law has required veterinary oversight for the use of all dosage forms of medically important antimicrobials in livestock; therefore, access to antibiograms was limited to licensed veterinarians. Respondents were asked to provide a full name, email address, veterinary license number, practice name (if applicable), and the species/production class(es) of interest. If no species/production class was selected, the respondent received all antibiograms. Respondents were required to indicate that they read and understood the following statement: “CAHFS antibiograms are intended for veterinary use only and are not to be distributed to clients/producers without an existing veterinarian-client-patient relationship. It is the veterinarian’s responsibility to use clinical judgment and adhere to label and legal restrictions for use of drugs depicted in CAHFS antibiograms.” Veterinary license numbers were matched to the name using the California Department of Consumer Affairs’ online license search feature to verify the recipient was a veterinarian before sharing the antibiograms.
Education

To optimize antibiogram utility, the CDFA AUS and CAHFS created educational resources. An outline of laboratory procedures associated with AST, including MIC values and how they relate to antibiograms (Supplementary Figure S2) was published online, in addition to a user guide designed to explain the antibiograms, including key formatting and content features. Two 1-hour virtual and interactive continuing education sessions were provided through local veterinary medical associations. Sessions described AST from sample collection to interpretation of the MIC report, creation of CAHFS antibiograms, the utility of antibiograms for guiding initial therapy, and limitations of currently available livestock antibiograms.

Enrolled veterinarians received links to the aforementioned published resources, in addition to a digital copy of CLSI’s VET09: Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings, First Edition, purchased by the CDFA. VET09 contains detailed information to guide AST interpretation and complemented the CDFA AUS’s published outreach.

**Antibiogram content and format**

The final antibiogram content and format were shaped by CLSI standards and incorporated feedback from the focus group and species experts. Included notations identified where data or methods deviated from CLSI guidelines. Three different formats were created and sent as a PDF to registered veterinarians. The majority opinion from focus group members was that a simple table and bar graph would be useful for quick reference by clinicians but that a detailed table would add valuable information.

A minimum of 30 isolates from a 1-year time span were included for each antibiogram, when possible; if not, the time frame was extended to 2 years, which was noted on the antibiogram for user awareness. When < 30 isolates were available during a 2-year period, a disclaimer was provided indicating that < 30 isolates reduce the statistical validity of the percent susceptible estimates.

Antimicrobial drugs were categorized on the basis of mechanism of action. For host-drug combinations in which drug use is illegal (e.g., no fluoroquinolones are licensed in sheep and goats, and extralabel drug use is illegal for fluoroquinolones), susceptibility data were excluded and replaced with a statement that extralabel drug use of that drug is prohibited in the host. For drugs that are contraindicated in the host species (e.g., tilmicosin in goats), no data were provided and a warning was given not to use the drug in that species. Although CLSI M39 recommends only indicating percent susceptible, the intermediate category may predict clinical efficacy in anatomical sites where the drug is known to concentrate or when the dosage may be safely increased. Therefore, this information was thought to be useful for clinical decision-making and was included. Furthermore, given inherent variability in test outcomes, inclusion of intermediates was intended to avoid overinterpretation of susceptible or resistant categories.

Up to 3 formats were provided for each host-bacteria combination: a bar chart, a simple table (Figure 3), and a detailed table (Figure 4). The detailed table

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**Table: CAHFS Bovine Respiratory Pathogens Antibiogram July 2020 - June 2021**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Mannheimia haemolytica (n=97)</th>
<th>Pasteurella multocida (n=82)</th>
<th>Histophilus somni (n=41)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>21%</td>
<td>27%</td>
<td>32%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>7%</td>
<td>2%</td>
<td>20%</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>90%</td>
<td>59%</td>
<td>70%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>7%</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>2%</td>
<td>26%</td>
<td>10%</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>48%</td>
<td>45%</td>
<td>77%</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>40%</td>
<td>23%</td>
<td>24%</td>
</tr>
<tr>
<td>Macrolides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>63%</td>
<td>43%</td>
<td>76%</td>
</tr>
<tr>
<td>Tildiprosin</td>
<td>56%</td>
<td>67%</td>
<td>63%</td>
</tr>
<tr>
<td>Tularosin</td>
<td>45%</td>
<td>43%</td>
<td>61%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>65%</td>
<td>65%</td>
<td>90%</td>
</tr>
<tr>
<td>Phenicols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flomfenicol</td>
<td>58%</td>
<td>65%</td>
<td>60%</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>40%</td>
<td>34%</td>
<td>47%</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>85%</td>
<td>82%</td>
<td>54%</td>
</tr>
</tbody>
</table>

* S (Susceptible), Int (Intermediate) and R (Resistant) based on CLSI cattle-specific breakpoints for each drug/pathogen combination.

New criteria indicates this isolate is susceptible to ampicillin/amoxicillin (IF MIC < 0.06) which current methods can’t evaluate.

No CLSI breakpoint for Histophilus somni in cattle, extrapolated from Mannheimia haemolytica CLSI breakpoints.

No CLSI breakpoint for Pasteurella multocida or Histophilus somni in cattle, extrapolated from Mannheimia haemolytica CLSI breakpoints.

To achieve > 30 isolates included in analysis, isolate recovery timeframe extended (January 2020 - June 30, 2021).

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**Figure 3** — Example simple antibiogram created by the California Animal Health and Food Safety Laboratory using mock data. Actual data not shown. Do not use this mock data for clinical decision-making.
contained several additional components not found in the simple table, including number and distribution of isolates at each MIC value for the drugs tested. Shading represented the range of antimicrobial concentrations tested, and different colors depicted interpretations of susceptible, intermediate, and resistant, with the darkest color used for resistant. A color palette of light, medium, and dark gold was chosen to enhance visual accessibility.
Breakpoints for susceptible, intermediate, and resistant were described for each antimicrobial agent tested. Solid bold lines represented resistant breakpoints along the ranges of antimicrobial concentrations. The distribution of isolates with an MIC at each antimicrobial concentration tested was shown as a percent of the total number included.

Factors impacting interpretation were added to the bottom of each antibiogram, including specification of extrapolated breakpoints. Additionally, a general disclaimer was included with each antibiogram stating that breakpoints are established using specific administration routes, drug formulations, and dosing concentrations and frequencies (all of which may impact the therapeutic drug concentrations and distribution to site[s] of infection), with a reference and link provided for Appendix D of CLSI Vet 01S, Edition 5.

Results

National survey of veterinary antibiogram development

A brief survey description and link were emailed to 96 individuals representing 53 VDLs. Representatives from 35 (66%) VDLs responded, of which 19 (54%) reported creating veterinary antibiograms (Table 1; Supplementary Table S1). Almost half of the participating VDLs did not create antibiograms, demonstrating underutilization of AST data. The majority (79%) of laboratories that reported creating antibiograms also provide education to veterinarians on how to use the antibiograms, either through footnotes, presentations, or individual consultation. Reported pathogen-host combinations included bovine respiratory pathogens, bovine Salmonella spp, canine and feline skin/soft tissue and urinary tract pathogens, and equine skin and respiratory pathogens.

California livestock antibiograms

This project provided antibiogram access and education to veterinarians, thereby supporting a one-health approach to antimicrobial stewardship. As of April 2023, antibiograms were created and distributed to 43 California-licensed veterinarians. Updated antibiograms based on samples from 2021 to 2022 are under development.

Discussion

The role of antibiograms in antimicrobial stewardship programs and the successful process developed by the CDFA AUS and CAHFS to create, promote, and disseminate livestock antibiograms is described. In result, antibiograms were created and distributed to veterinarians practicing in California, publicly available educational resources to help veterinarians optimally use the antibiograms were generated and distributed, and a process for ongoing updated antibiogram publication and distribution was established. By sharing our process, we seek to support antimicrobial susceptibility monitoring and veterinary antibiogram generation.

Together, the CDFA AUS and CAHFS developed processes and identified future directions that may be applicable to other states or institutions, though some limitations were encountered. A proportion of sample submission forms were incomplete. It was therefore unknown whether the sampled case patient had received antimicrobial therapy, which may have impacted culture and AST results. This also meant that the available, aggregated data could not be stratified on the basis of production class (eg, beef and dairy data were combined into a single “bovine” antibiogram). While most samples were from clinically ill animals, incomplete submission forms that omitted whether clinical signs were present at the time of sampling meant that samples from healthy animals may have been included in the analysis. A general disclaimer was included, stating the following: “these antibiograms include samples of all age groups and production class received by CAHFS and may include both clinical and surveillance samples.” The term surveillance sample was specifically identified to describe samples that were submitted without clinical history or with health surveillance listed as reason for submission; therefore, surveillance purposes could not be ruled out. Thorough completion of sample submission forms would allow for data stratification and more specific antibiograms in the future.

The pathogen-host combinations reflected in the antibiograms were limited by samples submitted to CAHFS within the preceding 1 to 2 years. Antibiograms are influenced by the samples they include and may be biased toward more severe clinical presentations since clinicians may be more likely to collect and submit these samples for culture and AST or, in the case of necropsy specimens, may originate from animals that were euthanized or succumbed to their disease after failed treatment attempts. Consequently, the available antibiograms may not represent the full range of pathogens of clinical interest in the state. Overrepresentation of 1 region, farm, or production class within the state could create bias. Isolates included in the antibiograms may not be truly representative of the statewide population since samples may have been submitted to other laboratories due to geographic proximity, price difference, turnaround time, and/or familiarity. However, the ability to create antibiograms specific to a narrower population is limited by low sample submission for AST. The CDFA AUS will continue to encourage sample submission to support additional antibiograms and annual updates to existing antibiograms, where sample submission makes updates possible.

Antibiograms showcase a large overview of AMR for specific pathogens and, as such, may influence antimicrobial usage to improve antimicrobial stewardship among veterinarians. Trends identified by antibiograms may reveal areas for further intervention by antimicrobial stewardship programs. For example, if high or increasing levels of resistance are noted for a certain drug-pathogen combination, clinicians may focus increased attention on preventing that condition or
utilizing alternative therapeutics. Trends should be reviewed considering the proportion testing positive, since fluctuations in sample numbers or the proportion of ill compared to healthy surveillance samples can affect the prevalence of resistance.

Buy-in and engagement by veterinarians, laboratorians, and animal owners are crucial for antibiogram development. Animal owners must see the value of AST to pay for such testing in order for laboratories to obtain adequate sample sizes for continued antibiogram production. Multiple factors may influence the number and types of samples that are tested and included in antibiograms. Beginning in June 2023, all dosage forms of medically important antimicrobial drugs will be under veterinary oversight in the US. Therefore, more clients will rely on veterinarians for antimicrobial access and concomitant AST. The cost of engaging a veterinarian, in addition to the cost of susceptibility testing, may be a barrier to sample submission and may also affect representation. Veterinary access and perception of need, or financial considerations, may influence client decisions to pursue AST, especially in rural or lower socioeconomic areas. This selection bias may result in reduced applicability of the antibiogram if animal populations are disproportionately represented.

Offering support for voluntary, farm-specific antibiograms is the next phase of statewide stewardship for California’s livestock. A pilot project is currently underway to generate and evaluate farm-specific antibiograms for bovine respiratory disease pathogens on California dairies. Farm-specific antibiograms more closely reflect resistance trends of an individual farm, as they are created from and used on the same population of animals, similar to human hospital-level antibiograms. When collected over multiple years, they can be used to assess resistance trends and develop disease prevention and treatment protocols.

Development of farm-specific antibiograms may be limited by the cost of AST, the need for at least 30 isolates, and data confidentiality concerns. State-subsidized susceptibility testing could be used to address cost concerns if test results generated for farm-specific antibiograms are also captured in statewide AMR monitoring programs. Training clients in sample collection and transport protocols could reduce the costs of repeated veterinary visits to sample individual animals. However, great care must be taken by the veterinarian to ensure client training and protocol adherence. Inappropriate sampling may result in the collection of normal flora or contaminants instead of the target pathogen; incorrect shipping methods may result in reduced yields. The isolate number needed to generate an antibiogram may not be attainable in a timely manner when the disease is uncommon or infrequent in the target population. Antibiograms released publicly should maintain strict data confidentiality, the provisions of which should be clearly communicated to the client to help ensure confidence in laboratories and testing programs.

Antibiograms may vary in their host-pathogen combinations, formatting, and distribution; however, to provide veterinarians with the context needed for antimicrobial decision-making, laboratories should provide transparency regarding the data source(s) and collection methods used. Antibiograms may improve client compliance in antimicrobial stewardship by empowering veterinarians with a visual data tool to explain the rationale for drug selection when advising initial therapy. Furthermore, antibiograms released to practicing veterinarians should include access to an educational component, such as that provided by the CDFA AUS and CAHFS, to help clinical veterinarians understand and optimally utilize antibiograms in their practice. Having a committee of experts, including practicing veterinarians, academics, and other stakeholders, provide feedback on antibiogram scope and structure can help increase uptake by clinical veterinarians. Veterinarians should continue submitting samples for AST, including treatment history, production class, and clinical signs on submission forms, to enable laboratories to create robust, specific antibiograms. While antibiograms are promoted as a useful tool for antimicrobial stewardship, it should be emphasized that they complement rather than replace AST and consultation with laboratorians to maximize their impact and reduce misapplication.

Additional detail regarding veterinary-specific antibiogram development is addressed in the companion Currents in One Health article by Burbick et al, AJVR, September 2023.

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