Background and Introduction

Antimicrobial resistance (AMR) is one of the most significant one-health challenges to combating infectious disease globally. Actions taken to preserve the effectiveness and availability of antimicrobial drugs, including responsible use and reduced use through management and disease prevention, are termed antimicrobial stewardship and should be practiced in all one-health sectors. Antimicrobial susceptibility testing (AST) is a critical stewardship tool to help medical practitioners select antimicrobials judiciously.

The use of AST requires the measurement of either minimum inhibitory concentrations (MICs) via dilution series or zones of inhibition via disk diffusion. This measurement is an in vitro evaluation of an antimicrobial drug's effect and has no clinical significance.
Antibiogram Development and Applications in Veterinary Medicine

Veterinary diagnostic laboratory antibiogram sample sources

Accreditation is not required for veterinary diagnostic laboratories (VDLs), and there is a diversity of accrediting bodies and standards to which VDLs may adhere. Most state-sponsored VDLs are accredited by the American Association of Veterinary Laboratory Diagnosticians; private or smaller laboratories may have no accreditation or may follow standards set by the International Organization for Standardization (ISO) guidelines.

Variations in patient populations may affect susceptibility trends; antibiograms from a referral hospital VDL may differ from a VDL serving community veterinarians since referral hospital patients often have prior antimicrobial exposure or may have chronic or critical conditions. Antibiograms at referral hospitals or intensive care units (ICUs) may include bacteria from hospital-acquired infections or recurrent or chronic infections, which tend to be less susceptible than bacteria from community-acquired infections. Patterns of susceptibility, even within different hospital units (eg, ICU, surgery), may differ.

Isolates included in antibiograms

To minimize the impact of a few outliers, a minimum of 30 isolates is recommended for antibiogram development. This count may be difficult for some VDLs to achieve in a relevant timeframe; therefore, to increase isolate numbers, closely related organisms (eg, members of the same order or family, such as Enterobacterales) are sometimes combined, as are samples from similar host species (eg, combining sheep and goat isolates) or collection sites with clinical applicability (eg, skin/soft tissue and respiratory). Extending the data collection timeframe can be a solution but may be complicated by AST interpretation changes, as discussed later.

Demographics and other factors that may impact data

When curating isolates, the laboratory must determine their relevance to veterinarians’ clinical decision-making. Optimally, antibiograms for clinical application should include diagnostic isolates from a single animal species, as breakpoints for individual drugs are approved for one host species and specific bacterial species. To ensure consistency, MIC values should be analyzed collectively, applying current breakpoints for all isolates included, as breakpoints may be updated over time. As approved breakpoints for all host-bacteria-drug combinations are lacking, a breakpoint may be extrapolated, following CLSI guidelines, from another animal species (eg, breakpoints established for clinical use in canine disease conditions applied to feline isolates), another bacterial species (eg, Escherichia coli [E coli] breakpoint for Klebsiella spp) or for different anatomic locations (eg, breakpoint developed based on soft tissue isolates applied to infection of a bone). Alternatively, laboratories may extrapolate from breakpoints established for isolates from humans, but the clinical relevancy is uncertain, which may be less useful in guiding therapeutic decisions. Because different breakpoint interpretations produce different antibiograms with the same MIC data, knowledge of the breakpoint origin is critical and should be included with the antibiogram. Other resources discuss and make recommendations for breakpoint extrapolation and presentation options.

Applying antibiogram data collected from one animal production class to another within the same species may be problematic, as subpopulations of hosts or risk factors that predispose to resistance alterations, such as previous antimicrobial exposure or vastly different husbandry practices, may introduce bias. For example, AMR trends in bovine respiratory disease pathogens from an operation exclusively
isolates. They are used to identify emerging resistors AMR, including from retail meat and food animal products intended for human consumption (e.g., retail animal biospecimens, such as feces or animal products from animals with or without clinical disease or from healthy animals at slaughter and with retail meat samples).

Prior antimicrobial therapy should be considered when including isolates in an antibiogram, whenever possible, since antimicrobial exposure could lead to overestimation of initial resistance. Menard et al demonstrated that, in critically ill dogs treated with 2 to 7 drugs, multidrug resistant (MDR) isolates increased by day 7 and started to decrease by day 60; however, it is unknown if this applies to all drug classes or if exposure timeframes impact susceptibility. Inability to stratify isolates based on treatment history is a shared challenge between human and veterinary antibiogram development due to insufficient information provided with sample submission. Even when provided, many laboratory information systems are not designed to manage such information in a retrievable manner to inform antibiogram development.

The absence of unique patient identification, both within and between different diagnostic and care facilities, hampers the ability to only include one isolate per patient per analysis period. It may not be possible to include only a single isolate per animal, especially for uncommon organisms, when individuals cannot be reliably identified or when one lab receives samples from the same animal via multiple facilities. Sorting isolate data by farm or owner is resource intensive and not likely to be feasible.

Additional uses for cumulative susceptibility testing data

In addition to the clinical application of antibiograms to empirically manage individual patients and groups of animals, susceptibility trends from common pathogens and indicator organisms can be used to evaluate broader trends affecting human and animal health. AST may be applied to isolates from animals with or without clinical disease or from animal biospecimens, such as feces or animal products intended for human consumption (e.g., retail meat). Many bacterial species routinely tested and reported are used as indicator organisms to monitor AMR, including from retail meat and food animal isolates. They are used to identify emerging resistance patterns and specific resistance genes using both phenotypic and genomic data. While published susceptibility summaries of bacteria of public health or zoonotic concern are common in both peer-reviewed literature and through public health agencies, their content and use may differ from those intended for clinical case management in animals.

Because disease-causing isolates may differ in resistance patterns from isolates collected from healthy animals or the environment, antibiograms intended to guide clinicians on empirical treatment should only include diagnostic isolates. If the goal is to monitor resistance patterns for human disease risk, breakpoints based on regimens and pharmacokinetics of antimicrobials in humans should be used to categorize susceptibility. For example, the National Antimicrobial Resistance Monitoring System monitors drugs used in humans and applies breakpoints established for isolates from humans, with the results reported for foodborne pathogens (Campylobacter, Salmonella) and indicator organisms (Enterococcus, E coli) associated with animal ceca collected from healthy animals at slaughter and with retail meat samples.

The traditional antibiogram table of bacteria and the percentage of isolates susceptible to different antimicrobials are generally sufficient to guide empiric antimicrobial therapy decisions. However, the data may also be used to address other clinical inquiries, such as the proportion of MDR isolates or patterns of coresistance. Addressing these questions may require different data sources and presentations. Antibiogram developers should seek feedback from end users regarding specific data presentations.

Accessibility and distribution

Transparency in, and adherence to, standard testing methodology and breakpoints are critical for the evaluation and use of antibiogram data. Information about the test source (commercial or in-house) and validation (e.g., CLSI reference method) may be stated with the data, and, if not, should be available upon inquiry to the source laboratory. In general, commercial platforms should validate their tests against the CLSI reference method. Established, standardized procedures across laboratories for bacterial culture and identification as well as AST methods, including quality control, provide confidence in individual lab results and allow for greater comparability across laboratories.

Antibiograms have been released publicly, distributed to veterinarians and/or clients, or published in peer-reviewed literature. National monitoring programs, such as VetPath and GermVet have published cumulative susceptibility data on a national scale. As medically important antimicrobials transition from over-the-counter to prescription, VDLs might disseminate antibiograms exclusively to veterinarians to further reinforce veterinary oversight of these drugs. Although there is pressure to publish data in peer-reviewed journals, publication in non-open-access journals limits the availability and application of the data by clinical veterinarians. Antibiograms should be released with information and tools necessary to use the data.

Limitations

Limitations associated with breakpoint development, selection, and extrapolation

Interpretive categories (S, SDD, I, and R) are developed through review of microbiological, pharmacokinetic, and clinical data. Categories are created by assessing microbiological properties of a bacterial family, genus, or species in combination with data on pharmacodynamics and host animal
species pharmacokinetics. Ideally, clinical outcome data would be incorporated to ensure the categories' relevance; however, these data are not always available. Since breakpoints are based on a specific drug dose, route, and frequency of administration in a particular animal species (and sometimes disease indication), they may not be applicable where those factors differ.4

Due to the extent of pharmacologic and microbiologic data needed, breakpoint development is challenging and suboptimal for many animal and bacterial species. No breakpoints exist for bacteria isolated from small ruminants, wild mammals, amphibians, or reptiles. Breakpoints for isolates from bovine, swine, and equine hosts are limited. Available breakpoints for bovine mastitis pathogens are few and specific for intramammary drug delivery, while other breakpoints used for bovine, and many swine, isolates are limited to respiratory disease pathogens. The only breakpoint that exists for Enterobacterales isolated from poultry is for enrofloxacin, a drug illegal to use in US poultry.22 Breakpoints for canine- and feline-source isolates are more widely available, including common pathogens from urinary tract and skin/soft tissue infections.3 The lack of breakpoints in veterinary medicine presents a significant challenge to a one-health antimicrobial stewardship.

Limited funding poses a significant impediment to breakpoint development in veterinary medicine. While support has increased for AMR monitoring and antimicrobial use and stewardship initiatives, gaps in breakpoints and clinical outcome data have largely been ignored by funding agencies; a recent federal funding opportunity for breakpoint development did not include veterinary-specific breakpoints (Marshall, DVM, MPVM, DACVPM, California Department of Food and Agriculture, Sacramento, CA, e-mail communication, December 2022).23 As there has not been widespread demand from the veterinary community for solutions to significant gaps in data needed to establish breakpoints, funding agencies have not allocated appropriate resources to make significant research progress.

Financial support is also needed for veterinary specialty training in pharmacology and microbiology and antimicrobial use overall. Without sufficient training and support, there will not be knowledgeable personnel to produce the data needed for breakpoint development and application. Currently, only 6 (18%) accredited US veterinary schools offer microbiology residency programs and only 11 (33%) offer pharmacology residency programs; lack of funding prevents the establishment of additional programs.24-26

Limitations in data interpretation due to types of resistance

Interpreting AST data requires understanding how the type of resistance is defined. Acquired resistance occurs when a bacterium either acquires foreign DNA or it develops a chromosomal mutation that alters the drug's inhibitory activity. For example, resistance to fluoroquinolone drugs commonly develops through chromosomal mutation,27 whereas methicillin resistance occurs via the acquisition of the mec gene cassette.28 Ideally, the designation of "resistant" on a report would indicate the presence of an acquired resistance mechanism.

In contrast, intrinsic resistance is present in all bacterial isolates of a particular species or genus, based on inherent bacterial properties. As examples, all Enterococcus spp have penicillin-binding proteins that confer resistance to cephalosporins; all Pseudomonas aeruginosa isolates have efflux pumps, reduced cell permeability, and inactivating enzymes that negate numerous antimicrobial agents’ effects.29 Laboratory software settings vary, and software may not automatically adjust for intrinsic resistance. Developers of antibiograms should account for intrinsic resistance, either through the exclusion of drugs for which the organism has known intrinsic resistance or by distinctly denoting it in the final report to prevent overestimation of resistance.

The last, and least understood, resistance occurs when a commonly used drug regimen is insufficient in a particular host species to achieve the drug concentrations necessary to kill or inhibit the growth of the bacterium at the infection site. For example, the ampicillin breakpoint used for E coli isolated from dog and cat infections outside the lower urinary tract (≤ 0.25 μg/ml) is based on serum concentrations of drug achieved in non-urinary sites with the typical dose regimen.30 Because this concentration fails to inhibit typical E coli growth in vitro, all E coli infections in dogs and cats outside the lower urinary tract are reported as resistant to ampicillin, which can be mistakenly interpreted as a significant stewardship issue and may make bacteria look MDR despite the lack of acquired resistance. CLSI's VET01S identifies this situation as a scenario in which to provide clinical guidance to veterinarians to avoid this drug for a non-lower urinary tract infection.4 For lower urinary tract infections, the veterinary-specific breakpoint for susceptibility is ≤ 8 μg/ml, since ampicillin concentrates to high levels in urine, and means that many lower urinary tract E coli isolates will be categorized as susceptible.

Laboratory technology limitations

A 2017 survey conducted by the United States Department of Agriculture (USDA) indicated that of the 52 participating VDLs, the majority (71%) used disk diffusion for AST, with a combination of manual and automated zone of inhibition measurement.30 As more laboratories transition to the MIC methodology, projects such as those supported by USDA National Animal Health Laboratory Network and the Food and Drug Administration's Veterinary Laboratory Investigation and Response Network drive adoption of standard operating procedures and quality control. These highly collaborative projects have advanced standardization practices; however, significant data integration and sharing challenges remain due to multiple manufacturers and testing platforms.

While organizations, such as CLSI and the European Committee on Antimicrobial Susceptibility Testing, approve breakpoints and interpretations,31,32
the addition or revision of breakpoints does not result in immediate updates to lab infrastructure, software, and AST interpretation protocols. Manufacturers may offer AST panels containing a defined set of drugs and concentration ranges tailored to the species of animal and/or bacterial characteristics (eg, gram positive, gram negative), but the limited space for MIC dilutions means suppliers must make compromises between the number of antimicrobial agents and their respective dilutions.

When new breakpoints are established, it is possible that the breakpoint is no longer captured in the range of concentrations tested for the drug on the commercial panel used. In these cases, delays in implementation may be attributed to the time it takes for modification of AST panels by manufacturers and for laboratories to use current stocks of panels. This may warrant custom panel development, which is more resource intensive than standard commercial panels.

Information technology (IT) to mine data and construct antibiograms is a significant limitation in veterinary medicine. Differences in AST platforms, breakpoints/interpretive categories, and reporting algorithms, as well as the lack of laboratory system integration, mean that data from separate laboratory systems may be difficult or impossible to combine or merge; evaluations of historical results need to consider updated breakpoints. Although using MIC values to compare trends is more precise than interpretive categories alone, this will not resolve the fundamental limitation of dilution space on a panel. In cases where a breakpoint shifts to be outside the previous range of dilutions, no quantitative comparison of MICs will be possible. The development of more refined and comprehensive panels could allow laboratories to better address changes in breakpoints.

Performing whole genome sequencing (WGS) offers another way to evaluate the susceptibility of an isolate, independent of AST. While literature reports on correlations of MIC and WGS data for enteric pathogens shared with humans, studies on veterinary-specific pathogens are limited and recent. As the cost, complexity, and time required to perform WGS decrease, the clinical utility increases, with some caveats. Resistance gene presence does not necessarily confer clinical resistance and should be considered alongside other testing methods. Similarly, the absence of resistance genes does not confirm clinical susceptibility nor predict treatment outcomes. An additional benefit of WGS is the ability to compare strains at single nucleotide resolution to help optimize autogenous vaccines or inform infection control and prevention programs on farm. Despite these benefits, WGS cannot recognize new resistance genes, reducing its utility for emerging resistance, and should not replace or substitute for foundational testing methodologies. Culture-independent testing also has many limitations beyond the scope of discussion but that warrant consideration.

**Funding limitations**

Only a minority of responding VDLs routinely performed AST on all bacterial isolates. Many veterinary clients are not able or willing to pay for diagnostic testing. A recent pilot project at the Cornell Animal Hospital to assess the feasibility of subsidizing small animal AST found that subsidies needed to be hard coded into medical records systems for optimal uptake since an initial opt-in approach
increased the administrative burden on clinicians and IT teams and reduced participation. Future subsidy programs should implement automatic enrollment or otherwise increase efficiency through dedicated support staff, additional training, and incentives.

Since VDLs often do not have adequate resources for IT or staffing necessary to generate antibiograms, sustained investment in laboratory and technological infrastructure for AST and antibiogram development through funds furnished by federal, state, and local agencies are needed to support veterinary stewardship and a one-health approach.

**Conclusion**

**Educational needs for practicing veterinarians**

Laboratory outreach to veterinarians is necessary to provide education on how antibiograms complement other diagnostic testing in empiric antimicrobial selection and how to identify an antibiotic that is appropriate to guide therapy for their patient or patient population. Antibiograms should contain sufficient information about the source population to help the veterinarian determine its applicability, including the number of isolates incorporated. Recognizing the dosage regimen used to develop the breakpoints may also help veterinarians achieve treatment success, as underdosing or using a different dose frequency may result in treatment failure of a pathogen deemed susceptible on the antibiogram. Whenever possible, the laboratory should direct veterinarians to educational resources to aid with optimal clinical application.

**Opportunities and future development**

While integration of antibiograms continues to shape veterinary stewardship, building on existing infrastructure and exploring opportunities can encourage steady improvement and novel applications. Establishment of standardized procedures for antibiogram development and distribution allows laboratories to efficiently share cumulative susceptibility data with relevant clinical and public health stakeholders (Figure 1).

Guidelines for creating antibiograms have been published. However, entities planning to regularly publish antibiograms must be transparent about their approaches, including how often they will publish, how many isolates are needed, which breakpoints will be used, how often they will be updated or incorporate new data, how data curation will happen (eg, for removing duplicates from the same animal), and whether the raw, deidentified data will be deposited in publicly accessible data repositories. Maintaining quantitative test results allows for future analysis once anonymization and deidentification reduce confidentiality concerns. Given that breakpoints change over time, raw MIC or disk diffusion data should be retained with antibiograms to provide interpretive context. Additionally, access to raw AST reports may be valuable for future researchers’ reanalysis or aggregation, including to determine MDR among individual isolates.

Although regional or national antibiograms expand data breadth, the perceived potential misapplication of a national antibiogram may impede implementation. Comparing antibiograms between countries, for example, might be used to justify regulatory efforts to limit antimicrobial use or be offered as evidence of injudicious use. Interest in these antibiograms, however, may still drive development; regional disease and husbandry practices should be considered in such cases.

Producers may be reluctant to authorize the contribution of their data to antibiogram development; however, the value of stewardship programs warrants proactive mitigation of these concerns. Through guidelines and national standards, antibiogram users and developers should implement protocols to anonymize and deidentify data to promote participation and prevent the abuse of antibiograms to infer causes of change in antimicrobial susceptibility patterns. Regulations exempting or shielding these data from public requests could provide additional comfort with data sharing.

The total number, capabilities, and types of laboratories performing veterinary AST are unknown and variable. Sharing antibiograms and providing education on their use could provide added value to entities providing AST. One approach could be a laboratory antibiogram repository with user access for those who have created antibiograms (eg, laboratories, hospitals) and/or supplied cases. Although current infrastructure is lacking, demand from veterinarians and pressure from regulators and legislators to demonstrate the veterinary profession’s commitment to antimicrobial stewardship could be the impetus needed.

With adequate funding and participation from all stakeholders, further work can advance the development of antibiograms in veterinary medicine as a tool for antimicrobial drug selection and monitoring of local, regional, and national AMR trends in support of one-health goals for antimicrobial stewardship.

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